

Inhibition of [³H]-naloxone binding by opiate agonists

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It has been shown that the relative receptor affinities of most opiates, as measured by their inhibition of [³H]-naloxone binding to rat brain homogenates, correlate well with their pharmacological actions in the guinea-pig ileum, which is a good predictor of their analgesic action in man (Kosterlitz & Waterfield, 1975; Creese & Snyder, 1975). Of particular interest is the fact that Na⁺ decreases the binding of agonists whilst not affecting, or slightly enhancing, that of antagonists (Pert & Snyder, 1974). The purpose of this communication is to draw attention to the great variability of the sodium shift.

A modification of the method of Pert & Snyder was used. Guinea-pig brain homogenates, prepared by means of an Ultraturrax, were spun at 600 g to remove cell debris. The supernatant was centrifuged at 49,000 g and the resulting pellets resuspended in either 50 mM Tris buffer (pH 7.4 at 37°C) or in Krebs-Tris. Aliquots of homogenate (2 ml) were incubated with 1 nM [³H]-naloxone (4 Ci/mmol, NIDA) plus the inhibiting drug at 37°C for 20 min, the samples then being cooled on ice for 30 min before filtration through Whatman GF/B filters. Stereospecific naloxone binding was determined by subtracting the binding in the presence of 50 nM MR 2266 {(–)-α-5,9-diethyl-2-(3-furymethyl)-2'-hydroxy-6,7-benzomorphane} from that in its absence. For each drug, the concentration required to produce a 50% inhibition of binding was calculated from log-probit regression lines.

The degree of sodium shift was not constant for all agonists since a 7 to 150 fold decrease in potency was observed. Pert & Snyder (1974) found that agonists with an antagonist component have a small sodium shift but the great variation found in this study cannot be explained on this basis, as only pure agonists were used. It is of interest that the three drugs with the

smallest sodium shifts belong to a group of benzomorphans which are unusual in that they do not cause morphine-like dependence in monkeys and require more naloxone than does normorphine to antagonize their actions in the guinea-pig ileum and mouse vas deferens (Hutchinson, Kosterlitz, Leslie, Waterfield & Terenius, 1975). They are (–)-α-(1R, 5R, 9R)-5,9-dimethyl-2-(L-tetrahydrofurfuryl)-2'-hydroxy-6,7-benzomorphan (MR 2034, C.H. Boehringer Sohn), (±)-α-5,9-dimethyl-2-(3-methyl-furfuryl)-2'-hydroxy-6,7-benzomorphan (MR 1353) and (±)-9-methyl-5-ethyl-8-oxo-2-cyclopropyl-methyl-2'-hydroxy-6,7-benzomorphan (ethylketocyclazocine, Win 35,197-2, Sterling-Winthrop).

For most of the agonists studied there is good correlation between the relative potencies of inhibition of binding and those found in the pharmacological models, the guinea-pig ileum and mouse vas deferens. However, some compounds, notably Win 35,197-2, were less potent in the binding assay than in the pharmacological models. The reason for this discrepancy is, as yet, unclear. It would, however, appear to be advisable to employ several parallel *in vitro* assays, using pharmacological responses and inhibition of binding, for the prediction of potencies *in vivo*.

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Dopamine utilization in the posterior pituitary gland of the rat

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Dopamine is present in the posterior lobe of the pituitary gland where it is thought to be contained

in nerve fibres (Björklund, 1968; Saavedra, Palkovits, Kizer, Brownstein & Zivin, 1975). As a first step in the study of the function of dopamine (DA) in this tissue, its utilization was investigated. Albino rats (Wistar, Porton strain) were injected with α-methyl-p-tyrosine (AMPT), an inhibitor of tyrosine hydroxylase and were killed 30, 60 and 120 min later. Control rats were injected with a 0.9% NaCl solution and killed after the same time intervals. The pituitary gland was dissected out, the anterior lobe separated from the

intermediate and posterior lobe and the latter tissues analysed together for their DA content since Saavedra *et al.* (1975) have shown that the posterior lobe contains the majority of the dopamine in the pituitary gland. The tissues from 3 rats were pooled for each sample. The DA content was determined by a radio enzymatic assay (Fry, House & Sharman, 1974). In untreated female rats (200-300 g body weight) 2.12 ± 0.14 ng DA/posterior lobe ($n=5$ samples) were found, in untreated male rats (250-300 g body weight) the value was 2.19 ± 0.10 ng ($n=6$ samples). The quantity of DA contained in the anterior lobe was about 0.2 ng/gland. In Table 1 some results on the effect of AMPT on pituitary DA are listed. In a group of female rats (mean body weight 200 g) there occurred a significant reduction in pituitary DA by 28% 30 min after AMPT; after 60 min it was reduced by 63% but no further decrease occurred after 120 minutes. In male rats (mean body weight 320 g) the reduction in pituitary DA 30 min and 60 min after AMPT was not statistically significant but after

120 min the DA content was significantly decreased by 44%. In the same rats a similar rate of fall in the DA concentration of the striatum was observed.

These experiments demonstrate that the DA in the posterior pituitary gland is being utilized. Experiments to study the concentration of pituitary DA under conditions which result in changes in the secretion of pituitary hormones are in progress.

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Table 1 Effect of α -methyl-*p*-tyrosine (AMPT) on the dopamine content of the posterior pituitary and the striatum in the rat

Rats age	Time after injection (min)	Dopamine content					
		Posterior pituitary (ng/posterior lobe)		Difference between control AMPT	Striatum (μ g/g tissue)		Difference between control and AMPT
		Control*	AMPT**		Control*	AMPT**	
Female 9 weeks	30	1.8 ± 0.07 (2)	1.30 ± 0.08 (3)	-28%, $P < 0.02$			
	60	2.01 ± 0.40 (3)	0.75 ± 0.13 (3)	-63% $P < 0.05$	—	—	—
	120	1.88 ± 0.15 (3)	0.81 ± 0.12 (3)	-57%, $P < 0.01$			
Male 16 weeks	30	3.07 ± 0.61 (5)	2.00 ± 0.29 (5)	-35% n.s.	9.07 ± 0.59 (10)	7.86 ± 0.60 (9)	-13%, n.s.
	60	2.50 ± 0.34 (5)	1.84 ± 0.42 (5)	-26%, n.s.	10.04 ± 0.51 (10)	7.11 ± 0.33 (10)	-29%, $P < 0.01$
	120	2.55 ± 0.09 (5)	1.44 ± 0.33 (5)	-44%, $P < 0.01$	9.16 ± 0.48 (10)	5.88 ± 0.52 (9)	-36%, $P < 0.01$

*: 10 ml 0.9% sodium chloride i.p./kg body weight; **: 200 mg/kg body weight i.p.; number in brackets: number of samples analysed. Posterior lobe: each sample consisted of three posterior lobes. Striatum: each sample contained the left and right striatum of one rat. Statistical analysis by Student's *t*-test; n.s.: not significant ($P > 0.05$).