Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 468 (2003) 103–108
www.elsevier.com/locate/ejphar

Potentiation of metabotropic $GABA_B$ receptors by L-amino acids and dipeptides in rat neocortex

David I.B. Kerr, Jennifer Ong*

Department of Anaesthesia and Intensive Care, The University of Adelaide, Adelaide, South Australia 5005, Australia

Received 5 December 2002; received in revised form 20 March 2003; accepted 28 March 2003

Abstract

Selected neutral L-α-amino acids, and their dipeptides, were reversible, stereospecific, potentiators of $GABA_B$ receptor-mediated hyperpolarizing responses to baclofen (3–100 μM) in rat neocortical slices. These responses were sensitive to the $GABA_B$ receptor antagonist (+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid (Sch50911) (30 μM). Most potent were L-Leu, L-Ile and L-Phe, as were the dipeptides L-Phe-Phe and L-Phe-Leu, and less potent were L-Met, L-Val, L-Cys, L-Cystine, L-Tyr, L-Thr, L-Arg and L-Ser. Inactive were L-Trp, L-His, L-Lys and L-Pro. These potentiators gave leftward shifts of the baclofen concentration–response curves with a Hill slope of 2, and a marked increase in the maximal hyperpolarizing responses. Selected L-amino acids and dipeptides are a class of naturally occurring $GABA_B$ potentiators, which may be allosteric modulators.

Keywords: $GABA_B$ receptor; L-Amino acid; Dipeptide; Baclofen; Neocortex, rat; Potentiator

1. Introduction

Metabotropic $GABA_B$ receptors, for the inhibitory neurotransmitter γ-aminobutyric acid (GABA), belong to Family 3 of G-protein-coupled receptors, together with metabotropic glutamate receptors, extracellular Ca^{2+}-sensing receptors and some pheromone receptors, as well as taste receptors (Kerr and Ong, 2001; Bowery et al., 2002). All members of this group share a degree of sequence similarity, but not identity, and contain an extracellular amino-terminal domain that is related to the ancestral bacterial periplasmic amino acid binding protein, leucine/isoleucine/valine binding protein (LIVBP), upon which the ligand binding sites for these receptors has been modelled (O'Hara et al., 1993; Conklin and Bourne, 1994; Couve et al., 2000). $GABA_B$ receptors are heterodimers of two subunits $GABA_{B1}$ and $GABA_{B2}$, which are essential for proper functional expression and maturation, as well as receptor activation and ligand specificity (for review, see Kerr and Ong, 2001). Curiously, only $GABA_{B1}$ binds the known agonist and antagonist ligands, but does not directly mediate any second messenger actions. Instead, the G-protein-coupled effects of $GABA_B$ receptor activation are mediated through the $GABA_{B2}$ subunit, which also plays a positive modulatory allosteric role in the heterodimer complex. $GABA_{B2}$, for which any ligands remain totally unknown, evidently is only activated indirectly by the $GABA_{B1}$ subunit, but plays a pivotal role in G-protein coupling of the heterodimer (Kaupmann et al., 1998; Margeta-Mitrovic et al., 2001a,b; Robbins et al., 2001; Kniazeff et al., 2002).

Allosteric modulators are known for members of Family 3 receptors, particularly mglu1 receptors (Knoflach et al., 2001) and extracellular Ca^{2+}-sensing receptors (Hammerland et al., 1998; Conigrave et al., 2000). Previously, Urwyler et al. (2001) showed that 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) is an allosteric modulator that not only increases the affinity of agonists at $GABA_{B1}$, but also the maximum efficacy of the heterodimer in several functional tests. Most recently, we have described a class of phenylalkylamines that are effective potentiators at $GABA_B$ receptors, enhancing $GABA_B$ receptor-induced hyperpolarizations in rat neocortical slices (Kerr et al., 2002). We here report that a specific series of L-α-amino acids, and their dipeptides, are

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

0014-2999/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0014-2999(03)01675-3

also potentiators of $GABA_B$ receptor-mediated hyperpolarizing responses in rat neocortical slices. A preliminary account of this work was presented at the 5th International $GABA_B$ Symposium (Kerr and Ong, 2002).

