The receptor mediating the apomorphine vasodilatation in the hindleg of the dog

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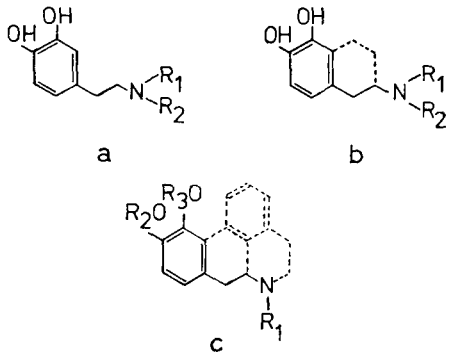
In the innervated femoral bed of the anaesthetized dog, local injection of the dopaminergic agent apomorphine causes a marked increase in femoral flow without a change in blood pressure. This vasodilatation is selectively blocked by haloperidol, suggesting that a "dopamine receptor" is involved. It was postulated that this dopamine receptor is located on sympathetic nerve endings and mediates an inhibition of the release of noradrenaline (Buylaert, Willems & Bogaert, 1977; Laubie, Schmitt & Falq, 1977). In view of the current interest in dopamine receptors (Iversen, 1975), we studied various dopamine agonists and antagonists in the femoral bed of the dog.

The experimental preparation has been described by Buylaert & others (1977). Mean blood flow was monitored electromagnetically in both femoral arteries of dogs (15–25 kg) anaesthetized with pentobarbitone (30 mg kg^{-1}). The systemic blood pressure was measured in a brachial artery. Drugs were administered into the femoral artery via a catheter in the arteria profunda femoris; bolus injections of 0·2 ml were used. The changes in femoral blood flow were calculated as percentage of the pre-existing flow. In the doses studied, the drugs had no effect on systemic blood pressure or heart rate. The agonists tested are listed in Table 1. The antagonists used were: haloperidol and pimozide (Janssen Pharmaceutica), thioridazine HCl (Sandoz), (+)- and (−)-butaclamol HCl (Ayerst laboratories) and *cis*- and *trans*-flupenthixol $(HCl)_2$ (Lundbeck). Solutions of haloperidol and pimozide were prepared as previously described (Willems, 1973). Butaclamol was suspended in distilled water with a few drops of Tween 20. Appropriate dilutions of the other drugs were made in saline on the day of the experiment and stored in ice.

Like haloperidol (Buylaert & others, 1977), the neuroleptic drugs (+)-butaclamol, *cis*-flupenthixol, pimozide and thioridazine, in doses that had no lasting effect on femoral flow, antagonized the vasodilatory effect of apomorphine but they were less potent than haloperidol. Their relative potencies were estimated by studying the dose dependent inhibition of the response to $2·5 \times 10^{-9}$ mol of apomorphine, a dose producing a submaximal effect (Buylaert & others, 1977). The results are shown in Fig. 1.

The isomers (−)-butaclamol and *trans*-flupenthixol which have no or only negligible neuroleptic activity (Møller-Nielsen, Pedersen & others, 1973; Voith & Cummings, 1976) did not antagonize the apomorphine vasodilatation: each substance was tested in 5 animals and the responses to apomorphine were 97 ± 15% of the controls after 14×10^{-8} mol of (−)-butaclamol, and 105 ± 5% after $5·4 \times 10^{-7}$ mol of *trans*-flupenthixol.

Table 1. *Structural formulas of the dopamine analogues studied in the innervated hindleg of the dog.*



	Name	Basic Structure	R_1	R_2	R_3
I	Dopamine	a	H	H	—
II	Epinine	a	H	CH_3	—
III	*NN*-Dipropyldopamine	a	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	—
IV	*NN*-Di Me-5,6-diOHATN†	b	CH_3	CH_3	—
V	*NN*-Di Pr-5,6-diOHATN	b	$CH_2CH_2CH_3$	$CH_2CH_2CH_3$	—
VI	Apomorphine	c	CH_3	H	H
VII	*N*-n-Propylnorapomorphine	c	$CH_2CH_2CH_3$	H	H
VIII	10,11-Dimethoxyapomorphine	c	CH_3	CH_3	CH_3

* The dotted line in the basic structure represents the segment that is different from the dopamine skeleton.
† ATN = 2-amino-1,2,3,4-tetrahydronaphthalene.

