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MODIFICATION BY DRUGS OF THE SECRETAGOGUE EFFECT OF DOPAMINE ON THE PANCREAS

Y. FURUTA, K. HASHIMOTO, Y. ISHII & K. IWATSUKI
Department of Pharmacology,
Tohoku University School of Medicine, Sendai, Japan 980

1 The specific stimulating action of dopamine and L-DOPA on exocrine pancreatic secretion was further investigated in the isolated blood-perfused canine pancreas.

2 6-Hydroxydopamine (100 μg, i.a.) stimulated the secretion but was far less potent than dopamine. Epinine (0.3-1mg. i.a.), α-methyldopamine (10-300 μg, i.a.) and octopamine (10-300 μg, i.a.) were ineffective.

3 α-Methyldopa (30 mg, i.a.) inhibited the stimulating effect of L-DOPA (1-3 mg) on pancreatic secretion.

4 Apomorphine (1 mg, i.a.) attenuated dopamine-induced (1-30 μg) pancreatic secretion but did not antagonize secretin-induced (0.03-0.3 units) or pancreozymin-induced (0.3-1 units) secretion.

5 These observations suggest that L-DOPA is probably converted to dopamine to stimulate the exocrine pancreas, and that dopamine interacts with the specific receptors.

6 The noradrenaline and dopamine content of the canine pancreas was estimated in controls and in dogs treated with secretin, reserpine, L-DOPA or α-methyldopa. The values for dopamine and noradrenaline in controls were 139 ± 6 and 375 ± 40 ng/g tissue (n=13), respectively. Reserpine reduced the noradrenaline content of the pancreatic tissue without affecting the dopamine content. L-DOPA or secretin caused a significant increase in the dopamine, but not in the noradrenaline content. It is suggested that dopamine has a physiological function in the pancreas which is independent of that of the noradrenaline-containing nerve fibres.

Introduction

It is well established that the exocrine secretion of the pancreas is controlled by two hormones, secretin (Bayliss & Starling, 1902) and pancreozymin (Harper & Raper, 1943). In 1942 Greengard, Roback & Ivy reported that pancreatic secretion can also be stimulated by epinine, dopamine and L-DOPA. This observation gained new interest when Alm, Ehinger & Falck (1969) observed a rapid turnover of 3-(3,4-dihydroxyphenyl)-L-alanine (L-DOPA) to 3-hydroxytyramine (dopamine) in the pancreas and Hashimoto, Satoh & Takeuchi (1971) confirmed the potent effect of L-DOPA and dopamine on exocrine pancreatic secretion. Furthermore it was found (Furuta, Hashimoto, Iwatsuki & Takeuchi, 1973) that the effect of L-DOPA was depressed by inhibitors of dopa decarboxylase whereas the effect of dopamine was potentiated by inhibitors of monoamine oxidase and diminished by haloperidol, a dopamine receptor blocking drug. No changes in pancreatic secretion were observed after injections of ephedrine, phenylephrine, methoxamine and tyramine (Takeuchi, Satoh & Hashimoto, 1973).

In the present studies a number of other compounds were tested for their ability to stimulate pancreatic secretion or to modify the effects of dopamine and L-DOPA. In addition the dopamine and noradrenaline content of the pancreas was measured in dogs treated with reserpine, α-methyldopa, secretin and L-DOPA.

Methods

Operative procedure

Mongrel dogs of either sex (8-17 kg) which had been fasted for 24 h were anaesthetized with pentobarbitone sodium 30 mg/kg intravenously. The pancreas was perfused with the animal's own blood from the femoral artery as described

previously (Hashimoto *et al.*, 1971; Furuta *et al.*, 1973). The rate of the exocrine secretion of the pancreas was measured by a drop counter and the blood flow was monitored by a magnetic flowmeter (Nihon Kohden MF-46). Drug solutions were injected over a period of 4 s into a rubber tube connected to the arterial cannula by a microinjecter (Jintan Terumo).

