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Functional Pharmacology in Human Brain

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***Abstract*——Most neurological and psychiatric disorders involve selective or preferential impairments of neurotransmitter systems. Therefore, studies of functional transmitter pathophysiology in human brain are of unique importance in view of the development of effective, mechanism-based, therapeutic modalities. It is well known that central nervous system functional proteins, including receptors, transporters, ion channels, and enzymes, can exhibit high heterogeneity in terms of structure, function, and pharmacological profile. If the existence of types and subtypes of functional proteins amplifies the possibility of developing selective drugs, such heterogeneity certainly increases the likelihood of interspecies differences. It is therefore essential, before choosing animal models to be used in preclinical pharmacology experimentation, to establish whether functionally corresponding proteins in men and animals also display identical pharmacological profiles. Because of evidence that scaffolding proteins, trafficking between plasma membrane and intracellular pools, phosphorylation and allosteric modulators can affect the function of receptors and transporters, experiments with human clones expressed in host cells where the environment of native receptors is rarely reproduced should be interpreted with caution. Thus, the use of neurosurgically removed fresh human brain tissue samples in which receptors, transporters, ion channels, and enzymes essentially retain their natural environment represents a unique experimental approach to enlarge our understanding of human brain processes and to help in the choice of appropriate animal models. Using this experimental approach, many human brain functional proteins, in particular transmitter receptors, have been characterized in terms of localization, function, and pharmacological properties.**

I. Introduction

Receptors, transporters, ion channels, and enzymes in the central nervous system (CNS[1]) exhibit high heterogeneity in terms of structure, function, and pharmacology. These protein isoforms, particularly neurotransmitter receptors, have traditionally been classified by pharmacological differences in the affinities of antagonists/inhibitors. The use of techniques of functional pharmacology, together with the availability of selective ligands, has led to the identification of multiple receptive sites in which the same neurotransmitter binds to elicit various responses. Studies of molecular biology have confirmed the existence of receptor types and subtypes within a single species by showing that pharmacological differences reflect differences in primary structure. It has subsequently become apparent that functionally equivalent receptors between species can display distinct pharmacological profiles and molecular biologists have identified key amino acids responsible for imparting ligand specificity. Clearly, this high receptor heterogeneity increases the likelihood of interspecies differences, thus making of unique importance studies on human brain functional proteins.

Mechanisms by which synaptic strength is modified play critical roles in neurotransmission. In the CNS, modification of the strength of synapses can be produced through neurotransmitters/neuromodulators released by neurons and glia. A classic mechanism by which the strength of synapses can be modulated involves the activation of receptors localized on neuronal axon terminals and termed presynaptic receptors (Starke et al., 1989; Bonanno and Raiteri, 1993; Langer, 1993; Miller, 1998; Raiteri, 2001). Presynaptic receptors can be activated by transmitters/modulators released by the terminals on which the receptors are localized (presynaptic autoreceptors) or by transmitters/modulators originating from neighboring structures (presynaptic heteroreceptors). Although the classic presynaptic modulatory systems involve metabotropic G protein-coupled receptors (GPCRs), presynaptic ionotropic receptors also can modify transmitter release [see Engelman and MacDermott (2004) for a review].

As to neurotransmitter transporters, their roles in brain physiology and pathology are only understood in part. These proteins represent highly sophisticated systems able to play more refined roles than simple transmitter (re)uptake. All known transmitter transporters can, under some conditions, mediate transmitter release directly from the cytosol (Attwell et al., 1993; Levi and Raiteri, 1993). Transporters may regulate the time course of synaptic events by modifying the extent of activation of receptors and the level of their desensitization (Seal and Amara, 1999; Sims and Robinson, 1999). Transporters for different transmitters can coexist and interact with each other on the same neuron [see Raiteri et al. (2002a) for a review]. Interactions between transporters and presynaptic receptors, as well as trafficking of transporters between the plasma membrane and intracellular pools, have been described previously (Reith, 2002).

It is now well accepted that native neurotransmitter receptors and transporters rarely exist as isolated entities; instead, they more frequently consist of "complexes," sometimes composed of several proteins (see, for instance, Husi et al., 2000; Couve et al., 2004). There is convincing evidence that GPCRs can exist as homo- or heterooligomers. Oligomerizations seem to occur physiologically or pathologically and may determine the pharmacology of GPCRs (Bouvier, 2001; Agnati et al., 2003; Milligan, 2004). Functional changes in receptor properties can also originate from the trafficking of GPCRs as well as of ionotropic receptors which takes place under a variety of conditions [see Collingridge and Isaac (2003) and Tan et al. (2004) for reviews]. Another important factor that can elicit changes in the function and pharmacology of native receptors is represented by the interactions between two different receptors or between a

[1] Abbreviations: CNS, central nervous system; GPCR, G protein-coupled receptor; ACh, acetylcholine; nAChR, nicotinic ACh receptor; NE, norepinephrine; NMDA, *N*-methyl-D-aspartate; VSCC, voltage-sensitive Ca^{2+} channel; NO, nitric oxide; ARC239, 2-(2,4-(*O*-methoxyphenyl)-piperazin-1-yl)ethyl-4,4-dimethyl-1,3-(2*H*,4*H*)-isoquinolindione; DA, dopamine; SKF38393, 2,3,4,5-tetrahydro-7,8-dihydroxy-1-phenyl-1*H*-3-benzazepine; DAT, dopamine transporter; 5-HT, 5-hydroxytryptamine (serotonin); h5-HT_{1B}, human 5-HT_{1B}; h5-HT_{1D}, human 5-HT_{1D}; CCK, cholecystokinin; CCK-LI, CCK-like immunoreactivity; SRIF, somatostatin; SRIF-LI, SRIF-like immunoreactivity; AMPA, α-amino-3-hydroxy-5-methylisoxazide-4-propionic acid; DPDPE, [D-Pen^2,D-Pen^5]-enkephalin; N/OFQ, nociceptin/orphanin FQ; NE, norepinephrine; ORL1, opioid receptor-like 1; TLE, temporal lobe epilepsy; 4-AP, 4-aminopyridine; AHS, Ammon's horn sclerosis; CB, calbindin D_{28K}; EAA, excitatory amino acid; PD, Parkinson's disease; DBS, deep brain stimulation; GPi, internal globus pallidus; STN, subthalamic nucleus; GPe, external globus pallidus; L-745,870, 3-[[4-(4-chlorophenyl)piperazin-1-yl]methyl]-1-1*H*-pyrollo[2,3-*b*]pyridine; S14297, 7-*N*,*N*-dipropylamino-5,6,7,8-tetrahydro-naphtho(2,3*b*)dihydro,2,3-furane; SB-236057, 1*-ethyl-5-(2*-methyl-4*-(5-methyl-1,3,4-oxadiazol-2-yl)biphenyl-4-carbonyl)-2,3,6,7-tetrahydrospiro[furo[2,3-*f*]indole-3,4*-piperidine]; GR127935, *N*-[methoxy-3-(4-methyl-1-piperazinyl)phenyl]-2′-methyl-4′-(5-methyl-1,2,4-oxadiazol-3-yl) [1,1-biphenyl]-4-carboxamide hydrochloride; Ro60-0175, (*S*)-2-(6-chloro-5-fluoroindol-1-yl)-1-methylethylamine; CGP35348, 3-aminopropyl(diethoxymethyl)-phosphinic acid; CGP52432, 3[[(3,4-(dichlorophenyl)methyl]amino)-propyl] (diethoxymethyl)phosphinic acid; CGP47656, 3-aminopropyl-(difluoromethyl)phosphinic acid; CGP36742, (3-aminopropyl)*n*-butylphosphinic acid; CR2249, (*S*)-4-amino[(4,4-dimethylcyclohexyl)amino]oxopentanoic acid; CR2361, enantiomer of CR2249; U50488, (±)-*trans*-3,4-dichloro-*N*-methyl-*N*-[2-(1-pyrrolidinyl)-cyclohexyl]-benzeneacetamide; J-113397, 1-[(3*R*,4*R*)-1-cyclooctylmethyl-3-hydroxymethyl-4-piperidyl]-3-ethyl-1,3-dihydro-2*H*-benzimidazol-2-one; WIN55,212-2, *R*-(+)-[2,3-dihydro-5-methyl-3-[(morpholinyl)methyl]pyrrolo-[1,2,3-*de*]-1,4-benzoxazinyl]-(1-naphthalenyl)methanone mesylate; SR141716, *N*-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamidehydrochloride; AM404, *N*-(4 hydroxyphenyl)-arachidonamide; CP55940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)-phenyl]-*trans*-4-(3-hydroxy-propyl)-cyclohexanol; AM251, *N*-(piperidin-1-1yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide.

receptor and a transporter localized on the same plasma membrane. All these events might provide molecular explanations for native receptor pharmacology that is not easily explained by heterologous expression of human receptors, which are unlikely to find, in a foreign environment, the ingredients present in their natural milieu. Native functional proteins (receptors, transporters, ion channels, and enzymes) represent therefore the best systems when investigating synaptic receptor function, secretory and transport processes and their modulations. Unfortunately, due to serious degradative postmortem changes, functional studies in human brain generally require the availability of fresh brain tissue samples, which represents a major problem in following this approach.

The present article is essentially focused on functional studies performed with fresh human brain tissue not considered in my previous review (Raiteri, 1994). Data from laboratory animal experiments are here discussed only in comparative terms to help in the choice of the animal species possibly representing appropriate models to be used in preclinical studies.

II. Cholinergic Receptors

Muscarinic receptors and their subtypes were the first cholinergic receptors to be functionally identified and characterized in human brain. In particular, a number of studies were published in the early 1990s dealing with muscarinic autoreceptors mediating inhibition of acetylcholine (ACh) release from human neocortex tissues (Feuerstein et al., 1990, 1992; Marchi et al., 1990; Beani et al., 1992). These works have already been considered in my previous review (Raiteri, 1994). Curiously, to my knowledge, no new reports of functional studies on human brain muscarinic receptors have appeared in the last decade. In contrast, there has been increasing interest in the identification and pharmacological characterization of native nicotinic ACh receptors (nAChRs) through functional studies with samples of human brain obtained in neurosurgery.

Nicotine produces several central effects, some of which seem to be beneficial, e.g., increased alertness, reduced anxiety, analgesia, and facilitation of cognitive processes. Subtypes of the nAChR have been proposed as potential targets for drugs to be used in Alzheimer's disease and in other pathological conditions (Lloyd and Williams, 2000; Paterson and Nordberg, 2000); the knowledge of their function, structural subunit composition, and pharmacology in the human CNS is therefore of critical importance for the development of mechanism-based therapeutic approaches.

A. Norepinephrine Release Regulation by Nicotinic Receptors

Nicotinic receptors are largely localized on presynaptic axon terminals where they mediate regulation of neurotransmitter release (Wonnacott, 1997). Nicotine was found to cause norepinephrine (NE) release from human neocortex slices through activation of mecamylamine-sensitive mechanisms (Pittaluga et al., 1999). Although, based on experiments with rat brain synaptosomes, nAChRs mediating an increase in NE release can be thought to exist on noradrenergic nerve terminals (Clarke and Reuben, 1996; Luo et al., 1998; Vizi, 1998; Risso et al., 2004), to what extent nicotinic agonists directly act at noradrenergic terminals in human cortex to evoke NE release remains to be established; in fact, the release of NE elicited by nicotine in human cortical slices was in part prevented by glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonists, suggesting an indirect mechanism whereby nicotine can provoke glutamate release onto NMDA receptors mediating release of NE (Pittaluga et al., 1999). Nicotine has indeed been reported to increase glutamate release in various animal preparations (Gray et al., 1996; Fedele et al., 1998) as well as in human cerebrocortex (Marchi et al., 2002b; see below).

The release of NE evoked by nicotine from human cerebrocortical slices prepared from specimens removed during neurosurgery for intractable severe epilepsy was external Ca^{2+}-dependent and attenuated by the N-type voltage-sensitive calcium channel (VSCC) blocker ω-conotoxin GVIA (Woo et al., 2002), suggesting activation of VSCCs following depolarization provoked by nicotine. As the nAChRs involved are insensitive to α-bungarotoxin, the authors tend to exclude the theory that $\alpha 7$ subunit-containing nAChRs play a relevant role, although the use of some other selective antagonist would be advisable. Interestingly, Woo et al. (2002) found that the nicotine-evoked release of NE was prevented by nitric-oxide synthase inhibitors, indicating the involvement of nitric oxide (NO). Moreover, the nicotine effect was attenuated by guanylyl cyclase inhibitors and potentiated by the phosphodiesterase inhibitor zaprinast, consistent with the involvement of cyclic GMP. Although the authors, based on a few results with glutamate receptor antagonists, proposed that endogenous glutamate does not play a role in the nicotine effect, the stimulation by nicotine of the NO/cyclic GMP pathway would seem compatible with the idea that, in human neocortical slices, nicotine causes release of glutamate onto NMDA receptors mediating both NO production (Maura et al., 2000) and NE release (Fink et al., 1992a).

Nicotinic receptors mediating NE release have recently been characterized by Amtage et al. (2004) using fresh specimens of human neocortex obtained during surgical access to remove epileptic or neoplastic tissue. Slices were prepared, prelabeled with [^{3}H]NE, and exposed in superfusion to nicotinic receptor ligands. The authors compared the release of [^{3}H]NE from human neocortex slices to that from rat neocortex slices. The nicotine-evoked release of [^{3}H]NE from human slices was found to be reduced in part by glutamate receptor

antagonists, consistent with the findings and interpretation given by Pittaluga et al. (1999) that only part of the nAChRs involved in NE release are located on noradrenergic neurons, whereas some are on glutamatergic neurons from which glutamate is released onto noradrenergic terminals. Of note, Amtage et al. (2004) report that nicotinic agonists seem unable to evoke release of [^{3}H]NE from rat neocortical slices, which would make it inappropriate to use the rat cortex as a model to screen for NE release-enhancing nicotinic receptor agonists.

In their work Amtage et al. (2004) characterized pharmacologically the subunit composition of the nAChRs-mediating enhancement of NE release in the human neocortex by using a number of selective nAChR antagonists. The nicotine-evoked [^{3}H]NE release was sensitive to α-conotoxin MII, an antagonist that selectively blocks $\alpha3\beta2$- and $\alpha6$-containing nAChRs. On the other hand, α-conotoxin AuIB, a selective $\alpha3\beta4$-subtype antagonist, failed to block the nicotine effect, thus excluding the involvement of $\alpha3\beta4$ subunit-containing nAChRs, in contrast to some observations on rat brain tissue (Vizi and Lendvai, 1999; Anderson et al., 2000).

Surprisingly, Amtage et al. (2004) found no evidence for the presence of nicotinic autoreceptors on cholinergic terminals in both human and rat neocortex. The authors admit that their "conclusion is in contrast to previous studies with mice and rats which suggest the occurrence of presynaptic nicotinic autoreceptors in the rodent brain." But the negative findings of Amtage et al. (2004) particularly contrast with results by Marchi et al. (2002b) showing the presence of release-enhancing nicotinic autoreceptors on cholinergic axon terminals isolated from human neocortical tissue (see below). The reasons for these discrepancies may be manifold, although it seems that the use of human (or rat) slices prevents in some way the identification of presynaptic receptors that can be clearly observed in superfused isolated axon terminal preparations.

B. Nicotinic α7 Receptors and Glutamate Release

The existence of presynaptic nAChRs able to mediate glutamate release had been suggested by different authors, based on electrophysiological and neurochemical studies in the laboratory animals (McGehee and Role, 1995; Gray et al., 1996; Radcliffe and Dani, 1998). However, direct demonstration that release-regulating nAChRs exist on glutamatergic axon terminals both in rat and human brain was obtained only recently (Marchi et al., 2002b). Experiments performed with synaptosomes prepared from fresh specimens of human cerebral cortex showed that nAChR agonists evoked release of glutamate, an effect prevented by α-bungarotoxin or by methyllycaconitine at concentrations compatible with their selective blockade of nAChRs containing $\alpha7$ subunits. Similar results have been obtained using synaptosomes from the rat corpus striatum (Marchi et al., 2002b) and, more recently, the rat hippocampus and cortex (unpublished results). It may be added that the $\alpha7$ nAChRs involved are presynaptic heteroreceptors localized on glutamatergic axon terminals because the superfusion system used only shows release modulations of a given transmitter when they are consequent to direct actions on the synaptosomes releasing that transmitter, whereas indirect effects are prevented (see Raiteri and Raiteri, 2000).

The $\alpha7$ nicotinic receptors able to mediate glutamate release in the human cerebral cortex might be involved in the cognition-enhancing activity of nicotine (see Levin and Simon, 1998). The pharmacological similarity between human and rat nicotinic heteroreceptors mediating potentiation of glutamate release suggests that the rodent receptor could represent a useful model in the development of new $\alpha7$ nAChR ligands.

C. Nicotinic Autoreceptors

When isolated human neocortex nerve endings, prelabeled with [^{3}H]choline, were exposed in superfusion to nicotine or to ACh (in the presence of atropine to block inhibitory muscarinic autoreceptors), an increase in the release of [^{3}H]ACh was observed (Marchi et al., 2002b). The releasing effect of ACh + atropine was insensitive to α-bungarotoxin or to methyllycaconitine, thus excluding the involvement of $\alpha7$ nAChRs. The following considerations support the view that these autoreceptors mediating positive feedback regulation of ACh release are $\alpha4\beta2$ nAChRs as previously suggested for the rat nicotinic autoreceptor (Wilkie et al., 1996): 1) dihydro-β-erythroidine, which completely blocked the effect of ACh at the autoreceptor, has been reported to be reasonably selective for the $\alpha4\beta2$ subtype (Albuquerque et al., 2000); 2) the EC_{50} values for (−)-nicotine (1 μM) and ACh (5 μM) in activating human autoreceptors are in keeping with those found at $\alpha4\beta2$ receptors heterologously expressed in different systems (McGehee and Role, 1995; Gopalakrishnan et al., 1997); 3) nicotine was significantly more potent on human $\alpha4\beta2$ than on human $\alpha4\beta4$, $\alpha2\beta4$,$\alpha3\beta2$, and $\alpha3\beta4$ receptors (Stauderman et al., 1998); 4) $\alpha4\beta2$ nAChRs constitute the major subtype lost in the cortex and hippocampus of Alzheimer's patients (Warpman and Nordberg, 1995), compatible with their presence on degenerating cholinergic axon terminals.

It seems important that the nicotinic autoreceptors localized on cholinergic terminals represent distinct nAChRs compared with the nicotinic heteroreceptors present on glutamatergic nerve endings: these two native subtypes of nAChR have different neuronal localization, subunit composition, function, and pharmacology (Marchi et al., 2002b). The pharmacological diversity offers a number of opportunities in terms of therapeutic intervention: autoreceptor ($\alpha4\beta2$) agonists and heteroreceptor ($\alpha7$) agonists are expected to reinforce the acetylcholine-glutamate pathway by increasing, respectively, ACh release and glutamate release.

Moreover, the pharmacology of these native human nAChRs seems very similar to that of the corresponding native rat receptors. Thus, monitoring glutamate and ACh release from rat brain synaptosomes may represent a convenient approach to functionally evaluate novel ligands at $\alpha7$- or $\alpha4\beta2$-containing nAChRs.

III. Adrenergic Receptors

The existence of several types and subtypes of adrenergic receptors and the consequent possibility of species differences would require extensive characterization of human adrenoceptors before appropriate animal models are chosen. However, functional studies of adrenergic receptors in human brain have focused exclusively on α_2-receptors regulating neurotransmitter release. Of these studies, those describing α_2-adrenergic regulation of ACh and glutamate release (Beani et al., 1992), of GABA release (Ferraro et al., 1993) and of serotonin release (Raiteri et al., 1990; Feuerstein et al., 1993) from human cerebral cortex tissues have already been considered in my previous review (Raiteri, 1994).

A. Norepinephrine Release Regulation through Autoreceptors

Similarly to what had been shown in several laboratory animal species [see Starke et al. (1989) for a review], human neocortex autoreceptors exhibit an α_2-adrenoceptor pharmacology (Raiteri et al., 1992). Experiments with human neocortical slices prelabeled with [^{3}H]NE and stimulated electrically showed that the Ca^{2+}-dependent and tetrodotoxin-sensitive release of the catecholamine was inhibited by clonidine (an α_2 agonist) and by oxymetazoline (an α_2 agonist that displays high-affinity for α_{2A}/α_{2D} receptors and low affinity for the α_{2B}/α_{2C} subtypes). Moreover, the human autoreceptors were insensitive to prazosin and to ARC239 (antagonists that exhibit high affinity for α_{2B} and α_{2C} adrenoceptors but low affinity for α_{2A}/α_{2D} subtypes). Based on these results, obtained with the few selective ligands available in the early 1990s, it was concluded that the presynaptic α_2-autoreceptors that modulate NE release in the human neocortex are not α_{2B}/α_{2C} but are either α_{2A} or α_{2D} (Raiteri et al., 1992).

It was subsequently established that α_{2A}- and α_{2D}-adrenoceptors are species orthologs, of which only one occurs in a given species and that humans possess the α_{2A} version, whereas rodents possess the α_{2D} version (Sastre and García-Sevilla, 1994; Bylund, 1995). Human neocortical α_2-autoreceptors therefore belong to the α_{2A} subtype of the adrenoceptor.

In 2000, Feuerstein et al. reinvestigated the subtype to which the α_2-autoreceptors belong in the human neocortex by using nine antagonists, including prazosin and ARC239, of which the authors could evaluate the dissociation constants (pK_B values) at the autoreceptors. The pK_B values of prazosin and ARC239 were the lowest (more than 2 orders of magnitude lower than that of rauwolscine), in keeping with the previous findings of Raiteri et al. (1992) showing insensitivity of the human autoreceptors to the two compounds. Compared with binding or functional results from the literature, the pK_B values of the nine antagonists tested by Feuerstein et al. (2000) correlated best with the antagonist affinities at α_{2A} binding sites, leading the authors to the definitive conclusion that the human neocortex presynaptic autoreceptors are α_{2A}. In considering that the rodent release-regulating α_2-autoreceptors belong to the α_{2D} subtype (Limberger et al., 1995), whereas the rabbit and pig cortical autoreceptors are α_{2A} (Limberger et al., 1995; Trendelenburg et al., 1997), it seems appropriate to use brain cortical tissues from guinea pigs or pigs as human autoreceptor models.

IV. Dopamine Receptors and Transporters

A. Dopamine Autoreceptors

The existence of release-regulating dopamine (DA) autoreceptors in human brain was investigated in slices from fresh specimens of human neocortex that were labeled with [^{3}H]DA and stimulated electrically (Fedele et al., 1993). Whereas the selective DA-1 receptor agonist SKF38393 did not affect the electrically evoked release of DA, quinpirole, an agonist at the receptors of the DA-2 family, with preference for the DA-2/DA-3 subtypes, inhibited the evoked release in a concentration-dependent manner. Quinpirole was antagonized by (−)-sulpiride, a DA-2 receptor antagonist with preference for the DA-2/DA-3 subtypes.

In a subsequent work, Fedele et al. (1999) investigated the pharmacological profile of DA autoreceptors in human neocortex further, to establish the subtype (DA-2, DA-3, or DA-4) to which the receptors belong. The quinpirole inhibition of the electrically evoked DA release was unaffected by the selective DA-4 receptor antagonist L-745,870 and by the selective DA-3 receptor antagonist S14297, leading to the conclusion that, in human neocortex, the release of DA in the terminal region of midbrain dopaminergic neurons is regulated through autoreceptors of the DA-2 subtype.

To identify a readily accessible model to be used in the development of selective DA-2 receptor ligands, Fedele et al. (1999) compared the results from human neocortical slices with those obtained in three animal systems: rat neocortical slices, rat striatal slices, and rat mesencephalic neuronal cultures, from which DA release was electrically stimulated. Results from rat striatal slices, mesencephalic neurons and human neocortical slices were superimposable, whereas quantitative differences emerged in the case of rat cortical slices.

B. Drugs of Abuse and Dopamine Release

Drug addiction is believed to result from the reinforcing properties of drugs of abuse on central reward sys-

tems, in particular on mesolimbic DA pathways (Di Chiara and Imperato, 1988; Koob et al., 1998). Although microdialysis experiments in rodents indicate that ethanol can evoke DA release in the nucleus accumbens (Di Chiara and Imperato, 1988; Rossetti et al., 1992; Weiss et al., 1993), studies on the effect of alcohol consumption by humans on dopaminergic transmission are very rare. Boileau et al. (2003) measured DA release in the ventral striatum/nucleus accumbens of six healthy subjects in response to alcohol oral ingestion using positron emission tomography and the DA receptor ligand [^{11}C]raclopride. Previous experiments in primates had shown that the binding of [^{11}C]raclopride is inversely proportional to the extracellular DA concentration in the striatum [see Laruelle (2000) for a review]. Boileau et al. (2003) observed a significant reduction in [^{11}C]raclopride binding potential bilaterally in the ventral striatum/nucleus accumbens, indicative of increased extracellular DA. The above technique had previously been used in humans to evaluate extracellular DA augmentation in response to various psychostimulants (Carson et al., 1997; Schlaepfer et al., 1997; Drevets et al., 2001; Volkow et al., 2001; Leyton et al., 2002; Martinez et al., 2003). Altogether, these findings support the idea that mesolimbic dopaminergic activation is a common property of abused drugs in humans.

