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Quantitative evaluation of the potencies of GABA-receptor agonists and antagonists using the rat hippocampal slice preparation

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1 CA1 population spikes recorded in the rat hippocampal slice were used to assess quantitatively the potencies of GABA-receptor agonists and antagonists on mammalian CNS neurones.

2 Apart from GABA itself, $GABA_A$-receptor agonists inhibited the CA1 population spikes with potencies that correlated closely ($r = 0.96$) with their ability to displace [^{3}H]-GABA from $GABA_A$-binding sites.

3 The low potency of GABA in this preparation was attributed to the action of uptake processes as the GABA uptake inhibitor, *cis*-4-hydroxynipecotic acid (2×10^{-4} M), produced an approximate 6 fold increase in the potency of GABA whilst having no effect on the potency of 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (THIP), a $GABA_A$-receptor agonist which is not a substrate for the GABA uptake system.

4 The inhibitory effects of the selective $GABA_A$-receptor agonists isoguvacine and muscimol were antagonized by bicuculline methochloride, which shifted the dose-response curves to the right in a parallel manner. The Schild plots for bicuculline methochloride against isoguvacine and muscimol had slopes of 1 and gave pA_2 values of 6.24 and 6.10, respectively. Picrotoxin also antagonized the inhibitory effects of isoguvacine and produced parallel shifts to the right of the dose-response curve. However, the Schild plot for picrotoxin had a slope significantly less than unity (0.82) and gave a pA_2 value of 6.89.

5 The novel $GABA_A$-receptor antagonist, pitrazepin, antagonized the inhibitory effects of isoguvacine in an apparently competitive manner. The Schild plot had a slope of 1 and gave a pA_2 of 6.69.

6 The inhibitory effects of baclofen, GABA and kojic amine were not antagonized by $GABA_A$-receptor antagonists and were presumed to be mediated by actions at $GABA_B$-receptors.

7 The inhibitory effects of THIP and isoguvacine were antagonized with the same potency by bicuculline methobromide. These results do not support the suggestion that THIP acts preferentially at a 'synaptic' bicuculline-sensitive, GABA receptor.

8 It is concluded that the CA1 population spike in the rat hippocampal slice is a useful test system for the quantitative analysis of both $GABA_A$- and $GABA_B$-receptor agonists and antagonists.

Introduction

γ-Aminobutyric acid (GABA) is considered to be the major inhibitory transmitter in the mammalian central nervous system (CNS) and it is known to act at two pharmacologically and functionally distinct receptors, termed $GABA_A$- and $GABA_B$-receptors (Bowery *et al.*, 1980; 1981; Hill & Bowery, 1981). The $GABA_A$-receptor is associated with a Cl^- ionophore/benzodiazepine receptor complex (Olsen, 1981) which mediates fast inhibitory postsynaptic potentials (i.p.s.ps) in many brain regions and is sensitive to blockade by bicuculline and picrotoxin (Krnjević, 1974; Curtis, 1979). In contrast, the $GABA_B$-receptor produces an increase in K^+ conductance and/or a decrease in Ca^{2+} potentials (Dunlap, 1981; Newberry & Nicoll, 1984; 1985; Gähwiler & Brown, 1985; Deisz & Lux, 1985) which may involve a second messenger system (Hill & Dolphin, 1984; Karbon *et al.*, 1984; Wojcik & Neff, 1984; Hill, 1985); is much less sensitive

to blockade by bicuculline and picrotoxin, and is selectively activated by the antispastic agent, baclofen (Bowery, 1982). In addition, it is possible that $GABA_B$-receptors may mediate late, slow i.p.s.ps as described in rat hippocampal cells (Nicoll & Alger, 1981; Newberry & Nicoll, 1985).

The ubiquitous nature of GABA as an inhibitory transmitter in the mammalian CNS and the therapeutic applications of drugs which interact with GABA receptors have resulted in considerable interest in GABA pharmacology (Bartholini, 1985). However, there have been relatively few attempts to quantify the potencies of GABA-mimetics and GABA-receptor antagonists in the mammalian CNS.

