Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 483 (2004) 147–153
www.elsevier.com/locate/ejphar

Selected amino acids, dipeptides and arylalkylamine derivatives do not act as allosteric modulators at $GABA_B$ receptors

Stephan Urwyler*, Tina Gjoni, Klemens Kaupmann, Mario F. Pozza, Johannes Mosbacher

Neuroscience Research, Novartis Institutes for BioMedical Research, Novartis Pharma AG, CH-4002 Basel, Switzerland

Received 31 July 2003; received in revised form 15 October 2003; accepted 17 October 2003

Abstract

Based on recent reports describing enhancing actions of arylalkylamines (fendiline [*N*-(3,3-diphenylpropyl)-α-methylbenzylamine] and prenylamine [*N*-(3,3-diphenylpropyl)-α-methylphenethylamine]), amino acids (L-phenylalanine, L-leucine and L-isoleucine), and dipeptides (L-Phe-Phe and L-Phe-Leu) on baclofen-induced responses in cortical slices, we have examined whether these compounds might act as positive allosteric modulators at $GABA_B$ receptors. Unlike the previously described allosteric $GABA_B$ receptor modulator CGP7930 (2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol), these compounds did not enhance $GABA_B$ receptor-mediated guanosine 5′-*O*-(3-thiotriphosphate) [GTP(γ)^{35}S] binding in native or recombinant cell membrane preparations. Similarly, in a competition binding assay using the antagonist radioligand [^{3}H]CGP62349, CGP7930, but not the other compounds, enhanced the affinities of γ-aminobutyric acid (GABA) for native $GABA_B$ receptors from rat brain cortex. Finally, in a cellular assay (Ca^{2+} signaling in a recombinant cell line), CGP7930 was again the only compound found to enhance the GABA response. It is concluded that the arylalkylamines, amino acids and dipeptides tested do not act as allosteric modulators at native and recombinant $GABA_B$ receptors.

Keywords: Arylalkylamine; Amino acid; Dipeptide; CGP7930; Allosteric modulation; $GABA_B$ receptor

1. Introduction

Allosteric modulators are drugs that interact with a site on a hormone or neurotransmitter receptor which is topographically distinct from the "orthosteric" binding pocket for agonists or competitive antagonists. As therapeutic agents, allosteric modulators might have several advantages over orthosteric ligands, such as an enhanced receptor subtype selectivity (Lazareno et al., 1998; Birdsall et al., 1999) or a lower propensity for inducing receptor desensitization when compared to agonists. Most importantly, they are effective only when and where an endogenous agonist is present, and therefore act more in line with physiological receptor activation than exogenous agonists. For these reasons, allosteric binding sites have recently attracted considerable interest as potential novel drug targets (Pin et al., 2001; Christopoulos, 2002). Specifically, different allosteric modulators have been reported in recent years for metabotropic glutamate (mGlu, Knoflach et al., 2001; Johnson et al., 2003) and $GABA_B$ receptors (Urwyler et al., 2001, 2003). Moreover, the extracellular Ca^{2+} sensing receptor, which, like mGlu and $GABA_B$ receptors, also belongs to "family 3" G-protein-coupled receptors, has been shown to be allosterically modulated by amino acids (Conigrave et al., 2000) and arylalkylamine-like molecules (Hammerland et al., 1998; Nemeth et al., 1998). This has prompted Kerr et al. (2002) to examine the effects of arylalkylamine derivatives on $GABA_B$ receptor-dependent responses. They found that baclofen-induced field potentials in rat neocortical slices were enhanced by fendiline [*N*-(3,3-diphenylpropyl)-α-methylbenzylamine], prenylamine [*N*-(3,3-diphenylpropyl)-α-methylphenethylamine] and F551 [*N*-(3,3-diphenylpropyl)-α-methyl-3-methoxybenzylamine]. Because the compounds were without effect when applied alone, the authors concluded that these three arylalkylamine derivatives are positive allosteric modulators of $GABA_B$ receptors. More recently, the same group has described similar effects for several amino acids and dipeptides (Kerr and Ong, 2003). However, this kind of potentiating effects

* Corresponding author. Tel.: +41-61-696-52-88; fax: +41-61-696-81-12.
E-mail address: stephan.urwyler@pharma.novartis.com (S. Urwyler).

0014-2999/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.10.024

in brain tissue slice preparations might also well be due, for example, to downstream effects or receptor–receptor interactions, without the involvement of true allosteric mechanisms at the molecular level. We have therefore re-examined the effects of selected arylalkylamines, amino acids and dipeptides and compared them with the known $GABA_B$ receptor modulator CGP7930 (2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol) (Urwyler et al., 2001) in different assay systems allowing to detect possible allosteric mechanisms of compounds at $GABA_B$ receptors.

