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Effects of endogenous glutamate on extracellular concentrations of taurine in striatum and nucleus accumbens of the awake rat: Involvement of NMDA and AMPA/kainate receptors

Short Communication

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Summary. Using microdialysis, the effects of endogenous glutamate on extracellular concentrations of taurine in striatum and nucleus accumbens of the awake rat were investigated. The glutamate uptake inhibitor L-*trans*-pyrrolidine-2,4-dicarboxylic acid (PDC) was used to increase the extracellular concentration of glutamate. PDC (1, 2 and 4 mM) produced a dose-related increase of extracellular concentrations of glutamate and taurine in striatum and nucleus accumbens. Increases of extracellular taurine were significantly correlated with increases of extracellular glutamate, but not with PDC doses, which suggests that endogenous glutamate produced the observed increases of extracellular taurine in striatum and nucleus accumbens. The role of ionotropic glutamate receptors on the increases of taurine was also studied. In striatum, perfusion of the antagonists of NMDA and AMPA/kainate glutamate receptors attenuated the increases of extracellular taurine. AMPA/kainate, but not NMDA receptors, also reduced the increases of extracellular taurine in nucleus accumbens. These results suggest that glutamate-taurine interactions exist in striatum and nucleus accumbens of the awake rat.

Keywords: Amino acids – Glutamate – Taurine – L-*trans*-PDC – Glutamate receptors – Striatum – Nucleus accumbens – Microdialysis – Rat

Introduction

The excitatory amino acid neurotransmitter glutamate has been shown to release taurine in different structures of the brain (Menéndez et al., 1990; Magnusson et al., 1991; Saransaari and Oja, 1992; Shibanoki et al., 1993; Del Arco and Mora, 1999). In turn it has been suggested that taurine may modulate the excitatory actions of glutamate (Menéndez et al., 1990; Shibanoki et al., 1993; Mora and Porras, 1994). In fact, taurine has been shown

to have inhibitory actions in the brain (Huxtable and Sebring, 1986; Huxtable, 1989), and to reduce the influx of calcium induced by the activation of NMDA glutamatergic receptors (Lehmann et al., 1984).

The striatum and nucleus accumbens contain glutamatergic terminals arising from cerebral cortex and from prefrontal cortex, hippocampus and amygdala, respectively (Smith and Bolam, 1990; Groenewegen et al., 1991). Taurine, which has been shown to be released from neurons and glia (Lehmann et al., 1985; Philibert et al., 1988; Holopainen et al., 1989; Levi et al., 1992), is also located in both structures of the brain (Palkovits et al., 1986; Strolin Benedetti et al., 1991). *In vivo* studies have shown that NMDA and AMPA/kainate agonists increase extracellular concentrations of taurine in striatum (Shibanoki et al., 1993; Bianchi et al., 1996). To our knowledge, no studies have reported the effects of glutamate on taurine in the nucleus accumbens.

The aim of the present study was to investigate the effects of an increase of endogenous glutamate on the extracellular concentrations of taurine in striatum and nucleus accumbens of the awake rat. The glutamate uptake blocker L-*trans*-pyrrolidin-3,4-dicarboxilic acid (PDC) was used to increase endogenous concentrations of glutamate. PDC produces a selective and potent inhibition of glutamate uptake without interfering with ionotropic or metabotropic glutamate receptor binding (Bridges et al., 1991; Thomsen et al., 1994) or producing neurotoxic damage *in vivo* in doses up to 100 mM (Massieu et al., 1995; Obrenovitch et al., 1996). The involvement of ionotropic glutamate receptors in the effects of endogenous glutamate was also investigated.

Materials and methods

Animals and surgery

Male Wistar rats (2–3 months, 250–350 g weight) were housed in individual wire mesh cages, provided with food and water *ad libitum*, and maintained in a temperature-controlled room under a light/dark cycle (lights on/off at 8:00 p.m./8:00 a.m.). All *in vivo* experiments, performed at the Universidad Complutense of Madrid, were conducted during the dark period of the light/dark cycle and followed the guidelines of the International Council for Laboratory Animal Science (ICLAS).

