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Effects of enzyme inhibitors of catecholamine metabolism and of haloperidol on the pancreatic secretion induced by L-DOPA and by dopamine in dogs

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Summary

1. Effects of inhibitors of DOPA decarboxylase, dopamine β-hydroxylase and monoamine oxidase, and haloperidol on the secretion of pancreatic juice induced by L-DOPA and dopamine were studied in preparations of the isolated blood-perfused canine pancreas.

2. The increased secretion induced by the infusion of L-DOPA (100 μg/min) was completely antagonized by Ro 4-4602 (300 μg), a DOPA decarboxylase inhibitor.

3. The secretogogue effect of dopamine (1–10 μg) intra-arterially was not affected by Ro 4-4602, but was enhanced by the infusion of fusaric acid (100 μg/min), a dopamine β-hydroxylase inhibitor.

4. The increase in the secretion induced by dopamine (1–10 μg) was enhanced by treatment with nialamide (100 mg/kg), a monoamine oxidase inhibitor, given intravenously.

5. Haloperidol (1 mg) intra-arterially attenuated the dopamine-induced pancreatic secretion.

6. It is concluded that L-DOPA is converted to dopamine in the acinar cells which causes an increase in the secretion of pancreatic juice, thus the intracellular level of dopamine may be controlled by enzymatic equilibrium.

Introduction

Dihydroxyphenylalanine (DOPA) and 3-hydroxytyramine (dopamine) are biochemical precursors in the biosynthesis of noradrenaline. However, a number of investigators have suggested that they may have important physiological functions as one of the active biogenic amines (Hornykiewicz, 1966). Previous studies in our laboratory demonstrated that L-DOPA and dopamine caused an increase in the secretion of pancreatic juice in the dog as did secretin. The effect of dopamine was not modified by treatment with either α- or β-adrenoceptor blocking agents or by atropine (Hashimoto, Satoh & Takeuchi, 1971). Biochemical analysis showed an induction of high bicarbonate but low chloride output by dopamine paralleling the increase in the rate of secretion (Furuta, Iwatsuki, Takeuchi & Hashimoto, 1972).

The present study was designed to analyse the action of L-DOPA and dopamine on the secretion of pancreatic juice in the presence of inhibitors of DOPA decarboxylase, dopamine β-hydroxylase or monoamine oxidase. The effect of haloperidol on the action of dopamine was also examined.

Methods

Twenty-eight mongrel dogs of either sex, weighing 10 to 18 kg, were used in this study. Anaesthesia was induced with 30 mg/kg of sodium pentobarbitone injected intravenously and it was maintained with 10 mg/kg of the agent which was given intra-muscularly at 1 h intervals. A tracheal tube was inserted and the animals were ventilated artificially with an Aika R-50 respirator. A polyethylene tube with an inner diameter of 0·8 mm and an outer diameter of 1·4 mm was inserted into the main pancreatic duct and the rate of secretion measured by a drop counter. The accessory pancreatic duct was ligated and cut. Polyethylene cannulae were inserted into the gastroduodenal and the splenic arteries through which the pancreas was perfused with the animal's own blood from the left femoral artery by means of a Harvard peristaltic pump (Model 500–1200). The details of the preparation were described in a previous paper (Hashimoto *et al.*, 1971). All experiments were performed under constant pressure at 100 mmHg (1 mmHg≡1·333 mbar). Blood flow was measured by a magnetic flowmeter (Nihon Kohden MF-2). Two electric manometers (Nihon Kohden RP-2) were used to measure perfusion pressure and systemic blood pressure in the right femoral artery.

A dose of 300 U/kg of sodium heparin was given at the beginning of the perfusion and a supplementary dose of 2,000 units was given intravenously at 1 h intervals. Drug solutions were injected into a rubber tube connected to the arterial cannula over a period of 4 s by a microinjector (Jintan Terumo). L-DOPA and fusaric acid were infused at a constant rate of 0·1 ml/min by a Harvard infusion pump (Model 600–900).

