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GABA and glutamate release affected by $GABA_B$ receptor antagonists with similar potency: no evidence for pharmacologically different presynaptic receptors

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1 The effects of a series of nine $GABA_B$ receptor antagonists of widely varying potencies on electrically stimulated release from cortical slices of [^{3}H]-GABA in the absence or presence of 10 μM of the $GABA_B$ agonist, (−)-baclofen and of endogenous glutamate in the presence of (−)-baclofen were compared.

2 The concentrations of the compounds half maximally increasing [^{3}H]-GABA release (EC_{50}'s) at a stimulation frequency of 2 Hz correlated well with the IC_{50} values obtained from the inhibition of the binding of the agonist, [^{3}H]-CGP 27492, to $GABA_B$ receptors in rat brain membranes (rank order of potency: CGP 56999 A ⩾ CGP 55845 A > CGP 52432 ⩾ CGP 56433 A > CGP 57034 A > CGP 57070 A ⩾ CGP 57976 > CGP 51176 > CGP 35348).

3 Likewise, the concentrations causing half-maximal increases of [^{3}H]-GABA in the absence or presence of (−)-baclofen, and of endogenous glutamate in the presence of (−)-baclofen, correlated well with each other. Reports in the literature suggesting the CGP 35348 exhibits a 70 fold preference for inhibition of (−)-baclofen's effects on glutamate over [^{3}H]-GABA release, and that CGP 52432 shows a 100 fold preference in the opposite sense, could not be confirmed in our model.

4 Therefore, our results suggest that, if there are pharmacological differences between $GABA_B$ autoreceptors and $GABA_B$ heteroreceptors on glutamatergic nerve endings in the rat cortex, they are not revealed by this series of compounds of widely different potencies.

5 In particular, our results with CGP 35348 and CGP 52432 do not support the hypothesis that $GABA_B$ autoreceptors and $GABA_B$ heteroreceptors on glutamatergic nerve endings represent subtypes with different pharmacology.

Keywords: $GABA_B$ autoreceptors; $GABA_B$ heteroreceptors; subtypes; [^{3}H]-GABA release; glutamate release; $GABA_B$ receptor antagonists

Introduction

Presynaptic $GABA_B$ receptors seem to modulate the release of several neurotransmitters. The $GABA_B$ agonist, baclofen, has been reported to inhibit the release of GABA itself via autoreceptors (Pittaluga *et al.*, 1987; Waldmeier *et al.*, 1988), and glutamate (Potashner, 1979), biogenic amines (Bowery *et al.*, 1980; Schlicker *et al.*, 1984; Gray & Green, 1987), cholecystokinin (Conzelmann *et al.*, 1986) and somatostatin (Bonanno *et al.*, 1991) via heteroreceptors. Recently, evidence for differential pharmacology of auto- and heteroreceptors has been presented. Thus, the $GABA_B$ receptor antagonist, phaclofen, was 10 times more potent in antagonizing the inhibitory effect of (−)-baclofen on the release of GABA and of somatostatin-like immunoreactivity (SRIF-LI) than of glutamate, whereas CGP 35348 was about 70 times more potent in inhibiting the effect of baclofen on glutamate and SRIF-LI than on GABA release (all from rat cortical synaptosomes and elicited by elevated K^+; Bonanno & Raiteri, 1992). The novel, more potent $GABA_B$ receptor antagonist, CGP 52432, inhibited the effect of baclofen on GABA release about 100 or 40 times more potently than its effects on glutamate or SRIF-LI release (Lanza *et al.*, 1993), respectively. From these results, the existence of multiple subtypes of $GABA_B$ receptors was inferred (Bonanno & Raiteri, 1993), an attractive hypothesis particularly with respect to drug development, because it offers the possibility of obtaining $GABA_B$ receptor antagonists which preferentially interact with the $GABA_B$ autoreceptor or certain heteroreceptors.

We have therefore investigated a series of new $GABA_B$ receptor antagonists, with markedly different potencies for the $GABA_B$ receptor as measured in *in vitro* radioligand binding assays, with respect to their relative potencies for antagonizing the inhibitory effect of (−)-baclofen on the electrically stimulated release of [^{3}H]-GABA and endogenous glutamate from rat cortical slices. The compounds were selected for this study because they are structurally related to CGP 35348 and CGP 52432, which reportedly affect GABA and glutamate release differentially. It seemed of interest to see whether relative potency differences with respect to $GABA_B$ auto- and heteroreceptors such as reported for the above two compounds, could also be found among structural relatives. CGP 35348 and CGP 52432 were included in the study for comparison.

Methods

Release experiments

Cross-chopped slices from rat (male Tif:RAlf(SPF) rats, Tierfarm Sisseln, Switzerland, weighing 180–240 g) cerebral cortex were prepared, loaded with [^{3}H]-GABA if appropriate and superfused as described previously (Baumann *et al.*, 1990; Waldmeier *et al.*, 1993a). Experiments in which [^{3}H]-GABA release was measured were done in the presence of the GABA transminase inhibitor, AOAA (50 μM), and the GABA uptake inhibitor, SK&F 89976 (10 μM), as described by Baumann *et al.* (1990). Three groups of 4 slice preparations each, were twice stimulated electrically at 2 Hz for 2 min (25 mA, 2 ms), at an interval of 36 min. One group

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served as a control; in the remaining two, the $GABA_B$ antagonists were added at appropriate concentrations, 2 fractions after the first stimulation (S_1). In one series of experiments, (−)-baclofen (10 μM) was present throughout, in the control as well as in the test group; again, the $GABA_B$ receptor antagonists were added at appropriate concentrations, 2 fractions after S_1.

