Baclofen and phaclofen modulate GABA release from slices of rat cerebral cortex and spinal cord but not from retina

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- 1 The effects of (–)-baclofen, muscimol and phaclofen on endogenous γ -aminobutyric acid (GABA) release from rat cortical slices, spinal cord slices and entire retinas were studied.
- 2 The spontaneous resting release of GABA from the three tissues was 3 to 6 pmol mg^{–1} wet wt 10 min^{–1}. Depolarization of cortical slices with KCl (50 mM) (high-K) produced an 8 fold increase in GABA release but high-K did not evoke an increased release of GABA from spinal slices or retinas.
- 3 When rats were injected with γ -vinyl-GABA (250 mg kg^{–1} i.p.) (GVG) 18 h before death, the tissue GABA stores were increased 3 to 6 fold and high-K then evoked striking Ca-dependent releases of GABA from all three tissues. Thus, in subsequent experiments, unless otherwise stated, the nervous tissues were taken from GVG-treated rats.
- 4 (–)-Baclofen (10 μ M) significantly reduced the K-evoked release of GABA from cortical and spinal slices but retinal release was not affected, even at a concentration of ($\pm$)-baclofen of 1 mM. For cortical slices, the IC₅₀ for baclofen was approximately 5.2 μ M. The inhibitory effect of baclofen on GABA release from cortical slices also occurred in slices prepared from saline-injected rats, indicating that GVG treatment did not qualitatively affect the results.
- 5 The inhibitory effect of (–)-baclofen on the K-evoked release of GABA from cortical and spinal slices was antagonised by phaclofen (500 μ M), confirming that baclofen was producing its effects by acting at the GABA_B-receptor.
- 6 Phaclofen (500 μ M) increased the spontaneous resting release of GABA from cortical slices taken from GVG-treated rats but not from saline-injected rats. Phaclofen did not increase GABA release from spinal slices or retinas taken from GVG-treated rats.
- 7 Baclofen (10 μ M) significantly reduced the K-evoked release from cortical slices of glutamate, aspartate, glycine and taurine.
- 8 Muscimol (10 μ M) and δ -aminolaevulinic acid (10 μ M) had no effect on either the resting or K-evoked release of GABA from cortical slices prepared from saline-injected or GVG-treated rats.
- 9 The results obtained with cortical and spinal slices are consistent with the presence of inhibitory GABA_B-autoreceptors. The phaclofen-induced increase in GABA release from cortical slices taken from GVG-treated rats, but not from saline-injected rats, implies that under conditions of high GABA release, considerable feedback inhibition is occurring via activation of the GABA_B inhibitory autoreceptors. No evidence was found for GABA_B-autoreceptors on retinal GABAergic amacrine cells or for GABA_A-autoreceptors in cortical slices or spinal cord slices.

Introduction

γ -Aminobutyric acid (GABA) is the major inhibitory transmitter substance in the central nervous system and it is now generally accepted that it acts on two types of receptor (Bowery, 1988). The GABA_A-

receptor, which is blocked by bicuculline and activated by muscimol, is linked to the chloride channel and is responsible for postsynaptic inhibition. The GABA_B-receptor is not blocked by bicuculline and is activated by baclofen. Until recently the absence of a specific GABA_B-antagonist has hindered our under-

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standing of the role of GABA_B-receptors. However, it is clear that many GABA_B-receptors are located on presynaptic nerve terminals and their activation inhibits transmitter release. Bowery *et al.* (1979) demonstrated that baclofen and GABA inhibited the release of [³H]-noradrenaline from rat isolated atria. Subsequently, inhibitory presynaptic GABA_B-receptors have been found in many peripheral tissues (see Hill & Bowery, 1986, for references) and in central neurones utilizing several transmitters, including dopamine (Bowery *et al.*, 1980), 5-hydroxytryptamine (5-HT, Gray & Green, 1987) and cholecystikinin (Conzelmann *et al.*, 1986). However, in the case of GABAergic neurones themselves, the acceptance of presynaptic inhibitory autoreceptors has been slow and there is still no consensus in the literature on the classification of these presumed GABA autoreceptors. In several studies, muscimol was found to decrease the K-evoked release of [³H]-GABA from brain slices or synaptosomes, suggesting the presence of GABA_A-autoreceptors on GABAergic nerve terminals (Mitchell & Martin, 1978; Brennan *et al.*, 1981; Kuriyama *et al.*, 1984). However, in similar experiments, others have found that baclofen, but not muscimol, decreased GABA release, suggesting the presence of GABA_B-autoreceptors (Anderson & Mitchell, 1985; Limberger *et al.*, 1986; Pittaluga *et al.*, 1987; Waldmeier *et al.*, 1988).

