Neurochemical Research, Vol. 28, No. 10, October 2003 (© 2003), pp. 1501–1515

Presynaptic Modulation Controlling Neuronal Excitability and Epileptogenesis: Role of Kainate, Adenosine and Neuropeptide Y Receptors*

João O. Malva,[1,2] Ana P. Silva,[1] and Rodrigo A. Cunha[1]

(Accepted March 25, 2003)

Based on the idea that seizures may arise from an overshoot of excitation over inhibition, all substances that may decrease glutamatergic function while having no effect or even increasing GABAergic neurotransmission are likely to be effective anticonvulsants. We now review the possible role of three such neuromodulators, kainate, adenosine, and neuropeptide Y receptors in controlling hyperexcitability and epileptogenesis. Particular emphasis is given on the robust neuromodulatory role of these three groups of receptors on the release of glutamate in the hippocampus, a main focus of epilepsy. Moreover, we also give special attention to the mechanisms of receptor activation and coupled signaling events that can be explored as attractive targets for the treatment of epilepsy and excitotoxicity. The present paper is a tribute to Arsélio Pato de Carvalho who has been the main driving force for the development of Neuroscience in Portugal, notably with a particular emphasis on the presynaptic mechanisms of modulation of neurotransmitter release.

KEY WORDS: Kainate receptors; adenosine receptors; neuropeptide Y receptors; glutamate; epilepsy; hippocampus.

INTRODUCTION

Epilepsy is probably a multietiological group of diseases that has a high incidence worldwide (1). However, therapy for epilepsy is mostly palliative because it is essentially aimed at alleviating the symptoms of epilepsy, that is, seizures (2). In fact, seizures are the phenotypic expression of a largely undefined cascade of events involving functional and structural changes, globally termed epileptogenesis (3), that may take years to result in clinical expression (4,5). Seizures result from the recurrent firing of excitatory neurons resulting from an imbalance toward a hyperexcitability state (6,7). This repetitive firing leads to excessive release of glutamate, the main excitatory neurotransmitter in the central nervous system, and ultimately to neuronal cell death (8–11), as illustrated by mesial temporal sclerosis, a hallmark of one of the most common adult forms of treatment-resistant epilepsy, temporal lobe epilepsy (12,13).

The more popular antiepileptic drugs have been developed as activity-dependent inhibitors of voltage-sensitive sodium channels (14,15). These antiepileptic drugs are mostly effective to control burst firing, which is characteristic of a seizure rather than non–burst-like nerve impulses. Incidentally, these drugs are also effective in attenuating the progression of seizures into status epilepticus (16), in accordance with Gower's concept of "seizures beget seizures." However, with progression

* Special issue dedicated to Dr. Arsélio Pato de Carvalho.

[1] Center for Neuroscience of Coimbra, Institute of Biochemistry, Faculty of Medicine, University of Coimbra, 3004-504 Coimbra, Portugal.

[2] Address reprint requests to: Tel: +351239833369; Fax: +3512398-22776; E-mail: jomalva@cnc.cj.uc.pt

0364-3190/03/1000–1501/0

of disease, there is a loss of efficiency of antiepileptic drugs (2), probably because these drugs may not have a direct effect on epileptogenesis or on neuronal cell death (16). Another possible target for therapeutic intervention to manage epilepsy might be restraining the excessive release of glutamate that occurs during seizures (17). This might be a promising strategy because glutamate has a triple role in (i) mediating firing on hyperexcitability of neuronal circuits (17–19); (ii) mediating synaptic plasticity phenomena that involve both functional and structural changes in neuronal circuits (20); and (iii) mediating excitotoxicity and neuronal cell death (21).

We are particularly interested in presynaptic modulatory systems that selectively modulate the release of glutamate rather than of inhibitory neurotransmitters such as GABA and that may be upregulated either by seizures or by the epileptogenic process. In this review, we consider three neuromodulatory systems that fulfil these two criteria, namely the neuromodulatory systems operated by kainate, adenosine, and neuropeptide Y (NPY) receptors.

Kainate Receptors

Glutamate is the principal excitatory neurotransmitter in the mammalian brain. In the hippocampus it plays a major role in synaptic transmission, but in situations of excessive glutamate release, such as epilepsy or excitotoxicity, glutamate may also be a key trigger of neuronal dysfunction (22,23). Presynaptic ionotropic and metabotropic glutamate receptors have been shown to modulate the release of important neurotransmitters in the brain (24,25). Among the identified presynaptic glutamate receptors, the role of kainate receptors has remained enigmatic for a long time. Now, an increasing number of reports have contributed to open new windows of knowledge about the functional properties of presynaptic kainate receptors, in particular in relation to hippocampal function and epilepsy. It has long been known that administration of kainate induces seizures, and indeed intraperitoneal or intracerebroventricular injection of kainate in rats has been used as an animal model of induction of temporal lobe epilepsy (26). On the other hand, it is known that kainate receptors are particularly abundant in the CA3 stratum lucidum (27,28) and that the selective lesion of the mossy fiber projections blocks kainate-induced seizures (28,29). In fact, kainate receptors are emerging as one of the key elements in hippocampal neuronal pacemaker activity and epileptogenesis (30).

Five different kainate receptor subunits were cloned (31,32). These subunits can form low-affinity kainate receptors, GluR5, GluR6, and GluR7, or high-affinity kainate receptors that includes KA1 and KA2 subunits. In addition, four GluR5 (GluR5-1, GluR5-2a, GluR5-2b, and GluR52c) and two GluR7 (GluR7a and GluR7b) alternative splice variants were also identified (31,32). Moreover, mRNA editing at the Q/R site, in the intracellular membrane loop between transmembrane domains 1 and 3, contributes to the generation of two alternative forms of GluR5, and editing at the same Q/R site and at the I/V and Y/C sites in the transmembrane domain 1 contribute to six different forms of GluR6 subunits (31,32).

Native ionotropic glutamate receptors, including kainate receptors, may be constituted by tetrameric organization of receptors subunits (33). The putative diversity of the subunit organization at native receptors can contribute to a significant variability of receptors with different pharmacological properties.

In contrast to AMPA receptors, kainate receptors are activated by lower concentrations of kainate as compared with AMPA. The general agonist potency order for kainate receptor activation is domoate>kainate>glutamate>AMPA for low-affinity kainate receptors, and kainate>domoate>glutamate>>AMPA for high-affinity kainate receptors (34). However, in contrast to the effect of kainate at AMPA receptors, the activation of kainate receptors with kainate results in a desensitizing response (35,36), and this desensitization is prevented by concanavalin A (36,37). In recent years, several agonists and antagonists selective for kainate receptor subunits have been actively produced, increasing the available tools for selective targeting of kainate receptors (38,39).

Presynaptic and Postsynaptic Kainate Receptors in the Hippocampus. The role of postsynaptic kainate receptors in synaptic transmission in the hippocampus was revealed as a result of the availability of efficient and selective AMPA receptor antagonists (40,41). However, the activity of these receptors is normally masked by the highly active AMPA receptors, and it is clearly difficult to find a role for kainate receptors in mediating fast neurotransmission (40–42). Similarly to what is known for ATP/P2 and nicotinic receptors signaling systems in the central nervous system, there is a paradox between robust receptor expression and inability to clearly identify a role in neurotransmission, suggesting that these receptors may behave as neuromodulatory systems (43). Interestingly, the activation of postsynaptic kainate receptors was also shown only to occur upon the stimulation at high-frequency of mossy fiber afferents

(44). This frequency-dependent activation of postsynaptic kainate receptors may suggest that kainate receptors are located at the periphery of the postsynaptic junction or also may be regarded as the result of presynaptic modulatory events initiated by glutamate-dependent retrograde mechanisms at the presynaptic terminal. Together, these observations strongly support the existence of a presynaptic target for kainate as a potential element responsible for the biological effects of kainate and domoate in the hippocampus. This putative kainate receptor located presynaptically at mossy fiber terminals is responsible for glutamate-induced glutamate release, which may be responsible for the strong epileptogenic and excitotoxic action of kainic acid at the CA3 area of the hippocampus, was visionarily hypothesized two decades ago (27).

Mossy Fiber Presynaptic Kainate Receptors, Glutamate Release, and Epileptogenesis. Presynaptic modulation of glutamatergic neurotransmission by kainate receptors has only recently reached relative consensus. For more than two decades the clear identification of the molecular target of the strong epileptogenic compound kainate, in the hippocampus, remained undetermined. However, in the last decade the cloning of kainate receptor subunits (31,45) and the identification of functional presynaptic kainate receptors by neurochemical (34,46–48) and by electrophysiological techniques (37,42,49–51) revealed an important molecular target for presynaptic modulation of glutamate and GABA release in the hippocampus.

The stratum lucidum of the CA3 area of the hippocampus is the brain area with the highest density of kainate receptors (30,52). Selective lesion of the mossy fiber projections and immunohistochemistry studies indicated that part of the kainate receptors in the stratum lucidum are presynaptically located at mossy fiber terminals (28,29,53). Following the selective lesion of the mossy fiber projections or following neonatal γ-irradiation, causing granule cell death, a significant decrease in the expression of high-affinity kainate binding sites and reduction of kainate-induced epileptiform discharges was observed (28–30). In agreement with the hippocampal expression of kainate receptor subunits (45,54) it may be expected that kainate receptors containing GluR6, KA1, and KA2 subunits may play a major role in mossy fiber synapses. Interestingly, GluR6 knockout mice are significantly less sensitive to kainate-induced epilepsy and excitotoxicity, indicating a major role for GluR6-containing receptors in the physiology of mossy fiber synapses (44).

Presynaptic kainate receptors in the CA3 area of the rat hippocampus directly modulate the intracellular free Ca^{2+} concentration (46,55) and induce the exocytotic release of glutamate (47,56–58), contributing to the generation of a positive feedback mechanism responsible for glutamate-induced glutamate release (27,34,58). This focus of excitability in the hippocampus may play a relevant role in LTP (56,59) and under pathological noncontrolled activation, for example, domoic acid intoxication (60), may contribute to the generation of epilepsy (30) and excitotoxicity (44) of CA3 pyramidal cells.

Subunit Composition of Mossy Fiber Presynaptic Kainate Receptors. In spite of the very important findings in the investigation of presynaptic kainate receptors at the mossy fiber synapses, another major issue still lacks consensus: the subunits involved in the organization of the native receptors. The identification of the subunit composition of the presynaptic and postsynaptic kainate receptors in hippocampal circuits may prove to be extremely important because the development of selective drugs differentially targeting presynaptic or postsynaptic receptors may play a key role in the future development of new antiepileptic strategies.

Studies with knockout mice for GluR6 and GluR5 indicate that GluR6 but not GluR5 subunits–containing receptors play a role in the presynaptic modulation of glutamate release from mossy fiber terminals (44,61). Supporting a role for GluR6 subunit–containing kainate receptors at CA3 nerve terminals, responsible for the modulation of intracellular Ca^{2+} and glutamate release, our group determined the agonist potency order in increasing the intracellular free Ca^{2+} concentration: domoate (EC_{50} 0.16 μM)>kainate (EC_{50} 0.86 μM)>AMPA (EC_{50} 43.04 μM) (34,46,47).

Other studies indicate a preferential role for GluR5 subunit–containing receptors in the presynaptic effects of kainate receptor agonists at mossy fiber synapses. It was shown that ATPA, a GluR5 kainate receptor agonist, depresses the mossy fiber–evoked synaptic transmission (41,62,63). On the other hand, the GluR5 antagonist LY382884 was able to inhibit the frequency-dependent facilitation of mossy fiber transmission (56,57,63). The latter studies indicate a role for GluR5 subunit–containing presynaptic autoreceptor, at the mossy fiber terminals, responsible for frequency-dependent facilitation of glutamate release. However, it is essential to keep in mind that the selectivity of kainate receptor agonists and antagonists was mainly tested in heterologous expression systems rather than in native receptors. Post-transcriptional region-specific modification of kainate receptor subunits, and the site-specific organization of kainate receptor subunits into functional native receptors, may hamper the interpretation about selectivity of the developed agonists

and antagonists. Moreover, the distribution of mRNA for the GluR5 subunit mainly in interneurons does not favor the involvement of GluR5 subunits in the assembly of presynaptic kainate receptors at mossy fiber terminals (64). However, the distribution of mRNA is not absolutely indicative of the location of the expression of the receptor proteins, especially in the case of synaptic receptors. So, one cannot exclude that the detected low levels of mRNA for GluR5 in dentate granular cells may account for the expression of GluR5 subunits at the mossy fiber nerve terminals.