2. Materials and methods

2.1. Guanosine 5′-O-(3-[^{35}S]thiotriphosphate) (GTP(γ)^{35}S) binding assays

Guanosine 5′-*O*-(3-[^{35}S]thiotriphosphate) (GTP(γ)^{35}S) binding assays with native or recombinant receptor preparations were carried out as described earlier (Urwyler et al., 2001, 2003). The composition of the assay mixtures (in a final volume of 250 μl in 96-well clear-bottom microtiter isoplates [PerkinElmer Wallac, Turku, Finland]) was as follows: 50 mM Tris–HCl buffer, pH 7.7; 10 mM $MgCl_2$; 0.2 mM EGTA; 2 mM $CaCl_2$; 100 mM NaCl; 10 μM guanosine 5′-diphosphate (30 μM with rat cortical membranes; Sigma, Buchs, Switzerland), 50 μl of the membrane suspension (approximately 10–20 μg of protein), 1.5 mg wheat germ agglutinin-coated scintillation proximity assay (SPA) beads (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK), 0.3 nM [^{35}S]GTP(γ)S (ca. 1000 Ci/mmol, stabilized solution, Amersham), and the test compounds at the appropriate concentrations. Nonspecific binding was measured in the presence of unlabelled GTP(γ)S (10 μM, Sigma). The samples were incubated at room temperature for 60 min, before the SPA beads were sedimented by centrifugation at 2600 rpm for 10 min. The plates were then counted in a Wallac 1450 Microbeta liquid scintillation counter.

