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The effects of benzodiazepine agonists, inverse agonists and Ro 15-1788 on the responses of the superior cervical ganglion to GABA *in vitro*

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1 The effects of benzodiazepines and their antagonists on the responses to γ-aminobutyric acid (GABA) of the superior cervical ganglion of the rat were examined using extracellular recording.

2 Chlordiazepoxide (1 μM to 28.9 μM) and flurazepam (145–725 nM) increased the responses of the ganglion to GABA and the increases were antagonized by Ro 15-1788, at 3.34 μM. The concentration of GABA used was 9.7 μM which gave half-maximal responses.

3 Chlordiazepoxide similarly increased the responses of the ganglion to GABA 38.8 μM in the presence of bicuculline 27.2 μM. This concentration of GABA gave, with bicuculline, responses of a similar magnitude as those to 9.7 μM in the absence of bicuculline. Bicuculline did not affect the actions of chlordiazepoxide or the antagonism by Ro 15-1788.

4 Ro 15-1788 did not affect the increases in GABA response caused by pentobarbitone or by phenobarbitone in the presence of bicuculline.

5 Ethyl β-carboline-3-carboxylate (βCCE) (207 nM to 1 μM) significantly decreased the responses to GABA in the presence and in the absence of bicuculline. The decreases were antagonized by Ro 15-1788 (3.34 μM). βCCE at 2.1 μM and above did not significantly change the responses to GABA.

6 Methyl β-carboline-3-carboxylate (βCCM) at 88 to 440 nM significantly decreased the responses to GABA. The decreases were antagonized by Ro 15-1788 (3.34 μM) and were also seen in the presence of bicuculline.

7 High concentrations of Ro 15-1788 decreased the responses to GABA, 9.7 μM, but increased the responses to GABA 38.8 μM in the presence of 27.2 μM bicuculline.

8 The pattern of effects of the benzodiazepines, β-carbolines and low doses of Ro 15-1788 on the responses to GABA was similar to the effects of these compounds on seizure threshold and anxiety-related behaviour *in vivo*.

Introduction

Following the discovery of benzodiazepine receptors (Squires & Braestrup 1977; Mohler & Okada, 1977) two groups of compounds have been found which compete for the binding sites and prevent the *in vivo* pharmacological effects of the benzodiazepines. The first group, including ethyl-β-carboline-3-carboxylate ethyl ester (βCCE), have intrinsic activity as they lower seizure thresholds or cause convulsions (Oakley & Jones, 1980; Cowen *et al.*, 1981) and have anxiogenic activity (File *et al.*, 1982). The second group, including the imidazobenzodiazepine Ro 15-1788, do not possess intrinsic activity at doses which show the antagonist actions (Nutt *et al.*, 1982). In order to distinguish these two types the first group have come to be known as 'inverse agonists' or 'contragonists'.

We showed recently that Ro 15-1788 not only antagonized the actions of the benzodiazepines but also prevented the pro-convulsant effects of βCCE (Nutt *et al.*, 1982). Antagonism of the anxiogenic effects of the β-carbolines by Ro 15-1788 has been demonstrated (File *et al.*, 1982) and the antagonism of their pro-convulsant and convulsant effects was confirmed by Braestrup *et al.* (1982). There have been many accounts in recent literature of the interactions found in binding studies between benzodiazepine receptors and γ-aminobutyric acid (GABA) receptors (e.g. Olsen, 1982) and these have

led to the suggestion that the benzodiazepine, barbiturate and GABA receptor sites all lie on a protein complex which includes the chloride ionophore, through which the effects of these drugs are expressed. Many studies have demonstrated potentiation of the actions of GABA by the benzodiazepines, both *in vivo* and *in vitro* (for reviews see Haefely *et al.*, 1981; Haefely, 1983) and this effect is thought to account for a large part of their actions *in vivo*. However, we do not yet have any proven explanation as to why there appear to be three types of compounds acting at the benzodiazepine receptor site—agonists, inverse agonists and antagonists. There has been much speculation on this topic and the suggestions have included the idea that either the β-carbolines or the benzodiazepines are acting as antagonists to an endogenous ligand controlling GABA activity, while Ro 15-1788 is a partial agonist; the idea that benzodiazepines and β-carbolines are acting on different types of receptors, while Ro 15-1788 happens to be an antagonist at both sites, and the new concept that the benzodiazepine receptor can regulate the effects of GABA in two directions, according to whether benzodiazepines or β-carbolines are bound, while Ro 15-1788 binds but produces no effect (Nutt *et al.*, 1982).

The majority of studies have been made *in vivo* or using receptor binding techniques and there have not been many electrophysiological studies. The present study was carried out to determine whether the actions of three types of compound seen *in vivo* are also seen in their effects on responses to applied GABA. The superior cervical ganaglion was used because it is not thought to contain nerves which normally use γ-aminobutyric acid (GABA) as a transmitter but contains receptors, which are similar to those in the central nervous system, at which it causes a depolarization by increasing chloride conductance (Bowery & Brown, 1974). In 1978, Bowery & Dray showed that the antagonism of this action of GABA by bicuculline was reversed by barbiturates and by chlordiazepoxide. We recently showed that this effect of chlordiazepoxide was antagonized by Ro 15-1788, which also blocked the decrease in GABA activity caused by βCCE in the same circumstances (Nutt *et al.*, 1982). In the present studies I have shown that the changes in the responses of the ganglia to GABA produced by the three types of compound parallel those seen *in vivo* and also that these effects are seen in the absence of bicuculline.

