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The affinities, potencies and efficacies of some benzodiazepine-receptor agonists, antagonists and inverse-agonists at rat hippocampal $GABA_A$-receptors

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1 The abilities of some benzodiazepine-receptor agonists, antagonists and inverse agonists to modulate the inhibitory potency of the γ-aminobutyric acid $(GABA)_A$-receptor agonist, isoguvacine, on the CA1 population spike recorded from slices of rat hippocampus, were determined.

2 Concentration-response curves were constructed of the extent to which the benzodiazepine-receptor ligands shifted the isoguvacine concentration-response curve to the left or right. These were compared to their displacement curves of [^{3}H]-Ro15-1788 binding to rat hippocampal membranes under near physiological assay conditions.

3 The above comparisons suggest that the effect on the potency of isoguvacine produced by the benzodiazepine-receptor agonists, diazepam and flunitrazepam, and the partial agonists, Ro16-6028 and Ro17-1812, closely parallels their degree of benzodiazepine-receptor occupancy. Thus, the partial agonists, Ro16-6028 and Ro17-1812, were unable to produce as large a maximum response as the full agonists, diazepam and flunitrazepam.

4 The maximum effects produced by diazepam, flunitrazepam, Ro16-6028, Ro17-1812, the antagonist, propyl- β-carboline-3-carboxylate, and the inverse agonist, methyl-6, 7-dimethyl-4-ethyl-β-carboline-3-carboxylate (DMCM), on the potency of isoguvacine in the hippocampal slice corresponded to the change in their affinities produced by the addition of GABA in the radioligand binding studies (GABA-shift). This suggests that the changes in affinity of benzodiazepine-receptor ligands produced by $GABA_A$-receptor activation reflects their ability to modify $GABA_A$-receptor function.

5 The benzodiazepine-receptor antagonists, Ro15-1788 and CGS 8216, had apparent agonist and inverse agonist effects, respectively, on the potency of isoguvacine. These effects occurred at concentrations above those required for saturation of the benzodiazepine-receptor, as labelled by [^{3}H]-Ro15-1788, and were not in agreement with the absence of any effect of $GABA_A$-receptor stimulation in the GABA-shift experiments. This indicates that these events are not mediated by an action at the classical benzodiazepine-receptor site.

6 It is concluded that hippocampal $GABA_A$-receptor function can be allosterically modulated in a manner consistent with the agonist/inverse-agonist model of benzodiazepine-receptor activation, and that compounds exist with varying efficacies throughout this range.

Introduction

Benzodiazepine receptors are an integral part of the γ-aminobutyric acid $(GABA)_A$-receptor/chloride ionophore complex and are involved in regulating the coupling between the $GABA_A$-agonist recognition site and the opening of the chloride ion channel (Olsen, 1982). It is now generally accepted that different ligands for benzodiazepine receptors can either enhance the effect of $GABA_A$-receptor activation (agonists, positive efficacy), reduce this effect (inverse agonists, negative efficacy) or have little direct effect but block the actions of both agonists and inverse agonists (antagonists, no efficacy) (Braestrup *et al.*, 1982; and see Haefely *et al.*, 1985 for extensive review). In addition, compounds possessing partial agonist and partial inverse-agonist properties have been described (Braestrup *et al.*, 1984; Haefely, 1984; Haefely & Polc,