The release of endogenous glutamate was measured by an *o*-phthaldialdehyde/h.p.l.c./fluorescence detection method in the presence of the glutamate uptake inhibitor, L-*trans*-PDC, as described by Waldmeier *et al.* (1993b). Three groups of 4 slice preparations each were twice stimulated electrically at 1 Hz for 4 min (25 mA, 2 ms), at an interval of 36 min. One group served as a 'baclofen control', (−)-baclofen (10 μM) being present throughout. In the two others, the $GABA_B$ receptor antagonists were added at appropriate concentrations, 2 fractions after the first stimulation (S_1).

Data were calculated as a percentage of fractional release for each fraction. From this, the release evoked by electrical stimulation in S_1 and S_2 was calculated by subtraction of the basal release, assuming a linear decline between the fractions before and after stimulation. This integration was done for one fraction in the case of [^{3}H]-GABA and, due to the longer stimulation period, for two fractions in the case of glutamate. The data shown in the graphs are the effects of the $GABA_B$ receptor antagonists as percentages of controls ± s.e.mean obtained from a comparison of the S_2/S_1 ratios in each experiment.

Binding experiments

[^{3}H]-CGP 27492 was used as a radioligand to measure interactions of the compounds with $GABA_B$ receptors (Bittiger *et al.*, 1988; Olpe *et al.*, 1990). Membranes from rat cerebral cortex were used and the assay was performed in 2 ml Krebs-Henseleit buffer pH 7.4, containing 20 mM Tris buffer, 200–300 μg membrane protein, 2 nM [^{3}H]-CGP 27492 (15 Ci mmol^{-1}) and the compounds to be tested. The incubation was performed at room temperature for 40 min and terminated by rapid filtration on Whatman GF/B glass fibre filters, which were washed twice with 5 ml ice-cold buffer. Filter-bound radioactivity was counted in Irgascint A 300 (Ciba-Geigy). Incubations were performed in triplicate and nonspecific binding was determined in the presence of (−)-baclofen (10 μM).

Estimations of IC_{50} and K_i values and Hill coefficients of the radioligand binding data, and EC_{50} values and maximal increases of release

These calculations were performed by nonlinear (sigmoidal) fitting using a commercially available PC software programme (GraphPad Prism 1.0, GraphPad Software Inc., San Diego, Ca, U.S.A.). K_i values were calculated from the IC_{50}'s by means of the Cheng-Prusoff equation (Cheng & Prusoff, 1973).

Chemicals

L-Glutamate, S-carboxymethylcysteine and amino-oxyacetic acid (AOAA) were obtained from FLUKA AG (Buchs, Switzerland), L-*trans*-pyrrolidine-2,4-dicarboxylic acid (L-*trans*-PDC) from Tocris (Bristol, U.K.), and *o*-phthaldialdehyde solution from Pierce Europe (Oud Beijerland, The Netherlands). The hydrochloride of SK&F 89976 (1-(4,4-diphenyl-3-butenyl)-3-piperidine carboxylic acid hydrochloride) was prepared by Dr H. Allgeier in our Chemistry Department. (−)-Baclofen, CGP 35348 (3-aminopropyl-diethoxy-methylphosphinic acid), CGP 51176 (3-amino-2-(**R**)-hydroxy-propyl-cyclohexyl-methyl-phosphinic acid), CGP 52432 ([3-[[(3,4-dichlorophenyl)methyl]amino]propyl] (diethoxymethyl) phosphinic acid), CGP 55845 A (3-[1-(**S**)-(3,4-dichlorophenyl)-ethyl]amino-2(**S**)-hydroxy-propyl-benzyl-phosphinic acid (hydrochloride), CGP 56433 A ([3-[[1-(**S**)-(3-carboxyphenyl) ethyl]amino] 2-(**S**)-hydroxy-propyl]-cyclohexyl-methyl-phosphinic acid (lithium salt)), CGP 56999 A ([3[[1-(**R**)-(3-carboxyphenyl)ethyl]amino] 2-(**S**)-hydroxy-propyl]-cyclohexyl-methyl-phosphinic acid (lithium salt)), CGP 57034 A ([3-[[1-(**S**)-(4-carboxy-phenyl)ethyl]amino] 2-(**S**)-hydroxy-propyl]-cyclohexylmethyl-phosphinic acid (lithium salt)), CGP 57070 A ([3-[[1-(**R**)-(4-carboxyphenyl) ethyl]amino] 2-(**S**)-hydroxy-propyl]-cyclohexylmethyl-phosphinic acid (lithium salt)) and CGP 57976 ([3-[[1-(**R**)-(3,4,5-trimethoxyphenyl) ethyl]amino] 2-(**S**)-hydroxy-propyl]-cyclohexylmethyl-phosphinic acid) are experimental compounds of Ciba-Geigy. The chemical structures are given in the left column of Table 1. The $GABA_B$ radioligand, [^{3}H]-CGP 27492 was synthesized by Ciba-Geigy Horsham, U.K. High purity water (MilliQ water purification system, Waters Assoc., Milford, MA, U.S.A.) was used for all drug dilutions, buffers, mobile phases etc. [2,3-^{3}H(N)-Aminobutyric acid ([^{3}H]-GABA; NET-191, specific activity 25–40 Ci mmol^{-1}) was obtained from New England Nuclear, Boston, Mass, U.S.A.

