Available online at www.sciencedirect.com

SCIENCE DIRECT

European Journal of Pharmacology 495 (2004) 137–143

www.elsevier.com/locate/ejphar

Gabapentin activates presynaptic $GABA_B$ heteroreceptors in rat cortical slices

David A.S. Parker[a], Jennifer Ong[b,*], Victor Marino[a], David I.B. Kerr[b]

[a] *Dental School, The University of Adelaide, Adelaide, South Australia 5005, Australia*
[b] *Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5005, Australia*

Received 19 February 2004; received in revised form 10 May 2004; accepted 14 May 2004
Available online 20 June 2004

Abstract

In electrically stimulated rat neocortical brain slices preloaded with [^{3}H]γ-aminobutyric acid (GABA) or [^{3}H]glutamic acid, the pharmacological actions of 1-(aminomethyl)-cyclohexaneacetic acid (gabapentin, Gp) were compared with the $GABA_B$ receptor agonists baclofen (Bac) and (3-amino-2-(*S*)-hydroxypropyl)-methylphosphinic acid (CGP 44532). Gabapentin, baclofen and CGP 44532 all reduced the electrically stimulated release of [^{3}H]glutamic acid (IC_{50} = 20 μM, 0.8 μM and 2 μM, respectively). These effects were sensitive to the $GABA_B$ receptor antagonists (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) or *N*-3-[[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino]-2-(*S*)-hydroxypropyl-*P*-(cyclo-hexylmethyl)-phosphinic acid (CGP 54626). By contrast, gabapentin was without effect on the release of [^{3}H]GABA, whilst baclofen (IC_{50} = 8 μM) and CGP 44532 (IC_{50} = 1 μM) inhibited [^{3}H]GABA release. It is concluded that gabapentin selectively activates presynaptic $GABA_B$ heteroreceptors, but not $GABA_B$ autoreceptors, and may be a useful ligand to discriminate between presynaptic $GABA_B$ receptor subtypes.

Keywords: GABA; Baclofen; Gabapentin

1. Introduction

1-(Aminomethyl)-cyclohexaneacetic acid (gabapentin; neurontin) is a novel lipophilic compound that readily enters the brain. It is a widely used anticonvulsant and antinociceptive drug that may possess antihyperalgesic and antiallodynic properties in inflammatory, surgical and neuropathic pain (Field et al., 1997, 2002; Hwang and Yaksh, 1997; Shimoyama et al., 1997); however, the mode of action of gabapentin remains somewhat of an enigma (Taylor et al., 1998). Gabapentin does not bind with high affinity to either γ-aminobutyric $acid_A$ ($GABA_A$) or $GABA_B$ receptors, nor does it influence GABA uptake, inhibit GABA-metabolic enzymes (Taylor, 1994) or affect GABA-induced neuronal responses (Rock et al., 1993). However, it binds with nanomolar affinity to the auxiliary α2δ subunit of voltage-dependent Ca^{2+} channels (Gee et al., 1996) and inhibits high-threshold neuronal $Ca2^+$ currents (Stefani et al., 1997), but whether this can account for the anticonvulsant and antinociceptive actions remains unknown.

$GABA_B$ receptors belong to Family 3 of the G-protein-coupled metabotropic receptors and are activated by the neurotransmitter GABA that mediates neuronal inhibition. These receptors are heterodimers of two subunits, $GABA_{B1}$ and $GABA_{B2}$, which are essential for functional expression, as well as receptor activation and ligand specificity (Bowery and Enna, 2000; Vacher and Bettler, 2003). The most abundant $GABA_B$ subunits are the two N-terminal splice variants, $GABA_{B1a}$ and $GABA_{B1b}$, and $GABA_{B2}$ subunits, which, together, mediate $GABA_B$ receptor functions in the brain. $GABA_B$ receptors either activate inwardly rectifying K^+ channels or inhibit voltage-gated Ca^{2+} channels in both pre- and postsynaptic components of excitatory and inhibitory neurons of the central nervous system (Kerr and Ong, 2001; Vacher and Bettler, 2003). Stimulation of presynaptic $GABA_B$ heteroreceptors decreases neurotransmitter release by reducing Ca^{2+} conductance, whilst activation of postsynaptic $GABA_B$ recep-

* Corresponding author. Tel.: +61-8-8303-5163; fax: +61-8-8303-3788.
E-mail address: jennifer.ong@adelaide.edu.au (J. Ong).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.05.029

tors increases a K^+ conductance (KIR) responsible for the long-lasting hyperpolarizing inhibitory potentials (Misgeld et al., 1995).