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1986). The actions of benzodiazepine-receptor ligands at the $GABA_A$-receptor complex have been studied in detail at the molecular level by radioligand binding techniques (Matsumoto & Fukuda, 1982; Korneyev, 1983; Skerritt & Johnston, 1983 and see Ehlert *et al.*, 1983; Martin, 1984 and Haefely *et al.*, 1985) and several electrophysiological investigations have examined the functional consequences of these interactions. However, most of these latter studies have been restricted to the examination of the effects of benzodiazepine-receptor ligands on the fractional change in the response to a single concentration of GABA (Choi *et al.*, 1977; Skerritt & MacDonald, 1984b; Jensen & Lambert, 1984; Chan & Farb, 1985). The more quantitative studies that have been carried out to date, by determining the extent to which benzodiazepines produce a lateral shift in the $GABA_A$-receptor agonist dose-response curve, have either been performed on non-mammalian preparations (Nistri & Constanti, 1978; Nistri & Berti, 1983) and/or have been restricted to only limited concentrations of benzodiazepine-receptor ligands (MacDonald & Barker, 1978; Choi *et al.*, 1981; Skerritt & MacDonald, 1984a; Simmonds, 1985). Furthermore, none of the studies described so far have made direct comparisons between the potencies and efficacies of benzodiazepine-receptor ligands measured electrophysiologically and their affinities and efficacies measured by binding studies.

The hippocampus is amenable to both electrophysiological and binding studies and the pharmacology of $GABA_A$-receptors on rat hipocampal neurones has recently been characterized in some detail (Kemp *et al.*, 1986). This makes it an ideal target for the investigation of compounds which interact with $GABA_A$-receptors in the mammalian central nervous system. In the present study we have attempted to quantify the potencies and relative efficacies of some benzodiazepine-receptor agonists, inverse agonists and antagonists at the $GABA_A$-receptor complex in the rat hippocampus. To do this we have constructed concentration-response curves for their abilities to modify the inhibitory effect of the selective $GABA_A$-receptor agonist, isoguvacine, on the CA1 population spike in the rat hippocampal slice preparation. This has been done by determining the extent to which the benzodiazepine-receptor ligands produced lateral shifts to the right or left of the isoguvacine concentration-response curve. These results have been compared to the ability of these compounds to displace the binding of the benzodiazepine-receptor antagonist, [^{3}H]-Ro15-1788 (Möhler & Richards, 1981; Brown & Martin, 1984) to hippocampal membranes and the change in their affinities induced by $GABA_A$-receptor activation ('GABA-shift', Baestrup *et al.*, 1984; Karobath & Supavilai, 1985), under near physiological assay conditions. [^{3}H]-Ro15-1788 was the radioligand of choice because of its reasonable affinity under the assay conditions used and its insensitivity to modulation by GABA (Möhler & Richards, 1981), thereby allowing an unbiased assessment of the GABA-shift.

Methods

Hippocampal slices were prepared and maintained in a small perfusion chamber as previously described (Kemp *et al.*, 1986). The Schaffer collateral-commissural pathway was stimulated every 30 s through metal bipolar stimulating electrodes made from two tungsten microelectrodes (TM25-5, Clark electromedical) glued together with a tip separation of 100–200 μm, and placed in the stratum radiatum. The stimulating parameters were a square wave pulse of less than 0.1 ms duration and up to 20 V amplitude. Glass micropipettes, filled with 3M NaCl and with resistances of 2–5 MΩ, were placed in the stratum pyramidal of the CA1 region and population spikes amplified and filtered using a Neurolog system (Digitimer Ltd). In most experiments two slices were placed in the perfusion chamber and their responses recorded in parallel. The recorded potentials were digitised by use of a Gould OS4040 digital oscilloscope and a BBC microcomputer based system used to average and measure the peak height of the population spikes. Cumulative concentration-response curves to isoguvacine were constructed as previously described (Kemp *et al.*, 1986). Each concentration was perfused for a 5 min period to ensure a maximum effect was achieved and the last four responses at each concentration averaged. The benzodiazepine-receptor ligands under study were perfused for 30 min before and during redetermination of the isoguvacine concentration-response curve. The dose-ratio between the control and drug-treated curves was always measured at the 50% inhibition level. It was found that increasing the concentration of the benzodiazepine and repeating the isoguvacine concentration-response curve led to inconsistent effects, possibly due to the development of acute tolerance. Therefore, only one concentration of one ligand was tested on each slice.

