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Indirect sympathomimetic activity of dopamine

M. J. KELLY, *School of Health and Applied Sciences, Leeds Polytechnic, Calverley Street, Leeds, LS1 3HE, U.K.*

Dopamine is a well established sympathomimetic with agonist activity at both α- (van Rossum 1965) and β-adrenoceptors (Tsai et al 1967; Kohli 1969). In addition, it has been reported to stimulate dopamine receptors in certain vascular tissues (Toda & Goldberg 1973, 1975; Toda 1976; Kelly 1981, 1982). Vascular dopamine receptors mediate a relaxation response which is unaffected by the presence of a β-adrenoceptor antagonist. However, it is possible that an indirect sympathomimetic component to dopamine's action, releasing endogenous noradrenaline from within the tissue could be responsible for producing part or all of the relaxation response, since the effects of endogenously released noradrenaline are more difficult to antagonize than those of exogenously applied material (Urquilla et al 1970).

Early studies showed dopamine to be a directly acting sympathomimetic (Fleckenstein & Burn 1953; Fleckenstein & Bass 1953) but subsequent investigations have confirmed the presence of an indirect component to dopamine's actions (Farmer 1965, 1966; Tsai et al 1967; Guimarães 1969; Heilman & Lum 1971; Gibson et al 1977). In view of these discrepancies it was thought that the indirect sympathomimetic activity of dopamine warranted further investigation.

Methods

Spiral strips of rabbit aorta, portal vein, renal artery and longitudinal muscle strips of jejunum were mounted vertically in tissue baths containing Krebs-Henseleit solution at 37 °C, bubbled with a 95% O_2/5% CO_2 gas mixture. α-Adrenoceptor-mediated responses to dopamine were measured isometrically as contractions for the vascular tissue and relaxations for intestinal tissue. Concentration-effect curves were constructed to provide EC50 values so that the potency of dopamine could be assessed for each tissue. In all experiments the tissues were incubated with propranolol (0·5 μM) so that β-adrenoceptor-mediated actions could be excluded from the assessment.

The effectiveness of pre-incubation with the uptake inhibitors cocaine (100 μM), guanethidine (10 μM) and desmethylimipramine (10 nM) on the uptake of [^{3}H]-noradrenaline into the tissues was measured. Tissues were cross-chopped into 1 mm segments and were incubated with 5 ml of Krebs-Henseleit solution alone or with inhibitor for the periods of time shown in the Results section. [^{3}H]Noradrenaline (5 p mol ml^{-1}) was added and the incubation continued for a further 10 min. The mixture was then filtered and the tissue pieces were retained, homogenized in 2 ml perchloric acid (0·4 M), centrifuged and an aliquot (1 ml) of the supernatant was taken for counting. The same volume of incubation solution was also taken for counting. Controls consisted of samples that had gone through the procedure in the absence of inhibitor and net uptake was then calculated by subtracting the uptake at 0 °C from the total uptake.

Some rabbits were pre-treated with the monoamine oxidase inhibitor pargyline (100 mg kg^{-1} i.p.) 16–18 h before they were used. The extent of monoamine oxidase inhibition was checked by measuring enzyme activity in the aorta, using the method of Krajl (1965). Other rabbits were pretreated with 6-hydroxydopamine, which was given in doses of 30 mg kg^{-1} i.v. at 1700 h on day 1, 20 mg kg^{-1} i.v. at 1300 h and 1700 h on day 2, before the tissues were taken for experiments at 1000 h on day 3. The degree of noradrenaline depletion caused by 6-hydroxydopamine was assessed by measuring the noradrenaline content of each tissue from pre-treated and untreated animals using the assay method of Welch & Welch (1969).

Results and discussion

The sympathomimetic potency of dopamine was studied by measuring α-adrenoceptor-mediated responses in rabbit isolated aorta, portal vein, renal artery and jejunum. The indirect sympathomimetic component of dopamine's responses was investigated by using pre-treatments designed to modify the functioning or structure of the sympathetic nerve ending.

