Naunyn-Schmiedeberg's Arch Pharmacol (2001) 363 : 537–542
DOI 10.1007/s002100100408

ORIGINAL ARTICLE

Thomas M. Freiman · Juraj Kukolja · Jan Heinemeyer
Klaus Eckhardt · Heike Aranda · Axel Rominger
David J. Dooley · Josef Zentner · Thomas J. Feuerstein

Modulation of K^+-evoked [^{3}H]-noradrenaline release from rat and human brain slices by gabapentin: involvement of K_{ATP} channels

Received: 15 September 2000 / Accepted: 11 January 2001 / Published online: 1 March 2001
© Springer-Verlag 2001

Abstract To elucidate the mechanism of action of the anticonvulsant gabapentin (GBP), we compared its effects on K^+-evoked [^{3}H]-noradrenaline ([^{3}H]-NA) release from rat hippocampal and human neocortical slices with those of the K_{ATP} channel opener pinacidil and the Na^+ channel blockers phenytoin, carbamazepine and lamotrigine. Rat hippocampal and human neocortical slices were loaded with [^{3}H]-NA and superfused. [^{3}H]-NA release was evoked by increasing the extracellular [K^+] from 3 to 15 mM. GBP decreased [^{3}H]-NA release from rat hippocampal with a pIC_{50} of 5.59 and a maximum inhibition of 44%. Concentration-dependent inhibition was also seen in human neocortical slices (39% inhibition with 100 µM GBP). These inhibitory effects were antagonized by the K_{ATP} channel antagonist glibenclamide, yielding a pA_2 of 7.50 in the rat. The K_{ATP} channel opener pinacidil (10 µM), like GBP, decreased [^{3}H]-NA release from rat hippocampal slices by 27% and this effect was also antagonized by glibenclamide. In human neocortical slices the inhibition by pinacidil (10 µM) was 31%. Although phenytoin (10 µM), carbamazepine (100 µM) and lamotrigine (10 µM) also decreased [^{3}H]-NA release (by 25%, 57% and 22%, respectively), glibenclamide did not antagonize the effects of these classical Na^+ channel blockers. These findings suggest that GBP inhibits K^+-evoked [^{3}H]-NA release through activation of K_{ATP} channels. To establish whether the K_{ATP} channels under investigation were located on noradrenergic nerve terminals or on other neuronal elements, the effects of GBP were compared in the absence and in the presence of tetrodotoxin (TTX 0.32 µM) throughout superfusion. Since the functional elimination of the perikarya of interneurons by TTX reduced the inhibitory effect of GBP, the K_{ATP} channels mediating the effect of GBP may be located on nerve terminals, probably on both noradrenergic and glutamatergic nerve endings.

Key words Gabapentin · K_{ATP} channel · Noradrenaline · Epilepsy · Anticonvulsants

T. M. Freiman and J. Kukolja contributed equally to this work.

J. Kukolja · J. Heinemeyer · K. Eckhardt · H. Aranda
A. Rominger · T. J. Feuerstein (✉)
Sektion Klinische Neuropharmakologie
der Neurologischen Universitätsklinik, Neurozentrum,
Breisacher Straße 64, 79106 Freiburg, Germany
e-mail: feuer@ukl.uni-freiburg.de,
Tel.: +49-761-2705280, Fax: +49-761-2705281

T. M. Freiman · J. Zentner
Neurochirurgische Universitätsklinik, Neurozentrum,
Breisacher Straße 64, 79106 Freiburg, Germany

D. J. Dooley
Department of CNS Pharmacology,
Pfizer Global Research and Development,
Ann Arbor, MI 48105, USA

Introduction

Gabapentin (GBP) is a novel anticonvulsant drug with proven efficacy in the treatment of focal seizures (Anhut et al. 1994). In contrast to other agents used in add-on combinations in epilepsy, GBP does not interact with standard anticonvulsants (e.g. phenytoin, carbamazepine and valproate) and has rather mild, if any, adverse effects (Anhut et al. 1995). Recently, monotherapy with GBP has also been recommended on the basis of clinical studies (Beydoun et al. 1998). Another important indication is neuropathic pain, for example diabetic neuropathy (Backonja et al. 1998), post-herpetic neuralgia (Segal and Rordorf 1996, Rowbotham et al. 1998) and sympathetic reflex dystrophy (Mellick and Mellick 1997).

GBP was designed originally as a structural analogue of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). Subsequent studies, however, have failed to demonstrate an action of GBP at GABA receptors (Taylor et al. 1998). Recent evidence suggests that, under certain conditions, GBP can increase the extracellular concentration of GABA by reversed transport (Fichter et al. 1996, Kocsis and Honmou 1994). GBP is transported actively through biological membranes (e.g. blood-brain barrier)

by the L-amino acid transporter system and accumulates in the cytosol of neurons (Su et al. 1995, Stewart et al. 1995). A high-affinity binding site for GBP has been identified on the auxiliary $\alpha_2\delta$-subunit of voltage-sensitive Ca^{2+} channels (VSCC) (Gee et al. 1996); the binding of [^{3}H]-GBP to this site is, moreover, displaceable by the non-selective VSCC antagonist and polyamine spermine (Dissanayake et al. 1997). Diverse reports have implied that GBP modulates neuronal VSCC. Stefani et al. (1998) have demonstrated an inhibition of VSCC (predominately L-type) in rat brain neurons by GBP; Dooley et al. (2000a) have reported inhibition of K^+-evoked glutamate release from rat brain slices, presumably reflecting non-L-type VSCC modulation. On the other hand, Schumacher et al. (1998) have found that the anticonvulsants carbamazepine and phenytoin, but not GBP, inhibit VSCC in human epileptic hippocampal granule cells.