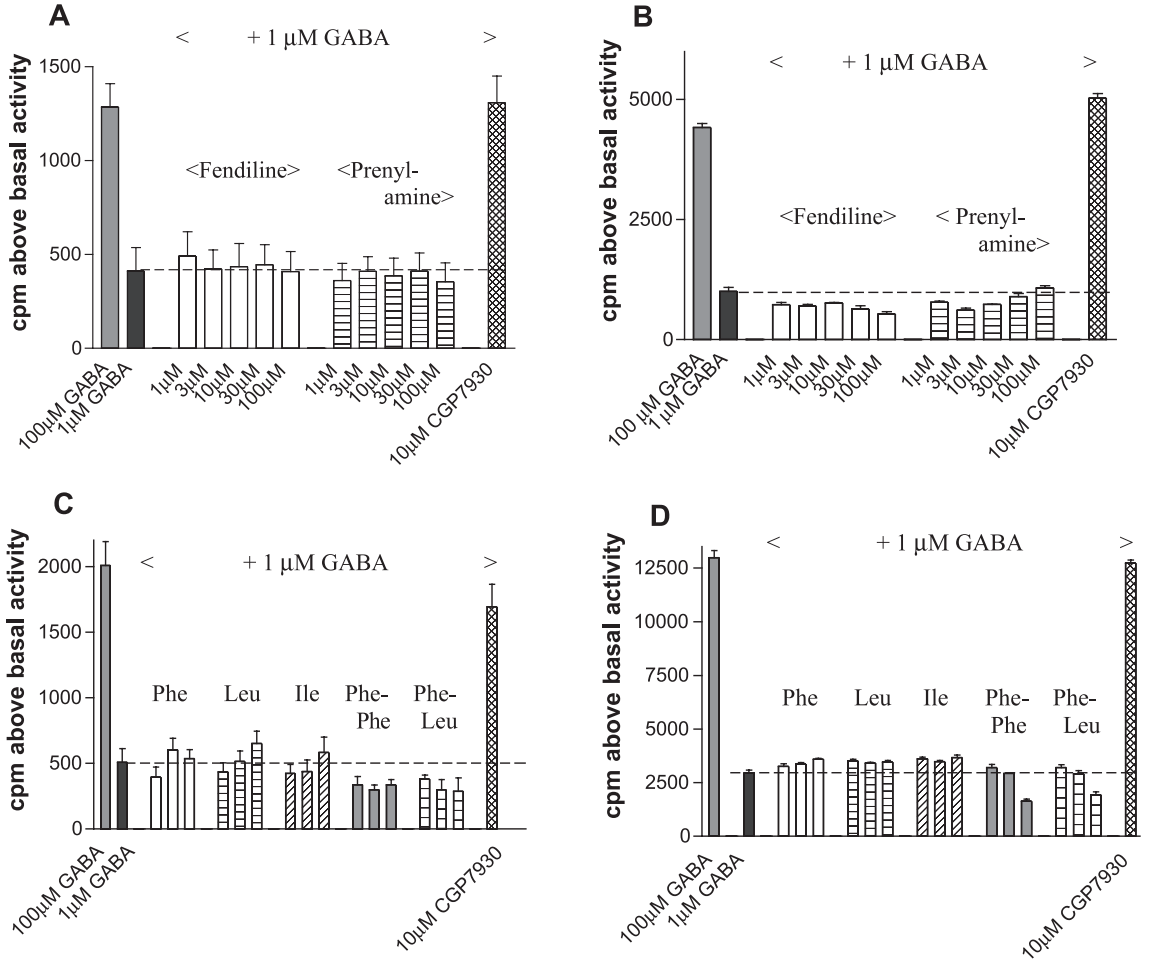

Fig. 1. Lack of allosteric effects of arylalkylamines (panels A and B) and amino acids and dipeptides (panels C and D) on the stimulation of GTP(γ)^{35}S binding via $GABA_B$ receptors in membranes from a recombinant CHO cell line stably expressing $GABA_{B(1b/2)}$ (panels A and C) or from rat brain cortex (panels B and D). The stimulation of GTP(γ)^{35}S binding was measured at a GABA concentration of 1 μM in the absence and in the presence of the given concentrations of the test compounds. For the amino acids and dipeptides, test concentrations were 10, 30 and 100 μM (from left to right in each group). For comparison, the enhancement of the GABA response by the known allosteric $GABA_B$ receptor modulator CGP7930 is also shown.

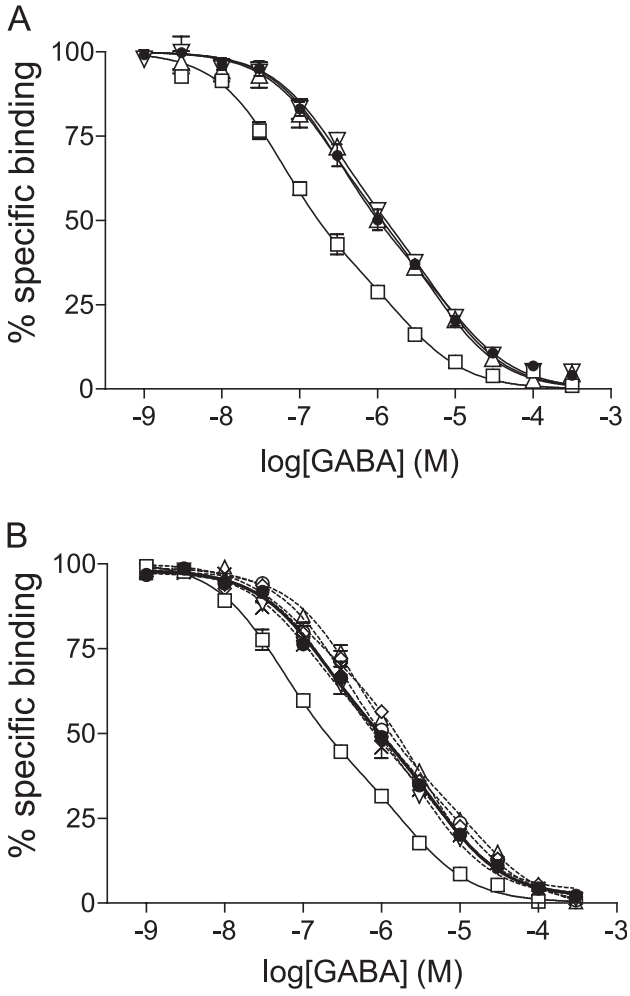

Fig. 2. Effects of compounds on the displacement of [^{3}H]CGP62349 from native $GABA_B$ receptors in rat cortical membranes by GABA. Top panel: Effects of fendiline (△), prenylamine (▽) and CGP7930 (□) (30 μM each). Bottom panel: Effects of L-Phe (◇), L-Leu (×), L-Ile (▽), L-Phe-Phe (Δ), L-Phe-Leu (○) (100 μM each) and CGP7930 (□, 30 μM). The symbol ● represents the control curve with GABA alone in both panels. The pK_i values for the high and low affinity components for such displacement curves from several experiments are given in Table 1.

