ELSEVIER

European Journal of Pharmacology 318 (1996) 295–300

ejp

In vivo blockade of thalamic $GABA_B$ receptors increases excitatory amino-acid levels

Gabriella Nyitrai [a], Zsuzsa Emri [b], Vincenzo Crunelli [b,*], Katalin A. Kékesi [a], Árpád Dobolyi [a], Gabor Juhász [a]

[a] *Department of Comparative Physiology, Eötvös Loránd University, Budapest, Hungary*
[b] *Physiology Unit, School of Molecular and Medical Biosciences, University of Wales, Museum Avenue, Cardiff, UK*

Received 3 October 1996; accepted 8 October 1996

Abstract

The effect of intrathalamic application of $GABA_B$ receptor antagonists on the basal excitatory amino-acid levels was studied using microdialysis probes implanted in the dorsal lateral geniculate nucleus and in the ventrobasal complex. In both nuclei, continuous perfusion of the $GABA_B$ receptor antagonist 3-aminopropyl-(diethoxymethyl)-phosphinic acid (CGP 35348) produced an increase in the extracellular concentration of aspartate and (to a lesser extent) glutamate, but no change was observed in the level of taurine, the main amino acid involved in the regulation of brain osmolarity processes. In contrast, 3-amino-2-hydroxy-2-(4-chlorophenyl)-propanesulphonic acid (2-hydroxy-saclofen), another $GABA_B$ receptor antagonist, failed to affect the extracellular concentration of aspartate, glutamate and taurine. Thus, the basal level of excitatory amino acids in the thalamus in vivo is under the control of CGP 35348-sensitive $GABA_B$ receptors.

Keywords: $GABA_B$ receptor; Microdialysis; Thalamus; Aspartate; Glutamate

1. Introduction

A high number of $GABA_B$ receptors occurs in the thalamus, in particular within the dorsal lateral geniculate nucleus and the ventrobasal complex (Bowery et al., 1987; Chu et al., 1990), where they are located both pre- and postsynaptically (Soltesz et al., 1988; Crunelli and Leresche, 1991; Lee et al., 1994; Emri et al., 1996). Presynaptic $GABA_B$ receptors have been suggested to play an important role in the regulation of transmitter release (Bowery et al., 1980; Bonanno and Raiteri, 1993; Pende et al., 1993), and in the thalamus their activation has been shown to reduce the amplitude of inhibitory postsynaptic currents and excitatory postsynaptic potentials in vitro (Le Feuvre et al., 1995; Emri et al., 1996) and transmitter release in vivo (Banerjee and Snead, 1995). Studies using selective $GABA_B$ receptor agonists and antagonists have suggested the existence of $GABA_B$ receptor subtypes (Bonanno and Raiteri, 1993; Misgeld et al., 1995; Kerr and Ong, 1995). In particular, the release of glutamate in cortical synaptosomes has been shown to be blocked by 3-aminopropyl-(diethoxymethyl)-phosphinic acid (CGP 35348), while phaclofen was inactive (Bonanno and Raiteri, 1993). 3-Amino-2-hydroxy-2-(4-chlorophenyl)-propanesulphonic acid (2-hydroxy-saclofen), the more potent structural analogue of phaclofen (Kerr et al., 1988; Misgeld et al., 1995), has been found to antagonize the effect of baclofen on excitatory postsynaptic potentials in the dorsal lateral geniculate nucleus in vitro (Emri et al., 1996), and to act as a partial agonist in the thalamus as well as in other brain areas (Nathan et al., 1990; Caddick et al., 1995; Emri et al., 1996).

The physiological relevance of $GABA_B$ receptor-mediated regulation of transmitter release, however, is still not fully understood. Biochemical and electrophysiological studies in vitro have suggested the presence of a tonic activation of presynaptic $GABA_B$ receptors that control glutamate and GABA release (Bernasconi et al., 1992; Emri et al., 1996), but no direct evidence in support of this hypothesis is available in vivo. Such a process might underlie the blockade of absence seizures (Liu et al., 1992; Snead, 1992) and the changes in the sleep-waking cycle (Juhász et al., 1994) produced by direct application of the $GABA_B$ receptor antagonist CGP 35348 into the thalamus.