GBP causes modest inhibition of the release of dopamine (Reimann 1983), serotonin and noradrenaline (NA) (Schlicker et al. 1985) from mammalian brain slices after electrical stimulation. Dooley et al. (2000b) have demonstrated that GBP produces more marked inhibition of NA release from rat neocortical slices after depolarization by elevation of extracellular [K^+]. We therefore investigated the effects of GBP on K^+-evoked [^{3}H]-NA release from axon terminals of rat hippocampus and human neocortex. Alzheimer and ten Bruggencate (1988) and Gandolfo et al. (1989) have shown previously that K_{ATP} channel activators decrease epileptic discharges in vitro and in vivo. Thus, we addressed the question whether these channels may be involved in the mechanism of action of GBP.

Methods

Male Wistar rats (200–300 g) were maintained according to institutional policies and guidelines. The animals were decapitated and the brains rapidly removed and rinsed with ice-cold buffer. Unless indicated otherwise, the buffer contained (in mM): NaCl 121, KCl 1.8, $CaCl_2$ 1.3, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, glucose 10 and ascorbic acid 0.06. Before use, the buffer was saturated with 95% O_2/5% CO_2. The hippocampi were isolated and sliced transversally (350-µm slices, McIllwain Tissue Chopper, Bachofer, Reutlingen, Germany).

Human neocortical tissue was obtained from resections of subcortical brain tumours. Every patient was informed and signed a declaration of consent. Tissue from patients with seizures was excluded when the cortical areas to be removed were identified as epileptic by presurgical epilepsy electroencephalogram (EEG) diagnosis. Human tissue infiltrated macroscopically with tumour was excluded also. Retrospectively, microscopic tumour penetration of the tissue used in the experiments could be detected after evaluating the results, since such tissue responded poorly to stimulation. Results from such slices, in which the response to the first stimulation (S_1, see below) was lower than a defined minimum, were excluded (see Results). The subsequent treatment of rat and human tissue slices was the same.

After rinsing, slices were incubated in 4 ml buffer containing 0.1 µM [^{3}H]-NA for 45 min at 37 °C. After incubation, each slice was transferred to a superfusion chamber (volume 100 µl) maintained at 37 °C by a water bath. The slices were held in position in the chambers by a nylon mesh and perfused continuously with buffer (37 °C) at 0.4 ml/min. The α_2-adrenoreceptor antagonist idazoxan (1 µM) was present in the superfusion buffer throughout the experiment to prevent autoinhibition due to the released NA. After a 60-min pre-perfusion period to equilibrate basal [^{3}H] outflow, the superfusate was collected in 5-min fractions (2 ml) in polyethylene vials for determination of the [^{3}H] efflux from the slices. At the end of the experiment, the slices were solubilized in 0.5 ml Soluene (Packard Instruments, Frankfurt, Germany) and radioactivity determined by liquid scintillation spectrometry after addition of scintillation fluid (Ultima Gold, Packard Instruments). During superfusion, the slices were stimulated twice after 75 min (S_1) and 125 min (S_2) by elevation of [K^+] from 3 to 15 mM for 8 min. The concentration of Na^+ in the buffer was reduced accordingly.

The outflow of [^{3}H] was calculated as fraction of the [^{3}H] content of the slice at the onset of the respective collection period (fractional rate). The stimulation-evoked [^{3}H] overflow, expressed as the fractional rate of the total radioactivity present in the tissue at the time of stimulation, was calculated as the difference between total [^{3}H] outflow (during stimulation and the subsequent 12 min) and the basal [^{3}H] outflow, which was assumed to decline linearly from the collection period before to that 20 min after onset of stimulation. The evoked [^{3}H] overflow was assumed to represent the release of NA (Taube et al. 1977). Drugs to be tested were added from 20 min before S_2 onwards. None of the substances, at the concentrations used, affected the basal [^{3}H] outflow, except lamotrigine (see Results). In some experiments, a drug was present throughout superfusion (in addition to idazoxan, see above) to determine interactions with a second drug given before S_2. Appropriate control slices were always run in parallel with drug-treated slices. The effects of drugs were evaluated by calculating the ratio S_2/S_1 of the [^{3}H] overflow evoked by the two stimulation periods. S_2/S_1 ratios were used to reduce the variation in S_1 values.

Results are given as arithmetic means or estimates with 95% confidence intervals (CI_{95}) to indicate probability (Altman 1991, Gardner and Altman 1986). Thus, significant differences between means or other parameters are obvious when their CI_{95} ranges do not overlap. If we were interested in whether two means with slightly overlapping CI_{95} ranges were still significantly different, Student's *t*-test was applied. The number of slices used was *n*; in rats evaluable data from *n*=10–12 single slices were obtained per animal; in humans, *n*=6–10 slices were evaluable from each patient. A mean S_2/S_1 ratio was only calculated when the single S_2/S_1 ratios originated from two or more rats or humans.