Radioligand binding assay

Rat hippocampal membranes were prepared and subject to extensive washing and freezing as described by Bowery *et al.* (1983). On the day of the experiment, the membranes were thawed and mixed with 30 volumes of a modified Krebs buffer (in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $NaHCO_3$ 5, HEPES 20, KH_2PO_4 1.2, $CaCl_2$ 2.5 and D-glucose 11; pH 7.4) and left at room temperature (23°C) for 30 min, then centrifuged at 49000 *g* for 10 min. The pellet was resuspended in 30

volumes of Krebs-HEPES buffer and incubated at room temperature for 15 min before centrifugation. This washing step was repeated three more times. For the [^{3}H]-Ro15-1788 binding assay, the washed membranes were homogenized in 0.75 ml of Krebs-HEPES buffer per sample, each containing 0.2–0.5 mg of protein (measured by the method of Lowry *et al.*, 1951) and added to polycarbonate test tubes containing 100 µl of 5 nM [^{3}H]-Ro15-1788, 100 µl of displacer or buffer (for determination of total binding) and 50 µl of buffer or of 6 mM GABA solution (300 µM, final concentration). To control for possible uptake of GABA, nipecotic acid (10^{-4}M), was included in the incubation medium in all experiments. Nonspecific binding of [^{3}H]-Ro15-1788 was defined by 3 µM clonazepam, giving 90–95% displaceable binding. Duplicate samples were incubated at 30°C for 30 min and terminated by rapid filtration through Whatman GB/B filters in a Brandel M24-R cell-harvester followed by 2 × 4 ml washes with ice-cold assay buffer. The filters were soaked in 10 ml Hydrofluor overnight and radioactivity determined by liquid scintillation counting at 41% counting efficiency. Potencies for displacement (K_i) and Hill Coefficients (nH) were determined from data obtained using at least 5 concentrations of the displacer, on at least 3 separate occasions, by computer-assisted iterative curve fitting.

Source of drugs

Diazepam, flunitrazepam and Ro15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-6-oxo-4H-imidazo [1,5-a] [1,4] benzodiazepine-3-carboxylate) were kindly provided by Dr H. Mohler, Hoffman La Roche. CGS 8216 (2,5-dihydro-2-phenyl-3H-pyrazolo [4,3-c] quinolin-3-one) was a gift from Ciba-Geigy. Propyl-β-carboline-3-carboxylate (β-CCPr) and methyl-6, 7-dimethyl-4-ethyl-β-carboline-3-carboxylate (DMCM) were provided by Ferrosan. Ro16-6028 (t-butyl(s)-8-bromo-11,12,13,13a-tetrahydro-9-oxo-9H-imidazo [1,5-a]-pyrrolo-[2,1-c] [1,4] benzodiazepine-1-carboxylate) and Ro17-1812 (cyclopropylmethyl (s)-8-chloro-12, 12a-dihydro-9-oxo-9H,11H-aceto[2,1-c] imidazo[1,5-a] [1,4] benzodiazepine-1-carboxylate were synthesized at MSDRL, Terlings Park.

Benzodiazepine-receptor ligands were initially dissolved in absolute ethanol at a concentration of 10^{-2}M or 10^{-3}M and diluted directly into artificial cerebrospinal fluid (aCSF) or Krebs buffer to the final concentration used. Ethanol at 0.1% vol/vol, the highest concentration added, had no effect on the potency of isoguvacine, log dose-ratio = 0.016 ± 0.019 (mean ± s.e.mean, n = 8 slices).