2. Methods

2.1. Preparation of rat neocortical wedges

All experiments were conducted in strict accordance with the guidelines of the ''Principles of Laboratory Animal Care'' (NIH publication no. 85-23, revised 1985), the Australian Code of Practice for the care and use of animals for scientific purposes of the National Health and Medical Research Council and The University of Adelaide Animal Ethics Committee. Rat neocortical slices were prepared from halothane anaesthetized, outbred male adult Sprague–Dawley rats (250–350 g), which were decapitated, using established procedures described previously (Ong et al., 2001). The brains were rapidly dissected out and immersed for 30 min in ice-cold oxygenated Krebs solution gassed with 95% O_2/5% CO_2 (pH 7.4) of the following composition (in mM): NaCl 118, KCl 2.1, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, glucose 11 and $MgSO_4$ 1.3. Cerebral cortical slices (400 μm thick) were prepared by cutting coronal sections from the anterior face of a 1-cm segment of the cerebrum containing the anterior cortex. This segment was fixed at its posterior face to the tissue block of the vibroslice microtome (Campden Instruments, UK) using acrylic glue. The slices were immediately immersed in ice-cold Krebs solution and radial wedges were then taken from each slice, at either side of the dorsal midline, to yield wedges of cingulate cortex and corpus callosum 2–3 mm wide. These were subsequently equilibrated in gassed Krebs solution at room temperature (20–23 °C) for 1 h prior to experimentation.

2.2. Grease-gap experiments

Following the equilibration period, using a superfusion method based on a grease-gap system as described previously (Horne et al., 1986; see Smart, 2001), the wedge-shaped slices from the neocortex were placed in a two-compartment perspex perfusion chamber, where each wedge was placed across a septum which separated pools containing the cortex and white matter by a grease seal. The grey matter was then continuously superfused with gassed Krebs medium at 25 °C delivered by a peristaltic pump at 1 ml/min, while the white matter was maintained in a stationary pool of the same medium. Differential recordings (mV) between the cortex and white matter, on either side of the septum, were measured with Ag/AgCl electrodes, and the DC potentials were monitored on a chart recorder using a high input-impedance DC amplifier. Here, Mg^{2+}-containing Krebs medium was used throughout the experiments to eliminate any spontaneous discharges, since the latter tended to complicate the hyperpolarizing responses.

After 60 min equilibration, the $GABA_B$ receptor agonist baclofen was added to the superfusing medium and applied to the cortical side of the tissue for 3 min to achieve steady-state concentrations within the recording chamber; the solution change required 20 s. The baclofen response was a downward deflection of the chart recorder, indicative of a hyperpolarizing population potential, being sensitive to Ba^{2+} (0.1 mM) or Cs^+ (1 mM), and mediated through activation of inwardly rectifying K^+ channels (Ong et al., 2001). Each preparation was allowed a minimum of 30 min recovery between drug applications; but in some experiments, after application of the amino acids, as much as 60 min was required before the recovery of the control baclofen responses could be re-established. When examining the modulatory effects of a potentiator, the latter was first superfused for 5 min to ensure that the potentiator alone had no action on the membrane potential. Following this, the modulator was then added together with the agonist for a further 3 min before wash-out. In some experiments, where the $GABA_B$ receptor antagonist was used, it was first superfused alone for 3 min, and then added together with the test compounds and agonist. In each experiment, control responses to the agonist were re-established, after each drug application.

2.3. Data analysis

In order to test the potentiating activity of the amino acids and their dipeptides, concentration–response curves for the agonist baclofen were constructed, in the absence and presence of differing concentrations of each potentiator. Results were quantified and values expressed as a percentage of the maximum amplitude of the hyperpolarization, obtained with the agonist alone at 100 μM, measured from the chart recordings. Responses obtained with different concentrations of baclofen were normalized to those with this maximum concentration of the agonist. Concentration–response curves were constructed in the absence and presence of the test agents. The EC_{50} values for baclofen were then taken from log-probit plots and cross-checked by calculations using the logistic function with variable Hill slope, implemented with MicroMath Scientist, for each concentration–response curve generated in the presence of the amino acid potentiators. Here, the EC_{50} is the concentration giving a response equal to 50% of that at the maximally effective baclofen concentration (100 μM), the plot confirming that this response is on the maximum plateau (i.e. saturation is achieved by 100 μM baclofen). All numerical data on the concentration–response curves were expressed as means±S.E.M. Each experiment was repeated on slices obtained from 6 to 12 different animals. Comparison of the data was made using Student's *t*-test with $P<0.05$ being significant.