Under Equithesin (2 ml/kg i.p.) anaesthesia, bilateral guide-cannulae were stereotaxically implanted in the brain to accomodate microdialysis probes in striatum and nucleus accumbens of the rats. Guide-cannulae assembly (Segovia et al., 1997b) were then fixed to the skull by means of two anchorage screws and application of dental cement. When inserted, the tip of the microdialysis probe was placed in: 0.6 mm rostral, 2.5 mm lateral from Bregma and 5 mm ventral from dura mater for striatum; and 1.6 mm rostral, 1.6 mm lateral from Bregma and 7.5 mm from dura mater for nucleus accumbens (König and Klippel, 1967).

In vivo microdialysis

Three-four days after surgery, microdialysis probes were inserted and experiments were performed on the freely moving rat. Probes of concentric design with an active dialyzing length of 2 mm (Segovia et al., 1997b) were perfused with artificial CSF (composition in

mM: NaCl, 137; $CaCl_2$, 1.2; KCl, 3; $MgSO_4$, 1; NaH_2PO_4, 0.5; Na_2HPO_4, 2; glucose, 3; pH = 7.3) at a flow rate of 2.5 μl/min. The average relative *in vitro* recovery obtained was (at room temperature): glutamate = 8.2 ± 1.4%; taurine = 8.6 ± 0.4% (mean ± SEM, n = 8).

After basal concentrations of amino acids were established, 15-min samples were collected during 240 min and immediately stored at −80°C until analysed. The first four samples were used as control. The change in perfusion medium during the experiments was done by a liquid switch (Harvard Apparatus). PDC (1, 2, 4 mM) was perfused for 60 min. When used, CPP (1 mM) or DNQX (1 mM) were perfused 60 min before coperfusion with PDC. The effective extracellular concentration of drugs, extrapolated from the relative recovery of the microdialysis probes, was <10% of the artificial CSF concentration. PDC, CPP and DNQX, purchased from Tocris Cookson Ltd. (Bristol, U.K.), were dissolved in CSF before infusion through the microdialysis probe. DNQX was first dissolved in dimethylsulfoxide (DMSO) so that when diluted in CSF, the proportion of DMSO was 1.25%.

At the end of experiments, the microdialysis probes were removed and stored in distilled water. The placement of the microdialysis probe was verified with a cryostat microtome and viewing lens.

Amino acid analysis

The amino acid content of samples was analysed by reverse-phase HPLC and fluorometric detection according to a method used previously in our laboratory (Segovia et al., 1997a; Segovia et al., 1997b; Del Arco et al., 1998). Briefly, precolumn derivatization of 5 μl-samples was performed with an o-phthalaldehyde solution. Derivatized samples were injected in a Rheodyne injector (20 μl loop) running first in a C18 precolumn (Tracer) and then in a C18 column of 5 μm particles and 4 × 150 mm (Tracer). A gradient program of two mobile phases at a flow rate of 1 ml/min was used. Solution A was 95:5 (v/v) mixture of 50 mM sodium acetate buffer (pH = 5.67) and methanol, to which 12.5 ml of isopropyl alcohol per litre was added; solution B was a 70:30 (v/v) methanol/water mixture. These conditions allowed amino acids to be detected within 15 min.

The amino acids were measured by a fluorescence detector (Waters 474). The excitation filter was set at 340 nm, and the emission filter at 460 nm. Amino acids were quantified using the MAXIMA 820 (Waters) software by the internal standard procedure. The internal standard used was 0.05 mM homoserine. The detection limit in our 5 μl-samples was 0.05 μM for all amino acids.