* Corresponding author. Tel.: (44-1222) 874-801; Fax: (44-1222) 874-986; e-mail: crunelli@cardiff.ac.uk

0014-2999/96/$15.00 Copyright © 1996 Elsevier Science B.V. All rights reserved.
PII S0014-2999(96)00813-8

In this study, therefore, the effect of intrathalamic injection of two $GABA_B$ receptor antagonists, CGP 35348 and 2-hydroxy-saclofen, on the basal extracellular concentration of excitatory amino acids was investigated in the ventrobasal complex and dorsal lateral geniculate nucleus using the in vivo microdialysis technique in anaesthetized rats.

2. Materials and methods

Male Wistar rats (200–300 g) were anaesthetized with halothane (1%) and microdialysis probes, constructed as previously described (Juhász et al., 1989), were implanted in the thalamus. Briefly, hollow fibers (Travenol, cut off: 5000 Da, outside diameter: 0.2 mm, length: 3 mm) were implanted in the ventrobasal complex, while, because of its smaller size, Terrenius HR hollow fibers with a 50000 D cut-off and 1 mm long active surface were used in the dorsal lateral geniculate nucleus. The probes were inserted into a 27-Gauge stainless steel tubing and implanted bilaterally in the ventrobasal complex (A: −1.8, L: 2.5, V: −7 mm) and dorsal lateral geniculate nucleus (A: −2.8, L: 4, V: −6.5 mm) (Pellegrino and Cushman, 1967).

Artificial cerebrospinal fluid (ACSF), containing 140 mEq Na^+, 3 mEq K^+, 1.2 mEq Ca^{2+}, 2 mEq Mg^{2+} and 144 mEq Cl^-, was perfused at a rate of 1 μl/min. The perfusates (20 μl) were collected every 20 min, frozen immediately and stored at −20°C. The first sample was collected 40 min after the end of implantation and two other samples were collected (control period) before perfusion with the $GABA_B$ receptor antagonists started. The $GABA_B$ receptor antagonists CGP 35348 (1, 2 and 5 mM) and 2-hydroxy-saclofen (1–3 mM) were dissolved in ACSF and applied unilaterally via the microdialysis probe for 2 h subsequent to the control period. On the basis of the results of in vitro calibrations of the probes (Juhász et al., 1989), the estimated concentration of $GABA_B$ receptor antagonist in the tissue surrounding the dialysis probe is 20–30% of the concentration inside the probe (Juhász et al., 1994). Thus, the effective concentration around the probes was estimated as 0.2–1.5 mM and 0.2–1 mM, for CGP 35348 and 2-hydroxy-saclofen, respectively. As an additional control experiment, 9 rats were perfused with ACSF alone