Results

Inhibition of ^{3}H-CGP 27492 binding

All nine $GABA_B$ receptor antagonists displaced [^{3}H]-CGP 27492 concentration-dependently in a monophasic manner, and to completeness. The IC_{50} values and the corresponding Hill coefficients were estimated by sigmoidal fitting. Because the Hill coefficients were all close to unity, the corresponding K_i values were calculated by means of the Cheng-Prusoff equation (radioligand concentration used in the experiments = 2 nM, K_D = 7.4 nM, cf. Bittiger *et al.*, 1988). The negative logarithms of the K_i values (pK_i) are given in Table 1.

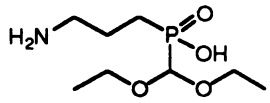
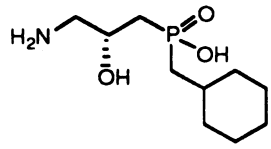
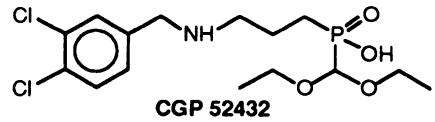
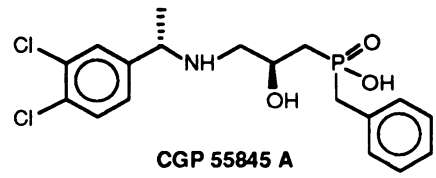
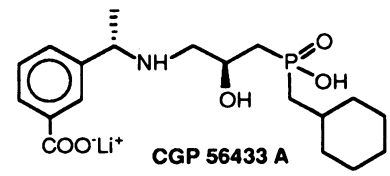
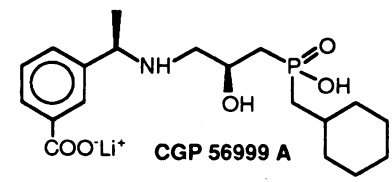
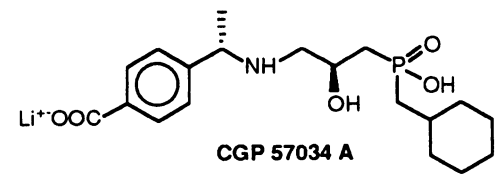
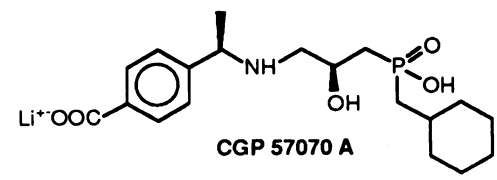
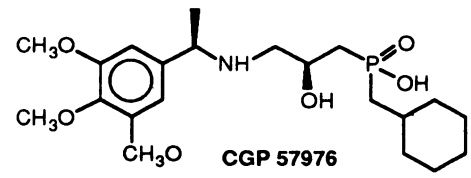
Effects on [^{3}H]-GABA release in the absence of (−)-baclofen

A typical example of an experiment, in this case with 1 μM CGP 56433 A, is given in Figure 1a. The increase in [^{3}H]-GABA release caused by this concentration of the drug was calculated as a percentage of control, comparing S_2/S_1 ratios. All the investigated compounds concentration-dependently increased the release of [^{3}H]-GABA elicited by electrical stimulation at 2 Hz, at which sufficient endogenous GABA is released to activate markedly the autoreceptor (Figures 2–4). The corresponding pEC_{50} values, (concentrations which increase control release half maximally), are summarized in Table 1. The data show that the selection of $GABA_B$ receptor antagonists investigated in this study covers a range of four orders of magnitude with respect to potency, from low nanomolar (CGP 56999 A) to micromolar (CGP 35348). The maximal increases of [^{3}H]-GABA release caused by these agents were approximately 300% of controls. This is in keeping with previous experience with a number of other $GABA_B$ receptor antagonists (Waldmeier *et al.*, 1993a).

Effects on [^{3}H]-GABA release in the presence of (−)-baclofen

Concentration-response curves of the effects of the $GABA_B$ receptor antagonists on [^{3}H]-GABA release were made in the presence of (−)-baclofen (10 μM), at a stimulation frequency of 2 Hz as above. A typical example of an experiment, in this case with 1 μM CGP 56433 A, is given in Figure 1b. The concentration-response data, also shown in Figures 2–4, differ little from those in the absence of (−)-baclofen, except that the maximal percentual increases are higher (approximately 450–500% of controls).

