## Chronic amphetamine administration and central dopamine receptor sensitivity

P. JENNER, C.D. MARSDEN & C.J. PYCOCK

*University Department of Neurology, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5*

Some psychotic disturbances in man may be associated with overstimulation of cerebral dopamine (DA) receptors and the chronic administration of amphetamine to animals may provide a model of these disorders (Snyder, 1973). Such administration potentiates stereotyped behaviour in rodents (Klawans, Margolin, Dana & Crossett, 1975) but it is difficult to explain why continued receptor stimulation should evoke an apparent hypersensitive response. We have therefore used the circling mouse, a model of dopamine receptor imbalance, to assess possible changes in DA receptor sensitivity during chronic (+)-amphetamine sulphate administration and following its withdrawal.

Male 'Swiss S' mice (20–25 g) showing consistent ipsiversive turning to (+)-amphetamine sulphate (4 mg/kg i.p.) and contraversive turning to apomorphine hydrochloride (0.25 mg/kg s.c.) two weeks following a 6-hydroxydopamine (16 μg in 4 μl 0.9% saline) induced lesion of one nigro-striatal pathway (von Voigtlander & Moore, 1973) were utilized. Animals were randomly divided into two groups ($n=30$). One group received increasing amounts of (+)-amphetamine sulphate in the drinking water until an approximate daily dosage of 20 mg/kg was reached after 1 month. This dosage level was continued for a further 2 months and then withdrawn. Control animals received drug-free drinking water during the experiment.

Chronic amphetamine administration was associated with an increase in spontaneous locomotor activity ($P<0.05$ at 2 and 3 months), as judged in Animex activity meters, although little (<1 turn/min) spontaneous circling behaviour was observed. Apomorphine-induced circling (0.01–0.5 mg/kg s.c.) when tested immediately prior to amphetamine administration and then at 1 week, 1, 2 and 3 months following drug administration was progressively reduced in comparison to control animals ($P<0.05$ at 2 and 3 months).

Spontaneous locomotor activity was decreased ($P<0.05$) up to 1 month following drug withdrawal and the circling response to apomorphine (0.01–0.5 mg/kg s.c.) remained depressed in comparison to control animals ($P<0.05$). Two months after drug withdrawal, spontaneous locomotor activity had returned to control values, although apomorphine-induced circling remained depressed.

Forebrain DA was decreased by 43% on the intact side after 2 months of chronic amphetamine administration and by 20% 1 month following drug withdrawal ($P<0.05$). The DA content of the lesioned side (27% of the intact side; $P<0.05$) was not further reduced by amphetamine administration.

Thus, chronic amphetamine administration inhibits apomorphine-induced circling, which might be due to increased stimulation of the intact striatal dopamine receptors. On the basis of this explanation the continued reduction in apomorphine-induced circling after amphetamine withdrawal would presume the development of supersensitivity of innervated striatal receptors. However, an alternative explanation might be that amphetamine administration decreases the response of nucleus accumbens to apomorphine and its withdrawal leaves accumbens receptors sub-sensitive to apomorphine.

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## Transport of dopamine by rat blood platelets

J.L. GORDON & H.J. OLVERMAN

*A.R.C. Institute of Animal Physiology, Babraham, Cambridge, and Department of Pathology, University of Cambridge*

Blood platelets have been proposed as models for studying the uptake mechanisms in central aminergic neurones (Sneddon, 1973). Under conditions where initial rates of uptake are measured (i.e. using low substrate concentrations and short incubation times) the kinetics of platelet 5-hydroxytryptamine (5-HT) uptake and its pharmacological inhibition closely resemble the uptake of 5-HT by synaptosomes (Tuomisto, 1974). Human blood platelets also accumulate dopamine against a concentration gradient by a mechanism which is energy dependent and temperature sensitive (Boullin & O'Brien, 1970; Solomon, Spirt & Abrams, 1970). It is, however,

unclear if this is a specific dopamine uptake mechanism and whether it has the same characteristics as the uptake mechanism in dopaminergic neurones in the corpus striatum.

Since most work on dopaminergic neurones has been carried out in rats (Snyder & Coyle, 1969; Horn, Coyle & Snyder, 1971) we have studied the uptake of dopamine by rat blood platelets to determine whether the platelet could be used as a model for the transport mechanism in dopaminergic neurones.

Uptake of dopamine by rat blood platelets was measured by a method similar to that described by Drummond & Gordon (1976) for uptake of 5-HT. Uptake of dopamine was linear for approximately 5 min at substrate concentrations of 1 μM or 20 μM. A biphasic curve was obtained when uptake was plotted against concentration (1 to 400 μM). This curve could be resolved into a saturable and a non-saturable component. The non-saturable component (which presumably represents diffusion) had a value of 40 pmol $10^8$ cells$^{-1}$ min$^{-1}$ at 100 μM dopamine. Lineweaver-Burk analysis of the active uptake (i.e. after subtraction of the diffusion component) gave values of about 70 μM for the $K_m$ and about 400 pmol $10^8$ cells$^{-1}$ 5 min$^{-1}$ for $V_{max}$.

The active uptake of dopamine was abolished in the presence of an equimolar concentration of 5-HT but the diffusion was unaffected. Inhibition by 5-HT was not increased by preincubation, indicating that 5-HT exerted its inhibitory effect primarily at the platelet plasma membrane and not at the intracellular amine storage granule. Inhibition by low concentrations of 5-HT was decreased by preincubation; this is consistent with the rapid uptake of 5-HT by rat platelets (Gordon & Olverman, 1976). Kinetic analysis showed the inhibition to be competitive, with a $K_i$ value of about 1 μM for 5-HT; this value is similar to the $K_m$ for 5-HT uptake by rat platelets (Gordon & Olverman, 1976).

Chlorimipramine, desmethylimipramine and benztropine, which are respectively selective inhibitors of uptake into serotonergic, noradrenergic and dopaminergic neurones (Horn *et al.*, 1971; Horn, 1976) were tested for their ability to inhibit the uptake of dopamine and 5-HT by platelets. Substrate concentrations used were 0.8 μM for 5-HT and 20 μM for dopamine. Each drug was virtually equipotent against uptake of dopamine and 5-HT, although the absolute potency of the drugs varied greatly; $IC_{50}$ values were about 0.1 μM for chlorimipramine, 3 μM for desmethylimipramine and 60 μM for benzotropine. Trenchard, Turner, Pare & Hills (1975) found that chlorimpramine had little effect on platelet dopamine uptake. However, in their study high substrate concentrations and long incubation times were used, and under these conditions diffusion (which is unaffected by chlorimipramine) would predominate.

We conclude that rat platelets do not have a specific active transport mechanism for dopamine, but that dopamine is transported into rat platelets via the 5-HT uptake mechanism, albeit with a much lower affinity. Consequently platelets cannot be regarded as models for studying the uptake of dopamine into central dopaminergic neurones.

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