Recently we found that in the CA3 area of the hippocampus GluR5, GluR6, and KA2 (but not KA1) subunits are present in significant levels in the presynaptic fraction of the synaptic junctions, and so may eventually account for the assembly of functional kainate receptors in CA3 nerve terminals (Pinheiro et al., unpublished observations). This study was based on a method of separation of presynaptic and postsynaptic proteins from the synaptic junctions (65) and identification of the receptor subunits by Western blotting. Using this strategy we recently identified surprisingly high levels of presynaptic AMPA receptor subunits in the rat hippocampus (66).

Depression of Excitatory Synaptic Transmission by Presynaptic Kainate Receptors. The prevalent view of kainate as a strong epileptogenic and excitotoxic compound in the hippocampus has been challenged by the finding that kainate receptor activation displays a biphasic response in CA1 Schaffer collateral synapses (42). At lower concentrations, kainate facilitates the isolated NMDA receptors–mediated $EPSC_S$, whereas higher concentrations of kainate cause a depression of the NMDA-mediated $EPSC_S$ through the activation of presynaptic kainate receptors (42). Moreover, the same authors also showed that activation of presynaptic kainate receptors in rat hippocampal synaptosomes inhibit the KCl-evoked release of glutamate (42). Accordingly, a presynaptic kainate-receptor mediated inhibition of the fEPSPs and EPSCs in the CA1 (62,67) and CA3 areas (68,69) was reported.

The puzzling presynaptic inhibition mediated by excitatory ionotropic receptors has been critically evaluated, and the mechanisms underlying depression of glutamatergic transmission and inhibition of glutamate release are still the subject of debate, and no consensus has yet been reached. Several different possibilities have been tested, including (i) the inhibition of presynaptic voltage-sensitive Ca^{2+} channels (67,68); (ii) hyperpolarization of nerve terminals as a result of influx of chloride through GluR6 containing subunits (70); (iii) intracellular coupling of presynaptic kainate receptors to Gi/Go proteins at glutamatergic nerve terminals (69); and (iv) decrease of the quantal release, probably because of sustained activation of presynaptic kainate receptors (61). The apparent contradictory findings may reflect cell and circuit variability in the hippocampus, as well as the likely multifactorial involvement of the identified processes in the inhibition of glutamate release.

Presynaptic Kainate Receptors and GABA Release. Presynaptic kainate receptors on GABAergic terminals that are able to reduce the evoked release of GABA and to depress the inhibitory synaptic transmission, in the hippocampus, have also been identified by several groups (48,50,51). However, the mechanism by which kainate receptors depress inhibitory synaptic transmission has been a matter of conflicting interpretation (30,32).

A group of reports attribute to presynaptic kainate receptors a direct presynaptic inhibition of the evoked release of GABA and depression of inhibitory transmission (32,48,50,51). The functional coupling between receptor activation and inhibition of GABA release inhibition was claimed to involve inhibitory G proteins (71–74), an interpretation based on the sensitivity to pertussis toxin of kainate receptor–induced depression of inhibitory synaptic transmission (71–73). Moreover, both protein kinase C and protein kinase A were shown to be involved in the downstream intracellular execution mechanisms leading to inhibition of GABA release (73,74). Other groups postulated that the kainate receptor–mediated depression of inhibitory synaptic transmission can be caused by a massive neuronal depolarization resulting from generation of ectopic action potential in axons (75) or, alternatively, caused by activation of GluR5 and GluR6-containing postsynaptic kainate receptors in interneurons (30,76–79), consistent with the expression of both subunits at a population of hippocampal interneurons (80). It was hypothesized that the resulting release of GABA from interneuronal terminals decreases the input membrane resistance of CA1 pyramids due to hyperactivation of $GABA_A$ receptors (30,76). Alternatively, at the presynaptic level GABA may activate presynaptic $GABA_B$ receptors (81) or the repetitive firing of interneurons may cause exhaustion of releasable synaptic vesicles containing GABA (30).

Recently, new findings challenged our knowledge about functional properties of presynaptic kainate receptors. It was shown that the direct activation of GluR6-containing (but not GluR5-containing) presynaptic kainate receptors increases the efficiency of GABAergic synapses (79,82–84). Interestingly, as shown for glutamatergic synapses, at GABAergic synapses a biphasic response of presynaptic kainate receptors was also found, with low agonist concentration potentiating and

high agonist concentration inhibiting the release of GABA (83).

A general consensus is now on the borderline of acceptance

A. "Presynaptic kainate receptors efficiently modulate the release of glutamate and GABA and synaptic transmission in a biphasic manner".

B. "Low concentrations of kainate receptor agonists potentiate, whereas high concentrations inhibit the release of neurotransmitters and synaptic transmission"

C. "Potentiation of the neurotransmitter release is caused by depolarization involving the influx of cations through the receptor channel."

D. "Inhibition of neurotransmitter release may be due to:

1. Interneuron depolarization caused by activation of postsynaptic kainate receptors and massive GABA release, which downregulates GABAergic synapses.
2. Activation of presynaptic kainate receptors may result in the inhibition of glutamate and GABA release by involving:
   a) Coupling to metabotropic inhibitory pathways.
   b) Hyperpolarization resulting from chloride influx.
   c) Inhibition of voltage-sensitive Ca^{2+} channels.
   d) Exhaustion of ready-to-fuse pool of synaptic vesicles.
3. A sum of some of the above indicated mechanisms in a regional and circuit-specific fashion."

E. Kainate is a potent excitatory neurotoxin. Although some mechanisms compatible with a kainate receptor–mediated decrease in excitability were identified, it is not clear how they are physiologically relevant. It is now clear that the prevalent effect of presynaptic kainate receptor activation in the CA3 area of the hippocampus is strongly excitatory and can be involved in synaptic plasticity and in dysfunction such as epileptogenesis and excitotoxicity. The development of new pharmacological tools that can selectively target CA3 presynaptic kainate receptors may represent an important step in the treatment of these disorders.

Adenosine Receptors

Adenosine is a prototypical neuromodulator because it controls the efficiency of synaptic transmission but is neither released in a transmitter-like fashion nor does it act vectorially to transmit information between neurons (85,86). The most evident effect of adenosine in the central nervous system is its ability to refrain synaptic transmission and neuronal excitability (87–89). This effect is due to the activation of inhibitory A_1 receptors, the most abundant of the four (A_1, A_{2A}, A_{2B}, and A_3) metabotropic adenosine receptors in the central nervous system (85,86).

Extracellular Adenosine Metabolism. The extracellular levels of adenosine are directly related with the degree of activity of neuronal circuits. Thus, with low frequencies of nerve stimulation (i.e., that do not trigger short-or long-term plasticity phenomena, typically below 0.1 Hz), there is a tonic on-going adenosine A_1 receptor-mediated inhibition of synaptic transmission (90) and the levels of extracellular adenosine raise with single pulsed stimulation of the preparations (91). Extracellular adenosine can result from the extracellular catabolism of ATP that can be released together with neurotransmitters and from other compartments in the brain (43). This released ATP can then be converted into adenosine through the action of a series of ectonucleotidases (92,93). Adenosine can also be released as such through bidirectional and non-concentrative nucleoside transporters (85,94) in stressful situations that cause a minor decrease in intracellular ATP concentrations (3–5 mM) resulting in dramatic changes in intracellular adenosine concentrations (basal concentration of about 50 nM) (85). Note that these metabolic imbalances may be localized in discrete subcellular compartments, such as dendrites, because it is well established that the activation of excitatory amino acid receptors increases the extracellular levels of adenosine (94). However, the main role of these nucleoside transporters is to clear up extracellular adenosine, which is then intracellularly either rephosphorylated into AMP, through adenosine kinase (in neurons), or deaminated into inosine, through adenosine deaminase (mainly in astrocytes) (85,94). Thus the existence of two pathways to form extracellular adenosine is probably due to the fact that adenosine fulfils two parallel functions, as a neuromodulator in nonstressful situations (in which extracellular adenosine is probably mainly formed from released ATP) and as a homeostatic modulator (where extracellular adenosine is probably released as such) (85).

Effects of Adenosine A_1 Receptors on Neuronal Excitability. The inhibitory effect of adenosine is particularly evident in excitatory rather than inhibitory neurons in the brain (85). In fact, in some brain areas, such as in the hippocampus, the activation of A_1 receptors only directly inhibits excitatory rather than inhibitory synpases (95,96). The inhibition by adenosine of synaptic transmission essentially results from a

presynaptic effect (97,98), namely to the ability of A_1 receptors to inhibit the evoked release of glutamate (99). This A_1 receptor–mediated control of glutamate release may involve either an inhibition of calcium entry through voltage-sensitive calcium channels (100–102) or a decreased affinity of the calcium sensor responsible for triggering neurotransmitter release (103–106).

The effects of adenosine on neuronal function are not limited to its ability to control the evoked release of glutamate. In fact, A_1 receptors are also located postsynaptically and nonsynaptically in neurons (107). Postsynaptically, A_1 receptors can inhibit NMDA receptors (108,109) and voltage-sensitive calcium channels (110,111). This is probably the basis for the ability of adenosine to control dendritic integration, namely synaptic plasticity phenomena (112). Furthermore, the A_1 receptor–mediated control of NMDA receptor function may be a key feature of the neuroprotective effects of adenosine (113,114). Adenosine also decreases neuronal firing (88,89) acting nonsynaptically mainly through activation of potassium channels leading to neuronal hyperpolarization (115–117). Probably the latter effect plays a key role in the A_1 receptor–mediated control of neuronal circuits, being more evident in situations of burst firing (88).

Anticonvulsant Properties of Adenosine A_1 Receptors. A series of studies have shown that administration of adenosine and of A_1 receptor agonists display anticonvulsant properties in different animal models of epilepsy (118–133). Likewise, adenosine and A_1 receptor agonists are also anticonvulsants in in vitro slice models of epilepsy (90,134). In vitro studies have been instrumental to support the idea that the anticonvulsant effect of adenosine may result in part from an inhibition of the evoked release of glutamate, but mainly from the hyperpolarizing action of adenosine mediated by activation of potassium channels (90,124,135). Thus adenosine is particularly effective in controlling secondary after-discharges and in refraining the rate of interictal spiking in brain slice models of epilepsy (132,135,136). Likewise, in animal models of epilepsy, adenosine and A_1 receptor agonists are able not only to increase the threshold for seizure induction but also contribute to seizure arrest (132,133).

The relevance of the adenosine neuromodulatory system for the control of seizures is best illustrated by the proposal that adenosine might be an endogenous anticonvulsant (137). This implies that endogenous adenosine would exert a tonic anticonvulsant effect. This has received direct experimental confirmation by the proconvulsant action of adenosine receptor antagonists (119,120,122,131,138–144). However, except in the CA3 region of the hippocampus where adenosine receptor antagonists cause a persistent paroxysmal activity (90,145–147), the blockade of adenosine receptors is by itself not sufficient to trigger seizures in naive animals or preparations (132), and adenosine receptor antagonists do not seem to alter the course of a seizure per se (144). However, adenosine receptor antagonists can prolong epileptic seizures (139,140,144) and can convert a pattern of recurrent seizures into status epilepticus (122,131).

The extracellular levels of adenosine raise abruptly with patterns of stimulation that induce seizures (148–154). This increase in extracellular adenosine starts within 10 s of stimulation and peaks at 60–180 min (144,148,150,153). Most importantly, the extracellular levels of adenosine remain elevated postictally and, in keeping with the hypothesis that adenosine is likely to be responsible for much of postictal depression, antagonists of adenosine receptors can reduce the duration of postictal depression (144,155).

