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PRELIMINARY COMMUNICATIONS

MORE MUSCIMOL BINDING SITES THAN GABA BINDING SITES IN A PARTICULATE FRACTION OF RAT BRAIN

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Muscimol (3-hydroxy-5-aminomethylisoxazole), a conformationally-restricted analogue of γ-aminobutyric acid (GABA), exerts pronounced bicuculline-sensitive depressant actions when applied iontophoretically to neurones of the mammalian CNS (1,2). Recent in vitro studies have revealed further that muscimol is about 5 - 10 times more potent than GABA itself at competing with [^{3}H]GABA or [^{3}H]bicuculline-methiodide for membrane binding sites of rat brain (3-5) and that [^{3}H]muscimol is bound to various CNS particulate fractions by mechanisms that involve one or two populations of high-affinity sites (6-9). However, except for one preliminary report (10), no studies have been aimed at comparing the maximal binding capacities (B_{max}) of muscimol and GABA in a CNS preparation. Herein, we present such data.

MATERIALS AND METHODS

Adult, male Wistar rats (175 - 225 g) were used to prepare crude membrane pellets of whole brain (11). Pellets were frozen at -25°C for 2 weeks - 3 months with no loss of binding activity for [^{3}H]GABA or [^{3}H]muscimol. For binding assays, frozen pellets (representing about 2 g original wet wt of tissue) were resuspended in 20 ml of deionized water, allowed to stand at 22°C for 20 min, and then centrifuged at 50,000 g, 20 min. This procedure was repeated, and then pellets were resuspended in 4.0 ml of ice-cold Na^+-free Tris-citrate buffer (50 mM; pH 7.1); all further operations were conducted at 0° - 4°C.

Aliquots (100 µl) of tissue suspension were mixed with 100 µl of Tris-citrate buffer, or with 100 µl of this same buffer containing enough unlabelled GABA to provide final concentrations of 10^{-3} M or 10^{-5} M, and allowed to stand for 10 min. (Both [^{3}H]GABA and [^{3}H]muscimol were maximally displaced by 10^{-5} M unlabelled GABA.) Then, 250 µl of buffer containing either [^{3}H]GABA ([2,3-^{3}H(N)]γ-aminobutyric acid; 36.12 Ci/mmole) or [^{3}H]muscimol ([methylene-^{3}H(N)]-3-hydroxy-5-aminomethylisoxazole; 13.68 Ci/mmole) at the same final concentrations of 6.2 - 30.8 nM plus tracer amounts of [^{14}C]sucrose ([U-^{14}C]sucrose; 673 mCi/mmole) were added. Samples were mixed, allowed to stand for 5 min, and centrifuged at 57,000 g for 5 min to obtain final pellet and supernatant fractions. [^{3}H]GABA binding to the particles was maximal under these conditions. Protein and radioactivity were determined as previously described (12). All radioactive products were from New England Nuclear.

RESULTS

As expected, pellet/supernatant distribution ratios (DR) for both [^{3}H]GABA and [^{3}H]muscimol were decreased in the presence of excess unlabelled GABA (Table 1). However, DRs for [^{3}H]muscimol were significantly greater than those for [^{3}H]GABA ($p < 0.001$), and excess unlabelled GABA displaced significantly more [^{3}H]muscimol than [^{3}H]GABA ($p < 0.001$) at all ligand concentrations tested (Table 1). The DR for [^{14}C]sucrose was 0.72 ± 0.04 (mean ± standard deviation; $n = 48$) and was not altered by the presence of excess GABA. Lineweaver-Burk plots of the differences in binding observed in the absence vs presence of excess unlabelled GABA revealed that the affinity of the particles for [^{3}H]muscimol was about 3 times that for [^{3}H]GABA and that the B_{max} of [^{3}H]muscimol binding was about twice that of [^{3}H]GABA (Fig. 1). Other experiments, performed by adding the particles to labelled ligand in the presence or absence of unlabelled GABA (10^{-3} M) and with a 30-min incubation period, also revealed this difference in B_{max} (data not shown).