There are conflicting reports on the pharmacological effects of gabapentin on $GABA_B$ receptors. For instance, it has been suggested that, in hippocampal neurons, gabapentin is a $GABA_B$ receptor subtype-selective agonist, activating postsynaptic K^+ currents without presynaptic actions (Bertrand et al., 2001, 2003; Ng et al., 2001); yet, gabapentin itself does not have any agonist activity on $GABA_B$ receptors, in a variety of preparations (Lanneau et al., 2001; Jensen et al., 2002). Although gabapentin has been shown to inhibit K^+-evoked glutamate release from rat brain neocortical slices, it is not known if this effect is mediated through $GABA_B$ receptors (Dooley et al., 2000). Moreover, any pharmacological actions of gabapentin on electrically stimulated release of [^{3}H]GABA and [^{3}H]glutamic acid from rat neocortical slices have not been examined previously on presynaptic $GABA_B$ auto- and heteroreceptors modulating transmitter release. In this study, we show that gabapentin reduced the electrically stimulated release of [^{3}H]glutamic acid but was without effect on the release of [^{3}H]GABA. Using the same neurochemical assays, we have also compared the pharmacological actions of gabapentin with those of the known $GABA_B$ receptor agonists baclofen (Bac) and the phosphinic acid analogue of β-hydroxy-GABA, (3-amino-2-(*S*)-hydroxypropyl)-methylphosphinic acid (CGP 44532; Froestl et al., 1995; Ong et al., 2001). It is concluded that, unlike the latter two agonists, at least in this paradigm, gabapentin activates presynaptic $GABA_B$ heteroreceptors, but not $GABA_B$ autoreceptors, and that it may be a useful ligand to discriminate between presynaptic $GABA_B$ receptor subtypes.

2. Materials and methods

Rat neocortical slices were prepared as described previously (Ong et al., 1990). Briefly, outbred male adult Sprague–Dawley rats (250–350 g) were anaesthetized with halothane and were decapitated. All animal experimentations were carried out according to the guidelines of the Australian Code of Practice for the care and use of animals for scientific purposes, of the National Health and Medical Research Council and of the University of Adelaide Animal Ethics Committee. The brains were removed rapidly and were then immersed for 30 min in ice-cold oxygenated Krebs solution (95% O_2:5% CO_2; pH 7.4), prior to the preparation of coronal slices (400 μm thick) using a vibraslice microtome (Campden Instruments, UK).

2.1. Incubation in [^{3}H]GABA

Slices of neocortex were equilibrated in warm, gassed Krebs solution (37 °C; 95% O_2:5% CO_2) for at least 40 min prior to incubation in Krebs solution containing GABA (0.05 μmol/l) and [^{3}H]GABA (0.05 μmol/l) for 30 min. Pairs of slices were rinsed, placed in small chambers and then superfused at 1 ml/min with Krebs solution containing the GABA uptake inhibitor 1-(2-(((diphenylmethylene)imino)oxy)ethyl)-1,2,5,6-tetrahydro-3-pyridinecarboxylic acid (NO-711; 5 μmol/l; 37 °C, gassed). Aliquots of superfusate were collected at 10-min intervals for the first four collections and for 4 min thereafter and their ^{3}H contents were assayed by liquid scintillation spectrometry. Slices were stimulated through platinum field electrodes by square wave pulses at 2 Hz (2.0 ms duration, 50 V) for 60 pulses at 10 min and for 300 pulses at 48 min (S_1) and 76 min (S_2) after superfusion commenced. At the end of each experiment, the residual ^{3}H content in the slices was extracted in 0.4 mol/l $HClO_4$ (containing EDTA, 3.0 mmol/l; Na_2SO_3, 10 mmol/l) at 4 °C for at least 16 h and was then assayed. From this data, the fractional overflow of ^{3}H during each collection period was calculated.

When required, the $GABA_B$ receptor antagonists, (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911) or *N*-3-[[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino]-2-(*S*)-hydroxypropyl-*P*-(cyclo-hexylmethyl)-phosphinic acid (CGP 54626), were added to the superfusion medium 20 min before S_2, and the agonists, either baclofen, gabapentin or CGP 44532, were added 10–12 min prior to S_2.

2.2. Incubation in [^{3}H]glutamic acid

Slices of neocortex were equilibrated in Krebs as described above and were incubated in Krebs solution containing glutamic acid (0.3 μmol/l) and [^{3}H]glutamic acid (0.3 μmol/l) for 45 min. During the equilibration and incubation periods, the Krebs solution contained semicarbazide (100 μmol/l) to inhibit the metabolism of glutamic acid. Pairs of slices were superfused at 1 ml/min with Krebs solution as described above. Aliquots of superfusate were collected at 8-min intervals and were assayed by liquid scintillation spectrometry. Slices were stimulated at 2 Hz (2.0 ms duration, 50 V) for 60 pulses at 16 min, 500 pulses at 64 min (S_1) and at 104 min (S_2), after superfusion commenced. Agonists (baclofen, gabapentin and CGP 44532) and antagonists (Sch 50911 and CGP 54626) were added to the superfusion medium prior to S_2 at the times described here. Amino acid analysis using *o*-phthaldialdehyde derivatization with high-performance liquid chromatography separation confirmed that an excess of 85% of the tritium, which overflowed in the 8 min from the commencement of stimulation, was glutamic acid.

2.3. Resting and stimulation-induced overflows

The resting overflow of ^{3}H (R_1 or R_2) is defined as the fractional overflow in the 8 min prior to stimulation (S_1 or

S_2). The effects of agonists and/or antagonists on the resting overflow of ^3H were determined by comparing the R_2/R_1 ratio with that in appropriate control slices.