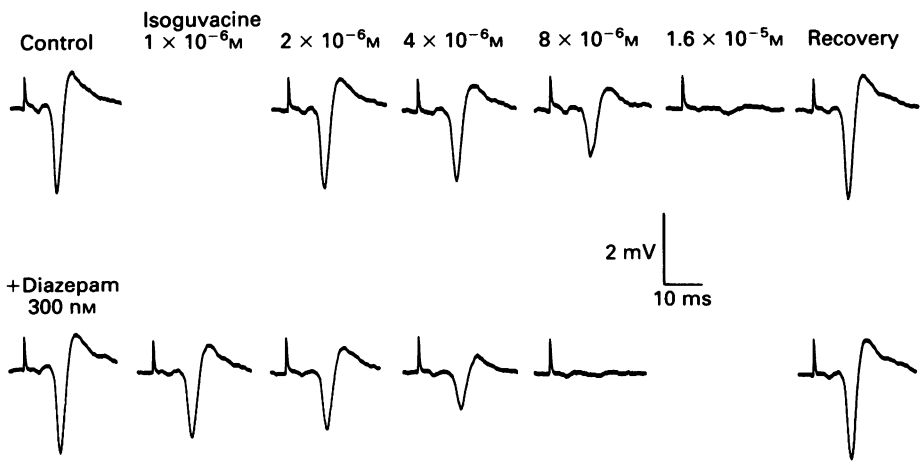

Figure 1 Potentiation of the inhibitory effect of isoguvacine on the CA1 population spike by diazepam. Traces show the effect of increasing concentrations of isoguvacine, added cumulatively, on the averaged CA1 population spike. The upper row shows control responses to isoguvacine and the lower row shows responses in the presence of, and following 30 min perfusion with, diazepam (300 nM). In the control, a concentration of 1 × 10^{-6}M isoguvacine was not tested, whilst in the presence of diazepam concentrations above 8 × 10^{-6}M were not applied because of complete inhibition of the population spike.

Results

Electrophysiological studies

The benzodiazepine-receptor agonists, diazepam and flunitrazepam, potently potentiated the inhibitory effect of isoguvacine on the CA1 population spike in a concentration-dependent manner (Figure 1). This resulted in parallel shifts to the left of the isoguvacine concentration-response curves (Figure 2a, b). The reversibility of this effect was studied in detail with 30 and 100 nM flunitrazepam ($n = 9$ slices). Partial recovery was usually apparent after 30 min of returning to normal aCSF but at least 60 min was generally required for a return to control values (Figure 2a). Complete concentration-response curves for the increase in potency of isoguvacine produced by diazepam and flunitrazepam are illustrated in Figure 3a. Maximum effects, expressed as log dose-ratios, of -0.41 ± 0.04 (mean ± s.e.mean, $n = 6$) and -0.43 ± 0.04 ($n = 9$), were produced by 1 μM diazepam and 0.1 μM flunitrazepam respectively. These maxima were not statistically different (unpaired t test) and correspond to 2.5 and 2.7 fold increases in the potency of isoguvacine. Higher concentrations of diazepam could not be used as they often had direct depressant effects on the size of the population spike. This effect appeared to be unrelated to the $GABA_A$-receptor potentiating effects of diazepam and may be due to block of uptake of endogenously released purines (Phillis *et al.*, 1979). Using the four parameter logistic equation, concentration-response curves were fitted to all the data points for diazepam and flunitrazepam ($n = 32$ and 24 slices, respectively) with the computer programme, Allfit (DeLean *et al.*, 1978). These curves also had maximum values which did not differ significantly, Hill coefficients not significantly different from unity and gave EC_{50} values of 38 nM for diazepam and 15 nM for flunitrazepam.

The reported benzodiazepine-receptor partial agonists, Ro16-6028 ($n = 23$) and Ro17-1812 ($n = 22$), (Haefely, 1984), also produced leftward shifts of the isoguvacine concentration-response curve, with threshold concentrations (10 nM) similar to that of diazepam. However, these effects reached immediate maxima at 30 nM, which were then maintained at a similar level over a large concentration range (up to 1 μM) (Figure 3b). The maximum shifts produced by Ro16-6028 and Ro17-1812 were significantly less than those caused by flunitrazepam and diazepam ($P < 0.01$, unpaired t test) (Figure 4) and were equivalent to 1.6 and 1.7 fold increases in the potency of isoguvacine, respectively.

