

# Pathophysiological Aspects of Temporal Lobe Epilepsy and the Role of P2X Receptors

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**Abstract:** In the central nervous system (CNS), ATP is released from vesicles at nerve terminals in a frequency-dependent manner and can activate P2 receptors widely expressed and distributed in the CNS. In addition to interacting with P2 receptors, ATP can be rapidly hydrolyzed to adenosine to activate P1 receptors modulating neuronal transmission. Thus, complex synaptic interactions in the CNS are modulated by P2 and P1 receptors. This review focuses on the role of P2X receptors in temporal lobe epilepsy. P2X receptors are cationic-selective channels gated by extracellular ATP. Seven subunits (P2X1-7) are expressed throughout the central nervous system and are involved with modulatory mechanisms of neurotransmitter release, hyperexcitability, intracellular calcium influx, cell-cell communication, neuroprotection, and cell death. This review discusses the current data regarding the involvement of P2 receptors in the pathophysiology of temporal lobe epilepsy (TLE).

**Keywords:** Temporal lobe epilepsy, purinergic receptors, P2X receptor, review.

## TEMPORAL LOBE EPILEPSY (TLE): DEFINITION, EPIDEMIOLOGY AND ETIOLOGY

Epilepsy is a common chronic brain disorder characterized by recurrent seizures due to excessive activation of cerebral neurons. TLE is the most frequent type of human epilepsy [1, 2]. In about 40% of patients, the TLE is refractory to medical therapy [3]. TLE syndrome is characterized by partial seizures that may or may not be secondarily generalized. Common symptoms include abdominal sensations and fear in patients with mesial temporal sclerosis or psychic symptoms (eg, *déjà vu*), abdominal sensation, tinnitus and vertigo in patients with lateral TLE [4].

Neuropathological studies indicate that TLE is frequently associated with hippocampal sclerosis [5]. Hippocampal sclerosis is detected routinely by image studies during pre-surgical evaluation of patients with intractable TLE. In a recent review, de Lenarolle and Lee [3] cited that about 70% of hippocampi removed surgically from patients with TLE show hippocampal sclerosis, and 30% do not show sclerosis, known as paradoxical temporal lobe epilepsy.

The etiology and the pathogenesis of this type of medial temporal lobe damage are not known. Several studies have shown a correlation between severe childhood illness (infection, febrile convulsions, *status epilepticus*, *SE*) and hippocampal atrophy in TLE [6, 7]. However, not all TLE patients exhibiting hippocampal damage have a history of initial insult. Some experimental and human data suggest that recurrent seizures may cause progressive damage to the hippo-

campus [5, 8]. Until now, it is unknown whether the damage found in the hippocampus is the cause or the consequence of TLE. However, surgical removal of the sclerotic hippocampus results in the best seizure-free outcome [3].

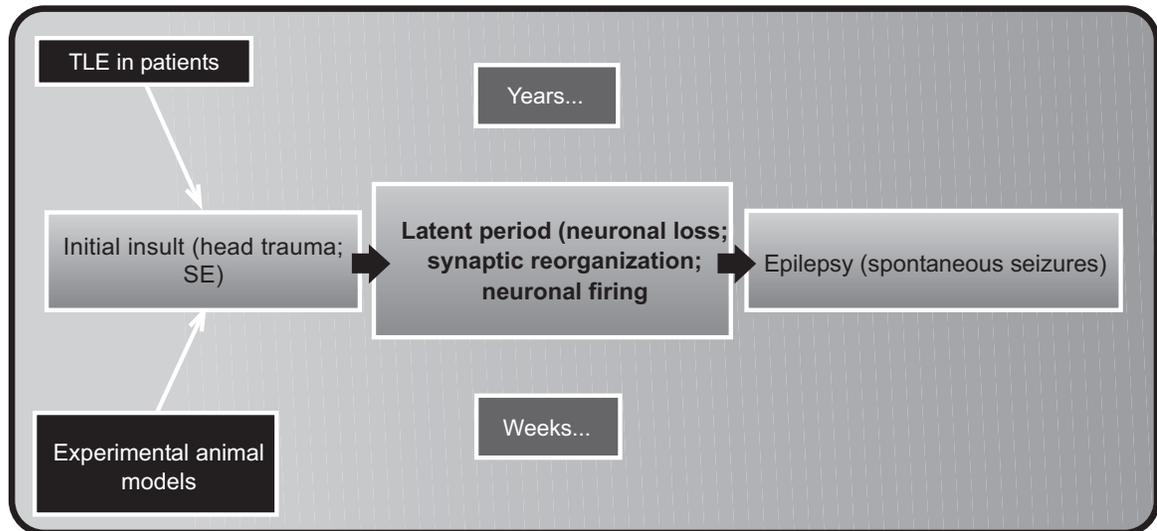
## EPILEPTOGENESIS

The development of an epileptic disorder involves a cascade of events activated by an initial insult in the brain. It is well known from studies using animal models [9, 10] as well as from studies from human patients [11] that there is a latent period between induction of a localized cerebral insult such as head trauma or *SE* and the appearance of a chronic epileptic condition. During the latent period, neuronal loss and abnormal synaptic reorganization occurs [12, 13]. This reorganization of the neuronal integration can take years in patients and weeks in animal models and ultimately leads to abnormally increased excitability and synchronization, which eventually cause spontaneous seizures (Fig. 1).