Table 1 IC_{50} values with respect to inhibition of binding of [^{3}H]-CGP 27492 to the GABA$_B$ receptor and concentrations causing half-maximal increases of the release of GABA, in the absence and presence of (−)-baclofen, and of glutamate in the presence of 10 μM (−)-baclofen

Compound		*[^{3}H]CGP 27492 binding* pKi A	*[^{3}H]-GABA release* pEC_{50} B	*[^{3}H]GABA rel. in presence of (−)-baclofen* pEC_{50} C	*Glu release in presence of (−)-baclofen* pEC_{50} D
	**CGP 35348**	4.71 ± 0.01 *Hill: 1.05 ± 0.03*	4.16 ± 0.33	3.98 ± 0.07	3.98 ± 0.11
	**CGP 51176**	5.47 ± 0.05 *Hill: 0.73 ± 0.06*	4.73 ± 0.38	4.83 ± 0.06	4.88 ± 0.10
	**CGP 52432**	7.35 ± 0.02 *Hill: 0.91 ± 0.04*	6.47 ± 0.07	6.49 ± 0.02	6.65 ± 0.25
	**CGP 55845 A**	8.35 ± 0.01 *Hill: 0.99 ± 0.02*	8.08 ± 0.26	7.60 ± 0.14	7.85 ± 0.08
	**CGP 56433 A**	7.20 ± 0.07 *Hill: 1.04 ± 0.17*	6.82 ± 0.01	6.59 ± 0.02	6.53 ± 0.14
	**CGP 56999 A**	8.70 ± 0.15 *Hill: 1.29 ± 0.04*	8.46 ± 0.09	8.20 ± 0.09	7.89 ± 0.15
	**CGP 57034 A**	6.75 ± 0.02 *Hill: 1.01 ± 0.04*	6.31 ± 0.01	6.31 ± 0.01	6.21 ± 0.01
	**CGP 57070 A**	6.27 ± 0.02 *Hill: 0.93 ± 0.04*	5.85 ± 0.01	5.66 ± 0.01	5.84 ± 0.25
	**CGP 57976**	6.05 ± 0.02 *Hill: 0.89 ± 0.03*	5.65 ± 0.01	5.55 ± 0.09	5.81 ± 0.04

Data are the negative logarithms ± s.e.mean of the K_i values (pK_i) of the compounds for the inhibition of [^{3}H]-CGP 27492 binding to GABA$_B$ receptors (**A**; Hill coefficients ± s.e.mean in italics) or of the half-maximal increases (pEC_{50}) of [^{3}H]-GABA release in the absence of (−)-baclofen (**B**), of [^{3}H]-GABA release in the presence of (−)-baclofen (**C**), and of the release of endogenous glutamate (Glu) in the presence of (−)-baclofen (**D**), respectively. They were determined by non-linear sigmoidal fitting of the binding data and the release data shown in Figures 2–4. Examination of the correlation between the different sets of data by means of linear regression yielded the following correlation coefficients: **A** vs. **B**: $r = 0.991$; **A** vs. **C**: $r = 0.994$; **A** vs. **D**: $r = 0.991$: **B** vs. **C**: $r = 0.994$; **B** vs. **D**: $r = 0.988$; **C** vs. **D**: $r = 0.990$.

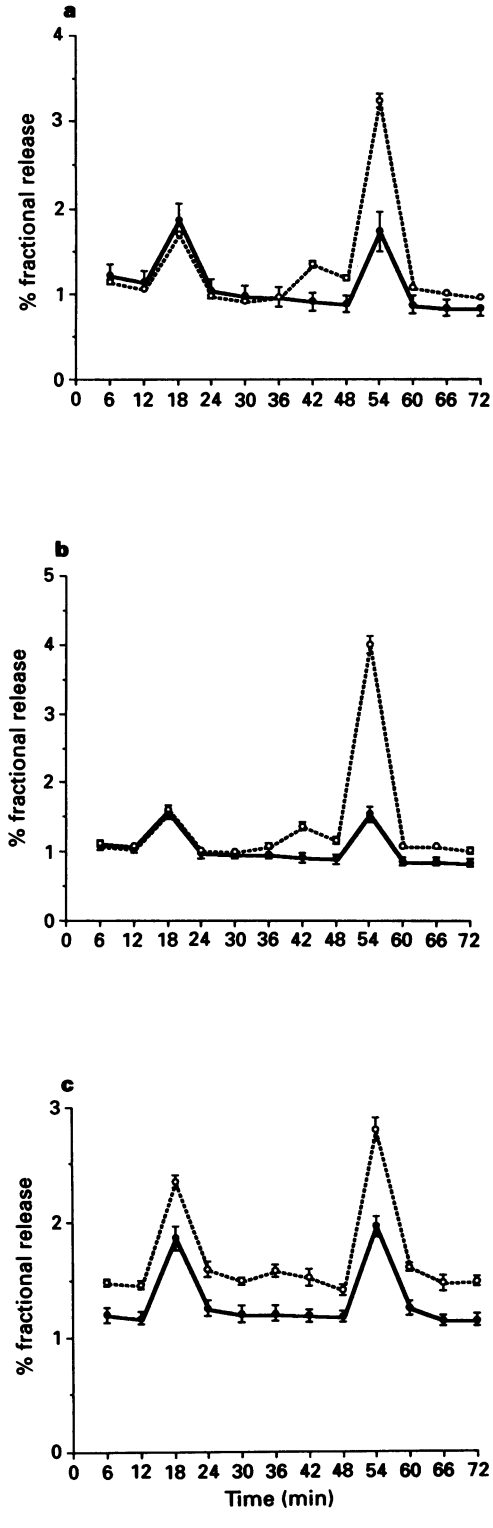


Figure 1 Examples of raw data used to calculate percentage increases given in Figures 2–4. Groups of 4 slice preparations were twice stimulated electrically, at 2 Hz for 2 min (25 mA, 2 ms) for measurements of the release of [^{3}H]-GABA, and at 1 Hz for 4 min (25 mA, 2 ms) for measurements of the release of endogenous glutamate (Glu). In the experiment with [^{3}H]-GABA in the absence (a) and presence of 10 μM (−)-baclofen (b), 50 μM AOAA and 10 μM SK&F 89976 were present as inhibitors of GABA transaminase and GABA uptake, respectively. In the experiments with endogenous glutamate (c), 100 μM *trans*-PDC was present as an inhibitor of glutamate uptake, and 10 μM (−)-baclofen was also present throughout. The test drug (in this case 1 μM CGP 56433 A) was added to the superfusion medium two fractions after the first stimulation (S_1). Data are means ± s.e.mean as a percentage of fractional release: (●—●) controls; (○—○) CGP 56433 A 1 μM.