The stimulation-induced overflows of ^3H at S_1 and S_2, SIO_1 and SIO_2 respectively, were calculated by subtracting the resting overflow (8 min) from the fractional overflow in the 8 min following the onset of stimulation. The effect of an agonist or antagonist was determined by comparing the SIO_2/SIO_1 ratio in the presence of the agent with that in control tissue.

2.4. Solutions

Krebs solution was of the following composition (mmol/l): NaCl (120), KCl (4.7), $NaHCO_3$ (25), KH_2PO_4 (1.0), $CaCl_2$ (2.0), $MgCl_2$ (1.3) and glucose (5.5); and contained aminooxyacetic acid (0.05 mmol/l) for the [^{3}H]GABA experiments.

2.5. Statistical analysis

The significance of the effects of an agonist, or antagonist, was assessed by unpaired Student's *t*-test, with significance levels at $p < 0.05$.

2.6. Drugs and chemicals

2, 3-[^{3}H][N]GABA, specific activity 1.06 TBq/mmol and L-[3,4-^{3}H]glutamic acid, specific activity 1.89 TBq/mmol, were obtained from New England Nuclear (Boston, MA, USA). CGP 54626 and CGP 44532 were gifts from Novartis (Basle, Switzerland). Aminooxyacetic acid hemihydrochloride and semicarbazide were purchased from Sigma (Missouri, USA), whilst the GABA uptake inhibitor NO-711 and baclofen were obtained from Research Biochemicals (Natick, MA, USA). Sch 50911 was a gift from Schering-Plough (USA) and gabapentin was a gift from Pfizer (Australia).

3. Results

3.1. Release of ^3H from neocortical slices incubated in [^{3}H]GABA

The overflow of ^3H from neocortical slices preincubated in [^{3}H]GABA resulted in an SIO_2/SIO_1 ratio of 0.94 ± 0.03 ($n = 12$). For comparative purposes, the SIO_2/SIO_1 ratios in the presence of either the agonists or the antagonists were standardised against an SIO_2/SIO_1 ratio of 1.0 in untreated slices, and were then expressed as a percentage.

As shown in the concentration–response curves, CGP 44532 was a more potent inhibitor of the release and overflow of ^3H than baclofen, in that the maximal inhibition (at 200 μM) was 61% as against 48% for baclofen, and the estimated EC_{50} was 1 μM as against 8 μM for baclofen (Fig. 1). Gabapentin had little or no effect on the SI overflow of [^{3}H]GABA, except at a concentration of 100 μM, which produced an inhibition of 12%.

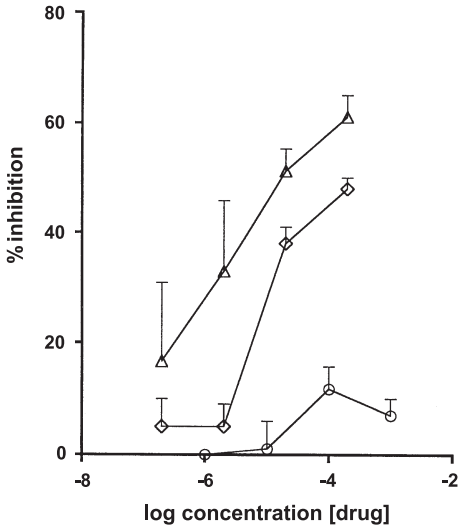

Fig. 1. Concentration–response curves for the inhibitory effects of baclofen (◇) gabapentin (○) and (3-amino-2-(*S*)-hydroxypropyl)-methylphosphinic acid [CGP 44532; (△)] on the stimulation-induced overflow of ^3H in neocortical slices preincubated in [^{3}H]GABA. Values shown represent the inhibitory effect of each agent on the overflow, expressed as a percentage of the overflow in the absence of the agent. Data are the means and standard errors of the means of at least four experiments, except baclofen (0.2 mM) and gabapentin (1.0 mM) where $n = 3$.

3.2. Release of ^3H from neocortical slices incubated in [^{3}H]glutamic acid

The SIO_2/SIO_1 ratio in untreated neocortical slices was 0.90 ± 0.06 ($n = 8$). As described above (see 3.1), the SIO_2/SIO_1 ratios in the presence of agonists or antagonists were standardised against that in the absence of agents, and were expressed as a percentage.

In these experiments, the maximal inhibition elicited by gabapentin on the overflow of ^3H was similar to that with baclofen, although less than with CGP 44532. From the concentration–response curves, the EC_{50} for each agent was 0.8 μM for baclofen, 2 μM for CGP 44532 and 20 μM for gabapentin (Fig. 2).

