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Influence of deaminated metabolites on the relaxing effect of dopamine on dog saphenous vein*

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The relaxing effect of dopamine was studied on the dog isolated saphenous vein contracted by prostaglandin $F_{2\alpha}$ after α-adrenoceptor blockade by phentolamine. This effect is partially inhibited by propranolol and haloperidol suggesting that dopamine has two types of receptors in this vessel: β- and dopaminergic receptors. However, the results obtained after treatment with drugs that interfere with deamination of catecholamines or after denervating the venous tissue led us to conclude that the agonist substance is not dopamine, but probably one of its deaminated metabolites or a secondary condensation product, namely tetrahydropapaveroline. Adrenaline has also been used to compare the behaviour of the two amines when interference in the deamination process is produced. The dissimilar results obtained with adrenaline are in good agreement with the hypothesis.

Dopamine produces a relaxing effect on the dog isolated saphenous vein contracted by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) after α-receptor blockade by phentolamine. We have shown (Macedo et al 1978) that this relaxation is partially blocked by propranolol or haloperidol and that the association of these drugs induces complete blockade. This led us to conclude that dopamine acts on two types of specific post-synaptic receptors in this vessel: β- and dopaminergic receptors.

However, further studies using drugs that interfere with catecholamine uptake and metabolism have raised doubts about this interpretation: the results obtained after pretreatment with iproniazid alone or with propranolol or haloperidol suggested that the agonist substance is not dopamine itself but rather one of its deaminated metabolites or a secondary product (Macedo et al 1980; Teixeira et al 1980).

The present study was undertaken to test this hypothesis by using drugs or procedures that interfere with the deamination of dopamine. Adrenaline was also used in order to compare the behaviour of the two amines.

MATERIALS AND METHODS

Mongrel dogs of 10–18 kg body weight and of either sex were anaesthetized with pentobarbitone sodium (30 mg kg^{-1} i.v. injected in the forelimb). Segments of both saphenous veins were removed and helically cut strips about 2·5–3 cm long were prepared and suspended in oxygenated Krebs-Henseleit solution (millimolar concentrations: NaCl, 118·6; KCl, 4·7; $CaCl_2$, 2·5; KH_2PO_4, 1·2; $MgSO_4 7H_2O$, 1·2; $NaHCO_3$, 25·0; glucose, 10·0) at 37 ± 0·5 °C, with added sodium EDTA ($2·7 \times 10^{-5}$ M) and ascorbic acid ($5·6 \times 10^{-5}$ M), in order to avoid autoxidation of catecholamines. The strips were subjected to a resting tension of 1·5 g, and isotonic responses recorded on a smoked drum with a frontal lever, adjusted to give an approximately 10-fold magnification. Each strip was allowed to stabilize during an equilibrium period of 1 h.

The strips were subjected to submaximal contraction (about 75%) with $PGF_{2\alpha}$ (3×10^{-6} M), after treatment for 30 min with phentolamine (7×10^{-5} M), and cumulative dose response curves of dopamine ($6·5 \times 10^{-6}$ to $1·5 \times 10^{-3}$ M) or adrenaline ($2·2 \times 10^{-8}$ to $1·6 \times 10^{-5}$ M) were obtained. (Control experiments showed that the pretreatment with phentolamine resulted in α-adrenoceptor blockade which was unchanged for a period of more than 90 min, whereathe duration of the experiments was of 70 min). Response to dopamine or adrenaline were also obtained from preparations additionally treated with the following drugs: propranolol ($2·8 \times 10^{-6}$ M), haloperidol (6×10^{-6} M), atropine (10^{-7} M), hexamethonium (10^{-5} M), cocaine ($1·4 \times 10^{-5}$ M), desoxycorticosterone (6×10^{-5} M), NAD (nicotinamide dinucleotide, 10^{-5} M), disulfiram ($2·8 \times 10^{-5}$ M), U-0521 (3'4-dihydroxy-2-methyl propiophenone, 10^{-4} M), pargyline (10^{-3}M), added to the bath 20 min before the addition of $PGF_{2\alpha}$. In some experiments, dogs were injected with iproniazid (65 mg kg^{-1} i.v.)

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24 h before removing the saphenous veins. In other animals, denervation was achieved by clamping the two extremities of the exposed lateral saphenous vein for 5 min; 6 days later the segments were removed. This procedure results in complete or almost complete denervation, according to Branco et al (1980) and personal experiments (unpublished results): the removed segment shows (a) a content in noradrenaline of less than 5% of the sham-operated, control vein; (b) absence of effects of cocaine $1{\cdot}4 \times 10^{-5}$ M; (c) ultrastructural signs of degeneration of adrenergic nerve terminals.

