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HIGH-AFFINITY STEREOSPECIFIC BINDING OF ^{3}H-DOPAMINE IN RAT BRAIN: INTERACTION WITH ENDOGENOUS DOPAMINE

Nicholas G. Bacopoulos

Departments of Pharmacology and Psychiatry,

Dartmouth Medical School,

Hanover, New Hampshire, U.S.A.

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The in vitro characterization of dopamine receptors and their pharmacologic properties has been achieved mainly through the use of radiolabelled dopamine antagonists such as ^{3}H-spiroperidol or ^{3}H-haloperidol, or agonists such as ^{3}H-apomorphine. There is abundant evidence that these radioligands bind to dopamine receptors in particulate fractions of rat brain regions which possess significant amounts of dopaminergic innervation (1,2,3).

However the sites labelled by ^{3}H-spiroperidol stereospecifically in rat caudate nucleus are far more numerous than the sites labelled by ^{3}H-apomorphine (2,4) although both sites are thought to represent dopamine receptors. Moreover the affinity of the ^{3}H-spiroperidol binding site for dopamine is 1,000-fold lower than the affinity of the ^{3}H-apomorphine binding site for this neurotransmitter. These discrepancies have led investigators to hypothesize that agonists and antagonists bind to different subunits (4) or different allosteric states (3) of the dopamine receptor. One may hypothesize that ^{3}H-dopamine, by definition, would bind to all the states or subunits of its receptor. This is not supported by the available experimental evidence. Few reports have appeared on ^{3}H-dopamine binding in rat brain regions, one stating that in rat caudate nucleus ^{3}H-dopamine binds to a site similar to the less numerous ^{3}H-apomorphine sites (2) rather than to a site the density of which equals the sum of agonist and antagonist sites or subunits. That study however was not investigating stereospecific ^{3}H-dopamine binding sites. Another report (1) implied that high-affinity stereospecific sites of ^{3}H-dopamine are absent from rat caudate nucleus.

Given the very high levels of dopamine in rat caudate nucleus, we reasoned that ^{3}H-dopamine binding in this brain region may be low or undetectable due to an interference by endogenous dopamine.

In this study we have used a sensitive radioenzymatic method (5) to monitor endogenous dopamine in particulate fractions of rat caudate nucleus and compared the level of remaining dopamine to the amount of ^{3}H-dopamine binding. Adult male Sprague-Dawley rats were used

in all experiments. Fresh caudate nuclei were homogenized in 20 mM Tris-HCl buffer pH 7.4 (1:50 w/vol) and centrifuged at 20,000 X g for 15 min. The pellet was resuspended in fresh buffer (1:50 w/vol) and recentrifuged. Pellet "A" thus obtained was resuspended as stated above and divided into 3 aliquots, two of which were incubated at 37 degrees C for 15 or 30 min and the third placed on ice for 30 min. All three aliquots were centrifuged at 20,000 X g for 15 min, the supernates were discarded, and the pellets were resuspended in buffer, to a final concentration of 2.0 mg protein/ml (Pellet "B").

Stereospecific binding and dopamine content were measured in aliquots of the tissue suspensions obtained through the various steps of the procedure described above. Tissue suspensions (0.2 mg protein) were incubated at 23 degrees C for 30 min in a volume of 1.0 ml, containing, in final concentration, 20 mM Tris HCl buffer, pH 7.4, 5.0 mM tetrasodium ethylene diamine tetraacetate (EDTA), 1.1 mM ascorbic acid, 15 μM pargyline and 0.2 - 15.0 nM ^{3}H-dopamine (40 Ci/mmole, New England Nuclear, Boston, MA) added in 1.1 mM ascorbate. The mixture was rapidly passed through glass fiber (GF/B) filters under suction. The filters were washed with 15 ml 20 mM Tris-HCl buffer, pH 7.4, containing 5.0 mM EDTA and 15 μM pargyline, and suspended in 10 ml 3% Protosol-Econofluor (New England Nuclear). Radioactivity was measured 24 hr later with a Beckman LS7500 scintillation counter. Stereospecific binding was the difference between binding in the absence and the presence of 10 μM d-butaclamol, evaluated for statistical significance by group t-test. The same concentration of the inactive stereoisomer l-butaclamol had no effect on ^{3}H-dopamine binding.

The amount of stereospecific binding of ^{3}H-dopamine varied greatly between the different preparations of rat caudate nucleus and appeared to be negatively correlated to the content of endogenous dopamine (Table 1). Preincubation at 37 degrees C resulted in a drastic increase in stereospecific binding accompanied by a reduction in dopamine content which could not be induced by successive washes of the particulate fraction with buffer at 4 degrees C. The omission of EDTA and pargyline from the preincubation buffer is essential for the induction of increased ^{3}H-dopamine binding. In contrast, the addition of these compounds to the binding assay is necessary for the demonstration of stereospecific binding.