In other experiments, the potentiators were applied at ascending concentrations, with a fixed concentration of the agonist baclofen (10 μM, EC_{50}), and concentration–response profiles for the potentiators were constructed by measuring the maximum hyperpolarization during co-application of the amino acids or dipeptides. Values are expressed as a percentage of the maximum hyperpolarization induced by the modulator at its highest concentration, relative to that for baclofen alone, and the data plotted as a function of potentiator concentration; the EC_{50} for each amino acid was then obtained, as above.

2.4. Drugs

Racemic (±)-baclofen and (+)-(*S*)-5,5-dimethylmorpholinyl-2-acetic acid (Sch50911) were purchased from Tocris Cookson (Bristol, UK). GABA, and the amino acids L-Arg, L-Cys and its dimer L-Cystine, L-His, L-Ile, L-Leu, L-Lys, L-Met, L-Phe, L-Pro, L-Ser, L-Thr, L-Trp, L-Tyr and L-Val, their respective D-isomers and the peptides L-Phe-Phe and L-Phe-Leu were all purchased from Sigma (St. Louis, MO, USA). CGP7930 was a gift from Novartis (Basel).

3. Results

As previously shown with the grease-gap method (Ong et al., 2001; Kerr et al., 2002), the concentration–response curve for hyperpolarization of neocortical slices by the $GABA_B$ receptor agonist baclofen, over a concentration range of 3–200 μM, gave an EC_{50} value of 10 μM, with a maximal effect (saturation, 100% response) at 100 μM, which falls on the plateau of the curves (n=12). Full recovery of baclofen-induced responses was obtained only after 30 min of tissue wash-out. Such hyperpolarizing responses were reversibly antagonised by the selective $GABA_B$ receptor antagonist, Sch50911 (5, 10 and 30 μM; n=6–10).

As seen from Fig. 1, in a typical example of $GABA_B$ receptor potentiation by a branched chain amino acid (L-Leu), the agonist baclofen at 10 μM (which is the EC_{50}), applied alone for 3 min induced a control hyperpolarization. In this preparation, 30 min after wash-out, subsequent application of the L-Leu (100 μM) alone for 5 min had no significant effect on the membrane potential, but when co-superfused with baclofen (10 μM) for 3 min, there was a marked potentiation of the baclofen-induced hyperpolarizing response, giving an initial spike followed by a more prolonged recovery. Notably, the hyperpolarization persisted for 15–20 min, as against 5 min control, in the absence of L-Leu. The control response then required some 45–60 min wash-out before it could be re-established; indeed, we emphasise that potentiation of responses to re-applied baclofen was still seen during the ensuing 30–40 min after wash-out of the potentiator. Also, the $GABA_B$ receptor antagonist Sch50911 (30 μM) abolished the potentiated response, indicating that $GABA_B$ receptors were involved (Fig. 1). As a reference compound, the known modulator CGP7930 (10 μM) was active in the low micromolar range, potentiating $GABA_B$ receptors with an EC_{50} of 10 μM (data not shown; n=6–8). At 10 μM, it likewise reversibly potentiated the response to baclofen (10 μM), the resultant hyperpolarization closely resembling that seen with L-Leu (Fig. 1). Recovery of the membrane potential to baseline level occurred within 10 min after wash-out, while the control baclofen response was restored within 30 min after wash-out.