Method

The method of Brown & Marsh, (1974) for recording surface potentials from the ganglion was used in the experiments. Male Wistar rats, 250–350 g, were killed by vertebral dislocation and the superior cervical ganglia dissected out. The connective sheath was removed, the tissues were suspended vertically and continuously superfused with Krebs solution (bubbled with 95% O_2, 5% CO_2 at 1 ml min^{-1}). The electrodes used were Ag/AgCl in 3 M KCl, with balsa wood wicks. The output was fed into a Tekman potentiometric recorder, with a 100 μF capacitor across the terminals to remove high frequency noise. Preliminary tests showed that this value of capacitor did not alter the amplitude of the recorded responses. In the majority of experiments the ganglia were removed the day before and stored overnight at 4°C in Krebs solution to produce more stable recordings (Brown & Galvan, 1977). Doses of GABA were applied by switching the perfusing fluid from the Krebs solution reservoir to Krebs solution containing GABA, by means of a solenoid valve. The optimum agonist contact time was found, in preliminary experiments, to be 2 min so this time was used throughout these experiments. When GABA doses were applied at 15 min intervals, consistent responses were obtained so this time interval was used throughout. The treatment drugs were added to both the superfusing Krebs and the GABA solutions and a minimum pretreatment time of 15 min allowed before continuing the application of doses of GABA. The preparations were maintained at 22°C throughout.

Most of the studies described here were planned to demonstrate the effects of the drug treatments on one concentration of GABA, 9.7 μM, in the absence of bicuculline and one concentration, 38.8 μM, in the presence of 27.2 μM bicuculline. The former GABA concentration was used because previous experiments (Little, 1982) showed this to be the ED_{50} as derived from the log dose-response curve. The latter concentration was chosen because, in the presence of 27.2 μM bicuculline, the amplitude of the responses produced was approximately 50% of maximum, so comparison could be made with the former situation. The effects of chlordiazepoxide and of βCCM, alone and combined with Ro 15-1788 on the dose-response curves to GABA were also studied.

The experimental schedules in the former set of experiments were planned so that four steady control responses were obtained to GABA (to give an indication of control variation) before the treatment drug(s) was added, after which three or four GABA doses were applied, to demonstrate any changes in the effects of the treatment drugs over 1 h. Control responses in experiments using methyl-β-carboline-3-carboxylate (βCCM) or βCCE were established in the same concentration of acid as used for the drug solutions. This acid concentration did not alter the responses. The results were expressed in the figures as percentages of the first control response amplitude for each preparation, with mean and s.e.mean calcu-

lated, but the original data (in μV) was used for statistical analysis. The absolute amplitudes of the responses to GABA varied between preparations (mean ± s.e.mean value for 9.7 μM GABA = 860 ± 72 μV, $n = 12$). Analysis of variance was performed, according to Armitage (1971), on the original data, i.e. the amplitudes of the depolarizations. In the experiments on the dose-response curves to GABA a control dose-response curve was established in each preparation and then this was repeated in the presence of the treatment drug(s).

The drugs used were obtained from the following sources: γ-aminobutyric acid and bicuculline, Sigma; chlordiazepoxide hydrochloride, flurazepam and Ro 15-1788 (ethyl-8-fluoro-5,6-dihydro-5-methyl-7-oxo-4H-imidazo-(1,5-a) (1,4)-benxodiazepine-3-carboxylate) Hoffman-la-Roche & Co.; ethyl- and methyl-β-carboline-3-carboxylate esters, Glaxo pharmaceuticals; pentobarbitone and phenobarbitone, B.D.H. The composition of Krebs solution was (mM) NaCl 118, $NaHCO_3$ 25, KCl 4.75, $CaCl_2$ 2.55, $MgSo_4$ 1.2, KH_2PO_4 1.19, D-glucose 11. All solutions were made up immediately before use. Chlordiazepoxide and flurazepam were dissolved in distilled water and GABA in sterile 0.9% w/v NaCl solution. Bicuculline was dissolved in 0.1 M HCl, then titrated to pH 3 with NaOH. Ro 15-1788 was dissolved directly in Krebs solution and βCCE and βCCM were dissolved in 0.3 M HCl. Subsequent dilutions, in all cases, were then made using Krebs solution.