The development of brain slice techniques now allows for quantitative pharmacological analysis to be performed on CNS tissue and this has been done with considerable success by Simmonds (1978; 1981; 1982) for $GABA_A$-receptor antagonists and modulators using a cuneate nucleus slice.

The hippocampus has become the most extensively used brain slice preparation and is ideal for pharmacological studies (see Dunwiddie *et al.*, 1983). It contains GABAergic interneurones (Ribak *et al.*, 1978; Somogyi *et al.*, 1983; 1985) which probably mediate their effects through both $GABA_A$ and $GABA_B$ receptors, and previous studies have shown that GABA-mimetic drugs depress the synaptically evoked CA1 population spike recorded in the rat hippocampal slice (Ault & Nadler, 1982; 1983). Therefore, we have used this preparation to assess quantitatively the potencies of a range of GABA-receptor agonists and antagonists on mammalian CNS neurones. Abstracts containing some of these results have been published (Kemp *et al.*, 1984; 1985).

Methods

Preparation of slices and recording of population spikes

Male Sprague-Dawley rats (approximately 100 g) were killed by decapitation and their brains rapidly removed. Slices, 350 μm thick, from the dorso-medial part of the hippocampus were cut in artifical cerebrospinal fluid (aCSF), at room temperature (20°C), using an Oxford vibratome. A single slice was placed on a nylon mesh and completely submerged in a small superfusion chamber, which was essentially the same as the 'Scottish Chamber' of Williams *et al.* (1984). The slice was continuously superfused with oxygenated aCSF at a rate of approximately 1.5 ml min^{-1}, at room temperature. The aCSF had the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.25, $MgSO_4$ 2, $CaCl_2$ 2, $NaHCO_3$ 25, glucose 11.

The Schaffer collateral-commissural pathway was stimulated every 30 s with either a glass micropipette filled with 3 M NaCl (resistance 2–10 MΩ) or a metal bipolar electrode made from two tungsten microelectrodes (TM25-5, Clark electromedical), placed in the stratum radiatum. Population spikes were recorded from the cell body layer of the CA1 pyramidal cells using glass micropipettes filled with 3 M NaCl and having resistances of 2–10 MΩ. The population spikes were recorded and averaged using a Neurolog system (Digitimer Ltd).

Construction of dose-response curves

The average of four, submaximal, control responses was taken and then the perfusing medium changed to one containing a drug by means of a three way tap. Drugs were perfused for 5 min periods to ensure that a maximal effect was achieved and the last four responses at each dose level were averaged and plotted by a pen recorder. Drug doses were added cumulatively and the dose-response curve was generated by plotting drug concentration against % reduction of the population spike.

Antagonist studies

Antagonists were perfused for 15 min prior to, and then continuously with, the GABA agonist. When several concentrations of antagonist were studied on the same slice, recovery from the inhibitory response to the agonist was obtained in the presence of the antagonist and then the antagonist concentration raised and a further 15 min allowed for equilibration. Following the observations of Ault & Nadler (1983), that bicuculline reduced the stimulus intensity required to produce a maximal response, we took care to ensure that the population spikes remained submaximal in the presence of the antagonists. Therefore, we did not change the stimulus intensity between the control and the antagonist runs.

Sources of drugs and chemicals

Muscimol, γ-aminobutyric acid, picrotoxin (Sigma); isoguvacine, piperidine-4-sulphonic acid, imidazole acetic acid (IAA), bicuculline methobromide (CRB, Cambridge); (±)-baclofen (Ciba-Geigy, Basel); 3-aminopropanesulphonic acid (Aldrich); bicuculline methochloride (Pierce); 4,5,6,7 -tetrahydroisoxazolo [5,4-c]pyridin-3-ol (THIP) (Lundbeck, Copenhagen); pitrazepin (Sandoz, Basel); thiomuscimol, *cis*-4-hydroxynipecotic acid (P. Krogsgaard-Larsen, Royal Danish School of Pharmacy, Copenhagen).