Thus, epilepsy can be considered as an active process which results in both ictal phenomena and permanent interictal functional and structural changes in the brain [14]. Patients who develop TLE demonstrate progression in both the number of seizures and in neurological symptoms related to seizure such as cognitive and behavioral disorders [15, 16]. The long latency before TLE develops suggests that therapeutic intervention is a good alternative to prevent the appearance of seizures.

The Fig. (1) shows a schematic view of the development of an epileptic disorder in patients and in animal models. Following an insult, the brain reorganizes networks that predispose the brain to the development of spontaneous seizures.

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**Fig. (1).** Schematic diagram of epileptogenesis in patients and in experimental model. An initial insult can cause brain damage, and the brain reorganizes during the latent period, which lasts weeks in animal models or years in patients, in a manner to predispose to the development of spontaneous seizures.

## NEUROPATHOLOGIC FINDINGS IN TLE

The hippocampus or Ammon's horn is one of the most vulnerable areas of the temporal lobe to develop cell loss after seizures. The histological pattern of hippocampal sclerosis in TLE patients is characterized by the loss of pyramidal cells in the prosubiculum and CA1 field of the hippocampus [5]. The findings also include neuronal loss in the hilus of the dentate gyrus and the adjacent CA3 field of the hippocampus [17, 18]. In many cases, hippocampal damage in TLE is accompanied by mossy fibers reorganization. Mossy fibers from the dentate granule cells which normally innervate the hilar mossy cells and CA3 pyramidal cells and interneurons become reorganized and project into the inner third of the molecular layer of the dentate gyrus [19-21]. The term "mesial temporal sclerosis" has been introduced to describe cellular damage in the hippocampus, amygdala, and entorhinal cortex [3].

## EXPERIMENTAL ANIMAL MODELS

Experimental animal models provide a useful approach to assess the mechanisms involved with epileptogenesis. The damage precedes the appearance of seizures in several animal models of human partial epilepsies. *SE* induced by systemic injection of pilocarpine or kainic acid causes structural brain damage in rats. Cell loss is observed in the hilus and CA3 region of the hippocampus as well as in the amygdala, entorhinal cortex, thalamus and cerebral cortex [9]. Moreover, prominent mossy fiber sprouting occurs [12]. According to Olney *et al.* [22], kainic acid and other analogues of glutamate are toxic because they activate glutamate receptors on neuronal membranes, resulting in prolonged depolarization, neuronal swelling and death. By activating M1 muscarinic receptors, pilocarpine activates phospholipase C which in turn produces diacylglycerol (DG) and inositol triphosphate (IP3), resulting in alterations in calcium and potassium, leading to enhanced excitability [23-25].

Increased excitability in the hippocampus results from decreased activity of ATPases that are unable to repolarize

the membrane [26, 27]. High intracellular calcium can promote glutamate release which by activating glutamate receptors allows the entrance of additional calcium to induce *SE*, excitotoxicity and cell death [24, 26]. In these experimental models, recurrent spontaneous seizures occur after a latent period, which is reminiscent of human TLE [12].