Statistical analysis

Data are reported as percentages of basal dialysate concentrations. The effects of PDC were calculated as the difference between the average of the samples in which PDC was perfused and the average of four control samples. A two factorial test (time-dose; time-treatment) with repeated measures followed by Dunnett's t-test was performed for multiple comparisons. Pearson's coefficient and independence test was used for the study of correlations among dialysate concentrations of glutamate and taurine.

Results

Effects of PDC on dialysate concentrations of glutamate and taurine in striatum and nucleus accumbens

Local infusion of PDC (1, 2 and 4 mM) in striatum and nucleus accumbens produced a dose-related increase of dialysate concentrations of glutamate [in striatum $r = 0.54$ ($n = 26$; $p < 0.01$) and in nucleus accumbens $r = 0.77$

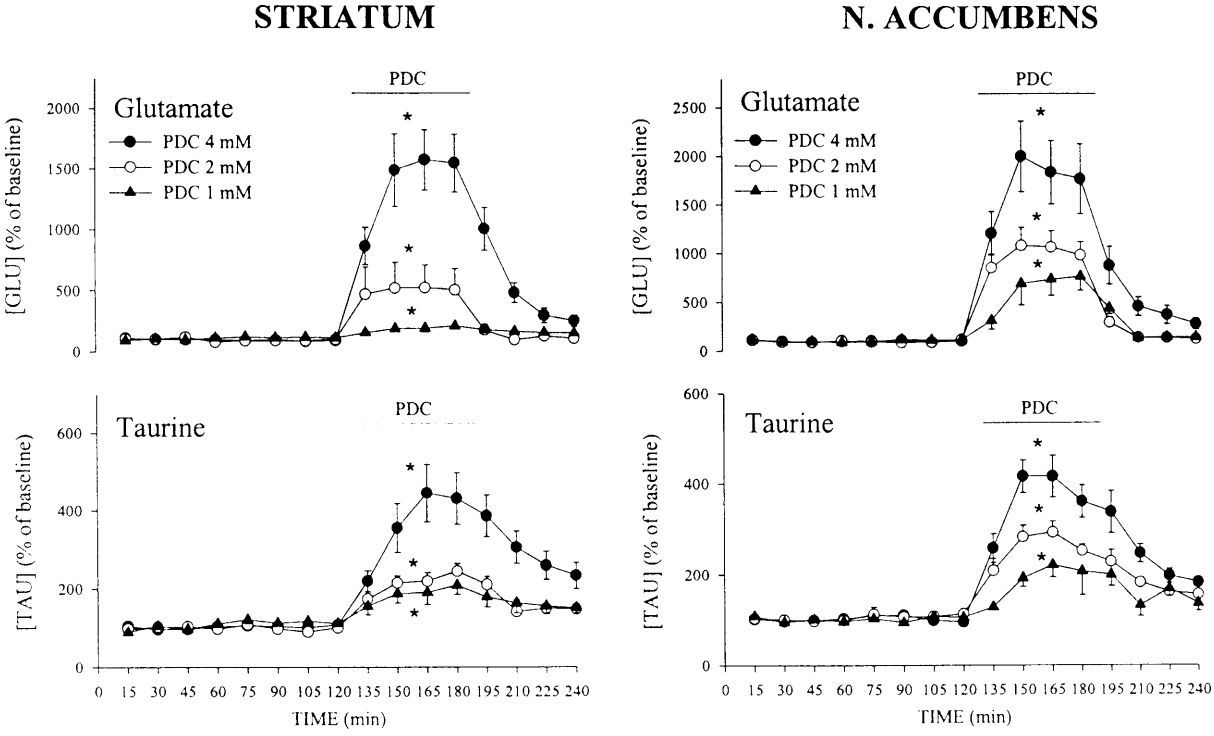

Fig. 1. Effects of intracerebral perfusion of PDC (1, 2, and 4 mM) on dialysate concentrations of glutamate and taurine in striatum and nucleus accumbens of the awake rat. Data (mean ± SEM) are percentage of baseline concentration. *$p < 0.05$ compared with basal concentration