Effects on endogenous glutamate release in the presence of (−)-baclofen

In analogy to the above, concentration-response curves of the effects of the $GABA_B$ receptor antagonists on the release of endogenous glutamate were made in the presence of (−)-baclofen (10 μM), at a stimulation frequency of 1 Hz. A typical example of an experiment, in this case with 1 μM CGP 56433 A, is given in Figure 1c. The data of the whole series of experiments are summarized in Figures 2–4. All the compounds concentration-dependently increased glutamate release as compared with that observed in the presence of (−)-baclofen alone. The maximal increases were approximately 170% of controls. It is important to note that there were no major differences between the concentration-response curves with respect to the release of [^{3}H]-GABA or endogenous glutamate (both in the presence of 10 μM (−)-baclofen) with any of the nine $GABA_B$ receptor antagonists studied.

The data of Figures 2–4 were used to estimate the concentrations of the compounds causing half-maximal responses (EC_{50}'s) with respect to [^{3}H]-GABA release in the absence or presence of (−)-baclofen, and to the release of endogenous glutamate in the presence of (−)-baclofen. The negative logarithms of the values obtained (pEC_{50}'s) are listed in Table 1. On the average, EC_{50}'s for [^{3}H]-GABA release without (−)-baclofen were about half those in its presence. The ratios [EC_{50} for [^{3}H]-GABA release]/[EC_{50} for glutamate release] in the presence of (−)-baclofen were on the average close to 1. The pEC_{50}'s all correlated with each other as well as with the pK_i's with respect to inhibition of [^{3}H]-CGP 27492 binding with correlation coefficients approaching unity (see footnote to Table 1). As examples, the correlation plots of the pK_i's with respect to [^{3}H]-CGP 27492 binding vs. pEC_{50}'s with respect to [^{3}H]-GABA release in the absence of (−)-baclofen (a) and of the pEC_{50}'s with respect to [^{3}H]-GABA vs. those of glutamate release, both in the presence of (−)-baclofen (b), are shown in Figure 5.

Discussion

There was an almost perfect correlation between the potencies of the nine $GABA_B$ receptor antagonists tested for inhibition of [^{3}H]-CGP 27492 binding to $GABA_B$ receptors in rat cortical membranes and for antagonism of the autoreceptor-mediated suppressing effect of endogenously released GABA on its own release, as measured by the overflow of [^{3}H]-GABA from preloaded rat cortical slices. Incidentally, the potencies of a different selection of $GABA_B$ receptor antagonists for increasing [^{3}H]-GABA release at 2 Hz have previously been found to correlate well with their potencies for inhibition of the binding of the agonist radioligand, [^{3}H]-CGP 27492 to $GABA_B$ receptors in rat brain membrane preparations (Waldmeier *et al.*, 1992). Such a result would be expected if (a) neither the agonist radioligand nor the antagonists discriminate between possible $GABA_B$ receptor subtypes, or (b) they are specific for a certain subtype.

Likewise, the correlation between binding data and those with respect to increasing [^{3}H]-GABA release in the presence of a large concentration of (−)-baclofen was almost perfect. Although the latter experimental conditions are complex, because the $GABA_B$ autoreceptor is activated both by endogenously released GABA and by the exogenous agonist, (−)-baclofen, these data were necessary to allow comparison with those on the effects of the $GABA_B$ receptor antagonists on glutamate release. The maximal increase of glutamate release from rat cortical slices elicited by a $GABA_B$ receptor antagonist is rather small, probably because there is little tonic activation by endogenous GABA of $GABA_B$ heteroreceptors on glutamatergic nerve endings in this area (Waldmeier *et al.*, 1993b). This makes potency comparisons with the effects of the compounds on [^{3}H]-GABA release difficult,