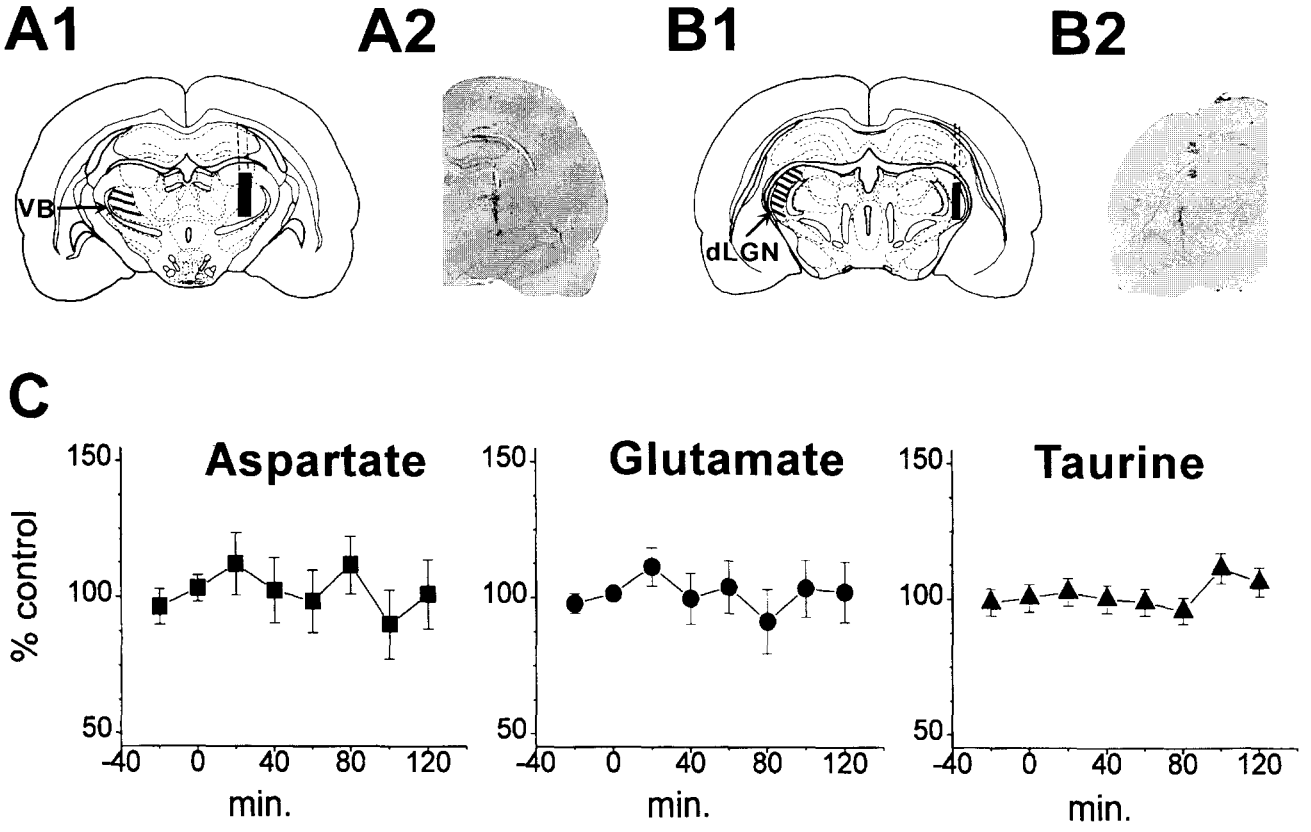

Fig. 1. Position of the microdialysis probes and basal level of aspartate, glutamate and taurine during perfusion with ACSF alone. A schematic diagram of the position of the probe in the ventrobasal complex (VB) and in the dorsal lateral geniculate nucleus (dLGN) is shown in A_1 and B_1, respectively. The black portion indicates the active surface of the probe, that starts 1 mm above its tip. Drawings adapted from Pellegrino and Cushman (1967). Nissl-stained coronal sections showing the probe tracts in the ventrobasal complex and in the dorsal lateral geniculate nucleus are shown in A_2 and B_2, respectively. Note that the active surface of the probe is 3 mm long and starts 1 mm above the probe tip. (C) Aspartate, glutamate and taurine concentrations do not show any change during continuous perfusion with ACSF alone. Concentrations are expressed as mean percentage (±S.E.M.) ($n = 9$) of control values (calculated from the −20 min and 0 min data points).