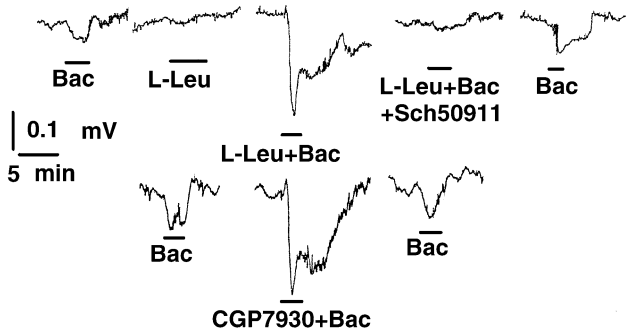

Fig. 1. Discontinuous records of the hyperpolarizing effects of baclofen (Bac; 10 μM) in a rat neocortical slice and the potentiating effect of the branched chain amino acid L-Leu (100 μM) on the baclofen-induced response, which was abolished by Sch50911 (30 μM). L-Leu (100 μM) when applied on its own had no significant effect on the membrane potential. The control response to baclofen was subsequently re-established upon tissue wash-out within 60 min. The interval between drug applications was at least 30 min. For comparison, the more potent modulator CGP7930 (10 μM) was shown to significantly and reversibly potentiate the response to baclofen (10 μM) in a separate slice.

In Fig. 2, concentration–response curves for baclofen, in the presence of L-Leu at two concentrations (50 and 100 μM), revealed an increase of agonist potency as well as maximal efficacy for each concentration (n=6). The baclofen concentration–response curves were shifted to the left in the presence of L-Leu, with a Hill slope of 2, denoting cooperativity. The potency for baclofen was increased by three- and eight-fold (EC_{50}=4.0 and 1.3 μM, respectively), and the corresponding maximum hyperpolarizing responses to baclofen (100 μM) were increased to 120±6.5% and 138±8.5% (Fig. 2; n=6). Using the modulator CGP7930 (10 μM), the concentration response curve for baclofen was again left-shifted five-fold, with a Hill slope of 2, as for the amino acids (data not shown). From curves for the enhancement effects of L-Leu (30, 56, 100 and 150 μM) and L-Phe (30, 56, 100, 300 and 500 μM) on responses to a fixed concentration of baclofen (10 μM), the estimated EC_{50} values for L-Leu and L-Phe in potentiating baclofen responses were both 50 μM (Fig. 3A,B; n=6–8). The maximal effects for L-Leu were reached at 150 μM and for L-Phe at 300 μM. The Hill slopes for both were 3.0. Although in most preparations, L-Leu itself when applied alone did not hyperpolarize the membrane; nevertheless, hyperpolarizing responses could be observed occasionally,

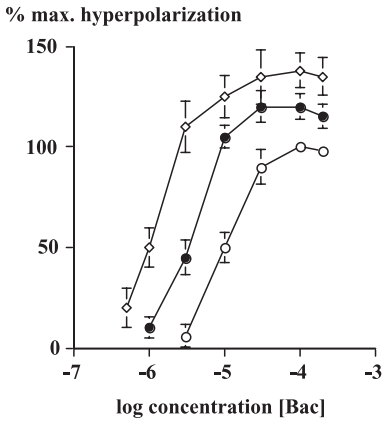

Fig. 2. Concentration–response curves for baclofen-induced hyperpolarizations recorded from rat neocortical slices, in the absence and presence of the amino acid potentiator L-Leu. Complete concentration–response curve for baclofen alone (○) and the leftward shifts of the curve by two concentrations of L-Leu (●: 50 and ◇: 100 μM), showing the increased maximal responses in the presence of L-Leu. Values are expressed as a percentage of the maximum hyperpolarization achieved by the agonist alone, and each point represents the mean and standard error of the mean of 6–12 determinations.