Drugs used in this study were L-DOPA (Nippon Kayaku), dopamine hydrochloride (ICN), Ro 4-4602, N^1-(DL-seryl)-N^2-(2,3,4, trihydroxybenzyl) hydrazine hydrochloride (Hoffmann-La Roche), nialamide hydrochloride (Pfizer), fusaric acid (kindly supplied by Banyu) haloperidol (Dainihon), (±)-propranolol hydrochloride (Sumitomo Chemicals) and phentolamine mesylate (Ciba). Each drug was freshly dissolved in a 0·9% w/v NaCl (saline) solution.

Statistical analysis was by means of Student's *t* test.

Results

Effect of Ro 4-4602 on the secretion of pancreatic juice induced by L-DOPA and dopamine

When L-DOPA was infused at a rate of 100 μg/min for 10 min, the secretion of pancreatic juice began to increase after a delay of about 2 min and reached a maximum about 5 or 6 min later. The increased secretion continued for about 60 min even after the discontinuation of the infusion. Neither arterial blood flow nor systemic blood pressure was affected by L-DOPA. The effect of a DOPA decarboxylase inhibitor, Ro 4-4602, was examined on the secretion of pancreatic juice induced by L-DOPA. An injection of 100 μg of Ro 4-4602 resulted in a significant reduction of the secretion induced by L-DOPA (Fig. 1a). The secretion was completely inhibited by 300 μg of Ro 4-4602, which itself exerted no action on the secretion of pancreatic juice or the arterial blood flow. The inhibitory effect of Ro 4-4602 on L-DOPA continued for over 4 hours.

Dopamine caused an increase in the secretion of pancreatic juice (Fig. 1b). The onset of the response was quick, but its duration was short. Its action disappeared after 10 minutes. The arterial blood flow first decreased, and then increased slightly in a majority of cases, but a simple reduction was observed in a few cases. Unlike L-DOPA, the effects of dopamine on the secretion of pancreatic juice and the arterial blood flow were not modified by 100 μg of Ro 4-4602.

