methionine-enkephalin amide. The corresponding $K_D$'s were 2.52 ± 0.48 and 1.96 ± 0.17 nM ($n = 5$ and the numbers of binding sites were 12.4 ± 0.93 and 12.8 ± 2.22 pmol/g wet wt ($n = 5$). Etorphine was found to have a similar number of binding sites (15.4 + 2.4 pmol/g wet wt ($n = 3$)) but a higher affinity (0.37 ± 0.03 nM ($n = 3$)) than the D-Ala$^2$-enkephalin amide. However, with both morphine and dihydromorphine two binding sites were found; the total numbers of binding sites were 3.7 ± 0.59 and 4.3 ± 0.36 pmol/g wet wt ($n = 4$), respectively.

When the binding of D-Ala$^2$-D-Leu$^5$-enkephalin, dihydromorphine and a mixture of the two ligands in a ratio of 3:1 was compared in the same homogenate, it was found that the mixture yielded a larger number of cpm's (10232 ± 366) than the maximal number of cpm's produced by either D-Ala$^2$-D-Leu$^5$-enkephalin (7812 ± 237) or dihydromorphine (6585 ± 267) alone ($n = 3$, $P<0.025$).

The above evidence suggests that there are at least two different binding sites, one of which recognizes morphine-like ligands and one which recognizes enkephalin-like ligands. The larger number of binding sites found for the D-Ala$^2$-enkephalin amides and etorphine may indicate that these compounds can interact with both binding sites.

**Reference**

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## The use of ADTN (2-amino-6, 7-dihydroxy-1, 2, 3, 4-tetrahydronaphthalene) as a ligand for brain dopamine receptors

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Dopamine (DA) receptors in human brain have been assessed *in vitro* using [$^3$H]-apomorphine (APO) as ligand (Lee, Seeman, Tourtellotte, Farley & Hornykiewicz, 1978), although the high proportion of non-specific binding encountered makes APO an unsatisfactory ligand. We have therefore, evaluated ADTN, a DA agonist, as a ligand for DA receptors, and compared [$^3$H]-ADTN binding in preparations of putamen from schizophrenics and controls.

Initial studies with calf striatal membranes (Burt, Creese & Snyder, 1976) showed that the relative potencies of DA agonists and antagonists in displacing high affinity [$^3$H]-ADTN binding (Table 1) were similar to those reported for [$^3$H]-DA and [$^3$H]-APO binding (Burt *et al.*, 1976; Seeman, Lee, Chau-Wong, Tedesco & Wong, 1976). Incubations contained membrane preparation (1mg protein), [$^3$H]-ADTN (10Ci/m mol) 7.5nM, and drugs in 50 mM tris/HCl pH 7.4 + 100 mM NaCl + 0.1% ascorbic acid. After incubation for 20 min at 37°C, bound radioactivity was separated by filtration and quantified by scintillation counting. Specific binding was defined as that displaced by DA (1μM) or (+)-butaclamol (1μM).

In calf striatal preparations specific [$^3$H]-ADTN binding (approximately 50% of total binding) was saturable and of high affinity. Scatchard analysis of saturation data yielded a $K_D$ of 9nM and $B_{MAX}$ of 180 fmol/mg protein.

In human putamen preparations specific binding was 25–30% of total binding. [$^3$H]-ADTN binding in preparations of putamen from controls ($n = 17$) and schizophrenics ($n = 19$) was (mean ± s.e. mean) 42 ± 6 fmol/mg and 49 ± 4 fmol/mg respectively.

Diagnostic criteria and details of storage data and neuroleptic medication have been described elsewhere (Owen, *et al.*, 1978).

**Table 1** Inhibition of [$^3$H]-ADTN binding by drugs

| | *Drug* | *Ki(nM)* | *Hill coefficient* |
|---|---|---|---|
| Agonists: | Dopamine | 11 | 1.4 |
| | Apomorphine | 5 | 1.4 |
| | ADTN | 7 | 1.1 |
| Antagonists: | (+)-Butaclamol | 45 | 0.61 |
| | (–)-Butaclamol | >10,000 | 0.6 |
| | Fluphenazine | 100 | 0.48 |
| | Haloperidol | 240 | 0.61 |
| | Spiperone | 360 | 0.65 |
| | Chlorpromazine | 530 | 0.48 |

Our results suggest that (a) ADTN, DA and APO bind to similar sites *in vitro* (b) with human brain preparations ADTN has advantages over APO as a ligand, and (c) the increased binding of DA antagonists in striata of schizophrenics (Owen, Cross, Crow, Longden, Poulter & Riley, 1978) is not associated with increased binding of agonists.