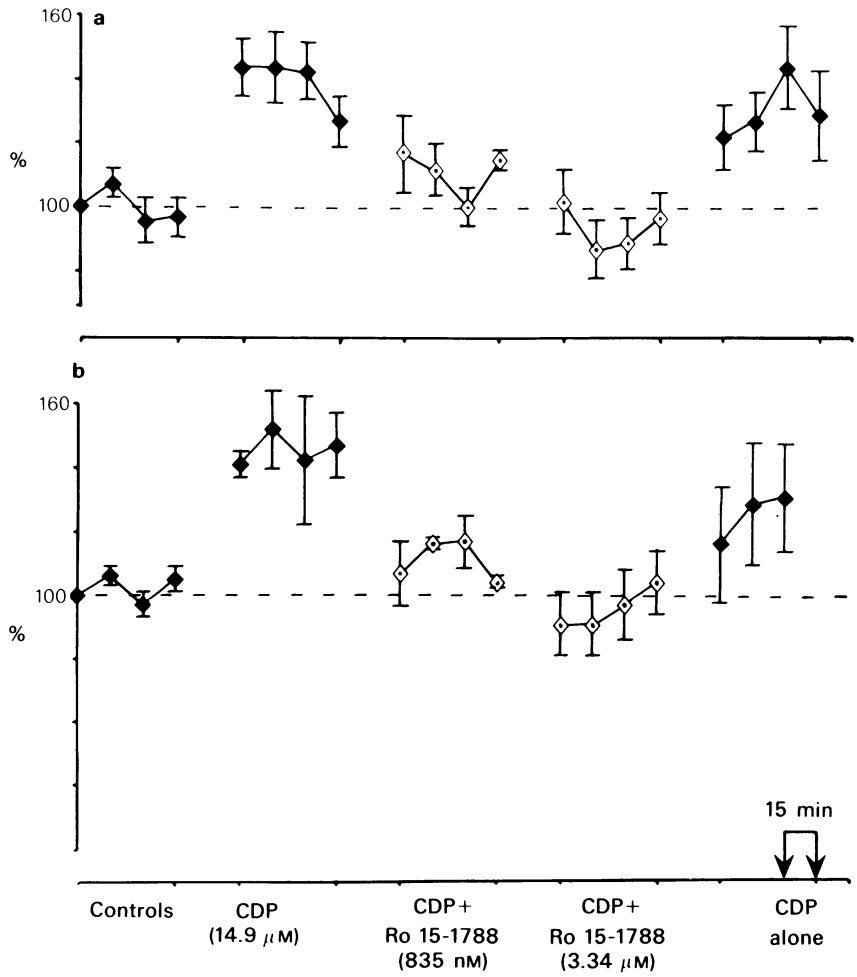

Figure 1 The effects of chlordiazepoxide (CDP) on the responses of (a) 9.7 μM GABA and (b) 38.8 μM GABA with 27.2 μM bicuculline. The results are expressed as percentages of the first control response for each preparation, mean ± s.e.mean (vertical line); (a) $n = 8$, (b) $n = 5$. Significant ($P < 0.05$) increases in the responses to GABA were seen with chlordiazepoxide in both (a) and (b) but in the presence of Ro 15-1788 no significant changes were seen ($P > 0.05$). The concentrations of chlordiazepoxide and Ro 15-1788 at the base of the figure refer to both (a) and (b).

Table 1 The effects of chlordiazepoxide on the responses to 9.7 μM GABA

	Chlordiazepoxide			
Controls	*1.5 μM*	*3 μM*	*14.9 μM*	*29.8 μM*
100				
100 ± 5	127 ± 10	131 ± 17	149 ± 13	142 ± 10
105 ± 6	130 ± 12	120 ± 12	153 ± 13	140 ± 11
102 ± 9	133 ± 12	130 ± 12	146 ± 9	138 ± 11

Doses of GABA were applied at 15 min intervals. Results are expressed as percentages of the first control response for each preparation, mean ± s.e.mean, $n = 6$. The effects of chlordiazepoxide were significant at all of the above concentrations ($P < 0.05$). The order in which results were obtained, in this and subsequent tables was: controls first, then values down each column in succession.

Results

(1) *Chlordiazepoxide*

Chlordiazepoxide increased the amplitude of the responses to 9.7 μM GABA, showing significant changes at 1.5, 3, 14.9 and 29.8 μM ($P < 0.05$), with the greatest increase at 14.9 μM (Table 1). The effect did not appear to alter over 1 h. Figure 1 shows that a similar increase was seen with 14.9 μM chlordiazepoxide whether 9.7 μM GABA was used, or 38.8 μM GABA in the presence of 27.2 μM bicuculline. In both instances the change was antagonized by Ro 15-1788, 835 nM and 3.34 μM. These concentrations of Ro 15-1788 had no effects alone. (see section (6) and Figure 6.). The antagonist action of Ro 15-1788 was rapidly reversible, as illustrated in Figure 1, but the effect of chlordiazepoxide alone was long lasting, needing over 3 h washing with normal Krebs solution before a decrease was seen.

(2) *Flurazepam*

Flurazepam also increased the responses to 9.7 μM GABA (Table 2) but the maximum change seen was smaller than that with chlordiazepoxide and considerable variation was seen in the extent of the effect between preparations, although the changes within each preparation were very consistent. The effects of 290 nM flurazepam were abolished by the addition of 3.34 μM Ro 15-1788 (Table 2).

(3) *Barbiturates*

The effects of Ro 15-1788 on the potentiation of GABA responses were studied for comparison (Table 3 and Figure 2). The increases produced by both pentobarbitone and phenobarbitone varied considerably between preparations, as shown by the large standard errors in Table 3 but were very consistent within each preparation, as illustrated in Figure 2. There was no effect of Ro 15-1788 on the actions of the barbiturates, either in the presence or the absence of bicuculline (Table 3).

(4) *Ethyl β-carboline-3-carboxylate (βCCE)*

βCCE, over a range of concentrations from 207 nM to 1 μM significantly ($P < 0.05$) decreased the effects of 9.7 μM GABA (Figure 3). Higher concentrations, such as 2.1 μM, however, did not cause significant changes. When Ro 15-1788 was added to the superfusion medium βCCE did not cause any significant changes at any of the concentrations, whether the antagonist was added after the effect of βCCE had been recorded (as for 1 μM βCCE) or in separate experiments (as for 413 nM βCCE).

Figure 3 also shows that a similar pattern of changes was seen in the effects of βCCE on the

Table 2 The effects of flurazepam on the responses to GABA (9.7 μM)

			Flurazepam	
a	*Controls*	*145 nM*	*290 nM*	*725 nM*
	100	113 ± 3	127 ± 18	121 ± 5
	98 ± 5	122 ± 5	133 ± 12	118 ± 5
	100 ± 1	120 ± 5	136 ± 18	108 ± 7
	98 ± 2	124 ± 7	129 ± 21	111 ± 10

			Flurazepam (290 nM)
b	*Controls*	*Flurazepam (290 nM)*	*+ Ro 15-1788 (3.34 μM)*
	100	154 ± 21	116 ± 14
	95 ± 6	150 ± 20	114 ± 10
	109 ± 6	147 ± 17	101 ± 9
	98 ± 5	136 ± 16	102 ± 10

Doses were applied at 15 min intervals. Results are expressed as percentages of the first control response of each preparation, mean ± s.e.mean $n = 6$. The changes in (a) were significant at 145 and 290 nM ($P < 0.05$) but not at 725 nM ($P > 0.1$) or at higher concentrations than 725 nM. See Table 1 for order of obtaining results.