DISCUSSION

Over an identical concentration range of labelled ligand, there existed about twice as many [^{3}H]muscimol as [^{3}H]GABA binding sites in particles of rat brain. This finding suggests that [^{3}H]muscimol binding occurs not only to those membranous sites that can be occupied by GABA itself, but also to another population of sites that are present in brain particles. Further consideration of these other sites could be useful for explain-

Table 1. Pellet/supernatant distribution ratios for [^{3}H]GABA and [^{3}H]muscimol in a particulate fraction of rat whole brain in the presence and absence of excess unlabelled GABA

Substance and concentration (nM)	Distribution ratio[a]		
	[^{3}H]GABA		
[^{3}H]GABA	Control (A)	+10^{-3} M GABA (B)	A-B
30.8	1.01 ± 0.04	0.85 ± 0.01	0.16
15.4	1.00 ± 0.03	0.85 ± 0.01	0.15
9.2	0.99 ± 0.02	0.85 ± 0.04	0.14
6.2	1.00 ± 0.01	0.84 ± 0.05	0.16
	[^{3}H]Muscimol		
[^{3}H]Muscimol	Control (A)	+10^{-5} M GABA (B)	A-B
30.8	1.87 ± 0.06	0.97 ± 0.02	0.90
15.4	2.07 ± 0.09	1.01 ± 0.06	1.06
9.2	2.25 ± 0.13	0.87 ± 0.05	1.38
6.2	2.18 ± 0.10	0.89 ± 0.04	1.29

Means ± standard deviations of 3 samples in all cases, or calculated differences; all values for [^{3}H]GABA and [^{3}H]muscimol were significantly greater under control conditions than in the presence of excess unlabelled GABA ($p < 0.001$; Student's t-test; one-tailed).

[a] Distribution ratio = $\frac{\text{dis/min per g pellet}}{\text{dis/min per g supernatant}}$.

ing the extra-synaptic binding of [^{3}H]muscimol that has been observed in recent autoradiographic (13) and membrane binding studies (8,9). Although the cellular origin(s) of these "extra" [^{3}H]muscimol binding sites remains unknown, it seems clear that the densities of cerebral GABA-receptors will be overestimated if [^{3}H]muscimol binding is used as the basis for such determinations.

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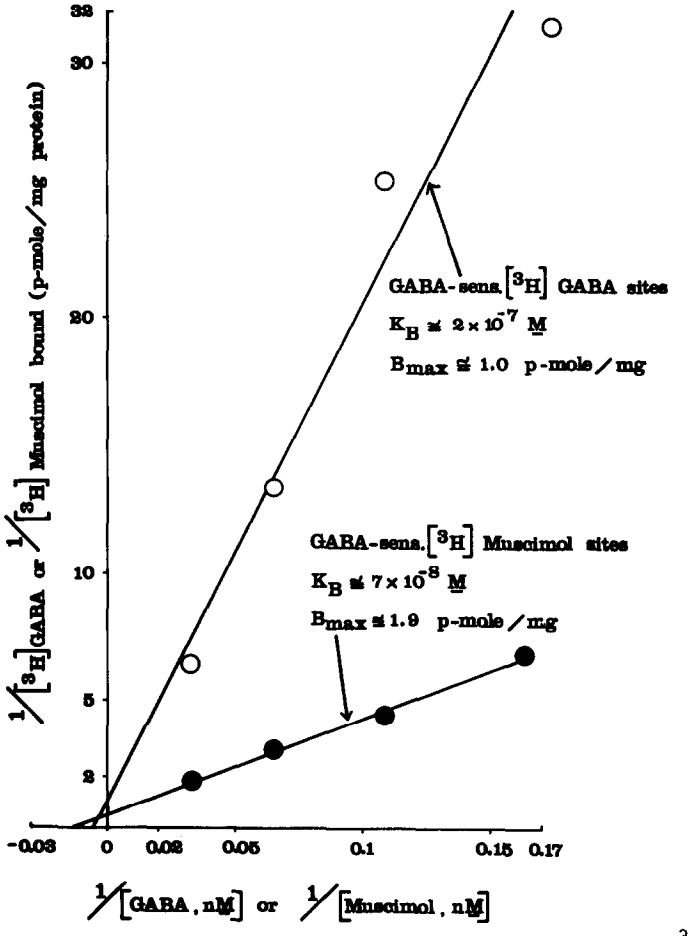

Fig. 1. Lineweaver-Burk plots of the high-affinity binding of [^{3}H]GABA and [^{3}H]muscimol to a particulate fraction of rat whole brain. Values represent reciprocals of the differences between the total amounts of labelled ligand found in the pellet in the absence and presence of excess unlabelled GABA (3 closely-agreeing samples in all cases). K_B and B_{max} values, determined by least-squares regression analyses, are indicated. Note that the capacity (B_{max}) for [^{3}H]muscimol sites is about twice that for [^{3}H]GABA sites.