2.2. Radioligand binding experiments

The procedure to measure the binding of [^{3}H]CGP62349 to rat cortical membranes was based on that described by Bittiger et al. (1996); it was, however, conducted in the SPA format. The assay mixture in a final volume of 250 μl contained 20 mM Tris–HCl buffer (pH 7.4), 118 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$, 5 mM D-glucose, 1 nM [^{3}H]CGP62349, the test compounds at the desired concentrations, rat cortical membranes (ca. 15 μg protein), and 1.5 mg wheat germ agglutinin-coated SPA beads (Amersham). Nonspecific binding was assessed in the presence of 5 μM CGP56999A. The samples were incubated for 90 min at room temperature, before being counted in a Wallac 1450 Microbeta liquid scintillation counter.

2.3. Measurement of intracellular Ca^{2+} concentrations by fluorimetry

For the measurement of changes in intracellular Ca^{2+} concentrations ($\Delta[Ca^{2+}]_i$) (Pagano et al., 2001; Urwyler et al., 2001, 2003), we loaded Chinese hamster ovary (CHO) cells stably transfected with human $GABA_{B(1b)}$, rat $GABA_{B(2)}$ and $G\alpha_{qo5}$ for 45 min with 2 μM fluo-4 acetoxymethyl ester (Molecular Probes, Eugene, OR) in Hank's balanced salt solution (Invitrogen, Basel, Switzerland) containing 20 mM HEPES and 50 μM probenecid (Sigma). Cells were washed and transferred to a fluorimetric image plate reader (FLIPR, Molecular Devices, Sunnyvale, CA, USA). Compounds were added during recording and 5 min prior to addition of 0.3 μM γ-aminobutyric acid (GABA). Relative fluorescence changes over baseline ($\Delta F/F$) were determined and averaged (quadruplicates within one experiment, three individual experiments).

2.4. Chemicals

Fendiline [*N*-(3,3-diphenylpropyl)-α-methylbenzylamine] hydrochloride, prenylamine [*N*-(3,3-diphenylpropyl)-α-methylphenethylamine] lactate salt, and the amino acids L-phenylalanine (Phe), L-leucine (Leu) and L-isoleucine (Ile) were purchased from Sigma. The dipeptides L-di-phenylalanine (L-Phe-Phe) and L-phenylalanyl-L-leucine (L-Phe-Leu) were from ICN Biomedicals (Eschwege, Germany). [^{35}S]GTP(γ)S (ca. 1000 Ci/mmol, stabilized solution) was from Amersham Biosciences, and [^{3}H]CGP62349 (85Ci/mMol) was obtained from American Radiolabeled Chemicals (St. Louis, MO). CGP7930 (2,6-Di-*tert*-butyl-4-(3-hydroxy-2,2-dimethyl-propyl)-phenol) was available in house.

Table 1
Effects of test compounds on the affinities of GABA as a displacer of [^{3}H]CGP62349 from native $GABA_B$ receptors in a rat cortical membrane preparation

Test compound	pK_i GABA, high affinity site (− log *M*)	pK_i GABA, low affinity site (− log *M*)	Proportion, high affinity site (%)	*N*
None (control)	7.42 ± 0.05	5.69 ± 0.04	48 ± 1.4	11
CGP7930	7.64 ± 0.05[a]	5.93 ± 0.05	58 ± 1.6[b]	7
Fendiline	7.38 ± 0.12	5.69 ± 0.07	51 ± 4	4
Prenylamine	7.37 ± 0.14	5.74 ± 0.06	49 ± 2.1	4
L-Phenylalanine	7.31 ± 0.11	5.69 ± 0.14	42 ± 4.9	3
L-Leucine	7.26 ± 0.05	5.72 ± 0.05	54 ± 1.6	3
L-Isoleucine	7.39 ± 0.05	5.88 ± 0.05	48 ± 1.4	3
L-Phe-Phe	7.19 ± 0.19	5.69 ± 0.25	47 ± 7.7	3
L-Phe-Leu	7.29 ± 0.16	5.64 ± 0.14	52 ± 6.7	3

The results shown were obtained from displacement curves as shown in Fig. 2. The test compounds were added at a concentration of 30 μM (CGP7930, fendiline and prenylamine) or 100 μM (amino acids and dipeptides). Best curve fits were obtained with a two-site model in all cases. The results shown are means ± S.E.M. from *N* independent experiments.

[a] $P = 0.06$ compared to the corresponding control values (ANOVA, Dunnett's test).

[b] $P < 0.05$ compared to the corresponding control values (ANOVA, Dunnett's test).

