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Short communication

Comparative potencies of CGP 47654A and CGP 46165A as $GABA_B$ receptor antagonists in rat brain

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Abstract

In rat neocortical slices maintained in Mg^{2+}-free Krebs medium, the γ-aminobutyric acid ($GABA_B$) receptor agonist baclofen concentration-dependently depressed the frequency of spontaneous discharges ($EC_{50} = 6.1$ μM). This was reversibly antagonised by 3-aminopropyl-(1,1-difluoro-*n*-butyl)-phosphinic acid (25, 100, 500 μM) (CGP 47654A) and 3-aminopropyl-*P*-(α-hydroxybenzyl)-phosphinic acid (CGP 46165A) (50, 100, 400 μM) which produced rightwards shifts of the baclofen concentration–response curves, with respective pA_2 values of 4.9 ± 0.2 and 4.6 ± 0.15. Although relatively potent on $GABA_B$ heteroreceptors studied here, CGP 47654A and CGP 46165A were 5 and 50 times weaker, respectively, as $GABA_B$ autoreceptor antagonists [Froestl, W., Mickel, S.J., Von Sprecher, G., Diel, P.J., Hall, R.G., Maier, L., Strub, D., Melillo, V., Baumann, P.A., Bernasconi, R., Gentsch, C., Hauser, K., Jaekel, J., Karlsson, G., Klebs, K., Maitre, L., Marescaux, C., Pozza, M.F., Schmutz, M., Steinmann, M.W., Van Riezen, H., Vassout, A., Mondadori, C., Olpe, H.R., Waldmeier, P.C., Bittiger, H., 1995. Phosphinic acid analogues of GABA. 2. Selective, orally active $GABA_B$ antagonists. J. Med. Chem. 38: 3313–3331.], representing potentially useful ligands for differentiating $GABA_B$ hetero- and autoreceptors.

Keywords: $GABA_B$ receptor antagonist; Brain slice, rat; Baclofen; CGP 47654A; CGP 46165A

1. Introduction

During the course of the development of antagonists active at $GABA_B$ receptors for the inhibitory neurotransmitter GABA, a variety of compounds were synthesised by replacing the carboxylic acid residues in GABA and baclofen derivatives with various *P*-substituted phosphinic acid groups (Froestl et al., 1995). These substituents provide accessory binding adjacent to the anionic recognition site of the $GABA_B$ receptor, which imparts improved binding at the receptor complex, resulting in a variety of 3-aminopropyl-*P*-substituted phosphinic acid congeners that are effective antagonists of $GABA_B$ receptor-mediated actions in functional studies (Davies et al., 1993; Olpe et al., 1993; Froestl and Mickel, 1997). By far, the majority of these show comparable affinities at $GABA_B$ auto- and heteroreceptors, correlating well with their affinities for inhibition of [^{3}H]3-aminopropylphosphinic acid ([^{3}H]CGP 27492) binding to $GABA_B$ receptors on rat cortical membranes (for example see Fig. 5 in Waldmeier et al., 1994). Interestingly, however, some of the congeners prepared by Froestl et al. (1995) display relatively more potent binding affinities for $GABA_B$ receptors than antagonist activity at $GABA_B$ autoreceptors modulating evoked [^{3}H]GABA release in electrically-stimulated rat brain slices (see Tables 1 and 2 in Froestl et al., 1995). Some of these are five or more times as potent in binding at $GABA_B$ receptors than as antagonists at the autoreceptors.

In this study, we have now examined the activities of 3-aminopropyl-(1,1-difluoro-*n*-butyl)-phosphinic acid (CGP 47654A) and 3-aminopropyl-*P*-(α-hydroxybenzyl)-phosphinic acid (CGP 46165A) (Fig. 1) as antagonists of $GABA_B$ heteroreceptors, in a functional assay using spontaneously discharging rat neocortical slices. These compounds have been selected for testing as they exhibit respectively several fold higher potency in $GABA_B$ receptor binding than as $GABA_B$ autoreceptor antagonists (Froestl et al., 1995), and we here show that CGP 46165A