In the present study, we have attempted to clarify the status of GABA autoreceptors by examining the effects of GABA_A and GABA_B agonists and antagonists on the release of endogenous GABA from three areas in the central nervous system. Our experiments provide support for the presence of GABA_B-autoreceptors in cerebral cortex and spinal cord but not in the retina.

Part of this work has been published previously in a preliminary form (Shah & Neal, 1989).

Methods

Preparation of tissues

Male Wistar rats (150–200 g) were killed by cervical dislocation. The eyes were enucleated and the retinas dissected in Krebs-bicarbonate medium at room temperature. The brains were removed and slices of cerebral cortex (0.2 mm thick) were prepared by means of a McIlwain tissue chopper as described previously (Cunningham & Neal, 1981). The spinal cord was removed following an extensive laminectomy and transverse (0.3 mm thick) slices were again prepared by a McIlwain chopper (Hopkin & Neal, 1971). In most experiments, tissues were taken from animals that had been treated with the irreversible GABA-T inhibitor, γ -vinyl-GABA (GVG) (250 mg kg⁻¹ i.p.) 18 h before death.

Incubation of tissues

In order to measure the release of GABA from the tissues, slices of cortex (5–6 slices, weight 15 mg), spinal cord (5–6 slices, weight 15 mg), or two entire retinas (approx 20 mg) were placed in a small perspex chamber (volume 0.5 ml) containing Krebs-bicarbonate medium (250 μ l) at room temperature and were gently agitated by a stream of 5% CO₂ in oxygen.

At 10 min intervals, the medium was drained off and replaced with fresh medium. The GABA and other amino acids in each of the resulting 10 min samples were measured by high performance liquid chromatography (h.p.l.c.) (Yazulla *et al.*, 1985). Potassium-evoked release of GABA was achieved by exposing the tissues for one 10 min period to KCl (50 mM). The effects of drugs on the K-evoked release of GABA was usually examined by exposing the tissue to the drug in the collection period immediately preceding the addition of KCl and during exposure to KCl.

High performance liquid chromatography

The samples were analysed on a Varian 5000 h.p.l.c. by use of precolumn derivatisation with o-phthalaldehyde (Turnell & Cooper, 1981).

Statistical analysis

Differences between the resting release of GABA and the K-evoked release of GABA were tested for significance by use of Student's *t* test.

Materials

Incubation medium Krebs Ringer bicarbonate of the following composition was used (mM): NaCl 118, KCl 4.8, CaCl₂ 2.4, MgSO₄ 1.2, NaHCO₃ 25, KH₂PO₄ 1.2, glucose 9.5. The medium was continuously gassed with 95% O₂ plus 5% CO₂.

Drugs γ -Vinyl-GABA (vigabatrin) was a gift from Merrell-Dow Pharmaceuticals Ltd. (–)-Baclofen was a gift from Professor N.G. Bowery. Phaclofen was obtained from Cambridge Research Biochemicals. Other chemicals (Analar Grade) were obtained from Sigma Chemical Company Ltd.

Results

Effect of high-K on GABA release

When cortical slices were exposed for 10 min to medium containing KCl (50 mM) (high-K) the release of GABA increased from approximately 3 to 26

Figure 1 Effect of high-K on GABA release from rat cerebral cortex, spinal cord and retina. In this and subsequent figures, p represents release in sample preceding stimulation and s, release during exposure to KCl (50 mM). Each result is the mean of 8 experiments and bars show s.e.mean. High-K evoked a significant release of GABA only from cortical slices, s significantly different from p: * $P < 0.001$ (Student's *t* test).

Figure 2 Effect of high-K on GABA release from nervous tissues taken from rats treated with γ -vinyl-GABA (GVG) 18 h before death. Note that GVG increases the resting release of GABA and enables a K-evoked release of GABA from cord and retina to be measured. Each result is the mean of 4–9 experiments and bars show s.e.mean; K-evoked release (s) significantly different from resting release (p): * $P < 0.001$, ** $P < 0.002$.