In the experiments in which the catecholamine content of the pancreas was measured, the abdomen of the anaesthetized dog was opened by a midline incision and the main pancreatic duct was carefully isolated from the duodenum. The duodenal wall was incised longitudinally and a polyethylene tube (i.d., 0.8 mm; o.d., 1.4 mm) was inserted into the main pancreatic duct in order to measure the secretion rate. The accessory pancreatic duct was ligated. After the resting rate of the pancreatic secretion had become constant, the animals were quickly bled from the carotid artery (control dogs, $n = 13$). A duodenal portion of the pancreas was removed 1.5 min later, rinsed with cold Ringer solution and immediately frozen in a freezer at -20° C.

In five dogs preparated in a similar fashion secretin was infused intravenously over 10 min at a rate of 0.1 unit kg^{-1} min^{-1}. In another 11 dogs L-DOPA was injected in a single dose of 5 mg/kg, intravenously. In the dogs treated with secretin and in five dogs given L-DOPA, the pancreas was removed when the increase in the secretion of the pancreatic juice reached a plateau; in six dogs treated with L-DOPA the pancreas was taken after the secretion had returned to the control rate.

Five dogs were pretreated with reserpine (0.1 mg/kg) daily for 5 days, three dogs with α-methyldopa (200 mg/kg) daily for 4 days before removal of the pancreas.

Chemical methods

The tissue was homogenized for 1 min in 10 volumes 0.4 N perchloric acid with an Ultra-turrax homogenizer. The catecholamines were absorbed on activated alumina, eluted with 0.05 N perchloric acid and converted to their fluorescent trihydroxyindole derivatives by oxidation with iodine. The fluorescence was read with a spectrofluorometer (Hitachi MPF-3). The activation and fluorescence wave lengths were 380 and 480 nm for noradrenaline and 325 and 370 nm for dopamine.

Drugs

The drugs were obtained from the following companies: dopamine hydrochloride, ICN; epinine hydrochloride, Sandoz (kindly provided by Dr K. Saameli); 6-hydroxydopamine hydrobromide, Sigma; α-methyldopamine hydrochloride, Nippon Roche (kindly provided by Dr L.M. Jampolsky) and Merck (Dr C.A. Stone); DL-octopamine hydrochloride, Sigma; apomorphine hydrochloride, Sandoz Yakuhin (kindly provided by Dr R. Toyoshima); secretin, Boots; pancreozymin, Boots; L-DOPA, Nippon Kayaku: α-methyldopa, kindly provided by Merck Banyu; reserpine, Ciba. All drugs were dissolved in 0.9% w/v NaCl solution immediately before use.

Statistical analysis

Student's t test was used.

Results

Effect of phenylethylamine derivatives on the pancreatic secretion

Epinine. Epinine injected intra-arterially caused a decrease in the pancreatic blood flow but its potency was only about one-tenth that of dopamine. No stimulation of the pancreatic secretion by epinine could be observed even at doses as large as 1 mg intra-arterially. A dose of 300 μg caused a slight inhibition of the spontaneous secretion, which lasted for a few minutes (Figure 1).

α-Methyldopamine. α-Methyldopamine (10 μg) caused a greater reduction in blood flow than dopamine (10 μg), and inhibited the spontaneous secretion for a few minutes at a dose of 300 μg as shown in Figure 1. After 300 μg α-methyldopamine, dopamine-induced secretion was slightly attenuated, but it soon returned to the initial rate.

Octopamine. Octopamine (10-300 μg) caused a reduction in blood flow but never modified the pancreatic secretion.

6-Hydroxydopamine. 6-Hydroxydopamine (10 μg) caused a decrease in blood flow and a slight stimulation of pancreatic secretion. The rate of secretion induced by 100 μg 6-hydroxydopamine was approximately equal to that of 3 μg dopamine (see Figure 1).