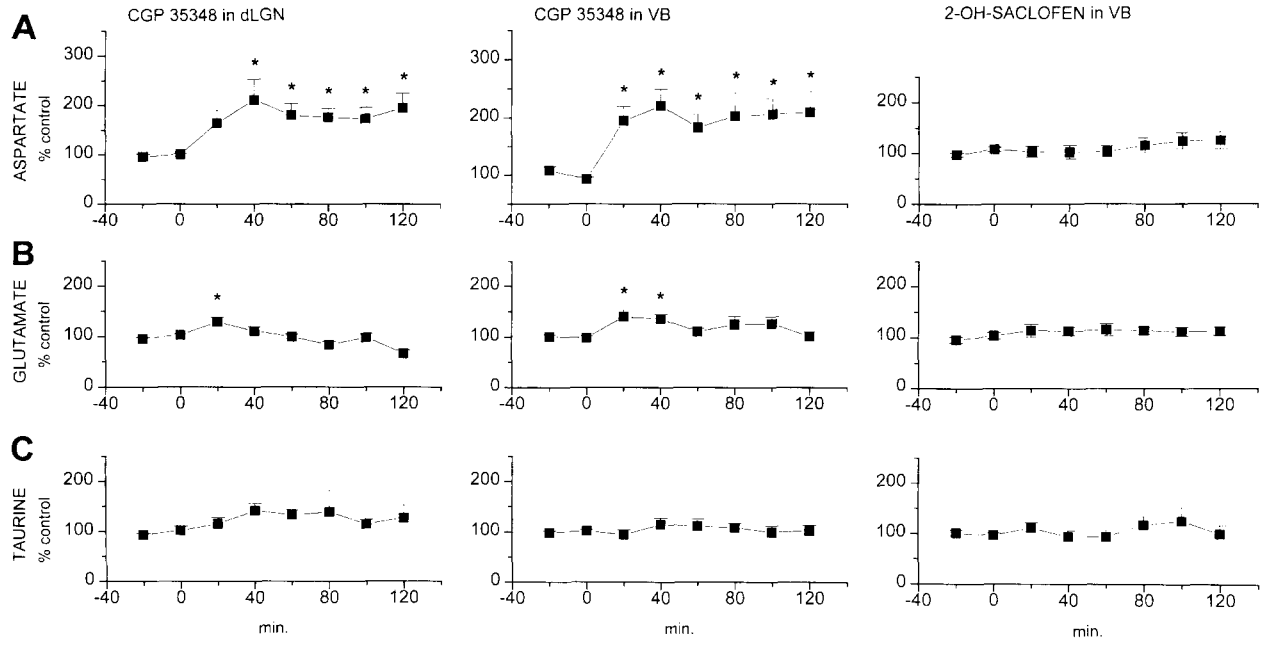

Fig. 2. Effect of intrathalamic application of CGP 35348 (1, 2 and 5 mM) and 2-hydroxy-saclofen (1–3 mM) on extracellular concentration of aspartate (A), glutamate (B) and taurine (C) in the ventrobasal complex (VB) and the dorsal lateral geniculate nucleus (dLGN). Data points are expressed as mean percentage (±S.E.M.) of the control values calculated from the last two samples before drug perfusion (i.e., −20 min and 0 min data points). Values for the aspartate data points in the ventrobasal complex are: 20 min: 195 ± 25%, 40 min: 221 ± 29%, 60 min:183 ± 23%, 80 min: 203 ± 40%, 100 min: 206 ± 27%, 120 min: 209 ± 36%, * $P < 0.05$ ($n = 19$). Values for the aspartate data points in the dorsal lateral geniculate nucleus are: 20 min: 164 ± 27%, 40 min: 212 ± 42%, 60 min: 180 ± 23%, 80 min: 177 ± 18%, 100 min: 173 ± 23%, 120 min 195 ± 30%. Values for glutamate in the ventrobasal thalamus at 20 and 40 min are 140 ± 13% and 136 ± 9%, respectively. Glutamate concentration at 20 and 40 min in the dorsal lateral geniculate nucleus is 129 ± 10%, 111 ± 12%, respectively.

for 3.5 h from the end of the surgery (Fig. 1C). The location of the dialysis probes was verified using Nissl-stained coronal sections (Fig. 1A2,B2). Results are expressed in the text and figure as percentage (means ± S.E.M.) of the last two control samples (40 min in total) collected before injection of the drug commenced (i.e., −20 min and 0 min data points in Fig. 2), and statistical analysis of the data was performed by Student's *t*-test.