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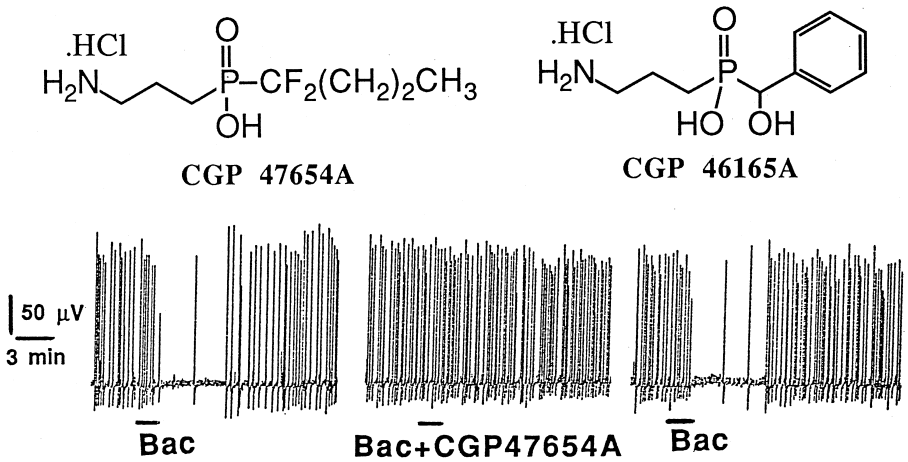


Fig. 1. Chemical structures of 3-aminopropyl-(1,1-difluoro-*n*-butyl)-phosphinic acid (CGP 47654A) and 3-aminopropyl-*P*-(α-hydroxybenzyl)-phosphinic acid (CGP 46165A). The representative record from a typical experiment shows the effect of CGP 47654A on the responses to baclofen (Bac) in the rat neocortical slice preparation, maintained in Mg^{2+}-free Krebs medium. Baclofen (Bac; 6 μM), applied for 2 min suppressed the spontaneous discharges for 5 min, with slowing for a further 5 min. Application of CGP 47654A (100 μM) reversibly antagonised these effects, with the control response to baclofen being re-established upon wash-out of the test compounds.

and CGP 47654A represent novel examples of ligands with corresponding higher potencies at $GABA_B$ hetero- as against autoreceptors.

2. Materials and methods

2.1. Rat neocortical slice preparations

The 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. 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, $MgSO_4$ 1.3. Cerebral cortical slices (400 μM thick) were prepared by cutting coronal sections using a vibraslice microtome (Campden Instruments, UK) and a radial wedge was cut from each side of the dorsal mid-line to yield slices of cingulate cortex and corpus callosum 2–3 mm wide. The slices were subsequently equilibrated in gassed Krebs solution at room temperature (20–23°C) for 60 min prior to experimentation.

Using a superfusion method based on a grease–gap system as described previously (Horne et al., 1986; Ong et al., 1990), the slices from the neocortex were superfused with gassed Mg^{2+}-free Krebs medium at 25°C delivered by a peristaltic pump at 1 ml/min. $MgSO_4$ was omitted in the Mg^{2+}-free medium. DC potentials between the cingulate cortex and corpus callosum were monitored on a chart recorder using Ag/AgCl electrodes, agar/saline bridges and a high input-impedance DC amplifier. The neocortical slices developed spontaneous paroxysmal discharges after equilibration in Mg^{2+}-free Krebs medium for 15 min. The $GABA_B$ receptor agonist baclofen, added to the superfusing medium, was applied to the cortical side of the tissue for 2 min and the preparation was allowed 30 min recovery between drug applications. The antagonist was first superfused for 2 min and then added together with the agonist.

Results were quantified by counting the number of spontaneous discharges in 10 min epochs, in the absence and presence of test compounds, and the values expressed as a percentage depression of the average control discharge rate during the 10 min immediately before the addition of drugs. Concentration–response curves for the agonist were constructed, in the absence and presence of the antagonist. The EC_{50} value, that is the concentration which produced 50% inhibition of the discharge rate, was calculated from the concentration–response curve, and estimates of apparent pA_2 values were made. The pA_2 value was derived from the relationship $pA_2 = \log (CR\text{-}1) - \log [B]$, where (CR-1) is the concentration ratio-1, and $[B]$ the antagonist concentration. All numerical data on the concentration–response curves were expressed as means ± S.E.M. Each experiment was repeated on 8 slices obtained from 4 different animals.