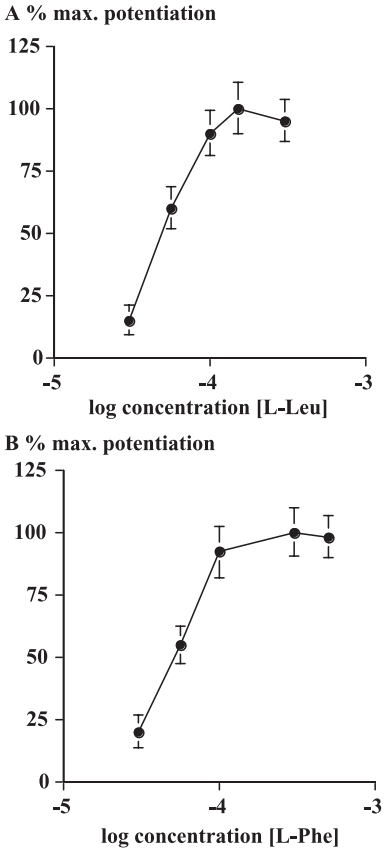

Fig. 3. Concentration–response profiles for the amino acid modulators (A) L-Leu (●) and (B) L-Phe (●) in potentiating the hyperpolarizing responses induced by a fixed concentration (EC_{50}) of baclofen (10 μM), in rat neocortical slices, maintained in Mg^{2+}-containing Krebs solution. Values are expressed as a percentage of the maximum hyperpolarization induced by the modulator at its highest concentration, and each point represents the mean and standard error of the mean of 6–8 determinations.

when using L-Leu at the highest concentration of 150 μM. For this reason, even higher concentrations were avoided.

To see which of the twenty coded amino acids were capable of potentiating baclofen-induced hyperpolarizations, ascending concentrations of amino acids were applied in the presence of a fixed concentration of baclofen (10 μM) and the EC_{50} for potentiation by each was obtained. As can be seen from Table 1, the hydrophobic branched chain amino acids, L-Leu and L-Ile, as well as L-Phe, were the most potent, in potentiating the baclofen (10 μM)-induced hyperpolarizing responses (n=6 for each drug), while the amino acids with more polar side chains were less active in potentiating baclofen responses. However, none of these used alone had any effect on the membrane potential. The corresponding D-amino acids were uniformly inactive as potentiators. Moreover, the active potentiating L-amino acids, at their highest concentrations, were without effect on depolarizing responses to GABA (100 μM) involving $GABA_A$ receptor-mediated actions (data not shown; n=6), indicating that they were selective for $GABA_B$ receptor sites.

We also examined if dipeptides, formed from the most potent amino acid potentiators, would similarly modulate baclofen responses in our preparations. Both L-Phe-Phe (L-di-phenylalanine) and L-Phe-Leu (L-phenylalanyl-leucine) were rather effective potentiators of the baclofen (10 μM) response. Quantitating such enhancing effects of L-Phe-Phe and L-Phe-Leu (5, 10, 30 and 100 μM) on a fixed concentration of baclofen (10 μM) gave an estimated EC_{50} of 10 μM for each dipeptide (Table 1; n=8). Neither of these

Table 1
EC_{50} values of the known modulator CGP7930 and L-amino acids and dipeptides in potentiating baclofen (10 μM)-induced hyperpolarizing responses in rat neocortical slices

Potentiators	EC_{50} (μM)
CGP7930	10
L-Leu	50
L-Ile	50
L-Phe	50
L-Met	80
L-Cys	90
L-Cystine	90
L-Val	100
L-Thr	100
L-Arg	100
L-Tyr	100
L-Ser	200
L-Trp	inactive
L-His	inactive
L-Lys	inactive
L-Pro	inactive
L-Phe-Phe	10
L-Phe-Leu	10

EC_{50} is the concentration that gives 50% of the maximum potentiation induced by the potentiator, derived from the concentration–response curve against a fixed concentration of baclofen (10 μM). The Hill slopes of the potentiators were all between 2.0 and 3.0, and the number of experiments performed for each potentiator was n=6–8.