3.3. Effects of either agonists or antagonists alone, or in combination, on the overflow of ^3H from neocortical slices preincubated with [^{3}H]GABA or [^{3}H]glutamic acid

3.3.1. Baclofen, Sch 50911 and CGP 54626

At the higher concentration of baclofen used in this study (GABA series, 20 μM; glutamic acid series, 10 μM), the agonist inhibited the SI overflow of ^3H by approximately 40% (Fig. 3). In contrast, while the $GABA_B$ receptor antagonist, Sch 50911 (10 μM), enhanced the release of [^{3}H]GABA by 140%, it had little effect on the SI overflow in tissues preincubated in [^{3}H]glutamic acid. Sch 50911

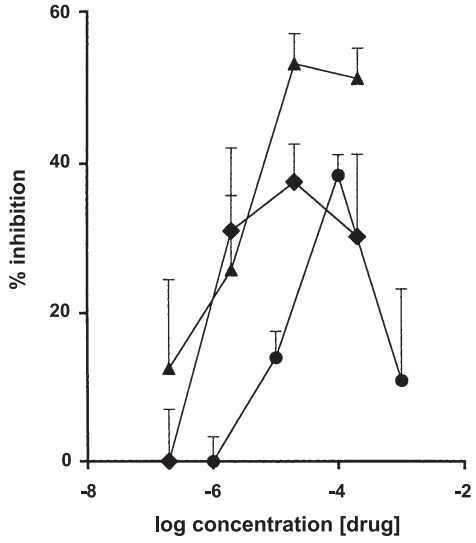

Fig. 2. Concentration–response curves for the inhibitory effects of baclofen (♦), gabapentin (●) and (3-amino-2-(*S*)-hydroxypropyl)-methylphosphinic acid [CGP 44532; (▲)] on the stimulation-induced overflow of ^{3}H in neocortical slices preincubated in [^{3}H]glutamic acid. Values shown represent the inhibitory effect of each agent on the overflow, expressed as a percentage of the overflow in the absence of the agent. Data are the means and standard errors of the means of at least four experiments, except CGP 44532 (0.2 mM) where $n = 3$.

reversed the inhibitory effects of baclofen on the release of both [^{3}H]GABA and [^{3}H]glutamic acid; although in the latter experiments, the SI overflow remained slightly less than that in untreated tissues. The other antagonist, CGP 54626 (0.1 μM), increased the SI overflow of ^{3}H by 71% in the GABA experiments but had no effect in the glutamic

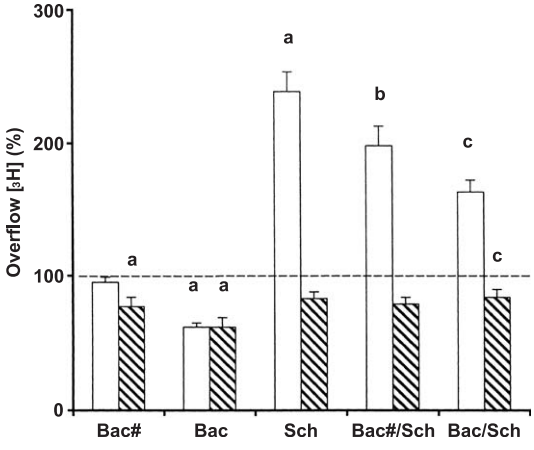

Fig. 3. The effects of baclofen (Bac)* and (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911; Sch; 10 μM) on the stimulation-induced overflow of ^{3}H from rat neocortical slices preincubated with either [^{3}H]GABA (0.1 μM, open columns) or [^{3}H]glutamic acid (0.6 μM, hatched columns). Results are expressed as a percentage of the overflow in untreated slices (100%) represented by the broken line ($n = 11$). *The concentration of baclofen was 2(#) or 20 μM in the [^{3}H]GABA experiments and 1(#) or 10 μM in the [^{3}H]glutamic acid experiments. [a]Effects of baclofen or Sch 50911 in untreated slices (unpaired Students' *t*-test; $p < 0.05$). [b,c]Effects of Sch 50911 in baclofen-treated slices (unpaired Students' *t*-test; $p < 0.05$; $n =$ at least five experiments).

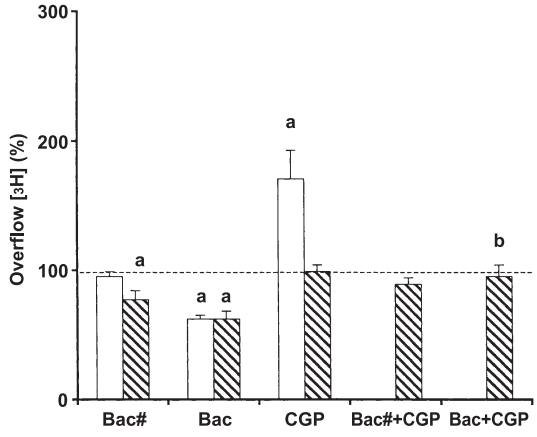

Fig. 4. The effects of *N*-3-[[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino]-2-(*S*)-hydroxypropyl-*P*-(cyclo-hexylmethyl)-phosphinic acid (CGP 54626; CGP; 0.1 μM), in the presence and absence of baclofen (Bac)*, on the stimulation-induced overflow of ^{3}H from rat neocortical slices preincubated with either [^{3}H]GABA (0.1 μM, open columns) or [^{3}H]glutamic acid (0.6 μM, hatched columns). Results are expressed as a percentage of the overflow in untreated slices (100%) represented by the broken line ($n = 11$). The effect of CGP 54626 on the release of [^{3}H]GABA in baclofen-treated slices was not examined. *The concentration of baclofen was 2(#) or 20 μM in the [^{3}H]GABA experiments and 1(#) or 10 μM in the [^{3}H]glutamic acid experiments. [a]Effects of baclofen or CGP 54626 in untreated tissue (unpaired Students' *t*-test; $p < 0.05$). [b]Effects of CGP 54626 in baclofen-treated tissue (unpaired Students' *t*-test; $p < 0.05$; $n =$ at least five experiments).

acid series (Fig. 4). While not tested in the GABA series, CGP 54626 prevented the inhibitory effect of baclofen (10 μM) on the SI overflow of ^{3}H in the glutamic acid series.