Table 3 The lack of effect of Ro 15-1788 on the potentiation of GABA responses by barbiturates

a *GABA (9.7 μM)*

Controls	*Pentobarbitone (50 μM)*	*Pentobarbitone (50 μM) + Ro 15-1788 (3.3 μM)*
100		
95 ± 5	146 ± 13	144 ± 14
92 ± 5	144 ± 8	127 ± 19
90 ± 6	135 ± 14	131 ± 15

b *GABA (38.8 μM) + bicuculline (27.2 μM)*

Controls	*Phenobarbitone (787 μM)*	*Phenobarbitone (787 μM) + Ro 15-1788 (3.34 μM)*
100	166 ± 17	168 ± 16
100 ± 2	166 ± 21	165 ± 17
102 ± 4	166 ± 17	176 ± 19
108 ± 5	172 ± 18	180 ± 24

Results are expressed as in Tables 1 and 2. (a) $n = 5$, (b) $n = 7$. The increases caused by pentobarbitone and by phenobarbitone were significant ($P < 0.05$) but the addition of Ro 15-1788 caused no significant changes.

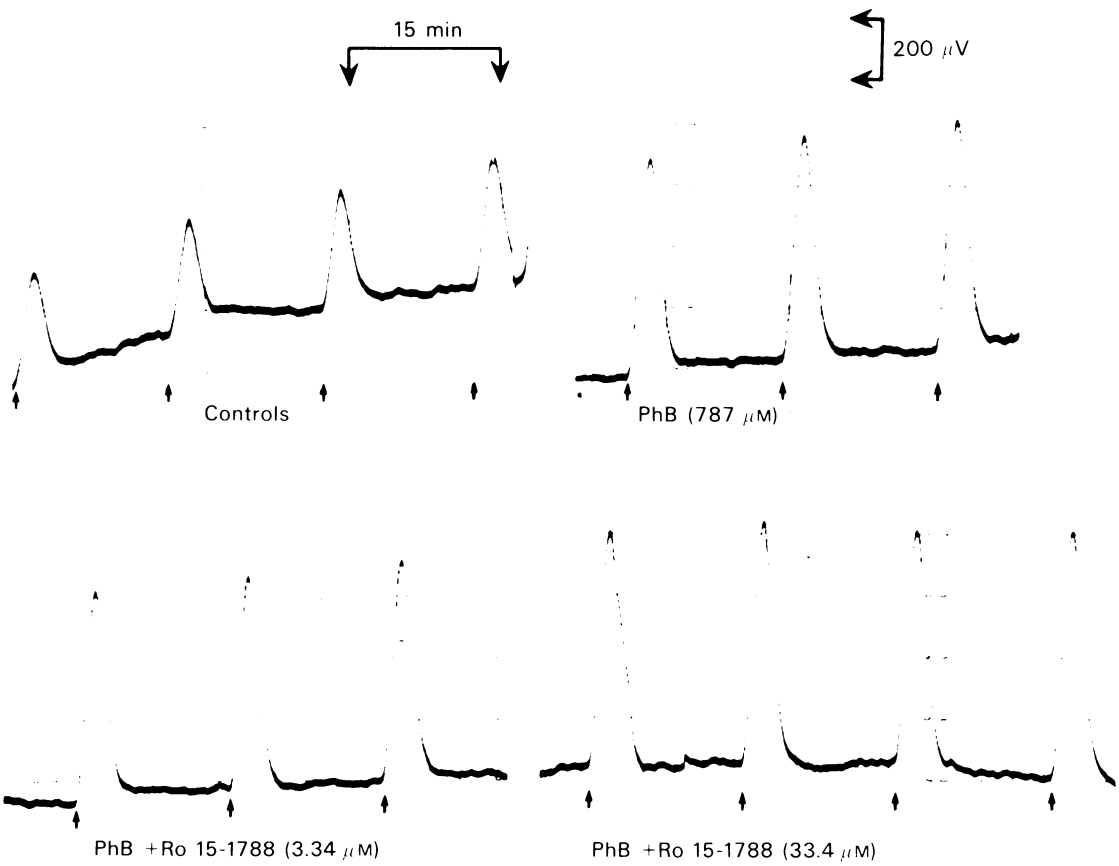

Figure 2 An example of the traces obtained, showing the increase in the responses to GABA, 38.8 μM in the presence of bicuculline 27.2 μM, by phenobarbitone (PhB) 787 μM. The doses of GABA were applied at 15 min intervals. Ro 15-1788, at 3.4 or 33.4 μM did not cause any change in the action of phenobarbitone.