C. Drugs of Abuse and Dopamine Transporters

Dopamine transporters (DATs) are targets for cocaine and other psychostimulants (Amara and Kuhar, 1993). In particular, cocaine potentiates dopaminergic transmission by blocking the reuptake of DA, leading to elevations in the synaptic level of the neurotransmitter. In vivo imaging studies in humans and in vitro binding studies in postmortem human brain have shown that chronic cocaine abuse results in an increase in DAT binding site density in the nucleus accumbens [see Mash et al. (2002) for references]. These data do not indicate, however, whether the increase in DAT binding sites reflects and increase in the function of DA transporters. Mash et al. (2002) measured uptake of [^{3}H]DA in metabolically active synaptosomes isolated from cryoprotected human brain specimens. The uptake of DA was elevated 2-fold in the ventral striatum from cocaine users compared with age-matched drug-free control subjects. This seems to be the first demonstration of adaptations in DA uptake in postmortem human brain. Based on the results, it is likely that chronic cocaine use, by causing an increase in DAT levels, results in a reduced amount of synaptic DA available to stimulate postsynaptic receptors. According to Mash et al. (2002), "the need to maintain homeostasis within central dopaminergic systems may be one of the factors that drives the compulsive use of cocaine."

V. Serotoninergic Receptors

Pharmacological studies as well as molecular cloning of 5-hydroxytryptamine (5-HT; serotonin) receptors have revealed high receptor heterogeneity. Seven major types of the 5-HT receptor have been identified and termed 5-HT$_1$ to 5-HT$_7$. Most of these receptor types are heterogeneous and occur as subtypes. Species homologs of the same receptor subtype may exist which, despite high structural homology, may display pronounced pharmacological differences (Hoyer and Middlemiss, 1989; Hoyer et al., 1994; Martin and Humphrey, 1994; Hartig et al., 1996).

Among the functions that have been attributed to 5-HT receptors, animal studies have shown that 5-HT receptor activation can mediate modulation of the release of various neurotransmitters. A number of functional studies with native human brain 5-HT receptors have been performed and deal with both autoreceptors and heteroreceptors.

A. Release of Serotonin and Control by Human 5-Hydroxytryptamine 1B Autoreceptors

Human neocortical slices, prelabeled with [^{3}H]5-HT, were found to release the [^{3}H]indoleamine upon electrical stimulation. This release was Ca^{2+}-dependent and could be inhibited by exogenous 5-HT. The inhibition was prevented by methiothepin, a broad spectrum 5-HT receptor antagonist (Schlicker et al., 1985). These results show that release-inhibiting presynaptic 5-HT autoreceptors, previously found to be present in the rat brain (Cerrito and Raiteri, 1979; Göthert and Weinheimer, 1979), also exist in the human brain.

Based on pharmacological studies, rat brain 5-HT autoreceptors had originally been classified as 5-HT$_{1B}$ subtype (Engel et al., 1986; Maura et al., 1986). However, 5-HT$_{1B}$ binding sites could not be detected in the human brain (Hoyer et al., 1986); therefore, it was expected that the human autoreceptors would have been pharmacologically different from those in the rat brain.

A subtype of the 5-HT binding site, termed 5-HT$_{1D}$, was then identified in human brain (Herrick-Davis et al., 1988; Hoyer et al., 1988) as well as in the brain of other species lacking the 5-HT$_{1B}$ site (Bruinvels et al., 1992) and found to display a regional distribution similar to that of the 5-HT$_{1B}$ site in rodents (Waeber et al., 1989). On the basis of this new information, receptors regulating the release of [^{3}H]5-HT from human neocortex slices (Galzin et al., 1992) and human neocortex synaptosomes (Maura et al., 1993) were proposed to belong to the 5-HT$_{1D}$ subtype.

Subsequent cloning studies revealed that 5-HT$_{1D}$ receptors are heterogeneous: two members of the subfamily, which were called 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$, were identified in the human brain (Hartig et al., 1992). Interestingly, replacement by site-directed mutagenesis of the threonine residue present at position 355 in the

human 5-$HT_{1D\beta}$ receptor with the corresponding asparagine found in rodent 5-HT_{1B} receptors was found to render the pharmacology of the receptors, originally quite different, essentially identical (Oksenberg et al., 1992; Parker et al., 1993). The human 5-$HT_{1D\beta}$ and the rodent 5-HT_{1B} receptors are encoded by a corresponding gene and may have the same biological functions in the two species. Having assumed this, it was proposed that the human autoreceptor be classified as 5-$HT_{1D\beta}$ (now termed h5-HT_{1B}) (Maura et al., 1993; Fink et al., 1995).

A clear confirmation that, in human cerebral cortex, the terminal 5-HT autoreceptor is of the h5-HT_{1B} subtype came from experiments with an h5-HT_{1B} selective ligand, SB-236057, which was shown to block the human terminal autoreceptor in a release study with human neocortex slices stimulated electrically (Middlemiss et al., 1999).

The available literature shows that h5-HT_{1B} receptors differ pharmacologically from the rodent r5-HT_{1B} receptors (Oksenberg et al., 1992; Parker et al., 1993). Therefore, the rodent autoreceptor cannot be useful as a model for the human counterpart. Other animal species, including guinea pig, pig, and rabbit, possess 5-HT autoreceptors that pharmacologically resemble the h5-HT_{1B} subtype and may represent appropriate models for the human 5-HT autoreceptor.

B. Glutamate Release and Modulation by Human 5-Hydroxytryptamine 1D Receptors

Animal studies suggest that 5-HT can interact with glutamate through multiple receptors to inhibit excitatory transmission in the CNS [see Maura et al. (1998) and references therein]. Because excessive glutamate release has been implicated in a number of pathophysiological conditions, understanding how the release of the excitatory transmitter can be regulated in the human CNS may lead to novel therapeutic avenues.

Several years ago we found that the release of glutamate from rat cerebellar synaptosomes could be potently inhibited through the activation of an unknown subtype of the 5-HT_1 receptor that we suggested be named 5-HT_{1D} (Raiteri et al., 1986). When the rat 5-HT_{1D} (now termed r5-HT_{1D}) was cloned, it was proposed to represent the species homolog of the human 5-HT_{1D} (now h5-HT_{1D}) receptor (Hamblin and Metcalf, 1991; Hartig et al., 1996).

To investigate whether 5-HT could directly modulate the release of glutamate in human brain, the efflux of glutamic acid and its modulation by 5-HT were studied in synaptosomal preparations from fresh neocortical samples obtained from patients undergoing neurosurgery to reach deeply sited tumors. Depolarization with high-K^+ elicited Ca^{2+}-dependent release of endogenous glutamate, which was inhibited by exogenous 5-HT and by the 5-$HT_{1B/1D}$ selective agonist sumatriptan. The agonist effects were blocked by concentrations of ketanserin known to antagonize preferentially the h5-HT_{1D} versus the h5-HT_{1B} receptors (Maura et al., 1998).

These results represent the first functional characterization of a 5-HT_{1D} receptor in the adult human brain. In considering that the human terminal autoreceptor is h5-HT_{1B}, the emerging pharmacological differences between h5-HT_{1B} auto- and h5-HT_{1D} heteroreceptors (see below) may lead to the development of novel therapeutic agents. Inhibition by 5-HT of glutamatergic transmission may be useful in a variety of pathological conditions including epilepsy [see Clough et al. (1996) and references therein], ischemia (Marcoli et al., 2004) (see also section XIII.C.) and depression [see Bonanno et al. (2005) and references therein].

C. Pharmacological Diversity between Human 5-Hydroxytryptamine 1B and Human 5-Hydroxytryptamine 1D Receptors

h5-HT_{1B} and h5-HT_{1D} receptors have been reported to have relatively low (63%) overall amino acid homology; in contrast, for a series of 19 structurally diverse compounds, the two cloned human receptors were originally found to be nearly indistinguishable in their binding affinities (Hartig et al., 1992; Weinshank et al., 1992). This view, based on radioligand binding experiments with cell lines expressing the cloned h5-HT_{1B} and h5-HT_{1D} receptors, is no longer tenable in the light of the functional studies of native receptors carried out with human neocortex synaptosomes (Marcoli et al., 1999). This study analyzed the pharmacological profiles of the presynaptic h5-HT_{1B} autoreceptors regulating the depolarization-evoked release of [^{3}H]5-HT and of the presynaptic h5-HT_{1D} heteroreceptors regulating the depolarization-evoked release of endogenous glutamate from human cerebral cortex synaptosomes. The nerve terminals were exposed in superfusion to eight serotonergic ligands during depolarization. Whereas two compounds, sumatriptan and methiothepin, behaved similarly at the auto- and at the heteroreceptors, the behavior of the other six compounds at the h5-HT_{1B} autoreceptor differed strikingly from that at the h5-HT_{1D} heteroreceptor (Table 1). Of note, some drugs known as 5-HT receptor antagonists, in particular metergoline and GR127935,

TABLE 1
Pharmacological diversity between h5-HT_{1B} autoreceptors regulating 5-HT release and h5-HT_{1D} heteroreceptors regulating glutamate release in human neocortex

Data taken from Marcoli et al. (1999).

Drug	h5-HT_{1B}	h5-HT_{1D}
Sumatriptan	Agonist	Agonist
Methiothepin	Antagonist	Antagonist
Ketanserin	Inactive	Antagonist
GR127935	Antagonist	Agonist
Metergoline	Antagonist	Agonist
SB-224289	Antagonist	Inactive
BRL-15572	Inactive	Antagonist
(+)-WAY 100135	Inactive	Agonist

SB-224289, 2,3,6,7-tetrahydro-1′-methyl-5-[2′-methyl-4′(5-methyl-1,2,4-oxadiazol-3-yl)biphenyl-4-carbonyl]furo[2,3*f*]indole-3-spiro-4′-piperidine hydrochloride; BRL-15572, 3-[4-(4-chlorophenyl)piperazin-1-yl]-1,1-diphenyl-2-propanol HCl; (+)-WAY 100135, *N*-tert-butyl-3-4-(2-methoxyphenyl) piperazin-1-yl-2-phenylpropanamide.

behaved as blockers at the autoreceptors but exhibited potent intrinsic activity at the heteroreceptors, thus strongly inhibiting glutamate release.

To conclude, our functional studies of native h5-HT_{1B} and h5-HT_{1D} receptors in the cerebral cortex show that the two receptors, originally reported to be pharmacologically identical (Weinshank et al., 1992), exhibit in fact a strikingly different pharmacology. The finding that compounds known to behave as pure 5-HT receptor antagonists in experiments with laboratory animals become full agonists at native 5-HT_{1D} receptors in human brain may be due to several reasons, including the existence of a high reserve of h5-HT_{1D} receptors. Whatever the explanation, considering the variability of the results obtained with cell systems expressing recombinant receptors, as well as the species variations in ligand affinities previously observed (see Zgombick et al., 1997), it is evident that functional studies with native human receptors remain of unique importance.

The pharmacological diversity of human 5-HT_{1B} and 5-HT_{1D} receptors augurs well for the potential development of receptor-selective drugs. In particular, the clear differences existing between release-regulating h5-HT_{1B} terminal autoreceptors and h5-HT_{1D} terminal heteroreceptors in human brain open the possibility for development of drugs that are selective autoreceptor antagonists or heteroreceptor agonists or both and that may represent novel mechanism-based therapeutic approaches potentially useful in conditions characterized by defective serotonergic transmission or/and excessive glutamatergic transmission including depression, epilepsy, and neurodegenerative diseases.

D. GABA Release and Modulation by 5-Hydroxytryptamine

Feuerstein et al. (1996a) investigated the presence of 5-HT receptors regulating the release of GABA provoked by electrical stimulation of human and rabbit neocortical slices. The slices were prelabeled with [^{3}H]glutamine to study the release of endogenously formed [^{3}H]GABA. The release of [^{3}H]GABA elicited by electrical stimulation was external Ca^{2+}-dependent and tetrodotoxin-sensitive. The addition of sumatriptan, a 5-$HT_{1B/1D}$ receptor agonist, decreased the release of [^{3}H]GABA, whereas methiothepin, a broad spectrum 5-HT receptor antagonist, prevented the sumatriptan effect. The use of slices does not permit a precise localization of the receptor, whether on the soma/dendrites or the terminals of GABA neurons or on both. As to the subtype of the 5-receptor involved, the authors proposed the 5-HT_{1D} as the most likely. As discussed above, in 1996, the human 5-HT_{1D} nomenclature comprised two receptors termed 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (now h5-HT_{1D} and h5-HT_{1B}); accordingly Feuerstein et al. (1996a) proposed that the 5-HT_{1D} receptor regulating GABA release in human neocortex was the 5-$HT_{1D\alpha}$ or 5-$HT_{1D\beta}$ subtype. The pharmacology of this receptor could be characterized more precisely by using the tools now available (see Marcoli et al., 1999).

Interestingly, in rabbit neocortex slices, the release of GABA could not be inhibited by sumatriptan. In addition, 5-carboxamidotryptamine, a broad spectrum 5-HT_1 agonist, was unable to affect the release of GABA evoked by electrical stimulation of rabbit neocortex, suggesting that receptors of the 5-HT_1 type regulating GABA release may not exist on GABAergic neurons of the rabbit neocortex.

E. Serotonin Inhibition of the N-Methyl-D-aspartate Receptor/Nitric Oxide/Cyclic GMP Pathway

The NMDA receptor/NO/cyclic GMP pathway and its possible modulation by 5-HT were studied in slices of human neocortex (Maura et al., 2000). Exposure of slices to NMDA caused cyclic GMP elevation, which was blocked by NO synthase and guanylate cyclase inhibitors. The NMDA effect was potently prevented by 5-HT or by the 5-HT_2 receptor agonist (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. Further pharmacological analysis with 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptor antagonists and the novel selective 5-HT_{2C} agonist Ro60-0175 showed that the 5-HT_2 receptor involved belongs to the 5-HT_{2C} subtype.

The 5-HT_{2C} receptor is not the only serotonergic receptor involved, however. In fact, the 5-HT_{1A} agonist 8-OH-DPAT inhibited the NMDA-evoked cyclic GMP response, an effect blocked by selective 5-HT_{1A} antagonists.

The work by Maura et al. (2000) also described the ability of the antidepressant trazodone to inhibit the NMDA receptor/NO/cyclic GMP pathway through the activation of 5-HT_{2C} receptors. The mechanism of action of trazodone as an antidepressant is not well understood, although a number of authors have proposed interactions with the serotonergic system [see Maura et al. (2000) for references]. The finding that trazodone, at concentrations compatible with those reached during antidepressant treatment, inhibited the NMDA receptor-mediated production of NO and cyclic GMP in human neocortical slices through receptors of the 5-HT_{2C} subtype suggests that activation of 5-HT_{2C} receptors could be relevant to the antidepressant activity of trazodone and, possibly, of selective serotonin reuptake inhibitors that also indirectly activate 5-HT_{2C} receptors. It should be noted that antidepressants were reported to produce adaptive changes, generally inhibitory, of NMDA receptor functions (Leonard, 1997; Nowak et al., 1998).

Altogether, the results available from experiments with fresh human brain tissues indicate that 5-HT is a major inhibitory agent of glutamatergic transmission in the human cerebral cortex able to act at multiple sites (Fig. 1). Not only can serotonin inhibit the evoked release of glutamate from nerve terminals by acting at presynaptic h5-HT_{1D} receptors (Maura et al., 1998); it

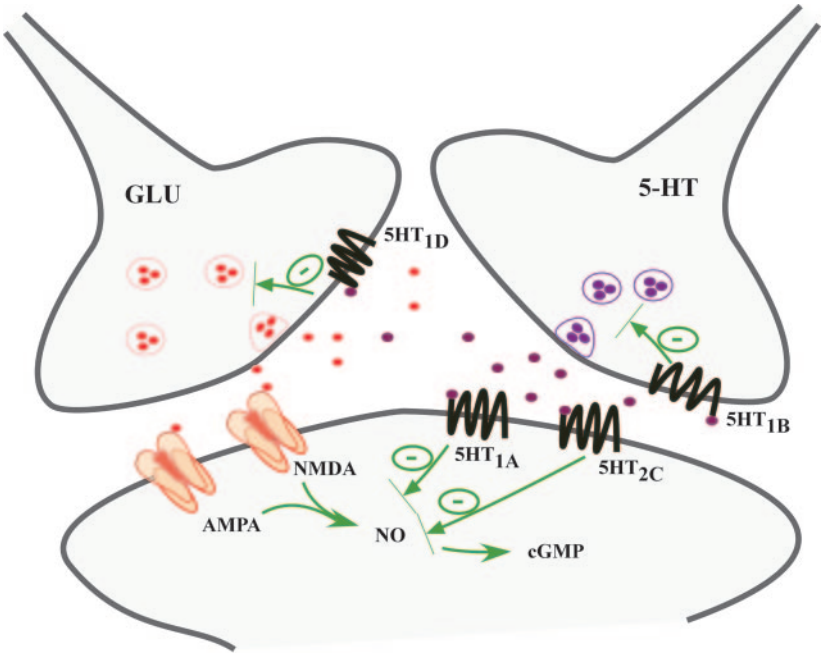

FIG. 1. Multiple 5-HT receptors regulate glutamatergic transmission in the human neocortex. Serotonin released from 5-HT terminals can activate 5-HT_{1B} autoreceptors mediating negative feedback regulation of 5-HT exocytosis and 5-HT_{1D} heteroreceptors mediating inhibition of release from glutamate (GLU) terminals. Serotonin, acting at postsynaptic 5-HT_{1A} and 5-HT_{2C} receptors, can also depress the NO/cyclic GMP production elicited by activation of postsynaptic ionotropic glutamate receptors.

also can inhibit events triggered by glutamate release, i.e. the NMDA receptor/NO/cyclic GMP pathway, by acting at postsynaptic receptors of the 5-HT_{2C} and 5-HT_{1A} subtype. Agonists at human 5-HT_{1D}, 5-HT_{2C}, and 5-HT_{1A} receptors or antagonists at 5-HT_{1B} autoreceptors, deserve attention as potentially useful drugs in neuropathologies with underlying defective serotonergic or excessive glutamatergic transmission.

VI. GABA Receptors

Ionotropic $GABA_A$ receptors have been the object of a number of functional studies with human brain tissue removed to treat drug-resistant epilepsies (see section XII.A.1.). As to receptors of the $GABA_B$ type, several reports deal with these metabotropic GABA receptors in human brain and are mainly focused on the existence of $GABA_B$ receptor subtypes [see Bonanno and Raiteri (1993) and Bowery et al. (2002) for reviews].

A. $GABA_B$ Receptor Subtypes: Pharmacological Evidence

1. $GABA_B$ Autoreceptors. The release of GABA from human cerebral cortex synaptosomes depolarized in superfusion with high-K^+ was inhibited by the $GABA_B$ receptor agonist (−)-baclofen, but not by the $GABA_A$ agonist muscimol, suggesting the presence of release-regulating $GABA_B$ autoreceptors in human neocortex GABAergic terminals (Bonanno et al., 1989). The existence of $GABA_B$ autoreceptors in human CNS was confirmed when the introduction of potent, selective $GABA_B$ receptor antagonists (Froestl et al., 1995) facilitated studies of $GABA_B$ receptors. In particular, the human $GABA_B$ autoreceptor was characterized using three antagonists: phaclofen, CGP35348 and CGP52432 (Fassio et al., 1994). The effect of (−)-baclofen on the evoked release of GABA from human neocortex synaptosomes was differentially reduced by the three antagonists: CGP52432 was by far the most potent (IC_{50} = 0.09 μM); phaclofen was much less potent (IC_{50} = 70 μM); CGP35348 was ineffective up to 100 μM. These results parallel those previously obtained with the neocortex of the rat (Bonanno and Raiteri, 1993; Lanza et al., 1993), indicating that human and rat cortical autoreceptors are pharmacologically very similar (Table 2).

TABLE 2
$GABA_B$ receptor subtypes regulating transmitter release in human and rat neocortex

Reported are IC_{50} values of $GABA_B$ receptor antagonists tested against (−)-baclofen. Reproduced from Bowery et al. (2002).

Drug	IC_{50} for Neurotransmitter			
	GABA	GLU	SRIF	CCK
		μM		
Rat neocortex				
Phaclofen	79.2	>300	62.6	66.1
CGP35348	>300	4.2	3.6	3.5
CGP52432	0.08	9.3	3.4	0.11
CGP47656	3.1	(Ago)	Ago	>300
CGP36742	>100	>100	0.14	>100
Human neocortex				
Phaclofen	~100	>300	N.D.	N.D.
CGP35348	>100	~10	24.4	13.9
CGP52432	≪1	>1; <30	0.06	0.08
CGP47656	<10	N.D.	Ago	Ago
CGP36742	>100	>100	~5	>100

GLU, glutamate; SRIF, somatostatin; Ago, agonist; (Ago), partial agonist; CCK, cholecystokinin; N.D., not determined.

2. $GABA_B$ Heteroreceptors Regulating Neuropeptide Release. Human neocortex synaptosomes released cholecystokinin-like immunoreactivity (CCK-LI) and somatostatin-like immunoreactivity (SRIF-LI) in a Ca^{2+}-dependent manner when depolarized in superfusion with

high-K^+. The $GABA_B$ receptor agonist (−)-baclofen inhibited both the evoked release of CCK-LI and SRIF-LI. The effect of (−)-baclofen was mimicked by CGP47656 and blocked by CGP35348 or CGP52432. Interestingly, in experiments of GABA release, CGP47656 behaved as an antagonist at the $GABA_B$ autoreceptors (Bonanno et al., 1996; Raiteri et al., 1996). Together, these results indicate that 1) CCK- and SRIF-releasing terminals in human neocortex possess release-inhibiting $GABA_B$ heteroreceptors; 2) these receptors differ pharmacologically from the human $GABA_B$ autoreceptors since the latter are CGP35348-insensitive (Fassio et al., 1994) and can be blocked by CGP47656; 3) human heteroreceptors regulating CCK and SRIF release seem to be pharmacologically similar to the corresponding receptors found in rats (Gemignani et al., 1994; Table 2). Cholecystokinin has been implicated in anxiety (Woodruff and Hughes, 1991; Harro et al., 1993); thus, the finding that inhibitory $GABA_B$ heteroreceptors present on CCK-releasing terminals differ pharmacologically from the $GABA_B$ autoreceptors mediating inhibition of GABA release suggests that $GABA_B$ heteroreceptor-selective agonists may have therapeutic potential as novel anxiolytics. As to the somatostatinergic system, reduced release of SRIF seems to play a role in cognitive impairments typical of Alzheimer's disease (Gabriel et al., 1993) or accompanying other pathologies; selective $GABA_B$ heteroreceptor antagonists could lead to increased SRIF release and consequently to cognitive improvement (see below).

A $GABA_B$ receptor antagonist, CGP36742, had been reported to improve the performances of mice, rats, and monkeys in tests covering diverse manifestations of learning and memory (Mondadori et al., 1993; Froestl et al., 1995). We examined the ability of CGP36742 to block release-regulating $GABA_B$ receptors. In particular, CGP36742 was tested against the inhibition of the depolarization-evoked release of GABA, glutamate, CCK, and SRIF produced by (−)-baclofen in rat and human neocortex axon terminals (Bonanno et al., 1999). It was found (Table 2) that CGP36742 potently antagonized the inhibition by (−)-baclofen of the release of SRIF from both rat (IC_{50} = 0.14 μM) and human (IC_{50} ~ 5 μM) neocortex synaptosomes. In contrast, the effects of (−)-baclofen on GABA, glutamate, and cholecystokinin release were insensitive to CGP36742 at concentrations up to 100 μM. CGP36742 is the first $GABA_B$ receptor antagonist displaying great selectivity for the $GABA_B$ presynaptic receptors regulating SRIF release. Considering the implication of the neuropeptide in cognitive processes, disinhibition of SRIF release with activation of SRIF receptors may represent one of the mechanisms involved in the behavioral activity of CGP36742. In the rat brain, the CGP36742-induced somatostatin (sst5) receptor activation was found to facilitate the function of NMDA receptors (Pittaluga et al., 2000, 2001a), which are known to play a primary role in cognitive processes. If humans respond as rats, CGP36742 (now termed SGS742) could act according to the scheme shown in Fig. 2 Interestingly, CGP36742/SGS742 is the first $GABA_B$ receptor antagonist entered into clinical trials. In a phase II double-blind, placebo-controlled study in 110 patients with mild cognitive impairment, oral adminis-

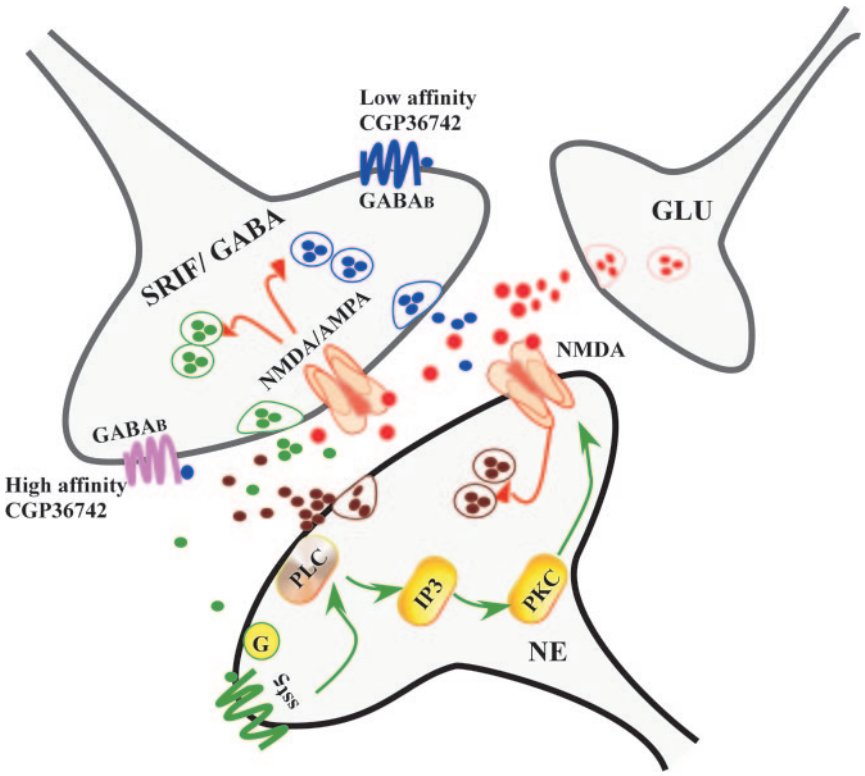

FIG. 2. Schematic representation of the neurotransmitter interactions that may underlie the cognitive enhancing activity of CGP36742 (now termed SGS742). GABA and somatostatin (SRIF) are often colocalized in interneurons, although purely GABAergic terminals (not shown) also exist. Glutamate (GLU; red dots) evokes release of norepinephrine (NE; brown dots), GABA (blue dots), and SRIF (green dots) by activating ionotropic NMDA/AMPA receptors located on the respective axon terminals. GABA can inhibit its own release and SRIF release through different $GABA_B$ receptor subtypes. CGP36742 (SGS742) binds with high affinity to the $GABA_B$ heteroreceptor (violet) modulating SRIF release but with very low affinity to the $GABA_B$ autoreceptor (blue) modulating GABA release (Bonanno et al., 1999). SRIF positively modulates NE release by activating sst_5 receptors (green) colocalized with NMDA receptors on noradrenergic terminals. When NMDA is added to slices, GABA is released and inhibits the release of SRIF. CGP36742 (SGS742) prevents this GABAergic inhibition, allowing the neuropeptide to reach the sst_5 receptors on NE terminals. These SRIF receptors trigger activation of NMDA receptors through the phospholipase C (PLC)-inositol trisphosphate (IP_3)-protein kinase C (PKC) pathway (Pittaluga et al., 2005).

tration of the drug significantly improved attention as well as working memory. A second phase II clinical trial in 280 patients with Alzheimer's disease is underway (Froestl et al., 2004).