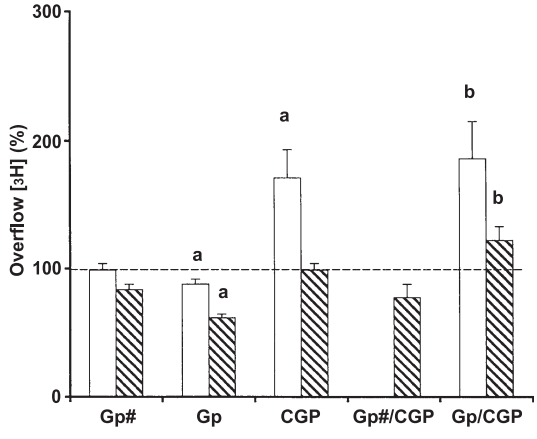

Fig. 5. The effects of gabapentin [Gp; 10(#) or 100 μM] and *N*-3-[[1-(*S*)-(3,4-dichlorophenyl)ethyl]amino]-2-(*S*)-hydroxypropyl-*P*-(cyclo-hexyl-methyl)-phosphinic acid (CGP 54626; CGP; 0.1 μM) on the stimulation-induced overflow of ^{3}H from rat neocortical slices preincubated with either [^{3}H]GABA (0.1 μM, open columns) or [^{3}H]glutamic acid (0.6 μM, hatched columns). Results are expressed as a percentage of the overflow in untreated slices (100%) represented by the broken line ($n = 11$). The effect of CGP 54626 in gabapentin-treated slices in the [^{3}H]GABA series was not tested. [a]Effects of gabapentin or CGP 54626 in untreated neocortical slices (unpaired Students' *t*-test; $p < 0.05$). [b]Effects of CGP 54626 in gabapentin (100 μM)-treated tissues (unpaired Students' *t*-test; $p < 0.05$; $n =$ at least five experiments).

In slices pretreated with [^{3}H]GABA, while baclofen (2 μM) did not affect the SI overflow of ^{3}H, Sch 50911, in the presence of baclofen, enhanced the overflow by 98% (Fig. 3). In contrast, in the glutamic acid experiments, baclofen (1 μM) inhibited the overflow of ^{3}H by 23%; an inhibition that was not reversed by either Sch 50911 (10 μM) or CGP 54626 (0.1 μM; Figs. 3 and 4).

3.3.2. Gabapentin, CGP 44532, SCH 50911 and CGP 54626

The effects of gabapentin (10 and 100 μM) and of CGP 44532 (2 and 20 μM) on the SI overflow of ^{3}H from slices pretreated with either [^{3}H]GABA or [^{3}H]glutamic acid, in the presence and absence of the $GABA_B$ receptor antagonists Sch 50911 and CGP 54626, are shown in Figs. 5 and 6. Gabapentin (100 μM) inhibited the overflow of ^{3}H in both series of experiments, although the magnitude of the inhibition in the GABA experiments was small (12%; Fig. 5). At the lower concentration (10 μM), gabapentin had little or no effect. Whilst the agonist CGP 44532 (10 and 100 μM) inhibited the SI overflow, inhibition was greater at the higher concentration (Fig. 6). Sch 50911 (10 μM) reversed the effects of CGP 44532 in the GABA experiments, but only at the higher concentration of CGP 44532 (20 μM) in the glutamic acid series. An alternate $GABA_B$ receptor antagonist, CGP 54626 (0.1 μM), enhanced the SI overflow of ^{3}H in slices preincubated in [^{3}H]GABA, but not [^{3}H]glutamic acid, and reversed the inhibitory action of gabapentin (100 μM) in both series of experiments (Fig. 5).

The resting overflows of ^{3}H in neocortical slices preincubated with [^{3}H]glutamic acid were unaffected by any of the agents tested; however, in those tissues incubated with [^{3}H]GABA, the antagonists Sch 50911 (10 μM) and CGP 54626 (0.1 μM) increased the mean resting overflows by 24% and 52%, respectively. These effects of the antagonists were also noted in neocortical slices that were treated with the agonists.