Table 1 Effect of γ -vinyl-GABA (GVG) treatment on the GABA content of rat cerebral cortex, spinal cord and retina

Tissue	GABA content ($\mu\text{mol g}^{-1}$ wet wt)		GVG/saline
	Saline injected	GVG injected	
Cerebral cortex	2.17 ± 0.29	5.85 ± 0.42	2.7
Spinal cord	0.89 ± 0.095	2.37 ± 0.16	2.7
Retina	2.77 ± 0.23	17.77 ± 1.05	6.4

Rats were injected (i.p.) with either normal saline or GVG (250 mg kg^{-1}) 18 h before death. Each result is the mean $\pm$ s.e. mean of 10 experiments. Values obtained from saline-injected animals were not significantly different from those obtained from uninjected animals (not shown).

pmol mg^{-1} wet wt 10 min^{-1} (Figure 1). However, exposure of spinal slices and retinas to high-K did not evoke any measurable increase in GABA release although the spontaneous resting release of GABA from these tissues was similar to that from cortex (Figure 1).

Effect of γ -vinyl-GABA on GABA release

In order to increase the storage levels of GABA, rats were treated with GVG (250 mg kg^{-1} i.p.) 18 h before death. The effect of this treatment on GABA levels in the cerebral cortex, spinal cord and retina is shown in Table 1. The GABA content of the retina was increased more than 6 fold, with the cortex and spinal cord showing less striking increases in GABA

Table 2 Ca-dependence of K-evoked GABA release

Tissue	GABA release (pmol mg^{-1} wet wt 10 min^{-1})					
	Normal medium			High-Mg/low-Ca medium		
	p	s	s/p	p	s	s/p
Cortical slices (S) (5)	2.7 ± 0.34	$26.9 \pm 3.04^*$	10	3.2 ± 0.52	2.4 ± 0.27	0.75
Cortical slices (GVG) (6)	7.6 ± 1.03	$58.5 \pm 2.88^*$	7.7	4.6 ± 0.63	4.6 ± 1.03	1.0
Spinal slices (GVG) (4)	7.6 ± 0.69	$20.3 \pm 2.35^*$	2.7	8.5 ± 1.04	7.1 ± 0.83	0.84
Retina (GVG) (6)	21.2 ± 2.42	$52.6 \pm 12.32^\dagger$	2.5	4.6 ± 0.63	4.6 ± 1.04	1.0

(S) and (GVG) indicate rats were injected (i.p.) with normal saline or γ -vinyl-GABA 18 h before death, respectively. Each result is the mean $\pm$ s.e.mean of the number of experiments indicated in parentheses. p and s indicate resting release and K-evoked release, respectively. Significant evoked release * $P < 0.001$, $^\dagger P < 0.05$. High-Mg/low-Ca medium contained MgCl_2 (20 mM) and CaCl_2 (0.2 mM).

levels. The effect of GVG treatment on GABA release is illustrated in Figure 2. The spontaneous, resting release of GABA was increased from all three tissues but more importantly, high-K now evoked striking releases of GABA from the retina and spinal slices. In the case of cortical slices, not only was the resting release of GABA increased but the K-evoked release was almost doubled in slices from GVG-treated rats compared with controls (treated: 13 fold increase; untreated: 8 fold increase). GVG has been shown to cause the accumulation of GABA in glial cells (Neal *et al.*, 1989). However, release from glia seems unlikely in the present experiments because the K-evoked release from all three tissues was found to be highly Ca-dependent (Table 2). Since GVG treatment enabled us to measure a K-evoked, Ca-dependent GABA release from cord, retina and cortex, it was used in most of the subsequent experiments. Note that in the figures, solid columns indicate results obtained from untreated rats whilst open columns indicate results obtained from GVG-treated rats.

Effect of baclofen on GABA release from cortical slices

The effect of (–)-baclofen on GABA release in cortical slices from GVG-treated rats is shown in Figure 3. (–)-Baclofen (10 μM) did not affect the resting release of GABA but the K-evoked release was reduced by more than two thirds. It might be argued that the effects of baclofen could be abnormal because of the treatment of the animals with GVG. However, baclofen (10 μM) halved the K-evoked

Figure 3 Effect of (–)-baclofen (Bac, 10 μM) on the K-evoked release of GABA from cortical slices prepared from brains of γ -vinyl-GABA (GVG)-treated rats (a) and saline-injected rats (b). In both cases baclofen significantly depressed the K-evoked GABA release ($P < 0.01$). Each result is the mean of 4–8 experiments; bars show s.e.mean.

release of GABA in slices taken from animals not treated with GVG (Figure 3). Thus, it seems unlikely that GVG treatment fundamentally changes the mechanism of GABA release from nervous tissues. The EC_{50} of (–)-baclofen in inhibiting the K-evoked release of GABA was approximately 5.2 μM and 80% inhibition was produced by 20 μM baclofen.