($n = 21$; $p < 0.001$)] (Fig. 1). PDC also increased dialysate concentations of taurine (Fig. 1). At the dose of 4 mM, PDC increased dialysate concentrations of glutamate from 1.07 ± 0.28 to 5.39 ± 0.7 μM and taurine from 1.08 ± 0.11 to 3.01 ± 0.32 μM in striatum; in nucleus accumbens this same dose of PDC increased glutamate from 0.29 ± 0.05 to 4.06 ± 0.41 μM and taurine from 0.65 ± 0.08 to 2.19 ± 0.23 μM.

Increases of dialysate concentrations of taurine were significantly correlated with increases of dialysate concentrations of glutamate (Fig. 2). Partial correlation coefficients also showed a correlation between the increases of taurine and glutamate independently of PDC dose [in striatum $r = 0.89$ ($n = 26$; $p < 0.001$) and in nucleus accumbens $r = 0.80$ ($n = 21$; $p < 0.001$)].

Effects of NMDA (CPP) and AMPA/KA (DNQX) glutamate receptor antagonists on the effects of PDC on dialysate concentrations of taurine

Intracerebral perfusion of CPP (1 mM) did not change basal dialysate concentrations of glutamate or taurine in striatum nor in nucleus accumbens. CPP attenuated by 54% ($p < 0.01$, $n = 15$) increases of dialysate taurine

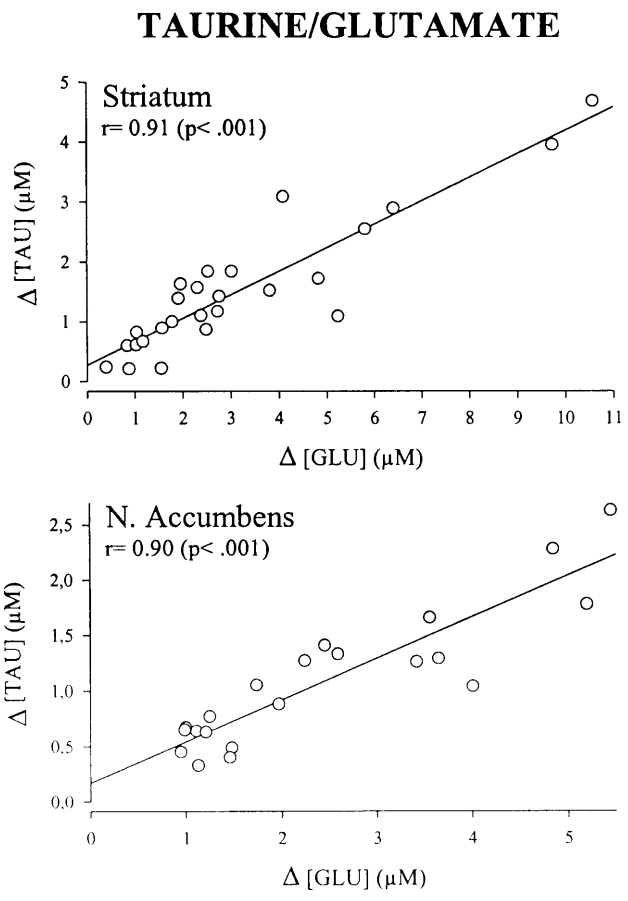

Fig. 2. Correlation between increases of dialysate concentration of taurine and glutamate induced by perfusion of PDC (1, 2, and 4 mM) in striatum and nucleus accumbens of the awake rat. Data are calculated as the difference between the average of samples in which PDC was perfused and the average of four basal samples

produced by PDC (4 mM) in striatum but not in nucleus accumbens (Fig. 3).