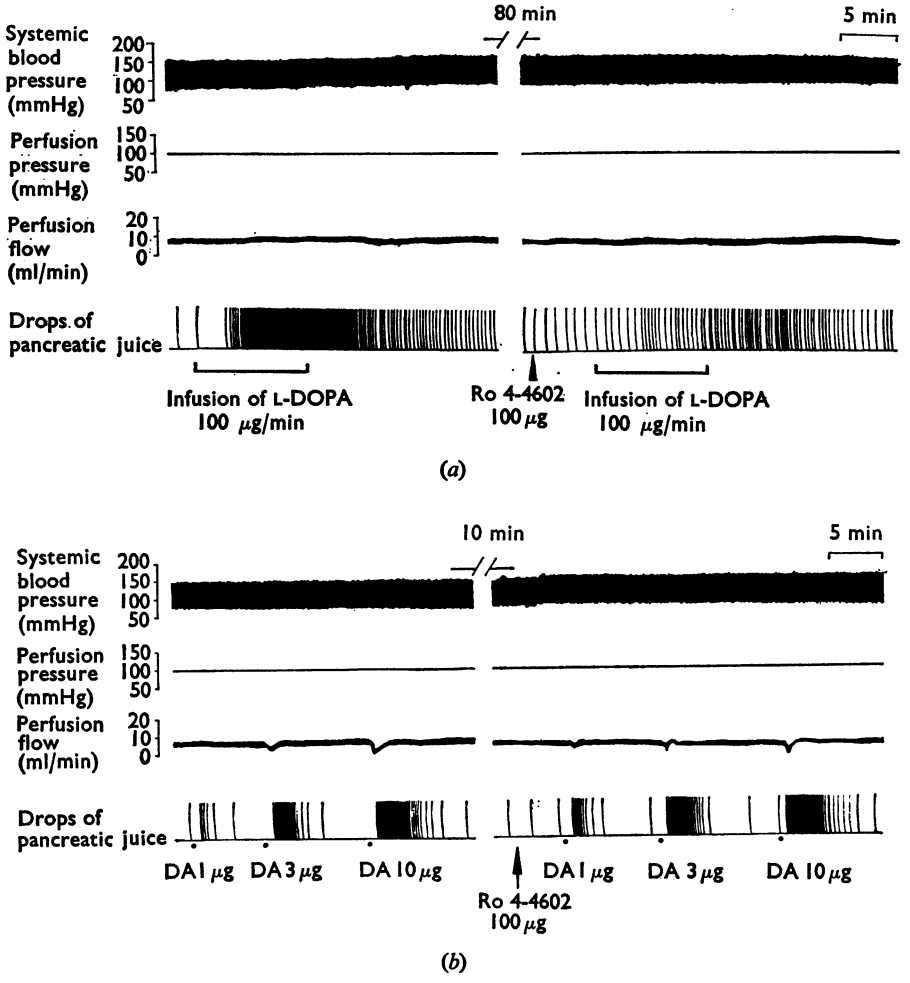

FIG. 1. (a) Inhibitory effect of Ro 4-4602 on the secretion of pancreatic juice induced by L-DOPA. L-DOPA was infused at a rate of 100 μg/min for 10 min and Ro 4-4602 (100 μg) was injected intra-arterially. (b) Absence of an inhibitory effect of Ro 4-4602 on the secretion of pancreatic juice induced by dopamine (DA). Dopamine (1, 3 and 10 μg) was injected intra-arterially.

Figure 2 shows the dose-inhibition curve for Ro 4-4602 on the secretion of pancreatic juice induced by 1 mg of L-DOPA. The figures are the means from 5 dogs. The percentage inhibition produced by doses of 10, 30, 100 and 300 μg of Ro 4-4602 was $19{\cdot}5 \pm 5{\cdot}9$, $45{\cdot}6 \pm 6{\cdot}7$, $77{\cdot}5 \pm 7{\cdot}6$ and $96{\cdot}5 \pm 4{\cdot}5$, respectively. The 50% inhibition dose (ID50) derived from these data is 36 μg.

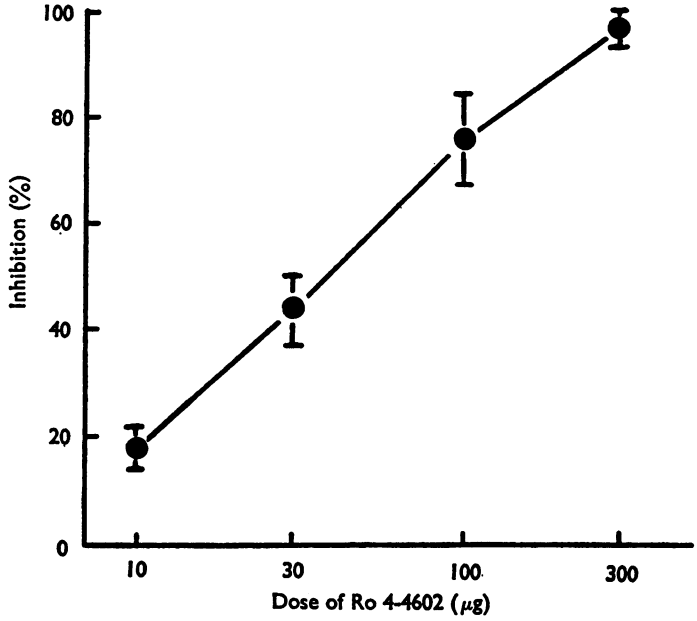

FIG. 2. Dose-inhibition curve for pancreatic secretion induced by L-DOPA caused by treatment with Ro 4-4602. Ro 4-4602 was injected intra-arterially. L-DOPA was infused at a rate of 100 μg/min for 10 min intra-arterially 5 min after Ro 4-4602. Each point represents the mean and the vertical bars indicate the standard error ($n=5$).

