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The conformation of dopamine at its uptake site; further studies with rigid analogues

Recently various attempts have been made to obtain information about the conformation at the uptake site of inhibitors of the neuronal catecholamine transport system by the use of less flexible analogues (Horn & Snyder, 1972; Miller, Fowble & Patil, 1973; Tuomisto, Tuomisto & Swissman, 1974). In the case of dopamine the three most important conformations to be considered are the *trans* or *anti* form, Fig. 1a, and the two *gauche* forms, Fig. 1b and c. Through the use of *cis*- and *trans*-2-phenylcyclopropylamine and 1- and 2-aminoindane it was suggested that in homogenates of the rat brain corpus striatum and hypothalamus the preferred conformation for a non-catechol inhibitor of dopamine or noradrenaline uptake, such as amphetamine, was *trans* rather than *gauche* (Horn & Snyder, 1972). This conclusion was supported by studies on the inhibition of noradrenaline uptake in the rat vas deferens by *cis*- and *trans*-3-phenyl-2-methylazetidin-3-ol and *cis*- and *trans*-2-phenylcyclopropylamine (Miller & others, 1973). Tuomisto & others (1974) have prepared and tested various *trans*-decalin derivatives which contain the catecholamine moiety and have published evidence that the preferred conformation for the interaction of catecholamines with the uptake site in rat brain is *gauche*. In order to obtain further information on this topic two other rigid analogues of dopamine, 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN), Fig. 1d, and 6,7-dihydroxytetrahydroisoquinoline (norsalsolinol), Fig. 1e, have been examined as inhibitors of [^{3}H]dopamine (^{3}H-DA) uptake in synaptosome rich homogenates of the rat corpus striatum.

Adult male Sprague-Dawley rats were pretreated 18 h previously with reserpine (5 mg kg^{-1}) to inactivate the granular catecholamine storage mechanism. The effect

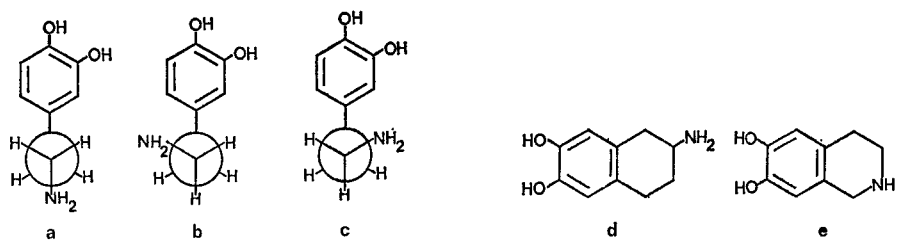

FIG. 1. Newman projections of the *trans* conformation of dopamine (a) and the two gauche conformations (b and c). Structural formulae for 2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) (d) and 6,7-dihydroxytetrahydroisoquinoline (e).

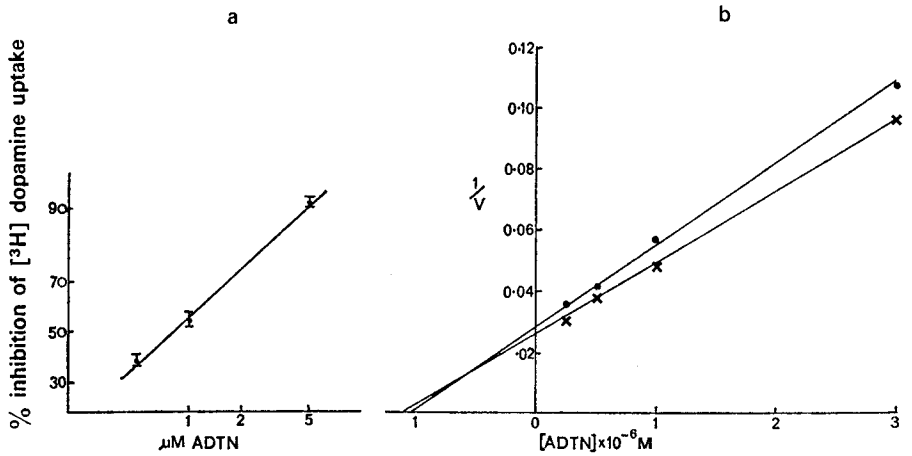

FIG. 2. a. Inhibition of ^{3}H-DA uptake into rat corpus striatum homogenates by various concentrations of ADTN. Results are the means ± s.e. for 5–8 determinations and are expressed as a per cent of control values.
b. A kinetic analysis of the inhibition of ^{3}H-DA uptake (0·1 and 0·2 μM) by various concentrations of ADTN. The method of graphical analysis is that of Dixon. The reciprocal of the tissue-to-medium ratio (T/M), 1/V, is plotted against the concentration of ADTN. Each point is the mean of 3–4 determinations. The point of intersection of the two lines is between the axes showing that the inhibition is competitive with a Ki of 0·7 μM. ● = 0·1 μM. X = 0·2 μM.