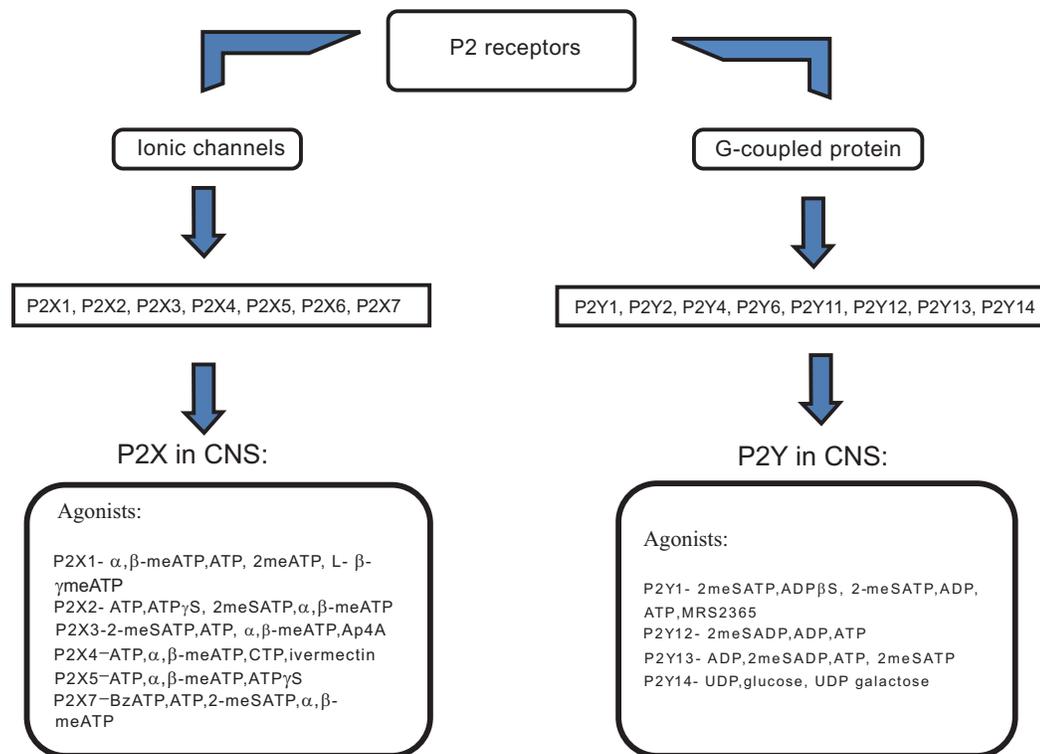
Kindling is an animal model of TLE, where seizures are induced by repetitively applying subthreshold stimulation to the undamaged brain [28]. Repeated seizures induce progressive cellular alterations not only in the hippocampus, but also in the amygdala, and the entorhinal cortex [29]. Furthermore, other studies have demonstrated that the neuronal loss is accompanied by aberrant mossy fiber axonal growth of dentate granule cells in the hippocampus [30].

## MOLECULAR CHANGES IN EPILEPTOGENESIS

Significant cell death and reorganization occurs in CA1, and studies have pointed to intense synaptic reorganization by calbindin and parvalbumin-positive neurons, which are presumably GABAergic neurons, that can result in the inhibition of inhibitory neurons leading to abnormal synchrony and seizure activity [31]. This is an evidence that hyperexcitability is not due to the loss of  $\gamma$ -aminobutyric acid (GABA) but involves other mechanisms that are related to increased excitatory neurotransmission.

There is evidence that mossy cell in the hilus and pyramidal neurons in CA3 show increased expression of GluR1 that in turn promote the excitation of granule cells [32].

To date, much attention has been given to the increased gliosis in this region, mainly of astrocytes, which could also contribute to hyperexcitability. There is evidence that astrocytes contribute with high levels of glutamate in hippocampal areas where neurons are sparse [3]. Some changes in astrocytes, for example, high sodium channels expression, reduced potassium inward rectifying channel and elevated expression of GluR1 and downregulation of glutamine synthetase, an enzyme responsible for the conversion of gluta-



**Fig. (2).** Schematic diagram of P2 receptors subtypes for purines and pyrimidines. P2X family of ligand-gated ion channel receptors and P2Y family of G-protein-coupled receptors as well as agonists rank order of potency for principal P2 receptors subtypes expressed in central nervous system (CNS) are shown. Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; Ap4A, diadenosine tetraphosphate; BzATP, 2'-3'-O-(4-benzoyl-benzoyl)-ATP;  $\alpha, \beta$ meATP,  $\alpha, \beta$ -methylene ATP; 2-meSATP, 2-methylthio ADP; 2-meSATP, 2-methiothio ATP; CTP, cytosine triphosphate; ATP $\gamma$ S, adenosine 5'-[ $\gamma$ -thio]triphosphate; MRS 2365, [(1R,2R,3S,4R,5S)-4-[6-amino-2-(methylthio)-9H-purin-9-yl] 2,3-dihydroxybicyclo[3.1.0]hex-1-yl]methyl] diphosphoric acid mono ester trisodium salt; UDP, uridine 5'-diphosphate. Data from Burnstock [46] and Illes & Ribeiro [48].

mate to glutamine, represent potential mechanisms by which astrocytes can release glutamate [33-36].

Astrocytes can modulate inflammatory reactions through the expression of the transcription factor nuclear factor kB (NF-kB) and activation of prostaglandin E2 (PGE2) in response to interleukin-1 $\beta$  (IL-1 $\beta$ ) [37]. PGE2 increases calcium levels within astrocytes and contributes to glutamate release [38].