Another potential benefit of adenosine and adenosine A_1 receptor agonists for the management of epileptic complications is their neuroprotective properties (113,156). In fact, under different stressful situations, adenosine and adenosine A_1 receptor agonists can attenuate the outcome of stress-induced neuronal cell death (113,156), an effect that may be of interest in view of the extensive neuronal cell death occuring as a consequence of seizure activity (8–11).

Impact of Seizures and Epilepsy on Adenosine Neuromodulation. The above presented evidence indicates that adenosine may exert a tonic anticonvulsant effect but also shows that exogenously added adenosine or A_1 receptor agonists can produce an even greater anticonvulsant effect. This suggests that the adenosine neuromodulatory system has a potential therapeutic interest to control seizures. However, because adenosine receptor agonists do not efficiently cross the blood brain barrier (157) and display evident side effects, including profound reductions in blood pressure and heart rate, hypothermia, sedation, and motor depression (121,158,159), it seems more reasonable to attempt to increase the extracellular levels of adenosine by controlling its clearance and metabolism rather than directly activating A_1 receptors. In fact, in spite of the large seizure-induced increase in the extracellular levels of adenosine, further increasing the levels of extracellular adenosine (160) by inhibition of adenosine transporters (134,141,161,162), adenosine kinase (162,163), or adenosine deaminase (162) increases the threshold for seizure induction. Likewise, increasing the number (164) or the affinity of A_1 receptors (165) also causes

the same effect. These observations also lead to the idea that repeated seizures may influence the adenosine neuromodulatory system.

In fact, the density of adenosine A_1 receptors is enhanced after acute seizure induction (170–172) but is reduced after chronic seizure induction (128,166–169). This is in agreement with the requirement of higher doses of A_1 receptor agonists to produce anticonvulsant effects as status epilepticus progresses (131), possibly because of the reduction in the efficacy of A_1 receptor agonists observed in fully kindled rats (173). In parallel, there is a complex change in the extracellular metabolism of purines. Thus, after seizures, there is an increased release of ATP (174), which may also play a prominent role in seizure control (175), a decrease in ecto-ATPase (and/or ecto-ATP diphosphohydrolase) activity(ies) (173,176–178), and a marked increase in the activity of ecto-5′-nucleotidase (173,179), the enzyme responsible for the formation of adenosine from adenine nucleotides (92). Finally, both the density (180) and the efficiency of nucleoside transporters (173) are decreased upon seizure induction. Overall, these modifications would lead one to anticipate a greater formation and longer half-life of ATP-derived adenosine. However, it is precisely the opposite that is observed, that is, the tonic inhibition by adenosine of hippocampal synaptic transmission or synaptic plasticity has a considerably lower amplitude in fully kindled rats (173). This makes sense in light of the hypothesis that ATP-derived adenosine leads to a preferential activation of facilitatory A_{2A} rather than inhibitory A_1 receptors (85,181).

Adenosine A_{2A} Receptors: A New Target for Epilepsy? The role of A_{2A} receptors in epilepsy is still ill-defined, mainly because the more selective adenosine A_{2A} receptor agonist CGS 21680 has a poor selectivity toward extrastriatal A_{2A} receptors (182). In fact, mixed effects of purported A_{2A} receptor agonists on seizure outcome have been reported (183–189). However, recent reports described robust anticonvulsant effects of A_{2A} receptor antagonists in the threshold of seizure induction (190,191). Likewise, adenosine A_{2A} receptor knockout mice also display a higher threshold for seizure induction in different models of epilepsy (190,191). This protective effect of A_{2A} receptor blockade or inactivation parallels the general neuroprotective effects of A_{2A} receptor blockade in different stressful situations (192–199).

The mechanism by which A_{2A} receptors control neuronal dysfunction is still poorly understood, especially because of the low density of A_{2A} receptors in extrastriatal regions of the brain (200). However, the recent observations that stressful situations lead to an increased expression and density of A_{2A} receptors (201) may help in understanding the efficiency of A_{2A} receptor antagonists in stressful situations based on the hypothesis of stress-induced gain of function of A_{2A} receptors. Interestingly, we have recently observed that there is an increased density of A_{2A} receptors in the cerebral cortex and hippocampus of fully kindled rats (202). A particular impact of A_{2A} receptor blockade in epilepsy may also be anticipated based on the ability of A_{2A} receptor blockade to blunt the effect of brain-derived neurotrophic factor (BDNF) (203). A and conversely in the ability of A_{2A} receptor agonists to trigger TrkB receptor activation even in the absence of BDNF (204). In fact, seizures induce a robust increase in the expression of the BDNF gene (205), and a striking reduction of development of kindling was found in BDNF heterozygotes (206). Also, infusion of TrkB receptor bodies (proteins that selectively bind ligands of TrkB receptors such as BDNF) in the ventricles of mature animals limit the development of kindling (207).

Although still requiring considerable experimental confirmation, these preliminary observations open a new conceptual possibility to interfere with seizure induction, and eventually epileptogenesis, and seizure-induced neuronal degeneration based on the blockade of adenosine A_{2A} receptors. This is particularly interesting given that lower doses of A_{2A} receptor antagonists seem to be required to hit central versus peripheral A_{2A} receptors (193,197,198), that the central effects of A_{2A} receptors antagonists seem to stable over time (208–210), and that there is an increased density and functional relevance of A_{2A} receptors in the neocortex and in the limbic cortex upon aging (211,212) when epilepsy complications are prevalent.

Neuropeptide Y and Its Receptors

Neuropeptide Y (NPY) is a member of a peptide family that also includes peptide YY (PYY) and pancreatic polypeptide (PP). This family of peptides is sometimes referred as the pancreatic polypeptide family, because PP was the first of these peptides to be discovered (213). However, NPY has remained much more conserved during evolution than PP, and this family of peptides should be more appropriately called NPY family (214,215). In mammals, NPY is mainly expressed in neuronal tissue (216), whereas PYY is primarily expressed in the gut endocrine cells (217). In contrast, PP seems to be exclusively pancreatic (217). Indeed, NPY has neurotransmitter properties (218), while PYY and PP act as hormones in an endocrine and exocrine fashion, that is, by regulating pancreatic and gastric secretion (217).

The members of the NPY family (NPY, PYY, and PP) act upon the same family of NPY receptors (219). Five distinct NPY receptors have been cloned, and sequence comparisons show that receptors Y_1, Y_4, and Y_6 are more closely related to each other than to the receptors Y_2 and Y_5 (220). Among the cloned receptors, the Y_1, Y_2, Y_4, and Y_5 receptors represent fully defined subtypes, whereas no functional correlate of the cloned y_6 receptor has been reported to date. Moreover, the subtypes Y_1, Y_2, and Y_5 preferentially bind NPY and PYY, and Y_4 preferentially binds PP. All the receptors of the family share the characteristics of seven transmembrane domain-G–protein coupled receptors. In particular, Y receptors act via pertussis toxin–sensitive G-proteins, such as members of the G_i and G_o family, and are capable of mediating the inhibition of adenylate cyclase and, consequently, the inhibition of cAMP accumulation in tissues and cells. However, the resulting functional effects depend on the system being studied.

NPY and Epilepsy. Emerging evidence points to an important role for NPY in the regulation of neuronal activity both under physiological conditions and during pathological hyperactivity such as that occurring during seizures. Following acute seizures, there is an increase in NPY levels in the neurons of some cortical and limbic areas, particularly in the hippocampus, amygdala, and frontal, pyriform, and entorhinal cortices (221,222). In the hippocampus, NPY is constitutively expressed in GABA interneurons. Seizures have been repeatedly shown to enhance NPY levels both in these inhibitory interneurons and in the excitatory granule cells and mossy fibers that do not normally contain the peptide (221–224). Indeed, expression of NPY in granule cells is induced by stimulation of metabotropic and ionotropic glutamate receptors, which suggests that excitatory glutamatergic neurotransmission regulates the expression of NPY. This neuropeptide can inhibit epileptiform activity in the rat hippocampus in vitro, which can be due to inhibition of glutamate-mediated synaptic transmission in areas CA1 and CA3 (225), or in epileptic humans dentate gyrus (226). Moreover, Van den Pol et al. (227) have demonstrated that glutamate-dependent activity is necessary for NPY to depress the intracellular Ca^{2+} concentration and the electrical activity of neurons. In animals lacking NPY, seizures induced by excessive excitation (e.g., kainic acid) were not controlled in a "normal" manner, resulting in prolonged seizure activity and death in most NPY-deficient mice (228). Also, transgenic rats overexpressing NPY, especially in the CA1 area of the hippocampus, were less susceptible to epileptogenesis because they showed a significant reduction in the number and duration of electroencephalographic seizure activity induced by kainate (229). Given that NPY inhibits excitatory neurotransmission in normal hippocampus (230) and that exogenous administration of NPY prominently suppresses limbic seizure activity induced by kainic acid (231), one possibility is that NPY acts as an endogenous anticonvulsant agent by dampening the excessive excitation associated with seizures.

Following seizures, the increase of Y_1 receptor mRNA in the hippocampus is widespread, rapid, and very transient, whereas there is a major increase of Y_2 receptor mRNA expression and a rapid and transient increase of Y_5 receptor mRNA in the dentate granule cell layer (232). Indeed, the increase in Y_2 receptor binding in the hilus of the dentate gyrus is associated with an enhanced NPY release after kainate injection (233). However, this upregulation is always dependent on the duration and spread of the epileptiform activity. Some studies preceding the discovery of Y_5 receptors have only implicated Y_2 or Y_1 receptors in the modulation of anticonvulsant actions of NPY in the rat hippocampus (234,235). There is some evidence showing that the activation of Y_2 or blockade of Y_1 receptors, respectively, reduce kainate-induced seizures (236), but the pharmacological profile of centrally administered NPY analogues capable of inhibiting kainate-induced seizures in rats also suggests the involvement of Y_5 receptors (231).

NPY and Excitotoxicity. NPY acting on Y_1 receptors causes inhibition of Ca^{2+} influx through N-type channels into the somata and dendrites of granule cells in the hippocampus (237), whereas an excitatory component of NPY appears to be mediated by postsynaptic Y_1 receptors (238). The activation of postsynaptic Y_1 receptors has a depolarizing action on granule neurons when applied to their dendritic projection in the stratum moleculare (239). Moreover, NPY acting on Y_2 receptors inhibits excitatory (glutamatergic) synaptic transmission (230) onto CA3 pyramidal cells (237). Recently, a presynaptic Y_2 receptor was also identified as the NPY receptor responsible for the NPY-mediated inhibition of glutamate release in the CA1 subregion (240,241).

Electrophysiological and pharmacological studies have revealed that NPY acts predominantly through Y_2 receptors and inhibits glutamate release (221,241,242) by inhibiting Ca^{2+} influx into presynaptic nerve terminals through several types of Ca^{2+} channels (243). Moreover, we recently showed that the inhibition of glutamate release in the hippocampus is mediated by the activation of Y_1, Y_2, and Y_5 receptors in the dentate gyrus and CA3 subregion and by the activation of Y_2

receptors in the CA1 subregion (241,243). Our results also strongly suggest the involvement of L-, N-, and P/Q-type channels in the inhibition of $[Ca^{2+}]_i$ and glutamate release mediated by NPY receptors in the hippocampus, showing that the intracellular mechanism of coupling between NPY receptor activation and inhibition of exocytosis involves the influx of Ca^{2+} through different voltage-gated calcium channels (243). Moreover, in the hippocampus the Y_1 receptors seem to act both presynaptically and postsynaptically, whereas the Y_2 receptors act mainly at presynaptic nerve terminals, showing however activity at dendrites (243). Y_5 receptors also seem to play an important role in the control of the glutamatergic mechanism in the hippocampus. Woldbye et al. (231) showed that these receptors inhibit kainic acid seizures, but others demonstrated that Y_5 receptor–deficient mice do not exhibit spontaneous seizure-like activity (244). Recently, Guo et al. (245) also showed that the Y_5 receptor subtype plays a critical role in modulation of hippocampal excitatory transmission at the hilar-to-CA3 synapse in the mouse, but does not suppress epileptiform activity in the CA3 hippocampal area. Moreover, we have also presented functional evidence for the interaction between Y_1 and Y_2 or Y_2 and Y_5 receptors. The simultaneous activation of both receptors did not result in a potentiation of the inhibition of glutamate release and $[Ca^{2+}]_i$ changes mediated by each receptor individually (243). These observations strongly suggest the formation of oligomers of Y_1 and Y_2 or Y_2 and Y_5 receptors, with a pharmacology very similar to that observed for the Y_2 receptors. It was also recently shown that NPY receptors may associate in dimers (246), further supporting our proposal of direct functional and physical interaction between different NPY receptors.