The β-carboline, β-CCPr, and the imidazobenzodiazepine, Ro15-1788, are both antagonists with

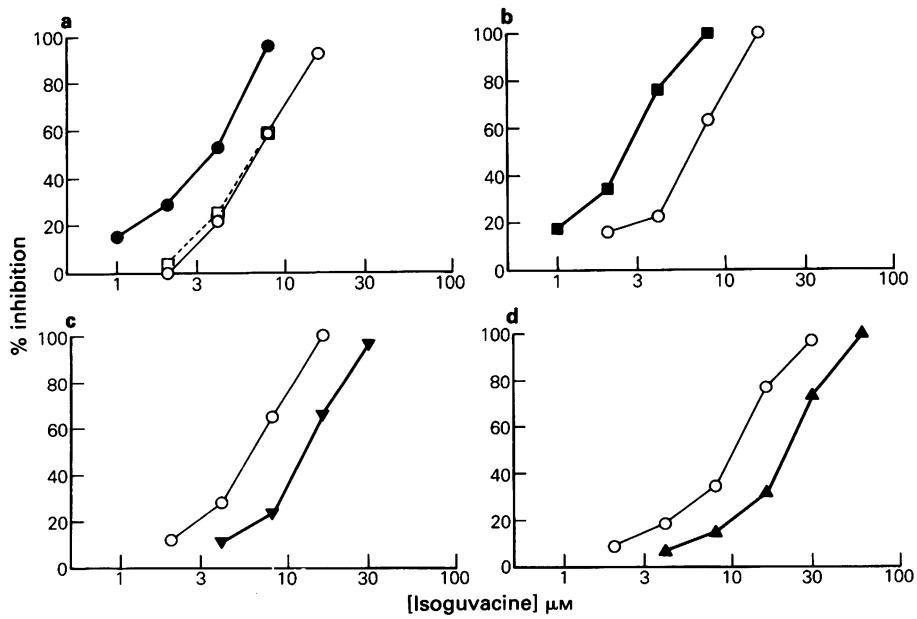

Figure 2 Examples of concentration-response curves for isoguvacine from 4 separate slices before (○) and following 30 min perfusion with (a) flunitrazepam 30 nM (●), (b) diazepam 300 nM (■), (c) CGS 8216 300 nM (▼) and (d) methyl-6, 7-dimethyl-4-ethyl-β-carboline-3-carboxylate 100 nM (▲). In (a) (□) show recovery from flunitrazepam 55 min after returning to control artifical CSF.