2.2. Drugs

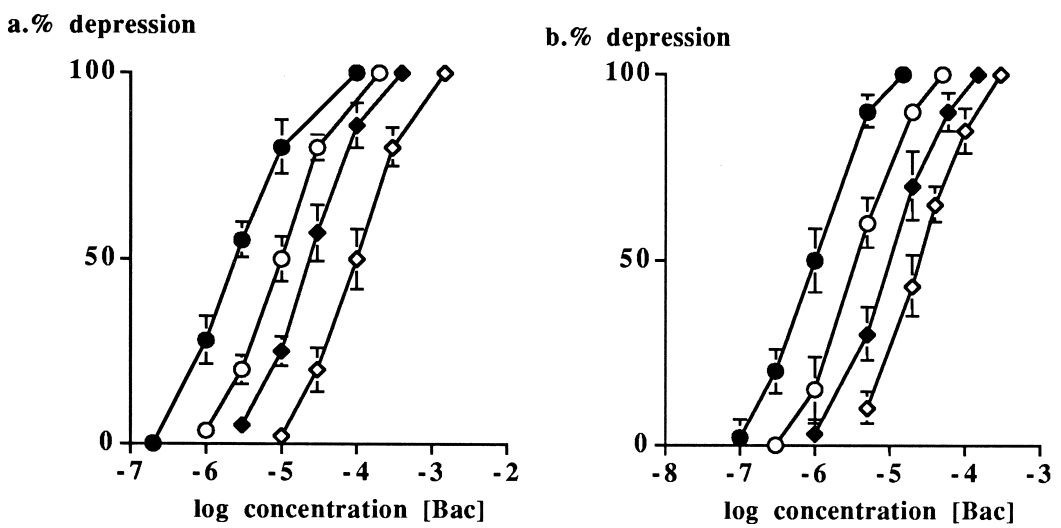
Racemic baclofen, CGP 47654A and CGP 46165A were synthesised at Novartis Pharmac. (Basel, Switzerland).

3. Results

Baclofen depressed the frequency of spontaneous depolarisations in neocortical slices superfused with Mg^{2+}-free Krebs medium in a consistent and reversible manner. Fig. 1 shows a representative experiment in which baclofen (Bac; 6 μM) abolished the spontaneous activity for 5 min, with a subsequent slowing of the discharges for a further 5 min; these effects generally lasted some 10 min and returned to baseline levels within 20 min following the initial wash-out of the drug. This depression was concentration-dependent, with an approximate EC_{50} of 6.1 μM (Fig. 2). Pre-treatment with CGP 47654A (100 μM) alone for 2 min did not affect the discharge rate or amplitude, but in combination with baclofen (6 μM) for 2 min, reversibly antagonised the baclofen-induced suppression of spontaneous discharges (Fig. 1). Following wash-out of the compounds, there was a complete recovery of the spontaneous activity and the depressant response to baclofen (6 μM; Fig. 1) within 15 min.

In order to quantify the antagonist potencies of CGP 47654A and CGP 46165A, the effects of three concentrations of CGP 47654A (25, 100, 500 μM) and of CGP 46165A (50, 100, 400 μM) on the baclofen concentration–response curve were measured. Increasing concentrations of both compounds caused a progressive shift of the baclofen concentration–response curve to the right, without depression of the maximum response. Using the ratio method and averaging, this yielded apparent pA_2 values of 4.9 ± 0.2 and 4.6 ± 0.15 for CGP 47654A and CGP 46165A, respectively (Fig. 2; $n = 8$). On their own, neither compound affected the discharge rate, or amplitude at the concentrations tested.

A comparison of the potencies of CGP 47654A and CGP 46165A on baclofen-induced responses in the spontaneously discharging slices, inhibition of binding of the $GABA_B$ agonist ligand [^{3}H]CGP 27492 to $GABA_B$ receptors in rat cerebral cortical membranes, and enhancement of electrically-evoked [^{3}H]GABA release from rat brain slices is shown in the table in Fig. 2. Here, the potencies of CGP 47654A and CGP 46165A as $GABA_B$ autoreceptor antagonists were considerably weaker than their binding affinities for $GABA_B$ receptors, or their antagonist activities on $GABA_B$ heteroreceptors in the spontaneously discharging slices. The EC_{150} of CGP 47654A was 115 μM, whilst 100 μM CGP 46165A was required to induce a 10% increase of [^{3}H]GABA release (Froestl et al., 1995), indicating that CGP 47654A was more potent than CGP



	pA_2[a]	IC_{50} (μM)[b]	EC_{150} (μM)[c]
CGP 47654A	4.9 ± 0.2	22	115
CGP 46165A	4.6 ± 0.15	8	10%[d]