The strong efficiency of Y_1, Y_2, and Y_5 receptors in modulating the exocytotic release of glutamate led us to investigate a neuroprotective role of NPY receptor activation against excitotoxic insults. Recently, we identified a robust neuroprotective effect of Y_1, Y_2, and Y_5 receptor activation against neuronal cell death caused by aggression associated with selective activation of AMPA or kainate receptors in organotypic cultures of rat hippocampal slices (247).

So, it seems that Y_1, Y_2, and Y_5 receptors play an important neuroprotective role during excitotoxic conditions and epilepsy, and the identification of which NPY receptors mediate the inhibitory or excitatory effects of NPY may be important for pharmacological targeting in several pathological conditions associated with glutamate receptor hyperactivation and dysfunction. Moreover, the putative formation of oligomers of NPY receptors can give us additional and important information relevant to the development of new drugs for the treatment of epilepsy.

REFERENCES

1. deLorenzo, R. J., Towne, A. R., Pellock, J. M., and Ko, D. J. 1992. Status epilepticus in children, adults and the elderly. Epilepsia 33(Suppl. 4):S15–S25.
2. Schmidt, D. and Krämer, G. 1994. The new anticonvulsant drugs: Implications for avoidance of adverse effects. Drug Safety 11:422–431.
3. Dalby, N. O. and Mody, I. 2001. The process of epileptogenesis: A pathophysiological approach. Curr. Opin. Neurol. 14:187–192.
4. Mathern, G. W., Babb, T. L., Leite, J. P., Pretorius, K., Yeoman, K. M., and Kuhlman, P. A. 1996. The pathogenic and progressive features of chronic human epilepsy. Epilepsy Res. 26:151–161.
5. Cole, A. J. 2000. Is epilepsy a progressive disease? The neurobiological consequences of epilepsy. Epilepsia 41(Suppl. 2): S13–S22.
6. Racine, R. J. 1972. Modification of seizure activity by electrical stimulation. I. Afterdischarge threshold. Electroencephalogr. Clin. Neurophysiol. 32:269–279.
7. Racine, R. J. 1972. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalogr. Clin. Neurophysiol. 32:281–294.
8. Cavazos, J. E. and Sutula, T. P. 1990. Progressive neuronal loss induced by kindling: A possible mechanism for mossy fiber synaptic reorganization and hippocampal sclerosis. Brain Res. 527:1–6.
9. Cavazos, J. E., Das, I., and Sutula, T. P. 1994. Neuronal loss induced in limbic pathways by kindling: Evidence for induction of hippocampal sclerosis by repeated brief seizures. J. Neurosci. 14:106–121.
10. Bengzon, J., Kokaia, Z., Elmer, E., Nanobashvili, A., Kokaia, M., and Lindvall, O. 1997. Apoptosis and proliferation of dentate gyrus neurons after single and intermitent limbic seizures. Proc. Natl. Acad. Sci. USA 94:10432–10437.
11. Tasch, E., Cendes, F., Li, L. M., Dubeau, F., Andermann, F., and Arnold, D. L. 1999. Neuroimaging evidence of progressive neuronal loss and dysfunction in temporal lobe epilepsy. Ann. Neurol. 45:568–576.
12. DeGiorgio, C. M., Tomiyasu, U., Gott, P. S., and Treiman, D. M. 1992. Hippocampal pyramidal cell loss in human status epilepticus. Epilepsia 33:23–27.
13. Wieshmann, U. C., Woermann, F. G., Lemieux, L., Free, S. L., Bartlett, P. A., Smith, S. J., Duncan, J. S., Stevens, J. M., and Shorvon, S. D. 1997. Development of hippocampal atrophy: A serial magnetic resonance imaging study in a patient who developed epilepsy after generalized status epilepticus. Epilepsia 38:1238–1241.
14. Willow, M., Gonoi, T., and Catterall, W. A. 1985. Voltage clamp analysis of the inhibitory actions of diphenylhydantoin and carbamazepine on voltage-sensitive sodium channels in neuroblastoma cells. Mol. Pharmacol. 27:549–558.
15. Bonifácio, M. J., Sheridan, R. D., Parada, A., Cunha, R. A., Patmore, L., and Soaresda-Silva, P. 2001. Interaction of the novel anticonvulsant, BIA 2-093, with voltage-gated sodium channels: Comparison with carbamazepine. Epilepsia 42:600–608.
16. Reynolds, E. H. 1995. Do anticonvulsants alter the natural time course of epilepsy? Treatment should be started as early as possible. Br. Med. J. 301:176–180.
17. Coutinho-Netto, J. C., Abdul-Ghani, A. S., Collins, J. F., and Bradford, H. F. 1981. Is glutamate a trigger factor in epileptic hyperactivity? Epilepsia 22:289–296.

18. Chapman, A. G. 1998. Glutamate receptors in epilepsy. Prog. Brain Res. 116:371–383.
19. Löscher, W. 1998. Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Prog. Neurobiol. 54:721–741.
20. Cain, D. P. 1989. Long-term potentiation and kindling: How similar are the mechanisms? Trends Neurosci. 12:6–10.
21. Lipton, P. 1999. Ischemic cell death in brain neurons. Physiol. Rev. 79:1431–1568.
22. Michaelis, E. K. 1998. Molecular biology of glutamate receptors in the central nervous system and their role in excitotoxicity, oxidative stress and aging. Prog. Neurobiol. 54:369–415.
23. Lees, G. J. 2000. Pharmacology of AMPA/kainate receptor ligands and their therapeutic potential in neurological and psychiatric disorders. Drugs 59:33–78.
24. MacDermott, A. B., Role, L. W., and Siegelbaum, S. A. 1999. Presynaptic ionotropic receptors and the control of transmitter release. Ann. Rev. Neurosci. 22:443–485.
25. Schoepp, D. D. 2001. Unveiling the functions of presynaptic metabotropic glutamate receptors in the central nervous system. J. Pharmacol. Exp. Ther. 299:12–20.
26. Ben-Ari, Y. 1985. Limbic seizure and brain damage produced by kainic acid: Mechanisms and relevance to human temporal lobe epilepsy. Neuroscience 14:375–403.
27. Coyle, J. T. 1983. Neurotoxic action of kainic acid. J. Neurochem. 41:1–11.
28. Represa, A., Tremblay, E., and Ben-Ari, Y. 1987. Kainate binding sites in the hippocampal mossy fibers: Localization and plasticity. Neuroscience 20:739–748.
29. Gaiarsa, J.-L., Zagrean, L., and Ben-Ari, Y. 1994. Neonatal irradiation prevents the formation of hippocampal mossy fibers and the epileptic action of kainate on rat CA3 pyramidal neurons. J. Neurophysiol. 71:204–215.
30. Ben-Ari, Y. and Cossart, R. 2000. Kainate: A double agent that generates seizures—Two decades of progress. Trends Neurosci. 23:580–587.
31. Chittajallu, R., Braithwaite, S. P., Clarke, V. R. J., and Henley, J. M. 1999. Kainate receptor: Subunits, synaptic localization and function. Trends Pharmacol. Sci. 20:26–35.
32. Lerma, J., Paternain, A. V., Rodríguez-Moreno, A., López-García, J. C. 2001. Molecular physiology of kainate receptors. Physiol. Rev. 81:971–998.
33. Rosenmund, C., Stern-Bach, Y., and Stevens, C. F. 1998. The tetrameric structure of a glutamate receptor channel. Science 280:1596–1599.
34. Malva, J. O., Carvalho, A. P., and Carvalho, C. M. 1998. Kainate receptors in hippocampal CA3 subregion: Evidences for a role in regulating neurotransmitter release. Neurochem. Int. 32:1–6.
35. Herb, A., Burnashev, N., Werner, P., Sakmann, B., Wisden, W., and Seeburg, P. H. 1992. The KA-2 subunit of excitatory amino acid receptors shows widespread expression in brain and forms ion channels with distantly related subunits. Neuron 8:775–785.
36. Patneau, D. K., Wright, P. W., Winters, C., Mayer, M. L., and Gallo, V. 1994. Glial cells of the oligodendrocyte lineage express both kainate-and AMPA-preferring subtypes of glutamate receptor. Neuron 12:357–371.
37. Huettner, J. E. 1990. Glutamate receptor channels in rat DRG neurons: Activation by kainate and quisqualate and blockade of desensitization by Con A. Neuron 5:255–266.
38. Bleakman, D. and Lodge, D. 1998. Neuropharmacology of AMPA and kainate receptors. Neuropharmacol. 37:1187–1204.
39. Bleakman, D. 1999. Kainate receptor pharmacology and physiology. Cell. Mol. Life Sci. 56:558–566.
40. Castillo, P. E., Malenka, R. C., and Nicoll, R. A. 1997. Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature 388:182–186.
41. Vignes, M. and Collingridge, G. L. 1997. The synaptic activation of kainate receptors. Nature 388:179–182.
42. Chittajallu, R., Vignes, M., Dev, K. K., Barnes, J. M., Collingridge, G. L., and Henley, J. M. 1996. Regulation of glutamate release by presynaptic kainate receptors in the hippocampus. Nature 379:78–81.
43. Cunha, R. A. and Ribeiro, J. A. 2001. ATP as a presynaptic modulator. Life Sci. 68:119–137.
44. Mulle, C., Sailer, A., Pérez-Otaño, I., Bureau, I., Maron, C., Gage, F. H., Mann, J. R., Bettler, B., and Heinemann, S. F. 1998. Altered synaptic physiology and reduced susceptibility to kainate-induced seizures in GluR6-deficient mice. Nature 392:601–605.
45. Hollmann, M. and Heinemann, S. 1994. Cloned glutamate receptors. Annu. Rev. Neurosci. 17:31–108.
46. Malva, J. O., Ambrósio, A. F., Cunha, R. A., Ribeiro, J. A., Carvalho, A. P., and Carvalho, C. M. 1995. A functionally active presynaptic high-affinity kainate receptor in the rat hippocampal CA3 subregion. Neurosci. Lett. 185:83–86.
47. Malva, J. O., Carvalho, A. P., and Carvalho, C. M. 1996. Domoic acid induces the release of glutamate in the rat CA3 sub-region. Neuroreport 7:1330–1334.
48. Cunha, R. A., Constantino, M. D., and Ribeiro, J. A. 1997 Inhibition of H3-gamma-aminobutyric acid release by kainate receptor activation in rat hippocampal synaptosomes. Eur. J. Pharmacol. 323:167–172.
49. Lerma, J., Partenain, A. V., Naranjo, J. R., and Mellström, B. 1993. Functional kainate-selective glutamate receptors in cultured hippocampal neurons. Proc. Natl. Acad. Sci. USA 90:11688–11692.
50. Clarke, V. R. J., Ballyk, B. A., Hoo, K. H., Mandelzys, A., Pellizzari, A., Bath, C. P., Thomas, J., Sharpe, E. F., Davies, C. H., Ornstein, P. L., Schoepp, D. D., Kamboj, R. K., Collingridge, G. L., Lodge, D., and Bleakman, D. 1997. A hippocampal GluR5 kainate receptor regulating inhibitory synaptic transmission. Nature 389:599–603.
51. Rodríguez-Moreno, A., Herreras, O., and Lerma, J. 1997. Kainate receptors presynaptically downregulate GABAergic inhibition in the rat hippocampus. Neuron 19:893–901.
52. Monaghan, D. T. and Cotman, C. W. 1982. The distribution of [^{3}H]kainic acid binding sites in rat CNS as determined by autoradiography. Brain Res. 252:91–100.
53. Petralia, R. S., Wang, Y. X., and Wenthold, R. J. 1994. Histological and ultrastructural localization of the kainate receptors, KA2 and GluR6/7, in the rat nervous system using selective antipeptide antibodies. J. Comp. Neurol. 349:85–110.
54. Bettler, B. and Mulle, C. 1995. Neurotransmitter receptors II: AMPA and kainate receptors. Neuropharmacol. 34:123–139.
55. Kamiya H., Ozawa, S., and Manabe, T. 2002. Kainate receptor-dependent short-term plasticity of presynaptic Ca^{2+} influx at the hippocampal mossy fiber synapses. J. Neurosci. 22:9237–9243.
56. Lauri, S. E., Bortolotto, Z. A., Bleakman, D., Ornstein, P. L., Lodge, D., Isaac, J. T. R., and Collingridge, G. L. 2001. A critical role of a facilitatory presynaptic kainate receptor in mossy fiber LTP. Neuron 32:697–709.
57. Lauri, S. E., Delany, C., Clarke, V. R. J., Bortolotto, Z. A., Ornstein, P. L., Isaac, J. T. R., and Collingridge, G. L. 2001. Synaptic activation of a presynaptic kainate receptor facilitates AMPA receptor-mediated synaptic transmission at hippocampal mossy fiber synapses. Neuropharmacol. 41:907–915.
58. Schmitz, D., Mellor, J., Frerking, M., and Nicoll, R. A. 2001. Presynaptic kainate receptors at hippocampal mossy fiber synapses. Proc. Natl. Acad. Sci. USA 98:11003–11008.
59. Schmitz, D., Mellor, J., and Nicoll, R. A. 2001. Presynaptic kainate receptor mediation of frequency facilitation at hippocampal mossy fiber synapses. Science 291:1972–1976.
60. Teitelbaum, J. S., Zatorre, R. J., Carpenter, S., Gendron, D., Evans, A. C., Gjedde, A., and Cashman, N. R. 1990. Neurologic sequelae of domoic acid intoxication due to the ingestion of contaminated mussels. N. Engl. J. Med. 322:1781–1787.
61. Contractor, A., Swanson, G. T., Sailer, A., O'Gorman, S., and Heinemann, S. F. 2000. Identification of the kainate receptor