The *N*-propylderivatives of dopamine, of 5,6-dihydroxy-2-amino-1,2,3,4-tetrahydronaphthalene (*NN*-diPr-5,6-diOHATN) and of apomorphine are potent agonists for central dopamine receptors (Cannon, 1975). Like apomorphine, they produced an increase in femoral blood flow and, as shown in Fig. 2, *NN*-diPr-5,6-diOHATN and *N*-propylnorapomorphine are more potent than apomorphine. The increases in blood flow produced by doses of the *N*-propylderivatives equipotent with $2·5 \times 10^{-9}$ mol apomorphine were completely blocked by haloperidol ($5·4 \times 10^{-8}$ mol; 3 experiments for each agonist) and (+)-butaclamol (14×10^{-8} mol; 2 experiments for each agonist), but not influenced by (−)-butaclamol (14×10^{-8} mol; 2 experiments for each agonist).

The *NN*-dimethyl derivative of 5,6-dihydroxy-2-amino-1,2,3,4-tetrahydronaphthalene (*NN*-diMe-5,6-diOHATN) has been shown to stimulate central

* Correspondence.

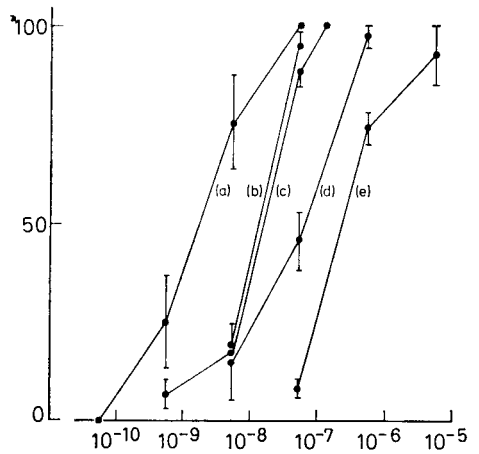


FIG. 1. Inhibition by increasing doses of the intra-arterially administered neuroleptics haloperidol (a), *cis*-flupenthixol (b), (+)-butaclamol (c), pimozide (d) and thioridazine (e) of the increments in femoral blood flow produced by local injection of $2{\cdot}5 \times 10^{-9}$ mol apomorphine. Cumulative doses of each neuroleptic were given at intervals of 7 min to 5 animals. The response to apomorphine before the administration of antagonists was taken as 100%, and the degree of inhibition was evaluated 5 min after injection of the antagonist. Vertical bars indicate the s.e.m. Ordinate: % inhibition of the response to $2{\cdot}5 \times 10^{-9}$ mol apomorphine. Abscissa: mol.

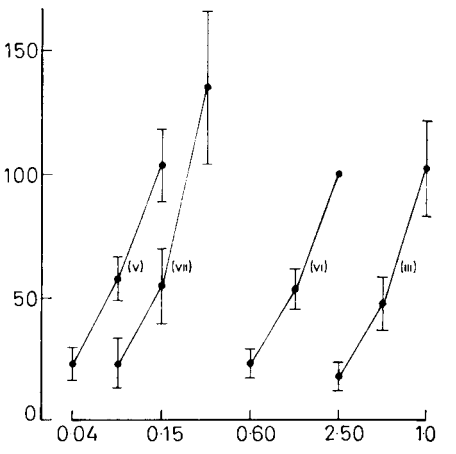


FIG. 2. Increases in femoral blood flow (in % of the increase by $2{\cdot}5 \times 10^{-9}$ mol apomorphine) produced by intra-arterial injection of increasing doses of apomorphine (VI), *NN*diPr-5,6-diOHATN (V), *N*-n-propylnorapomorphine (VII) and *NN*-dipropyldopamine (III). Each agonist was compared with apomorphine in 5 animals and the apomorphine curve represents the mean values obtained in all 15 experiments. Vertical bars indicate the s.e.m. Ordinate: % of the response to $2{\cdot}5 \times 10^{-9}$ mol apomorphine. Abscissa: $\times 10^{-9}$ mol.

(Cannon, 1975) and peripheral (Long, Heintz & others, 1975) dopamine receptors. When administered in a dose of $0{\cdot}6 \times 10^{-9}$ mol or higher (n = 6), this compound produced a dose dependent decrease in femoral blood flow. In 3 of these experiments a vasodilatation followed the vasoconstriction and this vasodilatation was completely blocked by haloperidol ($5{\cdot}4 \times 10^{-8}$ mol). 10,11-Dimethoxy-apomorphine is inactive on central dopamine receptors (Cannon, 1975) and, in doses up to 160×10^{-9} mol, had no effect on femoral blood flow in 5 animals.

Dopamine and epinine, a dopamine agonist in the renal vasculature (Goldberg, 1972), produced a dose dependent decrease in femoral blood flow occurring with doses of respectively 10 and $2{\cdot}5 \times 10^{-9}$ mol or higher. The vasoconstriction produced by dopamine was followed occasionally by a vasodilatation, which was not antagonized by haloperidol (54×10^{-8} mol). Propranolol (5×10^{-6} mol kg^{-1}, i.v.) likewise had no influence on this dilatation, an observation also made by Higgins, Millard & others (1973).