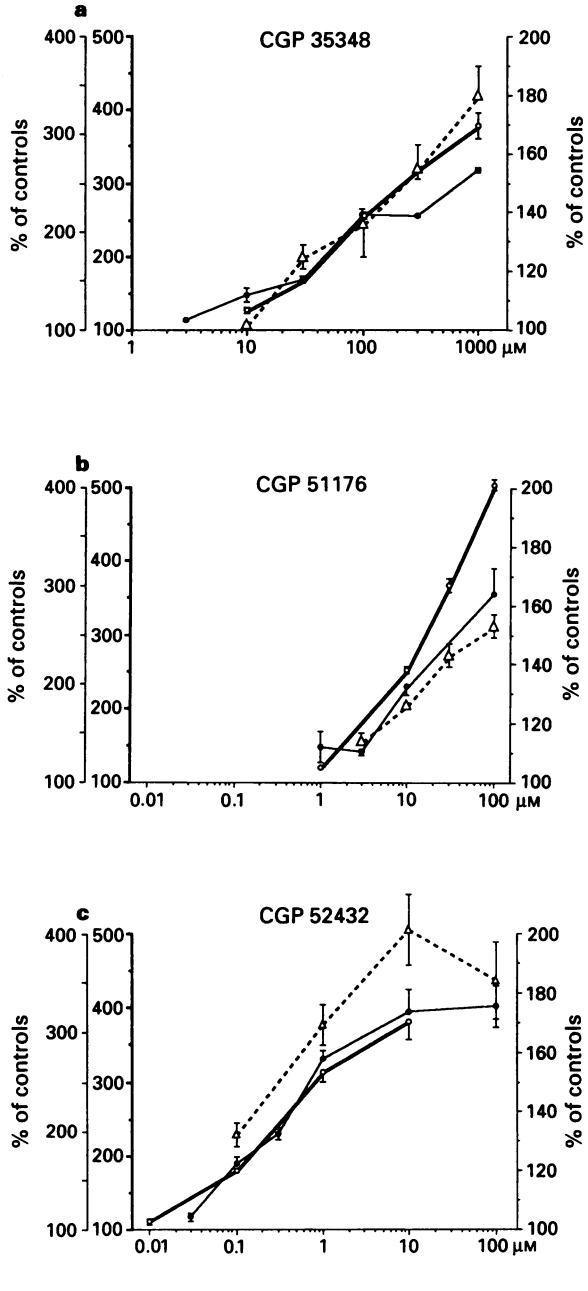


Figure 2 Effects of (a) CGP 35348, (b) CGP 51176 and (c) CGP 52432 on the release of [^{3}H]-GABA in the absence and presence of (−)-baclofen, and of endogenous glutamate (glutamate) in the presence of (−)-baclofen. Groups of 4 slice preparations were twice stimulated electrically, at 2 Hz for 2 min (25 mA, 2 ms) for measurements of the release of [^{3}H]-GABA, and at 1 Hz for 4 min (25 mA, 2 ms) for measurements of the release of endogenous glutamate. In the experiments with [^{3}H]-GABA, 50 μM AOAA and 10 μM SK&F 89976 were present as inhibitors of GABA transaminase and GABA uptake, respectively. In one series of experiments, 10 μM (−)-baclofen was also present throughout. In the experiments with endogenous glutamate, 100 μM *trans*-PDC was present as an inhibitor of glutamate uptake, and 10 μM (−)-baclofen was also present throughout.

Each experiment consisted of a control of 4 slice preparations which were stimulated twice under identical conditions, and two groups of 4 slice preparations where the test drugs were added to the superfusion medium two fractions after the first stimulation (S_1). The effects of the drugs were evaluated by expressing the corresponding S_2/S_1 ratios as a percentage of those of the controls ± s.e.mean ($n = 4$). When only the effects on [^{3}H]-GABA release were measured, these controls were not exposed to drugs other than AOAA and SK&F 89976. Otherwise, all groups in an experiment were exposed to 10 μM (−)-baclofen throughout (designated by 'GABA/baclofen' and 'glutamate/baclofen').

