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Does brain 3,4-dihydroxyphenylacetic acid reflect dopamine release?

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After sulpiride (75 mg kg^{-1}) administration, a significant increase in dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) occurred in the rat striatum. No attenuation in the sulpiride-induced dopamine and DOPAC increase was seen in the striatum from rats previously treated with benztropine (25 mg kg^{-1}). On the other hand, in the benztropine + sulpiride-treated group, HVA levels were significantly lower compared with those of rats to which only sulpiride had been given. The results presented suggest that under appropriate conditions, as when sulpiride is used, dopamine can be deaminated before its release has occurred.

It has been shown that antipsychotic drugs, by blocking dopamine receptors, enhance the synthesis and release of endogenous dopamine in brain dopaminergic neurons (Andén 1972; Zetterström et al 1984). Measurement of 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in brain after blockade of dopamine receptors is frequently used as an index of dopamine release or utilization (Roth et al 1976; Westerink & Korf 1976; Nicolaou 1980; Zetterström et al 1984). However, the use of dopamine metabolites as an indirect measure of the status of dopaminergic transmission is open to criticism since, depending on the mechanism by which dopamine is released from the presynaptic neuron, the levels of DOPAC and HVA may reflect very different neurochemical processes. At least three conditions must be met for this assumption to be valid; dopamine is released by a mechanism which prevents its metabolism until after release; re-uptake occurs into a cellular compartment where metabolism can occur; and dopamine is deaminated by monoamine oxidase (MAO) only after release into the synapse.

The release of dopamine by exocytosis would satisfy all three criteria since any vesicle-bound dopamine would be protected from MAO. Although it is generally believed that dopamine release may occur through an exocytotic process (Holz 1975; Mulder & Snyder 1976) there is also evidence to suggest it may be released from a cytoplasmic pool or in a non-exocytotic manner through a membrane transport system, especially when its synthesis is increased above the storage capacity of synaptic vesicles (Raiteri et al 1979; Niddam et al 1985). Therefore whether the DOPAC level reflects dopamine release or simply provides an index of dopamine synthesis is not resolved.

In the present experiments the question of whether DOPAC in the striatum reflects dopamine release or its utilization has been addressed by studying the neurochemical responses of rats treated with sulpiride. To see if dopamine is deaminated only after release into the synapse, experiments have also been performed in rats previously treated with the dopamine uptake blocker benztropine, which is also a blocker of the carrier-mediated release of dopamine (Raiteri et al 1979).

Materials and methods

Male Wistar rats, 250–300 g, housed in group cages with free access to food and water, were treated i.p. with either 0·9% NaCl (saline), 75 mg kg^{-1} sulpiride or 25 mg kg^{-1} benztropine + 75 mg kg^{-1} sulpiride (benztropine was given 90 min before the sulpiride). Forty or 80 min after sulpiride treatment the rats were decapitated, brains removed and placed on an ice-cold porcelain plate and the corpus striatum dissected, weighed and stored frozen in 0·1 M perchloric acid until analysed, usually within one week. The assay of dopamine, DOPAC and HVA was by high pressure liquid chromatography with electrochemical detection (HPLC-ECD). After gentle thawing, tissues were homogenized in 2 mL 0·1 M perchloric acid with a Duall-Kontes homogenizer. The homogenates were centrifuged (6000*g*, 20 min, 0 °C) and the supernatants decanted. For dopamine and DOPAC, aliquots of 1 mL of supernatant were placed in 5 mL conical-based glass vials with 50 mg alumina and pH adjusted to 8·4. Mechanical shaking for 10 min was followed by centrifugation and the supernatants were then discarded. Elution of alumina from Millipore (MF 1) microfilters was effected with 300 μL 0·1 M perchloric acid; 200 μL of the eluate was injected into a high pressure liquid chromatograph (BAS model 304). The assay for HVA was on another aliquot of the supernatant injected directly into the HPLC column. A 5 μM ODS column of 25 cm length was used. The mobile phase was a degassed solution of monochloracetic acid (0·15 M), sodium octylsulphate (0·3 mM) and EDTA (2 mM), pH 3·0, pumped at a rate of 1·8 mL min^{-1}. A carbon paste electrode was used, and the detector potential was +0·65 V. Dihydroxybenzylamine was used as an internal standard and dopamine, DOPAC and HVA were injected in different concentrations. Peak height increased linearly with the concentration of dopamine, DOPAC or HVA. The interassay coefficient of variation was less than 5%. Under our conditions the lower limits of detection for dopamine, DOPAC and HVA were 100, 40 and 300 pg mL^{-1}, respectively.