All values presented are shown as mean ± s.e.m. (% of $PGF_{2\alpha}$ induced contraction). Statistical analysis of the results was performed using Student's *t*-test.

RESULTS

After α-receptor blockade by phentolamine, dopamine or adrenaline produced a relaxing effect on the dog isolated saphenous vein contracted by prostaglandin $F_{2\alpha}$. The maximum value of this effect expressed as a percentage of the induced contraction during the steady state plateau was 30·1 ± 3·0% (mean ± s.e.m.; n = 13) or 49·5 ± 4·0% (mean ± s.e.m.; n = 7) for a $1{\cdot}76 \times 10^{-6}$ M dopamine concentration or $5{\cdot}94 \pm 10^{-7}$ M adrenaline concentration, respectively (Fig. 1).

Influence of drugs or denervation on the relaxing effect of dopamine

Table 1, which includes results of previous work (Macedo et al 1978, 1980; Teixeira et al 1980), shows that the relaxing effect of dopamine was partially antagonized by propranolol (80% of blockade) and by haloperidol (68% of blockade) and completely blocked by a combination of the two drugs.

In contrast, the blockade of muscarinic or nicotinic receptors by atropine or hexamethonium, respectively, had no influence on the responses to dopamine.

The value for the maximum relaxing effect exerted

Table 1. Maximum relaxing effect of dopamine on the dog isolated saphenous vein pretreated with phentolamine and contracted by $PGF_{2\alpha}$ in absence and presence of various drugs or denervation (see Methods). The results are expressed in mean ± s.e.m. (% of inhibition of $PGF_{2\alpha}$ induced contraction).

Drugs	Max. Eff. (%) (Dopamine concn = $1{\cdot}76 \times 10^{-6}$ M)	n
Control	30·1 ± 3·0	13
Propranolol ($2{\cdot}8 \times 10^{-6}$ M)	6·1 ± 3·5**	16
Haloperidol (6×10^{-6} M)	9·5 ± 0·7**(1)	8
Propranolol + haloperidol	0·8 ± 1·3**	13
Atropine (10^{-7} M)	34·0 ± 4·6	5
Hexamethonium (10^{-5} M)	30·4 ± 3·8	5
Cocaine ($1{\cdot}4 \times 10^{-5}$ M)	29·3 ± 5·9	6
Desoxycorticosterone (6×10^{-6} M)	33·7 ± 5·9	6
Coc. + desoxycortic.	38·5 ± 6·4	6
U − 0521 (10^{-4} M)	49·5 ± 5·7**	7
U − 0521 + haloperidol	21·0 ± 4·4*(1)	6
U − 0521 + propranolol	1·2 ± 0·5**(2)	7
Iproniazid (65 mg kg^{-1}, i.v. 24 h before)	19·0 ± 2·8**	10
Iproniazid + haloperidol	18·9 ± 2·0**	6
Iproniazid + propranolol	5·5 ± 3·5**(2)	6
Pargyline (10^{-5} M)	4·2 ± 3·7**(2)	5
NAD (10^{-6} M)	3·0 ± 2·5**	7
Disulfiram ($2{\cdot}8 \times 10^{-6}$ M)	11·0 ± 1·9**	6
Denervation	10·9 ± 2·9**(1)	10

* $P < 0{\cdot}01$: ** $P < 0{\cdot}001$ from control.
(1) Dopamine concentration = $5{\cdot}27 \times 10^{-4}$ M.
(2) Dopamine concentration = $5{\cdot}85 \times 10^{-6}$ M.