Anti-dopamine effect of apomorphine

Apomorphine (1 mg) intra-arterially caused a small, but long-lasting (about 2 h) stimulation of the pancreatic secretion and a transient increase in blood flow. When the rate of secretion returned to the initial value, the stimulating effect of

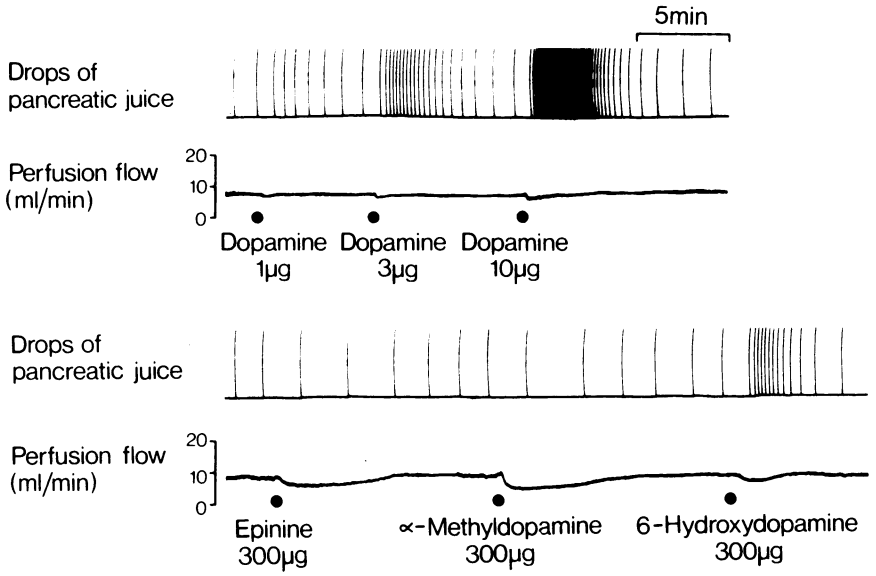

Fig. 1 Effects of dopamine, epinine, α-methyldopamine and 6-hydroxydopamine on the exocrine secretion of the dog isolated blood-perfused pancreas.