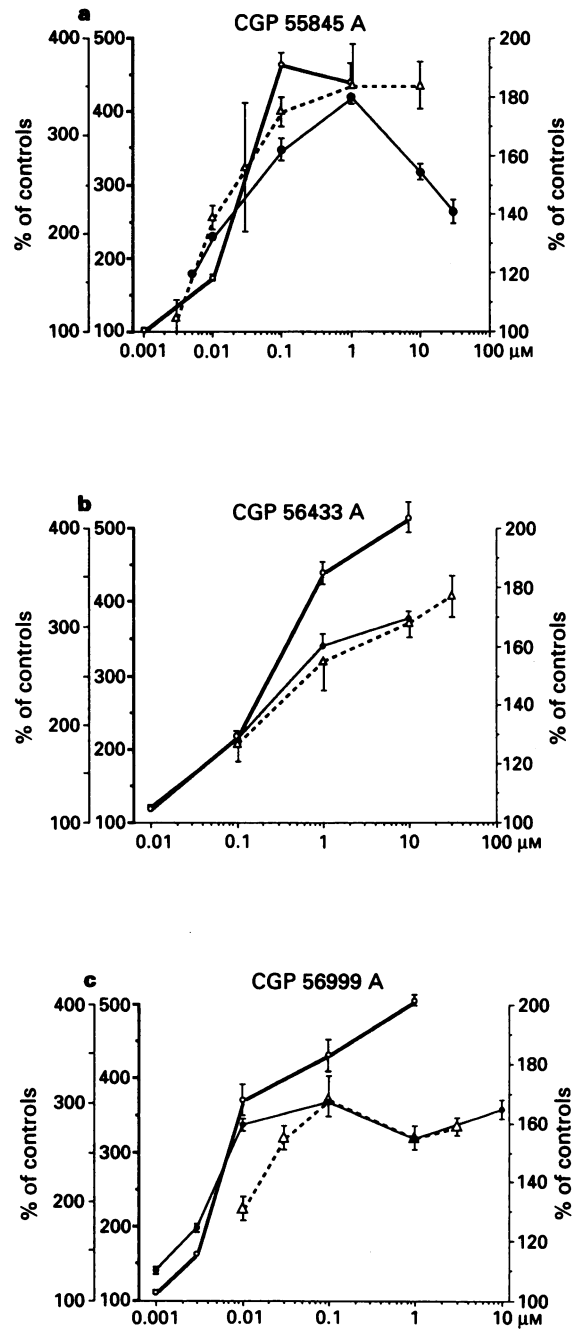


Figure 3 Effects of (a) CGP 55845 A, (b) CGP 56433 A and (c) CGP 56999 A on the release of [^{3}H]-GABA in the absence and presence of (−)-baclofen, and of endogenous glutamate in the presence of (−)-baclofen. Data are means ± s.e.mean as percentage of controls. For details see legend of Figure 2; (●—●) GABA; (○—○) GABA/baclofen; (Δ---Δ) Glu/baclofen.

Basal [^{3}H]-GABA release in the fraction immediately before S_1, S_1 and S_2/S_1 were 0.90–1.20% (fractional release), 0.725–1.165% (fractional release), and 0.938 and 1.165, respectively, in the absence of (−)-baclofen. In its presence, the corresponding figures were 0.95–1.05%, 0.507–0.644% and 1.142–1.366, respectively. Basal glutamate release, S_1, and S_2/S_1 were 0.746–1.187% (fractional release; absolute amounts of glutamate being between about 250–500 pmol/fraction), 0.605–0.893% (fractional release), and 1.122–1.299, respectively. These figures represented the lower and upper limits found in the experiments depicted in Figures 2–4.

The ordinates from left to right refer to [^{3}H]-GABA release in the absence and presence of (−)-baclofen, and to glutamate (Glu) release in the presence of (−)-baclofen, respectively: (●—●) GABA; (○—○) GABA/baclofen; (Δ---Δ) Glu/baclofen.

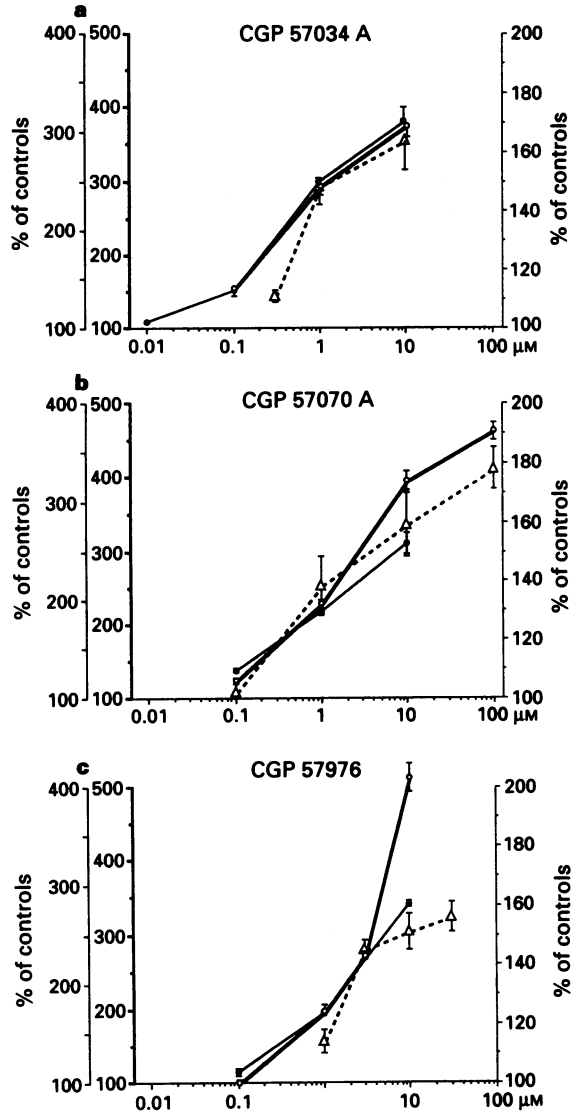


Figure 4 Effects of (a) CGP 57034 A, (b) CGP 57070 A and (c) CGP 57976 on the release of [^{3}H]-GABA in the absence and presence of (−)-baclofen, and of endogenous glutamate in the presence of (−)-baclofen. Data are means ± s.e.mean as percentage of controls. For details see legend of Figure 2; (●—●) GABA; (○—○) GABA/baclofen; (Δ---Δ) Glu/baclofen.