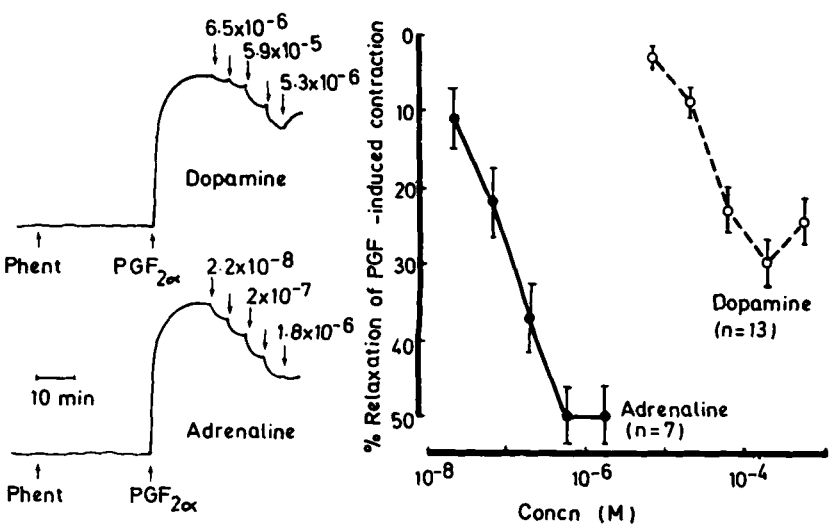

FIG. 1. Dose-response curves of dopamine and adrenaline on the dog isolated saphenous vein pretreated with phentolamine (7×10^{-5} M) and contracted by prostaglandin $F_{2\alpha}$ (3×10^{-6} M). Standard errors and n values are indicated.

by dopamine was not significantly modified either with neuronal or extraneuronal uptake blockade by cocaine or desoxycorticosterone, alone or in combination.

The inhibition by U-0521 of catechol-*O*-methyl transferase (COMT) was the only measure which resulted in a potentiation of the relaxing effect of dopamine. This was apparent for dopamine concentrations as low as $1{\cdot}95 \times 10^{-5}$ M, the maximal relaxation occurring with a $1{\cdot}76 \times 10^{-4}$ M concentration. Simultaneous use of U-0521 and propranolol completely blocked the relaxation responses to dopamine, whereas only a partial inhibition was observed after the combined use of U-0521 and haloperidol.

Table 1 and Fig. 2A show that there was a significant reduction in the maximum effect obtained in strips from iproniazid-pretreated dogs (about 40% of blockade). The addition of haloperidol to iproniazid did not alter the responses compared with iproniazid alone; propranolol plus iproniazid blocked relaxation by about 82%, i.e. the effect did not differ from that exerted by propranolol alone. Moreover, the relaxing effect of dopamine was almost abolished (86% of blockade) by pargyline, a more potent MAO inhibitor.

Figure 2B shows that NAD (which stimulates oxidation of 3,4-dihydroxyphenylacetaldehyde to DOPAC—3,4-dihydroxyphenylacetic acid) completely prevented the relaxing effect of dopamine; aldehyde deshydrogenase inhibition by disulfiram resulted in about 63% of blockade of the response to dopamine; denervated strips showed a much smaller response to dopamine (64% of blockade).