Effect of phaclofen on GABA release from cortical slices

Exposure of cortical slices (from GVG treated rats) to the GABA_B receptor antagonist phaclofen (Kerr *et al.*, 1987) increased the spontaneous resting release of GABA 4 fold and apparently blocked the depressant action of (–)-baclofen (10 μM) on the K-evoked release of GABA (Figure 4). However, phaclofen alone was found to increase significantly the K-evoked release of GABA (Figure 4). The effect of phaclofen on the resting and K-evoked release of GABA was not observed in cortical slices from saline injected rats (not illustrated).

Figure 4 Effect of phaclofen (Phac, 500 μM) on GABA release from cortical slices (from γ -vinyl-GABA-treated rats). (c and d) Phaclofen significantly increased the resting release of GABA ($P < 0.01$) and reduced the effect of (–)-baclofen (Bac, 10 μM) on the K-evoked release of GABA (cross-hatched column in (c)). The control response to high-K is shown on the left (a) and the control response to high-K in the presence of phaclofen is shown on the right (d). Each result is the mean of 4 experiments, bars show s.e.mean.

Figure 5 Effect of (–)-baclofen (Bac, 10 μ M) (b) and phaclofen (Phac, 500 μ M) (d) on the K-evoked release of GABA from spinal slices prepared from rats treated with γ -vinyl-GABA. In the presence of baclofen and phaclofen (c) the K-evoked release (s) was not significantly different from the control K-evoked release (a). Baclofen alone abolished the K-evoked release of GABA. Phaclofen alone did not increase either the resting or the K-evoked release of GABA (right). Each result is the mean of 5 experiments; bars show s.e.mean. K-evoked release (s) significantly different from resting release (p); * $P < 0.002$, ** $P < 0.02$.

Effect of (–)-baclofen and phaclofen on GABA release from spinal slices

The effect of (–)-baclofen (10 μ M) on GABA release in spinal slices from GVG-treated rats is illustrated in Figure 5. Baclofen completely inhibited the K-evoked release of GABA from spinal slices. This effect of baclofen was abolished by phaclofen, but unlike the effect of the antagonist on cortical slices, phaclofen did not affect either the resting or K-evoked release of GABA (Figure 5).

Effect of baclofen and phaclofen on GABA release from the retina

Neither ($\pm$)-baclofen (at concentrations up to 1 mM) nor phaclofen (500 μ M) had any observable effect on the spontaneous resting release or the K-evoked release of GABA from retinas taken from GVG-treated rats (Figure 6).

Effect of baclofen on the K-evoked release of other amino acids from cortical slices

Exposure of cortical slices to high-K more than doubled the release of aspartate and glutamate. The release of glycine and taurine was also significantly increased by high-K but the evoked release of these amino acids was small (Figure 7). Baclofen (10 μ M)

Figure 6 Effect of ($\pm$)-baclofen (Bac, 1 mM) and phaclofen (Phac, 500 μ M) on GABA release in the retinas from γ -vinyl-GABA-treated rats. Neither the resting (p) nor the K-evoked (s) release of GABA was affected by the drugs. Each result is the mean of 4–9 experiments; vertical bars show s.e.mean.

abolished the K-evoked release of all four amino acids (Figure 7).

Effect of muscimol and δ -aminolaevulinic acid on GABA release from cortical slices

Exposure of cortical slices from GVG-treated and saline-injected rats to the GABA_A agonists muscimol and δ -aminolaevulinic acid did not have any effect on the resting or K-evoked release of GABA (Figure 8).

Figure 7 Effect of (–)-baclofen (10 μ M hatched columns) on the release of (a) aspartate, (b) glutamate, (c) glycine and (d) taurine from cortical slices prepared from the brains of rats treated with γ -vinyl-GABA. Open columns: release in the absence of baclofen. Each result is the mean of 9 experiments; vertical bars show s.e.mean. K⁺-evoked release (s) significantly different from resting release (p) * $P < 0.001$, ** $P < 0.05$.