The observation that the neuroleptics studied antagonize the apomorphine vasodilatation, supports the idea that a 'dopamine receptor' is involved. Moreover, several substances known to stimulate dopamine receptors, mimick the effect of apomorphine on the hindleg.

That with dopamine and epinine this haloperidol sensitive dilatation is not seen, is perhaps due to their α-mediated vasoconstrictor effect. Such a direct action on vascular smooth muscle of agonists or antagonists interferes with their apomorphine-like effect and limits the usefulness of the preparation.

The renal vasculature has also been proposed as a model for the study of dopamine receptors (Crumly, Pinder & others, 1976), but a selective antagonism of the dopamine effect by haloperidol is difficult to demonstrate in that preparation (Yeh, McNay & Goldberg, 1969). We would propose the innervated femoral vasculature of the dog as a model for the study of dopamine agonists and antagonists. It allows demonstration of a clearcut and lasting antagonism of apomorphine by agents that are now generally accepted as acting on dopamine receptors.

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Enhancement of 5-hydroxytryptamine synthesis in brain by monoamine-depleting drugs

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The turnover of monoamines such as dopamine in the brain is regulated by feedback mechanisms. Drugs which activate pre- and/or postsynaptic dopamine receptors (e.g. apomorphine) decrease the cerebral dopamine turnover, whereas compounds which inhibit the dopamine receptors or reduce the number of chemical stimuli reaching them (e.g. neuroleptics, monoamine depletors like reserpine) enhance the turnover of dopamine (Andén, Roos & Werdinius, 1964; Carlsson & Lindqvist, 1963; Corrodi, Fuxe & Hökfelt, 1967; Clement-Cormier, Kebabian & others, 1974; Carlsson, 1975). For the estimation of the rate of synthesis of dopamine or 5-hydroxytryptamine (5-HT), inhibitors of cerebral decarboxylase such as NSD 1015 (3-hydroxybenzylhydrazine HCl) and benserazid (1-DL-seryl-2(2,3,4-trihydroxybenzyl)hydrazine HCl) have been used (Carlsson, Davis & others, 1972). As a result of decarboxylase inhibition, the concentration of the endogenous precursors of these amines, i.e. 3,4-dihydroxyphenylalanine (dopa) and 5-hydroxytryptophan (5-HTP) respectively, increases and in situations where dopamine or 5-HT synthesis is accelerated, the increase in precursors is enhanced. In fact, reserpine (as do neuroleptic drugs) causes an enhancement of the NSD 1015-induced rise of endogenous cerebral dopa (Carlsson, 1975) together with an increase of homovanillic acid (Andén & others, 1964), a major metabolite of dopamine. These long-lasting (over 24 h) changes are thought to be due to an acceleration of dopamine turnover. The action of reserpine on 5-hydroxytryptaminergic neurons seems to differ from that on dopaminergic neurons. Thus, while the drug induces a long-lasting increase of 5-hydroxyindoleacetic acid (5-HIAA) (Roos, Andén & Werdinius, 1964; Tozer, Neff & Brodie, 1966), the major metabolite of 5-HT in the brain, no marked enhancement of the rise of 5-HTP induced by NSD 1015 has been observed 7–8 and 5 h after administration of reserpine to mice and rats respectively (Carlsson & Lindqvist, 1972; Modigh, 1974).

The present paper deals with the effect of reserpine and of a benzoquinolizine derivative with a short-lasting, reserpine-like action (2-hydroxy-2-ethyl-3-isobutyl-9,10-dimethoxy-1,2,3,4,6,7-hexahydro-11bH-benzo[a]quinolizine HCl, Ro 4-1284; Pletscher & Da Prada, 1966) on 5-HT synthesis in rat brain.

Male albino rats (Füllinsdorf breed of Wistar origin, specified pathogen-free), 100 g, fasted for 16 h, were injected with 5 mg kg^{-1} reserpine or Ro 4-1284 (i.p.) and decapitated at various times thereafter. Some of the animals received NSD 1015 (100 mg kg^{-1}, i.p., calculated as base) 30 min before death. The rectal temperature of the rats was controlled by insertion of a flexible thermistor. Hypothermia was prevented by keeping the animals in boxes at 28–32°. Determinations of 5-HTP, 5-HT and 5-HIAA in whole brains without cerebellum were carried out using spectrophotofluorimetric methods (Giacalone & Valzelli, 1966; Lindqvist, 1971; Shellenberger & Gordon, 1971; Atack & Lindqvist, 1973). Estimations of 5-HTP were made in a pool of 4 brains, those of 5-HT and 5-HIAA on single brains.

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