3. $GABA_B$ Heteroreceptors Regulating Glutamate Release. In a comparative study on the release of endogenous glutamate and endogenous GABA from human neocortex synaptosomes, the effects of three selective $GABA_B$ receptor antagonists on the inhibition of the depolarization-evoked release of the two amino acids elicited by (−)-baclofen were investigated (Bonanno et al., 1997). Phaclofen antagonized the effect of (−)-baclofen on GABA release but did not modify that on the release of glutamate. The inhibition by (−)-baclofen of the release of GABA was insensitive to CGP35348 which, in contrast, blocked the heteroreceptors sited on glutamatergic terminals. Finally, CGP52432, added at 1 μM, blocked $GABA_B$ autoreceptors, but was ineffective at the heteroreceptors. The results (Table 2) show that the release of GABA and glutamate evoked by depolarization of human neocortex nerve terminals can be affected differentially through pharmacologically distinct $GABA_B$ receptors. These human receptors seem to be very similar to those present in the rat neocortex (Bonanno and Raiteri, 1992), suggesting that the rat receptors may be useful models in the development of selective ligands.

4. The Mystery of $GABA_B$ Receptor Subtypes. Receptor heterogeneity is a general phenomenon. Types and subtypes of receptors have often been identified pharmacologically on the basis of their differential blockade by antagonists. Looking at the data reported in Table 2, the existence of subtypes of the $GABA_B$ receptor seems therefore undeniable, with strong similarities between human and rat receptors.

The problem is that, as a rule, pharmacological heterogeneity has been found to reflect structural diversity. This seems not to be the case for $GABA_B$ receptors, however. The structure of $GABA_B$ receptors is rather peculiar, the receptors being heterodimers composed of two subunits, $GABA_{B1}$ and $GABA_{B2}$; heterodimer formation is obligate for functional expression of $GABA_B$ receptors [see Bowery et al. (2002) for a review]. The mystery lies in the so far unsuccessful attempts to identify other subunits and the increasing belief that $GABA_{B1}$ and $GABA_{B2}$ exclusively form all $GABA_B$ receptors and that there are no further subunits to be discovered. According to some authors [see, for instance, the review by Couve et al. (2004)], the long-standing controversy on the existence of multiple $GABA_B$ receptor subtypes in the CNS may be explained by assuming the existence and the different composition of "$GABA_B$ receptor complexes" and signaling machinery in different neurons. In other words, $GABA_{B1}$ and $GABA_{B2}$ subunits would associate with several proteins, giving rise to complexes (see Couve et al., 2004) that differ between neurons and may underlie the pharmacological diversity of the same heterodimer. Although the mystery remains unsolved, the differential blockade by $GABA_B$ antagonists of receptors (or receptor complexes), sited on different neurons and performing different functions, represents a pharmacological heterogeneity that can be exploited to obtain therapeutically useful $GABA_B$ receptor ligands.

VII. Glutamatergic Receptors

Excitatory amino acids have been the object of an impressive number of studies; however, functional glutamatergic transmission in human brain has been poorly investigated. Electrophysiological studies on glutamate receptors in human brain slices are described in section XII.A.1. in relation to epilepsy. Reports showing that activation of NMDA and non-NMDA ionotropic glutamate receptors can evoke release of NE from human neocortical slices (Fink et al., 1992a), that the NE release evoked by NMDA or kainic acid can be inhibited by ethanol (Fink et al., 1992b), and that high D-glucose, at concentrations compatible with hyperosmolar diabetic coma, differentially modifies the NMDA-evoked release of GABA and NE (Fink et al., 1994) were considered in my previous review (Raiteri, 1994). It should be noted that glutamate ionotropic and metabotropic receptors exist as subtypes having discrete pharmacological profiles, which makes interspecies differences quite likely; more intense investigation of functional glutamatergic transmission in human brain is therefore required.

A. Metabotropic Glutamate Receptors

1. Phosphatidylinositol Turnover. Glutamate metabotropic receptors linked to phosphatidylinositol hydrolysis have been identified in human cerebellum (Nicoletti et al., 1989) and neocortex (Morari et al., 1991; Dubeau et al., 1992). An interesting receptor-receptor interaction seems to occur in human cerebral cortical slices where the activation of the phosphatidylinositol turnover by quisqualate was found to be prevented by NMDA, suggesting that glutamate metabotropic receptor functions in the human neocortex are negatively modulated by NMDA receptor activation (Morari et al., 1991).

Morari et al. (1995) subsequently reported on the occurrence of another receptor-receptor interaction in the human brain. The authors observed that the stimulation of the phosphatidylinositol turnover brought about by glutamate metabotropic receptor agonists could be regulated through activation of ionotropic AMPA receptors. In particular, a submaximal AMPA and metabotropic receptor activation resulted in positive cooperation. Clearly, these early works were based on determinations of phosphatidylinositol hydrolysis in the presence of non-subtype-selective metabotropic receptor agonists and would therefore deserve further consideration taking advantage of the selective ligands now available.

2. Release of Acetylcholine. In a recent work (Feligioni et al., 2003) carried out with nerve terminals prepared from human neocortex, it was found that activation of metabotropic glutamate receptors of group I elicited release of ACh through the production of inositol trisphosphate in cholinergic nerve endings. In contrast, exposure of rat cholinergic nerve endings to a metabotropic group I agonist failed to evoke ACh release. Interestingly, the HIV-1 protein Tat provoked ACh release both from human and rat synaptosomes; however, whereas in human neocortex nerve terminals Tat activates inositol trisphosphate-linked metabotropic group I receptors to elicit ACh release, the viral protein acts in rats at an as yet unidentified receptor that mediates ACh release through internal Ca^{2+} mobilization triggered by cyclic adenosine diphosphoribose (Feligioni et al., 2003) (see section XIV.B. for more details).

B. N-Methyl-D-aspartate Glutamate Receptors

1. Release of Norepinephrine. The stimulation of NE release by NMDA/glycine observed in human neocortical slices by Fink et al. (1992a) could also be seen in synaptosomes isolated from human neocortical tissue (Pittaluga et al., 1996) indicating the existence of presynaptic release-enhancing NMDA receptors on human noradrenergic axon terminals. In the absence of glycine, the HIV-1 coat protein gp120 allowed NMDA to enhance the release of NE from human noradrenergic nerve endings (Pittaluga et al., 1996) (see section XIV.A.). Experiments with NMDA receptor antagonists led to the conclusion that gp120 mimics glycine at the receptor coagonist site on the NR1 subunit, being ~4 orders of magnitude more potent than the natural coagonist glycine. Of interest, gp120, which also acts at the NMDA receptors present on rat noradrenergic terminals (Pittaluga and Raiteri, 1994), is unable to mimic glycine at the NMDA receptors sited on rat striatal dopaminergic terminals (Pittaluga et al., 2001b). The gp120 potentiation of the NMDA-evoked NE release in human brain was blocked by memantine (Pittaluga et al., 1996), a low-affinity, uncompetitive, open-channel blocker of the NMDA receptor (Lipton and Chen, 2004) and this finding might have therapeutic implication (see section XVI.A.).

2. The "Kynurenate Test." A biochemical assay for putative cognition enhancers, termed the "kynurenate test," was introduced by Pittaluga et al. (1997). In this test, the NMDA-evoked release of NE in rat hippocampal slices is antagonized by kynurenic acid and putative cognition enhancers are tested for their ability to counteract the kynurenate antagonism. Several behaviorally active compounds were evaluated; based on the results, the kynurenate test seems to represent a useful assay for putative cognition enhancers acting through the glutamate system via NMDA receptors (Pittaluga et al., 1997).

It was important to establish whether compounds displaying activity in the rat brain responded similarly in a kynurenate test performed with human brain tissue. Slices from human neocortex were exposed to NMDA; the evoked release of NE was antagonized by kynurenic acid. This antagonism was potently prevented by behaviorally active compounds (aniracetam, oxiracetam, D-cycloserine, and the glutamate analog CR2249, but not its enantiomer CR2361) previously reported to positively respond in the kynurenate test performed with rat hippocampal slices (Pittaluga et al., 1999). The similarities between the data obtained with human neocortex slices and those obtained in the rat suggest that the kynurenate test performed with rat brain slices represents a useful assay to study cognition-enhancing drugs.

VIII. Neuropeptide Receptors

Neuropeptides are important modulators of neurotransmitter function, many of them being costored and coreleased with classic transmitters (Hökfelt et al., 2000). Although a number of studies have been performed in which release of neuropeptides from human brain tissue, particularly somatostatin and cholecystokinin, was characterized (see section VI.A.2.), very few reports deal with neuropeptide receptors in human CNS. Results from experiments with laboratory animals show that opioids can regulate neurotransmitter release, although with important species differences [see Feuerstein et al. (1996b) and references therein]. It is therefore important to investigate the modulation of release by opioids in human brain and, in particular, to characterize the subtypes of opioid receptor involved.

A. Release of Acetylcholine Mediated by Opioid Receptors

Feuerstein et al. (1996b) studied the effects of various opioid receptor ligands on the release of ACh evoked by electrical stimulation or by K^+ depolarization in slices of human neocortex. The evoked release of ACh was inhibited by the δ-opioid receptor agonist [D-Pen^2,D-Pen^5]-enkephalin and by the κ-opioid receptor agonist U50488. These effects were prevented, respectively, by the δ-opioid antagonist naltrindole and the κ-opioid antagonist norbinaltorphimine. Exposure to the μ-opioid receptor agonist D-Ala_2-NMePhe_4-Gly-ol_5-enkephalin also produced ACh release inhibition; however, this effect 1) was blocked by δ- and κ-opioid antagonists and 1) could not be seen with the μ-opioid agonists morphine and (+)-nortilidine. Interestingly, naltrindole augmented, on its own, the evoked release of ACh, suggesting activation of δ-opioid receptors by endogenous opioid peptides. Feuerstein et al. (1996b) also suggested a localization of δ- and κ-opioid receptors, based on experiments in which the release of ACh was elicited by high-K^+ in the presence of tetrodotoxin. Because the K^+-evoked ACh release was inhibited by U50488, but not by DPDPE, the authors proposed that κ-opioid receptors are presynaptic on cholinergic terminals, whereas δ-receptors are on cortical

interneurons. The nature of these interneurons remains to be established, however, because the lack of effect of $GABA_A$ and $GABA_B$ receptor agonists on ACh release tends to exclude the involvement of GABA interneurons.

In a subsequent work, Feuerstein et al. (1998) attempted to identify the transmitter of the interneuron acting through heteroreceptors on cholinergic terminals of the human neocortex. The authors found that the inhibition of the K^+-evoked ACh release by DPDPE disappeared in the presence of octreotide, a somatostatin$_2$ receptor agonist, suggesting that a somatostatinergic interneuron is responsible for the indirect effects of δ-opioid receptor activation. The latter receptor was subclassified as the δ_1 subtype, based on the finding that the δ_1-opioid receptor antagonist 7-benzylidennaltrexon prevented the DPDPE inhibition of ACh release. The authors concluded that somatodendritic δ_1-opioid receptors sited on somatostatin-containing interneurons mediate inhibition of somatostatin release onto cholinergic terminals provided with facilitatory somatostatin$_2$ receptors, resulting in δ_1 receptor-mediated (indirect) depression of ACh release.

The article made no mention of the neuronal distribution of somatostatin which is largely colocalized with GABA in interneurons (Hendry et al., 1984; Somogyi et al., 1984). Because a previous report (Feuerstein et al., 1996b) excludes the involvement of GABA receptors in the δ-opioid inhibition of ACh release, the findings raise the possibility that δ_1 receptor activation selectively affects the release of the cotransmitter somatostatin, although the existence of some "pure" somatostatinergic neurons should not be excluded.

B. Release of Norepinephrine Mediated by Opioid Receptor-Like 1 Receptors

Nociceptin/orphanin FQ (N/OFQ) is a neuropeptide identified as an endogenous ligand for the opioid receptor-like 1 (ORL1) receptor (Calò et al., 2000; Mogil and Pasternak, 2001). The peptide was reported to inhibit the release of NE from slices of various brain areas of mouse, rat, and guinea pig (Schlicker et al., 1998; Trendelenburg et al., 2000).

The effects of N/OFQ on the release of NE were comparatively studied in electrically stimulated slices of human and rat neocortex (Rominger et al., 2002). N/OFQ decreased the evoked release of [^{3}H]NE in both species; the effects of N/OFQ were prevented by the ORL1 antagonist J-113397 which, on its own, was unable to increase the evoked release of [^{3}H]NE suggesting the absence of a tonic activation by endogenous N/OFQ. Although the efficacy of N/OFQ was significantly lower in human than in rat slices, the affinities of the peptide for ORL1 receptors and the pA_2 values of the ORL1 antagonist seem to be very similar in the two species.

IX. Cannabinoids and Cannabinoid Receptors

The CNS effects of Δ^9-tetrahydrocannabinol, the main active constituent of marijuana and hashish and related compounds are mediated by cannabinoid receptors of the CB_1 type. These are G_i protein-coupled receptors, the activation of which leads to inhibition of adenylyl cyclase. CB_1 receptors exist on presynaptic axon terminals and their activation was shown in animal studies to cause inhibition of the release of neurotransmitters, possibly consequent to inhibition of VSCCs [see Schlicker and Kathmann (2001) for a review]. Cannabinoids have been reported to modulate neurotransmitter release from fresh human brain tissue, as summarized below.

A. Release of Norepinephrine

The effects of synthetic cannabinoid receptor ligands on the release of [^{3}H]NE were comparatively analyzed in slices of human, guinea pig, rat, and mouse hippocampal tissue (Schlicker et al., 1997). The cannabinoid receptor agonist WIN55,212-2 inhibited NE release in human and guinea pig hippocampal slices, but not in slices of rat and mouse hippocampus. The CB_1 receptor antagonist SR141716 markedly attenuated the WIN55,212-2-induced inhibition of NE release, suggesting that activation of CB_1 receptors mediate inhibition of NE release in human and guinea pig hippocampus. The modest augmentation of NE release provoked by SR141716, when added alone to human slices, suggests that CB_1 receptors may not be tonically activated in the human cerebrocortex.

The hippocampal formation is one of the brain regions with the highest level of CB_1 receptor expression (Herkenham et al., 1990). Cannabinoids are known to interfere with hippocampal functions; in particular, they cause impairments in memory consolidation and association in humans (Ameri, 1999; Hampson and Deadwyler, 1999). Noradrenergic transmission is positively involved in cognitive processes (Berridge and Waterhouse, 2003). Therefore, the observed inhibitory effect of CB_1 receptor activation on the release of NE in the hippocampus may be of some relevance to the cognitive impairments induced by cannabinoids.

B. Release of GABA

Starting from the reported existence of cannabinoid CB_1 receptors on the axon terminals of GABA interneurons of the rodent hippocampus (Tsou et al., 1999) and the inhibitory action of CB_1 receptor activation on the release of GABA (Katona et al., 1999), it was important to ascertain the existence of CB_1 receptors on GABAergic neurons in human hippocampus and to investigate cannabinoid effects on GABA release.

Using control postmortem and epileptic lobectomy tissue, Katona et al. (2000) found CB_1 receptors to be expressed on GABA interneurons and concentrated in axon terminals. In functional experiments with human

hippocampal slices showing no sclerotic damage, the CB_1 receptor agonist WIN55,212-2 strongly reduced [^{3}H]GABA release evoked by electrical stimulation; the effect of WIN55,212-2 was fully prevented by SR141716.

Thus, the localization on GABAergic axon terminals of hippocampal interneurons and the inhibitory function on GABA release of CB_1 receptors seem to be evolutionarily conserved, which makes the rat hippocampus an appropriate model of native CB_1 receptors useful to develop new CB_1 selective ligands. According the Katona et al (2000), the actions of exogenous cannabinoids and of endocannabinoids on GABAergic interneuronal networks may underlie some of their hippocampus-related behavioral effects.

C. Release of Acetylcholine

Steffens et al. (2003) compared the effects of cannabinoids on the release of ACh in human and mouse neocortex. Brain slices were labeled with [^{3}H]choline and stimulated electrically in superfusion conditions. The addition of WIN55,212-2 decreased ACh release; the CB_1 antagonist SR141716 prevented the inhibitory effect of WIN55,212-2, indicating that activation of CB_1 receptors may diminish ACh release in human neocortex.

The results of Steffens et al. (2003) also suggest that these CB_1 receptors are tonically activated by endocannabinoids released by depolarizing stimuli and which seem to accumulate in the biophase of CB_1 receptors depending on the stimulation conditions. The assumption of an endocannabinoid tone on CB_1 receptors modulating ACh release in human (but not in mouse) neocortex is justified by at least two observations: 1) SR141716 enhanced, on its own, the release of ACh in human (but not the mouse) neocortical slices and 2) the endocannabinoid uptake blocker AM404 inhibited the release of ACh in human (but not in mouse) brain tissue, probably due to increased endocannabinoid levels in the CB_1 receptor biophase.

Cannabinoids are known to impair cognition and memory, probably due to interference with various transmitter systems. Among these, the cholinergic transmission may play a role [see Sullivan (2000) for a review]. According to Steffens et al. (2003), the facilitatory effect of SR141716 on the release of ACh in the human neocortex suggests that cannabinoid CB_1 receptor antagonists may be useful in the treatment of dementia.

D. Release of Dopamine

Steffens et al. (2004) investigated the possible effects of cannabinoids on the release of dopamine (DA) from human neocortical slices and on the cyclic AMP accumulation in synaptosomes. The cannabinoid CB_1 receptor agonist CP55940 markedly depressed electrically evoked [^{3}H]DA release from human neocortical slices. The inhibitory effect of CP55940 was antagonized by the selective CB_1 receptor antagonist AM251, compatible with the involvement of cannabinoid receptors of the CB_1 type.

Surprisingly, CP55940 did not affect the release of [^{3}H]DA provoked by electrical stimulation of rat neocortical slices, suggesting the absence of a CB_1 receptor-mediated control of dopaminergic transmission in the rat neocortex. CB_1 receptors seem not to be present in the neocortex of mice either (Hermann et al., 2002), indicating that the rodent brain may not represent a suitable model to investigate cannabinoid-dopamine interactions in the neocortex.

When the CB_1 receptor antagonist AM251 was added alone to the human slices, a concentration-dependent increase in DA release was observed. Again AM251 was ineffective on DA release in the rat. One likely explanation for the AM251 effect is the disruption of an endocannabinoid tone that keeps the release of DA in human neocortex partly depressed (Steffens et al., 2004).

The addition of CP55940 to human neocortex synaptosomes reduced the forskolin-stimulated cyclic AMP accumulation, an effect mediated by CB_1 receptors because it was antagonized by AM251. Steffens et al (2004) speculated that CB_1 receptor activation may depress DA release not only by impairing the function of VSCCs but also by inhibiting adenylyl cyclase. Whether VSCCs and adenylyl cyclase are functionally connected in nerve terminals remains to be established.

To conclude, the work by Steffens et al. (2004) provides evidence for the presence of G_i protein-coupled CB_1 receptors modulating DA transmission in the human neocortex. These receptors seem not to exist in the rat neocortex. Moreover, the CB_1 receptors in human neocortex are tonically activated by endogenous cannabinoids or/and exhibit constitutive activity. If CB_1 receptor antagonists elicit DA release in the human neocortex, they may be used for the treatment of cognitive deficits associated with a decreased cortical dopaminergic transmission (Brever and Barch, 2002).

X. Calcium Channels and Intraterminal Calcium Pools

A. Voltage-Sensitive Calcium Channels

1. Influx of Calcium and Norepinephrine Release. Human cerebral cortical synaptosomes were used by Meder et al. (1999) to study VSCCs. Fura-2-loaded synaptosomes were depolarized by exposure to high-K^+ or to veratridine. Both agents increased intraterminal [Ca^{2+}], but the influx of external Ca^{2+} into nerve terminals involved distinct VSCCs. In particular, the increase of intraterminal [Ca^{2+}] caused by K^+ depolarization predominantly occurred by activation of P/Q-type Ca^{2+} channels and, less so, by N-type channels, whereas that caused by veratridine involved activation of P/Q-, N- and L-type Ca^{2+} channels. Moreover, a small portion of the veratridine-evoked increase of intraterminal [Ca^{2+}] was

resistant to VSCC blockade and might represent intraterminal Ca^{2+} accumulation via the Na^+/Ca^{2+} exchanger. Based on previous data with rat synaptosomes (Meder et al., 1997), the authors pointed out that there are considerable differences in presynaptic VSCCs between human and rat brain cortex.

Fink et al. (2002) subsequently tested the effect of gabapentin and pregabalin, two drugs efficacious in the treatment of epilepsy and neuropathic pain, on the K^+-induced intraterminal $[Ca^{2+}]$ increase in fura-2-loaded human neocortical synaptosomes. Both drugs inhibited the K^+-induced increase of intraterminal $[Ca^{2+}]$; the effect of gabapentin was prevented by the P/Q-type VSCC blocker ω-agatoxin IVA, indicating interaction with presynaptic VSCCs of the P/Q-type. As previously observed in rats, both gabapentin and pregabalin seem to bind to the $\alpha 2\delta$ subunit of VSCCs (Bryans and Wustrow, 1999); Fink et al. (2002) found $\alpha 2\delta$-1, $\alpha 2\delta$-2, and $\alpha 2\delta$-3 subunits to be present in human neocortical synaptosomes by immunoblot analysis. In neurotransmitter release experiments with human neocortical slices, gabapentin inhibited the K^+-evoked release of [^{3}H]NE, an effect that was prevented by blocking glutamate receptors of the AMPA type. The authors proposed that gabapentin and pregabalin may bind to the $\alpha 2\delta$ subunit of P/Q-type VSCCs to inhibit Ca^{2+} influx into and glutamate release from excitatory amino acid terminals that impinge on noradrenergic nerve endings, as previously shown to occur in rat neocortical slices (Doodley et al., 2000; Fink et al., 2000). The reduced cytosolic $[Ca^{2+}]$ and decreased glutamate release caused by gabapentin and pregabalin may effectively attenuate neuronal excitability to explain the clinical efficacy of these drugs.

2. Influx of Calcium and Nitric-Oxide Synthase Activity. Nitric-oxide synthase activity was studied in slices of human temporal cortex samples by measuring the conversion of tritiated L-arginine to L-citrulline (Fontana et al., 1997). Elevation of extracellular $[K^+]$, an event that can occur in a number of pathological conditions such as cerebral ischemia (Zetterström et al., 1995) and epilepsy (Louvel et al., 1994, 2001), augmented L-citrulline production. The response to high-K^+ was abolished by inhibiting NO synthase or blocking VSCCs with 10 mM Mg^{2+}. Glutamate receptors seem not to be involved because the K^+ effect was insensitive to selective antagonists. The K^+ (35 mM)-evoked activation of NO synthase was insensitive to ω-conotoxin GVIA and to nifedipine, but could be prevented in part by ω-agatoxin IVA. Interestingly, the inhibition caused by ω-agatoxin IVA was enhanced by adding ω-conotoxin GVIA or nifedipine. Further inhibition could be observed when the three VSCC blockers were added together. Fontana et al. (1997) concluded that elevation of extracellular $[K^+]$ causes NO synthase activation by external Ca^{2+} entering cells mainly through channels of the P/Q-type. Other VSCCs (L- and N-type) seem to contribute when P/Q-type channels are blocked (functional compensation?). Assuming that the present scenario reflects what occurs in some of the physiopathological conditions in which NO has been implicated, association of Ca^{2+} channel blockers or nonselective blockers may deserve better consideration than selective P/Q-type channel blockers as potential therapeutic agents.