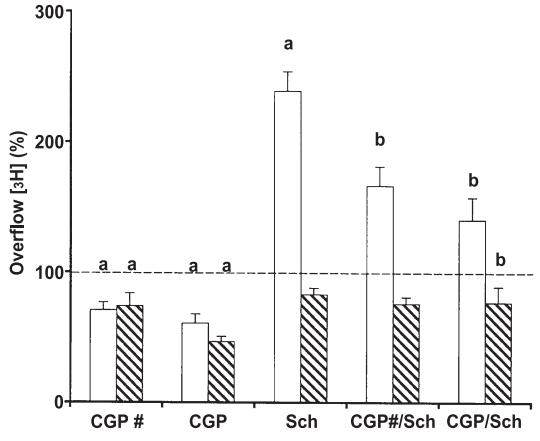

Fig. 6. The effects of (3-amino-2-(*S*)-hydroxypropyl)-methylphosphinic acid (CGP 44532; CGP; 2(#) and 20 μM) and (+)-(*S*)-5,5 dimethylmorpholinyl-2-acetic acid (Sch 50911; Sch; 10 μM) on the stimulation-induced overflow of ^{3}H from rat neocortical slices preincubated with either [^{3}H]GABA (0.1 μM, open columns) or [^{3}H]glutamic acid (0.6 μM, hatched columns). Results are expressed as a percentage of the overflow in untreated slices (100%) represented by the broken line ($n = 11$). [a]Effects of CGP 44532 or Sch 50911 in untreated neocortical slices (unpaired Students' *t*-test; $p < 0.05$). [b]Effects of Sch 50911 in neocortical slices treated with CGP 44532 (unpaired Students' *t*-test; $p < 0.05$; n = at least five experiments).

4. Discussion

Despite the conventional notion that gabapentin is a structural analogue of GABA (Satzinger, 1994), it is a spiro-compound that assumes a conformation where the acid and amine moieties mimic an α-amino acid and thus might not be expected to interact with any GABA receptor (Bryans et al., 1997). Yet, recent studies have demonstrated agonist actions of gabapentin on $GABA_{B1a}$ and $GABA_{B2}$ heterodimers coupled to Kir 3.1/3.2, inwardly rectifying K^+ channels in Xenopus oocytes, as well as on postsynaptic $GABA_B$ receptors in hippocampal neurons (Ng et al., 2001). Gabapentin also inhibits membrane Ca^{2+} mobilization in a mouse pituitary-intermediate malanotrope clonal cell line which expresses endogenous $GABA_{B1a}$ and $GABA_{B2}$ receptors. Likewise, it depresses voltage-dependent Ca^{2+}channels in hippocampal neurons (Bertrand et al., 2001). These results point to a subtype-selective agonist action of gabapentin on the $GABA_{B1a}$/$GABA_{B2}$ heterodimers without any effects on the $GABA_{B1b}$/$GABA_{B2}$ subunits (Bertrand et al., 2001, 2003). This is surprising because gabapentin does not appear to bind to $GABA_B$ receptors in radioligand binding studies (Taylor, 1995).

Gabapentin does bind to an α-amino acid binding site in the brain; a binding that is inhibited by branched-chain L-amino-acids such as L-leucine (Hill et al., 1993). Because the distribution of gabapentin binding overlaps with that of $GABA_B$ receptors in the central nervous system (see Ng et al., 2001), the above finding could be interpreted to mean that gabapentin acts as a modulator at such sites. Indeed, we have shown earlier that a range of L-α-amino acids are $GABA_B$ receptor modulators at $GABA_B$ receptor sites, potentiating $GABA_B$ receptor-mediated hyperpolarizing responses in rat brain (Kerr and Ong, 2003). Consistent with this hypothesis, our preliminary studies have shown a modulatory action of gabapentin on baclofen-induced hyperpolarizations (Ong and Kerr, unpublished observations). The pharmacological action of gabapentin on $GABA_{B1a}$/$GABA_{B2}$ heterodimers might then be that of modulation of the actions of endogenous GABA, mediated through gabapentin acting at these α-amino acid binding sites on the $GABA_B$ receptors. Although gabapentin does not appear to have any action on $GABA_B$ receptors in some preparations (Lanneau et al., 2001; Jensen et al., 2002), in this study, we found that gabapentin activates presynaptic $GABA_B$ heteroreceptors modulating electrically evoked [^{3}H]glutamic acid release from rat neocortical slices. Fur-

thermore, such agonist actions were found to be mediated through $GABA_B$ receptor sites because they were sensitive to the $GABA_B$ receptor antagonists Sch 50911 and CGP 54626 (Froestl et al., 1996; Ong et al., 1998).

When compared to the activities of baclofen and CGP 44532 on heteroreceptors, gabapentin was considerably less potent in reducing electrically stimulated [^{3}H]glutamic acid release. Here, the IC_{50} for gabapentin was 20 μM, close to the EC_{50} value of gabapentin (15 μM) acting on $GABA_{B1a}$/$GABA_{B2}$ heterodimeric receptors in Xenopus oocytes (Ng et al., 2001).