Intracerebral perfusion of DNQX (1 mM) did not change basal dialysate concentrations of glutamate or taurine in striatum nor in nucleus accumbens. DNQX attenuated by 77% in striatum ($p < 0.01$) and by 64% in nucleus accumbens, the increases of dialysate taurine produced by PDC (4 mM) (Fig. 3). DNQX also attenuated increases of dialysate glutamate in striatum and nucleus accumbens produced by this same dose of PDC by 45% and 48%, respectively.

Discussion

The present study shows that perfusion of the glutamate reuptake inhibitor PDC increases extracellular concentrations of glutamate and taurine in striatum and nucleus accumbens, and that the increases of extracellular

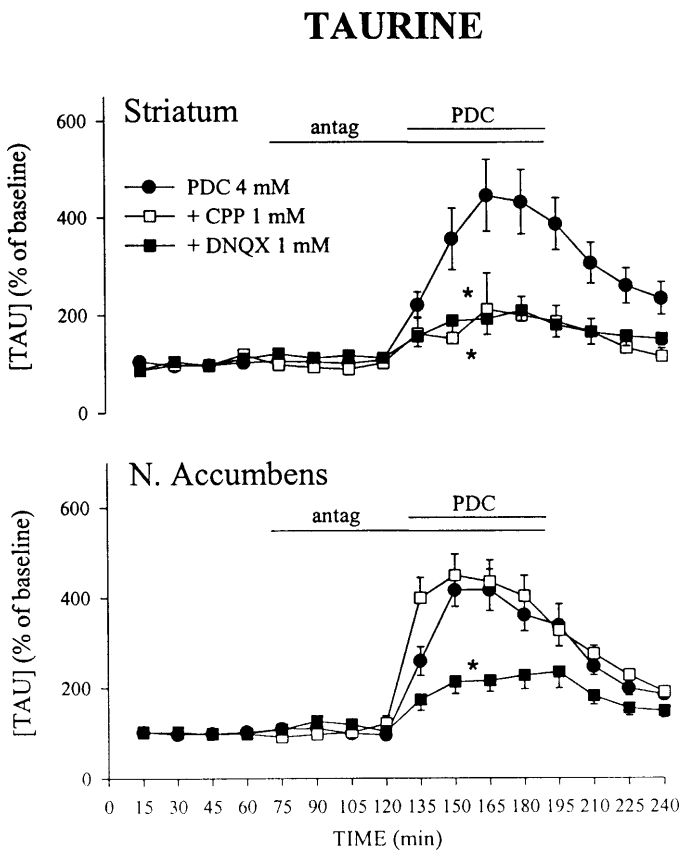

Fig. 3. Effects of intracerebral perfusion of PDC (4 mM), PDC plus CPP (1 mM), and PDC plus DNQX (1 mM) on dialysate concentrations of taurine in striatum and nucleus accumbens of the awake rat. Data (mean ± SEM) are percentage of baseline concentration. *$p < 0.01$ compared with PDC 4 mM increases

taurine are significantly correlated with increases of extracellular glutamate, but not with PDC dose, in both structures of the brain. This finding strongly suggests that the increases of extracellular taurine are produced by endogenous glutamate. This is further supported by the fact that ionotropic glutamate receptor antagonists attenuated increases of taurine produced by PDC in striatum and nucleus accumbens. Similar increases of taurine produced by endogenous glutamate and/or glutamate receptor agonists have been reported in striatum as well as in other structures of the brain such as prefrontal cortex, hippocampus and cerebellum (Muñoz et al., 1987; Menéndez et al., 1990; Magnusson et al., 1991; Shibanoki et al., 1993; Massieu et al., 1995; Bianchi et al., 1996; Fallgren and Paulsen, 1996; Semba and Wakuta, 1998; Del Arco and Mora, 1999).