Results

All the GABA-mimetics examined in this study produced a complete and dose-dependent inhibition

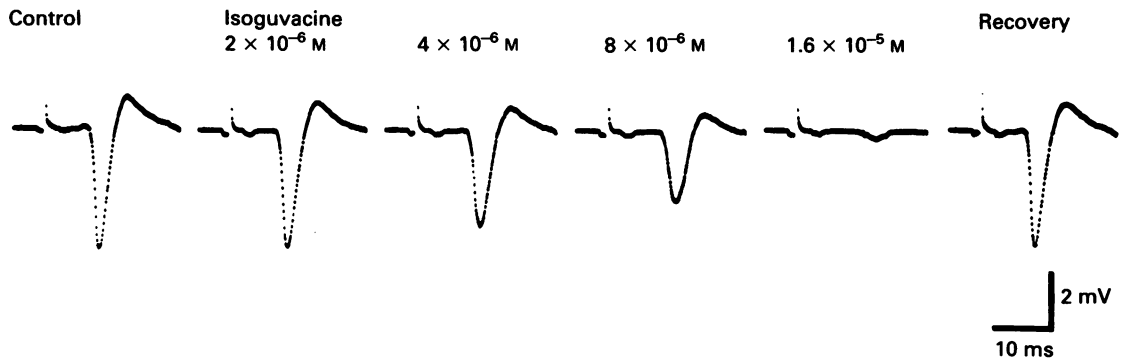

Figure 1 Effect of increasing concentrations of the selective $GABA_A$-receptor agonist, isoguvacine, on the averaged CA1 population spike. Recovery, 15 min after returning to aCSF.

of the CA1 population spike. An example of the depressant effect of the selective $GABA_A$ agonist, isoguvacine, on the population spike is shown in Figure 1. Concentration-response curves to some $GABA_A$ agonists, the $GABA_B$ agonist, baclofen, and GABA itself, are illustrated in Figure 2. The concentration of agonist required to produce 50% inhibition of the population spike (EC_{50} value) was estimated from the concentration-response curves and taken as a measure of agonist potency. With the exception of GABA itself, there was a close correlation ($r = 0.96$) between the rank order of potency of the ability of $GABA_A$ agonists to depress the CA1 population spike and to displace $GABA_A$-receptor binding (Figure 3). In four slices the GABA uptake inhibitor, *cis*-4-hydroxynipecotic acid (2×10^{-4} M) (Krogsgaard-Larsen *et al.*, 1981a), increased the potency of GABA (Figure 4) from $1.58 \pm 0.20 \times 10^{-4}$ M to $2.63 \pm 0.25 \times 10^{-5}$ M (EC_{50}, mean ± s.e.mean) without potentiating the effects of THIP ($2.95 \pm 0.96 \times 10^{-5}$ M to $4.57 \pm 0.29 \times 10^{-5}$ M), a GABA-receptor agonist which is not a substrate for GABA uptake (Krogsgaard-Larsen *et al.*, 1981a). The EC_{50} values for all the GABA-mimetics studied are summarized in Table 1.

As previously found (Ault & Nadler, 1983), the selective $GABA_B$-receptor agonist, baclofen, also produced a complete inhibition of the CA1 population

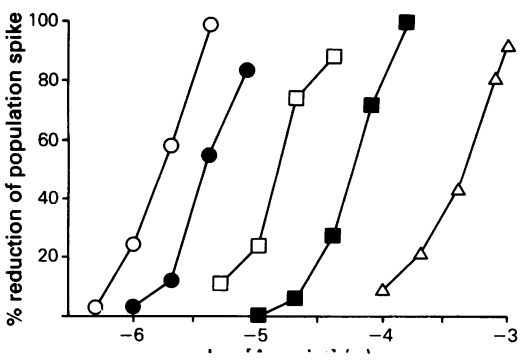

Figure 2 Examples of cumulative concentration-response curves for muscimol (○), (±)-baclofen (●), isoguvacine (□), 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (■) and GABA (△) on inhibition of the CA1 population spike. Data shown are from single experiments on different slices.