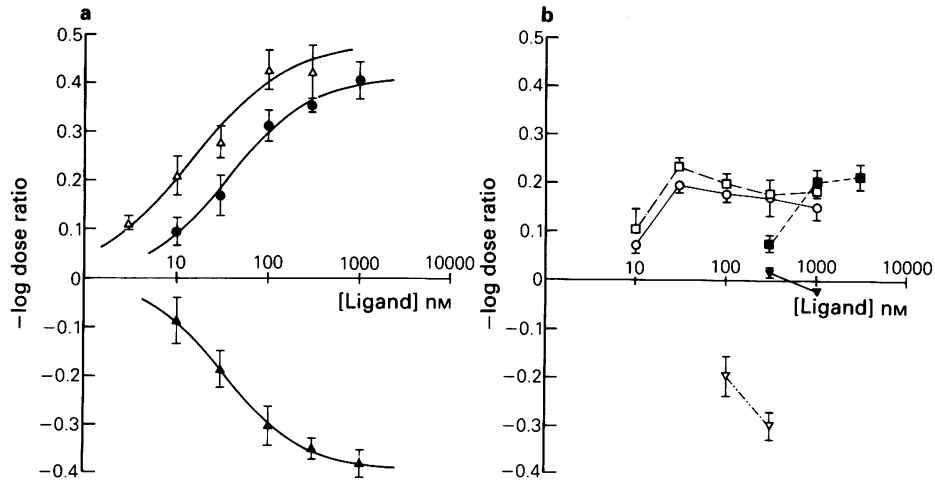

Figure 3 Concentration-response curves for the ability of benzodiazepine-receptor ligands to modulate the potency of isoguvacine on the hippocampal slice. The curves are plotted as log concentrations against −log dose ratio $(EC_{50} + drug)/EC_{50}$ control. (a) Concentration-response curves for the benzodiazepine-receptor agonists, flunitrazepam (△) and diazepam (●), and the inverse-agonist, methyl-6, 7-dimethyl-4-ethyl-β-carboline-3-carboxylate (△). The curves shown are computer generated best fits to all the data points for each compound, using the Allfit programme (see text). (b) The partial agonists RO17-1812 (□) and Ro16-6028 (○) and the antagonists propyl-β-carboline-3-carboxylate (▼), CGS 8216 (▽) and Ro15-1788 (■).

high affinity for the benzodiazepine recognition site (O'Brien *et al.*, 1981; Braestrup *et al.*, 1982; Polc *et al.*, 1982). At doses of 0.3 and 1 μM, β-CCPr ($n = 113$) produced no significant shift in the isoguvacine concentration-response curve (Figures 3b, 4). However, Ro15-1788, ($n = 15$) at doses of 0.3–3 μM, produced significant, concentration-related shifts to the left of the isoguvacine concentration-response curve (Figures 3b, 4). In contrast to the latter two compounds, the pyrazoloquinoline, CGS 8216 (Petrack *et al.*, 1986) at 0.1 and 0.3 μM ($n = 9$), caused concentration-related decreases in the potency of isoguvacine, resulting in significant shifts to the right of the isoguvacine concentration-response curves (Figures 2c, 3b). Similarly, the inverse-agonist, DMCM, produced the anticipated decrease in the potency of isoguvacine (Figure 2d). A maximum shift to the right of the isoguvacine concentration-response curve of 0.38 ± 0.04 ($n = 6$) was produced by 1 μM DMCM, which corresponds to a 2.4 fold decrease in the potency of isoguvacine. The complete concentration-response curve for DMCM is illustrated in Figure 3a and the best fit curve to all the data points ($n = 26$) had a maximum of 0.39, a Hill coefficient of 1.05 and an EC_{50} of 32 nM.

Binding studies

The affinities of the above compounds for benzodiazepine receptors, both in the absence and

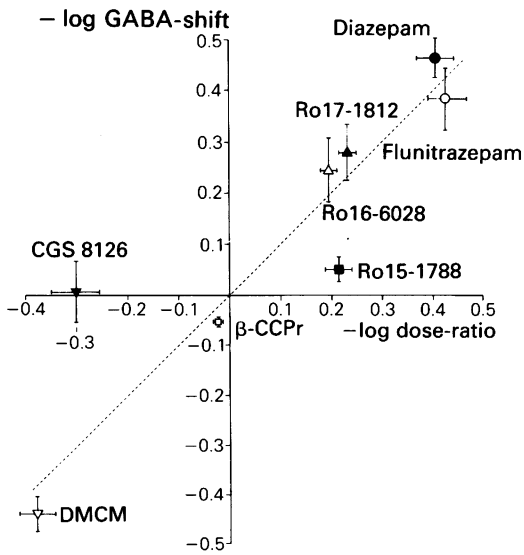

Figure 4 Comparison between the 'GABA-shift' seen with benzodiazepine-receptor ligands in the radioligand binding studies and the maximum effect they produced on the change of potency of isoguvacine in the hippocampal slice. (●) Diazepam, (○) flunitrazepam, (▲) Ro17-1812, (△) Ro16-6028, (■) Ro15-1788, (□) propyl-β-carboline-3-carboxylate, (▼) CGS 8216 and (▽) methyl-6, 7-dimethyl-4-ethyl-β-carboline-3-carboxylate DMCM. The dotted line indicates an exact correspondence between the two assays.

presence of GABA (3×10^{-4}M), were measured by their ability to displace [^{3}H]-Ro15-1788 binding from hippocampal membranes under near physiological assay conditions. The results are summarised in Table 1. The affinities of diazepam and flunitrazepam were increased in the presence of GABA, by 2.9 and 2.7 fold, respectively. However, the affinities of both Ro16-6028 and Ro17-1812 were increased to a smaller extent by the addition of GABA, only 1.8 and 1.9 fold, respectively. The three chemically different benzodiazepine-receptor antagonists all showed very little 'GABA-shift', whilst the affinity of the inverse agonist, DMCM, was reduced 2.8 fold by $GABA_A$-receptor stimulation. For comparison, in Figure 4, the 'GABA shift' of the above compounds has been plotted against the maximum effect they produced in the electrophysiological studies.