Regarding the extracellular glutamate concentrations reported in this study, the maximal concentrations reached at PDC 4 mM were 60–70 μM in

striatum and 40–50 μM in nucleus accumbens (after correcting for *in vitro* recovery of the probes). These values may be considered physiological since they are in agreement with the estimated affinity for ionotropic glutamate receptors (μM range), and also with the estimated 1–3 mM reported for the concentrations of glutamate in the synapses (Sands and Barish, 1989; Clements, 1996). Taking this into account the correlations between taurine and glutamate in striatum and nucleus accumbens reported here are an expression of physiological interactions between these neuroactive substances in these areas of the brain.

Taurine has been shown to be located in GABA projection neurons in striatum (Della Corte et al., 1990) so it might be possible that glutamate acts through neuronal glutamatergic receptors located on these GABA neurons to increase extracellular concentrations of taurine in striatum. A recent report showing that the infusion of glutamate agonists in striatum induces the release of GABA and taurine in striatum and substantia nigra agrees well with this possibility (Bianchi et al., 1996). The presence of NMDA and AMPA/kainate glutamate receptors on these neurons also support this hypothesis (Chen et al., 1996). However, since taurine has been shown to be released from glial cells (Philibert et al., 1988; Holopainen et al., 1989; Levi et al., 1992), glutamate could also act on glutamate receptors located in glia to produce the increases of taurine in the extracellular space.

In nucleus accumbens, no reports have previously indicated the release of taurine produced by glutamate. The present report indicating a positive correlation between increases of taurine and glutamate and the attenuation of the increases of taurine by the AMPA/kainate glutamatergic antagonist suggest for the first time the action of glutamate on taurine release in the nucleus accumbens of the awake rat. In this respect the presence in astrocytes mainly of AMPA/kainate receptors, but not NMDA receptors (Steinhäuser and Gallo, 1996) is of interest, and the differential effects of glutamatergic antagonists on the increases of taurine in striatum and nucleus accumbens found in this study could reasonably be attributed to a different action of glutamate on neurons and glia in these two structures of the brain.

It has been suggested that taurine may act as an inhibitory neuromodulator, increasing chloride conductance (Quinn, 1990; Häusser et al., 1992) and reducing the calcium influx to the cell (Lehmann et al., 1984; Schurr et al., 1987). It has also been shown that taurine can reduce the release of glutamate (Kamisaki et al., 1993) and modify the release of other neurotransmitters (Arzate et al., 1986; Oja and Kontro, 1988; Kamisaki et al., 1993; Ruotsalainen and Ahtee, 1996). Therefore the increases of extracellular taurine produced by glutamate in striatum and nucleus accumbens could modulate the excitatory effects of endogenous glutamate on other neurotransmitter systems and on glutamate itself. Accordingly, another possible role of taurine could be also to protect neurons against excitotoxicity produced by extracellular concentrations of glutamate in these structures of the brain (Menéndez et al., 1990; Magnusson et al., 1991).

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References

Arzate ME, Morán J, Pasantes-Morales H (1986) Inhibitory effect of taurine on 4-aminopyridine-stimulated release of labelled dopamine from striatal synaptosomes. Neuropharmacology 25: 689–694

Bianchi L, Galeffi F, Bartolini S, Bolam JP, Della Corte L (1996) The evoked release of eodogenous amino acids in the direct and indirect pathways of the basal ganglia. A dual microdialysis study in the freely moving rat. In: González-Mora JL, Borges R, Mas M (eds) Monitoring molecules in neurosciences. La Laguna, University of La Laguna, pp 176–177

Bridges RJ, Stanley MS, Anderson MW, Cotman CW, Camberlin AR (1991) Conformationally defined neurotransmitter analogues. Selective inhibition of glutamate uptake by one pyrrolidine-2,4-dicarboxylate diastereomer. J Med Chem 34: 717–725

Chen Q, Veenman CL, Reiner A (1996) Cellular expression of ionotropic glutamate receptor subunits on specific striatal neuron types and its implication for striatal vulnerability in glutamate receptor-medited excitotoxicity. Neuroscience 73: 715–731