To estimate parameters characterizing semi-logarithmic concentration/response curves individual S_2/S_1 data were evaluated by non-linear regression analysis using the logistic function:

$$S_2/S_1 = 1 - I_{\max} \cdot \frac{10^{\log[\mathrm{GBP}]}}{10^{-\mathrm{pIC}_{50}} + 10^{\log[\mathrm{GBP}]}}$$

with log[GBP] being the logarithm of the molar GBP concentration (independent variable), S_2/S_1 the dependent variable, pIC_{50} the negative logarithm of the concentration of GBP yielding half-maximum inhibition and I_{max} the maximum possible inhibition. pIC_{50} and I_{max} were estimated from the fitted concentration/response data as described in more detail by Feuerstein and Limberger (1999). An apparent pA_2 was calculated from the following:

$$pA_2 = \log(10^{\mathrm{pIC}_{50}' - \mathrm{pIC}_{50}} - 1) - \log[\mathrm{Ant}]$$

where pIC_{50}' is the pIC_{50} for GBP in the presence of 1 µM glibenclamide and, correspondingly, log[Ant]=–7

Drugs. [^{3}H]-NA (62.3 Ci/mmol; NEN, Bad Homburg, Germany); GBP (Parke-Davis, Ann Arbor, Mich., USA); glibenclamide, idazoxan HCl, phenytoin (RBI, Natick, Mass., USA); pinacidil, carbamazepine, tetrodotoxin (TTX, Sigma-Aldrich, Deisenhofen, Germany); lamotrigine (GlaxoWellcome, Bad Oldesloe, Germany). GBP, idazoxan, pinacidil and phenytoin were dissolved in distilled water before addition to the buffer. Glibenclamide, carbamazepine and lamotrigine were dissolved in ethanol; tetrodotoxin had to be solved in acidic buffer.

Results

The K^+-evoked release of [^{3}H]-NA from rat hippocampal slices resulted in a mean S_1 value of 4.3% of tissue [^{3}H] (CI_{95} 3.5–5.2%, *n*=73, presence of idazoxan only throughout superfusion). The corresponding mean S_1 in human neocortical tissue was 2.2% (CI_{95} 1.6–2.7%, *n*=50). The mean [^{3}H] content of rat slices was 155,962 dpm (CI_{95} 144,237–165,691 dpm, *n*=128); in human slices 102,278 dpm (CI_{95} 92,862–111,689 dpm, *n*=82). Rat experiments yielding S_1 values below 1% and experiments on human slices yielding S_1 values below 0.6% were omitted.

Effects of GBP on K^+-evoked [^{3}H]-NA release from rat hippocampal and human neocortical slices

GBP reduced [^{3}H]-NA release concentration dependently in both rat hippocampal and human neocortical slices (Fig. 1).

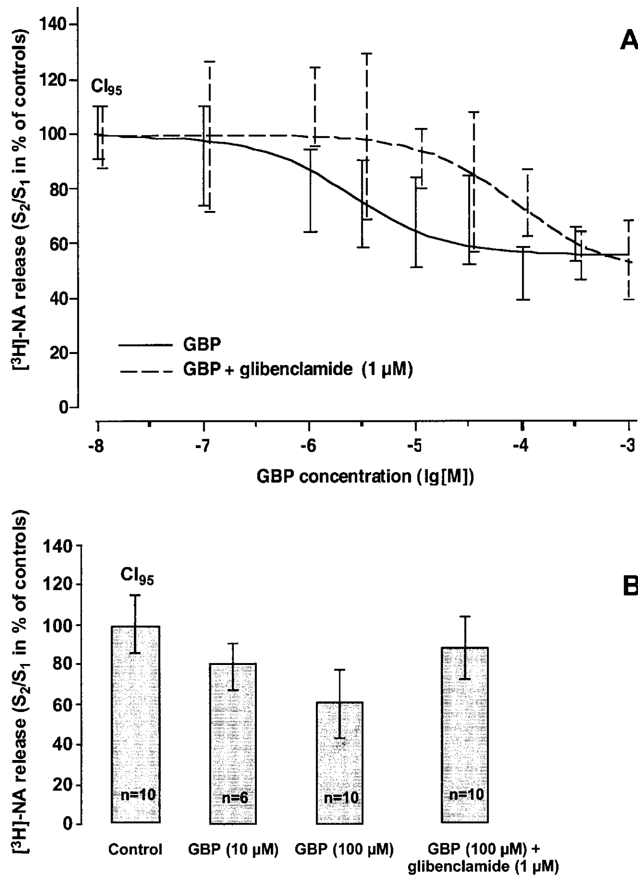

Fig. 1 Effects of gabapentin (*GBP*) on K^+-evoked [^{3}H]-noradrenaline (*NA*) release from rat hippocampal (**A**) and human neocortical (**B**) slices. Following incubation with [^{3}H]-NA slices were superfused and stimulated twice (S_1, S_2) by elevation of [K^+] from 3 to 15 mM for 8 min. GBP was added from 20 min before S_2 onwards. The effects are shown as mean S_2/S_1 ratios, expressed as a percentage of the corresponding controls with *error bars* representing 95% confidence intervals (*CI_{95}*); *n*=6–20 per GBP concentration. When given, glibenclamide was present throughout superfusion