3. Results

GTP[γ]^{35}S binding was used as a functional biochemical assay with membranes from a recombinant CHO cell line stably expressing $GABA_{B(1b/2)}$ (Urwyler et al., 2001). No enhancement of GABA-induced responses by the arylalkylamines fendiline and prenylamine (up to 100 μM) was observed, in contrast to CGP7930 (Fig. 1, panel A). Similarly, the amino acids L-Phe, L-Leu and L-Ile and the dipeptides L-Phe-Phe and L-Phe-Leu also produced no enhancement of the

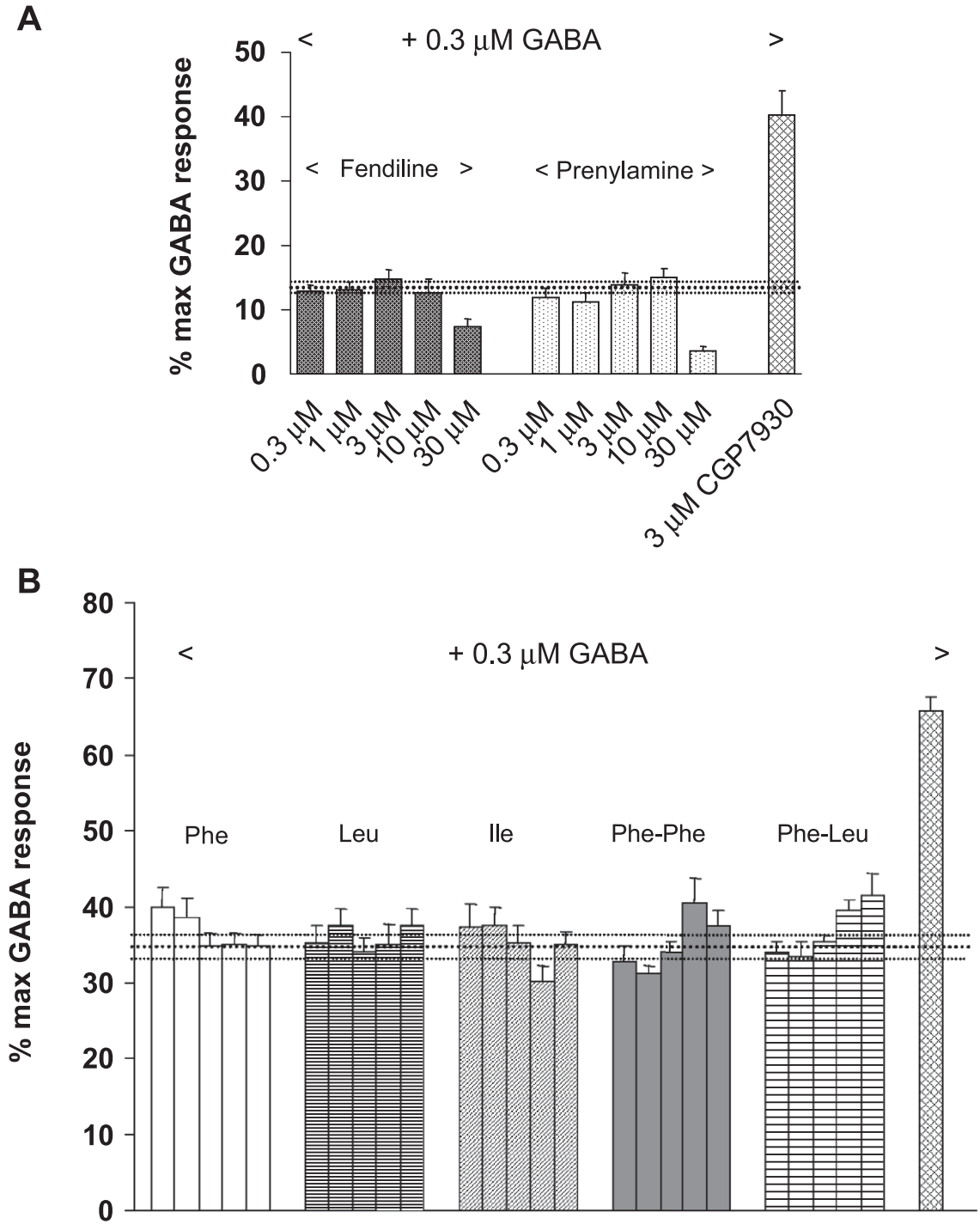

Fig. 3. Lack of effect of arylalkylamines (panel A) and amino acids and dipeptides (panel B) upon GABA-induced intracellular Ca^{2+} signals in CHO cells stably transfected with $GABA_{B(1b/2)}$ and $G\alpha_{qo5}$. For comparison, the enhancement of GABA responses by the known allosteric $GABA_B$ receptor modulator CGP7930 (3 μM) is also shown (last bar). The fluorescence signal is normalized to the effect of a saturating concentration (100 μM) of GABA alone; the data points shown represent means ± S.E.M. from quadruplicate determinations, control values ± S.E.M. are shown as dashed lines. The concentrations of amino acids and dipeptides in panel B were 1, 3, 10, 30 and 100 μM (from left to right in each group).