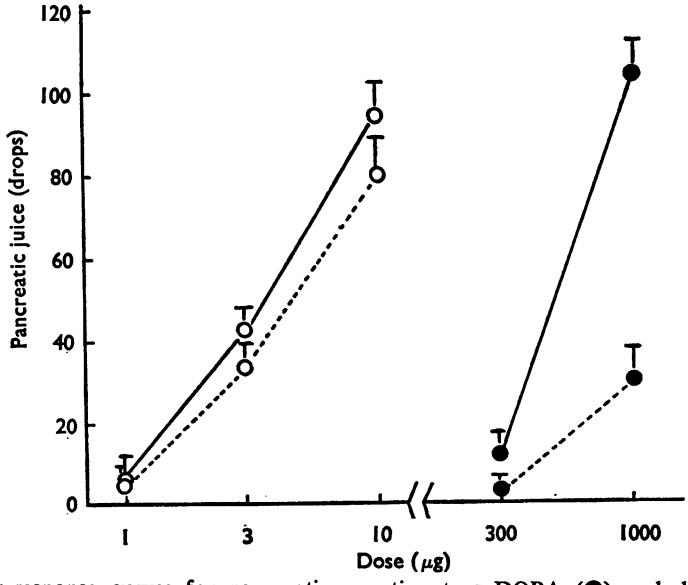

FIG. 3. Dose-response curves for pancreatic secretion to L-DOPA (●) and dopamine (○) before (——) and 5 min after (- - - - -) the administration of Ro 4-4602. L-DOPA was infused at a rate of 30 and 100 μg/min for 10 minutes. Dopamine (1, 3 and 10 μg) and Ro 4-4602 (100 μg) were injected intra-arterially. Each point represents the mean and the vertical bars indicate the standard error ($n=5$).

Figure 3 shows the dose-response curves for L-DOPA and dopamine before and 5 min after the administration of Ro 4-4602 (100 μg) in 5 dogs. The secretion induced by 1 mg of L-DOPA was significantly ($P<0.01$) reduced by 100 μg of Ro 4-4602, but the dose-response curve for dopamine was not influenced.

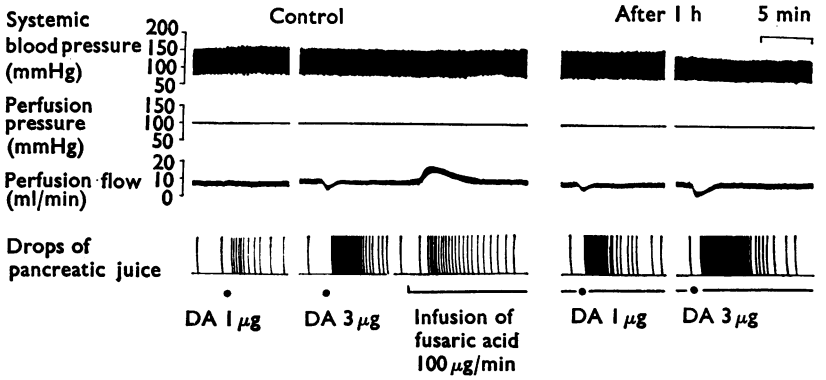

FIG. 4. Effect of fusaric acid on the secretion of pancreatic juice induced by dopamine (DA). Fusaric acid was infused at a rate of 100 μg/min and dopamine (1 and 3 μg) was injected intra-arterially.

Effect of fusaric acid on the secretion of pancreatic juice induced by dopamine

Figure 4 shows pancreatic secretion induced by dopamine before and during the infusion of fusaric acid (100 μg/minute). When fusaric acid was infused, the secretion of pancreatic juice first increased and then gradually fell to a constant level which, however, was a little higher than initially. The arterial blood flow also increased temporarily, returning to the initial rate of flow about 10 min later. The dopamine-induced secretion was enhanced by treatment with fusaric acid. Table 1 shows the results obtained in 5 dogs. A significant enhancement ($P<0.05$) was apparent 30 min after the infusion of fusaric acid, and the difference from the control responses was highly significant ($P<0.01$) after 60 minutes. The reduction in blood flow through the pancreas produced by dopamine was also enhanced by the infusion of fusaric acid.

Effect of nialamide on the secretion of pancreatic juice induced by dopamine

The results are shown in Table 2. Nialamide (100 mg/kg) i.v. did not cause any increase in the secretion of pancreatic juice, but the blood flow was increased,

TABLE 1. *Effect of infusion of fusaric acid on the secretion of pancreatic juice induced by dopamine*

Dose of dopamine	No. of experiments	Number of drops (mean ±S.E.M.) Control	After 30 min	After 60 min
1 μg	5	5·3±0·3	13·2±3·3*	15·5± 3·6†
3 μg	5	40·3±3·6	54·2±4·5*	68·2± 9·4†
10 μg	5	75·0±5·7	92·6±8·9	115·4±10·0†

Fusaric acid was infused at a rate of 100 μg/min and dopamine (1, 3 and 10 μg) was injected intra-arterially. Each value represents the mean ±S.E.M. Significance of difference $P<0.05$(*) and $P<0.01$(†).