The difference between our results and those of Titeler et al.(2) could be due to the absence of preincubation from the method of tissue preparation employed by those investigators. Creese et al.(1) comment that high-affinity binding sites of ^{3}H-dopamine cannot be measured in rat caudate nucleus, whereas the same group has demonstrated stereospecific ^{3}H-dopamine sites in bovine caudate nucleus (6). A possible explanation for this discrepancy is that rat caudate nucleus can be obtained and homogenized in a few minutes, thus preserving most of the endogenous dopamine, whereas bovine caudate nucleus might remain at room temperature for considerable periods of time after the animal is killed. Under those

conditions endogenous dopamine may be oxidized allowing the detection of ^{3}H-dopamine binding sites. This speculation can be tested by measuring dopamine levels in particulate fractions of bovine caudate nucleus such as those commonly used in radioreceptor assays.

TABLE 1. Stereospecific binding of ^{3}H-dopamine in particulate fractions of rat caudate nucleus*

Fraction	Total	Blank	SSB	fmol/mg protein	Dopamine nM
	cpm, X ± St. Dev. (N = 4)				
Pellet A	1156 ± 78	850 ± 56	306	34	32
Pellet B	1520 ± 107	926 ± 60	594	67	20
Pellet B, 15 min preincubation	2320 ± 112	1108 ± 120	1212	136	1.6
Pellet B, 30 min preincubation	2324 ± 185	1236 ± 112	1088	122	1.3

* Caudate nuclei from 5 rats were prepared as described in the text. Binding assays were carried out with 2.0 nM ^{3}H-dopamine and 0.2 mg tissue protein. SSB: stereospecific binding. Blank: measured with 10 μM d-butaclamol. Dopamine was measured radioenzymatically in perchloric acid extracts of the pellet suspensions. The final concentration of endogenous dopamine in the binding assay is shown. The difference between total and blank was significant in all cases (P less than 0.01).

Alternatively, preincubation may enhance binding via a mechanism independent of the removal of dopamine. Our results however suggest that endogenous dopamine can obscure the stereospecific binding sites of ^{3}H-dopamine. Conversely, the removal of the majority of residual dopamine revealed a large number of stereospecific sites. The longer incubation time (30 min) lowered stereospecific binding by increasing the blank.

A small portion of endogenous dopamine persisted even in tissue suspensions preincubated for 30 min. This dopamine may be contained in vesicles, in which case it would not interfere with the binding of ^{3}H-dopamine. It is also possible that the residual dopamine is in solution and may bind to a fraction of dopamine receptors which cannot be detected under the conditions described here.

The stereospecific binding sites of ^{3}H-dopamine in rat caudate nucleus, measured as described in pellets preincubated for 15 min, were saturable, with a K_d of 1.0 nM and a B_{max} of 200 fmoles/mg protein (Figure 1). Stereospecific binding was detected in the olfactory tubercles but not in cerebral cortex. The binding of ^{3}H-dopamine was inhibited by nonradioactive dopamine (IC_{50} = 1.0 nM) but unaffected by serotonin concentrations as

high as 1.0 μM. These results suggest that ^{3}H-dopamine binds to a single class of high-affinity receptors in rat caudate nucleus. The density of these receptors is much higher than what was previously reported (1,2) possibly due to the contamination of binding assays with endogenous dopamine. The relationship between sites labelled by ^{3}H-dopamine and sites labelled by 3H-apomorphine or ^{3}H-antipsychotic drugs needs to be reassessed. The pharmacologic properties of stereospecific ^{3}H-dopamine binding sites can be more reliably investigated by the method described here.

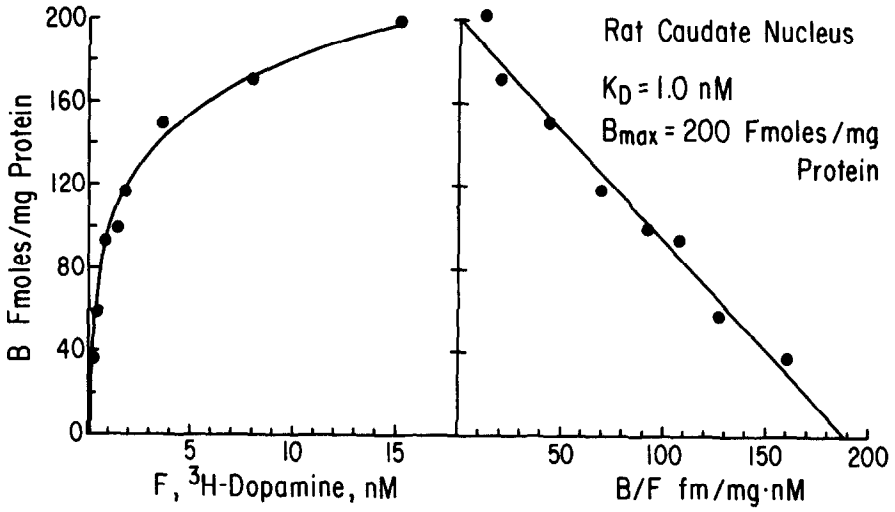

Figure 1. Saturation of ^{3}H-dopamine binding in preincubated particulate fraction of rat caudate nucleus. B: stereospecifically bound (Blank: 10 μM d-butaclamol). F: free ^{3}H-dopamine concentration in the binding assay. K_d (1.0 nM): the slope of the Scatchard plot, calculated by linear regression.

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