Fig. 2. Concentration–response curves for (*R,S*)-baclofen-induced suppression of the frequency of spontaneous discharges in the rat isolated neocortical slices, maintained in Mg^{2+}-free Krebs medium, in the absence and presence of 3-aminopropyl-(1,1-difluoro-*n*-butyl)-phosphinic acid (CGP 47654A) and 3-aminopropyl-*P*-(α-hydroxybenzyl)-phosphinic acid (CGP 46165A). The concentration–response curve for baclofen (●) was subsequently shifted to the right, in a parallel fashion by (a). CGP 47654A (○ 25, ♦ 100 and ◇ 500 μM) and by (b). CGP 46165A (○ 50, ♦ 100 and ◇ 400 μM). Values are expressed as a percentage depression of the control discharge rate. Each point represents the mean and standard error of the mean of 8 determinations. The table shows a comparison of the potencies of CGP 47654A and CGP 46165A as antagonists at $GABA_B$ receptors in rat brain. [a] pA_2 values are estimates of antagonist potencies in the spontaneously discharging rat neocortical slices using the relationship $pA_2 = \log (CR-1) - \log [B]$, where CR is the concentration ratio (CR) and [B] is the concentration of the antagonist. [b] IC_{50} is the estimated half maximal concentration of inhibition of [^{3}H]CGP 27492 binding to $GABA_B$ receptors in rat cerebral cortical membranes, and [c] EC_{150} is the concentration causing a 50% enhancement of electrically-induced release of [^{3}H]GABA from rat cortical slices (stimulation frequency 2 Hz). [d] 10% increase of [^{3}H]GABA release at a concentration of 100 μM. The binding and release data are obtained from Froestl et al. (1995).

46165A on autoreceptors. However, as previously reported in binding studies, their potencies in displacing [^{3}H]CGP 27492 binding to $GABA_B$ receptors were higher with IC_{50} values in the low micromolar concentrations (22 and 8 μM, respectively; Froestl et al., 1995).

4. Discussion

In the present study, the novel 3-aminopropyl-phosphinic acid derivatives CGP 47654A and CGP 46165A, with modified *P*-alkyl substituents at the phosphinic moiety (Fig. 1) reversibly antagonised the baclofen-induced depression of spontaneous discharges in rat neocortical slices maintained in Mg^{2+}-free Krebs medium. The *P*-1,1-difluoro-*n*-butyl derivative CGP 47654A displayed an apparent pA_2 value of 4.9 ± 0.2, whilst the α-hydroxybenzyl derivative CGP 46165A was twice less potent, with an apparent pA_2 of 4.6 ± 0.15. The corresponding affinities for inhibition of [^{3}H]CGP 27492 binding at $GABA_B$ receptors, with a pK_i of 4.7 for CGP 47654A and a pK_i of 5 for CGP 46165A (data from Froestl et al., 1995), give a reasonable correlation between binding and antagonism at heteroreceptors. However, the increase in electrically-evoked overflow of [^{3}H]GABA in rat cortical slices requires higher concentrations of CGP 47654A and CGP 46165A than their binding affinities would predict, the estimated EC_{150} values being 100 μM for CGP 47654A and 400 μM for CGP 46165A (unpublished observations; see also Tables 1 and 2 in Froestl et al., 1995). Thus, CGP 47654A was more potent than CGP 46165A as a $GABA_B$ autoreceptor antagonist, in increasing electrically-evoked overflow of [^{3}H]GABA in rat cortical slices (Froestl et al., 1995); moreover, higher concentrations of both CGP 47654A and CGP 46165A are required to block $GABA_B$ autoreceptors in the release studies, than to block heteroreceptors in the spontaneously discharging rat neocortex.

Whilst there are difficulties in making direct comparisons between the binding data and the data obtained from the functional studies, it is nevertheless intriguing that CGP 46165A, in particular, shows some 50 fold difference between its potencies at auto- as against heteroreceptors, whereas CGP 47654A shows at least a 5 fold less potency. This difference lies outside the correlation found by Waldmeier et al. (1994). In their Fig. 5, using a different series of *P*-substituted phosphinic analogues of GABA, an almost perfect correlation between their potencies for inhibition of [^{3}H]CGP 27492 binding and for antagonism of $GABA_B$ autoreceptor-mediated suppression of GABA release is found, suggesting that the latter $GABA_B$ receptor antagonists do not discriminate between $GABA_B$ receptor subtypes (Waldmeier et al., 1994).

In the current study, CGP 47654A and CGP 46165A displayed similar profiles in spontaneously discharging neocortical preparations and binding assays, but were weaker on autoreceptors in the release studies, CGP 47654A very much so. These results suggest that they may select between $GABA_B$ receptor subtypes. Indeed, with the recent cloning of two N-terminal splice variants of the $GABA_B$ receptors (Kaupmann et al., 1997; Morris et al., 1998), where the amino acid sequence shows similar homology to metabotropic glutamate receptor subtypes, it is perhaps not surprising that $GABA_B$ receptor subtypes may also exist. The development of ligands such as CGP 47654A and CGP 46165A might thus provide useful pharmacological tools for the investigation of $GABA_B$ receptor heterogeneity.

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