The noradrenaline uptake inhibitors, cocaine (100 μM), guanethidine (10 μM) and desmethylimipramine (10 nM) were incubated with the tissues for 30, 90 and 120 min respectively before potency measurements for dopamine were made. These agents, in the stated concentrations, greatly inhibited the uptake of [^{3}H]noradrenaline into the tissues (Table 1) and reduced the

Table 1. Effect of pretreatments on the [^{3}H]noradrenaline uptake into the rabbit tissues

Pretreatment	Tissue			
	Aorta	Renal artery	Portal vein	Jejunum
Cocaine	76 ± 6	84 ± 3	94 ± 2	99 ± 1 (n = 8)
Guanethidine	66 ± 15	86 ± 1	85 ± 1	80 ± 4 (n = 8)
Desmethylimipramine	57 ± 5	38 ± 2	65 ± 2	61 ± 6 (n = 8)
6-Hydroxydopamine	74 ± 11	77 ± 11	95 ± 12	85 ± 14 (n = 5)

Figures are % inhibition of mean of [^{3}H]noradrenaline uptake into untreated tissues.

Table 2. Measurement of the sympathomimetic potency of dopamine in control tissues and tissues pretreated with drugs which reveal indirect sympathomimetic activity

Pretreatment	Tissue: Aorta	Renal artery	Portal vein	Jejunum
Control	4·69±0·01 (8)	4·49±0·04 (5)	4·08±0·03 (6)	4·59±0·08 (31)
Cocaine	***4·48±0·04 (13)	4·45±0·04 (5)	*3·84±0·10 (7)	4·39±0·17 (9)
Guanethidine	***4·54±0·03 (4)	4·45±0·10 (4)	*3·94±0·04 (4)	4·52±0·05 (4)
Desmethylimipramine	**4·58±0·03 (4)	4·45±0·03 (4)	3·95±0·08 (4)	4·48±0·07 (4)
Pargyline	**5·14±0·13 (5)	4·52±0·05 (4)	***5·20±0·05 (10)	4·77±0·18 (5)
6-Hydroxydopamine	4·87±0·11 (11)	4·45±0·11 (4)	4·20±0·13 (6)	4·97±0·36 (6)

Mean negative logarithms of the EC50 values ± standard errors and numbers of determinations (brackets) are given. High figures indicate greater potency. The statistical significance of the differences between control and pretreatment values for each tissue is shown; ***P <0·001, **P <0·01, *P <0·05.

response of the portal vein to the indirectly acting amine, tyramine (100 μM), by over 80%.

Pargyline pretreatment produced a 93 ± 10% (n=5) reduction in monoamine oxidase activity in the aorta and a 65 ± 7% (n=5) increase in tyramine's (100 μM) response in the portal vein. 6-Hydroxydopamine pretreatment reduced the endogenous noradrenaline content of the aorta by 75 ± 13%, of the renal artery by 71 ± 18%, of the portal vein by 83 ± 22% and of the jejunum by 86 ± 10% (n=5 in each case). This pretreatment also inhibited the uptake of [^{3}H]noradrenaline into the tissues to the extent shown in Table 1, and abolished the response of the portal vein to tyramine (100 μM).

The potency of dopamine was tested on each of the tissues under control conditions and after the various pretreatments described above. The results are shown in Table 2. The neuronal uptake blockers, cocaine, guanethidine and desmethylimipramine reduced the sympathomimetic potency of dopamine in all of the tissues, but particularly in the aorta. This kind of modification of the response is characteristic of an indirect sympathomimetic action for the amine. The potency of methoxamine, a relatively pure directly acting sympathomimetic, was unaffected by these pretreatments. Unlike the findings of Tayo (1977) no differences were found between the effect of cocaine and desmethylimipramine on dopamine's responses in the tissues. In this study a low concentration of desmethylimipramine was chosen to avoid α-adrenoceptor antagonism.

Pargyline pretreatment increased the potency of dopamine but left that of methoxamine unaffected and this result again points to an indirect action. However, 6-hydroxydopamine pretreatment was unable to affect the responses to dopamine and although it increased the tissue sensitivity to methoxamine slightly it did not appear that a general increase in α-adrenoceptor sensitivity, produced by chemical sympathectomy, had masked a decreased responsiveness to dopamine.

Overall the results indicate a small indirect component to dopamine's vascular sympathomimetic actions which reaches statistically significant proportions in the aorta and portal vein. This magnitude of indirect sympathomimetic activity did not appear to be sufficient to account for the dopamine receptor activity previously described and was not present in the renal artery where dopamine receptors have been reported to exist (McNay et al 1965; McNay & Goldberg 1966; Yeh et al 1969; Toda & Goldberg 1973).

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