B. Calcium Pools and Dopamine Release

Bonanno et al. (2000) used human neocortex synaptosomes to study the release of DA provoked by various stimuli. Exposure in superfusion to 15 mM KCl, 100 μM 4-aminopyridine, 1 μM ionomycin or 30 mM caffeine elicited almost identical DA overflows. Removal of external Ca^{2+} abolished the overflows evoked by high-K^+ or ionomycin and largely prevented that caused by 4-aminopyridine, whereas the release evoked by caffeine remained unaffected. The Ca^{2+} chelator BAPTA reduced significantly the K^+- and the caffeine-evoked DA overflows. The effect of caffeine was attenuated by the ryanodine receptor blocker dantrolene or by the inositol trisphosphate receptor antagonist heparin and abolished by the combined addition of dantrolene and heparin. Tetanus toxin inhibited the K^+- or the 4-aminopyridine-evoked release of DA; in contrast, the releases stimulated by ionomycin and by caffeine were totally insensitive to the toxin. To conclude, DA release from human neocortex nerve terminals can be triggered by Ca^{2+} ions originating from various sources. It seems that stimuli not leading to activation of VSCCs elicit Ca^{2+}-dependent, probably exocytotic, release that, under the experimental conditions used, seems insensitive to tetanus toxin. A caffeine-stimulated DA release has rarely been observed in animal brain preparations. In fact, caffeine increased rat striatal DA release during *in vivo* microdialysis, but the effect was indirectly mediated through the known blocking activity of caffeine at adenosine receptors (Okada et al., 1997). The characteristics of the superfusion technique used to monitor DA release and the results with ryanodine and inositol trisphosphate receptor blockers permit to conclude that caffeine acts directly on human nerve terminals and causes release of DA by mobilizing Ca^{2+} ions from the endoplasmic reticulum of mesocortical dopaminergic neurons. The release of DA in the neocortex might be involved in the stimulatory and cognition enhancing properties of caffeine.

XI. Neurotransmitters in the Alzheimer's Brain

Alzheimer's disease is a progressive neurodegenerative disorder characterized by various mental dysfunctions including cognitive impairments, reduction in intellectual abilities, emotional instability and changes in personality. The most well-appreciated neuronal loss is in the cholinergic system (Coyle et al., 1983; Perry, 1986; Fibiger, 1991; Nordberg, 1999). However, other neuronal systems are affected, including noradrenergic, sero-

tonergic, GABAergic, and peptidergic neurons (Palmer, 1996).

In general, reliable release and uptake results can only be obtained by using fresh brain tissue preparations. The availability of fresh Alzheimer's tissue was an extremely rare event dating back to the 1980s, when small amounts of brain tissue were removed to provide a definitive diagnosis or to apply a therapeutic agent (Neary et al., 1986). Studies of functional neurochemistry with these bioptic specimens provided the greatest contribution to understanding of the pathogenesis of Alzheimer's disease (see below).

The vast majority of the studies on the Alzheimer's brain have been performed using postmortem tissues. Although meaningful data can be obtained from diseased postmortem human brain, interpretation of postmortem results requires careful consideration of potentially confounding factors including agonal state, possible tissue atrophy, energy failures due to postmortem delay, and so forth (see, for a detailed discussion, Palmer, 1996).

Because of the difficulties in performing functional experiments with postmortem tissues, studies on neurotransmitter receptors and transporters have been carried out with radioligand binding techniques. This approach may have some yet poorly considered drawbacks. It is now amply accepted that receptors and transporters travel from internal stores to the plasma membrane and vice versa; moreover, the proteins actively present on the plasma membrane may only represent a minority, the large majority being stored intracellularly. Thus, the possibility exists that classic radioligand techniques cannot easily discriminate between the two protein pools, leading to erroneous interpretations.

A. Functional Studies of Alzheimer's Brain Antemortem

Experiments with biopsy tissues have been important in the study of Alzheimer's disease for a number of reasons. The data obtained helped to separate changes related to the disease from the artefacts and epiphenomena associated with postmortem conditions. The results allowed identification of changes occurring early in the disease process. Moreover, antemortem tissue has offered the opportunity to relate neurochemistry to certain behavioral changes characteristic of Alzheimer's disease. Antemortem brain tissue suitable for functional studies will obviously not be available from patients with Alzheimer's disease. As recently reviewed by Cattabeni et al. (2004), platelets seem to provide human tissue useful to unravel the pathogenic mechanisms of Alzheimer's disease.

1. The Cholinergic System. Reduced activity of choline acetyltransferase in Alzheimer's disease was an early observation (Bowen, 1977). In a comparison of the enzyme activity in tissue of control subjects removed antemortem and postmortem, choline acetyltransferase seemed to be very stable postmortem (Palmer et al., 1988). Using crude synaptosomal preparations from Alzheimer's disease neocortex it was found that ACh synthesis and choline uptake were reduced with respect to controls (Sims et al., 1983; Palmer et al., 1987c). The antemortem biopsies used were obtained ~3.5 years after the onset of symptoms, which is approximately at the midpoint of the disease. These results indicate that neocortical cholinergic projections are lost at an early stage of the disease.

2. The Monoaminergic Systems. Histological data indicate loss of serotonergic perikarya in the dorsal raphe nucleus of the Alzheimer's brain (Mann et al., 1984). Antemortem studies show that the release and uptake of 5-HT in the Alzheimer's temporal cortex are both reduced with respect to controls (Palmer et al., 1987a,c). The damage to serotonergic neurons seems to occur early in the course of the disease since the loss of serotonergic markers was evident in a subset of patients that had displayed clinical symptoms for <2 years (Palmer et al., 1987a).

Studies of Alzheimer's tissue removed antemortem show that the concentration of NE was reduced both in temporal and frontal cortex. In addition, high-affinity uptake of the [^{3}H]catecholamine was lower in the pathological than in control tissue. Moreover, these changes were evident in a subset of patients who had displayed clinical symptoms for <2 years, suggesting that dysfunction of the noradrenergic system also occurs early in the course of the disease (Palmer et al., 1987b).

In a study of antemortem cortical tissue from subjects with Alzheimer's disease, the concentrations of DA and its metabolites were all unaffected, suggesting that DA nerve terminals in the neocortex are spared in Alzheimer's disease (Palmer et al., 1987b,c). In contrast to the cerebral cortex, reduced concentrations of homovanillic acid were observed in postmortem neostriatal tissue of patients with a clinical diagnosis of Alzheimer's disease (Palmer et al., 1984), suggesting a dysfunction in striatal DA transmission. Accordingly, parkinsonian symptoms commonly accompany Alzheimer's disease (Tyrrell et al., 1990; Joyce et al., 1998; Werber and Rabey, 2001). In a recent report (Zhang et al., 2004), it was observed that the use of cholinergic drugs for Alzheimer's disease, in particular the acetylcholinesterase inhibitors donepezil and galantamine, can enhance DA release by indirectly acting at, or allosterically modulating (Svensson and Nordberg, 1996), nicotinic AChRs present on DA terminals, which may underlie the influence of these drugs over some noncognitive symptoms of Alzheimer's disease, including motor dysfunctions.

3. Somatostatin. Some neuropeptides seem to be positively involved in cognitive processes. Among these, somatostatin, a neuropeptide particularly concentrated in the cerebral cortex and the hippocampus (Rubinow et al., 1995; Barton, 2003), has attracted considerable interest. Reduction of somatostatin levels in the cortical

areas is an autoptic finding typical of Alzheimer's disease (Davies et al., 1980; Beal et al., 1986; Dawbarn et al., 1986; Chan-Palay, 1987; Gabriel et al., 1993). Although the only study on somatostatin release with antemortem Alzheimer's temporal cortex tissue (Francis et al., 1987) showed only a nonsignificant decrease, the abundant results reporting significant reductions in the neuropeptide concentration also in the cerebrospinal fluid of patients (Minthon et al., 1997) suggest that, at least in the advanced stages of Alzheimer's disease, the somatostatinergic transmission is severely impaired (Palmer, 1996).

XII. Epilepsy: In Vitro and in Vivo Studies

A. The GABA and Glutamate Systems in Epilepsy

Temporal lobe epilepsy (TLE) is the most frequent and severe form of adult focal epilepsy. In many patients TLE is refractory to anticonvulsant drug therapy. A relevant proportion of these patients are treated by surgical removal of the epileptic focus, which usually comprises a portion of the temporal lobe including the hippocampus. Although the difficulty in obtaining control human tissue is obviously of major concern, studies using resected human focal epileptic tissues provide a unique opportunity to compare results derived from animal models of TLE with the human condition. Most of the studies described below have produced results that show clear similarities between the epileptic human and epileptic rat models, indicating that animal investigation can be of help in understanding the pathological aspects of the disease and in the development of novel mechanism-based, therapeutic modalities.

1. GABA and Glutamate Receptors: Electrophysiological Studies. Avoli et al. (1991) examined the epileptiform activity induced by low extracellular Mg^{2+} in slices of human epileptogenic neocortex obtained during neurosurgical procedures. Spontaneous epileptiform activity and episodes of spreading depression appeared within 1 to 2 hours of perfusion with Mg^{2+}-free artificial cerebrospinal fluid. Transient increases in extracellular $[K^+]$ were associated with each epileptiform discharge. Spreading depression episodes were accompanied by increases in external $[K^+]$ of up to 100 mM. Epileptiform discharges and spreading depressions were abolished when glutamate NMDA receptor antagonists were present, whereas blockade of ionotropic non-NMDA receptors did not influence the epileptiform activity elicited by lack of Mg^{2+} ions (Avoli et al., 1991). It was also shown that blockade of the $GABA_A$ receptors is sufficient to elicit epileptiform discharges in the human neocortex in vitro (Hwa et al., 1991).

Studies in the hippocampus and neocortex of rodents had reported that synchronous potentials could be recorded upon application of the K^+ channel blocker 4-aminopyridine (4-AP), even after excitatory amino acid receptors had been blocked by specific antagonists. Curiously, these phenomena seem to be mediated by the activities of $GABA_A$ receptors (Michelson and Wong, 1991; Perrault and Avoli, 1992). Similar to what was observed in normal rats, application of 4-AP to slices of human neocortex obtained in the course of neurosurgery for the relief of intractable seizures disclosed synchronous field potentials that correlate with a long-lasting depolarization and are mainly due to $GABA_A$ receptor activation. Of note, activation of glutamate receptors is not required for the generation and propagation of these GABA-mediated potentials (Avoli et al., 1994). The authors proposed that these potentials represent a novel mechanism for synchronization and spread of neuronal activity, including seizure-like discharges in the human neocortex.

In line with the results obtained by Avoli et al. (1994) with 4-AP, Williamson et al. (1995) reported that, in some situations, GABA could act as an excitatory neurotransmitter. These authors studied the responses to exogenously added GABA of dentate granule cells in slices isolated from patients with temporal lobe sclerosis. The cells examined exhibited reduced synaptic inhibition with concomitant hyperexcitability relative to the dentate granule cells in the hippocampi of patients with temporal lobe tumors in which cell sclerosis was not detectable and which were taken as "controls." To understand the reason for the disinhibition, Williamson et al. (1995) monitored the responses to exogenously applied GABA. The responses studied at the resting membrane potential were depolarizing and were mediated by $GABA_A$ receptors. In many cases, these depolarizing GABA responses could trigger action potentials. Interestingly, the $GABA_A$ receptor-mediated responses in the sclerotic hippocampi were ~80% longer than those in the comparison population. As one explanation, the authors suggest that the GABA transport system could be impaired in the sclerotic tissue (see below).

The glutamate-independent, $GABA_A$ receptor-mediated, synchronous activity that occurs in human neocortex slices exposed to 4-AP (Avoli et al., 1994) was further characterized by field potential and extracellular $[K^+]$ recordings (Louvel et al., 2001). These synchronous potentials are associated with elevations in extracellular $[K^+]$ that may contribute to their origin and spread within the neocortex. The synchronous potentials, previously found to be blocked by $GABA_A$ receptor antagonists, disappear following activation of μ-opioid receptors expected to block GABA release (Capogna et al., 1993), are significantly reduced by activation of $GABA_B$ receptors of undefined (probably presynaptic) localization, and seem to be modulated by metabotropic glutamate receptors of group I (Louvel et al., 2001).

Using patch-clamp recording techniques in dentate granule cells isolated from tissue resected from patients undergoing temporal lobectomy, Shumate et al. (1998) compared properties of human $GABA_A$ receptors to $GABA_A$ receptor properties in control and epileptic rats.

Of note, both the epileptic human and epileptic rat models exhibited the same pattern of neuropathology, in particular mesial temporal sclerosis and mossy fiber sprouting (Coulter et al., 1996). Both epileptic human and epileptic rat dentate granule cells exhibited $GABA_A$ receptor responses, which were strongly blocked by zinc, in contrast to the weak zinc sensitivity observed in control rat dentate granule cells. Zinc ions are released by the sprouted mossy fibers autoinnervating the dentate granule cells in the epileptic hippocampus. According to the authors, the enhanced zinc sensitivity exhibited by $GABA_A$ receptors may underlie an important failure of inhibition in the epileptic dentate gyrus where zinc is released from the mossy fiber terminals. Shumate et al. (1998) hypothesized that the enhanced zinc sensitivity may result from underlying shifts in subunit composition of $GABA_A$ receptors in the epileptic dentate gyrus. Considering the similarities between responses in human and rat isolated granule cells, useful information on the $GABA_A$ receptor subtypes involved could be obtained from animal models.

Intracranial electroencephalogram records from hippocampal structures in patients with TLE usually reveal interictal spikes, which occur between seizures. To study the origin and the mechanisms of this phenomenon, specimens resected from 21 patients that included the hippocampal formation were examined electrophysiologically (Cohen et al., 2002). The authors always detected rhythmic and synchronous extracellular spikes in records from the subiculum, an output hippocampal area that projects to the temporal cortex and is innervated by CA1 pyramidal cell axons. These synchronous events were similar to interictal discharges of patient electroencephalograms. Interestingly, synchronous discharges were suppressed by AMPA or NMDA glutamate receptor antagonists, as well as by $GABA_A$ receptor antagonists. Recording from different subicular sites indicates that the network of discharging neurons comprises both subicular interneurons and a subgroup of pyramidal cells. In these pyramidal cells, GABAergic signaling is depolarizing since GABAergic synaptic events reversed at depolarized potentials. The authors concluded that depolarizing GABAergic responses in neurons downstream to the sclerotic CA1 region contribute to human interictal activity. One possibility raised to explain the $GABA_A$ receptor-evoked depolarization is that the altered connectivity associated with damaged temporal lobe, including neuronal deafferentation, may lead to a switch of the neuron to an immature state in which $GABA_A$-mediated responses are known to be depolarizing (Cherubini et al., 1991).

It has to be noted that $GABA_A$ receptor-mediated depolarizing responses are not limited to immature CNS neurons or to pathological conditions such as epilepsy; indeed, there are numerous reports showing that GABA can exhibit "physiological" depolarizing actions in mature CNS neurons (Staley et al., 1995; Fassio et al., 1999; Gulledge and Stuart, 2003) [see Stein and Nicoll (2004) for a recent review].

Hippocampal specimens resected to cure intractable TLE can be useful to investigate the functional consequences of morphological alterations. In cases of hippocampal sclerosis, morphological investigation shows an atypical network of granule cells synaptically interconnected through aberrant supragranular mossy fibers. In a very recent work, Gabriel et al. (2004) investigated whether granule cell populations in slices from sclerotic and nonsclerotic hippocampi would develop epileptiform activity following low-frequency hilar stimulation in the presence of elevated extracellular $[K^+]$ and whether the experimental activity differs according to the presence of aberrant mossy fibers. The authors found that ictaform activity could be provoked in slices from both sclerotic and nonsclerotic hippocampi. However, the induction of ictaform discharges in slices from sclerotic hippocampi required lower concentrations of K^+ (i.e., lower depolarization) than in slices from nonsclerotic hippocampi, suggesting that synaptically coupled granule cells in the reorganized network, when depolarized by high-K^+, could cooperate to reach the threshold for seizure generation. Gabriel et al. (2004) reported that the two groups of patients also differed with respect to the pattern of epileptiform discharges. On the other hand, the different ictaform activities seemed to be synaptic in origin because they were dependent on external Ca^{2+} and disappeared in the presence of glutamate AMPA receptor blockade. According to the authors, the fact that it is possible to induce epileptiform activity in slices from neurosurgically removed human tissue permits investigation of mechanisms of drug resistance and evaluation of the advantages of newly developed drugs over presently available anticonvulsants.

2. In Vivo Microdialysis in Epileptic Patients: Glutamate/GABA Release and GABA Transporters. Glutamate is the major excitatory transmitter, and GABA is the major inhibitory transmitter in the CNS. Monitoring amino acid levels during intracerebral microdialysis in seven patients with medically intractable epilepsy, Ronne-Engstrom et al. (1992) had found dramatic increases of extracellular glutamate, aspartate, glycine, and serine in association with focal seizures. During and Spencer (1993) subsequently used bilateral intrahippocampal microdialysis to test the hypothesis that an increase in extracellular glutamate may trigger spontaneous seizures in the conscious human brain. The concentrations of glutamate and GABA were measured in the microdialysate before and during seizures in six patients with complex partial epilepsy investigated before surgery. Interestingly, a rise in glutamate was observed in the epileptogenic hippocampus just before the seizure. Because perfusion of glutamate into the cat hippocampus in vivo induces seizures (Biscoe and Straughan, 1966), the results with epileptic patients

suggest that an increase in the extracellular endogenous glutamate may do the same. Opposite to glutamate, the concentrations of GABA in the epileptogenic hippocampus were lower than those in the nonepileptogenic side. Moreover, whereas the increase in glutamate preceded seizures, GABA concentrations were unchanged before seizures, but increased during them and remained higher in the nonepileptogenic hippocampus. The glutamate/GABA ratio is raised in the epileptogenic hippocampus, particularly in the postseizure period, when glutamate remains high as the GABA concentration returns to normal, which might contribute to the lesions characteristic of TLE.

As compounds that block the synthesis, the release, or the postsynaptic receptors of GABA can induce convulsions, epilepsy may not only originate from an excess of excitatory transmission but also from alterations of the inhibitory effects of GABA, in particular a shortfall in the amount of GABA released. To test the hypothesis that the epileptogenic region in temporal lobe epilepsy is characterized by an alteration in the release of GABA, microdialysis was performed in eight awake patients (During et al., 1995). The local perfusion of glutamate induced an increase of GABA release which was independent of external Ca^{2+} ions in the nonepileptogenic hippocampus but much less so in the epileptogenic hippocampus. GABA release from neurons is of two types (Levi and Raiteri, 1974, 1993): physiological release is vesicular, external Ca^{2+}-dependent, and sensitive to clostridial toxins, and it can be evoked by depolarizing stimuli. The amino acid can also be released from neurons and glia (Gallo et al., 1991) by a Ca^{2+}-independent carrier-mediated process, whereby GABA is released through the GABA transporter working in the inside-outside direction; such a reversal in transport can be triggered by various stimuli, including glutamate and "pathologically" elevated extracellular K^+ concentrations (Drejer et al., 1987; Patrylo et al., 2001; Raiteri et al., 2002b) and is blocked by GABA transporter inhibitors.

In the work of During et al. (1995), it was reported that the number of GABA transporters is reduced in the epileptogenic hippocampus, which would imply that, if release of GABA during seizures occurs by reversal of GABA transport, the amounts of GABA released could be insufficient to counteract seizure activity. The possibility that the reduction in glutamate-induced GABA release is due to loss of ionotropic glutamate receptors is unlikely because both NMDA and ionotropic non-NMDA electrophysiological responses are maintained in resected epileptogenic tissue (Isokawa et al., 1991; Williamson, 1994). Following the oral administration of the GABA transporter blocker tiagabine in patients with mesial TLE, the extracellular GABA in the epileptogenic hippocampi was found to be diminished (During et al., 1992). Moreover, GABA-induced responses in granule cells recorded in vitro from epileptogenic hippocampal slices are significantly prolonged (Williamson et al., 1995). Altogether, these results support the hypothesis that GABA transporter function is abnormal in epileptogenic tissue. Accordingly, it was previously shown that the seizure-induced increase in extracellular GABA in epileptic patients is diminished in the epileptogenic hippocampus (During and Spencer, 1993). In conclusion, During et al. (1995) proposed that, during periods of intense neuronal and glial excitation, release of GABA can occur by transporter reversal triggered by depolarizing glutamate receptor activation (Fig. 3), which would represent a defense mechanism against excessive glutamatergic excitation. The loss of function of GABA transporters proposed by During et al. (1995) would diminish the carrier-mediated release of the inhibitory transmitter, thus contributing to the epileptogenic state in human mesial TLE.

3. The Glutamate-Glutamine Cycling in Epilepsy. If high extracellular glutamate concentrations are considered as a likely trigger of seizures in mesial TLE, the underlying mechanisms are not understood. As one possibility, Eid et al. (2004) investigated whether a deficiency in glutamine synthetase could explain the "hyperglutamatergicity" present in this pathological condition. It is well known that extracellular glutamate is largely taken up by astrocytes and converted by glutamine synthetase to glutamine. A glutamine synthetase deficiency would therefore be expected to cause increase in glutamate in the astrocytes, with consequent accumulation of extracellular excitatory amino acid (see Fig. 3).

As previously reported, During and Spencer (1993) had shown, in their in vivo microdialysis study, that extracellular glutamate concentrations in the epileptogenic hippocampus of patients increased just before a seizure. More recently, the glutamate-glutamine cycling was found to be slower in the hippocampus of patients with mesial TLE (Petroff et al., 2002). Eid et al. (2004) obtained hippocampal tissue from 29 patients; the surgically resected hippocampi were classified into two groups: mesial TLE and nonmesial TLE. In Western blots, the expression of glutamine synthetase was 40% lower in mesial TLE than in nonmesial TLE samples. The enzyme activity was 38% lower in mesial TLE versus nonmesial TLE. Of note, the reduction in glutamine synthetase was particularly pronounced in areas of the mesial TLE hippocampus with astroglial proliferation, which is surprising considering the fact that normal astrocytes are especially rich in glutamine synthetase. As the glutamate uptake seems not to be impaired (Eid et al., 2004), the deficiency in glutamine synthetase is likely to play a major role in the accumulation of extracellular glutamate observed in the epileptogenic hippocampus (During and Spencer, 1993).

It remains to be established how the impairment of enzyme activity occurs and whether a deficiency in glutamine synthetase can indeed represent a causative factor for the chronic seizures and the hippocampal sclero-

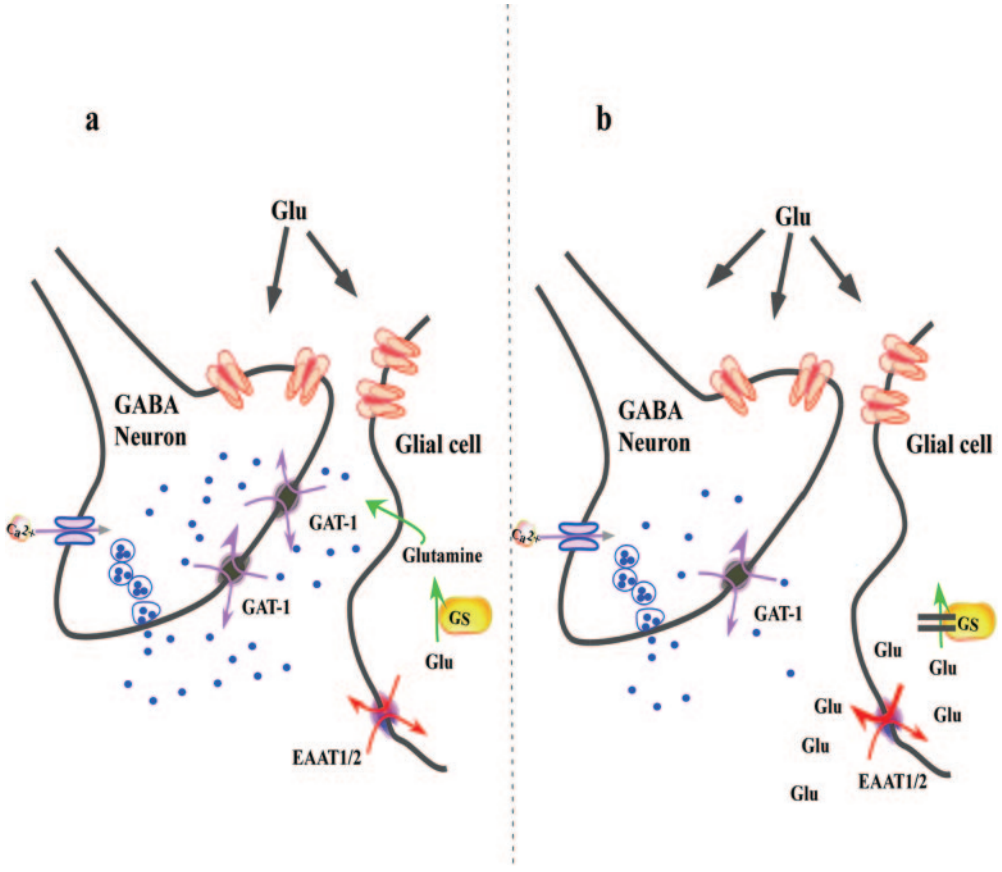

FIG. 3. A schematic diagram showing possible impirments of the GABA/glutamate equilibrium in the epileptogenic hippocampus. a, normal hippocampus. Neuronal firing causes Ca^{2+}-dependent exocytotic release of GABA. With moderate increases in extracellular glutamate, Na^{+} entry through ionotropic glutamate receptors triggers carrier-mediated release of GABA through GAT-1 transporters. Excess glutamate is captured by EAAT1/2 transporters into glial cells and transformed into glutamine by glutamine synthetase (GS). Moderate increases of extracellular $[K^{+}]$ potentiate GABA exocytosis. b, epileptogenic hippocampus. In response to elevated glutamate concentration, as may be seen with excessive excitation before seizure generation (During and Spencer, 1993), following glutamine synthetase deficiency (Eid et al., 2004), and/or consequent to $GABA_A$-receptor mediated depolarizing signals (Avoli et al., 1994), there is insufficient GABA efflux reflecting the loss of GABA transporters (During et al., 1995). The decreased availability of GABA transporters for carrier-mediated release becomes particularly relevant in presence of strong extracellular $[K^{+}]$ elevation (not shown), a condition in which the extra release of GABA may exclusively occur by transporter reversal (Raiteri et al., 2002b).

sis characteristic of mesial TLE. In the positive and animal studies suggesting that this may be the case [see Eid et al. (2004) for references], regulation of glutamine synthetase activity might constitute a novel approach in the therapy of temporal lobe epilepsy.