Besides IL-1 $\beta$ , astrocytes can also produce other immunological agents such as interleukins (IL-1, IL10 and IL-6); interferons (IFN)- $\alpha$  and  $\beta$ , tumor necrosis factor (TNF)- $\alpha$  and transforming growth factor (TGF)- $\beta$ , among others [37, 39]. It has been shown that IL-1 $\beta$  is up regulated in sclerotic hippocampus from patients with TLE and can worsen seizures through glutamate release [39]. Genes regulated by IL-1 $\beta$  are up regulated in sclerotic hippocampi from patients [39].

Growing evidence indicates that purines are widely involved in the molecular mechanisms underlying the various functions of astrocytes, either by modulating intracellular molecules involved in energy metabolism, nucleic acid synthesis, or by activating a variety of membrane receptors [40, 41]. Purines, by activating P2 receptors can also modulate calcium influx and there is substantial evidence that cellular cascades initiated by calcium influx and perturbed intracellular calcium homeostasis are involved in the excitotoxic cell

death produced by SE. In this paper we focus on recent data about the role of P2X receptors in CNS and in epilepsy.

## PURINES AS NEUROTRANSMITTER IN THE CENTRAL NERVOUS SYSTEM (CNS)

The concept that adenosine 5'-triphosphate (ATP) could be a neurotransmitter was proposed by Burnstock in 1972 [42]. This concept had considerable resistance for many years because ATP was considered only as an intracellular molecule. However, ATP is now recognized as a neurotransmitter in all nerve types in both the peripheral and central nervous systems [43].

In 1978 two subtypes of purinergic receptors were identified: the P1 receptor for adenosine and P2 for ATP and ADP [44]. Based on pharmacological characterization and molecular cloning, P2 receptors were divided into P2X and P2Y, based on whether they are ligand-gated ion-channels (P2X) or coupled to G proteins (P2Y) [45]. To date, seven mammalian P2X receptors, P2X1-7 and eight P2Y receptors have been cloned, including receptors activated by pyrimidines, purines and sugar nucleotides such as UDP-glucose and UDP-galactose [46]. P2 receptor function is involved in most physiological processes and participates in neurotransmission in the CNS during the development and in the adult brain [47]. The Fig. (2) shows a schematic view of P2 receptor subtypes expressed in CNS and its agonists rank order potency.

**Table 1. P2X Receptors in CNS**

Subtype	CNS Localization	Marker	Author
P2X2	CX, HPC, Hab, SNPc, HPT, SON, medulla, trigeminal nu	mRNA, protein	Xiang <i>et al.</i> , 1998 [50]; Atkinson <i>et al.</i> , 2000 [51]
P2X3	DRG, NTS, STN	mRNA, protein	Chen <i>et al.</i> , 2002 [52] Vulchanova <i>et al.</i> , 1996 [53]
P2X4	Cer, spinal cord, OB, Cx, HPC, HPT, Thal	mRNA, protein	Bo <i>et al.</i> , 1995 [54]; Buel <i>et al.</i> , 1996 [55]; Collo <i>et al.</i> , 1996 [56].
P2X6	Cer, OB, Cx, HPC, HPT, Thal, Str, SN, AMG, Ventricular area (ependymal layer)	mRNA	Collo <i>et al.</i> , 1996 [56]
P2X7	HPC, spinal cord	mRNA, protein	Rubio and Soto, 2001 [57]

Abbreviations: CNS, central nervous system; CX, cerebral cortex; HPC, hippocampus; Hab, habenula; SNPc, substantia nigra pars compacta; HPT, hypothalamus; Thal, thalamus; SON, supraoptic nucleus; DRG, dorsal root ganglia; NTS, nucleus tractus solitarius; STN, spinal trigeminal nucleus; Cer, cerebellum; Str, striatum; Acb, nucleus accumbens; GP, globus pallidus; SN, substantia nigra; OB, olfactory bulb.

Each P2Y receptor binds to a single heterotrimeric G protein (Gq/11 for P2Y1, P2Y2, P2Y4 and P2Y6 receptors, Gi for P2Y12 and P2Y13 and Gi/0 for P2Y14) although P2Y11 can couple to both Gs and Gq/11 proteins [43, 46]. Both P2X and P2Y receptors modulate the level of intracellular calcium ions [48, 49]. P2X is permeable to calcium whereas P2Y promotes release of calcium from intracellular stores by activating phospholipase C (PLC) and inositol triphosphate (IP3) (and/or modulation of adenylyl cyclase) [43].

The P2 receptor subtypes have a wide but heterogenous distribution in the CNS and are expressed in neurons and glial cells [43, 50-57].

P2X1 receptors are found in some regions such as cerebellum while P2X2 receptors are abundant in several brain areas such as cerebral cortex, hippocampus, habenula, substantia nigra pars compacta, ventromedial and arcuate hypothalamic nuclei, mesencephalic trigeminal nucleus, ventrolateral medulla [43, 50, 51]. P2X3 receptors are highly expressed in brain areas involved in pain transmission (nucleus tractus solitarii, spinal trigeminal nucleus).