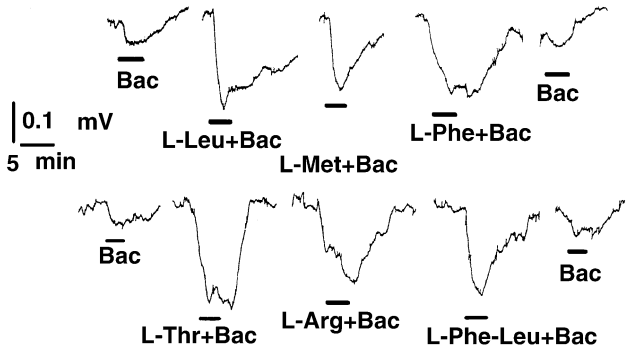

Fig. 4. A typical record showing the reversible potentiating effects of a series of amino acids on baclofen (10 μM)-induced hyperpolarizing potentials in neocortical slice preparations, with 45 min washing in between drug applications. Upper trace: L-Leu (100 μM), L-Met (100 μM), L-Phe (100 μM), all potentiated baclofen-induced responses. Lower trace: L-Thr (200 μM), L-Arg (200 μM) and the dipeptide L-Phe-Leu (20 μM) also enhanced the baclofen-induced hyperpolarizations. The control responses to baclofen were subsequently re-established upon tissue wash-out within 60 min.

peptides (5–100 μM) on their own had any effect on the membrane potential, or on $GABA_A$ receptor-mediated depolarizing responses to GABA (100 μM) (data not shown; n=6) (Fig. 4).

4. Discussion

We have identified, for the first time, that neutral L-α-amino acids with branched or aromatic side chains, together with their dipeptides, are robust potentiators of $GABA_B$ receptor-mediated hyperpolarizing responses in rat neocortical slices. The most potent were the neutral hydrophobic amino acids; those with more polar side chains were less active or inactive, the latter including the basic amino acids L-His, L-Lys and L-Pro, as well as the aromatic amino acid L-Trp. L-Val is an outlyer, relative to the other branched chain acids, possibly because it is the most hindered of the natural amino acids, which may partly interfere with its binding at the receptor. Others known to be involved in neurotransmission (Gly, Ala, Asp, Asn, Glu and Gln) were omitted from this study. On the other hand, all the D-isomers were inactive, which indicates that the modulatory actions of the L-amino acids on $GABA_B$ receptors are stereospecific. Interestingly, dipeptides such as L-Phe-Phe and L-Phe-Leu containing the most potent of these amino acids were even more effective potentiators, some 5 times as potent as the parent amino acids, equipotent with the known modulator CGP7930 (Urwyler et al., 2001).

Potentiating actions of the L-amino acids were selective to $GABA_B$ receptors, since they had no effect on $GABA_A$ receptor-mediated responses induced by GABA itself. In general, the amino acids themselves did not elicit any response in the absence of baclofen, but only potentiated agonist-dependent responses. However, L-Leu itself, at the highest concentration of 150 μM, did elicit a hyperpolarizing response occasionally, in a small number of preparations. The concentration–response curves for baclofen, in the presence of fixed concentrations of the active amino acids or peptides, showed concentration-dependent increases in both the potency and maximum efficacy (maximal responses) of the baclofen-induced hyperpolarizations, uniformly with a Hill slope of 2. On the other hand, the Hill slopes of L-Leu and L-Phe against a fixed concentration of baclofen were both 3.0. These Hill slopes indicate cooperativity, with binding at two distinct sites on the receptor complex, in the responses induced by baclofen in the presence of the amino acids, but cannot be analysed further with the present data. Furthermore, in the presence of these potentiators, the duration of these responses to baclofen became prolonged and potentiation of prolonged responses to re-applied baclofen persisted for some 30–45 min after wash-out of the amino acid, which suggests that they, in some way, prevented desensitization and delayed the dissociation of the agonist. All of the above properties are characteristic of positive modulators in general (Parmentier et al., 2002) and require further investigation.

It is, perhaps, not entirely unexpected that amino acids and peptides should potentiate agonist-induced responses of $GABA_B$ receptors. From among Family 3 G-protein-coupled receptors, to which $GABA_{B1/2}$ belong, Ca^{2+}-sensing receptors are also stimulated or modulated by amino acids, but with a totally different profile from that seen here with $GABA_B$ receptors; they also require much higher concentrations (in the mM range) to be effective (Conigrave et al., 2000). For example, the branched chain amino acids L-Leu and L-Ile are virtually inactive at the Ca^{2+}-sensing receptors, but were among the most potent amino acid potentiators of $GABA_B$ receptors. The binding site for amino acid allosteric modulators of Ca^{2+}-sensing receptors is known to be at serines adjacent to the binding site for Ca^{2+} itself, in the amino-terminal domain of the latter receptors (Zhang et al., 2002). However, it is not known where the amino acid potentiators bind at the $GABA_B$ heterodimer to potentiate agonist-induced responses.