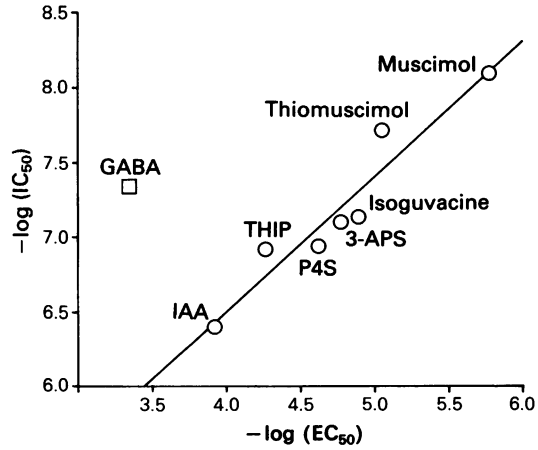

Figure 3 Correlation between the potencies of $GABA_A$-receptor agonists to inhibit the CA1 population spike (abscissa scale) and to displace [^{3}H]-GABA binding (ordinate scale). Binding data from Krogsgaard-Larsen *et al.* (1981b). GABA data not included in the correlation. Linear regression fitted by the method of least squares, $r = 0.96$. IAA = imidazoleacetic acid; THIP = 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol; P4S = piperidine-4-sulphonic acid; 3-APS = 3-aminopropanesulphonic acid.

Table 1 Potency of GABA-mimetics (EC_{50}) for inhibition of the CA1 population spike in the hippocampal slice

GABA agonist	EC_{50}(μM)	n
Muscimol	1.7 ± 0.3	16
(±)-Baclofen	3.8 ± 0.6	10
Thiomuscimol	8.9 ± 0.8	8
Isoguvacine	13.0 ± 1.0	26
3-Aminopropanesulphonic acid	17.0 ± 3.4	6
Piperidine-4-sulphonic acid	24.0 ± 3.2	8
THIP	55.0 ± 10	7
Kojic amine	90.0 ± 14	9
Imidazoleacetic acid	121.0 ± 59	4
GABA	460.0 ± 80	17

Data shown are means ± s.e.mean of *n* observations. THIP = 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol.

spike and this response was unaffected by the $GABA_A$-receptor antagonists bicuculline methochloride and picrotoxin. In addition, when applied in the bath to the hippocampal slice, the inhibitory effects of both kojic amine and GABA (Figure 5a) were also unaffected by bicuculline methochloride and picrotoxin.

In contrast, the responses to the selective $GABA_A$-receptor agonists isoguvacine and muscimol were blocked by $GABA_A$-receptor antagonists. Bicuculline methochloride (1×10^{-6} M to 1×10^{-4} M) produced parallel shifts to the right of the isoguvacine and muscimol concentration-response curves (Figure 5b). Dose-ratios were measured at the 50% inhibition level and Schild plots (Arunlakshana & Schild, 1959)

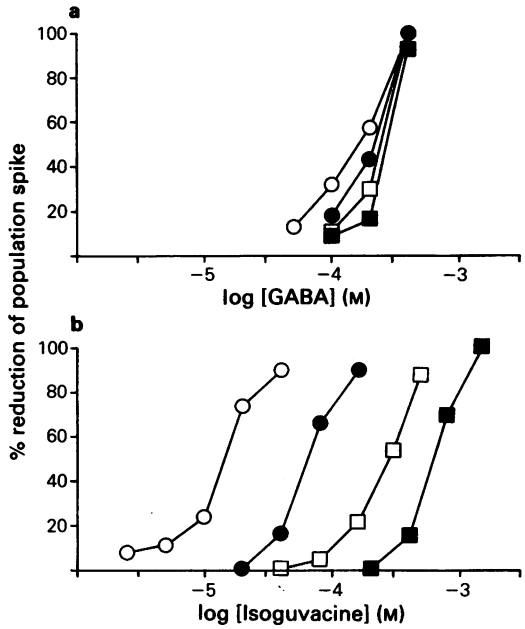

Figure 5 Effect of bicuculline methochloride on the inhibition of the CA1 population spike by GABA (a) and isoguvacine (b). Concentration-response curves in the absence (○) and presence of bicuculline methochloride 1 μM (●), 5 μM (□) and 10 μM (■). Data from single experiments on different slices are shown.