Effects of the K_{ATP} channel antagonist glibenclamide and the agonist pinacidil

Glibenclamide (1 µM) per se slightly, but significantly, elevated the mean S_2/S_1 ratio by 20% (data not shown). When given throughout superfusion at 1 µM this drug shifted the concentration/response curve for GBP (pIC_{50} 5.59, CI_{95} 4.93–6.36; I_{max} 44%, CI_{95} 35–55%) to the right (pIC_{50} 4.08, CI_{95} 3.52–4.75; I_{max} 51%, CI_{95} 33–79%, yielding a pA_2 for glibenclamide of 7.50, CI_{95} 6.69–8.26; Fig. 1). A similar effect was observed in human neocortical slices: the reduction of [^{3}H]-NA release by 100 µM GBP was diminished by glibenclamide (1 µM) to 11% (n.s. vs. control, CI_{95} –5 to 28%). The difference between the mean S_2/S_1 ratio of slices with GBP (100 µM) and that with GBP plus glibenclamide was significant (*P*=0.019). Since glibenclamide was able to attenuate the inhibitory effect of GBP on [^{3}H]-NA release, we expected the K_{ATP} channel agonist pinacidil (Schmid-Antomarchi et al. 1990) to mimic the effect of GBP. In rat hippocampal slices, pinacidil (10 µ M) reduced the [^{3}H]-NA release by 27% (Fig. 2). Again, this inhibitory effect was reversed by glibenclamide (1 µM): the decrease was only 4% (n.s. vs. control, CI_{95} –18 to 27%) (Fig. 2). The difference between the mean S_2/S_1 ratio of slices with pinacidil only and those with pinacidil plus glibenclamide was significant, despite overlapping CI_{95} ranges (*P*=0.026). In human neocortical slices, pinacidil reduced [^{3}H]-NA release by 31% (Fig. 2).

To investigate whether pinacidil and GBP acted at the same or at different sites, we added GBP (100 µM) and pinacidil (10 µM) together before S_2. The effect of this combination in reducing of [^{3}H]-NA release was not additive: [^{3}H]-NA release was inhibited by 50% (CI_{95} 40–59%, *n*=6) compared with 43% (CI_{95} 34–63%, *n*=11) reduction following GBP alone.

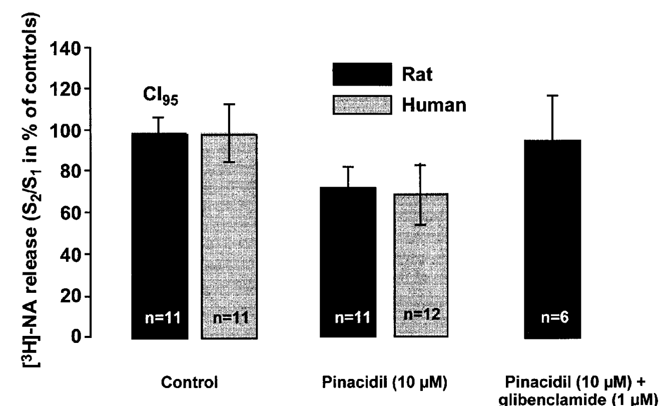

Fig. 2 Effects of pinacidil on K^+-evoked [^{3}H]-NA release from rat hippocampal and human neocortical slices. Following incubation with [^{3}H]-NA slices were superfused and stimulated twice by elevation of [K^+] from 3 to 15 mM for 8 min. Pinacidil was added from 20 min before S_2 onwards, its effects are shown as mean S_2/S_1 ratios expressed as a percentage of the corresponding controls. When given, glibenclamide was present given throughout superfusion

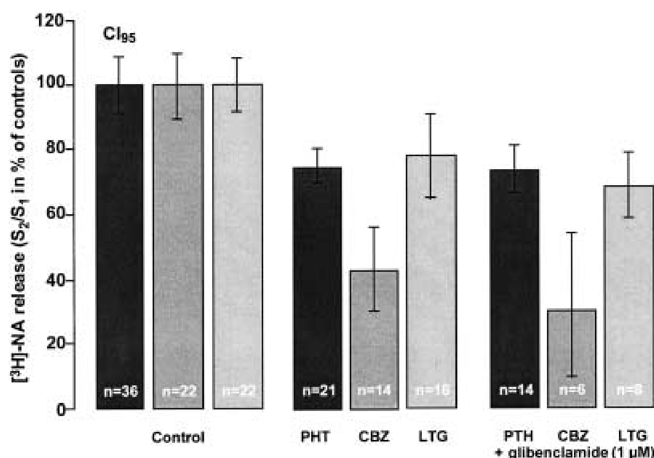

Fig. 3 Effects of phenytoin (*PHT*), carbamazepine (*CBZ*) and lamotrigine (*LTG*) on K^+-evoked [^{3}H]-NA release from rat hippocampal slices. Following incubation with [^{3}H]-NA slices were superfused and stimulated twice by elevation of [K^+] from 3 to 15 mM for 8 min. PHT, CBZ, or LTG was added from 20 min before S_2 onwards. Their effects are shown as mean S_2/S_1 ratios, expressed as a percentage of the corresponding controls. When given, glibenclamide was present throughout superfusion