Figure 8 Effect of (a and b) muscimol (10 μ M) and (c and d) δ -aminolaevulinic acid (δ -ALA) on the K-evoked release of GABA from cortical slices prepared from γ -vinyl-GABA treated rats (a and c) or saline-injected rats (b and d). Neither drug significantly reduced the K-evoked release of GABA. Each result is the mean of 4–6 experiments; vertical bars show s.e.mean.

Discussion

The present study clearly shows that the GABA_B agonist (–)-baclofen inhibits the K-evoked release of GABA from cortical slices and spinal slices prepared from rats treated with the irreversible GABA-T inhibitor GVG. The possibility that treatment of the animals with GVG fundamentally alters the GABA release process seems unlikely because baclofen had similar effects on the K-evoked release of GABA from cortical slices prepared from saline-injected animals.

The effect of baclofen on the K-evoked release of GABA is consistent with the idea that GABAergic nerve endings in the cortex and spinal cord possess inhibitory GABA_B-autoreceptors. This suggestion is strengthened, at least in the spinal cord, by the finding that the inhibitory action of baclofen on GABA release was completely abolished by the specific GABA_B-receptor antagonist, phaclofen (Kerr *et al.*, 1987; Dutar & Nicoll, 1988). Under 'normal' conditions, phaclofen did not affect the spontaneous resting release of GABA from cortical slices, but when the resting release was increased by treatment of the animals with GVG, phaclofen caused a striking further increase in both the resting and K-evoked release of GABA. This implies that in the cerebral cortex under conditions where the release of GABA is high, there is considerable feedback inhibition medi-

ated via the presumed GABA_B-autoreceptors. This result may have implications for the use of GVG as an antiepileptic drug, because an additional anti-convulsant action might be obtained by the concurrent administration of GVG with a GABA_B-receptor antagonist. However, for reasons which are unknown, phaclofen did not increase the resting release of GABA from spinal slices taken from GVG-treated rats, suggesting that a combination of these drugs may have additive effects only in certain brain regions.

In the present experiments, we cannot rule out the possibility that baclofen is acting on a feedback loop. However, further support for the suggestion that baclofen acts at GABA_B-receptors located on GABAergic nerve terminals has recently been provided by Bonanno *et al.* (1988). They showed that the K-evoked release of [³H]-GABA from rat cortical synaptosomes was inhibited by (–)-baclofen. This effect was antagonised by phaclofen but the conditions of their experiments were such that an effect on the spontaneous resting release of GABA was unlikely to be observed.

There have been several previous studies in which the effects of GABA agonists and antagonists on [³H]-GABA release from brain slices or synaptosomes have been examined. Some demonstrated that muscimol inhibited [³H]-GABA release, suggesting the presence of inhibitory GABA_A-autoreceptors (Mitchell & Martin, 1978; Brennan *et al.*, 1981; Kuriyama *et al.*, 1984). On the other hand, others found that muscimol was ineffective but that (–)-baclofen inhibited [³H]-GABA release, a result consistent with the presence of GABA_B-autoreceptors (Anderson & Mitchell, 1985; Limberger *et al.*, 1986; Pittaluga *et al.*, 1987). We have found no evidence for the presence of inhibitory GABA_A-autoreceptors in rat cerebral cortex or spinal cord. Neither muscimol nor δ -aminolaevulinic acid, at concentrations of 10 μ M, affected the release of endogenous GABA. It might be argued that this concentration was too low, but 10 μ M was the apparent IC₅₀ for the inhibitory action of δ -aminolaevulinic acid on the K-evoked release of [³H]-GABA from rat cortical synaptosomes (Brennan & Cantrill, 1979).

It is not clear why apparently similar experiments produce such different results. It may be that the use of [³H]-GABA, often in conjunction with amino-oxyacetic acid, resulted in the study of GABA release from inappropriate pools. It was for this reason that we chose to measure endogenous GABA release. Perhaps lower concentrations of K would have revealed an inhibitory action of muscimol. However, this seems unlikely as our results agree closely with those of Waldmeier *et al.* (1988), who found that baclofen, but not muscimol, decreased the

electrically-evoked release of endogenous GABA from slices of rat cerebral cortex, striatum and hippocampus. The suggestion that GABA autoreceptors are of the GABA_B-subtype has recently been greatly strengthened by electrophysiological studies, in which GABA-mediated monosynaptic inhibitory postsynaptic currents at synapses between embryonic rat hippocampal neurones in culture were measured. It was found that (–)-baclofen reduced inhibitory postsynaptic current amplitude but did not increase postsynaptic membrane conductance or block the postsynaptic response to GABA (Harrison, *et al.*, 1988).