To measure the amino-acid concentrations, precolumn derivatization of the amino acids with ortophthalaldehyde was used. Derivatization reactions were performed in the presence of mercaptoethanole at pH 10.5, and the ortophthalaldehyde-derivatized amino acids were detected using a fluorescent detector with 305–395 nm excitation and 430–470 nm emission filters. Due to the instability of the ortophthalaldehyde derivatives, the high pressure liquid chromatography technique was automated on a Pharmacia AminoSys chromatograph system for amino-acid analysis. Detection limits for amino acids were 0.5–5 pmol/10 μl. Separation of amino acids was performed by HP Hypersil ODS reversed phase columns (200 × 2.1 mm). Eluent A was 0.1 M phosphate buffer containing 0.5 (v/v) % tetrahydrofuran and 0.02 (v/v) % tetraethylammoniumhydroxide, pH 7.2; eluent B was 70% acetonitrile mixed with 0.1 M phosphate buffer adjusted to pH 7.2 with phosphoric acid. The gradient profile was: 0% B at 0 min, 11% B at 2 min, 22% B at 17 min, 50% B at 21 min, 100% B at 25 min, and 0% B at 35 min. Chromatograms were evaluated by PE Nelson 2000 software. External standards containing a concentration of 10 μM of the different amino acids were injected every 10 samples.

3. Results

3.1. Control levels of excitatory amino acids and taurine

Histological analysis of the position of the microdialysis probes showed that those implanted in the ventrobasal complex were all located close to the middle of this nucleus (Fig. 1A1,A2), and therefore the biochemical results obtained from these animals ($n = 28$) were all included in the analysis. Because of the smaller dimension of the dorsal lateral geniculate nucleus, however, it was difficult to locate the probe well within the centre of this nucleus (Fig. 1B1,B2). Thus, only data from 6 (out of 12) rats with probes implanted in the dorsal lateral geniculate nucleus were retained for subsequent amino-acid analysis.

Analysis of the amino-acid levels in animals that had been perfused with ACSF alone for 3.5 h showed no change in aspartate, glutamate and taurine 1 h onwards from the end of the implantation (−40 min data point in Fig. 1C). The average amino-acid concentration in the samples collected from the probes were as follows: ven-

trobasal complex: aspartate 0.53 ± 0.06 μM, glutamate: 2.42 ± 0.21 μM, taurine: 5.12 ± 1.1 μM; dorsal lateral geniculate nucleus: aspartate 0.45 ± 0.03 μM, glutamate 0.63 ± 0.08 μM, taurine 1.57 ± 0.63 μM.

3.2. Extracellular concentration of amino acids during CGP 35348 application in the ventrobasal complex

Application of different concentrations of CGP 35348 (1, 2 and 5 mM) produced similar changes in aspartate and glutamate concentration. The aspartate level in the ventrobasal complex increased significantly from the first sample collected after CGP 35348 application had begun (20 min data point in Fig. 2A) (195 ± 25%, $n = 19$, $P < 0.01$), and remained elevated throughout the experiment (2 h).

The extracellular glutamate concentration increased in the first two samples (140 ± 14%, 136 ± 9%, respectively; $n = 19$, both $P < 0.05$), but to a lesser degree than aspartate. Following this transient increase, the basal glutamate concentration returned to the control level (Fig. 2B). No change in the extracellular concentration of taurine was observed throughout the experiment (Fig. 2C).

3.3. Extracellular concentration of amino acids during CGP 35348 application in the dorsal lateral geniculate nucleus

Application of 5 mM CGP 35348 in the dorsal lateral geniculate nucleus increased the aspartate level throughout the test period, although no statistical significance was observed in the first sample collected (20 min data point in Fig. 2A) (165 ± 26%, $n = 6$). Thus, the concentration of aspartate reached its peak in the 2nd sample (212 ± 42%, $n = 6$, $P < 0.05$) and remained elevated throughout the course of the experiment (Fig. 2A). The change in the extracellular glutamate level only reached statistical significance in the first sample (128 ± 10%, $n = 6$, $P < 0.05$) (Fig. 2B). The extracellular taurine concentration in the dorsal lateral geniculate nucleus did not show any change during a 2 h perfusion with CGP 35348 (Fig. 2B).