Effects of other anticonvulsants on K^+-evoked [^{3}H]-NA release in rat hippocampus

Three classical anticonvulsant drugs without a known action on K_{ATP} channels were tested both alone and in combination with glibenclamide for effects on K^+-evoked [^{3}H]-NA release from rat hippocampal slices. Phenytoin (10 µM) decreased the K^+-evoked [^{3}H]-NA release by 25%. Glibenclamide (1 µM) did not affect the phenytoin-induced inhibition (Fig. 3). Carbamazepine (100 µM) inhibited [^{3}H]-NA release by 57%. Again, this reduction was not diminished by glibenclamide (Fig. 3). Lamotrigine (10 µ M) decreased [^{3}H]-NA release by 22%. At 10 µM this drug also slightly reduced the basal [^{3}H] outflow, i.e. the outflow of deaminated metabolites of [^{3}H]-NA. This (concentration-dependent) effect of lamotrigine was reduced in the presence of 10 µM pargyline and completely abolished by 100 µM pargyline, suggesting an inhibition of the enzyme monoamine oxidase by this drug (which will be the subject of a subsequent study). Glibenclamide did not reduce the inhibitory effect on K^+-evoked [^{3}H]-NA release of lamotrigine, which amounted to 32% in the presence of the K_{ATP} channel antagonist (Fig. 3). To compare the effects of these anticonvulsants, which block voltage-dependent Na^+ channels, TTX, a selective and potent inhibitor of these channels, was also tested at 1 µM. The ensuing inhibition of [^{3}H]-NA release was large (75%, CI_{95} 65–85%, *n*=7).

Effects of GBP in the presence of TTX

To establish whether the K_{ATP} channels under investigation were located on the noradrenergic nerve terminals and/or on other neuronal elements within the rat tissue under investigation, the effects of GBP (100 µM) were compared in the absence and in the presence of TTX (0.32 µM) throughout superfusion. In this series of experiments GBP alone reduced the mean S_2/S_1 ratio of controls by 56.1% (CI_{95} 41.4–70.8%, *n*=6); in the presence of TTX the GBP-elicited reduction was significantly less (30.8%, CI_{95} 19.4–42.2%, *n*=6, *P*=0.01). TTX per se depressed the mean S_1 value to 50% (CI_{95} 22.0–86.8%, *n*=12).

Discussion

The present study indicates that GBP inhibits [^{3}H]-NA release elicited by a rather low [K^+] (15 mM) through opening of K_{ATP} channels since, firstly, the inhibition was blocked by the K_{ATP} channel antagonist glibenclamide with a pA_2 of 7.50, similar to that found recently in another system (8.28, Jehle et al. 2000), secondly, the inhibition was mimicked by the K_{ATP} channel agonist pinacidil and, thirdly, the combination of GBP and pinacidil did not produce an additive or synergistic effect, suggesting a common site of pharmacological action. The action of GBP thus differs from the inhibitory effects of the anticonvulsants phenytoin, carbamazepine and lamotrigine on [^{3}H]-NA release. The inhibitory effects of these drugs were not reversed by glibenclamide. The conclusion that GBP acts through K_{ATP} channels is probably also valid for human brain tissue, although not all experiments made in the rat could be repeated on human slices due to the latter's very limited availability.

The mild and prolonged elevation of extracellular [K^+] applied in our experiments seems to correspond to the pathophysiological conditions propagating epileptic discharges in brain tissue (Jensen and Yaari 1997, Korn et al. 1987) and such increases in [K^+] are known to evoke [^{3}H]-NA release (e.g. Dooley et al. 2000b). According to those authors, the most robust inhibition by GBP of K^+-evoked [^{3}H]-NA release occurs at low [K^+] or, in other words, an increased stimulus intensity attenuates the modulatory effects of GBP on [^{3}H]-NA release. Dooley et al. (2000b) have also obtained concentration/response data in the rat using a K^+ stimulus of 25 mM, which we repeated using 15 mM K^+ in the absence and presence of glibenclamide, since the involvement of K_{ATP} channels may depend on the extracellular [K^+] used (Quast 1993). In experiments on human neocortex tissue we employed 10 and 100 µM GBP, which corresponds to the 10–100 µM achieved in brain tissue with therapeutic doses (Ben-Menachem et al. 1992, Welty et al. 1993). The possibility of eliciting [^{3}H]-NA release by mild K^+ depolarization was the first reason for choosing the model of NA release for the investigation into the mode of action of GBP in the possible context of the involvement of K^+ channels. In addition, GBP may modulate the synthesis and release of catecholamines (Pugsley et al. 1998) and NA propagates epileptiform discharges in rat hippocampal tissue at elevated [K^+] levels through β-adrenoceptors (Rutecki 1995). The activation of these receptors is believed to be the reason for the inhibition of the after-