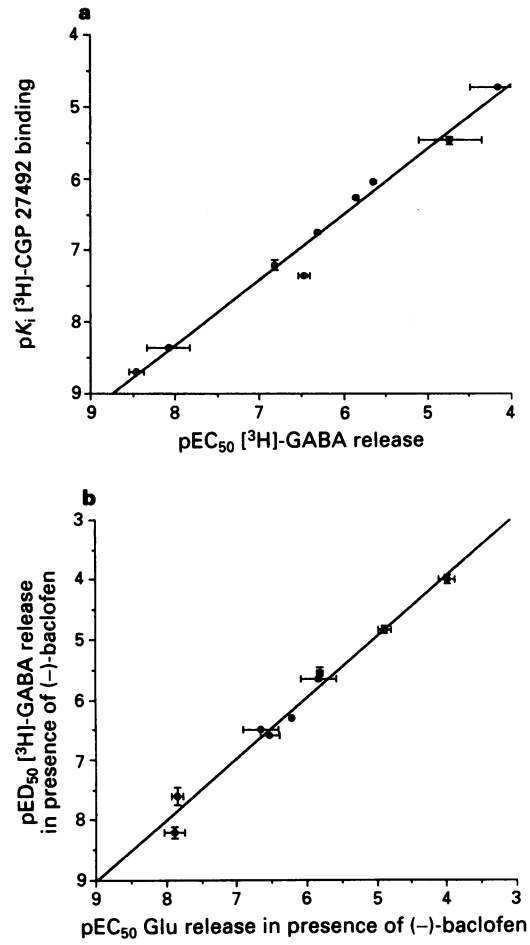


Figure 5 Examples of correlation plots: the negative logarithms ± s.e.mean of the K_i values (pK_i) of the compounds to inhibit [^{3}H]-CGP 27492 binding to the $GABA_B$ receptor were plotted against those of their EC_{50} values (pEC_{50}) to increase [^{3}H]-GABA release in the absence of (−)-baclofen (a) and the pEC_{50} values to increase [^{3}H]-GABA release in the presence of (−)-baclofen were plotted against those to increase glutamate (Glu) release in the presence of (−)-baclofen (b). Data taken from Table 1. The graphs serve to illustrate the correlations. Wherever error bars are absent, they are too small to be visible.

if not impossible. Much more useful data are obtained if antagonism by the compounds of the inhibitory effect of (−)-baclofen on glutamate release is considered. Consequently, a comparable set of data with respect to [^{3}H]-GABA release had to be generated.

There was little difference between the EC_{50}'s with respect to [^{3}H]-GABA and endogeneous glutamate release in the presence of (−)-baclofen given in Table 1. It seems even less impressive if one examines the concentration response-curves depicted in Figures 2–4. A less than twofold difference was observed with CGP 52432, in favour of increasing glutamate release. Interestingly, this compound was reported to exhibit a very marked, 100 fold specificity for the antagonism of the effects of (−)-baclofen on [^{3}H]-GABA vs endogenous glutamate release (Lanza *et al.*, 1993; Bonanno & Raiteri, 1993). Another prototypic compound on which these authors based their suggestion of the existence of multiple $GABA_B$ receptors is CGP 35348, which they found to be about 70 times more potent in antagonizing the effects of (−)-baclofen on glutamate than on [^{3}H]-GABA release (Bonanno & Raiteri, 1992; 1993). In our experiments, this factor was 1. Thus, some fundamental elements of Bonanno of Raiteri's hypothesis concerning multiple $GABA_B$ receptors could not be confirmed under our conditions. Moreover, a set of 9 $GABA_B$ receptor antagonists, structurally related to CGP 35348 and CGP 52432, did not show clearcut differences with respect to the antagonism of the effects of (−)-baclofen on [^{3}H]-GABA and glutamate release.

These differences between the findings of Raiteri's group and ours are unexpected. Notwithstanding the differences in the models (K^+ stimulation in synaptosomes vs. electrical stimulation in slices), there is reasonable agreement about the potencies of the inhibitory effects of the agonist, (−)-baclofen, on [^{3}H]-GABA (Baumann *et al.*, 1990; Bonanno & Raiteri, 1993) and glutamate release (Bonanno & Raiteri, 1993; Waldmeier *et al.*, 1993b). The differences begin with the effect of the antagonist CGP 35348 on [^{3}H]-GABA release in the presence of (−)-baclofen, where the Italian group finds a somewhat lower potency than we do. On the contrary, in their model, CGP 52432 is about 10 times more potent than in ours. The differences with respect to glutamate release in the presence of (−)-baclofen are even more marked: Bonanno & Raiteri (1993) find CGP 35348 about 30 times more potent and Lanza *et al.* (1993) find CGP 52432 about 60 times less potent than we do. The reasons for these

discrepancies between the results of Raiteri's group and ours are not clear.

Certainly, there are the above-mentioned experimental differences: i.e. K^+ vs. electrical stimulation, synaptosomes vs. slices. It is likely that, at the stimulation frequency of 2 Hz which we used for GABA, the antagonists must compete not only with the exogenous agonist but in addition with endogenously released GABA, and this may lead to somewhat higher EC_{50}'s than in synaptosomes. Also, the relations between effects on GABA and glutamate release might be systematically altered by a small factor because competition of the $GABA_B$ receptor antagonists with the endogenous ligand may play a lesser role in the case of glutamate release. This is because there seems to be little tonic activation of $GABA_B$ heteroreceptors on glutamate nerve endings (Waldmeier *et al.*, 1993b). However, these factors would affect the EC_{50}'s of every compound in the same manner and thus cause only a parallel shift of the correlations.

We have attempted to study the effects of the prototype drugs, CGP 35348 and CGP 52432, with respect to antagonism of the inhibitory effect of (−)-baclofen on K^+-evoked GABA and glutamate release in synaptosomes in much the same way as Raiteri's group. However, in our hands the maximal inhibition obtained with (−)-baclofen was too small (between 30 and 40%) and the variability too high to allow appropriate conclusions. Therefore, we are left at present with a quite unsatisfactory discrepancy with the Italian group, whose hypothesis on the pharmacological heterogenity of $GABA_B$ autoreceptors and heteroreceptors regulating glutamate release we cannot confirm in our model.

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