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Possible anomalous behaviour of apomorphine in relation to other dopamine receptor agonists

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Among the large number of dopamine (DA) receptor agonists available, are compounds derived from apomorphine, 2-aminotetralin (2-aminotetrahydronaphthalene), DA itself and various ergoline and octahydrobenzoquinoline structures (Seeman 1980). Besides covering a wide range of activities, these compounds show large variations in physico-chemical properties and in their susceptibility to metabolic degradation. This makes the comparative assessment of the central dopaminergic potency of these DA agonists in-vivo a difficult problem which can only be solved by measuring the concentrations in the central nervous system.

We have recently reported on the rat brain concentrations of some aminotetralin derivatives, i.e. 5,6- and 6,7-dihydroxy-2-aminotetralin (5,6- and 6,7-ADTN) (Westerink et al 1980) and their *N,N*-dipropyl analogues (DiPr-5,6- and -6,7-ADTN) (Feenstra et al 1983a). It was shown that in equimolar doses the 5,6-derivatives produce higher brain concentrations than their 6,7-isomers and that this was largely due to a difference in their susceptibility to undergo *O*-methylation by catechol-*O*-methyl transferase (COMT) (Rollema et al 1980). The ratio of in-vivo activities of the 5,6- and the 6,7-isomers (measured as the potency to decrease the concentrations of the DA metabolites dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum) when corrected for the differences in brain concentrations, was for both the primary amines and the dipropylamino derivatives the same as that reported for their in-vitro dopaminergic activity (inhibition of [^{3}H-]apomorphine and [^{3}H]spiperone binding; activation of DA-sensitive adenylate cyclase (Seeman 1980; Cannon et al 1978)). Here we report the extension of this approach to include apomorphine, which contains in its structure the 5,6- rather than the 6,7-dihydroxy-aminotetralin nucleus.

NN-Dipropyl-2-amino-5,6-dihydroxytetralin HCl and *NN*-dipropyl-2-amino-6,7-dihydroxytetralin HBr were synthesized in this laboratory. Apomorphine HCl was obtained from Brocacef.

Methods

Female Wistar rats (150–200 g) (CDL, Groningen) were injected intraperitoneally (i.p. 2 ml kg^{-1}), with solutions of the drugs in 0·9% NaCl with 0·1% sodium metabisulphite. Rats were killed after 45 min and the striatum was dissected and used for the determination of apomorphine (Westerink & Horn 1979), DiPr-5,6-ADTN or DiPr-6,7-ADTN (Feenstra et al 1982, 1983a).

Results

Fig. 1 shows that apomorphine reaches higher brain concentrations at 45 min after injection than does DiPr-5,6-ADTN. For the striatal levels the ratio apomorphine/DiPr-5,6-ADTN is 3–6 after doses of 1 μmol kg^{-1} or more, while after lower doses this ratio is even higher. This ratio is 24 for DiPr-5,6-ADTN and DiPr-6,7-ADTN, after doses of 16 μmol kg^{-1}. The difference between the brain concentrations of apomorphine and the aminotetralins may be explained by the higher lipophilicity of apomorphine, as can be seen from Table 1. The ratio of the in-vivo potencies after peripheral administration of apomorphine and DiPr-5,6-ADTN is 0·04 for the effect on striatal HVA levels and lower for other measures of dopaminergic activity such as the induction of stereotypy in rats or the decrease in L-dopa accumulation in reserpinized rats (See Feenstra et al 1980). Correction for the difference in striatal concentrations results in a ratio of about 0·01 (Table 1). However, the ratio of the in-vitro potencies is

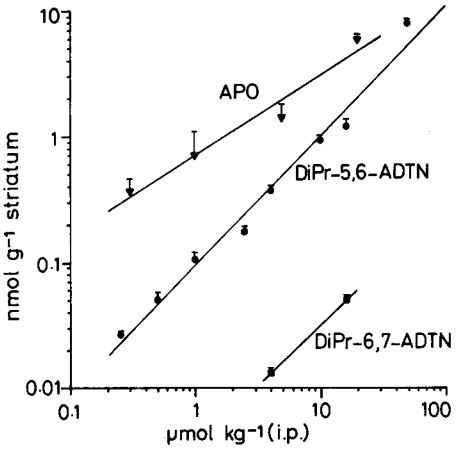

FIG. 1. Rat striatal concentrations in nmol g^{-1} (± s.e.m. n = 3–6) of apomorphine, DiPr-5,6-ADTN and DiPr-6,7-ADTN 45 min after i.p. injection.