P2X4 receptors show strong expression in the cerebellum, spinal cord, cerebral cortex, hippocampus, thalamus and brainstem. Both P2X2 and P2X4 receptors localize to postsynaptic specializations of parallel fiber synapses in the cerebellum and at Schaffer collateral synapses in the hippocampus [57]. Immunohistochemical studies have revealed that P2X1-6 receptor subtypes are expressed predominantly in neurons, whereas the P2X7 receptor is expressed in glial cells (microglia and astrocytes), lymphocytes, macrophages as well as in neurons, indicating its participation in inflammatory or immune insult processes and cell-to-cell communication [58, 59]. Evidence for participation of P2X receptors in different developmental processes such as neurite outgrowth (P2X3), postnatal neurogenesis (P2X4 and P2X5 receptors), and cell death (maybe involving P2X7 receptor) have been described [47]. A pattern of expression of P2X receptors in CNS determined by *in situ* hybridization and immunohistochemistry is shown in Table 1.

In neurons, P2X receptors mediate fast synaptic responses to ATP, and P2Y receptors mediate slow responses. Postsynaptic currents mediated by release of endogenous ATP have been shown in CA1 and CA3 hippocampal subfields [60]. There is evidence that P2X4 and P2X6 subunits can increase glutamate-mediated synaptic transmission (NMDA receptor) and intensify long-term potentiation [61].

The P2X7 receptor is an atypical member of the P2X receptor family that has been extensively studied in CNS. This receptor exhibit very low affinity for ATP (EC50=300-400  $\mu$ M) and has a long (240 amino acid) intracellular C-terminal region that permits to form a large non-selective cytolitic pore that is permeable to Na<sup>+</sup>, K<sup>+</sup> and Ca<sup>++</sup> upon prolonged or repeated agonist stimulation [62].

Functional, pharmacological and immunohistochemical studies have shown that P2X7 receptors are present also in presynaptic excitatory terminals in the hippocampus innervating both excitatory and inhibitory cells and their activation can evoke glutamate and GABA release [59, 63]. On the other hand, Sim *et al.*, [64] did not detect the presence of P2X7 receptors in hippocampal neurons of the adult rodent. According to the authors, the P2X7 receptor can be detected in neurons only after insults such as ischemic damage or other inflammatory diseases of the brain. We have demonstrated the expression of P2X7 in the hippocampus of patients with TLE and in epileptic rats (see discussions later).

Some authors have demonstrated that P2X receptors can modify the balance between inhibition and excitation by regulating the functioning of GABA-gated channels [65]. Co-activation of P2X2 and GABA-A receptors expressed in *Xenopus* oocytes leads to a functional cross-inhibition dependent on GABA-A subunit composition. GABA-A receptors containing  $\gamma$  subunit, for example, are not inhibited by P2X2. The relationship between the P2X receptor and the GABA or GABA-A receptor was also reported in the rat spinal cord [66] and dorsal root ganglia [67], indicating that, at least in these regions, P2X receptor may participate in neuronal transmission accompanied by GABA-mediated action.

Cross-talk between P2X receptor and GABA has been cited as one of the mechanisms involved in epileptogenesis [68]. P2X2 and P2X4 receptor expression was significantly reduced in the hippocampus of seizure-sensitive (SS) gerbils compared with seizure-resistant (SR) gerbils [68]. The downregulation of P2X receptors was closely related to the GABA concentration, which is lower in SS than in SR. In addition, P2X receptors expression was mediated by GABA-A receptor, but not GABA-B. However, further studies have reported that the expressions of P2X3 and P2X7 receptors are modulated by GABA-B receptor activation [69, 70]. Treatment with GABA-B receptor agonist baclofen and antagonist phaclofen resulted in increased and decreased P2X7 receptor expression in the hippocampus, respectively [70]. The P2X7 receptor may have different actions depending on ATP levels. When stimulated by high extracellular ATP concentration (micromolar), P2X7 receptor evokes long-lasting form of synaptic depression [71]. However, P2X7 can also be stimulated by low ATP concentration (nanomolar level) but only under presynaptic GABA-B receptors activation [70, 71]. In these conditions the P2X7 receptor does not cause synaptic depression [71].

The stimulation of P2X7 receptors on astrocytes mediates glutamate and GABA release providing a link between ATP and excitatory and inhibitory synaptic transmission [72]. ATP, glutamate and GABA may synergistically modulate synaptic transmission in neuronal systems as well as regulate the  $Ca^{++}$  wave propagation in astrocytes networks.