In the present study, gabapentin had little or no effect on $GABA_B$ autoreceptors modulating electrically stimulated [^{3}H]GABA release from rat neocortical slices, producing only a small inhibition (12%) at a concentration of 100 μM. This concentration lies within the upper range of the estimated therapeutic concentrations present in plasma and brain tissue (Dooley et al., 2000). Consistent with these findings, Ng et al. (2001) showed that gabapentin did not depress monosynaptic $GABA_A$ inhibitory postsynaptic currents, suggesting a lack of effect on presynaptic $GABA_B$ autoreceptors in hippocampal neurons; nor did it affect inhibitory postsynaptic currents in the hippocampus from any strains of mice (Bertrand et al., 2003). In addition, in adult superficial dorsal horn neurons, gabapentin (100 μM) did not alter the frequency or the amplitude of spontaneous excitatory and inhibitory postsynaptic currents, implying that presynaptic transmitter release was unaffected (Moore et al., 2002). However, gabapentin at 300 μM inhibited inhibitory postsynaptic currents recorded from CA1 pyramidal cells in rat hippocampus (van Hooft et al., 2002). In the present experiments, the $GABA_B$ receptor agonists, CGP 44532 and baclofen, reduced [^{3}H]GABA release with IC_{50} values of 1 μM and 8 μM, respectively, similar to our previous study (Ong et al., 2001), whereas gabapentin was rather inactive. This suggests that gabapentin is without effect on $GABA_B$ autoreceptors, whilst both CGP 44532 and baclofen inhibit [^{3}H]GABA release through activating $GABA_B$ autoreceptors sensitive to the antagonists Sch 50911 and CGP 54626. As previously reported, the binding affinity of CGP 44532 (IC_{50} = 45 nM) for $GABA_B$ receptors in rat cerebral cortex is higher than that of baclofen (IC_{50} = 109 nM) and about twice as active as baclofen in inhibiting electrically stimulated GABA release from cortical slices (Froestl et al., 1995).

In conclusion, presynaptic transmitter release in rat neocortical slices was differentially affected by gabapentin; the latter showing a preferential activation of presynaptic $GABA_B$ heteroreceptors regulating [^{3}H]glutamic acid release. By contrast, gabapentin did not have any significant effects on $GABA_B$ autoreceptors modulating [^{3}H]GABA release. Such discrimination by gabapentin between $GABA_B$ receptor subtypes is difficult to explain because gabapentin does not display any distinct pharmacological actions at presynaptic $GABA_B$ receptors in previous studies (Lanneau et al., 2001; Ng et al., 2001). Nevertheless, the mechanism(s) of actions of gabapentin are obviously complex, and further studies are required to clarify any effects of gabapentin at $GABA_B$ receptor-mediated transmission in relation to heteroreceptor control in nociception, spasticity and anticonvulsant actions.

Acknowledgements

We thank the following for the gifts of the various chemicals: Pfizer (Australia) for gabapentin, Dr. David Blythin (Schering-Plough, USA) for Sch 50911 and Dr. Wolfgang Froestl (Novartis, Switzerland) for CGP 44532 and CGP 54626.

References

Bertrand, S., Ng, Y.K., Purisai, M.G., Wolfe, S.E., Severidt, M.W., Nouel, D., Robitaille, R., Low, M.J., O'Neill, G.P., Metters, K., Lacaille, J.C., Chronwall, B.M., Morris, S.J., 2001. The anticonvulsant, antihyperalgesic agent gabapentin is an agonist at brain γ-aminobutyric acid type B receptors negatively coupled to voltage-dependent calcium channels. J. Pharmacol. Exp. Ther. 298, 15–24.

Bertrand, S., Nouel, D., Morin, F., Nagy, F., Lacaille, J.C., 2003. Gabapentin actions on Kir3 currents and *N*-type Ca^{2+} channels via $GABA_B$ receptors in hippocampal pyramidal cells. Synapse 50, 95–109.

Bowery, N.G., Enna, S.J., 2000. γ-Aminobutyric $acid_B$ receptors: first of the functional metabotropic heterodimers. J. Pharmacol. Exp. Ther. 292, 2–7.

Bryans, J.S., Davies, N., Gee, N.S., Horwell, D.C., Kneen, C.O., Morrell, A.I., O'Neill, J.A., Ratcliffe, G.S., 1997. Investigation into the preferred conformation of gabapentin for interaction with its binding site on the α2δ subunit of a calcium channel. Bioorg. Med. Chem. Lett. 7, 2481–2484.

Dooley, D.J., Mieske, C.A., Borosky, S.A., 2000. Inhibition of K^+-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. Neurosci. Lett. 280, 107–110.

Field, M.J., Oles, R.J., Lewis, A.S., McCleary, S., Hughes, J., Singh, L., 1997. Gabapentin (neurontin) and *S*-(+)-3-isobutylgaba represent a novel class of selective antihyperalgesia agents. Br. J. Pharmacol. 121, 1513–1522.

Field, M.J., Gonzalez, I., Tallarida, R.J., Singh, L., 2002. Gabapentin and the $neurokinin_1$ receptor antagonist CI-1021 act synergistically in two rat models of neuropathic pain. J. Pharmacol. Exp. Ther. 303, 730–735.

Froestl, W., Mickel, S.J., Hall, R.G., Von Sprecher, G., Strub, D., Baumann, P.A., Brugger, F., Gentsch, C., Jaekel, J., Olpe, H.R., Rihs, G., Vassout, A., Waldmeier, P.C., Bittiger, H., 1995. Phosphinic acid analogues of GABA: 1. New potent and selective $GABA_B$ agonists. J. Med. Chem. 38, 3297–3312.

Froestl, W., Mickel, S.J., Schmutz, M., Bittiger, H., 1996. Potent, orally active $GABA_B$ receptor antagonists. Pharmacol. Rev. Commun. 8, 127–133.

Gee, N.S., Brown, J.P., Dissanayake, V.U.K., Offord, J., Thurlow, R., Woodruff, G.N., 1996. The novel anticonvulsant drug, gabapentin (neurontin), binds to the α2δ subunit of a calcium channel. J. Biol. Chem. 271, 5768–5776.