B. Calcium Channels and Epilepsy

Dynorphin is a neuropeptide highly expressed in the dentate granule cells of the hippocampus, which acts on opiate receptors of the κ type (Chavkin et al., 1982). Dynorphin can have a number of inhibitory effects including inhibition of glutamate release (Weisskopf et al., 1993), modulation of NMDA receptors (Chen et al., 1995), and block of VSCCs (Ingram et al., 1997). It has been hypothesized therefore that dynorphin may act as an endogenous anticonvulsant (Snead and Simonato, 1991).

Jeub et al. (1999) investigated whether dynorphin can affect VSCC function in hippocampal granule cells isolated from two patient groups, one with a histopathological diagnosis of Ammon's horn sclerosis (AHS) and the other showing a lesion in the temporal lobe that did not involve the hippocampus proper. The patients in the AHS group showed strong dynorphin immunoreactivity in the inner molecular layer of the dentate gyrus, indicating recurrent mossy fiber sprouting. In contrast, no such dynorphin-immunoreactive structures were observed in the patients of the lesion group. In patients without mossy fiber sprouting, the application of dynorphin A caused a naloxone-sensitive concentration-dependent depression of VSCCs in most granule cells. In contrast, significantly less dentate granule cells displayed inhibition of VSCCs by dynorphin A in patients with mossy fiber sprouting. Because large amounts of dynorphin are present in recurrent mossy fiber collaterals, one possible explanation is that receptors undergo down-regulation secondary to a chronic stimulation by dynorphin. Whatever the reason, a modulatory feedback pathway linking neuronal activity with dynorphin-mediated inhibition of VSCCs seems not to be present in patients with AHS (Jeub et al., 1999).

If frequent and prolonged seizures can kill nerve cells, one possible mechanism involved in cell death is excessive accumulation of cytosolic Ca^{2+} from various origins. The role of calbindin D_{28K} (CB) in Ca^{2+} homeostasis and, in particular, in neuroprotection, is controversial (Cheng et al., 1994; Klapstein et al., 1998). Nägerl et al. (2000) addressed the regulation by CB of Ca^{2+} influx into human hippocampal neurons that survived AHS associated with mesial TLE, using acutely isolated dentate granule cells. It was found that, in patients with AHS, the loss of CB from granule cells markedly increased the Ca^{2+}-dependent inactivation of VSCCs, thereby decreasing the entry of Ca^{2+} during repetitive neuronal firing. Interestingly, when purified CB was introduced into granule cells, the inactivation of Ca^{2+} currents was restored to levels observed in cells with normal CB content obtained from patients with TLE but without AHS. These results support the idea that the loss of CB is neuroprotective. It is known that AHS is characterized by a dramatic loss of hippocampal neurons including the hilus and the CA1, CA3, and CA4 regions, whereas dentate gyrus granule cells are more resistant to the damage (Blümcke et al., 1999). According to Nägerl et al. (2000), the loss of CB may contribute to the selective resistance of these cells in AHS because it limits Ca^{2+} influx through an enhanced Ca^{2+}-dependent inactivation of VSCCs during prolonged neuronal discharges.

XIII. Brain Ischemia and Traumatic Injury

It is widely accepted that extracellular accumulation of excitatory amino acids, in particular glutamate, is an early event in brain ischemia, involved in membrane depolarization and in excitotoxic neuronal damage [see Choi (1988), Szatkowski and Attwell (1994), Nieber (1999), and references therein]. In patients with cerebral ischemia, but also with acute brain injury, a relation was indeed observed between the extracellular glutamate concentration and neurological deficits (Castillo et al., 1996; Persson et al., 1996; Bullock et al., 1998). Although the dependence of the excitotoxic damage on ionotropic glutamate receptor activation and the neuroprotective effects of glutamate receptor blockers (Szatkowski and Attwell, 1994; Lipton, 1999; Nieber, 1999) are consistent with neuroprotection being related to inhibition of excessive glutamate transmission, including elevated glutamate release (De Cristobal et al., 2001; Castillo et al., 2003), the question arises as to whether the control of glutamate efflux alone can be neuroprotectant in brain ischemia (Calabresi et al., 2000).

The possibility of controlling extracellular glutamate accumulation during ischemia rests on the knowledge of the mechanisms of glutamate efflux. Reversal of glutamate transporters (Levi and Raiteri, 1993; Szatkowski and Attwell, 1994; Jabaudon et al., 2000; Rossi et al., 2000), vesicular exocytosis (Drejer et al., 1985; Katchman and Hershkowitz, 1993; Jehle et al., 2000) and swelling-induced efflux through glial anion channels (Bednar et al., 1992) have been reported to occur in varying proportions during ischemia. Most likely, different modes of glutamate efflux become preponderant, depending on the ischemia model adopted and on the degree of oxygen/glucose deprivation reached in the tissue. Rapid and severe ischemia (such as oxygen/glucose deprivation plus inhibition of glycolysis and mitochondrial respiration) could mimic events occurring in the highly anoxic core of the ischemic area, with extreme energy failure in the mitochondria. The efflux of glutamate caused by such severe conditions applied to rat hippocampal slices was reported to take place almost entirely by carrier-mediated release (Rossi et al., 2000). It is conceivable that moderate ischemia better reflects events occurring in the penumbral zone surrounding the ischemic core, i.e. the zone that may be targeted by therapeutic intervention.

A. Glutamate Release during Ischemia

1. Mechanisms of Release. Glutamate release was studied using human neocortical slices obtained from patients undergoing neurosurgery to reach deeply sited tumors. Release of previously accumulated [^{3}H]D-aspartate (used as a marker of newly taken up glutamate/aspartate) and of endogenous glutamate was monitored during and after superfusion with "ischemic" (oxygen and glucose-deprived) medium. Both the efflux of [^{3}H]D-aspartate and that of endogenous glutamate significantly increased starting 18 min after exposure to the ischemic insult. After 24 or 36 min of ischemia, the excitatory amino acid efflux returned to basal values within 6 min of superfusion with physiological solution (Marcoli et al., 2004).

Superfusion with Ca^{2+}-free medium or with tetrodotoxin reduced by ~50 and 65%, respectively, the glutamate efflux, compatible with a vesicular exocytotic mode of exit of glutamate from human cerebrocortical slices exposed to ischemia. Vesicular glutamate release is usually considered to be of neuronal origin, although glial cells may well contribute to Ca^{2+}-dependent glutamate release during ischemia (Bezzi and Volterra, 2001). In apparent contrast with these results by Marcoli et al. (2004), Hegstad et al. (1996) found glutamate (as well as GABA and glycine) efflux from ischemic human cerebrocortical slices to be independent of extracellular Ca^{2+}. However, the Ca^{2+} dependency was evaluated by these authors during static incubation and by adding Ca^{2+}-free medium concomitantly with the ischemic insult, whereas Marcoli et al. (2004) worked under superfusion conditions and removed Ca^{2+} before the ischemic insult.

The Ca^{2+} dependence and the tetrodotoxin sensitivity of glutamate efflux, together with the return to basal levels after a few minutes of reperfusion, are consistent with a relatively good viability of glutamatergic axon terminals in human brain exposed to moderate ischemic

insult. It is known that extracellular K^+ concentrations can rise up to 60 mM during brain ischemia (Szatkowski and Attwell, 1994), which is compatible with glutamate release occurring in part by depolarization-evoked opening of VSCCs and consequent vesicular exocytosis; indeed, in human cerebrocortical tissues, the efflux of glutamate, aspartate, GABA, and glycine evoked by K^+ depolarization was found to be largely dependent on external Ca^{2+} (Hegstad et al., 1996; Bonanno et al., 1997).

Interestingly, the glutamate efflux from human cerebrocortical slices during oxygen and glucose deprivation (Marcoli et al., 2004) exhibited close similarities, as far as dependence on external Ca^{2+} and sensitivity to tetrodotoxin are concerned, with the glutamate efflux from rat cerebrocortical slices previously observed under the same experimental conditions (Marcoli et al., 2003), suggesting that rat ischemic cerebrocortical slices represent a useful experimental model to assess drug effects on glutamate efflux ultimately transferable to humans. Of note, it was observed that a longer oxygen/glucose deprivation is necessary to evoke glutamate release from human with respect to rat slices, consistent with an apparent greater resistance of the human tissue to ischemia (Jiang and Haddad, 1992).

2. Glutamate Release and Adenosine A_{2A} Receptors. If a consistent portion of the glutamate efflux caused by ischemia in human brain occurs by exocytosis, it should be possible to prevent it by activating presynaptic receptors, which can regulate the Ca^{2+}-dependent component of the depolarization-evoked neurotransmitter release from axon terminals (Langer, 1993; Levi and Raiteri, 1993).

The ischemia-evoked glutamate efflux from human cerebrocortex slices was significantly reduced by blocking adenosine receptors of the A_{2A} subtype with the selective compound SCH58261 (Zocchi et al., 1996). One interpretation is that glutamate release-enhancing A_{2A} receptors exist in human cerebral cortex, the activation of which by endogenously released adenosine participates in the glutamate efflux occurring during ischemia. Accordingly, presynaptic release-enhancing A_{2A} receptors had been found on rat cerebrocortex glutamatergic synaptosomes (Marchi et al., 2002a). Blockade of A_{2A} receptors was ineffective on the release of glutamate occurring in human cerebrocortical slices superfused in Ca^{2+}-free medium, which strengthens the view that adenosine endogenously released during ischemia activates exocytotic release-enhancing A_{2A} receptors present on glutamatergic terminals. Consistent with the idea that A_{2A} receptor block could prevent potentially detrimental activation of A_{2A} receptors, ischemic damage was reported to be reduced by A_{2A} receptor antagonists in rats (Phillis, 1995; Monopoli et al., 1998; von Lubitz, 1999) or in A_{2A} receptor knockout mice (Chen et al., 1999).

3. Glutamate Release and 5-Hydroxytryptamine Receptors. In human cerebral cortex, 5-HT seems to be an important regulator of glutamatergic transmission: the indoleamine was found to reduce the depolarization-evoked glutamate release (Maura et al., 1998) and the production of NO and cyclic GMP due to activation of ionotropic glutamate receptors (Maura et al., 2000) through multiple pre- and postsynaptic 5-HT receptors (see Fig. 1).

When 5-HT was added concomitantly with oxygen/glucose-deprived solution to human cerebrocortical slices in superfusion, the ischemia-evoked efflux of previously accumulated [^{3}H]D-aspartate or of endogenous glutamate was significantly reduced by ~60%. The effect of 5-HT was in part mimicked by the 5-HT_{1A} receptor agonist 8-OH-DPAT, which prevented the ischemia-provoked glutamate overflow by ~40% (Marcoli et al., 2004). Agonists at the 5-HT_{1A} receptor had been reported to prevent glutamate release in in vivo and in vitro rat models of ischemia (Mauler et al., 2001; Dawson et al., 2002). In slices of human cerebral cortex, postsynaptic 5-HT_{1A} receptors were found to hyperpolarize glutamatergic pyramidal neuron (Newberry et al., 1999) and to inhibit the NO synthase/guanylyl cyclase pathway linked to NMDA glutamate receptor activation (Maura et al., 2000). It would be interesting to explore in future studies receptor-receptor interactions between 5-HT and NMDA receptors possibly colocalized on glutamatergic neurons. In fact, hyperpolarizing 5-HT_{1A} receptors controlling NMDA-dependent excitation might directly protect neurons against excitotoxicity during and after ischemia, consistent with observations in in vitro and in vivo animal models (Azmitia, 2001; Harkany et al., 2001).

The 5-HT_{1A} agonist 8-OH-DPAT mimicked only in part the effect of 5-HT, suggesting the involvement of additional mechanisms. Inhibition by 5-HT of the ischemia-evoked glutamate efflux could also involve activation of release-inhibitory h5-HT_{1D} heteroreceptors previously found to be sited on human cerebrocortical glutamatergic axon terminals (Maura et al., 1998). Thus, it is reasonable to propose that presynaptic 5-HT_{1D} and postsynaptic 5-HT_{1A} receptors cooperate in reducing glutamatergic transmission when human cerebral cortex slices are exposed to ischemic insults. To conclude, selective 5-HT ligands may deserve to be considered in a multipharmacological approach to neuroprotection in brain ischemia.

B. Glutamatergic Transmission following Brain Injury: In Vivo Microdialysis Studies

Persson et al. (1996) measured glutamate in dialysate samples collected for periods ranging from 6 to 11 days after ictus in 10 patients with subarachnoid hemorrhage. The data were matched with tomography findings, clinical course, and outcome. According to the authors, the correlation between the dialysate levels of

glutamate and outcome supports the concept of glutamate receptor overactivation in acute human brain injury.

Bullock et al. (1998) investigated the role of glutamatergic transmission in patients who suffered severe traumatic head injury. Excessive amounts of excitatory amino acids (EAAs) in the extracellular space may lead to neurotoxicity and cell death. The authors placed a microdialysis probe into the grey matter for 4 days and monitored the levels of glutamate/aspartate in the dialysate. The levels of EAAs were dramatically increased in several patients; secondary ischemic brain injury and poor outcome were strongly correlated with high EAA levels. Thus, according to Bullock et al. (1998), these patients may be the best candidates for treatment with glutamate antagonists in the future.

Extracellular glutamate was monitored for 9 days in 17 patients following severe traumatic brain injury (Vespa et al., 1998). The glutamate levels were particularly increased on postinjury day 3. Transient elevations of dialysate glutamate were seen each day, in conjunction with seizure activity, in a number of patients. In many seizure-free patients, the increase in extracellular glutamate occurred when cerebral perfusion pressure was $<$70 mm Hg.

More recently, the amino acid content of microdialysis samples from nine children with severe head injuries was examined (Richards et al., 2003). Although the interpretation of changes in extracellular glutamate levels measured by microdialysis is complicated by many factors, well discussed by the authors, a link between high dialysate glutamate and less favorable outcome seems established both in adults (Bullock et al., 1998) and in children. Interestingly, the relationship between levels of glutamate and glutamine seems to have good prognostic value. In particular, an increased extracellular glutamine/glutamate ratio is associated with more favorable outcome so that estimation of this ratio may have some prognostic value in head trauma.

XIV. Effects of HIV-1 Proteins

The clinical manifestations of AIDS often include neurological and neuropsychiatric disturbances known as the AIDS dementia complex (Price et al., 1988). The origins of the motor, cognitive, and behavioral impairments accompanying HIV-1 infection, may be present in ~30% of adult patients but in ~70% of children, are poorly understood. Because neurons can rarely, if ever, be infected by the virus, it is hypothesized that indirect mechanisms are primarily involved in the neuronal deficits and in the neurotoxic events that often occur during AIDS infection (Kaul and Lipton, 1999; Valle et al., 2000; Bezzi et al., 2001; Kaul et al., 2001; Mollace et al., 2001), although direct effects of HIV-1 proteins have been reported as well (see below).

The occurrence of neurotoxic events is sustained by postmortem histological observations made in cerebral tissue from HIV-1-positive patients, in whom loss of neurons has clearly been described previously (Fox et al., 1997; Masliah et al., 1997; Everall et al., 1999). These dramatic effects may, however, not be necessary to explain the above-mentioned neuropsychiatric deficits; in fact, relatively subtle changes in central neurotransmission induced by HIV-1 proteins might be at the basis of various functional impairments.

A. Activation of Glutamate N-Methyl-D-aspartate Receptors by gp120

In the last decade, evidence has been provided suggesting that the HIV-1 envelope protein gp120 could directly influence central neuronal functions, causing changes in neurotransmitter release or affecting receptor-mediated mechanisms which may not be necessarily related to neuronal damage, but may underlie the cognitive and behavioral impairments characteristic of the AIDS dementia complex.

To investigate whether gp120 can directly affect receptor function in the human brain, the effect of the coat protein on the release of NE evoked by NMDA receptor activation in human cerebrocortical nerve ending preparations was studied (Pittaluga et al., 1996). This system was chosen because 1) NMDA receptors had been reported to be major agents in the neurotoxic effects of gp120 in various experimental animal models, very often in cell cultures (Brennemann et al., 1988; Dreyer et al., 1990; Müller et al., 1992; Dawson et al., 1993; Savio and Levi, 1993; Kaul et al., 2001), 2) NMDA receptors play a critical role in the processes of learning and memory (Collingridge and Bliss, 1987), 3) NE also is positively involved in cognition, particularly in attentive activities (Cahill et al., 1994; Aston-Jones, 2002; Berridge and Waterhouse, 2003), and 4) the human cerebral cortex was reported to contain NMDA receptors, the activation of which mediates increase of NE release (Fink et al., 1992a).

When added during superfusion to human cerebrocortical synaptosomes prelabeled with [^{3}H]NE, NMDA, in the presence of the coagonist glycine, enhanced the release of the [^{3}H]catecholamine. The HIV-1 protein gp120 mimicked glycine: added at 1 nM, gp120 potentiated the NMDA-evoked release of [^{3}H]NE. The release elicited by NMDA plus gp120 was prevented by 7-Cl-kynurenate, an antagonist at the glycine site of the NMDA receptor and by the open channel blockers dizocilpine and memantine. Interestingly, the effect of gp120 was retained by its peptide fragment termed the V3 loop. Finally, gp120 was able to reverse and surmount the antagonism by 7-Cl-kynurenate of the NMDA-evoked [^{3}H]NE release (Pittaluga et al., 1996).

These results show that a binding site for gp120 exists on the axon terminals of noradrenergic neurons in the human cerebral cortex. By acting at this site, probably

through the V3 loop, the HIV-1 coat protein would facilitate the NE-releasing activity of endogenous NMDA receptor agonists (glutamate, aspartate, and quinolinic acid). This site of binding for gp120 seems to be the glycine site of NMDA receptors where gp120 acts as a "false" glycinergic coagonist of glutamate. Pathologically relevant is the observation that gp120 (or V3) is 3 to 4 orders of magnitude more potent than the natural ligand glycine. The potency of these foreign agonists, together with their likely prolonged persistence at the NMDA receptors, might lead to serious impairments of NMDA receptor-mediated neurotransmission. In fact, only glycine, but not gp120 (or V3), is expected to be released phasically onto NMDA receptors and to be efficiently removed by reuptake. An abnormal and persistent reinforcement of the glutamate-dependent NE release due to the presence of gp120 (or its V3 loop) in the biophase of NMDA receptors might lead to unbalances in the processes underlying learning and memory, thus contributing to the cognitive deficits characteristic of AIDS dementia. It may be worth a reminder that intracerebral administration of gp120 in rats was reported to cause learning impairment (Glowa et al., 1992) in absence of signs of degeneration (Bagetta et al., 1994).

The finding that memantine was able to prevent the gp120 potentiation of the NMDA-evoked effect is of interest. Memantine is a low-affinity, uncompetitive, open-channel blocker of the NMDA receptor, which was reported not to substantially affect normal synaptic activity but to prevent excessive NMDA activity (Lipton and Chen, 2004). Memantine has recently been introduced for the treatment of Alzheimer's disease, but additional clinical studies of memantine for other forms of dementia, including the AIDS dementia complex, are currently underway.

Results almost identical to those obtained with human cerebral cortex nerve endings were obtained when gp120 and its V3 loop were studied using rat nerve ending preparations (Pittaluga and Raiteri, 1994; Pattarini et al., 1998), suggesting that the NMDA-evoked release of NE from rat nerve endings represents a convenient model to investigate the mechanistic aspects of the action of gp120 and its peptide fragments on NMDA receptors and to test compounds potentially useful in the treatment of AIDS dementia.

B. Activation of Glutamate Metabotropic Receptors by Tat

Increasing attention has been focused on Tat, a nonstructural viral protein known to be produced and actively released by HIV-1-infected cells (Ensoli et al., 1993). Tat has been detected in the brain of animals with viral encephalitis and its encoding mRNA has been found in the CNS of HIV-1-infected patients exhibiting AIDS dementia symptoms (Hudson et al., 2000; Valle et al., 2000).

Tat can apparently induce neurological/neuropsychiatric impairments and neurotoxicity because it was shown both to excite and kill human and rodent cerebrocortical neurons (Nath et al., 1996; Kruman et al., 1998) by mechanisms involving neuronal Ca^{2+} homeostasis (Haughey et al., 1999, 2001). The protein binds to and depolarizes neuronal membranes (Sabatier et al., 1991), allowing increases in cytosolic Ca^{2+} through receptor-operated channels and mobilization from inositol trisphosphate-sensitive internal stores (Nath et al., 1996; Haughey et al., 1999, 2001).

We investigated whether Tat can affect neurotransmission in human brain by modulating transmitter release from fresh human cerebrocortical nerve ending preparations (Feligioni et al., 2003). Experiments with corresponding preparations from rat brain were run in parallel. Tat was found to selectively enhance the release of ACh; the protein was in fact unable to affect the basal release of endogenous glutamate, aspartate, GABA, serine, and NE but, added at 1 to 3 nM, it evoked release of [^{3}H]ACh from human and rat cerebrocortical synaptosomes prelabeled with [^{3}H]choline. As illustrated schematically in Fig. 4, in human nerve endings, the Tat-evoked release of ACh depends on activation of group I metabotropic glutamate receptors, and mobilization of Ca^{2+} from inositol trisphosphate-sensitive intraterminal stores. In contrast, in rat nerve endings, the Tat-evoked ACh release seems to involve mobilization of Ca^{2+} from ryanodine-sensitive internal stores triggered by the binding of Tat to an as yet unidentified "receptor" on cholinergic neurons the activation of which leads to cyclic adenosine diphosphoribose production (Feligioni et al., 2003).

In conclusion, Tat can evoke release of ACh from human and rat cerebrocortex cholinergic axon terminals with remarkable neuronal selectivity. The mechanism involved in the release from human terminals differs strikingly from that in rodent terminals. Tat binds to different recognition sites on human versus rat cholinergic terminals, a view that is also supported by the finding that the releasing effect of Tat in human terminals is mimicked by peptide fragments different from those mimicking it in rat terminals. Therefore, differently from the case of gp120, the rodent model should be used with great caution when Tat effects on neurotransmission are studied.

The pathophysiological significance of the Tat effects on ACh release is presently unknown. In any case, once present in the brain extracellular fluid, Tat may not be easily inactivated and may therefore, as the intact protein or in the form of active peptide fragments, provoke release of ACh outside of the physiological range and with abnormal kinetic characteristics, potentially contributing to the behavioral disturbances occurring in AIDS dementia.

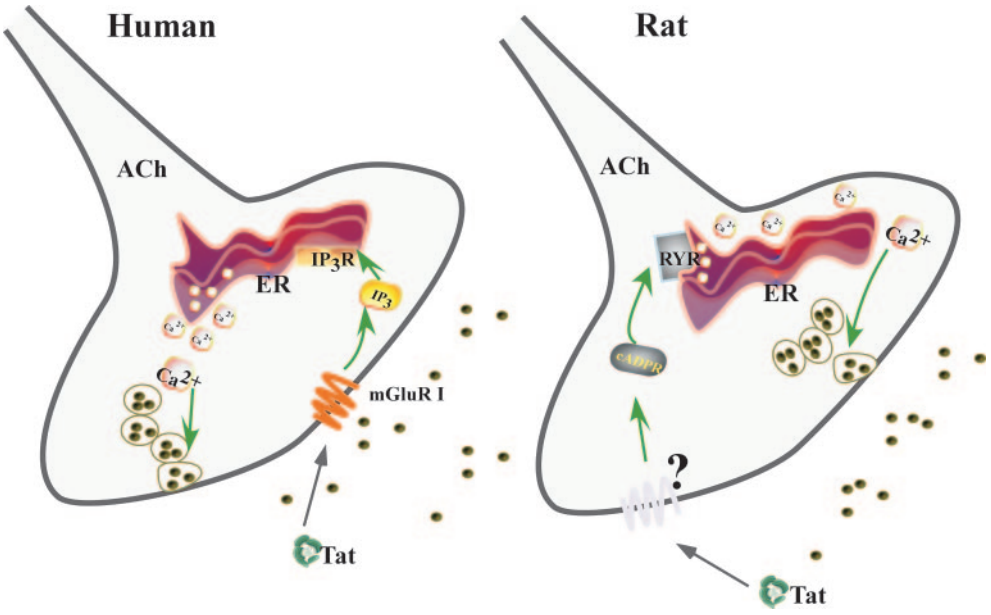

FIG. 4. Effects of the HIV-1 protein Tat on the release of ACh from human and rat neocortical cholinergic nerve endings. In human neocortex, Tat activates glutamate group I metabotropic receptors (mGluRI) on ACh terminals leading to inositol trisphosphate (IP_3) production, IP_3 receptor (IP_3R) activation, mobilization of Ca^{2+} from the endoplasmic reticulum (ER), and vesicular ACh release. In rat neocortex cholinergic terminals Tat binds to an unidentified receptor whose activation leads to ACh release. This release also is dependent on intraterminal Ca^{2+}, but this is mobilized by ryanodine receptor (RYR) activation via the endogenous agonist cADPR.