In $GABA_{B1}$ receptors, the GABA binding region is derived in evolution from the bacterial branched chain LIVBP (Galvez et al., 1999; Kniazeff et al., 2002), and it may be no accident that L-Leu and L-Ile are the most potent potentiators in the present series. We are thus tempted to speculate that the modulatory, naturally occurring amino acids and peptides might likewise bind at $GABA_{B2}$ sites, derived in some similar manner from other ancestral periplasmic amino acid binding proteins, including those for small amino acids and peptides (Nickitenko et al., 1995). Importantly, in fish, there are olfactory receptors, belonging to Family 3 and related to Ca^{2+}-sensing receptors, which respond specifically to amino acids that act as feeding cues (see Table 1 in Speca et al., 1999). The existence of such amino acid receptors indicates that broad-spectrum recognition of neutral amino acids is entirely possible in the

amino-terminal of specific Family 3 receptors. Could it be, then, that $GABA_{B2}$ is a further example of such amino acid sensitive receptors, but with a modulatory function at the $GABA_B$ receptor heterodimer?

In the present study, using intact functional native $GABA_B$ receptors, our results demonstrate that selected naturally occurring L-amino acids and peptides are effective potentiators of $GABA_B$ receptor-mediated actions in rat neocortical slices. The cooperativity revealed by the Hill slope of their concentration–response curves, along with the increased efficacy and maximal response in the presence of the amino acids, suggest that they could possibly be allosteric modulators at $GABA_B$ receptors. However, binding studies, and site-directed mutagenesis studies in expression systems, on the respective $GABA_B$ receptor subunits, are required to resolve the question of the site of action for these potentiators and of any allosteric actions they might induce.

Acknowledgements

We thank Dr. Froestl of Novartis (Basel) for the kind gift of CGP7930 and the University of Adelaide Small Research Grant Scheme for support.

References

Bowery, N.G., Bettler, B., Froestl, W., Gallagher, J.P., Marshall, F., Raiteri, M., Bonner, T.I., Enna, S.J., 2002. International union of pharmacology: XXXIII. Mammalian γ-aminobutyric $acid_B$ receptors: structure and function. Pharmacol. Rev. 54, 247–264.

Conigrave, A.D., Quinn, S.J., Brown, E.M., 2000. L-Amino acid sensing by the extracellular Ca^{2+}-sensing receptor. Proc. Natl. Acad. Sci. U. S. A. 97, 4814–4819.

Conklin, B.R., Bourne, H.R., 1994. Homeostatic signals. Marriage of the flytrap and the serpent. Nature 367, 22.

Couve, A., Moss, S.J., Pangalos, M.N., 2000. $GABA_B$ receptors: a new paradigm in G protein signaling. Mol. Cell. Neurosci. 16, 296–312.

Galvez, T., Parmentier, M.L., Joly, C., Malitschek, B., Kaupmann, K., Kuhn, R., Bittiger, H., Froestl, W., Bettler, B., Pin, J.P., 1999. Mutagenesis and modeling of the GABA-B receptor extracellular domain support a Venus flytrap mechanism for ligand binding. J. Biol. Chem. 274, 13362–13369.

Hammerland, L.G., Garrett, J.E., Hung, B.C.P., Levinthal, C., Nemeth, E.F., 1998. Allosteric activation of the Ca^{2+} receptor expressed in *Xenopus laevis* oocytes by NPS 467 or NPS 568. Mol. Pharmacol. 53, 1083–1088.

Horne, A.L., Harrison, N.L., Turner, J.P., Simmonds, M.A., 1986. Spontaneous paroxysmal activity induced by zero magnesium and bicuculline: suppression by NMDA antagonists and GABA mimetics. Eur. J. Pharmacol. 122, 231–238.

