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HIGH-AFFINITY ^{3}H-DOPAMINE RECEPTORS (D_3 SITES) IN HUMAN AND RAT BRAIN

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There are at least two dopaminergic binding sites in the calf brain. One site is labelled by ^{3}H-dopamine or ^{3}H-apomorphine and has a high affinity for dopamine (with a nanomolar K_D). The other site (the D_2 site) can be labelled by ^{3}H-neuroleptics or ^{3}H-dihydroergocryptine and has a high affinity for neuroleptics and ergots, but a lower affinity for dopamine itself [1,2]. While the D_2 dopamine receptor has been detected in rat, dog and human brain [3,4], high-affinity ^{3}H-dopamine binding in the rat "has proven virtually impossible" to detect by some workers [1,5].

We now report characterization of high-affinity ^{3}H-dopamine binding in the rat and the first demonstration and characterization of this site labelled by ^{3}H-dopamine in the human brain.

Dissected regions from brains of rat (Wistar; 250 g) and human (22-91 years of age; 3-25 hr post-mortem) were placed in 15 vol of cold TEAN buffer [15 mM Tris-HCl, pH 7.4, 5 mM Na_2EDTA, 0.02% (1.1 mM) ascorbate and 12.5 μM nialamide], briefly Polytron-homogenized (Brinkman PT-10; setting of 7; 10 sec), centrifuged (44,000 x g) and resuspended (15 vol) four times (in order to remove endogenous dopamine), and finally stored frozen (-20°, in concentrations of 100-200 mg original wet weight/ml). Before use, the suspension was diluted and further Polytron-homogenized (10 sec).

^{3}H-Dopamine binding assays were carried out using quadruplicate glass test tubes (12 x 75 mm) which received, in order, 200 μL buffer (with or without competing nonradioactive drug), 200 μL of ^{3}H-dopamine (15, 34 or 41 Ci/mmole; New England Nuclear, Boston) and 200 μL tissue (0.45-0.7 mg protein of human tissue or 0.5-0.6 mg protein of rat tissue). After incubation at 20-22° for 30 min, 0.5 ml aliquots were vacuum-filtered through Whatman GF/B filters, followed by a wash of 10 ml buffer.

Using ^{3}H-dopamine concentrations of 0.1 nM to 7 nM and 10^{-6}M cold apomorphine to define specific binding, Scatchard analyses of regions in 5 human brains revealed a high-affinity ^{3}H-dopamine binding site in the dopamine-rich areas, caudate (B_{max} = 63 ± 10 fmoles/mg protein; K_D = 2.2 ± 0.4 nM) and putamen (B_{max} = 57 ± 11 fmoles/mg protein; K_D = 2.8 ± 0.3 nM). The dopamine-poor areas (globus pallidus, thalamus and hippocampus) contained no detectable high-affinity ^{3}H-dopamine binding. Scatchard analyses in rat brain areas revealed high-affinity ^{3}H-dopamine sites in the corpus striatum (B_{max} = 81 fmoles/mg; K_D = 2.3 nM), while in dopamine-poor areas (cerebellum, hippocampus, hypothalamus and frontal cortex) no binding was detectable. These results indicate that the ^{3}H-dopamine binding site in the human and rat brain has a dopaminergic distribution.

A variety of drugs were tested for their ability to compete with the specific binding of 0.66 nM ^{3}H-dopamine. This concentration of ^{3}H-dopamine produced 40% specific binding in the rat (total binding of 400 cpm/filter) and 35%-60% specific binding in the human (total binding of 300-700 cpm/filter). Drug IC_{50} values were obtained in rat from at least three experiments on pooled striatum. In human caudate, IC_{50} values were obtained by averaging determinations from individual brains. The number of brains used is indicated by the value in parentheses. Dopamine, apomorphine, NPA and ADTN had high affinity for the ^{3}H-dopamine

binding site in the human caudate, with IC_{50} values (± S.E.M.) of 4.3 ± .7 nM (n=9), 3.4 ± .8 nM (n=9), 3.9 ± 1 nM (n=6), 5.1 ± 2 nM (n=5), respectively. Haloperidol, spiperone and chlorpromazine had a relatively low affinity for the site with IC_{50} values of 3000 ± 1300 nM (n=4), 5100 ± 1100 nM (n=4), 6500 ± 1600 nM (n=3), respectively. Stereospecificity was demonstrated as (+)-butaclamol had an IC_{50} of 720 ± 290 nM (n=5) while (-)-butaclamol had an IC_{50} of > 50,000 nM (n=5). The ergots, dihydroergocryptine and bromocryptine, had intermediate affinities for the site with IC_{50}'s of 60 ± 5 nM (n=2) and 437 ± 92 nM (n=5).

Fig. 1 compares the average human ^{3}H-dopamine drug IC_{50} results with ^{3}H-dopamine drug IC_{50}'s in the rat striatum and calf caudate.

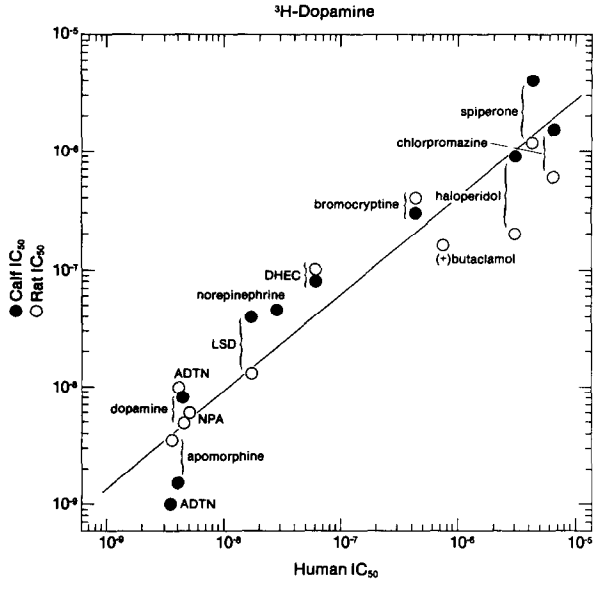

Fig. 1. The IC_{50} values for various drugs against the specific binding of ^{3}H-dopamine to human caudate (horizontal axis) correlate with the values obtained in the rat striatum and the calf caudate (vertical axis). The correlation coefficients of drug IC_{50} values for human vs calf and human vs rat were 0.96 and 0.95, respectively. Abbreviations: NPA, N-n-propylnorapomorphine; ADTN, 2-amino-6,7-dihydroxy-tetrahydronaphthalene.

These data indicate that the high-affinity dopamine site labelled by ^{3}H-dopamine in the rat and human brain is similar to the extensively studied high-affinity dopamine receptor labelled by ^{3}H-dopamine in the calf. Since this high-affinity dopamine receptor has now been shown to have a high affinity for dopaminergic agonists, to have a dopaminergic distribution and to be present in three separate species, we propose that this receptor be called the D_3 dopamine receptor.

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