subunits underlying modulation of excitatory synaptic transmission in the CA3 region of the hippocampus. J. Neurosci. 20:8269–8278.
62. Vignes, M., Clarke, V. R. J., Parry, M. J., Bleakman, D., Lodge, D., Ornstein, P. L., and Collingridge, G. L. 1998. The GluR5 subtype of kainate receptor regulates excitatory synaptic transmission in areas CA1 and CA3 of the rat hippocampus. Neuropharmacol. 37:1269–1277.
63. Bortolotto, Z. A., Clarke, V. R. J., Delany, C. M., Parry, M, C,, Smolders. I., Vignes, M., Ho, K. H., Miu, P., Brinton, B. T., Fantaske, R., Ogden, A., Gates, M., Ornstein, P. L., Lodge, D., Bleakman, D., and Collingridge, G. J. 1999. Kainate receptors are involved in synaptic plasticity. Nature 402:297–301.
64. Bahn, S., Volk, B., and Wisden, W. 1994. Kainate receptor gene expression in the developing rat brain. J. Neurosci. 14:5525–5547.
65. Phillips, G. R., Huang, J. K., Wang, Y., Tanaka, H., Shapiro, L., Zhang, W., Shan, W.-S., Arndt, K., Frank, M., Gordon, R. E., Gawinowicz, M. A., Zhao, Y., and Colman, D. R. 2001. The presynaptic particle web: Ultrastructure, composition, dissolution, and reconstitution. Neuron 32:1–20.
66. Pinheiro, P. S., Rodrigues, R. J., Silva, A. P., Cunha, R. A., Oliveira, C. R., and Malva, J. O. 2003. Solubilization and immunological identification of presynaptic alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors in the rat hippocampus. Neurosci. Lett. 336:97–100.
67. Kamiya, H. and Ozawa, S. 1998. Kainate receptor-mediated inhibition of presynaptic Ca^{2+} influx and EPSP in area CA1 of the rat hippocampus. J. Physiol. 509:833–845.
68. Kamiya H. and Ozawa, S. 2000. Kainate receptor-mediated presynaptic inhibition at the mouse hippocampal mossy fibre synapse. J. Physiol. 523:653–665.
69. Frerking, M., Schmitz, D., Zhou, Q., Johansen, J., and Nicoll, R. A. 2001. Kainate receptors depress excitatory synaptic transmission at CA3-CA1 synapses in the hippocampus via a direct presynaptic action. J. Neurosci. 21:2958–2966.
70. Burnashev, N., Villaroel, A., and Sackmann, B. 1996. Dimensions and ion selectivity of recombinant AMPA and kainate receptor channels and their dependence on Q/R site residues. J. Physiol. 496:165–173.
71. Rodrígues-Moreno, A. and Lerma, J. 1998. Kainate receptor modulation of GABA release involves a metabotropic function. Neuron 20:1211–1218.
72. Cunha, R. A., Malva, J. O., and Ribeiro, J. A. 1999. Kainate receptors coupled to Gi/Go proteins in the rat hippocampus. Mol. Pharmacol. 56:429–433.
73. Cunha, R. A., Malva, J. O. and Ribeiro, J. A. 2000. Presynaptic inhibition by kainate receptors of [^{3}H]GABA release is pertussis-toxin-sensitive in the rat hippocampus. FEBS Lett. 469:159–162.
74. Rodríguez-Moreno, A., López-Garcia, J. C., and Lerma, J. 2000. Two populations of kainate receptors with separate signaling mechanisms in hippocampal interneurons. Proc. Natl. Acad. Sci. USA 97:1293–1298.
75. Semyanov, A. and Kullmann, D. M. 2001. Kainate receptor-dependent axonal depolarization and action potential initiation in interneurons. Nat. Neurosci. 4:718–723.
76. Cossart, R., Esclapez, M., Hirsch, J. C., Bernard, C., and Ben-Ari, Y. 1998. GluR5 kainate receptor activation in interneurons increases tonic inhibition of pyramidal cells. Nat. Neurosci. 1:470–478.
77. Frerking, M., Malenka, R. C., and Nicoll, R. A. 1998. Synaptic activation of kainate receptors on hippocampal interneurons. Nat. Neurosci. 1:479–486.
78. Bureau, I., Bischoff, S., Heinemann, S. F., and Mulle, C. 1999. Kainate receptor-mediated response in the CA1 field of wild-type and GluR6-deficient mice. J. Neurosci. 19:653–663.
79. Mulle, C., Sailer, A., Swanson, G. T., Brana, C., O'Gorman, S., Bettler, B., and Heinemann, S. F. 2000. Subunit composition of kainate receptors in hippocampal interneurons. Neuron 28: 475–484.
80. Paternain, A. V., Herrera, M. T., Nieto, M. A., and Lerma, J. 2000. GluR5 and GluR6 kainate receptor subunits coexist in hippocampal neurons and coassemble to form functional receptors. J. Neurosci. 20:196–205.
81. Frerking, M., Petersen, C. C., and Nicoll, R. A. 1999. Mechanisms underlying kainate receptor-mediated disinhibition in the hippocampus. Proc. Natl. Acad. Sci. USA 96:12917–12922.
82. Cossart, R., Tyzio, R., Dinocourt, C., Esclapez, M., Hirsch, J. C., Ben-Ari, Y., and Bernard, C. 2001. Presynaptic kainate receptors that enhance the release of GABA on CA1 hippocampal interneurons. Neuron 29:497–508.
83. Jiang, L., Xu, J., Nedergaard, M., and Kang, J. 2001. A kainate receptor increases the efficacy of GABAergic synapses. Neuron 30:503–513.
84. Kullmann, D. M. and Semyanov, A. 2002. Glutamatergic modulation of GABAergic signaling among hippocampal interneurons: Novel mechanisms regulating hippocampal excitability. Epilepsia 43:174–178.
85. Cunha, R. A. 2001. Adenosine as a neuromodulator and as a homeostatic regulator in the nervous system: Different roles, different sources and different receptors. Neurochem. Int. 38:107–125.
86. Dunwiddie, T. V. and Masino, S. A. 2001. The role and regulation of adenosine in the central nervous system. Ann. Rev. Neurosci. 24:31–55.
87. Dunwiddie, T. V. 1985. The physiological role of adenosine in the central nervous system. Int. Rev. Neurobiol. 27:63–139.
88. Greene, R. W. and Haas H. L. 1991. The electrophysiology of adenosine in the mammalian central nervous system. Prog. Neurobiol. 36:329–341.
89. Phillis, J. W. and Wu P. H. 1981. The role of adenosine and its nucleotides in central synaptic transmission. Prog. Neurobiol. 16:187–239.
90. Dunwiddie, T. V. 1980. Endogenously released adenosine regulates excitability in the in vitro hippocampus. Epilepsia 21:541–548.
91. Mitchell, J. B., Lupica, C. R., and Dunwiddie, T. V. 1993. Activity-dependent release of endogenous adenosine modulates synaptic responses in the rat hippocampus. J. Neurosci. 13: 3439–3447.
92. Cunha, R. A. 2001. Regulation of the ecto-nucleotidase pathway in rat hippocampal nerve terminals. Neurochem. Res. 26: 979–991.
93. Zimmermann, H. and Braun, N. 1999. Ecto-nucleotidases: Molecular structures, catalytic properties, and functional roles in the nervous system. Prog. Brain Res. 120:371–385.
94. Latini, S. and Pedata, F. 2001. Adenosine in the central nervous system: Release mechanisms and extracellular concentrations. J. Neurochem. 79:463–484.
95. Lambert, N. A. and Tyler, T. J. 1991. Adenosine depresses excitatory but not fast inhibitory synaptic transmission in area CA1 of the rat hippocampus. Neurosci. Lett. 122:50–52.
96. Yoon, K. W. and Rothman, S. M. 1991. Adenosine inhibits excitatory but not inhibitory synaptic transmission in the hippocampus. J. Neurosci. 11:1375–1380.
97. Proctor, W. R. and Dunwiddie, T. V. 1987. Pre-and postsynaptic actions of adenosine in the in vitro hippocampus. Brain Res. 426:187–190.
98. Thompson, S. M., Hass, H. L., and Gahwiller, B. H. 1992. Comparison of the actions of adenosine at pre- and postsynaptic receptors in the rat hippocampus in vitro. J. Physiol. 451: 347–363.
99. Ambrósio, A. F., Malva, J. O., Carvalho, A. P., and Carvalho, A. M. 1997. Inhibition of N-, P/Q- and other types of Ca^{2+} channels in rat hippocampal nerve terminals by adenosine A_1 receptor. Eur. J. Pharmacol. 340:301–310.
100. Yawo, H. and Chuhma, N. 1993. Preferential inhibition of ω-conotoxin-sensitive presynaptic Ca^{2+} channels by adenosine autoreceptors. Nature 365:256–258.