3.4. Lack of effect of 2-hydroxy-saclofen on amino-acid levels in the ventrobasal complex

2-Hydroxy-saclofen (1–3 mM) was only tested in the ventrobasal complex of 9 rats ($n = 9$), and it did not have any effect on the extracellular concentration of aspartate, glutamate and taurine (Fig. 2A,B,C).

4. Discussion

The main conclusion of this investigation is that blockade of $GABA_B$ receptors by intrathalamic application of CGP 35348 increases the basal extracellular concentration of aspartate and (to a lesser extent) of glutamate in two sensory nuclei, the ventrobasal complex and the dorsal lateral geniculate nucleus.

4.1. Extracellular excitatory amino-acid levels are modulated by CGP 35348

No change in the concentration of taurine accompanied the increase in aspartate and glutamate levels produced by continuous perfusion of CGP 35348. Since taurine is the most potent amino acid in regulating osmolarity processes in the brain (Lehmann, 1990; Schousboe and Pasantes-Morales, 1992), we may therefore exclude the possibility of a non-specific amino-acid release produced by volume regulation by the neurones/glial cells around the dialysis probe during the 2 h application of CGP 35348.

Relatively high concentrations of CGP 35348 in the dialysis probes were used to achieve a concentration of the antagonist in the tissue around the probe similar to that achieved in previous in vivo experiments on the sleep-waking cycle (Juhász et al., 1994). In addition, because of the dilution factor, the effective concentration outside the probe reached in this experiment was similar to the concentration that has been shown in in vitro electrophysiological experiments to affect both pre- and postsynaptic $GABA_B$ receptors (Emri et al., 1996). Thus, the inability of CGP 34358 to change the extracellular levels of glutamate in the thalamus (Banerjee and Snead, 1995) and striatum (Waldmeier et al., 1992) could have been due to the much lower concentrations of CGP 35348 used in these studies.

The larger increase in aspartate (compared to glutamate) is in contrast with the results obtained with CGP 35348 in cortical synaptosomal preparations (Bonanno and Raiteri, 1993; Pende et al., 1993). A different sensitivity of aspartate and glutamate release to the modulation of presynaptic receptors in the hippocampus has been observed (Martin et al., 1991; Zhou et al., 1995), and the presence of a similar difference in the thalamus might explain the higher increase in aspartate levels observed in our experiments. Furthermore, glutamate and aspartate are taken up by high affinity glutamate/aspartate transporter systems present in neurones and glial cells (Currie and Kelly, 1981). The presence of several glutamate transporters with different affinities for glutamate and aspartate has been reported (Ferkaney and Coyle, 1986; Robinson et al., 1991; Balcar and Li, 1992), and it is therefore possible that the smaller and transient increase in glutamate concentration observed in this study might be the result of a more efficient removal of glutamate from the extracellular space.

Finally, the similarities of the effects produced by CGP 35348 application into the ventrobasal complex and the dorsal lateral geniculate nucleus indicate that the $GABA_B$ receptor-mediated processes regulating the extracellular concentration of aspartate and glutamate in these two sensory thalamic nuclei are virtually identical. It is also

worth noting that a tonic activation of presynaptic, but not of postsynaptic, $GABA_B$ receptors has been observed in vitro in the ventrobasal complex and the dorsal lateral geniculate nucleus. However, the ability of CGP 35348 to interact with pre- and postsynaptic $GABA_B$ receptors and the complicated thalamic network makes it impossible at present to distinguish between a tonic activation of either pre- and/or postsynaptic $GABA_B$ receptors as the mechanism underlying the increase in thalamic excitatory amino-acid concentration observed in this study.