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Table 1. Comparison of in-vivo and in-vitro potencies of DA agonists.

Compound	HVA[a]	Ratio brain concentrations	Corrected in vivo activity[b]	In-vitro activities c	d	e	Adenylate cyclase[f]	p (pH 7·4)[g]
(1) Apomorphine	1·35		6	50	650	1·3–3·5	(1·5)[h]	52·5
Ratio 1/2	23	3—6	100	3	1·3	0·1–0·3	(0·5)[h]	8·8
(2) DiPr-5,6-ADTN	0·06		0·06	16	490	11	3·3	6·0
Ratio 3/2	36	0·04—0·06	2	4	3·9	2	2	0·8
(3) DiPr-6,7-ADTN	2·14		0·11	63–76	1900	17	6·3	4·6

(a) Dose in μmol kg^{-1} to decrease rat striatal HVA to 70% (Feenstra et al 1980).
(b) Dose[a] corrected for the ratio of brain concentrations.
(c) IC50 in nmol litre^{-1} for the inhibition of [^{3}H]haloperidol binding to rat caudate nucleus (data from Leysen et al cited in Seeman 1980).
(d) IC50 in nmol litre^{-1} for the inhibition of [^{3}H]spiperone binding to calf caudate nucleus (data from Seeman et al cited in Seeman 1980).
(e) IC50 in nmol litre^{-1} for the inhibition of [^{3}H]apomorphine binding to calf caudate (data from Seeman et al cited in Seeman 1980).
(f) EC50 in μmol litre^{-1} for the stimulation of the DA-sensitive adenylate cyclase in rat striatum (Cannon et al 1978).
(g) Partition ratio between n-octanol and phosphate buffer pH 7.4 (Feenstra et al in preparation).
(h) Apomorphine is a mixed agonist/antagonist in this model. The maximal stimulation in only about 30% that of dopamine (see Seeman 1980).

0·3–10. Thus, when brain concentrations are taken into account, apomorphine is 100 times less potent than DiPr-5,6-ADTN, while on the basis of in-vitro data it should be as potent as the aminotetralin or even more so.

Discussion

Several explanations for this anomalous behaviour of apomorphine may be suggested, e.g.

1. The in-vitro receptor binding data only reflect the affinity of a drug for the receptor but not its pharmacological activity. Apomorphine has previously been shown to be a mixed agonist/antagonist in the DA sensitive adenylate cyclase model (see Seeman 1980). No such mixed activity has been reported for the aminotetralins, which behave like full agonists in the adenylate cyclase system (Cannon et al 1978). The mixed activity of apomorphine may result in a rather weak agonist effect in-vivo.

2. The behaviour of apomorphine may be looked upon in the light of the concept of multiple receptors for DA (Kebabian & Calne 1979; Seeman 1980; Horn et al 1981). Apomorphine might affect a receptor in addition to those that are activated by the aminotetralins and in this way limit its own activity. In this respect the discovery that D1 and D2 receptors have an opposing effect on the striatal cyclic AMP efflux is very interesting (Stoof & Kebabian 1981).

3. Apomorphine was shown to undergo a high non-stereospecific binding to brain membranes in-vitro (Leysen & Gommeren 1981). This has raised problems in the use of [^{3}H]apomorphine as a ligand for DA receptors and has excluded the use of apomorphine as an in-vivo DA receptor ligand like n-propylnorapomorphine (Köhler et al 1981) and DiPr-5,6-ADTN (Feenstra et al 1983b). It may be that this aspecific binding limits the amount of apomorphine that is available to interact with DA receptors.

Recently, Seeman (1980) compared the in-vitro and in-vivo activities of DA receptor agonists. He concluded that the ability to inhibit [^{3}H]spiperone binding correlates better with in-vivo potency than does the ability to inhibit [^{3}H]apomorphine binding. However, a correction of the in-vivo potencies for differences in lipid solubility with calculated factors improved the correlation of the inhibition of APO binding with the in-vivo inhibition of striatal L-dopa accumulation. It was not made clear why this would only be applicable to a correlation with apomorphine labelled (D_3) sites and not to a correlation with spiperone labelled (D_2) sites, this correlation is made worse by the correction. Seeman admits that it would be better to measure the actual brain concentrations of the DA receptor agonists and our results indicate why: calculated log p values do not reflect the lipophilicity at pH 7·4. DiPr-5,6- and -6,7-ADTN have the same calculated log p which is higher than that for apomorphine. However at physiological pH the measured log p for apomorphine is one order of magnitude larger than for the aminotetralins. Furthermore there is no relation whatsoever between calculated log p values and brain concentrations for the 3 compounds that we have studied.