XV. Parkinson's Disease: In Vivo Microdialysis Studies

Microdialysis measurements in the brain of patients with Parkinson's disease (PD) were performed for the first time in 1989 (Meyerson et al., 1990) during neurosurgical thalamotomy to relieve tremor. This was possible as the microdialysis probes were introduced through the same trajectory as the lesioning electrode, thus causing no additional damage to the brain. The main goal was to test the reliability of the microdialysis technique for biochemical characterization of the target area in the human brain during a routine operation. In this study, dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, 5-hydroxyindoleacetic acid, hypoxanthine, inosine, guanosine, adenosine, GABA, taurine, aspartate, and glutamate were measured in the dialysates from the thalamus. The results showed that the technique was safe, no additional damage was observed and consistent and levels of reproducible analytes could be measured, following 20 to 30 min of stabilization.

Later on, this technique was also used during neurosurgery of patients with PD for implantation of electrodes for deep brain stimulation (DBS) in the internal globus pallidus (GPi) and subthalamic nucleus (STN), after prolonged therapy washout (Fedele et al., 2001a, b). In these studies, the extracellular levels of aspartate, glutamate, glycine, and GABA in the external globus pallidus (GPe), GPi, and STN were measured before (off-state) and after (on-state) perioperative administration of a clinically effective dose of apomorphine. In the off-state, it has been found that GABA basal levels in the GPi were significantly higher than those measured in the GPe and STN. Considering the current view on basal ganglia pathophysiology, the higher GABA levels in the GPi should not be caused by the degenerative pathology, as both GABAergic pathways reaching this brain region (the direct pathway from the putamen and the indirect pathway via collaterals of the GPe fibers directed to the STN) are supposed to be less active in off-state PD patients (Albin et al., 1989; De Long, 1990; Lang and Lozano, 1998a,b). Therefore, this finding indicates that the GPi receives more GABAergic inputs than the other basal ganglia nuclei and could represent a biochemical marker for GPi target identification in PD surgery (Fedele et al., 2001a,b). Unexpectedly, administration of apomorphine failed to alter amino acids levels in all the brain structures investigated, despite the significant decrease of GPi and STN firings, measured by electrophysiological recordings and the clear-cut amelioration of clinical symptoms, suggesting that the acute effects of the dopaminergic therapy are not due to modulation of neuroactive amino acid release. However, as authors themselves acknowledge, such negative findings may be due to limitations of the microdialysis technique that can fail to detect physiologically relevant synaptic events if they do not sufficiently leak out of the synaptic cleft into the extracellular milieu. Moreover, microdialysis data from laboratory animals have shown that extracellular basal levels of amino acids, especially glutamate, might originate from metabolic pools, which can obscure possible changes in the levels of neuronal nature (Timmerman and Westerink, 1997).

Recently, to unravel the mechanisms underlying the beneficial effects of DBS of the STN (STN-DBS), the same research group has investigated the effects of this type of stimulation on the extracellular levels of cGMP in the GPi of PD patients (Stefani et al., 2005). Cyclic GMP has been monitored as a marker of glutamatergic transmission, given the possible limitations of the microdialysis technique in measuring variations of extracellular glutamate mentioned above. As a matter of fact, it has been extensively documented that the production

of this cyclic nucleotide is particularly linked to glutamatergic neurotransmission, via activation of NO/synthase, and can be measured in vivo by microdialysis in different brain regions of rodents (Vallebuona and Raiteri, 1994) [see Fedele and Raiteri (1999), Pepicelli et al. (2004), and references therein for reviews]. Authors found that extracellular cyclic GMP basal levels were significantly augmented during clinically effective STN-DBS, indicating an increased activity of the STN-GPi glutamatergic pathway. This result is at variance with the initial hypothesis suggesting that the beneficial effects of STN-DBS were due to the high frequency-mediated inactivation of STN cells (Benazzouz et al., 1995; Filali et al., 2004; Welter et al., 2004). However, it has also been shown that STN cells can fire at peculiarly high frequency rates, either spontaneously or during passive movement (Levy et al., 2001; Stefani et al., 2002). Moreover, STN stimulation leads to the increase in blood oxygenation level-dependent signals and blood flow in ipsilateral basal ganglia workstations, as assessed by functional magnetic resonance imaging and positron emission tomography techniques (Jech et al., 2001; Hershey et al., 2003), suggesting stimulation, and not inhibition, of GPi activity. Finally, GPi firing activity is increased during STN-DBS in a primate model of PD, demonstrating that the stimulation can reinforce and synchronize, but not suppress, the glutamatergic driving force from STN (Hashimoto et al., 2003). However, all these data conflict with the well-established concept that STN-DBS-induced relief of PD symptoms are mediated by disinhibition of the thalamocortical pathway, which, in turn, should be accomplished by a decrease and not increase in GPi activity. Therefore, additional studies will be necessary to untangle this paradox.

XVI. Conclusions

This article is a comprehensive review of the work carried out in the last decade on human brain functional proteins, particularly neurotransmitter receptors. The literature available on the topic shows that the large majority of the studies have been performed with fresh tissue samples obtained from neurosurgery. It seems clear that frozen brain samples from autopsies, certainly useful in experimental approaches such as radioligand binding, lend themselves poorly to studies aimed to understand the function of receptors, transporters, ion channels, and enzymes. Surprisingly, a large proportion of the reports deal with neurotransmitter release and release-regulating receptors, indicating that the physiology and pharmacology of these fundamental neuronal properties can be conveniently investigated in neurosurgically derived human brain tissue samples. Relatively less abundant are the reports regarding electrophysiological works; these were generally performed using slices of epileptogenic regions and almost exclusively focussed on transmitter amino acid receptors. Some publications describe changes in neurotransmitter amino acids and second messengers monitored during microdialysis in epileptic or parkinsonian patients undergoing neurosurgery. Besides the obvious importance of the information one can obtain when working with human brain, this approach seems unique for a number of reasons. Since the availability of fresh human tissue specimens is rare, it is critical to identify animal species in which functionally corresponding receptors exhibit pharmacological profiles identical or very similar to those in humans, so that they can be used as models in preclinical pharmacology. Moreover, heterologous expression of human receptors/transporters may not represent substantial alternatives to native proteins whose function and pharmacology is dependent on a number of interactions with agents that may exclusively be present in their natural environment.

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REFERENCES

Agnati LF, Ferré S, Lluis C, Franco R, and Fuxe K (2003) Molecular mechanisms and therapeutical implications of intramembrane receptor/receptor interactions among heptahelical receptors with examples from the striatopallidal GABA neurons. *Pharmacol Rev* **55:**509–550.

Albin RL, Young AB, and Penney JB (1989) The functional anatomy of basal ganglia disorders. *Trends Neurosci* **12:**366–375.

Albuquerque EX, Pereira EFR, Mike A, Eisenberg HM, Maelicke A, and Alkondon M (2000) Neuronal nicotinic receptors in synaptic functions in humans and rats: physiological and clinical relevance. *Behav Brain Res* **113:**131–141.

Amara S and Kuhar MJ (1993) Neurotransmitter transporters: recent progress. *Annu Rev Neurosci* **16:**73–93.

Ameri A (1999) The effects of cannabinoids on the brain. *Prog Neurobiol* **58:**315–348.

Amtage F, Neughebauer B, McIntosh JM, Freiman T, Zentner J, Feuerstein TJ, and Jackisch R (2004) Characterization of nicotinic receptors inducing noradrenaline release and absence of nicotinic autoreceptors in human neocortex. *Brain Res Bull* **62:**413–423.

Anderson DJ, Puttfarcken PS, Jacobs IC, and Faltynek C (2000) Assessment of nicotinic acetylcholine receptor-mediated release of [^{3}H]NE from rat brain slices using a new 96-well format assay. *Neuropharmacology* **39:**2663–2672.

Aston-Jones J (2002) Norepinephrine, in *Neuropsychopharmacology: The Fifth Generation of Progress* (Davis KL, Charney D, Coyle JT, and Nemeroff C eds) pp 47–58, Lippincott Williams and Wilkins, Baltimore.

Attwell D, Barbour B, and Szatkowski M (1993) Nonvesicular release of neurotransmitter. *Neuron* **11:**401–407.

Avoli M, Drapeau C, Louvel J, Pumain R, Olivier A, and Villemure JG (1991) Epileptiform activity induced by low extracellular magnesium in the human cortex maintained in vitro. *Ann Neurol* **30:**589–596.

Avoli M, Mattia D, Siniscalchi A, Perreault P, and Tomaiuolo F (1994) Pharmacology and electrophysiology of a synchronous GABA-mediated potential in the human neocortex. *Neuroscience* **62:**655–666.

Azmitia EC (2001) Modern views on an ancient chemical: serotonin effects on cell proliferation, maturation and apoptosis. *Brain Res Bull* **56:**413–424.

Bagetta G, Finazzi-Agrò A, Palma E, and Nisticò G (1994) Intracerebral injection of human immunodeficiency virus type I coat glycoprotein GP120 does not produce neurodegeneration in rats. *Neurosci Lett* **176:**97–100.

Barton JJ (2003) Disorders of face perception and recognition. *Neurol Clin* **21:**521–548.

Beal MF, Mazurek MF, Svendsen CN, Bird ED, and Martin JB (1986) Widespread reduction of somatostatin-like immunoreactivity in the cerebral cortex in Alzheimer's disease. *Ann Neurol* **20:**489–495.

Beani L, Bianchi C, Antonelli T, Calò G, Morari M, Ferioli V, and Gaist G (1992) Comparison of [^{3}H]choline and D-[^{3}H]aspartate efflux from guinea pig and human neocortex. *J Neurochem* **58:**1454–1459.

Bednar MM, Kohut JJ, Kimelberg HK, Gross JJ, and Gross CE (1992) In vitro evidence supporting two mechanisms of action for the anion transport inhibitor L-644,711 in cerebral ischemia. *Neurol Res* **14:**53–56.

Benazzouz A, Piallat B, Pollak P, and Benabid AL (1995) Responses of substantia nigra pars reticulata and globus pallidus complex to high frequency stimulation of the STN in rats: electrophysiological data. *Neurosci Lett* **189:**77–80.

Berridge CW and Waterhouse BD (2003) The locus coeruleus-noradrenergic system: modulation of behavioral state and state-dependent cognitive processes. *Brain Res* **42:**33–84.

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta

G, Kollias G, Meldolesi J, et al. (2001) CXCR4-activated astrocyte glutamate release via TNFα: amplification by microglia triggers neurotoxicity. *Nat Neurosci* **4:**702–710.
Bezzi P and Volterra A (2001) A neuron-glia signalling network in the active brain. *Curr Opin Neurobiol* **11:**387–394.
Biscoe TJ and Straughan DW (1966) Micro-electrophoretic studies of neurones in the cat hippocampus. *J Physiol (Lond)* **183:**341–359.
Blümcke I, Zuschratter W, Schewe J-C, Suter B, Lie AA, Riederer BM, Meyer B, Schramm J, Eiger CE, and Wiestler OD (1999) Cellular pathology of hilar neurons in Ammon's horn sclerosis. *J Comp Neurol* **414:**437–453.
Boileau I, Assaad J-M, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay RE, and Dagher A (2003) Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* **49:**226–231.
Bonanno G, Caritià F, Cavazzani P, Munari C, and Raiteri M (1999) Selective block of rat and human neocortex $GABA_B$ receptors regulating somatostatin release by a $GABA_B$ antagonist endowed with cognition enhancing activity. *Neuropharmacology* **38:**1789–1795.
Bonanno G, Cavazzani P, Andrioli GC, Asaro D, Pellegrini G, and Raiteri M (1989) Release-regulating autoreceptors of the $GABA_B$ type in human cerebral cortex. *Br J Pharmacol* **96:**341–346.
Bonanno G, Fassio A, Schmid G, Severi P, Sala R, and Raiteri M (1997) Pharmacologically distinct $GABA_B$ receptors that mediate inhibition of GABA and glutamate release in human neocortex. *Br J Pharmacol* **120:**60–64.
Bonanno G, Gemignani A, Schmid G, Severi P, Cavazzani P, and Raiteri M (1996) Human brain somatostatin release from isolated cortical nerve endings and its modulation through $GABA_B$ receptors. *Br J Pharmacol* **118:**1441–1446.
Bonanno G, Giambelli R, Raiteri L, Tiraboschi E, Zappettini S, Musazzi L, Raiteri M, Racagni G, and Popoli M (2005) Chronic antidepressants reduce depolarization-evoked glutamate release and protein interactions favoring formation of SNARE complex in hippocampus. *J Neurosci* **25:**3270–3279.
Bonanno G and Raiteri M (1992) Functional evidence for multiple γ-aminobutyric $acid_B$ receptor subtypes in the rat cerebral cortex. *J Pharmacol Exp Ther* **262:** 114–118.
Bonanno G and Raiteri M (1993) Multiple $GABA_B$ receptors. *Trends Pharmacol Sci* **14:**259–261.
Bonanno G, Sala R, Cancedda L, Cavazzani P, Cossu M, and Raiteri M (2000) Release of dopamine from human neocortex nerve terminals evoked by different stimuli involving extra- and intraterminal calcium. *Br J Pharmacol* **129:**1780–1786.
Bouvier M (2001) Oligomerization of G-protein-coupled transmitter receptors. *Nat Rev Neurosci* **2:**274–286.
Bowen DM (1977) Biochemistry of dementias. *Proc R Soc Med* **70:**351–352.
Bowery NG, Bettler B, Froestl W, Gallagher JP, Marshall F, Raiteri M, Bonner TI, and Enna SJ (2002) International union of pharmacology. XXXIII. Mammalian γ-aminobutyric $acid_B$ receptors: structure and function. *Pharmacol Rev* **54:**247–264.
Brennemann DE, Westbrook GL, Fitzgerald SP, Ennist DL, Elkins KL, Ruff MR, and Pert CB (1988) Neuronal cell killing by the envelope protein of HIV and its prevention by vasoactive intestinal peptide. *Nature (Lond)* **335:**639–642.
Brever TS and Barch DM (2002) A theory of cognitive control, aging cognition and neuromodulation. *Neurosci Biobehav Rev* **26:**809–817.
Bruinvels AT, Lery H, Nozulak J, Palacios JM, and Hoyer D (1992) 5-HT_{1D} binding sites in various species: similar pharmacological profile in dog, monkey, calf, guinea-pig and human brain membranes. *Naunyn-Schmiedeberg's Arch Pharmacol* **346:**243–248.
Bryans JS and Wustrow DJ (1999) 3-Substituted GABA analogs with central nervous system activity: a review. *Med Res Rev* **19:**149–177.
Bullock R, Zauner A, Woodward JJ, Myseros J, Choi SC, Ward JD, Marmarou A, and Young HF (1998) Factors affecting excitatory amino acid release following severe human head injury. *J Neurosurg* **89:**507–518.
Bylund DB (1995) Pharmacological characteristics of α_2 adrenergic receptor subtypes. *Ann NY Acad Sci* **763:**1–7.
Cahill L, Prins B, Weber M, and McGaugh JL (1994) Beta-adrenergic activation and memory for emotional events. *Nature (Lond)* **371:**702–704.
Calabresi P, Picconi B, Saulle E, Centonze D, Hainsworth AH, and Bernardi G (2000) Is pharmacological neuroprotection dependent on reduced glutamate release? *Stroke* **31:**766–772.
Calò G, Guerrini R, Rizzi A, Salvadori S, and Regoli D (2000) Pharmacology of nociceptin and its receptor: a novel therapeutic target. *Br J Pharmacol* **129:**1261–1283.
Capogna M, Gahwiller BH, and Thompson SM (1993) Mechanism of μ-opioid receptor-mediated presynaptic inhibition in the rat hippocampus in vitro. *J Physiol (Lond)* **470:**539–558.
Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, and Eckelman WC (1997) Quantification of amphetamine-induced changes in [^{11}C]raclopride binding with continuous infusion. *J Cereb Blood Flow Metab* **17:**437–447.
Castillo J, Davalos A, Naveiro J, and Noya M (1996) Neuroexcitatory amino acids and their relation to infarct size and neurological deficit in ischemic stroke. *Stroke* **27:**1060–1065.
Castillo J, Leira R, Moro MA, Lizasoain I, Serena J, and Davalos A (2003) Neuroprotective effects of aspirin in patients with acute cerebral infarction. *Neurosci Lett* **339:**248–250.
Cattabeni F, Colciaghi F, and Di Luca M (2004) Platelets provide human tissue to unravel pathogenic mechanisms of Alzheimer disease. *Prog Neuropsychopharmacol Biol Psychiatry* **28:**763–770.
Cerrito F and Raiteri M (1979) Serotonin release is modulated by presynaptic autoreceptors. *Eur J Pharmacol* **57:**427–430.
Chan-Palay V (1987) Somatostatin immunoreactive neurons in the human hippocampus and cortex shown by immunogold/silver intensification on vibratome sections: coexistence with neuropeptide Y neurons and effects in Alzheimer's type dementia. *J Comp Neurol* **260:**201–223.
Chavkin C, James JF, and Goldstein A (1982) Dynorphin is a specific endogenous ligand of the κ opioid receptor. *Science (Wash DC)* **215:**413–415.
Chen J-F, Huang Z, Ma J, Zhu JM, Moratalla R, Standaert D, Moskowitz MA, Fink JS, and Schwarzschild MA (1999) A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. *J Neurosci* **19:**9192–9200.
Chen L, Gu Y, and Mae Huang L-Y (1995) The mechanism of action for the block of NMDA receptor channels by the opioid peptide dynorphin. *J Neurosci* **15:**4602–4611.
Cheng B, Christakos S, and Mattson MP (1994) Tumor necrosis factors protect neurons against metabolic-excitotoxic insults and promote maintenance of calcium homeostasis. *Neuron* **12:**139–153.
Cherubini E, Gaiarsa JL, and Ben Ari Y (1991) GABA: an excitatory transmitter in early postnatal life. *Trends Neurosci* **14:**515–519.
Choi DW (1988) Calcium-mediated neurotoxicity: relationship to specific channel types and role in ischemic damage. *Trends Neurosci* **11:**465–469.
Clarke PBS and Reuben M (1996) Release of [^{3}H]noradrenaline from rat hippocampal synaptosomes by nicotine: mediation by different nicotinic receptor subtypes from striatal [^{3}H]dopamine release. *Br J Pharmacol* **117:**595–606.
Clough R, Statnick M, Maring-Smith M, Wang C, Eells J, Browning R, Dailey J, and Jobe P (1996) Fetal raphe transplants reduce seizure severity in serotonin-depleted GEPRs. *Neuroreport* **8:**341–346.
Cohen I, Navarro V, Clemenceau S, Baulac M, and Miles R (2002) On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science (Wash DC)* **298:**1418–1421.
Collingridge GL and Bliss TVP (1987) NMDA receptors: their role in long-term potentiation. *Trends Neurosci* **10:**288–293.
Collingridge GL and Isaac JT (2003) Functional roles of protein interactions with AMPA and kainate receptors. *Neurosci Res* **47:**3–15.
Coulter DA, Rafiq A, Shumate M, Gong QZ, DeLorenzo RJ, and Lyeth BG (1996) Brain injury-induced enhanced limbic epileptogenesis: anatomical and physiological parallels to an animal model of temporal lobe epilepsy. *Epilepsy Res* **26:**81–91.
Couve A, Calver AR, Fairfax B, Moss SJ, and Pangalos MN (2004) Unravelling the unusual signalling properties of the $GABA_B$ receptor. *Biochem Pharmacol* **68:** 1527–1536.
Coyle JT, Price DL, and DeLong MR (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. *Science (Wash DC)* **219:**1184–11190.
Davies P, Katzman R, and Terry RD (1980) Reduced somatostatin-like immunoreactivity in cerebral cortex from cases of Alzheimer disease and Alzheimer senile dementia. *Nature (Lond)* **288:**279–280.
Dawbarn D, Rosser MN, Mountjoy CQ, Roth M, and Emson PC (1986) Decreased somatostatin immunoreactivity but not neuropeptide Y immunoreactivity in cerebral cortex in senile dementia of the Alzheimer's type. *Neurosci Lett* **70:**154–159.
Dawson LA, Galandak J, and Djali S (2002) Attenuation of ischemic efflux of endogenous amino acids by the novel 5-HT_{1A}/5-HT_2 receptor ligand adatanserin. *Neurochem Int* **40:**203–209.
Dawson VL, Dawson TM, Uhl GR, and Snyder SH (1993) Human immunodeficiency virus type 1 coat protein neurotoxicity mediated by nitric oxide in primary cortical cultures. *Proc Natl Acad Sci USA* **90:**3256–3259.
De Cristobal J, Moro MA, Davalos A, Castillo J, Leza JC, Camarero J, Colado MI, Lorenzo P, and Lizasoain I (2001) Neuroprotective effect of aspirin by inhibition of glutamate release after permanent focal cerebral ischemia in rats. *J Neurochem* **79:**456–459.
De Long MR (1990) Primate models of movement disorders of basal ganglia origin. *Trends Neurosci* **13:**281–285.
Di Chiara G and Imperato A (1988) Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* **85:**5274–5278.
Doodley DJ, Mieske CA, and Borosky SA (2000) Inhibition of K^+-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. *Neurosci Lett* **280:**107–110.
Drejer J, Benveniste H, Diemer NH, and Schousboe A (1985) Cellular origin of ischemia-induced glutamate release from brain tissue in vivo and in vitro. *J Neurochem* **45:**145–151.
Drejer J, Honore T, and Schousboe A (1987) Excitatory amino acid-induced release of 3H-GABA from cultured mouse cerebral cortex interneurons. *J Neurosci* **7:**2910–2916.
Drevets WC, Gautier C, Price JC, Kupfer DJ, Kinahan PE, Grace AA, Price JL, and Mathis CA (2001) Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol Psychiatry* **49:**81–96.
Dreyer EB, Kaiser PK, Offermann JT, and Lipton SA (1990) HIV-1 coat protein neurotoxicity prevented by calcium channel antagonists. *Science (Wash DC)* **248:** 364–367.
Dubeau F, Sherwin A, Olivier A, Villemure J, Leblanc R, Quesney LF, Andermann E, and Andermann F (1992) Excitatory amino acids modulate phosphoinositide signal transduction in human epileptic neocortex. *Epilepsia* **33:**255–262.
During MJ, Mattson RH, Scheyer RD, Rask C, Pierce M, McKelvy J, and Thomas V (1992) The effect of tiagabine HCl on extracellular GABA levels in the human hippocampus. *Epilepsia* **33 (Suppl 3):**83.
During MJ, Ryder KM, and Spencer DD (1995) Hippocampal GABA transporter function in temporal-lobe epilepsy. *Nature (Lond)* **376:**174–177.
During MJ and Spencer DD (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. *Lancet* **341:**1607–1610.
Eid T, Thomas MJ, Spencer DD, Runden-Pran E, Lai JCK, Malthankar GV, Kim JH, Danbolt NC, Ottersen OP, and de Lanerolle NC (2004) Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. *Lancet* **363:**28–37.
Engel G, Göthert M, Hoyer D, Schlicker E, and Hillenbrand K (1986) Identity of inhibitory presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain

cortex with 5-HT_{1B} binding sites. *Naunyn-Schmiedeberg's Arch Pharmacol* **332:** 1–7.
Engelman HS and MacDermott AB (2004) Presynaptic ionotropic receptors and control of transmitter release. *Nat Rev* **5:**135–145.
Ensoli B, Buonaguro L, Barillari G, Fiorelli V, Gendelman R, Morgan RA, Wingfield P, and Gallo RC (1993) Release, uptake and effects of extracellular human immunodeficiency virus type 1 Tat protein on cell growth and viral transactivation. *J Virol* **67:**277–287.
Everall IP, Heaton RK, Marcotte TD, Ellis RJ, McCutchan JA, Atkinson JH, Grant I, Mallory M, and Masliah E (1999) Cortical synaptic density is reduced in mild to moderate human immunodeficiency virus neurocognitive disorder. HNRC Group. HIV Neurobehavioral Research Center. *Brain Pathol* **9:**209–217.
Fassio A, Bonanno G, Cavazzani P, and Raiteri M (1994) Characterization of the GABA autoreceptor in human neocortex as a pharmacological subtype of the $GABA_B$ receptor. *Eur J Pharmacol* **263:**311–314.
Fassio A, Rossi F, Bonanno G, and Raiteri M (1999) GABA induces norepinephrine exocytosis from hippocampal noradrenergic axon terminals by a dual mechanism involving different voltage-sensitive calcium channels. *J Neurosci Res* **57:**324–331.
Fedele E and Raiteri M (1999) In vivo studies of the cerebral glutamate receptor/NO/cGMP pathway. *Prog Neurobiol* **58:**89–120.
Fedele E, Andrioli GC, Ruelle A, and Raiteri M (1993) Release-regulating dopamine autoreceptors in human cerebral cortex. *Br J Pharmacol* **110:**20–22.
Fedele E, Fontana G, Munari C, Cossu M, and Raiteri M (1999) Native human neocortex release-regulating dopamine D2 type autoreceptors are dopamine D_2 subtype. *Eur J Neurosci* **11:**1–8.
Fedele E, Mazzone P, Stefani A, Bassi A, Ansaldo MA, Raiteri M, Altibrandi MG, Pierantozzi M, Giacomini P, Bernardi G, et al. (2001a) Microdialysis in Parkinsonian patient basal ganglia: acute apomorphine-induced clinical and electrophysiological effects not paralleled by changes in the release of neuroactive amino acids. *Exp Neurol* **167:**356–365.
Fedele E, Stefani A, Bassi A, Pepicelli O, Altibrandi MG, Frasca S, Giacomini P, Stanzione P, and Mazzone P (2001b) Clinical and electrophysiological effects of apomorphine in Parkinson's disease are not paralleled by amino acid release changes: a microdialysis study. *Funct Neurol* **16:**57–66.
Fedele E, Varnier G, Ansaldo MA, and Raiteri M (1998) Nicotine administration stimulates the in vivo *N*-methyl-D-aspartate receptor/nitric oxide/cyclic GMP pathway in rat hippocampus through glutamate release. *Br J Pharmacol* **125:**1042–1048.
Feligioni M, Raiteri L, Pattarini R, Grilli M, Bruzzone S, Cavazzani P, Raiteri M, and Pittaluga A (2003) The human immunodeficiency virus-1 protein Tat and its discrete fragments evoke selective release of acetylcholine from human and rat cerebrocortical terminals through species-specific mechanisms. *J Neurosci* **23:** 6810–6818.
Ferraro L, Tanganelli S, Calò G, Antonelli T, Fabrizi A, Acciarri N, Bianchi C, Beani L, and Simonato M (1993) Noradrenergic modulation of γ-aminobutyric acid outflow from the human cerebral cortex. *Brain Res* **629:**103–108.
Feuerstein TJ, Albrecht C, Wessles I, Zentner J, and Jackisch R (1998) δ_1-Opioid receptor-mediated control of acetylcholine (ACh) release in human neocortex slices. *Int J Dev Neurosci* **16:**795–802.
Feuerstein TJ, Dooley DJ, and Seeger W (1990) Inhibition of norepinephrine and acetylcholine release from human neocortex by ω-conotoxin GVIA. *J Pharmacol Exp Ther* **252:**778–785.
Feuerstein TJ, Gleichauf O, Peckys D, Landwehrmeyer GB, Scheremet R, and Jackisch R (1996b) Opioid receptor-mediated control of acetylcholine release in human neocortex tissue. *Naunyn-Schmiedeberg's Arch Pharmacol* **354:**586–592.
Feuerstein TJ, Huber B, Vetter J, Aranda H, Van Velthoven V, and Limberger N (2000) Characterization of the α_2-adrenoceptor subtype, which functions as α_2-autoreceptor in human neocortex. *J Pharmacol Exp Ther* **294:**356–362.
Feuerstein TJ, Hüring H, van Velthoven V, Lücking CH, and Lanwehrmeyer GB (1996a) 5-HT_{1D}-like receptors inhibit the release of endogenously formed [^{3}H]GABA in human, but not in rabbit, neocortex. *Neurosci Lett* **209:**210–214.
Feuerstein TJ, Lehmann J, Sauermann W, van Velthoven V, and Jackisch R (1992) The autoinhibitory feedback control of acetylcholine release in human neocortex tissue. *Brain Res* **572:**64–71.
Feuerstein TJ, Mutschler A, Lupp A, Van Velthovenm V, Schlicker E, and Göthert M (1993) Endogenous noradrenaline activates α_2-adrenoceptors on serotonergic nerve endings in human and rat neocortex. *J Neurochem* **61:**474–480.
Fibiger HC (1991) Cholinergic mechanisms in learning, memory and dementia: a review of recent evidence. *Trends Neurosci* **14:**220–223.
Filali M, Hutchison WD, Palter VN, Lozano AM, and Dostrowsky JO (2004) Stimulation-induced inhibition of neuronal firing in human subthalamic nucleus. *Exp Brain Res* **156:**274–281.
Fink K, Dooley DJ, Meder WP, Suman-Chauhan N, Duffy S, Clusmann H, and Göthert M (2002) Inhibition of neuronal Ca^{2+} influx by gabapentin and pregabalin in the human neocortex. *Neuropharmacology* **42:**229–236.
Fink K, Meder W, Dooley DJ, and Göthert M (2000) Inhibition of neuronal Ca^{2+} influx by gabapentin and subsequent reduction of neurotransmitter release from rat neocortical slices. *Br J Pharmacol* **130:**900–906.
Fink K, Schultheiss R, and Göthert M (1992a) Stimulation of noradrenaline release in human cerebral cortex mediated by *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors. *Br J Pharmacol* **106:**67–72.
Fink K, Schultheiss R, and Göthert M (1992b) Inhibition of *N*-methyl-D-aspartate- and kainate-evoked noradrenaline release in human cerebral cortex slices by ethanol. *Naunyn-Schmiedeberg's Arch Pharmacol* **345:**700–703.
Fink K, Zentner J, and Göthert M (1994) Increased GABA release in the human brain cortex as a potential pathogenetic basis of hyperosmolar diabetic coma. *J Neurochem* **62:**1476–1481.
Fink K, Zentner J, and Göthert M (1995) Subclassification of presynaptic 5-HT autoreceptors in the human cerebral cortex as 5-$HT_{1D\beta}$ receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:**451–454.
Fontana G, Fedele E, Cossu M, Munari C, and Raiteri M (1997) Activation of brain nitric oxide synthase in depolarized human temporal cortex slices: differential role of voltage-sensitive calcium channels. *Br J Pharmacol* **122:**930–934.
Fox L, Alford M, Achim C, Mallory M, and Masliah E (1997) Neurodegeneration of somatostatin-immunoreactive neurons in HIV encephalitis. *J Neuropathol Exp Neurol* **56:**360–368.
Francis PT, Bowen DM, Lowe SL, Neary D, Mann DMA, and Snowden JS (1987) Somatostatin content and release measured in cerebral biopsies from demented patients. *J Neurol Sci* **78:**1–16.
Froestl W, Gallagher M, Jenkins H, Madrid A, Melcher T, Teichman S, Mondadori CG, and Pearlman R (2004) SGS742: the first $GABA_B$ receptor antagonist in clinical trials. *Biochem Pharmacol* **68:**1479–1487.
Froestl W, Mickel SJ, Von Sprecher G, Diel PJ, Hall RG, Maier L, Strub D, Melillo V, Baumann PA, Bernasconi R, et al. (1995) Phosphinic acid analogues of GABA. 2. Selective, orally active $GABA_B$ antagonists. *J Med Chem* **38:**3313–3331.
Gabriel S, Njunting M, Pomper JK, Merschhemke M, Sanabria ERG, Eilers A, Kivi A, Zeller M, Meencke H-J, Cavalheiro EA, et al. (2004) Stimulus and potassium-induced epileptiform activity in the human dentate gyrus from patients with and without hippocampal sclerosis. *J Neurosci* **24:**10416–10430.
Gabriel SM, Biere LM, Haroutunian V, Purohit DP, Perl DP, and Davis KL (1993) Widespread deficits in somatostatin but not neuropeptide Y concentrations in Alzheimer's disease cerebral cortex. *Neurosci Lett* **155:**116–120.
Gallo V, Patrizio M, and Levi G (1991) GABA release triggered by the activation of neuron-like non-NMDA receptors in cultured type 2 astrocytes is carrier-mediated. *Glia* **4:**245–255.
Galzin AM, Poirier MF, Lista A, Chodkiewicz JP, Blier P, Ramdine R, Loô H, Roux FX, Redondo A, and Langer SZ (1992) Characterization of the 5-hydroxytryptamine receptor modulating the release of 5-[^{3}H]hydroxytryptamine in slices of the human neocortex. *J Neurochem* **59:**1293–1301.
Gemignani A, Paudice P, Bonanno G, and Raiteri M (1994) Pharmacological discrimination between γ-aminobutyric acid type B receptors regulating cholecystokinin and somatostatin release from rat neocortex synaptosomes. *Mol Pharmacol* **46:** 558–562.
Glowa JR, Panlilio LV, Brennemann DE, Gozes I, Fridkin M, and Hill JM (1992) Learning impairment following intracerebral administration of the HIV envelope protein gp120 or a VIP antagonist. *Brain Res* **570:**49–53.
Gopalakrishnan M, Molinari EJ, and Sullivan JP (1997) Regulation of human $\alpha4\beta2$ neuronal nicotinic acetylcholine receptors by cholinergic channel ligands and second messenger pathways. *Mol Pharmacol* **52:**524–534.
Göthert M and Weinheimer G (1979) Extracellular 5-hydroxytryptamine inhibits 5-hydroxytryptamine release from rat brain cortex slices. *Naunyn-Schmiedeberg's Arch Pharmacol* **310:**93–96.
Gray R, Rajan AS, Radcliffe KA, Yakehiro M, and Dani JA (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature (Lond)* **383:**713–716.
Gulledge AT and Stuart GJ (2003) Action potential initiation and propagation in Layer 5 pyramidal neurons of the rat prefrontal cortex: absence of dopamine modulation. *J Neurosci* **23:**11363–11372.
Hamblin MW and Metcalf MA (1991) Primary structure and functional characterization of a human 5-HT_{1D}-type serotonin receptor. *Mol Pharmacol* **40:**143–148.
Hampson RE and Deadwyler SA (1999) Cannabinoids, hippocampal function and memory. *Life Sci* **65:**715–723.
Harkany T, Mulder J, Horvath KM, Keijser J, Van Der Meeberg EK, Nyakas C, and Luiten PG (2001) Oral post lesion administration of 5-HT_{1A} receptor agonist repinotan hydrochloride (BAY x 3702) attenuates NMDA induced delayed neuronal death in rat magnocellular nucleus basalis. *Neuroscience* **108:**629–642.
Harro J, Vasar E, and Bradwein J (1993) CCK in animal and human research on anxiety. *Trends Pharmacol Sci* **14:**244–249.
Hartig PR, Branchek TA, and Weinshank RL (1992) A subfamily of 5-HT_{1D} receptor genes. *Trends Pharmacol Sci* **13:**152–159.
Hartig PR, Hoter D, Humphrey PPA, and Martin GR (1996) Alignment of receptor nomenclature with the human genome: classification of 5-HT_{1B} and 5-HT_{1D} receptor subtypes. *Trends Pharmacol Sci* **17:**103–105.
Hashimoto T, Elder CM, Okun MS, Patrick SK, and Vitek JL (2003) Stimulation of the subthalamic nucleus changes the firing pattern of pallidal neurons. *J Neurosci* **23:**1916–1923.
Haughey NJ, Holden CP, Nath A, and Geiger JD (1999) Involvement of inositol 1,4,5-trisphosphate-regulated stores of intracellular calcium in calcium dysregulation and neuron cell death caused by HIV-1 protein Tat. *J Neurochem* **73:**1363–1374.
Haughey NJ, Nath A, Mattson MP, Slevin JT, and Geiger JD (2001) HIV-1 Tat through phosphorylation of NMDA receptors potentiates glutamate excitotoxicity. *J Neurochem* **78:**457–467.
Hegstad F, Berg-Johnsen J, Haugstad TS, Hauglie-Hanssen E, and Langmoen IA (1996) Amino-acid release from human cerebral cortex during simulated ischaemia in vitro. *Acta Neurochir* **138:**234–241.
Hendry SHC, Jones EG, De Felipe J, Schmechel D, Brandon C, Beinfeld M, and Emson PC (1984) Neuropeptide containing neurones of the cerebral cortex are also GABAergic. *Proc Natl Acad Sci USA* **81:**6526–6530.
Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, and Rice KC (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci USA* **87:**1932–1936.
Hermann H, Marsicano G, and Lutz B (2002) Coexpression of the cannabinoid receptor type 1 with dopamine and serotonin receptors in distinct neuronal subpopulations of the adult mouse forebrain. *Neuroscience* **109:**451–460.
Herrick-Davis K, Titeler M, Leonhardt S, Struble R, and Price D (1988) Serotonin 5-HT_{1D} receptors in human prefrontal cortex and caudate: interaction with a GTP binding protein. *J Neurochem* **51:**1906–1912.
Hershey T, Revilla FJ, Wernle A, Gibson PS, Dowling JL, and Perlmutter JS (2003) Cortical and subcortical blood flow effects of subthalamic nucleus stimulation in PD. *Neurology* **23:**816–821.

Hökfelt T, Broberger C, Xu ZQ, Sergeyev V, Ubink R, and Diez M (2000) Neuropeptides-an overview. *Neuropharmacology* **39:**1337–1356.
Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, and Humphrey PA (1994) International Union of Pharmacology classification of receptors for 5-hydroxytryptamine (serotonin). *Pharmacol Rev* **2:**157–203.
Hoyer D and Middlemiss DN (1989) Species differences in the pharmacology of terminal 5-HT autoreceptors in mammalian brain. *Trends Pharmacol Sci* **10:**130–132.
Hoyer D, Pazos A, Probst A, and Palacios JM (1986) Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites: apparent absence of 5-HT_{1B}. *Brain Res* **376:**85–96.
Hoyer D, Waeber C, Pazos A, Probst A, and Palacios JM (1988) Identification of a 5-HT_1 recognition site in human brain membranes different from 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} sites. *Neurosci Lett* **85:**357–362.
Hudson L, Liu J, Nath A, Jones M, Raghavan R, Narayan O, Male D, and Everall I (2000) Detection of the human immunodeficiency virus regulatory protein tat in CNS tissues. *J Neurovirol* **6:**145–155.
Husi H, Ward MA, Choudhary JS, Blackstock WP, and Grant SG (2000) Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nat Neurosci* **3:**661–669.
Hwa GG, Avoli M, Oliver A, and Villemure JG (1991) Bicuculline-induced epileptogenesis in the human neocortex maintained in vitro. *Exp Brain Res* **83:**329–339.
Ingram S, Wilding TJ, McClesky EW, and Williams JT (1997) Efficacy and kinetics of opioid action on acutely dissociated neurons. *Mol Pharmacol* **52:**136–143.
Isokawa M, Avanzini G, Finch DM, Babb TL, and Levesque MF (1991) Physiologic properties of human dentate granule cells in slices prepared from epileptic patients. *Epilepsy Res* **9:**242–250.
Jabaudon D, Scanziani M, Gahwiler BH, and Gerber U (2000) Acute decrease in net glutamate uptake during energy deprivation. *Proc Natl Acad Sci USA* **97:**5610–5615.
Jech R, Urgosik D, Tintera J, Nebuzelsky A, Krasensky J, Liscak R, Roth J, and Ruzicka E (2001) Functional magnetic resonance imaging during deep brain stimulation: a pilot study in four patients with Parkinson's disease. *Movement Disorders* **16:**1126–1132.
Jehle T, Lagrèze WA, Blauth E, Knörle R, Schnierle P, Lücking CH, and Feuerstein TJ (2000) Gabapentin-lactam (8-aza-spiro[5,4]decan-9-on; GBP-L) inhibits oxygen glucose deprivation-induced [^{3}H]glutamate release and is a neuroprotective agent in a model of acute retinal ischemia. *Naunyn-Schmiedeberg's Arch Pharmacol* **362:**74–81.
Jeub M, Lie A, Blümcke I, Elger CE, and Beck H (1999) Loss of dynorphin-mediated inhibition of voltage-dependent Ca^{2+} currents in hippocampal granule cells isolated from epilepsy patients is associated with mossy fiber sprouting. *Neuroscience* **94:**465–471.
Jiang C and Haddad GG (1992) Differential responses of neocortical neurons to glucose and/or O_2 deprivation in the human and rat. *J Neurophysiol* **68:**2165–2173.
Joyce JN, Murray AM, Hurtig HI, Gottlieb GL, and Trojanowski J (1998) Loss of dopamine D2 receptors in Alzheimer's disease with parkinsonism but not Parkinson's or Alzheimer's disease. *Neuropsychopharmacology* **19:**472–480.
Katchman AN and Hershkowitz N (1993) Early anoxia-induced vesicular glutamate release results from mobilization of calcium from intracellular stores. *J Neurophysiol* **70:**1–7.
Katona I, Sperlágh B, Maglóczky Z, Sántha E, Köfalvi A, Czirják S, Mackie K, Vizi ES, and Freund TF (2000) GABAergic interneurons are the targets of cannabinoid actions in the human hippocampus. *Neuroscience* **100:**797–804.
Katona I, Sperlágh B, Sik A, Kofalvi A, Vizi ES, Mackie K, and Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* **19:**4544–4558.
Kaul M and Lipton SA (1999) Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. *Proc Natl Acad Sci USA* **96:**8212–8216.
Kaul M, Garden GA, and Lipton SA (2001) Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature (Lond)* **410:**988–994.
Klapstein GJ, Vietla S, Lieberman DN, Gray PA, Airaksinen MS, Thoenen H, Meyer M, and Mody I (1998) Calbindin-D28k fails to protect hippocampal neurons against ischemia in spite of its cytoplasmic calcium buffering properties: evidence from calbindin-D28k knockout mice. *Neuroscience* **85:**361–373.
Koob GF, Sanna PP, and Bloom FE (1998) Neuroscience of addiction. *Neuron* **21:**467–476.
Kruman II, Nath A, and Mattson MP (1998) HIV-1 protein Tat induces apoptosis of hippocampal neurons by a mechanism involving caspase activation, calcium overload and oxidative stress. *Exp Neurol* **154:**276–288.
Lang AE and Lozano AM (1998a) Parkinson's disease. First of two parts. *N Engl J Med* **339:**1044–1053.
Lang AE and Lozano AM (1998b) Parkinson's disease. Second of two parts. *N Engl J Med* **339:**1130–1143.
Langer SZ (1993) 25 years since the discovery of presynaptic receptors: present knowledge and future perspectives. *Trends Pharmacol Sci* **18:**95–99.
Lanza M, Fassio A, Gemignani A, Bonanno G, and Raiteri M (1993) CGP52432: a novel potent and selective $GABA_B$ autoreceptor antagonist in rat cerebral cortex. *Eur J Pharmacol* **237:**191–195.
Laruelle M (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. *J Cereb Blood Flow Metab* **20:**423–451.
Leonard BE (1997) The role of noradrenaline in depression: a review. *J Psychopharmacol* **11:**S39–S47.
Levi G and Raiteri M (1974) Exchange of neurotransmitter amino acid at nerve endings can simulate high affinity uptake. *Nature (Lond)* **250:**735–737.
Levi G and Raiteri M (1993) Carrier mediated release of neurotransmitters. *Trends Neurosci* **16:**415–419.
Levin ED and Simon BB (1998) Nicotinic acetylcholine involvement in cognitive function in animals. *Psychopharmacology* **138:**217–230.
Levy R, Dostrovsky JA, Lang AE, Hutchison WD, and Lozano AM (2001) Effects of apomorphine on subthalamic nucleus and globus pallidus internus neurons in patients with Parkinson's disease. *J Neurophysiol* **86:**249–260.
Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, and Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting and novelty seeking: a PET/[^{11}C]raclopride study in healthy men. *Neuropsychopharmacology* **27:**1027–1035.
Limberger N, Trendelenburg AU, and Starke K (1995) Subclassification of presynaptic α_2-adrenoceptors: α_{2D}-autoreceptors in mouse brain. *Naunyn-Schmiedeberg's Arch Pharmacol* **352:**43–48.
Lipton SA (1999) Neuronal protection and destruction by NO. *Cell Death Diff* **6:**943–951.
Lipton SA and Chen HS (2004) Paradigm shift in neuroprotective drug development: clinically tolerated NMDA receptor inhibition by memantine. *Cell Death Diff* **11:**18–20.
Lloyd GK and Williams M (2000) Neuronal nicotinic acetylcholine receptors as novel drug targets. *J Pharmacol Exp Ther* **292:**461–467.
Louvel J, Avoli M, Kurcewicz I, and Pumain R (1994) Extracellular free potassium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. *Neurosci Lett* **167:**97–100.
Louvel J, Papatheodoropoulos C, Siniscalchi A, Kurcewicz I, Pumain R, Devaux B, Turak B, Esposito V, Villemeure JG, and Avoli M (2001) GABA-mediated synchronization in the human neocortex: elevations in extracellular potassium and presynaptic mechanisms. *Neuroscience* **105:**803–813.
Luo S, Kulak JM, Cartier GE, Jacobsen RB, Yoshikami D, Olivera BM, and McIntosh JM (1998) α-Conotoxin AuIB selectively blocks $\alpha 2\beta 4$ nicotinic acetylcholine receptors and nicotine-evoked norepinephrine release. *J Neurosci* **18:**8571–8579.
Mann DMA, Yates PO, and Marcyniuk B (1984) Monoaminergic neurotransmitter systems in presenile Alzheimer's disease and in senile dementia of Alzheimer type. *Clin Neuropathol* **3:**199–205.
Marchi M, Raiteri L, Risso F, Vallarino A, Bonfanti A, Monopoli A, Ongini E, and Raiteri M (2002a) Effects of adenosine A_1 and A_{2A} receptor activation on the evoked release of glutamate from rat cerebrocortical synaptosomes. *Br J Pharmacol* **136:**434–440.
Marchi M, Risso F, Viola C, Cavazzani P, and Raiteri M (2002b) Direct evidence that release-stimulating $\alpha 7^*$ nicotinic cholinergic receptors are localized on human and rat brain glutamatergic axon terminals. *J Neurochem* **80:**1071–1078.
Marchi M, Ruelle A, Andrioli GC, and Raiteri M (1990) Pirenzepine-insensitive muscarinic autoreceptors regulate acetylcholine release in human neocortex. *Brain Res* **520:**347–350.
Marcoli M, Cervetto C, Castagnetta M, Sbaffi P, and Maura G (2004) 5-HT control of ischemia-evoked glutamate efflux from human cerebrocortical slices. *Neurochem Int* **45:**687–691.
Marcoli M, Maura G, Munari C, Ruelle A, and Raiteri M (1999) Pharmacological diversity between native human 5-HT_{1B} and 5-HT_{1D} receptors sited on different neurons and involved in different functions. *Br J Pharmacol* **126:**607–612.
Marcoli M, Raiteri L, Bonfanti A, Monopoli A, Ongini E, Raiteri M, and Maura G (2003) Sensitivity to selective adenosine A_1 and A_{2A} receptor antagonists of the release of glutamate induced by ischemia in rat cerebrocortical slices. *Neuropharmacology* **45:**201–210.
Martin GR and Humphrey PP (1994) Receptors for 5-hydroxytryptamine: current perspectives on classification and nomenclature. *Neuropharmacology* **33:**261–273.
Martinez D, Slifstein M, Broft A, Mawlawi O, Hwang DR, Huang Y, Cooper T, Kegeles L, Zarahn E, Abi-Dargham A, et al. (2003) Imaging human mesolimbic dopamine transmission with positron emission tomography. Part II: amphetamine-induced dopamine release in the functional subdivisions of the striatum. *J Cereb Blood Flow Metab* **23:**285–300.
Mash DC, Pablo J, Ouyang Q, Hearn WL, and Izenwasser S (2002) Dopamine transport function is elevated in cocaine users. *J Neurochem* **81:**292–300.
Masliah E, Heaton RK, Marcotte TD, Ellis RJ, Wiley CA, Mallory M, Achim CL, McCutchan JA, Nelson JA, Atkinson JH, et al. (1997) Dendritic injury is a pathological substrate for human immunodeficiency virus-related cognitive disorders. *Ann Neurol* **42:**963–972.
Mauler F, Fahrig T, Horvath E, and Jork R (2001) Inhibition of evoked glutamate release by the neuroprotective 5-HT_{1A} receptor agonist BAY x 3702 in vitro and in vivo. *Brain Res* **888:**150–157.
Maura G, Marcoli M, Pepicelli O, Rosu C, Viola C, and Raiteri M (2000) Serotonin inhibition of the NMDA receptor/nitric oxide/cyclic GMP pathway in human neocortex slices: involvement of 5-HT_{2C} and 5-HT_{1A} receptors. *Br J Pharmacol* **130:**1853–1858.
Maura G, Marcoli M, Tortarolo M, Andrioli GC, and Raiteri M (1998) Glutamate release in human cerebral cortex and its modulation by 5-hydroxytryptamine acting at h5-HT_{1D} receptors. *Br J Pharmacol* **123:**45–50.
Maura G, Roccatagliata E, and Raiteri M (1986) Serotonin autoreceptor in rat hippocampus: pharmacological characterization as a subtype of the 5-HT_1 receptor. *Naunyn-Schmiedeberg's Arch Pharmacol* **334:**323–326.
Maura G, Thellung S, Andrioli GC, Ruelle A, and Raiteri M (1993) Release-regulating serotonin 5-HT_{1D} autoreceptors in human cerebral cortex. *J Neurochem* **60:**1179–1182.
McGehee DS and Role LW (1995) Physiological diversity of nicotinic acetylcholine receptors expressed by vertebrate neurons. *Annu Rev Physiol* **57:**521–546.
Meder W, Fink K, and Göthert M (1997) Involvement of different calcium channels in K^+- and veratridine-induced increases of cytosolic calcium concentration in rat cerebral cortical synaptosomes. *Naunyn-Schmiedeberg's Arch Pharmacol* **356:**797–805.
Meder W, Fink K, Zentner J, and Göthert M (1999) Calcium channels involved in K^+- and veratridine-induced increase of cytosolic calcium concentration in human cerebral cortical synaptosomes. *J Pharmacol Exp Ther* **290:**1126–1131.
Meyerson BA, Linderoth B, Karlsson H, and Ungerstedt U (1990) Microdialysis in the human brain: extracellular measurements in the thalamus of parkinsonian patients. *Life Sci* **46:**301–308.

Michelson HB and Wong RK (1991) Excitatory synaptic responses mediated by $GABA_A$ receptors in the hippocampus. *Science (Wash DC)* **253:**1420–1423.

Middlemiss DN, Göthert M, Schlicker E, Scott CM, Selkirk JV, Watson J. Gaster LM, Wyman P, Riley G, and Price GW (1999) SB-236057, a selective 5-HT_{1B} receptor inverse agonist, blocks the 5-HT human terminal autoreceptor. *Eur J Pharmacol* **375:**359–365.

Miller RJ (1998) Presynaptic receptors. *Annu Rev Pharmacol Toxicol* **38:**201–227.

Milligan G (2004) G protein-coupled receptor dimerization: function and ligand pharmacology. *Mol Pharmacol* **66:**1–7.

Minthon L, Edvinsson L, and Gustafson L (1997) Somatostatin and neuropeptide Y in cerebrospinal fluid: correlations with severity of disease and clinical signs in Alzheimer's disease and frontotemporal dementia. *Dement Geriatr Cogn Disord* **8:**232–239.