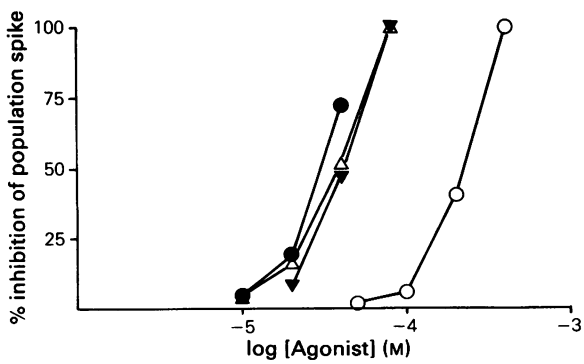

Figure 4 Effect of *cis*-4-hydroxynipecotic acid on the inhibition of the CA1 population spike by GABA and 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol (THIP). Concentration response curves to GABA (○, ●) and THIP (△, ▼) in the absence (open symbols) and presence (closed symbols) of *cis*-4-hydroxynipecotic acid (2×10^{-4} M).

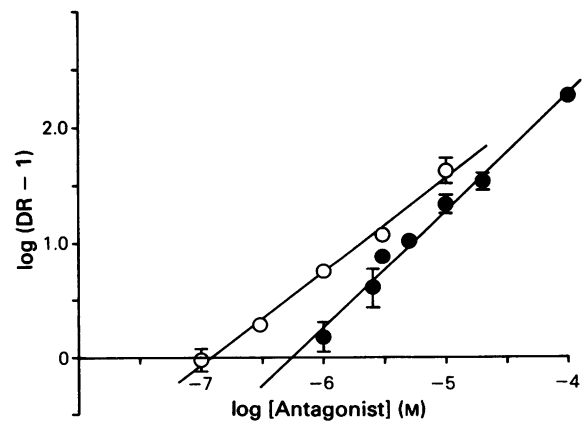

Figure 6 Schild regressions for the antagonism of isoguvacine by bicuculline methochloride (●) and picrotoxin (○). Vertical lines indicate s.e.mean where larger than symbol, $n > 3$ for each point; total $n = 35$ and 37 for bicuculline methochloride and picrotoxin, respectively. Bicuculline methochloride: slope = 1.03; $pA_2 = 6.24$. Picrotoxin: slope = 0.82; $pA_2 = 6.89$.

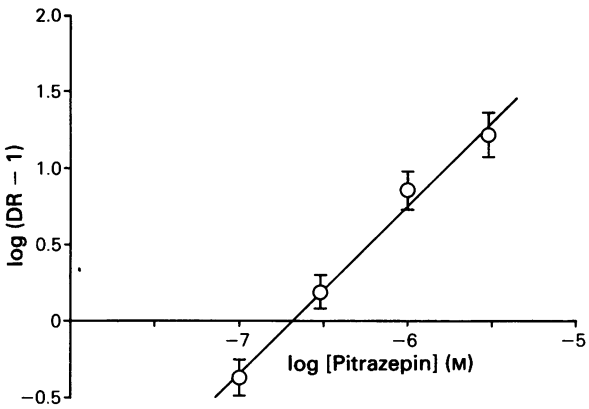

Figure 7 Schild regression for antagonism of isoguvacine by pitrazepin. Vertical lines indicate s.e.mean, $n = 5$ for each point. Slope = 1.09; $pA_2 = 6.69$.