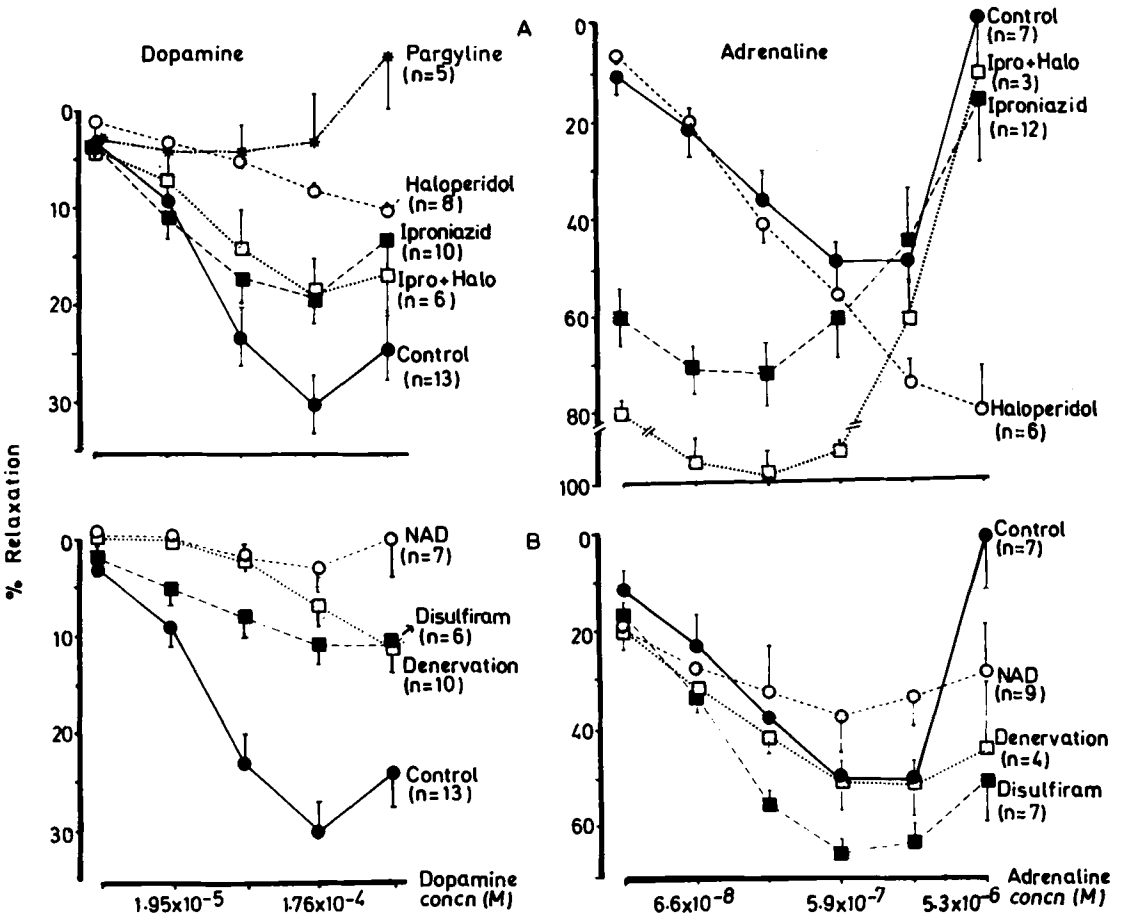

FIG. 2. Dose-response curves of dopamine and adrenaline in dog saphenous vein strips in the absence and presence of various inhibitors. Standard errors and n values are indicated.

Influence of drugs or denervation on the relaxing effect of adrenaline

The relaxation induced with adrenaline was completely blocked by propranolol, contractile effects occurring with concentrations of adrenaline higher than $1{\cdot}78 \times 10^{-6}$ M; haloperidol had no significant influence on the relaxing effect. However, while the maximal response seen with control occurred at $5{\cdot}90 \times 10^{-7}$ M adrenaline (max effect = 49·5 ± 4·0% of relaxation), in the haloperidol-treated strips the relaxation was 57·2 ± 6·4% at $5{\cdot}90 \times 10^{-7}$ M adrenaline but increased till 9 times higher concentrations were reached (max effect = 81·1 ± 4·1% at $5{\cdot}3 \times 10^{-6}$ M adrenaline) (Fig. 2A).

Fig. 2A shows that pretreatment with iproniazid enhanced the relaxing effect induced with adrenaline (max effect = 72·2 ± 4·9% at $6{\cdot}6 \times 10^{-8}$ M adrenaline concentration); moreover, this increased relaxation seen with adrenaline in the iproniazid-treated strips was significantly enhanced by haloperidol (max effect = 99·1 ± 4·5% at $1{\cdot}98 \times 10^{-7}$ M adrenaline). These results are at variance with those obtained with dopamine under the same conditions (see Fig. 2A).

The relaxing effect of adrenaline was not significantly antagonized by NAD (max effect = 37·1 ± 8·6%, n = 9), was slightly increased by disulfiram (max effect = 64·8 ± 2·9%, n = 7; i.e. about 30% of enhancement), and unaffected by denervation. Once more a different behaviour between the two amines is evident.

DISCUSSION

Our results show that both dopamine and adrenaline produce a relaxing effect on the dog isolated saphenous vein contracted by prostaglandin $F_{2\alpha}$ after α-receptor blockade by phentolamine. However, the preparations were about 500 times more sensitive to adrenaline than to dopamine, indicating that their affinities are different. On the other hand, the results obtained with propranolol and haloperidol clearly show that adrenaline relaxation is mediated exclusively by β-receptor stimulation whereas dopamine relaxation involves two types of receptors: β- and dopaminergic receptors. The enhancement of the relaxing effect of adrenaline after pretreatment with haloperidol, occurring with the higher adrenaline concentrations used, is probably due to the α-adrenoceptor blocking capacity of haloperidol (Hersom et al 1978).

It is not surprising that neither neuronal nor extraneuronal uptake blockade significantly modified the responses to dopamine. In fact, at high dopamine concentrations neuronal uptake is saturated (thus, the blockade of this uptake can only show modest effects) and extraneuronal uptake becomes dominant according to Hertting & Suko (1972); however, at 10^{-5} M or higher dopamine concentrations, desoxycorticosterone is ineffective as a blocking drug because it is a partial and competitive antagonist of extraneuronal uptake (Bönisch 1978).