Mogil JS and Pasternak GW (2001) The molecular and behavioral pharmacology of the orphanin FQ/nociceptin peptide and receptor family. *Pharmacol Rev* **53:**381–415.

Mollace V, Nottet HSLM, Clayetee P, Turco MC, Muscoli C, Salvemini D, and Perno CF (2001) Oxidative stress and neuroAIDS: triggers, modulators and novel antioxidants. *Trends Neurosci* **24:**411–416.

Mondadori C, Jaekel J, and Preiswerk G (1993) CGP 36742: the first orally active $GABA_B$ blocker improves the cognitive performance of mice, rats and rhesus monkeys. *Behav Neural Biol* **60:**62–681993.

Monopoli A, Lozza G, Forlani A, Mattavelli A, and Ongini E (1998) Blockade of adenosine A_{2A} receptors by SCH58261 results in neuroprotective effects in cerebral ischaemia in rats. *Neuroreport* **9:**3955–3959.

Morari M, Calò G, Antonelli T, Gaist G, Acciarri N, Fabrizi A, Bianchi C, and Beani L (1991) Inhibitory effect of NMDA receptor activation on quisqualate-stimulated phosphatidylinositol turnover in the human cerebral cortex. *Brain Res* **553:**14–17.

Morari M, Calò G, Ferraro L, Fabrizi A, Acciarri N, Piazza G, Bianchi C, and Beani L (1995) AMPA receptor activation regulates the glutamate metabotropic receptor stimulated phosphatidylinositol turnover in human cerebral cortex slices. *Neurochem Int* **26:**77–83.

Müller WEG, Schröder HC, Ushijima H, Dapper J, and Bormann J (1992) gp120 of HIV-1 induces apoptosis in rat cortical cell cultures: prevention by memantine. *Eur J Pharmacol Mol Pharmacol Sec* **226:**209–214.

Nägerl UV, Mody I, Jeub M, Lie AA, Elger CE, and Beck H (2000) Surviving granule cells of the sclerotic human hippocampus have reduced Ca^{2+} influx because of a loss of calbindin-D_{28K} in temporal lobe epilepsy. *J Neurosci* **20:**1831–1836.

Nath A, Psooy K, Martin C, Knudsen B, Magnuson DSK, Haughey N, and Geiger JD (1996) Identification of a human immunodeficiency virus type 1 Tat epitope that is neuroexcitatory and neurotoxic. *J Virol* **70:**1475–1480.

Neary D, Snowden JS, Bowen DM, Sims NR, Mann DMA, Yates PO, and Davison AN (1986) Cerebral biopsy in the investigation of presenile dementia due to cerebral atrophy. *J Neurol Neurosurg Psychiatry* **49:**157–162.

Newberry NR, Footitt DR, Papanastassiou V, and Reynolds DJ (1999) Actions of 5-HT on human neocortical neurones in vitro. *Brain Res* **833:**93–100.

Nicoletti F, Canonico PL, Favit A, Nicoletti G, and Albanese V (1989) Receptor-mediated stimulation of inositol phospholipid hydrolysis in human brain. *Eur J Pharmacol* **160:**299–301.

Nieber K (1999) Hypoxia and neuronal function under in vitro conditions. *Pharmacol Ther* **82:**71–86.

Nordberg A (1999) PET studies and cholinergic therapy in Alzheimer's disease. *Rev Neurol* (*Paris*) **155 (Suppl 4):**S53–S63.

Nowak G, Legutko B, Skolnick P, and Popik P (1998) Adaptation of cortical NMDA receptors by chronic treatment with specific serotonin reuptake inhibitors. *Eur J Pharmacol* **342:**367–370.

Okada M, Kiryu K, Kawata Y, Mizuno K, Wada K, Tasaki H, and Kaneko S (1997) Determination of the effects of caffeine and carbamazepine on striatal dopamine release by in vivo microdialysis. *Eur J Pharmacol* **321:**181–188.

Oksenberg D, Marsters SA, O'Dowd BF, Jin H, Havlik S, Peroutka SJ, and Ashkenazi A (1992) A single amino-acid difference confers major pharmacological variation between human and rodent 5-HT_{1B} receptors. *Nature (Lond)* **360:**161–163.

Palmer AM (1996) Neurochemical studies of Alzheimer's disease. *Neurodegeneration* **5:**381–391.

Palmer AM, Francis PT, Benton JS, Sims NR, Mann DMA, Neary D, Snowden JS, and Bowen DM (1987a) Presynaptic serotonergic dysfunction in patients with Alzheimer's disease. *J Neurochem* **48:**8–15.

Palmer AM, Francis PT, Bowen DM, Benton JS, Neary D, Mann DMA, and Snowden SJ (1987b) Catecholaminergic neurones assessed ante-mortem in Alzheimer's disease. *J Neurochem* **414:**365–375.

Palmer AM, Lowe SL, Frances PT, and Bowen DM (1988) Are postmortem biochemical studies of human brain worthwhile? *Biochem Soc Trans* **16:**472–475.

Palmer AM, Sims NR, Bowen DM, Neary D, Palo J, Wikstrom J, and Davison AN (1984) Monoamine metabolite concentrations in lumbar cerebrospinal fluid of patients with histologically verified Alzheimer's dementia. *J Neurol Neurosurg Psychiatry* **47:**481–484.

Palmer AM, Wilcock GK, Esiri MM, Francis PT, and Bowen DM (1987c) Monoaminergic innervation of the frontal and temporal lobes in Alzheimer's disease. *Brain Res* **401:**231–238.

Parker EM, Grisel DA, Iben LG, and Shapiro RA (1993) A single amino acid difference accounts for the pharmacological distinctions between the rat and human 5-hydroxytryptamine$_{1B}$ receptors. *J Neurochem* **60:**380–383.

Paterson D and Nordberg A (2000) Neuronal nicotinic receptors in the human brain. *Prog Neurobiol* **61:**75–111.

Patrylo PR, Spencer DD, and Williamson A (2001) GABA uptake and heterotransport are impaired in the dentate gyrus of epileptic rats and humans with temporal lobe sclerosis. *J Neurophysiol* **85:**1533–1542.

Pattarini R, Pittaluga A, and Raiteri M (1998) The human immunodeficiency virus-1 envelope protein gp120 binds through its V3 sequence to the glycine site of *N*-methyl-D-aspartate receptors mediating noradrenaline release in the hippocampus. *Neuroscience* **87:**147–157.

Pepicelli O, Raiteri M, and Fedele E (2004) The NOS/sGC pathway in the rat central nervous system: a microdialysis overview. *Neurochem Int* **45:**787–797.

Perrault F and Avoli M (1992) 4-Aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. *J Neurosci* **12:**104–115.

Perry EK (1986) The cholinergic hypothesis—ten years on. *Br Med Bull* **42:**63–69.

Persson L, Valtysson J, Enblad P, Warme PE, Cesarini K, Lewen A, and Hillered L (1996) Neurochemical monitoring using intracerebral microdialysis in patients with subarachnoid hemorrhage. *J Neurosurg* **84:**606–616.

Petroff OA, Errante LD, Rothman DL, Kim JH, and Spencer DD (2002) Glutamate-glutamine cycling in the epileptic human hippocampus. *Epilepsia* **43:**703–710.

Phillis JW (1995) The effects of selective A_1 and A_{2A} adenosine receptor antagonists on cerebral ischemic injury in the gerbil. *Brain Res* **705:**79–84.

Pittaluga A and Raiteri M (1994) HIV-1 envelope protein gp120 potentiates NMDA-evoked noradrenaline release by a direct action at rat hippocampal and cortical noradrenergic nerve endings. *Eur J Neurosci* **6:**1743–1749.

Pittaluga A, Bonfanti A, and Raiteri M (2000) Somatostatin potentiates NMDA receptor function via activation of $InsP_3$ receptors and PKC leading to removal of the Mg^{2+} block without depolarization. *Br J Pharmacol* **130:**557–566.

Pittaluga A, Feligioni M, Ghersi C, Gemignani A, and Raiteri M (2001a) Potentiation of NMDA receptor function through somatostatin release: a possible mechanism for the cognition-enhancing activity of $GABA_B$ receptor antagonists. *Neuropharmacology* **41:**301–310.

Pittaluga A, Feligioni M, Longordo F, Arvigo M, and Raiteri M (2005) Somatostatin-induced activation and upregulation of NMDA receptor function: mediation through CaMKII, PLC, PKC and Src in hippocampal noradrenergic nerve endings. *J Pharmacol Exp Ther* **313:**242–249.

Pittaluga A, Pattarini R, Andrioli GC, Viola C, Munari C, and Raiteri M (1999) Activity of putative cognition enhancers in kynurenate test performed with human neocortex slices. *J Pharmacol Exp Ther* **290:**423–428.

Pittaluga A, Pattarini R, Feligioni M, and Raiteri M (2001b) *N*-Methyl-D-aspartate receptors mediating hippocampal noradrenaline and striatal dopamine release display differential sensitivity to quinolinic acid, the HIV-1 envelope protein gp120, external pH and protein kinase C inhibition. *J Neurochem* **76:**139–148.

Pittaluga A, Pattarini R, Severi P, and Raiteri M (1996) Human brain *N*-methyl-D-aspartate receptors regulating noradrenaline release are positively modulated by HIV-1 coat protein gp120. *AIDS* **10:**463–468.

Pittaluga A, Vaccari D, and Raiteri M (1997) The "kynurenate test": a biochemical assay for putative cognition enhancers. *J Pharmacol Exp Ther* **283:**82–90.

Price RW, Brew B, Sidtis J, Rosenblum M, Scheck AC, and Cleary P (1988) The brain in AIDS: central nervous system HIV-1 infection and AIDS dementia complex. *Science (Wash DC)* **239:**586–592.

Radcliffe KA and Dani JA (1998) Nicotinic stimulation produces multiple forms of increased glutamatergic synaptic transmission. *J Neurosci* **18:**7075–7083.

Raiteri L and Raiteri M (2000) Synaptosomes still viable after 25 years of superfusion. *Neurochem Res* **25:**1265–1274.

Raiteri L, Raiteri M, and Bonanno G (2002a) Coexistence and function of different neurotransmitter transporters in the plasma membrane of CNS neurons. *Prog Neurobiol* **68:**287–309.

Raiteri L, Stigliani S, Zedda L, Raiteri M, and Bonanno G (2002b) Multiple mechanisms of transmitter release evoked by 'pathologically' elevated extracellular $[K^+]$: involvement of transporter reversal and mitochondrial calcium. *J Neurochem* **80:**706–714.

Raiteri M (1994) Functional studies of neurotransmitter receptors in human brain. *Life Sci* **54:**1635–1647.

Raiteri M (2001) Presynaptic autoreceptors. *J Neurochem* **78:**673–675.

Raiteri M, Bonanno G, Maura G, Pende M, Andrioli GC, and Ruelle A (1992) Subclassification of release-regulating α_2-autoreceptors in human brain cortex. *Br J Pharmacol* **107:**1146–1151.

Raiteri M, Bonanno G, Paudice P, Cavazzani P, and Schmid G (1996) Human brain cholecystokinin: release of cholecystokinin-like immunoreactivity (CCK-LI) from isolated cortical nerve endings and its modulation through $GABA_B$ receptors. *J Pharmacol Exp Ther* **278:**747–751.

Raiteri M, Maura G, Bonanno G, and Pittaluga A (1986) Differential pharmacology and function of two 5-HT_1 receptors modulating transmitter release in rat cerebellum. *J Pharmacol Exp Ther* **237:**644–648.

Raiteri M, Maura G, Folghera S, Cavazzani P, Andrioli GC, Schlicker E, Schalnus R, and Göthert M (1990) Modulation of 5-hydroxytryptamine release by presynaptic inhibitory α_2-adrenoceptors in the human cerebral cortex. *Naunyn-Schmiedeberg's Arch Pharmacol* **342:**508–512.

Reith MEA (2002) *Neurotransmitter Transporters: Structure, Function and Regulation*. Humana Press, Totowa, NJ.

Richards DA, Tolias CM, Sgouros S, and Bowery NG (2003) Extracellular glutamine to glutamate ratio may predict outcome in the injured brain: a clinical microdialysis study in children. *Pharmacol Res* **48:**101–109.

Risso F, Grilli M, Parodi M, Bado M, Raiteri M, and Marchi M (2004) Nicotine exerts a permissive role on NMDA receptor function in hippocampal noradrenergic terminals. *Neuropharmacology* **47:**65–71.

Rominger A, Förster S, Zentner J, Dooley DJ, McKnight AT, Feuerstein TJ, Jackisch R, and Vlaskovska M (2002) Comparison of the ORL1 receptor-mediated inhibition of noradrenaline release in human and rat neocortical slices. *Br J Pharmacol* **135:**800–806.

Ronne-Engstrom E, Hillered L, Flink R, Spannare B, Ungerstedt U, and Carlson H (1992) Intracerebral microdialysis of extracellular amino acids in the human epileptic focus. *J Cereb Blood Flow Metab* **12:**873–876.

Rossetti ZL, Hmaidan Y, and Gessa GL (1992) Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. *Eur J Pharmacol* **221:**227–234.

Rossi DJ, Oshima T, and Attwell D (2000) Glutamate release in severe brain ischemia is mainly by reversed uptake. *Nature (Lond)* **403:**316–321.
Rubinow DR, Davis CL, and Post RM (1995) Somatostatin in the central nervous system, in *Psychopharmacology: The Fourth Generation of Progress* (Bloom FE, Kupfer DJ eds) pp 553–596, Raven Press Ltd., New York.
Sabatier J-M, Vives E, Mabrouk K, Benjouad A, Rochat H, Duval A, Hue B, and Bhraoui E (1991) Evidence for neurotoxic activity of tat from human immunodeficiency virus type 1. *J Virol* **65:**961–967.
Sastre M and García-Sevilla JA (1994) α_2-Adrenoceptor subtypes identified by [^{3}H]RX821002 binding in the human brain: the agonist guanoxabenz does not discriminate different forms of the predominant α_{2A} subtype. *J Neurochem* **63:** 1077–1085.
Savio T and Levi G (1993) Neurotoxicity of HIV coat protein gp120, NMDA receptors and protein kinase C: a study with rat cerebellar granule cell cultures. *J Neurosci Res* **34:**265–272.
Schlaepfer TE, Pearlson GD, Wong DF, Marenco S, and Dannals RF (1997) PET study of competition between intravenous cocaine and [^{11}C]raclopride at dopamine receptors in human subjects. *Am J Psychiatry* **154:**1209–1213.
Schlicker E, Brandt F, Classen K, and Göthert M (1985) Serotonin release in human cerebral cortex and its modulation via serotonin receptors. *Brain Res* **331:**337–341.
Schlicker E and Kathmann M (2001) Modulation of transmitter release *via* presynaptic cannabinoid receptors. *Trends Pharmacol Sci* **22:**565–572.
Schlicker E, Timm J, Zentner J, and Göthert M (1997) Cannabinoid CB_1 receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedeberg's Arch Pharmacol* **356:**583–589.
Schlicker E, Werthwein S, Kathmann M, and Bauer U (1998) Nociceptin inhibits noradrenaline release in the mouse brain cortex via presynaptic ORL1 receptors. *Naunyn-Schmiedeberg's Arch Pharmacol* **358:**418–422.
Seal RP and Amara SG (1999) Excitatory amino acid transporters: a family in flux. *Annu Rev Pharmacol Toxicol* **39:**431–456.
Shumate MD, Lin DD, Gibbs JW III, Holloway KL, and Coulter DA (1998) $GABA_A$ receptor function in epileptic human dentate granule cells: comparison to epileptic and control rat. *Epilepsy Res* **32:**114–128.
Sims KD and Robinson MB (1999) Expression patterns and regulation of glutamate transporters in the developing and adult nervous system. *Crit Rev Neurobiol* **13:**169–197.
Sims NR, Bowen DM, Allen ST, Smith CCT, Neary D, Thomas DJ, and Davison AN (1983) Presynaptic cholinergic dysfunction in patients with dementia. *J Neurochem* **40:**503–509.
Snead OC and Simonato M (1991) Opioid peptides and seizures, in *Neurotransmitters and Epilepsy* (Fisher RS and Coyle J eds) pp 181–200, Wiley-Liss, New York.
Somogyi P, Hodgson AJ, Smith AD, Nunzi GM, Gorio A, and Jang-Yen WU (1984) Different populations of GABAergic neurons in the visual cortex and hippocampus of cat contain somatostatin- or cholecystokinin-immunoreactive material. *J Neurosci* **4:**2590–2603.
Staley KJ, Soldo BL, and Proctor WR (1995) Ionic mechanisms of neuronal excitation by inhibitory $GABA_A$ receptors. *Science (Wash DC)* **269:**977–981.
Starke K, Göthert M, and Kilbinger H (1989) Modulation of neurotransmitter release by presynaptic autoreceptors. *Physiol Rev* **69:**864–989.
Stauderman KA, Mahaffy LS, Akong M, Velicelebi G, Chavez-Noriega LE, Crona JH, Johnson EC, Elliott KJ, Gillespie A, Reid RT, et al. (1998) Characterization of human recombinant neuronal nicotinic acetylcholine receptor subunit combinations $\alpha2\beta4$, $\alpha3\beta4$ and $\alpha4\beta4$ stably expressed in HEK293 cells. *J Pharmacol Exp Ther* **284:**777–789.
Stefani A, Bassi A, Mazzone P, Pierantozzi M, Gattoni G, Altibrandi MG, Giacomini P, Peppe A, Bernardi G, and Stanzione P (2002) Subdyskinetic apomorphine responses in globus pallidus and subthalamus of parkinsonian patients: lack of clear evidence for the 'indirect pathway.' *Clin Neurophysiol* **113:**91–100.
Stefani A, Fedele E, Galati S, Pepicelli O, Frasca S, Pierantozzi M, Peppe A, Brusa L, Orlacchio A, Hainsworth AH, et al. (2005) Subthalamic stimulation activates internal pallidus: evidence from cGMP microdialysis in PD patients. *Ann Neurol* **57:**448–452.
Steffens M, Engler C, Zentner J, and Feuerstein TJ (2004) Cannabinoid CB_1 receptor-mediated modulation of evoked dopamine release and of adenylyl cyclase activity in the human neocortex. *Br J Pharmacol* **141:**1193–1203.
Steffens M, Szabo B, Klar M, Rominger A, Zentner J, and Feuerstein TJ (2003) Modulation of electrically-evoked acetylcholine release through cannabinoid CB_1 receptors: evidence for an endocannabinoid tone in the human neocortex. *Neuroscience* **120:**455–465.
Stein V and Nicoll RA (2004) GABA generates excitement. *Neuron* **37:**375–378.
Sullivan JM (2000) Cellular and molecular mechanisms underlying learning and memory impairments produced by cannabinoids. *Learning Memory* **7:**132–139.
Svensson AL and Nordberg A (1996) Tacrine interacts with an allosteric activator site on $\alpha4\beta2$ nAChRs in M10 cells. *Neuroreport* **7:**2201–2205.
Szatkowski M and Attwell D (1994) Triggering and execution of neuronal death in brain ischemia: two phases of glutamate release by different mechanisms. *Trends Neurosci* **17:**359–365.
Tan CM, Brady AE, Nickols HH, Wang Q, and Limbird LE (2004) Membrane trafficking of G protein-coupled receptors. *Annu Rev Pharmacol Toxicol* **44:**559–609.
Timmerman W and Westerink BH (1997) Brain microdialysis of GABA and glutamate: what does it signify? *Synapse* **27:**242–261.
Trendelenburg AU, Cox SL, Schelb V, Klebroff W, Khairallah L, and Starke K (2000) Modulation of ^{3}H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and β-adrenoceptors in mouse tissues. *Br J Pharmacol* **130:**321–330.
Trendelenburg AU, Sutej I, Wahl CA, Molderings GJ, Rump LC, and Starke K (1997) A re-investigation of questionable subclassifications of presynaptic $\alpha2$-autoreceptors: rat vena cava, rat atria, human kidney and guinea-pig urethra. *Naunyn-Schmiedeberg's Arch Pharmacol* **356:**721–737.
Tsou K, Mackie K, Sanudo-Pena MC, and Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* **93:**969–975.
Tyrrell PJ, Sawle GV, Ibañez V, Bloomfield PM, Leenders KL, Frackowiak RS, and Rossor MN (1990) Clinical and positive emission tomographic studies in the extrapyramidal syndrome of dementia of the Alzheimer type. *Arch Neurol* **47:** 1318–1323.
Valle LD, Croul S, Morgello S, Amini S, Rappaport J, and Khalili K (2000) Detection of HIV-1 Tat and JCV capsid protein, VP1, in AIDS brain with progressive multifocal leukoencephalopathy. *J Neurovirol* **6:**221–228.
Vallebuona F and Raiteri M (1994) Extracellular cGMP in the hippocampus of freely moving rats as an index of nitric oxide (NO) synthase activity. *J Neurosci* **14:**134–139.
Vespa P, Prins M, Ronne-Engstrom E, Caron M, Shalmon E, Hovda DA, Martin NA, and Becker DP (1998) Increase in extracellular glutamate caused by reduced cerebral perfusion pressure and seizures after human traumatic brain injury: a microdialysis study. *J Neurosurg* **89:**971–982.
Vizi ES (1998) Different temperature dependence of carrier-mediated (cytoplasmic) and stimulus-evoked (exocytotic) release of transmitter: a simple method to separate the two types of release. *Neurochem Int* **33:**359–366.
Vizi ES and Lendvai B (1999) Modulatory role of presynaptic nicotinic receptors in synaptic and non-synaptic chemical communication in the central nervous system. *Brain Res Rev* **30:**287–295.
Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L, Ding Y, Gatley SJ, Gifford A, and Franceschi D (2001) Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. *J Neurosci* **21:**RC121.
von Lubitz DKJE (1999) Adenosine and cerebral ischemia: therapeutic future or death of a brave concept? *Eur J Pharmacol* **371:**85–102.
Waeber C, Schoeffter P, Palacios JM, and Hoyer D (1989) 5-HT_{1D} receptors in guinea pig and pigeon: radioligand binding and biochemical studies. *Naunyn-Schmiedeberg's Arch Pharmacol* **340:**479–485.
Warpman U and Nordberg A (1995) Epibatidine and ABT 418 reveal selective losses of $\alpha4\beta2$ nicotinic receptors in Alzheimer brains. *Neuroreport* **6:**2419–2423.
Weinshank RL, Zgombick JM, Macchi MJ, Branchek TA, and Hartig PR (1992) Human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-HT_{1D} α and 5-$HT_{1D\beta}$. *Proc Natl Acad Sci USA* **89:**3630–3634.
Weiss F, Lorang MT, Bloom FE, and Koob GF (1993) Oral alcohol self-administration stimulates dopamine release in the rat nucleus accumbens: genetic and motivational determinants. *J Pharmacol Exp Ther* **267:**250–258.
Weisskopf MG, Zalutsky RA, and Nicoll RA (1993) The opioid peptide dynorphin mediates heterosynaptic depression of hippocampal mossy fibre synapses and modulates long-term potentiation. *Nature (Lond)* **362:**423–427.
Welter ML, Houeto JL, Bonnet AM, Bejjani PB, Mesnage V, Dormont D, Navarro S, Cornu P, Agid Y, and Pidoux B (2004) Effects of high-frequency stimulation on subthalamic neuronal activity in parkinsonian patients. *Arch Neurol* **61:**89–96.
Werber EA and Rabey JM (2001) The beneficial effect of cholinesterase inhibitors on patients suffering from Parkinson's disease and dementia. *J Neural Transm* **108:**1319–1325.
Wilkie GI, Hutson P, Sullivan JP, and Wonnacott S (1996) Pharmacological characterization of a nicotinic autoreceptor in rat hippocampal synaptosomes. *Neurochem Res* **21:**1141–1148.
Williamson A (1994) Electrophysiology of epileptic human neocortical and hippocampal neurons maintained in vitro. *Clin Neurosci* **2:**47–52.
Williamson A, Telfeian AE, and Spencer DD (1995) Prolonged GABA responses in dentate granule cells in slices isolated from patients with temporal lobe sclerosis. *J Neurophysiol* **74:**378–387.
Wonnacott S (1997) Presynaptic nicotinic ACh receptors. *Trends Neurosci* **20:**92–98.
Woo R-S, Park E-Y, Shin M-S, Jeong M-S, Zhao R-J, Shin B-S, Kim C-J, Park J-W, and Kim K-W (2002) Mechanism of nicotine-evoked release of [^{3}H]noradrenaline in human cerebral cortex slices. *Br J Pharmacol* **137:**1063–1070.
Woodruff GN and Hughes J (1991) Cholecytsokinin antagonists. *Annu Rev Pharmacol Toxicol* **31:**469–501.
Zetterström TSC, Vaughan-Jones RD, and Grahame-Smith DG (1995) A short period of hypoxia produces a rapid and transient rise in $[K^+]_c$ in rat hippocampus in vivo which is inhibited by certain K^+-channel blocking agents. *Neuroscience* **67:**815–821.
Zgombick JM, Bard JA, Kucharewicz SA, Urquhart DA, Weinshank RL, and Branchek TA (1997) Molecular cloning and pharmacological characterization of guinea pig 5-HT_{1B} and 5-HT_{1D} receptors. *Neuropharmacology* **36:**513–524.
Zhang L, Zhou F-M, and Dani JA (2004) Cholinergic drugs for Alzheimer's disease enhance in vitro dopamine release. *Mol Pharmacol* **66:**538–544.
Zocchi C, Ongini E, Conti A, Monopoli A, Negretti A, Baraldi PG, and Dionisotti S (1996) The non-xanthine heterocyclic compound, SCH58261, is a new potent and selective A_{2A} adenosine receptor antagonist. *J Pharmacol Exp Ther* **276:**398–404.