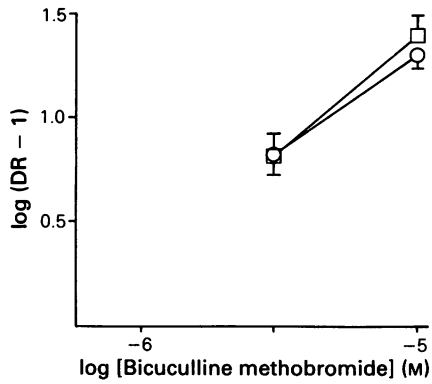

Figure 8 Schild plots for antagonism of isoguvacine (□) and 4,5,6,7-tetrahydroisoxazolo[5,4-c] pyridin-3-ol (○) by bicuculline methobromide on the same preparations. Vertical lines indicate s.e.mean, $n = 4$ for each point.

constructed from these values using linear regression analysis. The Schild regressions for bicuculline methochloride against isoguvacine and muscimol had slopes of 1.04 ± 0.14 ($\pm$ 95% confidence limits) and 1.03 ± 0.2 respectively and yielded pA_2 values of 6.24 and 6.10 (Figure 6) Picrotoxin also produced parallel displacements to the right of the isoguvacine dose-response curve with a pA_2 of 6.89. However, the Schild plot of these data had a slope of 0.82 ± 0.10, which is significantly less than unity ($P < 0.002$, t test) (Figure 6).

Pitrazepin, has recently been described as a $GABA_A$-receptor antagonist (Gähwiler *et al.*, 1984) but its potency had not previously been measured quantitatively in intact tissues. We found pitrazepin to be a potent antagonist of isoguvacine in the hippocampal slice. The Schild regression is shown in Figure 7 and had a slope of 1.09 ± 0.25 and gave a pA_2 value of 6.69.

It has been suggested that THIP acts preferentially at a 'synaptic' GABA receptor (Allan *et al.*, 1980; Alger & Nicoll, 1982) and that in the hippocampus, bicuculline has a lower potency at this 'synaptic' receptor than at the 'extrasynaptic' receptor (Alger & Nicoll, 1982). Therefore, we compared the potency of bicuculline methobromide as an antagonist of both THIP and isoguvacine, another selective $GABA_A$-receptor agonist, in the same preparations. Two doses of bicuculline methobromide, 3×10^{-6} M and 1×10^{-5} M, were used on each slice. There was no difference between the potency of bicuculline methobromide as an antagonist of the inhibitory effects of THIP or isoguvacine. The results from these experiments are illustrated in the form of a Schild plot in Figure 8.

Discussion

These results indicate that the CA1 population spike recorded from the hippocampal slice is a convenient and reliable test system for the quantitative analysis of both $GABA_A$- and $GABA_B$-receptor agonists and antagonists. All the GABA-mimetics studied were able to inhibit completely the population spike. However, the concentration-response curves were steep, covering only 1 decade of concentrations, and intracellular studies (Fernandez & Kemp, unpublished observations) suggest that $GABA_A$-receptor agonists, produce 100% inhibition of the population spike at concentrations well below those which induce maximum increases in membrane conductance (see also Okada & Ozawa, 1982). The rank order of potency of $GABA_A$-receptor agonists in the hippocampal slice correlates closely with their ability to displace [^{3}H]-GABA from $GABA_A$-receptor binding sites, which suggests that receptor affinity is the major determinant of agonist potency in this preparation. It is also of interest to note that a similar correlation exists between the affinity of $GABA_A$-receptor agonists and the average length of Cl^- ion channel lifetime they evoke (Barker & Mathers, 1981), as both of these factors would be expected to contribute to agonist potency.

The results with the $GABA_A$-receptor antagonists bicuculline methochloride and picrotoxin are in broad agreement with those of Simmonds (1982), although both antagonists were slightly more potent in the present study. In contrast to bicuculline, on dorsal funiculus fibres, bicuculline methochloride produced a Schild plot with a slope significantly less than 1, although studies with combinations of antagonists

revealed it to be acting at the same site as bicuculline (Simmonds, 1982). The Schild plot of 1 obtained in this study is in agreement with previous electrophysiological and biochemical evidence (Olsen & Snowman, 1983) that bicuculline methochloride acts as a competitive antagonist at the mammalian $GABA_A$-receptor site.