Another interesting point is the possible dissociation of activities of apomorphine and DiPr-5,6-ADTN. Both contain in their structures the α rotamer of DA (Cannon 1975), whereas DiPr-6,7-ADTN contains the β rotamer of DA. Suggestions that these two conformations activate two different DA receptors (Cannon 1975; Dandiya et al 1975; Cools 1981) are not in agreement with our results where the two aminotetralins are fully comparable and apomorphine behaves anomalously. In conclusion, we have shown that the higher in-vivo potency of DiPr-5,6-ADTN compared with apomorphine is not due to higher brain concentrations of the former compound. As it is also not caused by a higher in-vitro potency we therefore conclude that the in-vivo behaviour of apomorphine deviates from that of certain other DA receptor agonists.

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Chlorpromazine enhances haemolysis induced by haemin

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Chlorpromazine, a cationic phenothiazine drug generally used as an antipsychotic tranquillizer, binds to cell membranes and membrane lipids (Seeman 1972; Römer & Bickel 1979; Farah & Kellaway 1981). It can cause shape change in red blood cells (Deuticke 1968; Kanaho et al 1981). Between 10^{-4} and 10^{-5} M chlorpromazine protects human erythrocytes from hypotonic lysis. However at 10^{-3}M or higher, this drug can disrupt membranes (Seeman 1972; Seeman & Kwant 1969). Haemin or ferriprotoporphyrin IX has been reported to cause lysis of erythrocytes and blood stream forms of *Trypanosoma brucei* (Meshnick et al 1977; Chou & Fitch 1980). Here we report that haemin-induced lysis of human, mouse and rabbit erythrocytes is enhanced by chlorpromazine in the micromolar range.

Method

Blood was collected from healthy human donors, and mice and rabbits fed nutritionally sufficient diets. Blood was centrifuged, the buffy coat layer removed and the red blood cells were washed three times with 310 mOsm phosphate-buffer-saline (PBS). The erythrocytes were freed of reticulocytes by passage through a cellulose CF-11 column (Richards & Williams 1973). A 10% red blood cell suspension in PBS buffer pH 7·4, 4 °C, was the stock for the time-course and fixed time haemolysis experiments. Lysis of 0·25% red cell suspension was monitored at 540 nm after sedimentation of the cells and cell debris. Complete haemolysis was measured by the absorbance of haemoglobin at 540 nm after sedimentation of cell debris following freeze-thaw lysis of red blood cells. Mouse red blood cell membranes were prepared and extracted for lipids by the chloroform–methanol method (Bligh & Dyer 1959). Liposomes were made from the extracted lipid suspension (1 mg ml^{-1}) by sonication with Branson sonicator at 50 °C for 15 min.

Haemin was recrystallized from that sold by Sigma Chemical Co. (St Louis, Mo., U.S.A.). Chlorpromazine and phospholipids were also from Sigma, but perazine was donated by Yoshitomi Pharmaceutical Co. (Osaka, Japan). Difference spectroscopy was performed on a Shimadzu UV 240 spectrophotometer with a matched pair of quartz tandem cells (Hellma (England) Ltd.) in a thermostated cell holder. The procedure for two-component difference spectroscopy was as described previously (Jearnpipatkul & Panijpan 1980). For the three-component (haemin-chlorpromazine-lipid) difference spectrum, one chamber of the control cuvette had haemin and lipid, the other chlorpromazine, whereas the sample cuvette had the three components in one chamber and the other chamber contained buffer; all components must have the same concentration X pathlength in both cuvettes.

Results

Fig. 1 shows the lysis after 1 h incubation of human, mouse and rabbit red blood cells by haemin in the absence and presence of 5×10^{-6} M chlorpromazine. Fig. 2 shows the 1 h haemolysis of mouse red blood cells at 7 μM haemin and different concentrations of chlorpromazine. Chlorpromazine enhanced haemolysis due to haemin. That haemolysis induced by haemin was

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