TABLE 2. *Effect of nialamide on the secretion of pancreatic juice induced by dopamine*

Dose of dopamine	No. of experiments	Number of drops (mean ±S.E.M.) Control	After 60 min	After 120 min
1 μg	7	6·5±1·9	16·3±5·4*	19·5± 4·5†
3 μg	7	31·7±3·7	40·2±8·6	42·8±10·5*
10 μg	7	76·4±9·4	98·9±7·5	107·3± 5·4†

Nialamide was injected in a dose of 100 mg/kg intravenously and dopamine (1, 3 and 10 μg) was injected intra-arterially. Each value represents the mean ±S.E.M. Significance of difference $P<0.05$(*) and $P<0.01$(†).

returning to the initial level about 30 min later. The secretion induced by dopamine was enhanced by pretreatment with nialamide. The enhancement was apparent 60 min after the injection of nialamide and was highly significant 120 min after a dose of 1, 3 or 10 μg of dopamine ($P<0{\cdot}01$). The reduction in blood flow produced by dopamine was also enhanced by the treatment with nialamide.

Antagonism between haloperidol and dopamine on the secretion of pancreatic juice and the vascular response

Previously we observed that the dopamine-induced secretion was not modified by α- and β-adrenoceptor blocking agents (Hashimoto *et al.*, 1971). In the pancreas pretreated with phentolamine (300 μg) and propranolol (300 μg), haloperidol (1 mg) injected into the pancreatic artery significantly reduced dopamine-induced secretion. Haloperidol (3 mg) completely inhibited the action of 10 μg of dopamine. After treatment with phentolamine and propranolol, dopamine caused vasodilatation, which was also attenuated by haloperidol. Figure 5 shows the dose-response curves ($n=5$) for dopamine before and 2 min after the injection of 1 mg of haloperidol. Haloperidol significantly shifted the dose-response curves of dopamine-induced secretion and vasodilatation to the right ($P<0{\cdot}01$).

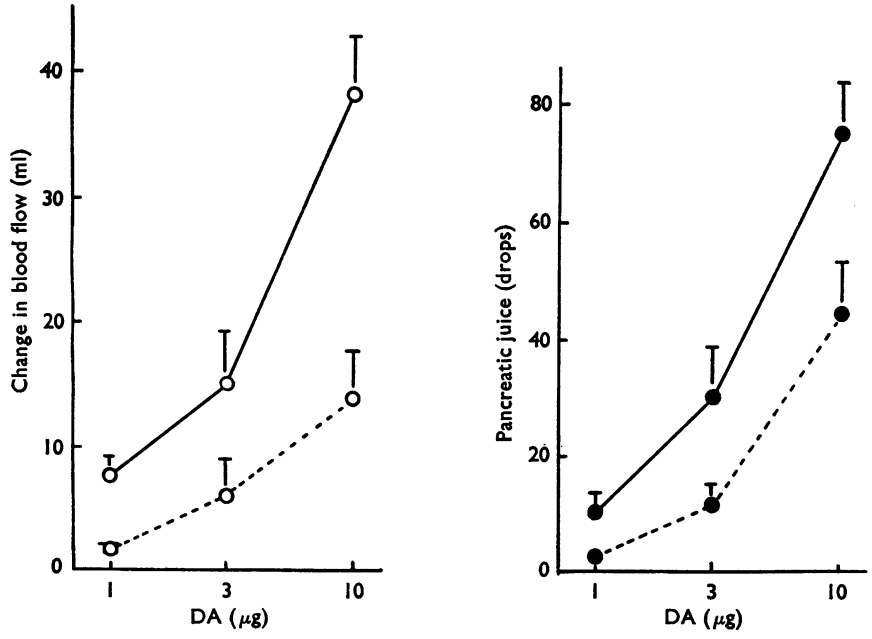

FIG. 5. Dose-response curves for dopamine-induced pancreatic secretion (●) and vasodilation (○) before (——) and 2 min after (- - - - -) the administration of haloperidol. Dopamine (DA) (1, 3 and 10 μg) and haloperidol (1 mg) were injected intra-arterially. Phentolamine and propranolol were injected into the pancreatic artery each in a dose of 300 μg prior to injection of dopamine and haloperidol. Each point represents the mean and the vertical bars indicate the standard error ($n=5$). Difference is significant ($P<0{\cdot}01$).