hyperpolarization (AHP), a K^+ current hyperpolarizing the neuron below the resting potential after action potentials (Haas and Konnerth 1983, Nishimura et al. 1995). However, anticonvulsant actions of NA have also been described; for example, knock-out mice deficient in NA (dopamine-β-hydroxylase null mutants) exhibit enhanced susceptibility to the convulsant kainic acid (Szot et al. 1999). Thus, NA may exhibit pro- and anticonvulsant activity, hence complicating the interpretation of a role for this neurotransmitter in the action of GBP as an antiepileptic drug. In the present study, therefore, evoked [^{3}H]-NA release served mainly as a model for investigating K_{ATP} channel functions in response to GBP.

K_{ATP} channels are distributed widely throughout the central nervous system (Dunn-Meynell et al. 1998) and may be located on axon terminals rather than on the soma of neurons (Lee et al. 1996). Drugs acting at these channels could conceivably modulate [^{3}H]-NA release if noradrenergic nerve terminals were also endowed with K_{ATP} channels. To investigate this possibility, we compared the effects of GBP in the absence and presence of the Na^+ channel blocker TTX. TTX is known to abolish action potentials in the tissue under investigation and, therefore, to eliminate interneurons functionally. Thus, if K_{ATP} channels were located exclusively on the somatodendritic region of interneurons activating noradrenergic terminals, TTX should abolish the depressant action of GBP on [^{3}H]-NA release. In contrast, if K_{ATP} channels were located exclusively on the noradrenergic terminals the relative effect of GBP should be the same in the presence and absence of TTX. Since the depressant action of GBP was only diminished, but not abolished, by 0.32 μM TTX, a concentration which should suffice to eliminate nearly all action potentials (see, for instance, Feuerstein et al. 1990), K_{ATP} channels as executors of GBP's action may be located on both noradrenergic nerve terminals and on the somatodendritic or terminal region of a further neuron linked to the release of [^{3}H]-NA. Since such a neuron should elicit or facilitate, but not diminish, [^{3}H]-NA release to mediate a depressant action of GBP through K_{ATP} channels, the most likely candidate may be the glutamatergic neuron, as glutamate is known to activate *N*-methyl-D-aspartate (NMDA) receptors eliciting [^{3}H]-NA release in both rat (Göthert and Fink 1991) and human (Fink et al. 1992, T.J Feuerstein, H, Aranda, unpublished results) neocortical tissue.

The anticonvulsant drugs phenytoin, carbamazepine and lamotrigine were also evaluated for comparison with GBP. These three reference compounds block voltage-dependent Na^+ channels in a voltage- and frequency-dependent manner (Cheung et al. 1992, Schwarz and Grigat 1989), thus preventing action potentials and possibly reducing neurotransmitter release. Accordingly, all three drugs depressed K^+-evoked [^{3}H]-NA release significantly. The reduction of [^{3}H]-NA release by TTX substantiated the role of voltage-dependent Na^+ channels in this process. Glibenclamide did not reverse the inhibitory effects of phenytoin, carbamazepine and lamotrigine as it did with GBP and pinacidil. Consequently, there is a clear distinction between the action of the Na^+ channel antagonists on the one hand and pinacidil and GBP on the other, which strengthens the conclusion of K_{ATP} channel activation by GBP.

Increasing the K^+ conductance clamps the membrane potential at sufficiently negative values to prevent depolarization-induced Ca^{2+} entry (Hamilton et al. 1986). The K^+ channel openers may therefore act indirectly as Ca^{2+} entry inhibitors, preventing Ca^{2+}-mediated transmitter release; thereby showing a pharmacological profile similar to that of Ca^{2+} channel antagonists. Stefani et al. (1998) has suggested that GBP inhibits VSCC, as demonstrated by a block of its inhibitory effect on VSCC in isolated adult rat brain neurons by nifedipine. The antagonists ω-conotoxin GVIA and ω-conotoxin MVIIC, which inhibit Ca^{2+} currents of $\alpha_1\beta$ and $\alpha_1\alpha$ VSCC, partially reduced the inhibitory effect of GBP. In contrast, Schumacher et al. (1998) have demonstrated that GBP, relative to phenytoin and carbamazepine, did not inhibit VSCC in human hippocampal granule cells. Dooley et al. (2000a) has suggested recently that GBP acts as a VSCC antagonist. Those authors demonstrated a decrease in K^+-evoked glutamate release from rat hippocampal and neocortical slices, an effect also seen with ω-conotoxin GVIA, ω-conotoxin MVIIC and agatoxin TK. This conclusion does not, however, necessarily imply that GBP shares the same mechanism of action as directly-acting VSCC modulators. At variance with the findings of Dooley et al. (2000b), who explained the effects of GBP mainly by actions on VSCC, our results implicate for the first time K_{ATP} channels in the mode of action of GBP. Further research on K_{ATP} channels modulating the release of other transmitters, e.g. glutamate, and on K_{ATP} channel function in electrophysiological experiments is desirable for a better understanding of the effects of GBP.