101. Wu, L. G. and Saggau, P. 1994. Adenosine inhibits evoked synaptic transmission primarily by reducing presynaptic calcium influx in area CA1 of hippocampus. Neuron 12:1139–1148.
102. Scholz, K. P. and Miller, R. J. 1996. Presynaptic inhibition at excitatory hippocampal synapses: Development and role of presynaptic Ca^{2+} channels. J. Neurophysiol. 76:39–46.
103. Scholz, K. P. and Miller, R. J. 1992. Inhibition of quantal transmitter release in the absence of calcium influx by a G protein-linked adenosine receptor at hippocampal synapses. Neuron 8:1139–1150.
104. Capogna, M., Gähwiler, B. H., and Thompson, S. M. 1996. Presynaptic inhibition of calcium-dependent and calcium-dependent and calcium-independent release elicited with ionomycin, gadolinium, and α-latrotoxin in the hippocampus. J. Neurophysiol. 75:2017–2028.
105. Dittman, J. S. and Regehr, W. G. 1996. Contributions of calcium-dependent and calcium-independent mechanisms to presynaptic inhibition at a cerebellar synapse. J. Neurosci. 16:1623–1633.
106. Silinsky, E. M., Hirsh, J. K., Searl, T. J., Redman, R. S., and Watanabe, M. 1999. Quantal ATP release from motor nerve endings and its role in neurally mediated depression. Prog. Brain Res. 120:145–158.
107. Tetzlaff, W., Schubert, G. W., and Kreutzberg, G. W. 1987. Synaptic and extrasynaptic localization of adenosine binding sites in the rat hippocampus. Neuroscience 21:869–875.
108. de Mendonça, A., Sebastião, A. M., and Ribeiro, J. A., 1995. Inhibition of NMDA receptor-mediated currents in isolated rat hippocampal neurons by adenosine A_1 receptor activation. Neuroreport 6:1097–1100.
109. Klishin, A., Tsintsadze, T., Lozovaya, N., and Krishtal, O. 1995. Latent *N*-methyl-D-aspartate receptors in the recurrent excitatory pathway between hippocampal CA1 pyramidal neurons: Ca^{2+}-dependent activation by blocking A_1 adenosine receptors. Proc. Natl. Acad. Sci. USA 92:12431–12435.
110. Mogul, D. J., Adams, M. E., and Fox, A. P. 1993. Differential activation of adenosine receptors decreases N-type but potentiates P-type Ca^{2+} currents in hippocampal CA3 neurons. Neuron 10:327–334.
111. McCool, B. A. and Farroni, J. S. 2001. A_1 adenosine receptors inhibit multiple voltage-gated Ca^{2+} channel subtypes in acutely isolated rat basolateral amygdala neurons. Br. J. Pharmacol. 132:879–888.
112. de Mendonça, A. and Ribeiro, J. A. 2001. Adenosine and synaptic plasticity. Drug Dev. Res. 52:283–290.
113. de Mendonca, A., Sebastião, A. M., and Ribeiro, J. A. 2000. Adenosine: Does it have a neuroprotective role after all? Brain Res. Rev. 33:258–274.
114. Sebastião, A. M., de Mendonça, A., Moreira, T., and Ribeiro, J. A. 2001. Activation of synaptic NMDA receptors by action potential-dependent release of transmitter during hypoxia impairs recovery of synaptic transmission on reoxygenation. J. Neurosci. 21:8564–8571.
115. Gerber, U. and Gahwiler, B. H. 1994. $GABA_B$ and adenosine receptors mediate enhancement of the K^+ current, IAHP, by reducing adenylyl cyclase activity in rat CA3 hippocampal neurons. J. Neurophysiol. 72:2360–2367.
116. Luscher, C., Jan, L. Y., Stoffel, M., Malenka, R. C., and Nicoll, R. A. 1997. G protein-coupled inwardly rectifying K^+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19:687–695.
117. Wetherington, J. P. and Lambert, N. A. 2002. Differential desensitisation of responses mediated by presynaptic and postsynaptic A_1 adenosine receptors. J. Neurosci. 22:1248–1255.
118. Barraco, R. A., Swanson, T. H., Phillis, J. W., and Berman, R. F. 1984. Anticonvulsant effects of adenosine analogues on amygdaloid-kindled seizures in rats. Neurosci. Lett. 46: 317–322.
119. Dragunow, M. and Goddard, G. V. 1984. Adenosine modulation of amygdala kindling. Exp. Neurol. 84:654–665.
120. Dragunow, M., Goddard, G. V. and Laverty, R. 1985. Is adenosine an endogenous anticonvulsant? Epilepsia 26:480–487.
121. Dunwiddie, T. V. and Worth, T. 1982. Sedative and anticonvulsant effects of adenosine analogs in mouse and rat. J. Pharmacol. Exp. Ther. 220:70–76.
122. Eldridge, F. L., Paydarfar, D., Scott, S. C., and Dowell, R. T. 1989. Role of endogenous adenosine in recurrent generalized seizures. Exp. Neurol. 103:179–185.
123. Franklin, P. H., Zhang, G., Tripp, E. D., and Murray, T. F. 1989. Adenosine A_1 receptor activation mediates suppresion of (−)-bicuculline methiodide-induced seizures in rat prepiriform cortex. J. Pharmacol. Exp. Ther. 251:1229–1236.
124. Khan, G. M., Smolders, I., Ebinger, G., and Michotte, Y. 2001 2-Chloro-N^6-cyclopentyladenosine-elicited attenuation of evoked glutamate release is not sufficient to give complete protection against pilocarpine-induced seizures in rats. Neuropharmacology 40:657–667.
125. Maitre, M., Chesielski, L., Lehmann, A., Kempf, E., and Mandel, P. 1974. Protective effect of adenosine and nicotinamide against audiogenic seizures. Biochem. Pharmacol. 23: 2807–2816.
126. Petersen, E. N. 1991. Selective protection by adenosine agonists of DMCM-induced seizures. Eur. J. Pharmacol. 195:256–261.
127. Rosen, L. B. and Berman, R. F. 1987. Differential effects of adenosine analogs on amygdala, hippocampus and caudate nucleus kindled seizures. Epilepsia 28:658–666.
128. Simonato, M., Varani, K., Muzzolini, A., Bianchi, C., Beani, L. and Borea, P. A. 1994. Adenosine A_1 receptors in the rat brain in the kindling model of epilepsy. Eur. J. Pharmacol. 265: 121–124.
129. Turski, W. A., Cavalheiro, E. A., Ikonomidou, C., Moraes-Mello, L. E. A., Bortolotto, Z. A., and Turski, L. 1985. Effects of aminophylline and 2-chloroadenosine on seizures produced by pilocarpine in rats: Morphological and electroencephalographic correlates. Brain Res. 361:309–323.
130. von Lubitz, D. K., Paul, I. A., Carter, M., and Jacobson, K. A. 1993. Effects of N^6-cyclopentyl adenosine and 8-cyclopentyl-1,3-dipropylxanthine on *N*-methyl-D-asparte-induced seizures in mice. Eur. J. Pharmacol. 249:265–270.
131. Young, D. and Dragunow, M. 1994. Status epilepticus may be caused by loss of adenosine anticonvulsant mechanisms. Neuroscience 58:245–261.
132. Dunwiddie, T. V. 1999. Adenosine and suppression of seizures. Pages 1001–1010, in Delgado-Escueta, A. V., Wilson, W. A., Olsen, R. W., and Porter, R. J. (eds.), Jasper's Basic Mechanisms of the Epilepsies, 3rd ed., Advances in Neurology, Vol. 79, Lippincott Williams & Wilkins, Philadelphia.
133. Dragunow, M. 1988. Purinergic mechanisms in epilepsy. Prog. Neurobiol. 31:85–108.
134. Ault, B. and Wang, C. M. 1986. Adenosine inhibits epileptiform activity arising in hippocampal area CA3. Br. J. Pharmacol. 87:695–703.
135. Lee, K. S., Schubert, P., and Heinemann, U. 1984. The anticonvulsant action of adenosine: A postsynaptic dendritic action by a possible endogenous anticonvulsant. Brain Res. 321:160–164.
136. Tancredi, V., D'Antuono, M., Nehlig, A., and Avoli, M. 1998. Modulation of epileptiform activity by adenosine A1 receptor-mediated mechanisms in the juvenile rat hippocampus. J. Pharmacol. Exp. Ther. 286:1412–1419.
137. Dragunow, M. 1986. Adenosine: The brain's natural anticonvulsant. Trends Pharmacol. Sci. 7:128–130.
138. Chu, N. S. 1981. Caffeine- and aminophylline-induced seizures. Epilepsia 22:85–94.
139. Dragunow, M. and Robertson, H. A. 1987. 8-Cyclopentyl-1,3-dimethylxanthine prolongs epileptic seizures in rats. Brain Res. 417:377–379.
140. Francis, A. and Fochtmann, L. 1994. Caffeine augmentation of electroconvulsive seizures. Psycopharmacology 115:320–324.