4.2. 2-Hydroxy-saclofen had no effect on excitatory amino-acid levels

The extracellular level of amino acids did not change during continuous perfusion with 2-hydroxy-saclofen. In vitro investigations have revealed a partial agonist action of 2-hydroxy-saclofen both on pre- and postsynaptic $GABA_B$ receptors (Nathan et al., 1990; Caddick et al., 1995; Emri et al., 1996), and phaclofen, a close structural analogue of 2-hydroxy-saclofen, has been shown to be unable to modulate glutamate release in vitro and in vivo (Bonanno and Raiteri, 1993; Banerjee and Snead, 1995; Misgeld et al., 1995). Although CGP 35348 and 2-hydroxy-saclofen have been shown to be 'about equiactive' in other preparations (cf., Kerr and Ong, 1995), the present results indicate that the thalamic mechanism(s) controlling the basal levels of aspartate and glutamate are not affected by 2-hydroxy-saclofen.

Acknowledgements

We wish to thank Bob Jones for the photography. This work was supported by the Hungarian Science Foundation (grants OTKA T016552 and F016560) and by the Wellcome Trust (grant 37089). Zs.E. was supported by a Royal Society-Foreign and Commonwealth Office Fellowship.

References

Balcar, V.J. and Y. Li, 1992, Heterogenity of high affinity uptake of L-glutamate and L-aspartate in the mammalian central nervous system, Life Sci. 51, 1467.

Banerjee, P.K. and O.C. Snead III, 1995, Presynaptic gamma-hydroxybutyric acid (GHB) and gamma-aminobutyric acidB ($GABA_B$) receptor-mediated release of GABA and glutamate (GLU) in rat thalamic ventrobasal nucleus (Ventrobasal thalamus): a possible mechanism for the generation of absence-like seizures induced by GHB, J. Pharmacol. Exp. Ther. 273, 1534.

Bernasconi, R., T. Leonhardt, K. Stoecklin and P. Martin, 1992, Blockade of $GABA_B$ receptors may increase excitatory amino acid-mediated transmission, Pharmacol. Commun. 2, 28.

Bonanno, G. and M. Raiteri, 1993, Multiple $GABA_B$ receptors, Trends Pharmacol. Sci. 14, 259.

Bowery, N.G., D.L. Hill, A.L. Hudson, A. Doble, D.N. Middlemiss, J. Shaw and M. Turnbull, 1980, (−) Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor, Nature 283, 92.

Bowery, N.G., A.L. Hudson and G.W. Price, 1987, $GABA_A$ and $GABA_B$ receptor site distribution in the rat central nervous system, Neuroscience 20, 365.

Caddick, S.J., I.M. Stanford and J.E. Chad, 1995, 2-Hydroxy-saclofen causes a phaclofen-reversible reduction in population spike amplitude in the rat hippocampal slice, Eur. J. Pharmacol. 274, 41.

Chu, D.C.M., R.L. Albin, A.B. Young and J.B. Penney, 1990, Distribution and kinetics of $GABA_B$ binding sites in rat central nervous system: a quantitative autoradiographic study, Neuroscience 34, 341.

Crunelli, V. and N. Leresche, 1991, A role for $GABA_B$ receptors in excitation and inhibition of thalamocortical cells, Trends Neurosci. 14, 16.

Currie, D.N. and J.S. Kelly, 1981, Glial versus neuronal uptake of glutamate, J. Exp. Biol. 95, 181.

Emri, Zs., J.P. Turner and V. Crunelli, 1996, Tonic activation of presynaptic $GABA_B$ receptors on thalamic sensory afferents, Neuroscience 72, 689.

Ferkaney, J. and J.T. Coyle, 1986, Heterogenity of sodium dependent excitatory amino acid uptake mechanisms in rat brain, J. Neurosci. Res. 16, 491.

Juhász, G., J. Tarcali, K. Pungor and E. Pungor, 1989, Electrochemical calibration of in vivo brain dialysis samplers, J. Neurosci. Methods 29, 131.

Juhász, G., Zs. Emri, K.A. Kekesi, O. Salfay and V. Crunelli, 1994, Blockade of thalamic $GABA_B$ receptors decreases EEG synchronization, Neurosci. Lett. 172, 155.