Acknowledgements We thank the Deutsche Forschungsgemeinschaft (SFB 505) and the Medical Faculty of the Universität Freiburg for providing financial support. We thank Parke-Davis, Ann Arbor, Michigan, USA, and Degussa AG, Darmstadt, Germany, for material support. We also thank David Thorn for experimental support and Sascha Schmidt and Thomas Günther for construction of the superfusion chambers.

References

Altman DG (1991) Statistics in medical journals: developments in the 1980s. Stat Med 10:1897–1913

Alzheimer C, Bruggencate G ten (1988) Actions of BRL 34915 (cromakalim) upon convulsive discharges in guinea pig hippocampal slices. Naunyn-Schmiedeberg's Arch Pharmacol 337: 429–434

Anhut H, Ashman P, Feuerstein T J, Sauermann W, Saunders M, Schmidt B (1994) Gabapentin (neurontin) as add-on therapy in patients with partial seizures: a double-blind, placebo-controlled study. The International Gabapentin Study Group. Epilepsia 35:795–801

Anhut H, Ashman P, Feuerstein TJ, Sauermann W, Saunders M, Schmidt B (1995) Long-term safety and efficacy of gabapentin (neurontin) as add-on therapy in patients with refractory partial seizures. J Epilepsy 8:44–50

Backonja M, Beydoun A, Edwards KR, Schwartz SL, Fonseca V, Hes M, LaMoreaux L, Garofalo E (1998) Gabapentin for the symptomatic treatment of painful neuropathy in patients with diabetes mellitus: a randomized controlled trial. Epilepsia 40: S57-S59

Ben-Menachem E, Persson LI, Hedner T (1992) Selected CSF biochemistry and gabapentin concentrations in the CSF and plasma in patients with partial seizures after a single oral dose of gabapentin. Epilepsy Res 11:45–49

Beydoun A, Fakhoury T, Nasreddine W, Abou-Khalil B (1998) Conversion to high dose gabapentin monotherapy in patients with medically refractory partial epilepsy. Epilepsia 39:188–193

Cheung H, Kamp D, Harris E (1992) An in vitro investigation of the action of lamotrigine on neuronal voltage-activated sodium channels. Epilepsy Res 13:107–112

Dissanayake VU, Gee NS, Brown JP, Woodruff GN (1997) Spermine modulation of specific [^{3}H]-gabapentin binding to the detergent-solubilized porcine cerebral cortex $\alpha_2\delta$ calcium channel subunit. Br J Pharmacol 120:833–840

Dooley DJ, Mieske CA, Borosky SA (2000a) Inhibition of K^+-evoked glutamate release from rat neocortical and hippocampal slices by gabapentin. Neurosci Lett 280:107–110

Dooley DJ, Mieske CA, Pugsley TA (2000b) Stimulus-dependent modulation of [^{3}H]-norepinephrine release from rat neocortical slices by gabapentin and pregabalin. J Pharmacol Exp Ther 295:1086–1093

Dunn-Meynell AA, Rawson NE, Levin BE (1998) Distribution and phenotype of neurons containing the ATP-sensitive K^+ channel in rat brain. Brain Res 814:41–54

Feuerstein TJ, Limberger N (1999) Mathematical analysis of the control of neurotransmitter release by presynaptic receptors as a supplement to experimental data. Naunyn-Schmiedeberg's Arch Pharmacol 359:349–359

Feuerstein TJ, Dooley DJ, Seeger W (1990) Inhibition of norepinephrine and acetylcholine release from human neocortex by ω-conotoxin GVIA. J Pharmacol Exp Ther 252:778–785

Fichter N, Taylor CP, Feuerstein TJ (1996) Pathophysiologically induced GABA release from slices of the rat caudato-putamen: effects of gabapentin (abstract). Naunyn-Schmiedeberg's Arch Pharmacol 354:R35

Fink K, Schultheiss R, Göthert M (1992) Stimulation of noradrenaline release in human cerebral cortex mediated by N-methyl-D-aspartate (NMDA) and non-NDMA receptors. Br J Pharmacol 106:67–72

Gandolfo G, Romettino S, Gottesmann C, Luijtelaar G van, Coenen A, Bidard JN, Lazdunski M (1989) K^+ channel openers decrease seizures in genetically epileptic rats. Eur J Pharmacol 167:181–183

Gardner MJ, Altman DG (1986) Confidence intervals rather than *P* values: estimation rather than hypothesis testing. BMJ 292: 746–750

Gee NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN (1996) The novel anticonvulsant drug, gabapentin (neurontin), binds to the a_2d subunit of a calcium channel. J Biol Chem 271:5768–5776

Göthert M, Fink K (1991) Stimulation of noradrenaline release in the cerebral cortex via presynaptic *N*-methyl-D-aspartate (NMDA) receptors and their pharmacological characterization. J Neural Transm Suppl 34:121–127

Haas HL, Konnerth A (1983) Histamine and noradrenaline decrease calcium-activated potassium conductance in hippocampal pyramidal cells. Nature 302:432–434

Hamilton TC, Weir SW, Weston AH (1986) Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. Br J Pharmacol 88:103–111