141. Kostopoulos, G., Drapeau, C., Avoli, M., Olivier, A., and Villemeure, J. G. 1989. Endogenous adenosine can reduce epileptiform activity in the human epileptogenic cortex maintained in vitro. Neurosci. Lett. 106:119–124.
142. Mori, H., Mizutani, T., Yoshimura, M., Yamanouchi, H., and Shimada, H. 1992. Unilateral brain damage after prolonged hemiconvulsions in the elderly associated with theophylline administration. J. Neurol. Neurosurg. Psychiatry 55: 466–469.
143. Peters, S. G., Wochos, D. N., and Peterson, G. C. 1984. Status epilepticus as a complication of concurrent electroconvulsive and theophylline therapy. Mayo Clin. Proc. 59:568–570.
144. Whitcomb, K., Lupica, C. R., Rosen, J. B., and Berman, R. F. 1990. Adenosine involvement in postictal events in amygdala-kindled rats. Epilepsy Res. 6:171–179.
145. Alzheimer, C., Sutor, B., and ten Bruggencate, G. 1993. Disinhibition of hippocampal CA3 neurons induced by suppression of an adenosine A_1 receptor-mediated inhibitory tonus: Pre- and postsynaptic components. Neuroscience 57:565–575.
146. Ault, B., Olney, M. A., Joyner, J. L., Boyer, C. E., Notrica, M. A., Soroko, F. E., and Wang, C. M. 1987. Pro-convulsant actions of theophylline and caffeine in the hippocampus: Implications for the management of temporal lobe epilepsy. Brain Res. 426:93–102.
147. Chesi, A. J. R. and Stone, T. W. 1997. Alkylxanthine adenosine antagonists and epileptiform activity in rat hippocampal slices in vitro. Exp. Brain Res. 113:303–310.
148. Berman, R. F., Fredholm, B. B., Aden, U., and O'Connor, W. T. 2000. Evidence for increased dorsal hippocampal adenosine release and metabolism during pharmacologically induced seizures in rats. Brain Res. 872:44–53.
149. During, M. J., and Spencer, D. D. 1992. Adenosine: A potential mediator of seizure arrest and postictal refractoriness. Ann. Neurol. 32:618–624.
150. Lewin, E. and Bleck, V. 1981. Electroshock seizures in mice: Effect on brain adenosine and its metabolites. Epilepsia 22:577–581.
151. Park, T. S., van Wylen, D. G. L., Rubio, R., and Berne, R. M. 1987. Interstitial fluid adenosine and sagital sinus blood flow during bicuculline-seizures in newborn piglets. J. Cereb. Blood Flow Metabol. 7:633–639.
152. Schrader, J., Wahl, M., Kuschinsky, W., and Kreutzberg, G. N. 1980. Increase of adenosine content in cerebral cortex of the cat during bicuculline-induced seizure. Pflügers Arch. 387:245–251.
153. Winn, H. R., Welsh, J. E., Bryner, C., Rubio, R., and Berne, R. N. 1979. Brain adenosine production during the initial 60 seconds of bicuculline seizures in rats. Acta Physiol. Scand. 72:536–537.
154. Winn, H. R., Welsh, J. E., Rubio, R., and Berne, R. N. 1980. Changes in brain adenosine during bicuculline-induced seizures in rats: Effects of hypoxia and altered systemic blood pressure. Circ. Res. 47:868–877.
155. Kulkarni, C., David, J., and Joseph, T. 1994. Involvement of adenosine in postictal events in rats given electroshock. Indian J. Physiol. Pharmacol. 38:39–43.
156. Fredholm, B. B. 1997. Adenosine and neuroprotection. Int. Rev. Neurobiol. 40:259–280.
157. Brodie, M. S., Lee, K. S., Fredholm, B. B., Stahle L., and Dunwiddie, T. V. 1987. Central versus peripheral mediation of responses to adenosine receptor agonists: Evidence against a central mode of action. Brain Res. 415:323–330.
158. Katims, J. J., Annau, Z., and Snyder, S. H. 1983. Interactions in the behavioral effects of methylxanthines and adenosine derivatives. J. Pharmacol. Exp. Ther. 227:167–173
159. Malhotra, J. and Gupta, Y. K. 1997. Effect of adenosine receptor modulation on pentylenetetrazole-induced seizures in rats. Br. J. Pharmacol. 120:282–288.
160. Huber, A., Padrum, V., Déglon, N., Aebischer, P., Mölher, H., and Boison, D. 2001. Grafts of adenosine-releasing cells suppress seizures in kindling epilepsy. Proc. Natl. Acad. Sci. USA 98:7611–7616.
161. Ashton, D., de Prins, E., Willems, R., van Belle, H., and Wauquier, A. 1988. Anticonvulsant action of the nucleoside transport inhibitor, soluflazine, on synaptic and non-synaptic epileptogenesis in the guinea-pig hippocampus. Epilepsy Res. 2:65–71.
162. Zhang, G., Franklin, P. H., and Murray, T. F. 1993. Manipulation of endogenous adenosine in the rat prepiriform cortex modulates seizure susceptibility. J. Pharmacol. Exp. Ther. 264:1415–1424.
163. Wiesner, J. B., Ugarkar, B. G., Castellino, A. J., Barankiewicz, J., Dumas, D. P., Gruber, H. E., Foster, A. C., and Erion, M. D. 1999. Adenosine kinase inhibitors as a novel approach to anticonvulsant therapy. J. Pharmacol. Exp. Ther. 289:1669–1677.
164. Szot, P., Sanders, R. C., and Murray, T. F. 1987. Theophylline-induced upregulation of A_1-adenosine receptors associated with reduced sensitivity to anticonvulsants. Neuropharmacology 26:1173–1180.
165. Janusz, C. A. and Berman, R. F. 1993. The adenosine binding enhancer, PD 81,723, inhibits epileptiform bursting in the hippocampal brain slice. Brain Res. 619:131–136.
166. Ekonomou, A., Angelatou, F., Vergnes, M., and Kostopoulos, G. 1998. Lower density of A_1 adenosine receptors in nucleus reticularis thalami in rats with genetic absence epilepsy. Neuroreport 9:2135–2140.
167. Ekonomou, A., Sperk, G., Kostopoulos, G., and Angelatou, F. 2000. Reduction of A_1 adenosine receptors in rat hippocampus after kainic acid-induced limbic seizures. Neurosci. Lett. 284:49–52.
168. Glass, M., Faull, R. L. M., Bullock, J. Y., Jansen, K., Mee, E. W., Walker, E. B., Synek, B. J. L., and Dragunow, M. 1996. Loss of A_1 adenosine receptors in human temporal lobe epilepsy. Brain Res. 710:56–68.
169. Ochiisshi, T., Takita, M., Ikemoto, M., Nakata, H., and Suzuki, S. S. (1999) Immunohistochemical analysis on the role of adenosine A_1 receptors in epilepsy. Neuroreport 10:3535–3541.
170. Angelatou, F., Pagonopoulou, O., and Kostopoulos, G. 1990. Alterations of A_1 adenosine receptors in different mouse brain areas afer pentylentetrazol-induced seizures, but not in the epileptic mutant mouse 'tottering.' Brain Res. 534:251–256.
171. Daval, J. L. and Werck, M. C. 1991. Autoradiographic changes in brain adenosine A_1 receptors and their coupling to G proteins following seizures in the developing rat. Dev. Brain Res. 59:237–247.
172. Vanore, G., Giraldez, L., Rodriguez de Lores Arnaiz, G., and Girardi, E. 2001. Seizure activity produces differential changes in adenosine A_1 receptors within rat hippocampus. Neurochem. Res. 26:225–230.
173. Rebola, N., Coelho, J. E., Costenla, A. R., Lopes, L. V., Parada, A., Oliveira, C. R., Soares-da-Silva, P., de Mendonça, A., and Cunha, R. A. 2003. Decrease of adenosine A_1 receptor density and of adenosine neuromodulation in the hippocampus of kindled rats. Eur. J. Neurosci. (in press).
174. Wieraszko, A. and Seyfried, T. N. 1989. Increased amount of extracellular ATP in stimulated hippocampal slices of seizure prone mice. Neurosci. Lett. 106:287–293.
175. Vianna, E. P., Ferreira, A. T., Naffah-Mazzacoratti, M. G., Sanabria, E. R., Funke, M., Cavalheiro, E. A., and Fernandes, M. J. 2002. Evidence that ATP participates in the pathophysiology of pilocarpine-induced temporal lobe epilepsy: Fluorimetric, immunohistochemical, and Western blot studies. Epilepsia 43(Suppl. 5):227–229.
176. Bonan, C. D., Amaral, O. B., Rockenbach, I. C., Walz, R., Battastini, A. M., Izquierdo, I., and Sarkis, J. J. 2000. Altered ATP hydrolysis induced by pentylenetetrazol kindling in rat brain synaptosomes. Neurochem. Res. 25:775–779.
177. Bonan, C. D., Walz, R., Pereira, G. S., Worm, P. V., Battastini, A. M., Cavalheiro, E. A., Izquierdo, I., and Sarkis, J. J. 2000. Changes in synaptosomal ectonucleotidase activities in two rat models of temporal lobe epilepsy. Epilepsy Res. 39:229–238.

178. Nagy, A. K., Houser, C. R., and Delgado-Escueta, A. V. 1990. Synaptosomal ATPase activities in temporal cortex and hippocampal formation of humans with focal epilepsy. Brain Res. 529:192–201.
179. Schoen, S. W., Ebert, U., and Loscher, W. 1999. 5′-Nucleotidase activity in mossy fibers in the dentate gyrus of normal and epileptic rats. Neuroscience 93:519–526.
180. Pagonopoulou, O. and Angelatou, F. 1998. Time development and regional distribution of [^{3}H]nitrobenzylthioinosine adenosine uptake binding in the mouse brain after acute pentylenetetrazol-induced seizures. J. Neurosci. Res. 53:433–442.
181. Cunha, R. A., Correia-de-Sá, P., Sebastião, A. M., and Ribeiro, J. A. 1996. Preferential activation of excitatory adenosine receptors at rat hippocampal and neuromuscular synapses by adenosine formed from released adenine nucleotides. Br. J. Pharmacol. 119:253–260.
182. Fredholm, B. B., Cunha, R. A., and Svenningsson, P. 2002. Pharmacology of adenosine A_{2A} receptors and therapeutic applications. Curr. Top. Med. Chem. 3:1349–1364.
183. Adami, M., Bertorelli, R., Ferri, N., Foddi, M. C., and Ongini, E. 1995. Effects of repeated administration of selective adenosine A_1 and A_{2A} receptor agonists on pentylenetetrazole-induced convulsions in the rat. Eur. J. Pharmacol. 294:383–389.
184. De Sarro, G., De Sarro, A., Paola, E. D., and Bertorelli, R. 1999. Effects of adenosine receptor agonists and antagonists on audiogenic seizure-sensible DBA/2 mice. Eur. J. Pharmacol. 371: 137–145.
185. Huber, A., Güttinger, M., Möhler, H., and Boison, D. 2002. Seizure suppression by adenosine A_{2A} receptor activation in a rat model of audiogenic brainstem epilepsy. Neurosci. Lett. 329:289–292.
186. Klitgaard, H., Knutsen, L. J. S., and Thomsen, C. 1993. Contrasting effects of adenosine A_1 and A_2 receptor ligands in different chemoconvulsive rodent models. Eur. J. Pharmacol. 242:221–228.
187. Morgan, P. F. and Durcan, M. J. 1990. Caffeine-induced seizures: Apparent proconvulsant action of *N*-ethylcarboxamidoadenosine (NECA). Life Sci. 47:1–8.
188. Zgodzinski, W., Rubaj, A., Kleinrok, Z., and Sieklucka-Dziuba, M. 2001. Effect of adenosine A_1 and A_2 receptor stimulation on hypoxia-induced convulsions in adult mice. Pol. J. Pharmacol. 53:83–92.
189. Zhang, G., Franklin, P. H., and Murray, T. F. 1994. Activation of adenosine A_1 receptor underlies anticonvulsant effect of CGS21680. Eur. J. Pharmacol. 255:239–243.
190. El Yacoubi, M., Ledent, C., Parmentier, M., Daoust, M., Costentin, J., Vaugeois, J. M. 2001. Absence of the adenosine A_{2A} receptor or its chronic blockade decrease ethanol withdrawal-induced seizures in mice. Neuropharmacology 40:424–432.
191. Vaugeois, J. M., Benmaamar, R., Depaulis, A., Ledent, C., Parmentier, M., Costentin, J., and El Yacoubi, M. 2002. Adenosine A_{2A} receptor deficient mice are more resistant to seizures. Proc. 3rd Forum Eur. Neurosci.
192. Behan, W. M. H. and Stone, T. W. 2002. Enhanced neuronal damage by co-administration of quinolinic acid and free radicals, and protection by adenosine A_{2A} receptor antagonists. Br. J. Pharmacol. 135:1435–1442.
193. Chen, J. F., Huang, Z., Ma, J., Zhu, J., Moratalla, R., Standaert, D., Moskowitz, M. A., Fink, J. S., and Schwarzschild, M. A. 1999. A_{2A} adenosine receptor deficiency attenuates brain injury induced by transient focal ischemia in mice. J. Neurosci. 19:9192–9200.
194. Chen, J. F., Xu, K., Petzer, J. P., Staal, R., Xu, Y. H., Beilstein, M., Sonsalla, P. K., Castagnoli, K., Castagnoli, N. JR., and Schwarzschild, M. A. 2001. Neuroprotection by caffeine and A_{2A} adenosine receptor inactivation in a model of Parkinson's disease. J. Neurosci. 21:RC143.
195. Dall'Igna, O. P., Porciúncula, L. O., Souza, D. O., Cunha, R. A., and Lara, D. R. 2003. Neuroprotection by caffeine and adenosine A_{2A} receptor blockade of β-amyloid neurotoxicity. Br. J. Pharmacol. 138:1207–1209.
196. Ikeda, K., Kurokawa, M., Aoyama, S., and Kuwana, Y. 2002. Neuroprotection by adenosine A_{2A} receptor blockade in experimental models of Parkinson's disease. J. Neurochem. 80:262–270.
197. Monopoli, A., Lozza, G., Forlani, A., Mattavelli, A., and Ongini, E. 1998. Blockade of adenosine A_{2A} receptors by SCH 58261 results in neuroprotective effects in cerebral ischaemia in rats. Neuroreport 9:3955–3959.
198. Popoli, P., Pintor, A., Domenici, M. R., Frank, C., Tebano, M. T., Pezzola, A., Scarchilli, L., Quarta, D., Reggio, R., Malchiodi-Albedi, F., Falchi, M., and Massotti, M. 2002. Blockade of striatal adenosine A_{2A} receptor reduces, through a presynaptic mechanism, quinolinic acid-induced excitotoxicity: Possible relevance to neuroprotective interventions in neurodegenerative diseases of the striatum. J. Neurosci. 22:1967–1975.
199. Reggio, R., Pezzola, A., and Popoli, P. 1999. The intrastriatal injection of an adenosine A_2 receptor antagonist prevents frontal cortex EEG abnomalities in a rat model of Huntington's disease. Brain Res. 831:315–318.
200. Cunha, R. A., Johansson, B., van der Ploeg, I., Sebastião, A. M., Riberio, J. A., and Fredholm, B. B. 1994. Evidence for functionally important adenosine A_{2a} receptors in the rat hippocampus. Brain Res. 649:208–216.
201. Kobayashi, S. and Millhorn, D. E. 1999. Stimulation of expression for the adenosine A_{2A} receptor gene by hypoxia in PC12 cells; A potential role in cell protection. J. Biol. Chem. 274:20358–20365.
202. Rebola, N., Soares-da-Silva, P., Oliveira, C. R., and Cunha, R. A. 2002. Increased density of adenosine A_{2A} receptors in the cerebral cortex of kindled rats. Proc. 23th Meet. Portuguese Pharmacol. Soc. C64.
203. Diógenes, M. J., Sebastião, A. M., and Ribeiro, J. A. 2002. Brain derived neurotrophic factor facilitates synaptic transmission in rat hippocampus through A_{2A} adenosine receptor activation. Proc. 23th Meet. Portuguese Pharmacol. Soc. C66.
204. Lee, F. S. and Chao, M. V. 2001. Activation of Trk neurotrophin receptors in the absence of neurotrophins. Proc. Natl. Acad. Sci. USA 98:3555–3560.
205. Ernfors, P., Bengzon, J., Kokais, Z., Persson, H., and Lindvall, O. 1991. Increased levels of messenger RNAs for neurotrophic factors in the brain during kindling epileptogenesis. Neuron 7:165–176.
206. Kokaia, M., Ernfors, P., Kokaia, Z., Elmer, E., Jaenisch, R., and Lindvall, O. 1995. Suppressed epileptogenesis in BDNF mutant mice. Exp. Neurol. 133:215–224.
207. Binder, D. K., Routbort, M. J., Ryan, T. E., Yancopoulos, G. D., and McNamara, J. O. 1999. Selective inhibition of kindling development by intraventricular administration of TrkB receptor body. J. Neurol. Sci. 19:1424–1436.
208. Halldner, L., Lozza, G., Lindström, K., and Fredholm, B. B. 2000. Lack of tolerance to motor stimulant effects of a selective adenosine A_{2A} receptor antagonist. Eur. J. Pharmacol. 406:345–354.
209. Pinna, A., Fenu, S., and Morelli, M. 2001. Motor stimulant effects of the adenosine A_{2A} receptor antagonist SCH 58261 do not develop tolerance after repeated treatments in 6-hydroxydopamine-lesioned rats. Synapse 39:233–238.
210. Popoli, P., Reggio, R., and Pezzola, A. 2000. Effects of SCH 58261, an adenosine A_{2A} receptor antagonist, on quinpirole-induced turning in 6-hydroxydopamine-lesioned rats: Lack of tolerance after chronic caffeine intake. Neuropsychopharmacology 22:522–529.
211. Lopes, L. V., Cunha, R. A., and Ribeiro, J. A. 1999. Increase in the number, G protein coupling, and efficiency of facilitatory adenosine A_{2A} receptors in the limbic cortex, but not striatum, of aged rats. J. Neurochem. 73:1733–1738.
212. Rebola, N., Sebastião, A. M., de Mendonça, A., Oliveira, C. R., Ribeiro, J. A., and Cunha, R. A. 2003. Enhanced adenosine A_{2A} receptor facilitation of synaptic transmission in the hippocampus of aged rats. J. Neurophysiol. in press.
213. Kimmel, J. R., Hayden, L. J., and Pollock, H. G. 1975. Isolation and characterization of a new pancreatic polypeptide hormone. J. Biol. Chem. 250:9369–9374.