Considerable evidence indicates that large amounts of ATP released from dying cells after insults might induce reactive astrogliosis, microglia proliferation and acts as a powerful chemoattractant at the site of the injury [73]. Both astrocytes and activated microglia are able to induce the release of cytokines such as IL-1 $\beta$ , TNF- $\alpha$ , IL-6, among others, which influence neuroinflammatory processes during neurodegeneration [74]. Activation of microglial P2X7 receptors by ATP induces TNF- $\alpha$  release and this effect is regulated by extracellular signal-regulated kinase (ERK) and p38 mitogen-activated protein (MAP) kinase [75]. In addition, data of our group have shown an increase in the expression and activation of MAPK in the hippocampus of rats during the early stages of pilocarpine-induced SE showing a relationship between both signalling pathways [76].

To date it is not clear whether microglia protects or damage neurons or whether TNF is beneficial or toxic. TNF may enhance injury induced by ischemia and trauma [77, 78] as well as provide neuroprotection due to its ability to induce the expression of anti-apoptotic and anti-oxidative proteins. The dual effect of TNF is mediated by different TNF receptors, with the p55 TNF receptor 1 (TNFR1) mediating neurotoxic effect, and p75 TNF receptor 2 (TNFR2) eliciting neuroprotection [79]. Intriguingly, co-culturing with microglia protects neurons against glutamate-induced excitotoxicity after stimulation with ATP or its analogue BzATP [75]. According to the authors, the effect was due to a neuroprotective factor released from P2X7-activated microglia, the TNF $\alpha$  (see detailed discussions later).

Recent study demonstrated that P2X7 receptors in the rat hippocampus are not able to process and release IL-1 $\beta$  upon P2X7 receptor activation. However, quantitative analysis of caspase-1 revealed that the lack of IL-1 $\beta$  release in hippo-

campal astrocytes is due a lack of processing of pro-caspase-1 and that signaling through P2X7 receptors can modulate the immune response of glial cells. Streit *et al.*, [80] have cited neuroinflammation as in injury-induced glial activation that contributes to neuropathologies. It has been suggested that prolonged inflammatory responses in CNS may significantly contribute to the pathology seen in epilepsy [81, 82].

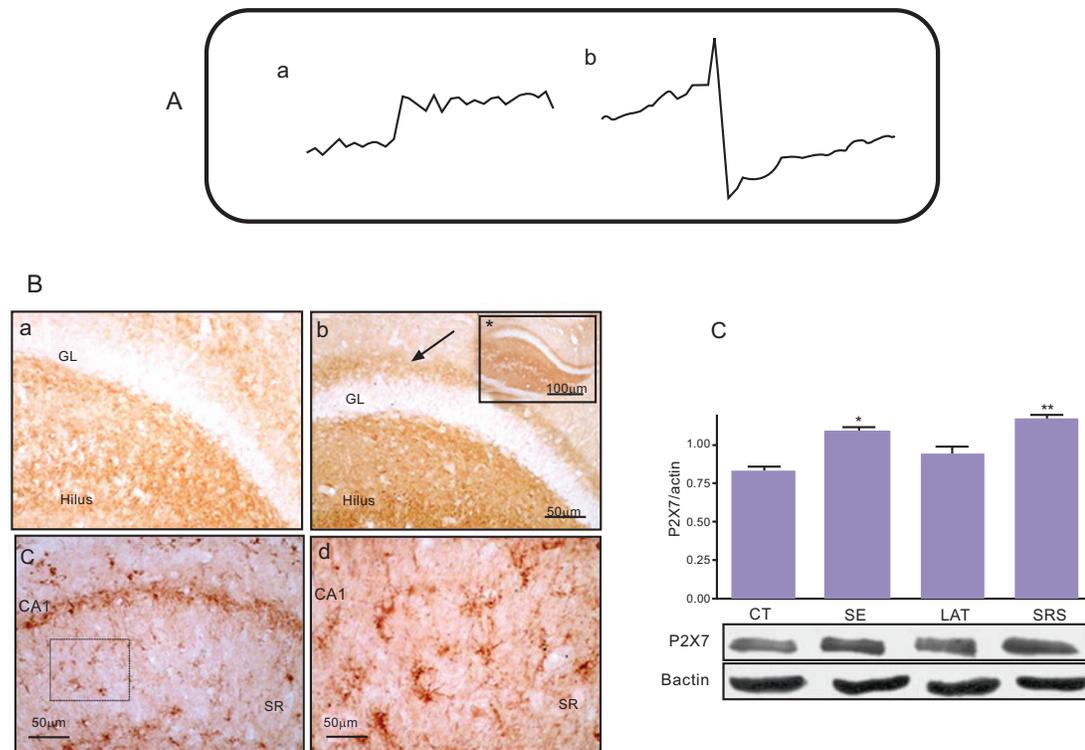
## PURINERGIC P2X AND TEMPORAL LOBE EPILEPSY

Some studies have shown the involvement of purinoceptor signaling in epilepsy [81, 83-86]. Microinjection of ATP analogues into the pre-piriform cortex induces generalized seizures, and extracellular ATP concentrations are significantly augmented after electrical stimulation of Schaffer collaterals in mice with inherited susceptibility to audiogenic seizures [83, 84].