Jehle T, Lagrèze WA, Blauth E, Knörle R, Schnierle P, Lücking CH, Feuerstein TJ (2000) Gabapentin-lactam (8-aza-spiro[5,4]decan-9-on, GBP-L) inhibits glucose oxygen deprivation-induced [^{3}H]-glutamate release and is a neuroprotective agent in a model of acute retinal ischemia. Naunyn-Schmiedeberg's Arch Pharmacol 362:74–81

Jensen MS, Yaari Y (1997) Role of intrinsic burst firing, potassium accumulation, and electrical coupling in the elevated potassium model of hippocampal epilepsy. J Neurophysiol 77: 1224–1233

Kocsis JD, Honmou O (1994) Gabapentin increases GABA-induced depolarization in rat neonatal optic nerve. Neurosci Lett 169:181–184

Korn SJ, Giacchino JL, Chamberlin NL, Dingledine R (1987) Epileptiform burst activity induced by potassium in the hippocampus and its regulation by GABA-mediated inhibition. J Neurophysiol 57:325–340

Lee K, Dixon AK, Rowe IC, Ashford ML, Richardson PJ (1996) The high-affinity sulphonylurea receptor regulates K_{ATP} channels in nerve terminals of the rat motor cortex. J Neurochem 66:2562–2571

Mellick GA, Mellick, LB (1997) Reflex sympathetic dystrophy treated with gabapentin. Arch Phys Med Rehabil 78:98–105

Nishimura Y, Muramatsu M, Asahara T, Tanaka T, Yamamoto T (1995) Electrophysiological properties and their modulation by norepinephrine in the ambiguus neurons of the guinea pig. Brain Res 702:213–222

Pugsley TA, Whetzel SZ, Dooley DJ (1998) Reduction of 3,4-diaminopyridine-induced biogenic amine synthesis and release in rat brain by gabapentin. Psychopharmacology (Berl) 137:74–80

Quast U (1993) Do the K^+ openers relax smooth muscle by opening K^+ channels? Trends Pharmacol Sci 14:332–337

Reimann W (1983) Inhibition by GABA, baclofen and gabapentin of dopamine release from rabbit caudate nucleus: are there common or different sites of action? Eur J Pharmacol 94:341–344

Rowbotham M, Harden N, Stacey B, Bernstein P, Magnus-Miller L (1998) Gabapentin for the treatment of postherpetic neuralgia: a randomized controlled trial. JAMA 280:1837–1842

Rutecki PA (1995) Noradrenergic modulation of epileptiform activity in the hippocampus. Epilepsy Res 20:125–136

Schlicker E, Reimann W, Göthert M (1985) Gabapentin decreases monoamine release without affecting acetylcholine release in the brain. Arzneimittelforschung 35:1347–1349

Schmid-Antomarchi H, Amoroso S, Fosset M, Lazdunski M (1990) K^+ channel openers activate brain sulfonylurea-sensitive K^+ channels and block neurosecretion. Proc Natl Acad Sci USA 87:3489–3492

Schumacher TB, Beck H, Steinhauser C, Schramm J, Elger CE (1998) Effects of phenytoin, carbamazepine, and gabapentin on calcium channels in hippocampal granule cells from patients with temporal lobe epilepsy. Epilepsia 39:355–363

Schwarz JR, Grigat G (1989) Phenytoin and carbamazepine: potential- and frequency-dependent block of Na^+ currents in mammalian myelinated nerve fibers. Epilepsia 30:286–294

Segal AZ, Rordorf G (1996) Gabapentin as a novel treatment for postherpetic neuralgia. Neurology 46:1175–1176

Stefani A, Spadoni F, Bernardi G (1998) Gabapentin inhibits calcium currents in isolated rat brain neurons. Neuropharmacology 37:83–91

Stewart BH, Chan OH, Lu RH, Reyner EL, Schmid HL, Hamilton HW, Steinbaugh BA, Taylor MD (1995) Comparison of intestinal permeabilities determined in multiple in vitro and in situ models: relationship to absorption in humans. Pharm Res 12:693–699

Su TZ, Lunney E, Campbell G, Oxender DL (1995) Transport of gabapentin, a gamma-amino acid drug, by system l alpha-amino acid transporters: a comparative study in astrocytes, synaptosomes, and CHO cells. J Neurochem 64:2125–2131

Szot P, Weinshenker D, White SS, Robbins CA, Rust NC, Schwartzkroin PA, Palmiter RD (1999) Norepinephrine-deficient mice have increased susceptibility to seizure-inducing stimuli. J Neurosci 19:10985–10992

Taube HD, Starke K, Borowski E (1977) Presynaptic receptor systems on the noradrenergic neurones of rat brain. Naunyn-Schmiedeberg's Arch Pharmacol 299:123–141

Taylor CP, Gee NS, Su TZ, Kocsis JD, Welty DF, Brown JP, Dooley DJ, Boden P, Singh L (1998) A summary of mechanistic hypotheses of gabapentin pharmacology. Epilepsy Res 29:233–249

Welty DF, Schielke GP, Vartanian MG, Taylor CP (1993) Gabapentin anticonvulsant action in rats: disequilibrium with peak drug concentrations in plasma and brain microdialysate. Epilepsy Res 16:175–181