stimulation of GTP[γ]^{35}S binding by GABA (Fig. 1, panel C). The same results were obtained with a native $GABA_B$ receptor preparation (membranes from rat brain cortex) (Fig. 1, panels B and D). Moreover, the same outcome was found in this assay when L-baclofen was used as an agonist instead of GABA (data not shown).

In a binding assay using [^{3}H]CGP62349 to label native $GABA_B$ receptors in rat cortical membranes (Bittiger et al., 1996), the curves describing the displacement of the radioligand by GABA were biphasic, consisting of a high affinity and a low affinity component (Fig. 2; Table 1). CGP7930 was the only compound shifting the displacement curve towards lower concentrations (Fig. 2), thereby increasing the pK_i values of GABA for both the high and low affinity sites (Table 1). The control binding of the radioligand [^{3}H]CGP62349 was somewhat lower in the presence of CGP7930, but not in that of the other compounds tested (not shown). This was due to a change in affinity of the antagonist radioligand induced by CGP7930, as observed in saturation experiments (not shown). The corresponding K_d values were taken into account for the calculation of the pK_i values shown in Table 1, resulting in less marked differences between the conditions in the absence (control) and in the presence of CGP7930 than might be expected from the curves shown in Fig. 2. Nevertheless, although the effects of CGP7930 shown in Table 1 were at the borderline of statistical significance (the difference for the low affinity site was significant in the pairwise *t*-test comparison), they were consistently found in all our experiments. Furthermore, the relative proportion of the high affinity component was also significantly increased by 30 μM CGP7930 (Table 1). On the other hand, the pK_i values for the high and low affinity GABA binding components and the relative proportions of high affinity sites remained unchanged in the presence of the arylalkylamines, amino acids and dipeptides listed in Table 1. When L-baclofen was used as a displacing agent instead of GABA, the results obtained led to the same conclusion (data not shown).

When $GABA_B$ receptor-mediated responses were recorded as a Ca^{2+} signal in intact CHO cells stably transfected with $GABA_{B(1b/2)}$ and the chimeric G protein $G\alpha_{qo5}$ to couple the $GABA_B$ receptor to the phospholipase C pathway (Franek et al., 1999), a clear potentiation of GABA-induced elevations in intracellular Ca^{2+} was only seen with CGP7930, but not with the arylalkylamines, amino acids and dipeptides tested (Fig. 3). At the highest concentration tested (30 μM), fendiline and prenylamine induced a $GABA_B$ receptor-independent increase in fluorescence that quenched the subsequent GABA-induced signal (Fig. 3).

4. Discussion

Based on the enhancement of baclofen-induced responses in rat neocortical slices, Kerr et al. (2002) have claimed that the arylalkylamine derivatives fendiline, prenylamine and F551 are allosteric modulators at $GABA_B$ receptors. In a subsequent report (Kerr and Ong, 2003), they have strongly suggested that certain amino acids and dipeptides might also act in this way. However, for the reasons outlined in the introduction, such electrophysiological effects in an intact tissue preparation are not sufficient proof of an allosteric mechanism of action of compounds. Since allosteric agents act by definition upon the same protein molecule as orthosteric ligands (such as natural or synthetic agonists), thereby modulating their affinity and/or efficacy, the demonstration of allosterism is best done by proving such interactions at the molecular level (Christopoulos and Kenakin, 2002), for example, in radioligand binding or functional biochemical assays.

The GTP(γ)^{35}S binding assay is a well-established method to assess the function of a number of G-protein-coupled receptors. We tested the arylalkylamines fendiline and prenylamine, as well as those amino acids and dipeptides which were the most active in the experiments of Kerr and Ong (2003), in this assay to examine whether they would enhance the activation of $GABA_B$ receptors by GABA or L-baclofen. With both agonists, and with recombinant as well as with native $GABA_B$ receptors, none of these compounds produced an increase in the GTP(γ)^{35}S signal (Fig. 1 for GABA). In contrast, the well-established allosteric $GABA_B$ modulator CGP7930 (Urwyler et al., 2001) strongly enhanced the stimulation of GTP(γ)^{35}S binding in all cases (Fig. 1).