There now seems to be a considerable amount of evidence from *in vitro* experiments that baclofen can decrease GABA release by an action on inhibitory GABA_B-autoreceptors. This has obvious implications for the mechanism of the antispastic action of baclofen, since it is difficult to see how a decrease in GABA release would produce this effect. It has been shown previously that baclofen can reduce the

release of glutamate and/or aspartate from a variety of CNS preparations (Potashner, 1979; Johnson *et al.*, 1980; Collins *et al.*, 1982; Kato *et al.*, 1982; Zhu & Chuang, 1987) and in the present study we have confirmed that the K-evoked release of these excitatory amino acid transmitters from cortical slices is inhibited by baclofen. In addition, we also found that the K-evoked release of glycine and taurine was reduced. We have not examined the effects of baclofen on glutamate/aspartate release from other areas of the CNS, but it seems that the drug may have widespread inhibitory actions on the release of both the main excitatory and inhibitory transmitters in the brain. Presumably, the antispastic effect of the drug occurs because the decrease in excitatory transmitter (glutamate/aspartate) release outweighs the decrease in inhibitory transmitter (GABA/glycine) release. We are at present trying to test this idea by examining the effects of baclofen on central amino acid release by use of *in vivo* dialysis.

References

- ANDERSON, R.A. & MITCHELL, R. (1985). Evidence for GABA_B autoreceptors in median eminence. *Eur. J. Pharmacol.*, **118**, 355–358.
- BONANNO, G., FONTANA, G. & RAITERI, M. (1988). Phaclofen antagonises GABA at autoreceptors regulating release in rat cerebral cortex. *Eur. J. Pharmacol.*, **154**, 223–224.
- BOWERY, N.G. (1988). Gamma-aminobutyric acid and benzodiazepine receptors. *Curr. Opin. Neurol. Neurosurg.*, **1**, 569–575.
- BOWERY, N.G., DOBLE, A., HILL, D.R., HUDSON, A.L., SHAW, J.S. & TURNBULL, N.G. (1979). Baclofen: a selective agonist for a novel type of GABA receptor. *Br. J. Pharmacol.*, **67**, 444P.
- BOWERY, N.G., HILL, D.R., HUDSON, A.L., DOBLE, A., MIDDELMISS, D.N., SHAW, J. & TURNBULL, M. (1980). (–)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel GABA receptor. *Nature*, **283**, 92–94.
- BRENNAN, M.J.W. & CANTRILL, R.C. (1979). δ -Aminolaevulinic acid is a potent agonist for GABA autoreceptor. *Nature*, **280**, 514–515.
- BRENNAN, M.J.W., CANTRILL, R.C., OLDFIELD, M. & KROGSGAARD-LARSEN, P. (1981). Inhibition of γ -aminobutyric acid release by γ -aminobutyric acid agonist drugs. *Pharmacology of the γ -aminobutyric acid receptor*. *Mol. Pharmacol.*, **19**, 27–30.
- COLLINS, G.G.S., ANSON, J. & KELLY, E.P. (1982). Baclofen: effects on evoked field potentials and amino acid neurotransmitter release in the rat olfactory cortex slice. *Brain Res.*, **238**, 371–383.
- CONZELMANN, U., MEYER, D.K. & SPERK, G. (1986). Stimulation of receptors of γ -aminobutyric acid modulates the release of cholecystokinin-like immunoreactivity from slices of rat neostriatum. *Br. J. Pharmacol.*, **89**, 845–852.
- CUNNINGHAM, J.R. & NEAL, M.J. (1981). On the mechanism by which veratridine causes a calcium-independent release of γ -aminobutyric acid from brain slices. *Br. J. Pharmacol.*, **73**, 655–667.
- DUTAR, P. & NICHOLL, R.A. (1988). A physiological role for GABA_B receptors in the central nervous system. *Nature*, **332**, 156–158.
- GRAY, J.A. & GREEN, A.R. (1987). GABA_B-receptor mediated inhibition of potassium-evoked release of endogenous 5-hydroxytryptamine from mouse frontal cortex. *Br. J. Pharmacol.*, **91**, 517–522.
- HARRISON, N.L., LANGE, G.D. & BARKER, J.L. (1988). (–)Baclofen activates presynaptic GABA_B receptors on GABAergic inhibitory neurones from embryonic rat hippocampus. *Neurosci. Lett.*, **85**, 105–109.
- HILL, D.R. & BOWERY, N.G. (1986). GABA-receptor-mediated functional responses in peripheral tissues. In *GABAergic Mechanisms in the Mammalian Periphery*. ed. Erdo, S.L. & Bowery, N.G. pp. 87–97. New York: Raven Press.
- HOPKIN, J. & NEAL, M.J. (1971). Effect of electrical stimulation and high potassium concentrations on the efflux of [¹⁴C] glycine from slices of spinal cord. *Br. J. Pharmacol.*, **42**, 215–223.
- JOHNSTON, G.A.R., HAILSTONE, M.H. & FREEMAN, C.G. (1980). Baclofen: stereoselective inhibition of excitant amino acid release. *J. Pharm. Pharmacol.*, **32**, 230–231.
- KATO, K., GOTO, M. & FUKADA, H. (1982). Baclofen: inhibition of the release of L-³H-glutamate and L-³H-aspartate from rat whole brain synaptosomes. *Gen. Pharmacol.*, **13**, 445–447.
- KERR, D.I.B., ONG, J., PRAGER, R.H., GYNTHNER, B.D. & CURTIS, D.R. (1987). Phaclofen: a peripheral and central baclofen antagonist. *Brain Res.*, **405**, 150–154.
- KURIYAMA, K., KANMORI, K., TAGUCHI, J. & YONEDA, Y. (1984). Stress-induced enhancement of suppression of