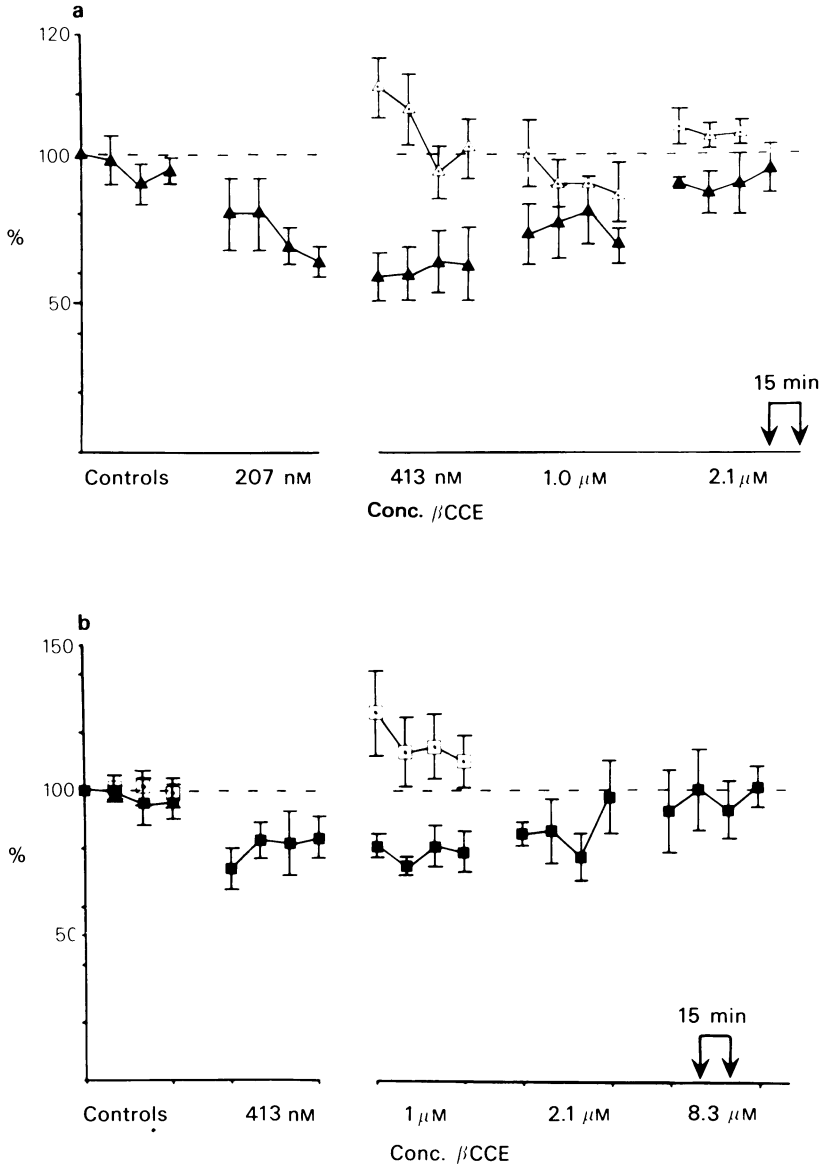

Figure 3 (a) The effects of ethyl β-carboline-3-carboxylate (βCCE) (closed symbols) on the responses to 9.7 μM GABA. The results are expressed as described for figure 1, $n = 6$. The changes were significant ($P < 0.05$) at concentrations of βCCE of 207 nM, 413 nM and 1 μM but not at higher concentrations ($P > 0.1$). When Ro 15-1788, 3.34 μM was included in the superfusion medium (open symbols) no significant changes were seen ($P > 0.1$). The break in the abscissa scale indicates that the results were obtained from separate experiments. For clarity only one set of control values has been included, those obtained in the study on 207 nM βCCE. The results for each concentration of βCCE were expressed as percentages of the first control response for each experiment. In each case the control responses were obtained in the presence of a concentration of HCl equivalent to that used for each concentration of βCCE. (b) The effects of βCCE on the responses to 38.8 μM GABA in the presence of 27.2 μM bicuculline. The control values for the concentrations of βCCE above 413 nM have been omitted. (■) Controls for βCCE, (□) controls for βCCE 1 μM plus Ro 15-1788 3.34 μM. The decreases in the responses to GABA seen with 413 nM and 1 μM βCCE were significant ($P < 0.05$) but 2.1 and 8.3 μM βCCE did not cause any significant changes.

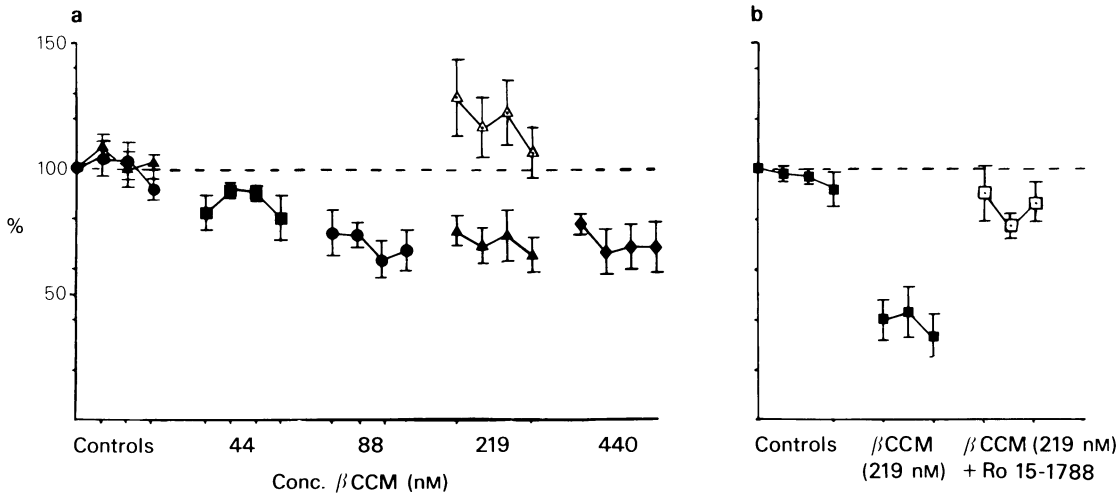

Figure 4 The effects of methyl β-carboline-3-carboxylate (βCCM) on the responses to GABA 9.7 μM (a) and GABA 38.8 μM plus, bicuculline 27.7 μM (b). The results are expressed as in Figure 1, $n = 5$. The decreases seen with 88, 219 and 440 nM βCCM in (a) and with 219 nM βCCM in (b) were significant ($P < 0.01$) but no significant changes were seen when Ro 15-1788 3.34 μM was present in addition ($P > 0.05$). In (a) the controls are indicated by (●) for 88 nM βCCM and (▲) for 219 nM βCCM (with and without Ro 15-1788). Control responses were obtained in the presence of the same concentration of HCl as used to dissolve the βCCM at each concentration. Open symbols indicate responses in the presence of βCCM plus Ro 15-1788 (3.34 μM).

responses to 38.8 μM GABA with 27.2 μM bicuculline. At 413 nM and 1 μM βCCE caused significant ($P < 0.05$) decreases in the amplitude of the GABA responses but 2.1 μM and 8.3 μM βCCE did not cause any significant changes. Ro 15-1788, 3.34 μM, prevented the effects of 1 μM βCCE. It is difficult to make a direct comparison between the extent of the changes produced by βCCE in the absence and the presence of bicuculline as the results were obtained in separate experiments, but it appears that in the former case the effects were slightly greater (decreases to approximately 60% of controls) than in the latter case (decreases to approximately 80% of control values).