Positive allosteric modulators usually enhance the affinities of agonists for a given receptor. We have previously shown that CGP7930 enhances the affinity of the agonist radioligand [^{3}H]3-aminopropylphosphinic acid ([^{3}H]APPA) for native $GABA_B$ receptors in saturation experiments (Urwyler et al., 2001). The recently described modulator GS39783 shifts the curves describing the displacement of the antagonist radioligand [^{3}H]CGP62349 by agonists, consisting of a high and a low affinity component, towards lower concentrations (Urwyler et al., 2003). At the same time, it also increases the relative proportion of high agonist affinity sites. In this study, we show that CGP7930 has similar effects (Fig. 2; Table 1), although they seem slightly less pronounced than those found previously for GS39783 (Urwyler et al., 2003). Again, these effects were not mimicked by the arylalkylamines, amino acids and dipeptides tested (Fig. 2; Table 1).

Finally, the same result was obtained in a cellular assay, namely the measurement of intracellular Ca^{2+} mobilization in CHO cells stably expressing $GABA_{B(1b/2)}$ and $G\alpha_{qo5}$. The co-transfection of the chimeric G protein enables $GABA_B$ receptors to couple to the phospholipase C pathway (Franek et al., 1999). Using this readout, we found again that CGP7930 was the only compound of those tested able to enhance the GABA-induced signal (Fig. 3). It should be noted that the GABA concentrations which we have chosen in our functional receptor assays (Figs. 1 and 3) produce control responses in the range of 15–35% of the maximal effects of GABA. Kerr et al. (2002) and Kerr and

Ong (2003) observed clear effects of the compounds under scrutiny at equivalent concentrations of baclofen or another $GABA_B$ receptor agonist, SKF97541 (in their assay system, the potencies of these agonists were considerably lower than that of GABA in our assays). Therefore, we would certainly have been able to detect enhancing effects of the compounds examined, if they were to be positive allosteric modulators.

In summary, we were not able to show any allosteric effects of selected arylalkylamines, amino acids and dipeptides on $GABA_B$ receptors, be it in membrane-based or cellular assays, using native or recombinant receptor preparations. Thus, our data allow excluding allosteric modulation of $GABA_B$ receptors as an explanation for the effects on baclofen-induced responses which have previously been observed with these compounds (Kerr et al., 2002; Kerr and Ong, 2003). It therefore rather seems that these compounds act at distinct sites in the complex circuitry in brain tissue slice preparations, possibly even at different cells (“downstream effects”). Alternatively, intracellular interactions at the effector level could also be at the origin of these previous findings. In fact, many examples of “receptor crosstalk” have been documented (for review, see Cordeaux and Hill, 2002), in particular also in the case of pathways involving $GABA_B$ receptors (reviewed by Enna, 2001). One such case is the enhancement of the stimulation of adenylyl cyclase activity by different neurotransmitter receptors when $GABA_B$ receptors are activated at the same time, likely mediated through the synergism of G protein β/γ-subunits (liberated from G_i/G_o upon $GABA_B$ receptor activation), and $G\alpha_s$ in stimulating adenylyl cyclase types II and IV (Olianas and Onali, 1999; Onali and Olianas, 2001). An example of “crosstalk” between $GABA_B$ and ionotropic, purinoceptors has also been published recently (Sokolova et al., 2003). Many other mechanisms are possible, and further studies at distinct interaction levels are necessary to elucidate the precise type of interactions between arylalkylamines or amino acids and $GABA_B$ receptor-mediated responses in brain slices.

Acknowledgements

The authors wish to thank Dr. Delphine Dupuis for her comments on the manuscript, and J. Heid, M. Horvath, C. Lampert, D. Monna and C. Schoeffel-Bernhard for their expert technical assistance.

References

Birdsall, N.J., Farries, T., Gharagozloo, P., Kobayashi, S., Lazareno, S., Sugimoto, M., 1999. Subtype-selective positive cooperative interactions between brucine analogs and acetylcholine at muscarinic receptors: functional studies. Mol. Pharmacol. 55, 778–786.

Bittiger, H., Bellouin, C., Froestl, W., Heid, J., Schmutz, M., Stampf, P., 1996. [^{3}H]CGP62349: a new potent $GABA_B$ receptor antagonist radioligand. Pharmacol. Rev. Commun. 8, 97–98.

Christopoulos, A., 2002. Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. Nat. Rev. Drug Discov. 1, 198–210.