Benztropine mesylate was kindly donated by Prof. F. Peres-Gomes (Merck, Sharp & Dohme, Lisbon, Portugal) and racemic sulpiride obtained from Sigma Chemical Company (St Louis, MO, USA). Sulpiride was dissolved in saline containing 1% acetic acid.

Statistical determination of differences of means was performed by the two-tailed Student's *t*-test.

Results

Control rats were non-treated animals matched with the saline-treated animals. Saline injections (1 mL at 40 and 80 min) did not induce a significant change in the dopamine, DOPAC or HVA levels (Table 1). Forty and 80 min after treatment with sulpiride a significant increase in dopamine occurred in the striatum. Previous treatment with benztropine did not significantly affect the sulpiride-induced increase in dopamine. The DOPAC content in rat striatum was also significantly increased after sulpiride at 40 and 80 min. No attenuation in the sulpiride-induced increase in DOPAC was seen in the striatum from rats previously treated with benztropine. HVA levels in rats treated with benztropine + sulpiride were significantly higher compared with controls, but significantly lower compared with values from rats given only sulpiride.

Table 1. Levels of dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) (in $\mu g\ g^{-1}$) in the rat striatum of control, sulpiride and benztropine + sulpiride-treated animals. (A) rats were killed 40 min and (B) 80 min after sulpiride administration. Values are means ± s.e.m. (n = 4).

	DA	DOPAC	HVA
A			
Control	7·8 ± 0·3	0·9 ± 0·05	0·6 ± 0·05
Sulpiride	15·2 ± 1·4†	1·9 ± 0·2†	1·7 ± 0·09†
Benztropine + sulpiride	14·9 ± 1·7†	1·8 ± 0·2†	1·3 ± 0·1*†
B			
Control	8·1 ± 0·5	0·8 ± 0·06	0·7 ± 0·06
Sulpiride	15·4 ± 0·8†	2·2 ± 0·2†	1·9 ± 0·1†
Benztropine + sulpiride	14·5 ± 1·3†	1·9 ± 0·1†	1·4 ± 0·1*†

* Statistically different from sulpiride values ($P < 0·05$).
† Statistically different from control values ($P < 0·001$).

Discussion

The present study was undertaken to address the question of whether, in rats treated with the neuroleptic drug sulpiride, dopamine is deaminated by MAO before it is released or after release has occurred. Although sulpiride has been considered an atypical antipsychotic drug, data from Zetterström et al (1984) show that sulpiride, haloperidol and *cis*-flupenthixol increase the release of dopamine as well as the formation of DOPAC and HVA in the rat striatum, with no obvious difference between the drugs. In the present study, we chose sulpiride on the basis that it is a more selective antagonist at dopamine D_2 receptors than haloperidol or *cis*-flupenthixol (Seeman 1980). We reasoned, as have others (Lloyd & Bartholini 1975) that neuroleptic drugs enhance the synthesis and release of endogenous dopamine, but also increase DOPAC and HVA levels in the striatum (Westerink & Korf 1976; Roth et al 1976: Nicolaou 1980; Zetterström et al 1984). If dopamine is first released into the synapse so that deamination occurs after re-uptake, a dopamine uptake blocker such as benztropine, which is devoid of any MAO inhibiting properties, should prevent, or at least attenuate, the increase in DOPAC levels caused by sulpiride (Coyle & Snyder 1969). Though benztropine is not as selective as nomifensine or buproprion for dopaminergic neurons, it is an adequate tool in these experiments because it has one of the lowest inhibitor constants for dopamine uptake (Richelson & Pfenning 1984) and because the noradrenergic innervation of the rat striatum is sparse.