Kaupmann, K., Malitschek, B., Schuler, V., Heid, J., Froestl, W., Beck, P., Mosbacher, J., Bischoff, S., Kulik, A., Shigemoto, R., Karschin, A., Bettler, B., 1998. $GABA_B$-receptor subtypes assemble into functional heteromeric complexes. Nature 396, 683–687.

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., 2002. Amino-acids and other modulators at $GABA_B$ receptors. The 5th International $GABA_B$ Symposium, Paris, 34.

Kerr, D.I.B., Ong, J., Puspawati, N.M., Prager, R.H., 2002. Arylalkylamines are a novel class of positive allosteric modulators at $GABA_B$ receptors in rat neocortex. Eur. J. Pharmacol. 451, 69–77.

Kniazeff, J., Galvez, T., Labesse, G., Pin, J.-P., 2002. No ligand binding in the GB2 subunit of the $GABA_B$ receptor is required for activation and allosteric interaction between the subunits. J. Neurosci. 22, 7352–7361.

Knoflach, F., Mutel, V., Jolidon, S., Kew, J.N.C., Malherbe, P., VIeira, E., Wuchmann, J., Kemp, J.A., 2001. Positive allosteric modulators of metabotropic glutamate 1 receptor: characterization, mechanism of action, and binding site. Proc. Natl. Acad. Sci. U. S. A. 98, 13402–13407.

Margeta-Mitrovic, M., Jan, Y.N., Jan, L.Y., 2001a. Ligand-induced signal transduction within heterodimeric $GABA_B$ receptor. Proc. Natl. Acad. Sci. U. S. A. 98, 14643–14648.

Margeta-Mitrovic, M., Jan, Y.N., Jan, L.Y., 2001b. Function of GB1 and GB2 subunits in G protein coupling of $GABA_B$ receptors. Proc. Natl. Acad. Sci. U. S. A. 98, 14649–14654.

Nickitenko, A.V., Trakhanov, S., Quiocho, F.A., 1995. 2A resolution structure of DppA, a periplasmic dipeptide transport/chemosensory receptor. Biochemistry 34, 16585–16595.

O'Hara, P.J., Sheppard, P.O., Thogersen, H., Venezia, D., Halderman, B.A., McGrane, V., Houamed, K.M., Thomsen, C., Gilbert, T.L., Mulvihill, E.R., 1993. The ligand-binding domain in metabotropic glutamate receptors is related to bacterial periplasmic binding proteins. Neuron 11, 41–52.

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.

Parmentier, M.-L., Prezeau, L., Bockaert, J., Pin, J.-P., 2002. A model for the functioning of family 3 GPCRs. Trends Pharmacol. Sci. 23, 268–274.

Robbins, M.J., Calver, A.R., Filippov, A.K., Hirst, W.D., Russell, R.B., Wood, M.D., Nasir, S., Couve, A., Brown, D.A., Moss, S.J., Pangalos, M.N., 2001. $GABA_{B2}$ is essential for G-protein coupling of the $GABA_B$ receptor heterodimer. J. Neurosci. 21, 8043–8052.

Smart, T.G., 2001. Michael Arthur Simmonds-recognition of 25 years as an editor of neuropharmacology. Neuropharmacology 40, 467–468.

Speca, D.J., Lin, D.M., Sorensen, P.W., Isacoff, E.Y., Ngai, J., Dittman, A.H., 1999. Functional identification of a goldfish odorant receptor. Neuron 23, 487–498.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., Bettler, B., Kaupmann, K., 2001. Positive allosteric modulation of native and recombinant γ-aminobutyric $acid_B$ receptors by 2,6-di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol (CGP7930) and its aldehyde analog CGP13503. Mol. Pharmacol. 60, 963–971.

Zhang, Z., Qiu, W., Quinn, S.J., Conigrave, A.D., Brown, E.M., Bai, M., 2002. Three adjacent serines in the extracellular domains of the CaR are required for L-amino acid-mediated potentiation of receptor function. J. Biol. Chem. 277, 33727–33735.