Our Lab was interested in studying purinergic signaling in temporal lobe epilepsy because many of the mechanisms involved in epileptogenesis are activated by intracellular calcium and P2 receptors are important modulators of calcium in the CNS both in neuron and glia. Using fluorometric technique, we were able to demonstrate a biphasic intracellular calcium increase in response to ATP in the hippocampus of chronic rats subjected to pilocarpine [85]. Different from control, a short increase followed by an abrupt decrease in the fluorescence was obtained when ATP was applied in hippocampal slices of epileptic rats, suggesting high expression of P2X7 receptors (see Fig. 3A). Our predictive observation was confirmed by immunohistochemistry and Western blot analysis which revealed high expression of P2X7 in mossy fiber at the proximal dentate gyrus and CA3 area of epileptic rats (Fig. 3B, C).

In a recent study we characterized the expression of P2X receptors in the hippocampus of rats at different phases of the epileptogenesis induced by pilocarpine. P2X2, P2X4 and P2X7 were studied through immunohistochemistry and Western blot analysis (hippocampus) at different times after the onset of SE, 12 h for acute period, 7 days for latent period and 90 days for epileptic condition [86]. Significant changes in P2X receptor expression were detected in acute and epileptic rats (Fig. 3). The main findings included a decrease in P2X4 receptor expression in the hippocampus of epileptic rats, with an increased level of P2X7 receptors during acute and chronic phases of TLE. In the acute phase, diffuse P2X7 receptor staining was found in cell bodies resembling glial cells while in latent and chronic periods, P2X7 receptors were almost exclusively located in nerve terminals in the CA3 region and dentate gyrus.

Reduction of P2X4 receptor levels in epileptic hippocampi of rats may reflect neuronal loss, which is intense in the CA1, CA3, dentate gyrus and hilus [10]. However, a reduction of P2X4 receptor levels was observed in areas that normally are not damaged in this model, indicating functional changes regarding this receptor in epilepsy. Reduced P2X4 receptor expression could also be a part of a compensatory mechanism in response to disturbed GABAergic neurotransmission [68, 86]. Indeed, a functional interaction between P2X2 and P2X4 with GABA-A receptors has been proposed for epileptic conditions [65, 68]. In our study, P2X2 remained unchanged in all studied periods.



**Fig. (3).** **A:** Fluorescence intensity of intracellular calcium in hippocampus slice. Arrows indicate 0,5 mM of adenosine triphosphate addition (**a**, control rat; **b**, epileptic rat). **B:** light microscopy micrographs showing the immunohistochemical for P2X7 receptors in rat hippocampus treated with saline (**a**) or pilocarpine (360 mg/kg, i.p., 15 min before 1 mg/kg of methylscopolamine s.c., **b**, **c**, **d**). Panel (**a**) and (**b**) refer to dentate gyrus of control group (**a**) and chronic group (**b**). Epileptic rat (**b**) showed strong punctate staining in the hilus and in sprouted mossy fibers in supragranular layer of dentate gyrus. Higher amplification of the dentate gyrus is shown in the box in **b**. Panel (**c**) shows the stratum pyramidale and stratum radiatum (SR) of CA1 of rat during status epilepticus with the presence of immunopositive glial cells. Panel (**d**) shows an amplification of the outlined area in (**c**). **C:** Western blot study of P2X7 in the hippocampus of control (CT), and pilocarpine injected groups (SE, rats with 12 h of SE; LAT, latent rats studied 7 days after status epilepticus onset, and SRS, rats with spontaneous recurrent seizures, studied 90 days after status epilepticus onset). Expression of P2X7 was increased in hippocampi of SE and SRS group compared to CT.

Several lines of evidence point to a modulatory role of P2X4 receptors in LTP [61, 83]. Indeed, P2X4 receptor knockout mice present reduced LTP, indicating the importance of this receptor in memory processes [87]. Special attention should be given to changes in the expression of P2X4 receptor in the hippocampus because it could be a mechanism involved in cognitive deficits associated with TLE. It is well established that patients with TLE often show impairments in attention, memory, mental processing speed with many factors contributing to these changes such as brain lesions, localization and lateralization, and antiepileptic drugs [4].