Clements JD (1996) Transmitter timecourse in the synaptic cleft: its role in central synaptic function. Trends Neurosci 19: 163–171

Del Arco A, Mora F (1999) Effects of endogenous glutamate on extracellular concentrations of GABA, dopamine and dopamine metabolites in the prefrontal cortex of the freely moving rat: involvement of NMDA and AMPA/kainate receptors. Neurochem Res 24: 1027–1035

Del Arco A, Castañeda TR, Mora F (1998) Amphetamine releases GABA in striatum of the freely moving rat: involvement of calcium and high affinity transporter mechanisms. Neuropharmacology 37: 199–205

Della Corte L, Bolam JP, Clarke DJ, Parry DM, Smith AD (1990) Sites of [3H]taurine uptake in the rat substantia nigra in relation to the release of taurine from the striatonigral pathway. Eur J Neurosci 2: 50–61

Fallgren AB, Paulsen RE (1996) A microdialysis study in rat brain of dihydrokainate, a glutamate uptake inhibitor. Neurochem Res 21: 19–25

Groenewegen HJ, Berendse HW, Meredith GE, Haber SN, Voorn P, Wolters JG, Lohman ASM (1991) Functional anatomy of the ventral, limbic system-innervated striatum. In: Willner P, Scheel-Krüger J (eds) The mesolimbic dopamine system: from motivation to action. John Wiley & Sons, Chichester, pp 19–59

Häusser MA, Yung WH, Lacey MG (1992) Taurine and glycine active the same Cl-conductance in substantia nigra dopamine neurones. Brain Res 571: 103–108

Holopainen I, Kontro P, Oja SS (1989) Release of taurine from cultured cerebellar granule cells and astrocytes: co-release with glutamate. Neuroscience 29: 425–432

Huxtable RJ (1989) Taurine in the central nervous system and mammalian actions of taurine. Prog Neurobiol 32: 471–533

Huxtable RJ, Sebring LA (1986) Towards a unifying theory for the actions of taurine. Trends Pharmacol Sci 7: 481–485

Kamisaki Y, Maeda K, Ishimura M, Omura H, Itoh T (1993) Effects of taurine on depolarization-evoked release of amino acids from rat cortical synaptosomes. Brain Res 627: 181–185

König JRF, Klippel RA (1967) The rat brain. Krieger R.E., New York

Lehmann A, Hagberg H, Hamberger A (1984) A role for taurine in the maintenance of homeostasis in the central nervous system during hyperexcitation? Neurosci Lett 52: 341–346

Lehmann A, Lazarewicz JW, Zeise M (1985) N-methylaspartate-evoked liberation of taurine and phosphoethanolamine in vivo: site of release. J Neurochem 45: 1172–1177

Levi G, Gallo V, Patrizio M (1992) Release of exogenous and endogenous neurotransmitter amino acids from cultured astrocytes. In: Yu ACH, Hertz L, Norenberg MD, Sykova E, Waxman SG (eds) Progress in brain research, vol 94. Neuronal-astrocytic interactions. Elsevier, Amsterdam, pp 243–250

Magnusson KR, Koerner JF, Larson AA, Smullin DH, Skilling SR, Beitz AJ (1991) NMDA-, kainate- and quisqualate-stimulated release of taurine from electrophysiologically monitored rat hippocampal slices. Brain Res 549: 1–8

Massieu L, Morales-Villagrán A, Tapia R (1995) Accumulation of extracellular glutamate by inhibition of its uptake is not sufficient for inducing neuronal damage: an in vivo microdialysis study. J Neurochem 64: 2262–2272

Menéndez N, Solís JM, Herreras O, Herranz AS, Del Rio RM (1990) Role of endogenous taurine on the glutamate analogue-induced neurotoxicity in the rat hippocampus *in vivo.* J Neurochem 55: 714–717