(5) *Methyl β-carboline-3-carboxylate (βCCM)*

The methyl β-carboline clearly decreased the responses to GABA (Figure 4). This effect was significant ($P < 0.01$) at βCCM concentrations of 88, 219 and 440 nM for 9.7 μM GABA and at 219 nM βCCM for 38.8 μM GABA with bicuculline. The effects of βCCM 219 nM when bicuculline was present (decreases to approximately 40% of control values) appeared greater than in the absence of this compound (decreases to approximately 70% of control values), but, as explained in (4), a direct comparison is not possible.

(6) *Ro 15-1788*

At the concentrations at which the antagonism of the effects of the benzodiazepines and the β-carbolines were seen, 3.34 μM, Ro 15-1788 alone did not alter the amplitude of the responses to GABA with or without bicuculline (Figure 5, $P > 0.1$). However, at considerably higher concentrations small but consistent changes were seen. When 9.7 μM GABA was used, Ro 15-1788 did not cause any significant ($P > 0.05$) changes at 167 or 334 μM, but 833 μM decreased the responses ($P < 0.05$, Figure 5a). A different pattern was seen in the effects of Ro 15-1788 on the responses to 38.8 μM GABA in the presence of bicuculline (Figure 5b). At 167, 334 and 833 μM Ro 15-1788 caused small, but significant ($P < 0.05$), increases in the responses to GABA. When the latter concentration was removed from the superfusate the effect disappeared within 15 min (Figure 5b).

(7) *Dose-response curves to GABA*

Figure 6 shows the effects of chlordiazepoxide and of βCCM on the dose-response curves to GABA and the antagonism of their effects by Ro 15-1788. Neither chlordiazepoxide nor βCCM affected the maximum responses obtained, the changes being

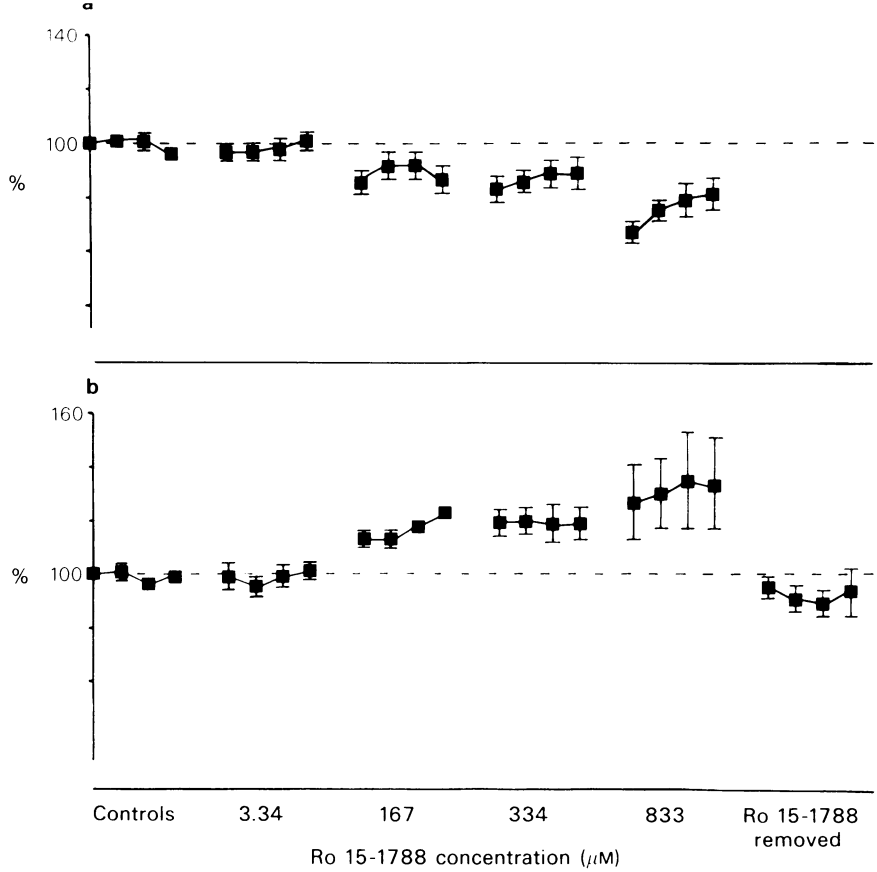

Figure 5 The effects of Ro 15-1788 on the responses to 9.7 μM GABA (a) and 38.8 μM GABA plus 27.2 μM bicuculline (b). The results are expressed as described for Figure 1; (a) $n = 6$ (b) $n = 5$ for 3.34 and 167 μM, $n = 7$ for 334 μM and $n = 4$ for 833 μM. The decrease due to 833 μM in (a) was significant ($P < 0.05$) and the increases due to 167, 334 and 833 μM in (b) were significant ($P < 0.05$). The concentrations of Ro 15-1788 at the base of the figure apply to both (a) and (b).