Under electrical stimulation of the nigrostriatal pathway or in basal conditions, benztropine reduced the amount of DOPAC in the striatum by 50% and 25%, respectively, thereby suggesting that deamination of dopamine occurred mainly after its release (Roth et al 1976). However, in the present situation the mechanism of dopamine release seems to be through an exocytotic process. More recent data on the mechanisms of dopamine release suggest that the amine can also be transferred to the extracellular space by the membrane transport system, a process which can be inhibited by blockers of the dopamine carrier (Raiteri et al 1979). The finding that pretreatment with benztropine did not significantly reduce DOPAC levels in sulpiride-treated rats raises a number of points. First, the benztropine failed to reduce DOPAC levels in the sulpiride-treated rats because dopamine synthesis had been increased above the storage capacity of synaptic vesicles, thus leading to increased concentrations of free dopamine in the cytoplasm that are unprotected from MAO. Secondly, dopamine may have been deaminated before release occurred because release of sulpiride-induced recently synthesized dopamine was through a non-exocytotic mechanism. Thirdly, the results concerning HVA in benztropine + sulpiride-treated rats are in agreement with the mechanism of dopamine release described by Raiteri et al (1979), as it was found that benztropine, possibly through inhibition of the carrier-mediated release of dopamine, significantly decreased the formation of HVA. This finding also provides evidence that the sulpiride-induced pool of recently synthesized dopamine was not stored entirely in synaptic vesicles, at least some being free in the cytoplasm.

In conclusion, the results suggest that under the appropriate conditions, when sulpiride is used, dopamine can be deaminated before its release has occurred; they also support the view of the relative unimportance of intraneuronal MAO in the metabolism of the released dopamine in sulpiride-treated animals. Although this conclusion cannot be generalized to other antipsychotic drugs, it would be interesting to extend

this kind of analysis to other drugs known to increase both dopamine and DOPAC brain levels.

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Erythrocyte binding of cephalosporins

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A rapid sample preparation and HPLC technique was used to study the erythrocyte binding properties of six cephalosporins at therapeutic concentrations. The negligible red blood cell partition coefficients indicated that only small amounts of the cephalosporins were taken up by the blood cells at all drug concentrations. Thus, plasma concentrations were about twice as high as the respective blood concentrations. Blood/plasma ratios solely depended on the haematocrit and were independent of the extent of protein binding.

All drugs that are distributed to their target sites in the body via the systemic circulation come into contact with blood cells. In contrast to extensive studies of the interaction of numerous drugs with plasma protein, very little information is available about drug-blood cell interaction. The significance of drug binding to plasma proteins is widely recognized and its investigation is a standard procedure for the characterization of new drugs. However, drug binding to blood cells has not been given the same attention, and there is no reason to assume it may be of less significance, since erythrocytes account for approximately half of the blood volume. Furthermore, drugs bound to erythrocytes may provide a novel drug delivery system (Lewis & Alpar 1984). In previous studies (Derendorf & Garrett 1983; Derendorf et al 1984; Garrett & Hunt 1974) it has been shown that drug binding of lipophilic drugs to erythrocytes can be described by a red blood cell partition coefficient:

$$D = \frac{C_{RBC}}{C_{pw}} = \frac{A_{tot} - C_{pw} \cdot V_{pw}}{C_{pw}(V_B - V_{pw})} \quad (1)$$

where D is the red blood cell partition coefficient, C_{RBC} is the concentration of the drug in the erythrocytes, C_{pw} is the concentration in plasma water (protein-free plasma), A_{tot} is the total amount of drug added to a red blood cell suspension, V_B is the volume of the red blood cell suspension and V_{pw} is the volume of plasma water. Drug binding to glass can be compensated for by preparing the calibration curve for C_{pw} in plasma water, i.e. under the same conditions as in the absence of erythrocytes. The volume V_{pw} can be calculated from the total volume V_B and the haematocrit H.

It can be assumed that only free drug in blood is able to diffuse into the blood cells. The results obtained can be confirmed with the separate evaluation of binding when drug is added to whole blood. The relation of drug concentration in blood (C_B) and plasma (C_p) after equilibration between blood cells, plasma proteins and plasma water is reached, can be described by equation 2:

$$\frac{C_B}{C_p} = D \cdot H \cdot (1 - f_b) + 1 - H \quad (2)$$

where f_b is the fraction bound to plasma protein in the concentration studied. This fraction can be determined by equilibrium dialysis, ultrafiltration or ultracentrifugation. A comparison of erythrocyte binding in the presence (eqn 2) and absence (eqn 1) of plasma protein after equilibrium is achieved will confirm the model if agreement is shown (Derendorf & Garrett 1983).

The aim of the present study was to investigate systematically the binding of a series of cephalosporins to erythrocytes.