Mora F, Porras A (1994) Interactions of dopamine, excitatory amino acids and inhibitory amino acids in the basal ganglia of the conscious rat. In: Percheron G, McKenzie JS, Féger J (eds) The basal ganglia IV. New ideas and data on structure and function. Plenum Press, New York, pp 441–447

Muñoz MD, Herreras O, Herranz AS, Solís JM, Martín del Río R, Lerma J (1987) Effects of dihydrokainic acid on extracellular amino acids and neuronal excitability in the in vivo rat hippocampus. Neuropharmacology 26: 1–8

Obrenovitch TP, Urenjak J, Zilkha E (1996) Evidence disputing the link between seizure activity and high extracellular glutamate. J Neurochem 66: 2446–2454

Oja SS, Kontro P (1988) Release of taurine gaba and dopamine from rat striatal slices: mutual interactions and developmental aspects. Neuroscience 24: 49–58

Palkovits M, Elekes I, Lang T, Patthy A (1986) Taurine levels in discrete brain nuclei of rats. J Neurochem 47: 1333–1335

Philibert RA, Rogers KL, Allen AJ, Dutton GR (1988) Dose-dependent, K+-stimulated efflux of endogenous taurine from primary astrocyte cultures is Ca2+-dependent. J Neurochem 51: 122–126

Quinn MR (1990) Taurine allosterically modulates binding sites of the GABAA receptor. In: Anonymous taurine: functional neurochemistry, physiology and cardiology. Wiley-Liss, New York, pp 121–127

Ruotsalainen M, Ahtee L (1996) Intrastriatal taurine increases striatal extracellular dopamine in a tetrodutoxin-sensitive manner in rats. Neurosci Lett 212: 175–178

Sands SB, Barish ME (1989) A quantitative description of excitatory amino acid neurotransmitter responses on cultured embryonic *Xenopus* spinal neurons. Brain Res 502: 375–386

Saransaari P, Oja SS (1992) Release of GABA and taurine from brain slices. Prog Neurobiol 38: 455–482

Schurr A, Tseng MT, West CA, Rigor BM (1987) Taurine improves the recovery of neuronal function following cerebral hypoxia: an *in vitro* study. Life Sci 40: 2059–2066

Segovia G, Del Arco A, Mora F (1997a) Endogenous glutamate increases extracellular concentrations of dopamine, GABA, and taurine through NMDA and AMPA/kainate receptors in striatum of the freely moving rat: a microdialysis study. J Neurochem 69: 1476–1483

Segovia G, Porras A, Mora F (1997b) Effects of 4-aminopyridine on extracellular concentrations of glutamate in striatum of the freely moving rat. Neurochem Res 22: 1491–1497

Semba J, Wakuta MS (1998) Regional differences in the effects of glutamate uptake inhibitor L-*trans*-pyrrolidine-2,4-dicarboxylic acid on extracellular amino acids and dopamine in rat brain: an *in vivo* microdialysis study. Gen Pharmac 31: 399–404

Shibanoki S, Kogure M, Sugahara M, Ishikawa K (1993) Effect of systemic administration of N-methyl-D-aspartic acid on extracellular taurine level measured by microdialysis in the hippocampal CA1 field and striatum of rats. J Neurochem 61: 1698–1704

Smith AD, Bolam JP (1990) The neural network of the basal ganglia as revealed by the study of synaptic connections of identified neurones. Trends Neurosci 13: 259–265

Steinhäuser C, Gallo V (1996) News on glutamate receptors in glial cells. Trends Neurosci 19: 339–345

Strolin Benedetti M, Russo PA, Marrari P, Dostert P (1991) Effects of ageing on the content in sulfur-containing amino acids in rat brain. J Neural Transm 86: 191–203

Thomsen C, Hansen L, Suzdak PD (1994) L-Glutamate uptake inhibitors may stimulate phosphoinositide hydrolysis in baby hamster kidney cells expressing mGluRla via heteroexchange with L-glutamate without direct activation of mGluRla. J Neurochem 63: 2038–2047

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