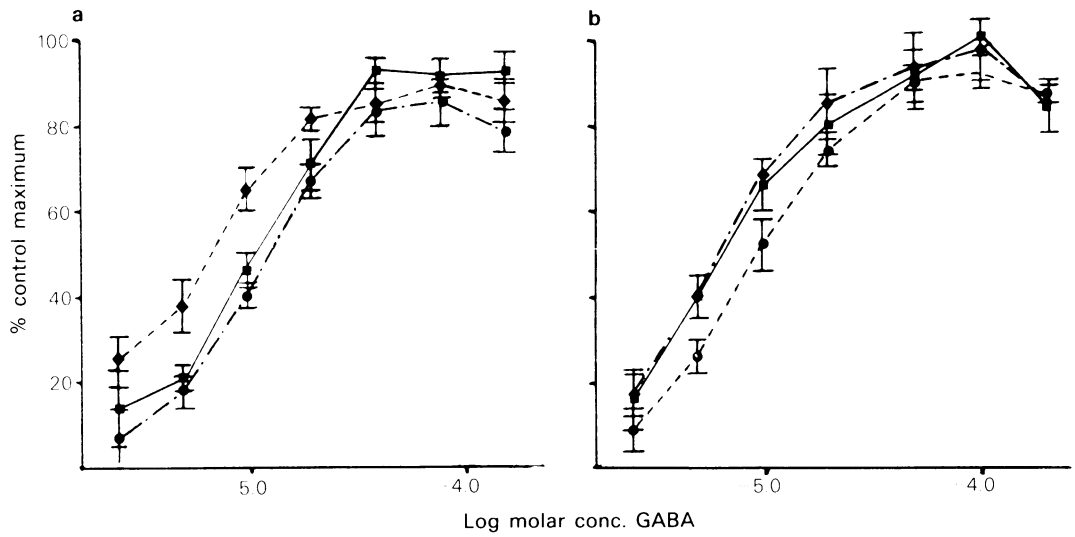

Figure 6 The effects of (a) chlordiazepoxide (CDP) with and without Ro 15-1788 and (b) methyl β-carboline-3-carboxylate (βCCM) with and without Ro 15-1788 on the dose-response curves to GABA. Results were expressed as percentages of the control maximum for each preparation, then the mean values calculated ($n = 6$). Vertical lines show s.e.mean. (a) Controls (■—■), CDP 14.9 μM (◆...◆), CDP plus Ro 15-1788 3.34 μM (●-.-.●). (b) Controls (■—■), βCCM 219 nM (●---●), βCCM plus Ro 15-1788 3.34 μM (◆-.-.◆).

confined to the lower doses of GABA. In this ganglion preparation, however, there is considerable desensitization to GABA when it is applied at high doses. The experimental schedule was designed to minimise this effect between doses but it is likely that desensitization limits the magnitude of each response obtained during the application of high doses. Hill plots of the dose-response curves gave the following slopes: controls 0.71, chlordiazepoxide 0.62; controls 0.68, βCCM 0.82. The fact that these values were all below 1.0 is likely to be due to the desensitization occuring at high doses. A further complication in these studies is that, because of the time schedule and the slow responses, comparison is being made between the effects of doses applied up to 3 h apart, thus leading to greater variation than in the earlier experiments.

Discussion

This discussion will be concerned firstly with the details of interpretation of the results and secondly with the implications of the results for the theories described in the Introduction.

The demonstration of the potentiation by benzodiazepines of the ganglionic responses to GABA in the absence of bicuculline is in agreement with the results of Schlosser & Franco, (1979) but disagrees with the results of Bowery & Dray, (1978) who did not see this effect unless bicuculline was included in the solutions. However, the extent of the changes and the concentration range for chlordiazepoxide required in the present studies was in agreement with the latter authors. The effects of Ro 15-1788 on the actions of the benzodiazepines was not examined in these earlier studies as the compound was not then available, but the current results show a clear antagonism of the effects of chlordiazepoxide and flurazepam in the presence or absence of bicuculline suggesting that the receptors involved are similar to those found in the CNS. Receptor binding studies have not so far demonstrated this type of receptor in the periphery (Haefely *et al.*, 1981; Regan *et al.*, 1981). The effective concentrations of chlordiazepoxide and of flurazepam were rather higher than their K_i values for benzodiazepine receptor binding (220 and 11 nM respectively, Mohler & Okada, 1977). Measured CSF concentrations of chlordiazepoxide in man have also been lower than the concentrations effective in the present studies (Stanski *et al.*, 1976). The latter comparison for flurazepam is difficult to make because of its extensive metabolism described below (Kaplan *et al.*, 1973). Other work *in vitro* has found similar effective concentrations as the present studies (e.g. Simmonds, 1980) but the corresponding data for the β-carbolines or Ro 15-1788 is minimal as most studies have used iontophoretic or intravenous administration. Mitchell & Martin (1980) showed effects of βCCE, *in vitro*, at concentrations of 25–200 nM. The K_i values for receptor binding for the β-carbolines are about 1 nM and for Ro 15-1788, 2 nM (Hunkeler *et al.*, 1981).

The effects of chlordiazepoxide and of βCCM on the dose-response curves to GABA suggest that greater effects are seen when low concentrations of GABA are present. As described in the Results, desensitization complicates the interpretation of these curves but it is apparent that βCCM did not decrease the maximum responses nor did chlordiazepoxide increase them. (It is possible to increase the maximum responses to GABA on this preparation, for example with ketamine; Little & Atkinson, 1983).

The small size of the changes in the GABA responses after the addition of flurazepam might at first sight be unexpected in view of the effectiveness of flurazepam *in vivo*. However, it has been found that flurazepam is rapidly converted to desalkyl flurazepam *in vivo* (Greenblatt *et al.*, 1981) and it is thought that the latter compound is responsible for most of its effects. It is unlikely that this compound would be formed in the ganglion so the effects seen would be only of the parent compound. Simmonds (1980) showed a small, but significant potentiation by flurazepam of the effects of muscimol on cuneate nucleus slices.