Only COMT inhibition induced a significant increase in the relaxing effect of dopamine. However, comparing the results obtained with dopamine alone or in association with propranolol or haloperidol with those after U-0521 we can speculate: (a) in the two series of results, dopamine relaxation was systematic and almost completely blocked by propranolol; (b) The relaxation seen with dopamine in the control and in the U-0521-treated strips is blocked to the same degree by haloperidol (about 68% and 60%, respectively), i.e. the difference between the results obtained with haloperidol alone (9·5 ± 0·7% of relaxation) or U-0521 + haloperidol (21·0 ± 4·4% of relaxation) seems to correspond to the U-0521 potentiation effect.

Thus, evidence has been produced that dopamine potentiation by the drug U-0521 is mediated not by specific dopaminergic receptors but by β-adrenoceptors. These results are in agreement with previous reports concerning isoprenaline, adrenaline and noradrenaline by Guimarães & Paiva (1977), who interpret the potentiation caused by U-0521 as a consequence of a higher concentration of the unchanged amine in close neighbourhood of β-adrenoceptors. However, a doubt arises about the extension of such an interpretation to dopamine. In fact, at concentrations of dopamine higher than 10^{-5} M, *O*-methylation becomes negligible (Hellmann et al 1971; Hertting & Suko 1972).

The results seen with dopamine and adrenaline in the strips obtained from iproniazid-treated dogs are also of importance. In fact, the results with dopamine suggest that the agonist relaxing substance, the effect of which was blocked by haloperidol, is not present when MAO is inhibited, i.e. the relaxing effect is not directly due to dopamine but to deaminated metabolites specifically antagonized by haloperidol. In the same sense, the results with pargyline show that in the concentration used it almost totally blocks both MAO-A and MAO-B. The converse results with adrenaline expand this hypothesis.

The fact that dopamine relaxation was abolished by NAD (which stimulates oxidation of 3,4-dihydroxyphenylacetaldehyde, the first metabolite of the

oxidative deamination pathway of dopamine, to DOPAC) seems to localize the agonist metabolite. In 1970, Walsh et al and Davis et al, using liver homogenates, showed that deamination was the predominant factor in dopamine metabolism (see also Hellmann et al 1971, with experiments in the perfused rat heart). They found about 8% as DOPAC, 9% as neutral metabolites (3,4-dihydroxyphenylacetaldehyde + DOPET—dihydroxyphenylethanol) and 56% as tetrahydropapaveroline (product of a non-enzymatic condensation of dopamine with its metabolite 3'4-dihydroxyphenylacetaldehyde); incorporation of NAD into incubation mixtures essentially abolished tetrahydropapaveroline production (3%) and markedly enhanced the formation of the acid (66% as DOPAC) or neutral metabolites (18%) without modification of the remaining dopamine. On the basis of these concepts our results suggest that the relaxation is primarily due to the tetrahydropapaveroline formed and not to the dopamine added.

In contrast, disulfiram induced a surprising influence on the relaxing effect of dopamine, since aldehyde dehydrogenase inhibition should result in an increase of that effect, as actually ocurred with adrenaline. However, we admit that our results for dopamine could be due to a change in the metabolic pathway of dopamine to give DOPET or a tetrahydroisoquinoline alkaloid (according to Davis et al 1970), with α-agonist (Osswald et al 1975) or dopamine-antagonist (Sheppard & Burghardt 1974) activities.

As shown above, denervation antagonized the relaxing effect of dopamine, whereas it had no effect on adrenaline effect. Therefore, these results confirm our belief that the deaminated metabolites of dopamine, or a secondary product, are involved in its relaxing effect; in fact, Branco et al (1980) have shown that denervation effects a marked reduction in MAO activity of both types A and B.

In conclusion, our results show that, in the relaxing effect of dopamine on the dog saphenous vein, two types of post-synaptic receptors are involved: β- and dopaminergic receptors. However, the specific 'dopaminergic' effect is due not to dopamine but probably to one of its deaminated metabolites or to a secondary condensation product. Although Halushka & Hoffmann (1968) failed to detect significant amounts of tetrahydropapaveroline in any tissue other than the liver, these results are in agreement with the concept postulated by Holtz et al (1963, 1964a, 1964b), that the vasodepressor effect of dopamine is due to the formation of a condensation product of dopamine, i.e., tetrahydropapaveroline. On the other hand, this opinion fits in with the results obtained by Walsh et al (1970) and Davis et al (1970) and, more recently, with the increasing importance attributed by numerous workers to tetrahydroisoquinoline alkaloids derived from catecholamines, although almost always referred to their central effects.

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