Discussion

The present results demonstrate that benzodiazepine-receptor ligands modulate the functional response of $GABA_A$-receptor stimulation on hippocampal neurones in a manner compatible with the proposed agonist/inverse-agonist model of benzodiazepine-receptor activation (Braestrup *et al.*, 1984). In agreement with previous studies, our results clearly show that $GABA_A$-receptor-mediated events are facilitated by benzodiazepine receptor agonists such as diazepam and flunitrazepam. Furthermore, in the present study the degree of this potentiation has been quantified and concentration-response curves to these agonists constructed. This enables a direct comparison to be made between the data from the concentration-response curves and the data from the displacement curve of [^{3}H]-Ro15-1788 binding produced by diazepam and flunitrazepam (Figure 3, Table 1). The similarities between these data suggest that there is a close correspondence between the response these agonists produce and their degree of benzodiazepine-receptor occupancy. Thus, for these full agonists the Hill coefficients of both types of curves are equal to 1, the EC_{50} and K_i values are similar and the maximum effects correspond to 90%, or greater, receptor occupancy. Similarly, for the partial agonists, Ro17-1812 and Ro16-6028, the concentrations required to produce threshold effects (10 nM) correspond to a greater than 60% receptor occupancy and then low maximum effects are reached upon receptor saturation. These results indicate that 100% receptor occupancy is required to produce a maximum modulation of the response to $GABA_A$ receptor stimulation. In general, the affinities of the benzodiazepine-receptor ligands tested in the present binding study were lower than those previously reported (Möhler & Richards, 1981; Möhler *et al.*, 1981). This is due to the use of a more physiological buffer and elevated temperature.

The apparent partial agonist nature of Ro15-1788 in the electrophysiological studies is in agreement with some previous functional and behavioural studies (Nutt *et al.*, 1982; Albertson *et al.*, 1982; Greckseh *et al.*, 1983; Jensen *et al.*, 1983; Vellucci & Webster, 1983; Robertson & Riives, 1983; Skerritt & MacDonald, 1983), although this is not reflected in the present and previous biochemical investigations (Hunkeler *et al.*, 1981; Korneyev, 1983; Skerritt & Johnston, 1983). However, whilst concentration-related, the potentiation of the effects of isoguvacine only occurred at high concentrations of Ro15-1788 compared to its affinity for the benzodiazepine recognition site. Studies using

Table 1 Displacement of [^{3}H]-Ro15-1788 binding by benzodiazepine receptor ligands and their sensitivities to GABA

	K_i(nM)	*nH*	*GABA-shift*	$\log\left(\frac{IC_{50}+GABA}{IC_{50}\ control}\right)$
Diazepam	58.8 ± 5.3	0.96 ± 0.04	2.89 ± 0.22	−0.46 ± 0.03
Flunitrazepam	12.5 ± 4.2	0.94 ± 0.01	2.73 ± 0.15	−0.39 ± 0.06
Ro16-6028	0.94 ± 0.21	0.99 ± 0.11	1.78 ± 0.27	−0.25 ± 0.06
Ro17-1812	5.17 ± 1.21	0.99 ± 0.12	1.91 ± 0.24	−0.28 ± 0.05
Ro15-1788	5.37 ± 0.64	1.05 ± 0.05	1.12 ± 0.07	−0.05 ± 0.03
β-CCPr	12.64 ± 2.09	0.74 ± 0.06	1.13 ± 0.02	−0.05 ± 0.01
CGS 8216	0.26 ± 0.08	0.86 ± 0.12	1.00 ± 0.15	0.00 ± 0.06
DMCM	3.41 ± 0.45	0.81 ± 0.07	0.36 ± 0.03	0.44 ± 0.04