Discussion

Although DOPA and dopamine are biochemical precursors of noradrenaline, dopamine is found in high concentration in some kinds of mammalian tissues. More than 50% of total catecholamines in the pancreas was reported to be dopamine (Schümann & Heller, 1959). Furthermore, DOPA decarboxylase was found in the pancreas of several species (Holz, Credner & Strübing, 1942). Recently it was shown that DOPA is taken up into the exocrine cells of the pancreas and converted to dopamine (Alm, Ehinger & Falck, 1969). The effect of DOPA and dopamine on the secretion of pancreatic juice was first reported by Greengard, Roback & Ivy (1942), and it was shown by Hashimoto *et al.* (1971) that secretion increased only after some delay in response to the intra-arterial infusion of L-DOPA, but the response to dopamine was prompt.

We have now shown that the increase in the secretion induced by L-DOPA is inhibited by Ro 4-4602, a selective inhibitor of DOPA decarboxylase (Porter, Watson, Titus, Toraro & Byer, 1962). The dose-response curve for L-DOPA was shifted to the right after 100 μg of Ro 4-4602 was injected into the pancreatic artery. Complete inhibition was obtained with a dose of 300 μg. On the other hand, the secretion induced by dopamine was not affected by Ro 4-4602. These results indicate that the increase in the secretion induced by DOPA is due to its conversion to dopamine.

The secretion of pancreatic juice induced by dopamine was enhanced by fusaric acid which is a potent inhibitor of dopamine β-hydroxylase *in vivo* as well as *in vitro* (Hidaka, Nagatsu, Takeya, Takeuchi, Suda, Kojiri, Matsuzaki & Umezawa, 1969). The reduction in blood flow produced by dopamine was also increased. Dopamine is taken up into the pancreatic tissue where it is converted to noradrenaline by dopamine β-hydroxylase (Symchowicz, Korduba, Veals & Tabachnik, 1968). Our observation that fusaric acid increases dopamine-induced pancreatic secretion could be explained if there were specific receptors for dopamine in the acinar cells. Then inhibition of the metabolic conversion of dopamine to noradrenaline would increase the dopamine in the exocrine cells. The increase in the resting secretion by fusaric acid would also be explained by such a mechanism.

Pretreatment with the monoamine oxidase (MAO) inhibitor nialamide (100 mg/kg) increased the secretion of pancreatic juice and the reduction in arterial blood flow induced by dopamine. Dopamine is metabolized by MAO more rapidly than noradrenaline (Blaschko, 1952). MAO has been shown to be present in the pancreas (Glenner, Burtner & Brown, 1957; West, 1958; Petkov, 1965). Thus, the effect of nialamide is easily explained especially as it has been reported to increase the dopamine content of the rat pancreas (Alm, Ehinger, Falck & Nordgren, 1971).

Other workers have shown dopamine to cause renal, mesentric and coronary vasodilation which was not mediated through the β-adrenoceptor but which was attenuated by haloperidol (McNay, McDonald & Goldberg, 1963; McNay & Goldberg, 1966; Brooks, Stein, Matson & Hyland, 1969; Yeh, McNay & Goldberg, 1969). The reports suggest that dopamine might act on a specific receptor in these vascular beds. In our experiments, it was observed that the dopamine-induced pancreatic secretion and vasodilatation were attenuated by 1 mg of haloperidol given intra-arterially, which suggests that a specific dopamine receptor is present in the pancreas. The pancreas has been shown to be innervated by sympa-

thetic and parasympathetic nerve fibres, but its secretory acini were demonstrated not to have a direct adrenergic nerve supply (Richins, 1945), and noradrenaline did not stimulate the secretion of pancreatic juice (Hashimoto *et al.*, 1971). They do not, therefore, possess receptors for noradrenaline.

Our results support the suggestion that the dog pancreas has a specific receptor for dopamine. Although its physiological role is not yet clear, these experiments show that some side effects of therapy with L-DOPA could be ascribed to an increase in pancreatic secretion.

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