Abundant expression of P2X7 in hippocampal neurons of CA1 and CA3 subfields during *SE* was considered critical because excitotoxic cascades are activated resulting in cell death. As cited above, both seizures and cell death can release high levels of ATP into the extracellular milieu, which can activate P2X7 leading to intensified cell death [88]. Activation of P2X7 results in the formation of large, hydrophilic, non selective membrane pores permeable to molecules >900 Da causing necrosis and apoptosis.

In addition, P2X7 receptor expression is intense in cell with characteristics of reactive astrocytes and microglia, in-

dicating the involvement of P2X7 receptor with inflammatory processes [86]. Our data are in agreement with those of other authors who also demonstrated inflammatory processes associated with P2X7 receptor in microglia after *SE* [81].

Neuroinflammation has been described as a form of injury-induced glial activation that contributes to the pathology of epilepsy [82]. Several authors have described the increased expression of IL-1 $\beta$ , TNF- $\alpha$ , IL-6 and iNOS after seizures, suggesting their participation in cell death [73, 89-91]. However, the contribution of these mediators in neurodegeneration during *SE* remains to be determined.

As described above, microglia can protect or harm neurons. Suzuki *et al.* [75] demonstrated that TNF- $\alpha$  released from ATP-activated microglia in co-culture system have neuroprotective effects on neurons exposed to glutamate. They proposed that ATP released by damaged cells or inflammation leads to chemotaxis of microglia to a damaged brain area, and activates P2X7 receptors to stimulate the microglia to secrete neuroprotective factor such as TNF. Thus, to determine the role of TNF in the damage cascade after *SE*, it is important to investigate the type of TNF receptor in the hippocampus. TNF released by P2X7-activated microglia have dual actions based on the receptor population that it

activates: TNFR1 is neurotoxic and TNFR2 elicit neuroprotection [79].

Increased P2X7 receptor expression in glial cells in the hippocampus after pilocarpine-induced *SE* indicates that this receptor can participate in the astrogliosis process resulting in disfunction of the adenosine system leading to additional seizures [90]. Astrogliosis is accompanied by overexpression of adenosine kinase, an enzyme involved with metabolism of adenosine to AMP, resulting in decrease in the adenosine level, which can predispose brain to seizure onset.

Other inflammatory mediators have been described in the hippocampus of rat subjected to pilocarpine, and molecules such as kinins and prostaglandins and seem to participate in the pathophysiology of TLE [92-94].

Finally, the predominant presence of P2X7 in mossy fiber terminals in the stratum lucidum of CA3 and dentate gyrus of epileptic rats from pilocarpine model suggests that this receptor can modulate the release of neurotransmitters, such as glutamate and GABA. P2X7 labeling was also shown in the stratum oriens and radiatum of CA1 [86]. Under control conditions, the presence of P2X7 receptors in presynaptic terminals can facilitate glutamate and GABA release from hippocampal nerves terminals [59, 63]. However, Rodrigues et al [95] demonstrated by molecular biology and functional experiments that whereas P2X1, P2X2/3 and P2X3 facilitate the release of glutamate, P2Y1, P2Y2 and P2Y4 receptors inhibit glutamate release from glutamatergic terminals in the hippocampus. P2X7 does not presynaptically control glutamate release [95].

Glutamate is the primary excitatory amino acid neurotransmitter in the central nervous system and its activity is carefully modulated in the synaptic cleft by glutamate transporters [96]. In previous studies we have demonstrated that rats with pilocarpine-induced epilepsy present high levels of hippocampal glutamate and calcium transporters (SERCAs), suggesting hyperexcitability [97-99].

Overexpression of neuronal glutamate transporter has been reported associated with high level of extracellular glutamate [100, 101].

According to our recent data, P2X2 and P2X7 receptors could be involved with hyperexcitability [86].

Currently, we are studying the participation of purinergic P2X and P2Y receptors in the hippocampal functions of patients with TLE, and preliminary results are in agreement with data obtained previously with rats. Indeed, high expression levels of P2X2, P2X7 and P2Y1 can be observed in cell bodies resembling glia and nerve terminals, confirming the modulatory role of these receptors in glutamate release and inflammatory processes (in preparation).

## CONCLUDING REMARKS

P2 receptors are involved in most physiological and pathological responses and modulate neurotransmitter release. The results obtained in pilocarpine model and in patients with TLE implicate that purinergic signaling mediated by different types of P2X and P2Y receptors, exerts important roles at different stages of epileptogenesis, especially for calcium influx, glutamate and GABA release, hyperexcitability, inflammation and cell death. Although the exact role

of P2 receptor in these mechanisms has remained elusive due to the complex nature of ATP and adenosine signaling in synapses and astrocytes, or of ATP and GABA cross-talk, the current literature suggests the importance of P2 receptors in TLE. Further research on P2 receptors in adult and in developing brain may provide novel mechanisms and therapeutic targets to control epileptogenesis.

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