The affinities of benzodiazepine-receptor ligands for displacement of [^{3}H]-Ro15-1788 (0.5 nM) binding to rat hippocampal membranes and their sensitivities to modulation by GABA. The values are expressed as the mean ± s.e.mean of > 3 separate determinations. The GABA-shift is control IC_{50}/IC_{50} in the presence of 3×10^{-4}M GABA. For direct comparison with the electrophysiological data the log (IC_{50} + GABA/IC_{50} control) is also given. β-CCPr = probyl-β-carboline-3-carboxylate; DMCM = methyl-6,7-dimethyl-4-ethyl-β-carboline-3-carboxylate; CGS8216 = 2,5-dihydro-2-phenyl-3H-pyrazolo[4,3-c] quinolin-3-one.

Ro15-1788 as an antagonist of the effects of diazepam gave a pA_2 of 8.9 (Kemp & Marshall, unpublished observations), which is in good agreement with its affinity measured in binding experiments (Table 1). This suggests that saturation of benzodiazepine-receptors by Ro15-1788 occurred before an effect on the potency of isoguvacine was seen, and yet this potentiation increased still more with further increases in the concentration of Ro15-1788. These data indicate that the increase in potency of isoguvacine produced by high concentrations of Ro15-1788 is unlikely to be mediated by the benzodiazepine-recognition site acted upon by diazepam and labelled by low concentrations of [^{3}H]-Ro-15-1788 (0.5 nM), and this suggestion is further supported by the inability of GABA to alter the affinity of Ro15-1788 for this site. Nevertheless, the fact that relatively high concentrations of Ro15-1788 facilitate $GABA_A$-receptor activity (the present results; Nutt *et al.*, 1982; Skerritt & MacDonald, 1983) may explain its apparent partial agonist effects seen with large doses in behavioural experiments. Similar arguments may also hold for the apparent inverse-agonist like activity of CGS 8216 which also has a high affinity for the benzodiazepine-recognition site and has been reported to possess some proconvulsant activity (Jensen *et al.*, 1983).

Apart from Ro15-1788 and CGS 8216, for the reasons outlined above, there was a good agreement between the maximum changes in potency of isoguvacine produced by the benzodiazepine-receptor ligands and the degree of 'GABA-shift' observed in the radioligand binding studies (Figure 4). These results are commensurate with the proposal that the changes in affinity of benzodiazepine-receptor ligands produced by GABA reflect their ability to modify $GABA_A$-receptor function (Braestrup *et al.*, 1982).

The inverse-agonist, DMCM, reduced the potency of isoguvacine with a maximum effect that was similar in magnitude to the maximum potentiations produced by diazepam and flunitrazepam. If DMCM is a full inverse-agonist, and diazepam and flunitrazepam full agonists at the benzodiazepine receptor, then these maxima may represent the extremes to which $GABA_A$-receptor function can be modified by allosteric interactions at the benzodiazepine-recognition site.

In conclusion, these studies provide further evidence for a modulatory coupling between the benzodiazepine-recognition site and the $GABA_A$-receptor. In addition, they have addressed two questions regarding the molecular mechanisms of benzodiazepine action (Haefely *et al.*, 1985). The present investigation suggests that the maximum shift of the $GABA_A$-receptor agonist concentration-response curve reflects the intrinsic activity (efficacy) of benzodiazepine-receptor agonists and inverse agonists and that this corresponds to their GABA-shift. They also indicate that 100% receptor occupancy is required for this maximal effect to occur. This is in keeping with the view that each functional $GABA_A$-receptor complex encompasses a benzodiazepine-receptor site and thus, for all the $GABA_A$-receptors to be influenced then all the benzodiazepine-receptor sites need to be occupied.

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