- [³H]GABA release from striatal slices by presynaptic autoreceptor. *J. Neurochem.*, **42**, 943–950.
- LIMBERGER, N., SPATH, L. & STARKE, K. (1986). A search for receptors modulating the release of γ -[³H]aminobutyric acid in rabbit caudate nucleus slices. *J. Neurochem.*, **46**, 1109–1117.
- MITCHELL, P.R. & MARTIN, I.L. (1978). Is GABA release modulated by presynaptic receptors? *Nature*, **274**, 904.
- NEAL, M.J., CUNNINGHAM, J.R., SHAH, M.A. & YAZULLA, S. (1989). Immunocytochemical evidence that vigabatrin in rats causes GABA accumulation in glial cells of the retina. *Neurosci. Lett.*, **98**, 29–32.
- PITTALUGA, A., ASARO, D., PELLEGRINI, G. & RAITERI, M. (1987). Studies on ³H-GABA and endogenous GABA release in rat cerebral cortex suggest the presence of autoreceptors of the GABA_B type. *Eur. J. Pharmacol.*, **144**, 45–52.
- POTASHNER, S.J. (1979). Baclofen: effects on amino acid release and metabolism in slices of guinea pig cerebral cortex. *J. Neurochem.*, **32**, 103–109.
- SHAH, M.A. & NEAL, M.J. (1989). Effect of baclofen on the release of GABA and other amino acids from rat cortex, spinal cord and retina. *Br. J. Pharmacol.*, **96**, 142P.
- TURNELL, D.C. & COOPER, J.D.H. (1981). Rapid assay for amino acids in serum or urine by precolumn derivatization and reversal phase liquid chromatography. *Clin. Chem.*, **28**, 101–118.
- WALDMEIER, P.C., WICKI, P., FELDTRAUER, J.J. & BAUMANN, P.A. (1988). Potential involvement of a baclofen-sensitive autoreceptor in the modulation of the release of endogenous GABA from rat brain slices *in vitro*. *Naunyn Schmiedebergs Arch. Pharmacol.*, **337**, 289–295.
- YAZULLA, S., CUNNINGHAM, J.R. & NEAL, M.J. (1985). Stimulated release of endogenous GABA and glycine from the goldfish retina. *Brain Res.*, **345**, 384–388.
- ZHU, X.Z. & CHUANG, D.M. (1987). Modulation of calcium uptake and D-aspartate release by GABA_B receptors in cultured cerebellar granule cells. *Eur. J. Pharmacol.*, **141**, 401–408.

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