214. Larhammar, D., Blomqvist, A. G., and Söderberg, C. 1993. Evolution of neuropeptide Y and its related peptides. Comp. Biochem. Physiol. 106C:743–752.
215. Larhammar, D., Söderberg, C., and Blomqvist, A. G. 1993. Evolution of neuropeptide Y family of peptides. Pages 1–41, in Colmers, W. F. and Wahlestedt, C. (eds.), The Biology of Neuropeptide Y and Related Peptides, Humana Press, Totowa, New Jersey.
216. Allen, J. M. and Baldi, D. 1993. Structure and expression of the neuropeptide Y gene. Pages 43–64, in Colmers, W. F. and Wahlestedt, C. (eds.). The Biology of Neuropeptide Y and Related Peptides, Humana Press, Totowa, New Jersey.
217. Sundler, F., Böttcher, G., Ekblad, E., and Håkanson, R. 1993. PP, PYY and NPY: Occurrence and distribution in the periphery. Pages 157–196, in Colmers, W. F. and Wahlestedt, C. (eds.), The Biology of Neuropeptide Y and Related Peptides, Humana Press, Totowa, New Jersey.
218. Lundberg, J. M. 1996. Pharmacology and cotransmission in the autonomic nervous system: Integrative aspects on amines, neuropeptides, adenosine triphosphate, amino acids and nitric oxide. Pharmacol. Rev. 48:113–178.
219. Michel, M. C., Beck-Sickinger, A., Cox, H., Doods, H. N., Herzog, H., Larhammar, D., Quirion, R., Schwartz, T., and Westfall, T. 1998. XVI. International union of pharmacology recommendations for the nomenclature of neuropeptide Y, peptide YY, and pancreatic polypeptide receptors. Pharmacol. Revi. 50:143–150.
220. Larhammar, D. 1996. Structural diversity of receptors for neuropeptide Y, peptide YY and pancreatic polypeptide. Regul. Pept. 65:165–174.
221. Schwarzer, C., Sperk, G., Rizzi, M., Gariboldi, M., and Vezzani, A. 1996. Neuropeptides-immunoractivity and their mRNA expression in kindling: Functional implications for limbic epileptogenesis. Brain Res. Rev. 22:27–50.
222. Vezzani, A., Sperk, G., and Colmers, W. F. 1999. Neuropeptide Y: Emerging evidence for a functional role in seizure modulation. Trends Neurosci. 22:25–30.
223. Gruber, G., Greber, S., Rupp, E., and Sperk, G. 1994. Differential NPY mRNA expression in granule cells and interneurons of the rat dentate gyrus after kainic acid injection. Hippocampus 4:474–482.
224. Takahashi, Y., Tsunashima, K., Sadamatsu, M., Schwarzer, C., Amano, S., Ihara, N., Sasa, M., Kato, N., and Sperk, G. 2000. Altered hippocampal expression of neuropeptide Y, somastotatin, and glutamate decarboxylase in Ihara's epileptic rats and spontaneously epileptic rats. Neurosci. Lett. 287:105–108.
225. Klapstein, G. J. and Colmers, W. F. 1997. Neuropeptide Y suppresses epileptiform activity in rat hippocampus in vitro. J. Neurophysiol. 78:1651–1661.
226. Patrylo, P. R., Van Den Pol, A. N., Spencer, D. D., and Williamson, A. 1999. NPY inhibits glutamatergic excitation in the epileptic human dentate gyrus. J. Neurophysiol. 82: 478–483.
227. Van den Pol, A. N., Obrietan, K., Chen, G., and Belousov, A. B. 1996. Neuropeptide Y-mediated long-term depression of excitatory activity in suprachiasmatic nucleus neurons. J. Neurosci. 16:5883–5895.
228. Baraban, S. C., Hollopeter, G., Erickson, J. C., Schwartzkroin, P. A., and Palmiter, R. D. 1997. Knock-out mice reveal a critical antiepileptic role for neuropeptide Y. J. Neurosci. 17:8927–8936.
229. Vezzani, A., Michalkiewicz, M., Michalkiewicz, T., Moneta, D., Ravizza, T., Richichi, C. et al. 2002. Seizure susceptibility and epileptogenesis are decreased in transgenic rats overexpressing neuropeptide Y. Neuroscience 110:237–243.
230. Colmers, W. F., Klapstein, G. J., Fournier, A., St-Pierre, S., and Treherne, K. A. 1991. Presynaptic inhibition by neuropeptide Y in rat hippocampal slice in vitro is mediated by a Y_2 receptor. Br. J. Pharmacol. 102:41–44.
231. Woldbye, D. P. D., Larsen, P. J., Mikkelsen, J. D., Klemp, K., Madsen, T. M., and Bolwig, T. G. 1997. Powerful inhibition of kainic acid seizures by neuropeptide Y via Y5-like receptors. Nat. Med. 3:761–764.
232. Kopp, J., Nanobashvili, A., Kokaia, Z., Lindvall, O., and Hökfelt, T. 1999. Differential regulation of mRNAs for neuropeptide Y and its receptor subtypes in widespread areas of the rat limbic system during kindling epileptogenesis. Mol. Brain Res. 72:17–29.
233. Röder, C., Schwarzer, C., Vezzani, A., Gobbi, M., Minnini, T., and Sperk, G. 1996. Autoradiographic analysis of neuropeptide Y receptor binding sites in the rat hippocampus after kainic acid-induced limbic seizures. Neuroscience 70:47–55.
234. Bleakman, D., Harrison, N. L., Colmers, W. F., and Miller, R. J. 1992. Investigations into neuropeptide Y-mediated presynaptic inhibition in cultured hippocampal neurones of the rat. Br. J. Pharmacol. 107:334–340.
235. McQuiston, A. R. and Colmers, W. F. 1996. Neuropeptide Y_2 receptors inhibit the frequency of spontaneous but not miniature EPSCs in CA3 pyramidal cells of rat hippocampus. J. Neurophysiol. 76:3159–3168.
236. Vezzani, A., Rizzi, M., Conti, M., and Samanin, R. 2000. Modulatory role of neuropeptide in seizures induced in rats by stimulation of glutamate receptors. Am. Soc. Nutri. Sci. 7402:1046S–1048S.
237. McQuiston, A. R., Petrozzino, J. J., Connor, J. A., and Colmers, W. F. 1996. Neuropeptide Y_1 receptors inhibit N-type calcium currents and reduce transient calcium increases in rat dentate granule cells. J. Neurosci. 16:1422–1429.
238. Gariboldi, M., Conti, M., Cavaleri, D., Samanin, R., and Vezzani, A. 1998. Anticonvulsant properties of BIBP3226, a non-peptide selective antagonist at neuropeptide Y Y_1 receptors. Eur. J. Neurosci. 10:757–759.
239. Brooks, P. A., Kelly, J. S., Allen, J. M., Smith, D. A. S., and Stone, T. W. 1987. Direct excitatory effects of neuropeptide Y (NPY) in rat hippocampal neurons in vitro. Brain Res. 408:295–298.
240. Weiser, T., Wieland, H. A., and Doods, H. N. 2000. Effects of the neuropeptide Y Y_2 receptor antagonist BIIE0246 on presynaptic inhibition by neuropeptide Y in rat hippocampal slices. Eur. J. Pharmacol. 404:133–136.
241. Silva, A. P., Carvalho, A. P., Carvalho, C. M., and Malva, J. O. 2001. Modulation of intracellular calcium changes and glutamate release by neuropeptide Y1 and Y2 receptors in the rat hippocampus: Differential effects in CA1, CA3 and dentate gyrus. J. Neurochem. 79:286–296.
242. Greber, S., Schwarzer, C., and Sperk, G. 1994. Neuropeptide Y inhibits potassium-stimulated glutamate release through Y_2 receptors in rat hippocampal slices in vitro. Br. J. Pharmacol. 113:737–740.
243. Silva, A. P., Carvalho, A. P., Carvalho, C. M., and Malva, J. O. 2003. Functional interaction between neuropeptide Y receptors and modulation of calcium channels in the rat hippocampus. Neuropharmacology 44:282–292.
244. Marsh, D. J., Baraban, S. C., Hollopeter, G., and Palmiter, R. D. 1999. Role of the Y5 neuropeptide Y receptor in limbic seizures. Proc. Natl. Acad. Sci. USA 96:13518–13523.
245. Guo, H., Castro, P. A., Palmiter, R. D., and Baraban, S. C. 2002. Y5 receptors mediate neuropeptide Y actions at excitatory synapses in area CA3 of the mouse hippocampus. J. Neurophysiol. 87:558–566.
246. Dinger, M. C., Bader, J. E., Kobor, A. D., Kretzschmar, A. K., and Beck-Sickinger, A. G. 2003. Homodimerization of neuropeptide Y receptors investigated by fluorescence resonance energy transfer in living cells. J. Biol. Chem. 278:10562–10571.
247. Silva, A. P., Pinheiro, P. S., Carvalho, A. P., Carvalho, C. M., Jakobsen, B., Zimmer, J. and Malva, J. O. 2003. Activation of neuropeptide Y receptors is neuroprotective against excitoxicity in organotypic hippocampal slice cultures. FASEB J. 17:1118–1120.