of various concentrations of the drugs as inhibitors of 0·1 μM ^{3}H-DA uptake into homogenates of the corpus striatum during a 5 min incubation period was estimated using a previously described method (Horn & Snyder, 1972; Horn, 1973; Horn, Cuello & Miller, 1974). Drugs were tested at three concentrations and the IC50 (the concentration required to produce a 50% inhibition in ^{3}H-DA uptake) was determined by a graphical method using log-probit paper, Fig. 2a. ADTN was the more potent of the two compounds tested having an IC50 of $8{\cdot}5 \times 10^{-7}$M, Fig. 2a, whereas norsalsolinol (IC50 = $1{\cdot}8 \times 10^{-4}$M) was more than 200 times weaker. Employing the method of Dixon (1953), it was shown that the inhibition by ADTN of ^{3}H-DA uptake was of a competitive nature (Fig. 2b) with a Ki of $7{\cdot}0 \times 10^{-7}$M. Using the relationship for competitive inhibition that IC50 = Ki (1+S/Km) (Cheng & Prusoff, 1973) where S = 0·1 μM and Km = 0·4 μM (Snyder & Coyle, 1969) the calculated Ki is $6{\cdot}8 \times 10^{-7}$M, which is in excellent agreement.

Certain tetrahydroisoquinoline derivatives have recently attracted attention as being possible side products in the metabolism of L-dopa in man (Sandler, Bonham-Carter & others, 1973) and for their possible involvement in the pharmacological effects of alcoholic beverages due to their formation from the resultant acetaldehyde (Heikkila, Cohen & Dembiec, 1971). 6,7-Dihydroxy-1,2,3,4-tetrahydroisoquinoline has a spectrum of pharmacological activity suggesting a stimulation of sympathetic nerve function (Hjort, DeBeer & Fassett, 1938; Fassett & Hjort, 1938) and has recently been shown *in vitro* to be taken up and stored in sympathetic nerves of the rat iris by a desipramine sensitive process (Cohen, Mytillineou & Barrett, 1972). It has also been postulated that its mode of action may involve the release of catecholamines from their storage sites (Cohen & others, 1972). From the data reported here and elsewhere (Heikkila & Cohen, 1974) it would appear unlikely that norsalsolinol produces its effects by an inhibition of dopamine uptake.

A molecular model of ADTN, which is a more or less rigid molecule, clearly shows that conformationally it is almost identical with the *trans*-form of dopamine. Norsalsolinol, however, may be regarded as an approximate analogue of the *gauche*

form of dopamine with respect to the distance of the amino group from the catechol function. Thus, it would appear that the *trans* form of dopamine is the preferred conformation for interaction with the uptake site. The *trans* conformation of dopamine has been shown to be the preferred form in the solid state by X-ray crystallography (Bergin & Carlstrom, 1968), in solution by nmr analysis and by theoretical calculations (Bustard & Egan, 1971). In the case of the *trans*-decalin derivatives of the catecholamines (Tuomisto & others, 1974) in contrast to the two compounds used here, a large hydrophobic moiety has been added to the catecholamine molecule in order to reduce flexibility, this may have undesirable conformational effects on the uptake site. A further complication is the fact that the presence of the bulky decalin moiety means that the molecule can only approach the uptake site from one side, rather than having a more or less unrestricted access as in the natural agonist or in simple rigid analogues. ADTN has recently been shown to produce a stimulation of locomotor activity in mice (Woodruff, Elkhawad & Pinder, 1974). This effect was blocked by haloperidol but not by the catecholamine synthesis inhibitor α-methyl-*p*-tyrosine. It was suggested that ADTN brought about this effect by acting directly on dopamine receptors rather than by releasing dopamine. Further evidence that ADTN is a dopamine receptor agonist has been obtained from *in vitro* studies on the dopamine sensitive adenylate cyclase from rat corpus striatum (Miller, Horn & others, 1974). In this assay system ADTN is as potent as dopamine in effecting a stimulation of cyclic AMP production, this result can be taken as further evidence of the validity of using ADTN to investigate the preferred conformation of dopamine. The present results clearly show, however, that as well as having a direct effect on the dopamine receptor ADTN is a potent inhibitor of dopamine uptake and that the preferred conformation for dopamine at the uptake site is *trans*.

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