A.J.C. is an MRC student.

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## Characterization of calf brain dopamine receptors

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The characteristics of brain dopamine receptors using tritiated agonists and antagonists have been reported from several laboratories. From such binding studies Creese, Burt & Snyder (1975) proposed a two-state convertible receptor whereas, more recently, Nagy, Lee, Seeman & Fibiger (1978) proposed that there were two receptors, i.e. pre- and postsynaptic receptors. In order to test the above hypothesis we have compared the potencies of several dopamine agonists and antagonists to displace [$^3$H]-dopamine, [$^3$H]-apomorphine and [$^3$H]-spiroperidol binding from calf caudate membranes in the presence and absence of saturating concentrations of non-radioactive dopamine agonists and antagonists.

Calf caudate membranes were prepared and incubated for 30 min at 25° in the manner described by Titeler, Weinreich & Seeman (1977) except that the incubation volume was 1.0 ml. To measure 'antagonist' potencies membranes (20 mg wet weight) were incubated with [$^3$H]-spiperone (0.25 nM, 23.6 Ci/mmole) in the presence and absence of dopamine (1 μM). To measure 'agonist' potencies membranes (20 mg wet weight) were incubated with [$^3$H]-dopamine (1.5 nM, 10 and 7 Ci/mmole) or [$^3$H]-apomorphine (1.5 nM, 10.2 Ci/mmole) in the presence and absence of fluspirilene (1 μM). The membranes were recovered by rapid filtration through GF/B filters followed by two 5 ml washes with fresh, ice-cold, incubation medium. Specific binding of [$^3$H]-spiperone was defined as that radioactivity displaced by (+)-butaclamol (1μM) and specific binding of [$^3$H]-dopamine and [$^3$H]-apomorphine as that radioactivity displaced by 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (1μM, ADTN).

The compounds tested fell into three categories in their relative potencies to displace the tritiated ligands used. The neuroleptic drugs, such as spiperone, chlorpromazine and fluspirilene were effective at nanomolar concentrations to displace [$^3$H]-spiperone ($IC_{50}^S$ = 3.5 nM, 78 nM and 95 nM respectively) but were only effective at micromolar concentrations to displace [$^3$H]-dopamine ($IC_{50}^S$ = 11.3 μM, 27 μM and >100 μM) and [$^3$H]-apomorphine ($IC_{50}^S$ = 13.4 μM, 14.4 μM and >100 μM). Conversely dopamine agonists, such as dopamine, apomorphine and ADTN, were effective at nanomolar concentrations, to displace [$^3$H]-dopamine ($IC_{50}^S$ = 1.8 nM, 4.0 nM and 3.7 nM) and [$^3$H]-apomorphine ($IC_{50}^S$ = 2.0 nM, 3.4 nM and 3.0 nM) but were only effective at micromolar concentrations to displace [$^3$H]-spiperone ($IC_{50}^S$ = 47 μM, 1.1 μM and 8.0 μM).

The *in vivo* dopamine agonists, bromocriptine and dihydroergocriptine however, were both more potent to displace the binding of [$^3$H]-spiperone ($IC_{50}^S$ = 240 nM and 68 nM) than either the binding of [$^3$H]-dopamine ($IC_{50}^S$ = 1347 nM and 254 nM) or the binding of [$^3$H]-apomorphine ($IC_{50}^S$ = 1121 nM and 317 nM). Thus there appears to be two classes of dopamine agonist, one having higher affinity for [$^3$H]-dopamine and [$^3$H]-apomorphine binding sites and the other having affinity for [$^3$H]-spiperone binding sites. These results are not consistent with the two-state convertible receptor hypothesis.

The addition to the incubation media of saturating concentrations of dopamine or fluspirilene had little or no effect on total binding in the absence of drugs nor did it significantly affect the potencies of dopamine agonists and antagonists. Therefore, occupancy of the sites to which ($^3$H]-spiperone binds has no effect on the binding of [$^3$H]-dopamine and [$^3$H]-apomorphine. Conversely, occupancy of the sites to which [$^3$H]-dopamine and [$^3$H]-apomorphine bind has no effect on the binding of [$^3$H]-spiperone. These results are also not consistent with the two-state convertible receptor hypothesis.

The above data support the hypothesis that in the calf caudate, there are two populations of dopamine receptors. One of these receptor populations may be labelled by [$^3$H]-dopamine and [$^3$H]-apomorphine and the other labelled by [$^3$H]-spiperone and probably by [$^3$H]-dihydroergocriptine (Titeler *et al.*, 1977). Whether these two populations represent pre- and postsynaptic receptors remains to be resolved.

**References**

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