Kerr, D.I.B., J. Ong, G.A.R. Johnston, J. Abbenante and R.H. Prager, 1988, 2-Hydroxy-saclofen: an improved antagonist at central and peripheral $GABA_B$ receptors, Neurosci. Lett. 92, 92.

Kerr, D.I.B. and J. Ong, 1995, $GABA_B$ receptors, Pharmacol. Ther. 67, 187.

Lee, S.M., M.H. Friedberg and F.F. Ebner, 1994, The role of GABA-mediated inhibition in the rat ventral posterior medial thalamus. II. Differential effects of $GABA_A$ and $GABA_B$ receptor antagonists on responses of VPM neurons, J. Neurophysiol. 71, 1716.

Le Feuvre, Y., A. Guyon and N. Leresche, 1995, $GABA_B$ autoreceptors modulate spontaneous $GABA_A$ IPSCs in thalamocortical neurones, Soc. Neurosci. Abstr. 21, 47. 11.

Lehmann, A., 1990, De-arrangements in cerebral osmohomeostasis: a common denominator for stimulation of taurine and phosphoethanolamine release, in: Taurine: Functional Neurochemistry, Physiology and Cardiology (Wiley-Liss, New York, NY) p. 337.

Liu, Z., M. Vergnes, A. Depalis and C. Marescaux, 1992, Involvement of intrathalamic $GABA_B$ neurotransmission in the control of absence seizures in the rat, Neuroscience 48, 87.

Martin, D., G.A. Bustos, M.A. Bowe, S.D. Bray and J.V. Nadler, 1991, Autoreceptor regulation of glutamate and aspartate release from slices of the hippocampal CA1 area, J. Neurochem. 56, 1647.

Misgeld, U., M. Bijak and W. Jarolimek, 1995, A physiological role for $GABA_B$ receptors and the effects of baclofen in the mammalian central nervous system, Prog. Neurobiol. 46, 423.

Nathan, T., M.S. Jensen and J.D.C. Lambert, 1990, $GABA_B$ receptors play a major role in paired-pulse facilitation in area CA1 of the rat hippocampus, Brain Res. 531, 55.

Pellegrino, L.J. and A.J. Cushman, 1967, A Stereotaxic Atlas of the Rat Brain (Appleton-Century-Crofts, New York, NY).

Pende, M., M. Lanza, G. Bonanno, M. Raiteri, 1993, Release of endogenous glutamic and aspartic acids from cerebrocortex synaptosomes and its modulation through activation of a gamma-aminobutyric acidB ($GABA_B$) receptor subtype, Brain Res. 604, 325.

Robinson, M.B., M. Hunter-Ensor and J. Sinor, 1991, Pharmacologically distinct sodium-dependent L[H3] glutamate transport process in rat brain, Brain Res. 544, 196.

Schousboe, A. and H. Pasantes-Morales, 1992, Role of taurine in neural cell volume regulation, Can. J. Physiol. Pharmacol. 70, S356.

Snead III, O.C., 1992, Evidence for $GABA_B$-mediated mechanisms in experimental generalised absence seizures, Eur. J. Pharmacol. 213, 343.

Soltesz, I., M. Haby, N. Leresche and V. Crunelli, 1988, The $GABA_B$ antagonist phaclofen inhibits the late K^+-dependent IPSP in cat and rat thalamic and hippocampal neurones, Brain Res. 448, 351.

Waldmeier, P.C., K. Stocklin and J.J. Feldtrauer, 1992, Systemic administration of baclofen and the $GABA_B$ antagonist, CGP 35348, does not affect GABA, glutamate or aspartate in microdialysates of the striatum of conscious rats, Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 548.

Zhou, M., C.L. Peterson, Y.B. Lu and J.V. Nadler, 1995, Release of glutamate and aspartate from CA1 synaptosomes: selective modulation of aspartate release by ionotropic glutamate receptor ligands, J. Neurochem. 64, 1556.