In contrast, although picrotoxin produced parallel displacements to the right of the isoguvacine dose-response curve (cf Simmonds, 1982), it produced a Schild plot with a slope significantly less than 1. This is in agreement with the suggestions that it acts at a site associated with the $GABA/Cl^-$ ionophore complex rather than the $GABA_A$-recognition site itself (see Simmonds, 1982). Indeed, studies with combinations of bicuculline methochloride and picrotoxin (unpublished observations) demonstrate that in the hippocampus picrotoxin also acts at a site separate from that occupied by bicuculline methochloride.

Pitrazepin gave a Schild plot with a slope not significantly different from unity, which is indicative of competitive antagonism. This is in keeping with the observation of Gähwiler *et al.* (1984) that pitrazepin displaces [^{3}H]-muscimol from $GABA_A$-binding sites with a Hill-slope close to 1. The pA_2 value of 6.69 indicates that pitrazepin is a potent antagonist at $GABA_A$-receptors, being some three times more potent than bicuculline methochloride (Kemp *et al.*, 1985).

Bicuculline methobromide failed to discriminate between responses to THIP and isoguvacine, both selective $GABA_A$-receptor agonists with low affinity for the GABA uptake system (Bowery *et al.*, 1981; Krogsgaard-Larsen *et al.*, 1981a). In our experiments direct comparisons with GABA were confounded by its susceptibility to uptake and its actions at $GABA_B$-receptors. However, it is likely that these factors, particularly uptake, also contributed to the observations of Allan *et al.* (1980) that GABA and THIP had different potencies in different preparations. Their results show that the potency of GABA was much higher on isolated spinal roots than on the hemisected spinal cord where the density of uptake sites is approximately ten times greater (Davies & Johnston, 1974). Indeed, if the potency of THIP is compared to that of muscimol and isoguvacine, other selective $GABA_A$ agonists which are also poor substrates for uptake processes, then there is little change in their relative potencies between these preparations (Allan *et al.*, 1980). The small effect of nipecotic acid on the response to GABA in the spinal cord seen by Allan *et al.* (1980) is similar to that found by Brown *et al.* (1980) and Brown & Scholfield (1984) and is unlikely to be due to the lack of influence of uptake processes (Brown & Scholfield, 1984). In our present experiments the GABAuptake inhibitor, *cis*-4-hydroxynipecotic acid, increased the potency of GABA by approximately 6 fold without potentiating the effect of THIP. This suggests that the low potency of GABA is the result of the action of uptake processes. Our data provide no evidence to support the suggestion that there are subtypes of the bicuculline-sensitive, $GABA_A$-receptor on hippocampal CA1 pyramidal cells.

It has previously been demonstrated that the selective $GABA_B$-receptor agonist, baclofen, inhibits the Schaffer collateral-commissural excitation of CA1 cells (Ault & Nadler, 1982; Olpe *et al.*, 1982) in a bicuculline insensitive manner (Ault & Nadler, 1983). This effect is probably mediated by a mixture of pre- and post-synaptic actions at $GABA_B$-receptors (Olpe *et al.*, 1982; Ault & Nadler, 1983; Newberry & Nicoll, 1984). In support of this and in agreement with earlier studies (Ault & Nadler, 1983), we found that $GABA_A$-receptor antagonists failed to block the depressant actions of baclofen or GABA on the CA1 population spike. In addition, the inhibitory effect of kojic amine was similarly unaffected by either bicuculline methochloride or picrotoxin. This is in keeping with recent observations (Karbon *et al.*, 1984) which indicate that kojic amine is a more potent agonist at $GABA_B$-receptors than at $GABA_A$-receptors (Yarbrough *et al.*, 1979). Our results suggest that in this preparation kojic amine is approximately 20 times less potent than (±)-baclofen at $GABA_B$-receptors.

In conclusion, these results indicate that the *in vitro* hippocampal slice preparation is well suited to evaluate the potency of GABA-receptor agonists on CNS neuronal activity and can also be used to determine the affinities of both $GABA_A$- and $GABA_B$-receptor antagonists.

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