Hill, D.R., Suman-Chauhan, N., Woodruff, G.N., 1993. Localization of [^{3}H]gabapentin to a novel site in rat brain: autoradiographic studies. Eur. J. Pharmacol., Mol. Pharmacol. Sect. 244, 303–309.

Hwang, J.H., Yaksh, T.L., 1997. Effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic model in the rat. Reg. Anesth. 22, 249–256.

Jensen, A.A., Mosbacher, J., Elg, S., Lingenhoehl, K., Lohmann, T., Johansen, T.N., Abrahamsen, B., Mattsson, J.P., Lehmann, A., Bettler, B., Brauner-Osborne, H., 2002. The anticonvulsant gabapentin (neurontin) does not act through γ-aminobutyric acid-B receptors. Mol. Pharmacol. 61, 1377–1384.

Kerr, D.I.B., Ong, J., 2001. Metabotropic $GABA_B$ receptors: new challenges in drug design. Curr. Med. Chem. CNS Agents 1, 27–42.

Kerr, D.I.B., Ong, J., 2003. Potentiation of metabotropic $GABA_B$ receptors by L-amino-acids and dipeptides in rat neocortex. Eur. J. Pharmacol. 468, 103–108.

Lanneau, C., Green, A., Hirst, W.D., Wise, A., Brown, J.T., Donnier, E., Charles, K.J., Wood, M., Davies, C.H., Pangalos, M.N., 2001. Gabapentin is not a $GABA_B$ receptor agonist. Neuropharmacology 41, 965–975.

Misgeld, U., Bijak, M., Jarolimek, W., 1995. A physiological role for $GABA_B$ receptors and the effects of baclofen in the mammalian central nervous system. Prog. Neurobiol. 46, 423–462.

Moore, K.A., Baba, H., Woolf, C.J., 2002. Gabapentin—actions on adult superficial dorsal horn neurons. Neuropharmacology 43, 1077–1081.

Ng, G.Y.K., Bertrand, S., Sullivan, R., Ethier, N., Wang, J., Yergey, J., Belley, M., Trimble, L., Bateman, K., Alder, L., Smith, A., Mckernan, R., Metters, K., O'Neill, G.P., Lacaille, J.-C., Hebert, T.E., 2001. γ-Aminobutyric acid type B receptors with specific heterodimer composition and postsynaptic actions in hippocampal neurons are targets of anticonvulsant gabapentin action. Mol. Pharmacol. 59, 144–152.

Ong, J., Kerr, D.I.B., Johnston, G.A.R., Hall, R.G., 1990. Differing actions of baclofen and 3-aminopropylphosphinic acid in rat neocortical slices. Neurosci. Lett. 109, 169–173.

Ong, J., Marino, V., Parker, D.A.S., Kerr, D.I.B., Blythin, D.J., 1998. The morpholino-acetic acid analogue Sch 50911 is a selective $GABA_B$ receptor antagonist in rat neocortical slices. Eur. J. Pharmacol. 362, 35–41.

Ong, J., Bexis, S., Marino, V., Parker, D.A.S., Kerr, D.I.B., Froestl, W., 2001. Comparative activities of the enantiomeric $GABA_B$ receptor agonists CGP 44532 and CGP 44533 in central and peripheral preparations. Eur. J. Pharmacol. 412, 27–37.

Rock, D.M., Kelly, K.M., Macdonald, R.L., 1993. Gabapentin actions on ligand- and voltage-gated responses in cultured rodent neurons. Epilepsy Res. 16, 89–98.

Satzinger, G., 1994. Antiepileptics from gamma-aminobutyric acid. Arzneimittelforschung 44, 261–266.

Shimoyama, N., Shimoyama, M., Davis, A.M., Inturrisi, C.E., Elliott, K.J., 1997. Spinal gabapentin is antinociceptive in the rat formalin test. Neurosci. Lett. 222, 65–67.

Stefani, A., Spadoni, F., Bernardi, G., 1997. Gabapentin inhibits calcium currents in isolated rat brain neurons. Neuropharmacology 37, 83–91.

Taylor, C.P., 1994. Emerging perspectives on the mechanism of action of gabapentin. Neurology 44, S10–S16.

Taylor, C.P., 1995. Gabapentin: mechanisms of action. In: Levy, R.H., Mattson, R.H., Meldrum, B.S. (Eds.), Antiepileptic Drugs. Raven Press, New York, pp. 829–841.

Taylor, C.P., Gee, N.S., Su, T.-Z., Kocsis, J.D., Welty, D.F., Brown, J.P., Dooley, D.J., Boden, P., Singh, L., 1998. A summary of mechanistic hypotheses of gabapentin pharmacology. Epilepsy Res. 29, 233–249.

Vacher, C.M., Bettler, B., 2003. $GABA_B$ receptors as potential therapeutic targets. Curr. Drug Target CNS Neurol. Disord. 2, 248–259.

van Hooft, J.A., Dougherty, J.J., Endeman, D., Nichols, R.A., Wadman, W.J., 2002. Gabapentin inhibits presynaptic Ca^{2+} influx and synaptic transmission in rat hippocampus and neocortex. Eur. J. Pharmacol. 449, 221–228.