As mentioned in the Results section there was a certain amount of variability in the extent of the responses of this preparation to benzodiazepines, particularly with flurazepam and the barbiturates (Tables 2 and 3). The changes were, however, very consistent within each preparation (Figure 2). The between-preparation variability did not appear to correlate with the age or sex of the animals, the absolute amplitude of the GABA responses which could be recorded, or the signal to noise ratio of the recordings, and was seen even between two preparations from the same animal. It may, therefore, have been due to differences in the number or, distribution of the receptors involved or, perhaps more likely, the receptor and associated structures may be very susceptible to damage. This variability may account for the lack of agreement in the literature, for instance concerning the effects of the barbiturates (Bowery & Dray, 1978; Evans, 1979). Simmonds (1983) noted variation in the extent of the potentiation by flurazepam of the responses of rat cuneate nucleus slices to muscimol.

The only difference between the effects of the drugs studied in the presence or absence of bicuculline was seen with the very high doses of Ro 15-1788. We have suggested that the potentiation seen when

bicuculline was present may have been due to a weak 'partial agonist' activity of Ro 15-1788 and this correlated very well with the small increase in seizure thresholds which we found *in vivo* as the same ratio of antagonist to agonist doses was seen (Nutt *et al.*, 1982). It is not clear why this was not seen in the absence of bicuculline and it may represent a change in the properties of the receptor caused by the presence of the latter drug. There have been some reports of intrinsic activities of Ro 15-1788 which can be either benzodiazepine-like or β-carboline-like (e.g. File *et al.*, 1982; Corda *et al.*, 1982; Nutt *et al.*, 1982) so it is possible that the effects of this compound can vary in different circumstances, for example according to the situation at the GABA receptors.

In the present studies there appeared to be a limit to the depression of GABA responses by the β-carbolines as the higher concentrations of βCCE did not cause any significant depression, while 219 and 440 nM βCCM did not have greater effects than 88 nM. *In vivo* the pro-convulsant effects of βCCE did not increase over a certain dose (Cowen *et al.*, 1981), while the convulsant effects of βCCM decreased when the dose was raised (Oakley & Jones, 1982), and the dose-response curve for the effects of the β-carboline FG 7142 on convulsion threshold shows a decreased effect at higher doses (Little & Nutt, unpublished observations). The effects of the β-carbolines in the current studies suggest that the pattern of effects of different concentrations *in vivo* may originate in their actions on GABA transmission rather than in pharmacokinetic or metabolic changes.

The importance of the present results to benzodiazepine receptor theories lies in the fact that the effects of the three types of compound, agonist, inverse agonists and antagonist, have been demonstrated on the responses to applied GABA in a preparation which does not contain endogenous GABAergic neurones. There have been no previous electrophysiological studies in which the effects of all three types of compound have been compared directly. Study & Barker (1981) have shown that benzodiazepines increase the frequency of opening of chloride channels in cultured cells, in reponse to GABA, while barbiturates increased the channel lifetime. The enhancement of central inhibition in the whole animal by benzodiazepines has been shown to be antagonized by Ro 15-1788 (Polc *et al.*, 1981). Patersen & Roberts (1983) showed that βCCM decreased the responses of hippocampal pyramidal cells to GABA but the effects of Ro 15-1788 were not studied. Polc *et al.*, (1982) showed that βCCE and βCCM depresses dorsal root potentials and enhances facilitation of pyramidal population spikes in the hippocampus, both effects were interpreted as being due to depression of GABA transmission and were antagonized by Ro 15-1788. All of these studies on central inhibitions were carried out in anaesthetized animals and the treatment drugs were applied intravenously or by pressure ejection so that exact concentrations were not known. A further, and more important, complication of these studies on central inhibition is the problem of the possible activity of endogenous ligands acting at benzodiazepine receptors. It has been suggested that, rather than having opposite effects at the same receptor type, either the benzodiazepines or the β-carbolines could be acting by antagonizing an endogenous ligand. The demonstration of both types of effect in the ganglion makes this unlikely, although of course an endogenous ligand could exist in the absence of GABA. In order to use the latter concept to explain the two types of action one would have to presuppose that the extent of activity of the hypothetical ligand was exactly the same in the ganglion, which does not contain GABA neurones, as in the whole animal. This is not to say that there is not an endogenous ligand normally active at these sites, only that this is not the explanation for the two types of pharmacological effect.

When we first showed that Ro 15-1788 antagonized the pro-convulsant effects of βCCE and the anti-convulsant effects of benzodiazepines (Nutt *et al.*, 1982) we suggested three possible explanations for the results: an increased 'partial agonist' action of Ro 15-1788 caused by βCCE, an unusual receptor at which ligands can have opposite pharmacological effects and the involvement of multiple benzodiazepine receptors. The first and third possibilities appear unlikely, as there is no evidence for the former and β-carbolines have now been shown to bind to both type I and type II benzodiazepine receptors (Braestrup *et al.*, 1982), hence the second concept remains the most appealing.

The concept of receptors at which ligands could produce two opposite types of action is new in pharmacology but the existence of several different types of receptor on one complex with effects exerted through one common neurotransmitter (such as GABA) is also new, so there is no precedent for the type of mechanism which may be involved. It appears that efficacy can vary in both directions, as βCCE appears to be a partial inverse agonist, compared with βCCM, *in vivo* (Oakley & Jones, 1982; Braestrup *et al.*, 1982). It is possible, however, that the benzodiazepines and the β-carbolines may be acting on adjacent, interacting receptors (Doble *et al.*, 1982). However, when it is remembered that receptors are not single binding sites but involve interactions between many chemical groups the distinction between 'the same' or 'different' receptor binding sites becomes less clear. The demonstration that the effects of the three types of compounds may be seen

on a comparatively simple peripheral preparation may enable a more direct comparison to be made between receptor interaction and physiological effects than has previously been possible.

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