Christopoulos, A., Kenakin, T., 2002. G protein-coupled receptor allosterism and complexing. Pharmacol. Rev. 54, 323–374.

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.

Cordeaux, Y., Hill, S.J., 2002. Mechanisms of cross-talk between G-protein-coupled receptors. NeuroSignals 11, 45–57.

Enna, S.J., 2001. $GABA_B$ receptor signalling. In: Möhler, H. (Ed.), Pharmacology of GABA and Glycine Neurotransmission. Handbook of Experimental Pharmacology, vol. 150. Springer, Berlin, pp. 329–342.

Franek, M., Pagano, A., Kaupmann, K., Bettler, B., Pin, J.P., Blahos, J., 1999. The heteromeric GABA-B receptor recognizes G-protein α subunit C-termini. Neuropharmacology 38, 1657–1666.

Hammerland, L.G., Garrett, J.E., Hung, B.C., 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.

Johnson, M.J., Baez, M., Jagdmann, G.E. Jr., Britton, T.C., Large, T.H., Callagaro, D.O., Tizzano, J.P., Monn, J.A., Schoepp, D.D., 2003. Discovery of allosteric potentiators for the metabotropic glutamate 2 receptor: synthesis and subtype selectivity of *N*-(4-(2-Methoxyphenoxy)phenyl)-*N*-(2,2,2-trifluoroethylsulfonyl)pyrid-3-ylmethylamine. J. Med. Chem. 46, 3189–3192.

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.

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.

Knoflach, F., Mutel, V., Kew, J.N.C., Malherbe, P., Vieira, E., Wichmann, 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.

Lazareno, S., Gharagozloo, P., Kuonen, D., Popham, A., Birdsall, N.J., 1998. Subtype-selective positive cooperative interactions between brucine analogues and acetylcholine at muscarinic receptors: radioligand binding studies. Mol. Pharmacol. 53, 573–589.

Nemeth, E.F., Steffey, M.E., Hammerland, L.G., Hung, B.C.P., Van Wagenen, B.C., Delmar, E.G., Balandrin, M.F., 1998. Calcimimetics with potent and selective activity on the parathyroid calcium receptor. Proc. Natl. Acad. Sci. U. S. A. 95, 4040–4045.

Olianas, M.C., Onali, P., 1999. $GABA_B$ receptor-mediated stimulation of adenylyl cyclase activity in membranes of rat olfactory bulb. Br. J. Pharmacol. 126, 657–664.

Onali, P., Olianas, M.C., 2001. βγ-Mediated enhancement of corticotrophin-releasing hormone-stimulated adenylyl cyclase activity by activation of γ-aminobutyric $acid_B$ receptors in membranes of rat frontal cortex. Biochem. Pharmacol. 62, 183–190.

Pagano, A., Rovelli, G., Mosbacher, J., Lohmann, T., Duthey, B., Stauffer, D., Ristig, D., Schuler, V., Meigel, I., Lampert, C., Stein, T., Prézeau, L., Blahos, J., Pin, J.P., Froestl, W., Kuhn, R., Heid, J., Kaupmann, K., Bettler, B., 2001. C-terminal interaction is essential for surface trafficking but not for heteromeric assembly of $GABA_B$ receptors. J. Neurosci. 21, 1189–1202.

Pin, J.P., Parmentier, M.L., Prézeau, L., 2001. Positive allosteric modulators for gamma-aminobutyric $Acid_B$ receptors open new routes for the development of drugs targeting family 3 G-protein-coupled receptors. Mol. Pharmacol. 60, 881–884.

Sokolova, E., Nistri, A., Giniatullin, R., 2003. The ATP-mediated fast current of rat dorsal root ganglion neurons is a novel effector for $GABA_B$ receptor activation. Neurosci. Lett. 338, 181–184.

Urwyler, S., Mosbacher, J., Lingenhoehl, K., Heid, J., Hofstetter, K., Froestl, W., Bettler, B., Kaupmann, K., 2001. Positive allosteric modu-

lation 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 CGP13501. Mol. Pharmacol. 60, 963–971.

Urwyler, S., Pozza, M.F., Lingenhoehl, K., Mosbacher, J., Lampert, C., Froestl, W., Koller, M., Kaupmann, K., 2003. GS39783 (*N*,*N*′-Dicyclopentyl-2-methylsulfanyl-5-nitro-pyrimidine-4,6-diamine) and structurally related compounds: Novel allosteric enhancers of γ-aminobutyric acid$_B$ receptor function. J. Pharmacol. Exp. Ther. 307, 322–330.