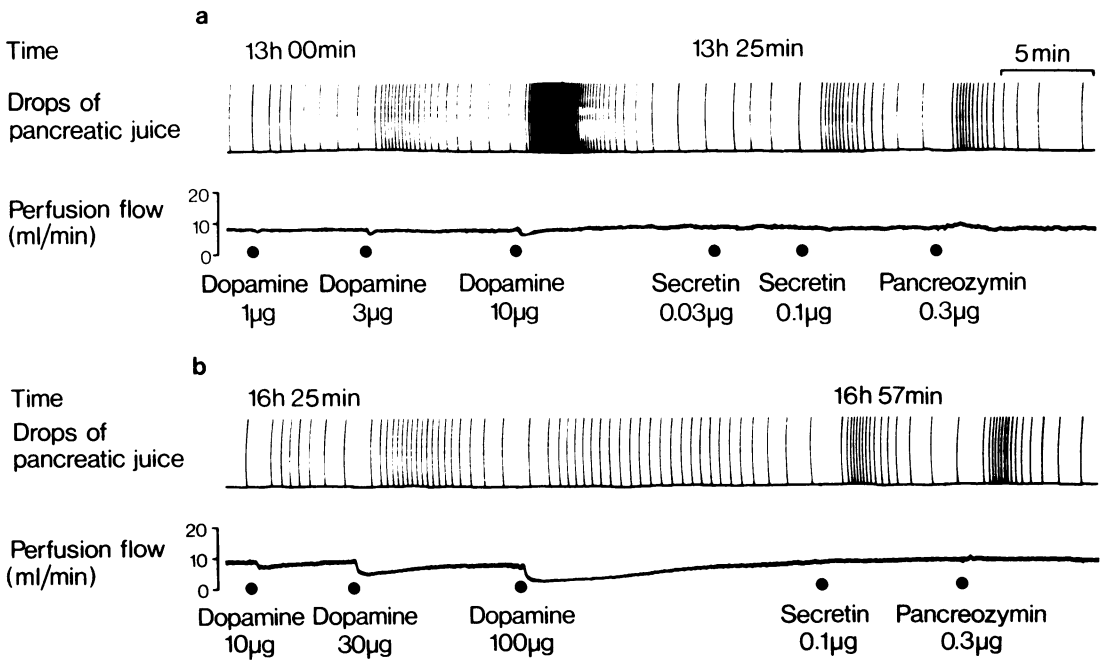

Fig. 2 Effects of apomorphine (1 mg i.a.) on pancreatic secretion induced by dopamine, secretin and pancreozymin. Each secretagogue was injected intra-arterially. (a) Control; (b) after apomorphine at 14 hours 25 minutes.

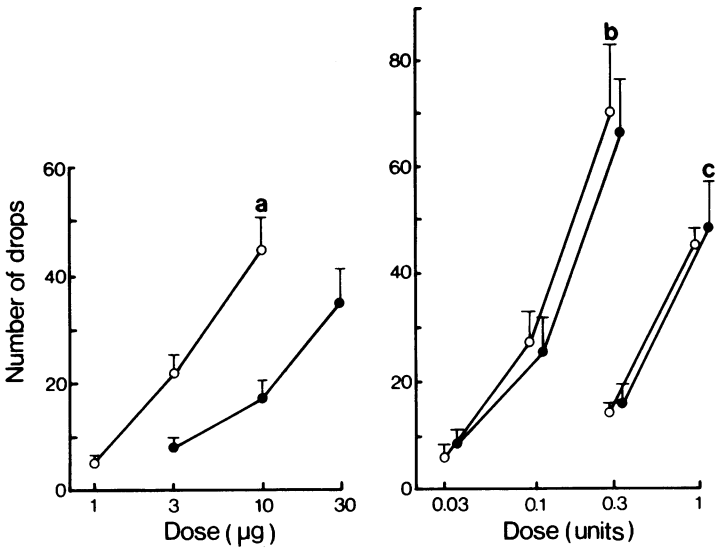

Fig. 3 Dose-response curves for pancreatic secretion in response to intra-arterial injections of (a) dopamine, (b) secretin and (c) pancreozymin before (○) and 2-3 h after (●) apomorphine (1 mg, i.a.). Each point represents the mean of 5 experiments in 5 dogs and vertical bars indicate s.e. mean. Apomorphine shifted the dose-response curve for dopamine significantly to the right ($P < 0.01$), but did not modify that for secretin or pancreozymin.

dopamine (3-30 μg) on the secretion was inhibited, while that of secretin (0.03-0.3 units) or pancreozymin (0.3-1 units) was not modified (see Figure 2). This anti-dopamine effect of apomorphine lasted for over 5 hours. Dose-response curves for the pancreatic secretion in response to each secretagogue before and after apomorphine (1 mg) are shown in Figure 3. Apomorphine shifted the dose-response curve for dopamine to the right ($P < 0.01$) but did not significantly alter that for secretin or pancreozymin.

Table 1 Catecholamines in the canine pancreas.

Treatment	*No. of animals*	*Dopamine (ng/g)*	*Noradrenaline (ng/g)*
None	13	139 ± 6	375 ± 40
Secretin (0.1 unit kg^{-1} min^{-1} for 10 min, i.v.)	5	221 ± 13*	431 ± 89
L-DOPA (5 mg/kg, i.v., at peak response)	5	818 ± 204*	563 ± 109
L-DOPA (3 mg/kg, i.v., after response)	6	543** ± 159*	380 ± 42
Reserpine (0.1 mg/kg daily for 5 days, s.c.)	5	135 ± 8	14 ± 5*
α-Methyldopa (200 mg/kg daily for 4 days, s.c.)	3	175 ± 31	100 ± 16*

Each value represents mean with s.e. mean.
* $P < 0.01$ when compared with no treatment.
** The lower dopamine content in the pancreas is probably due to the smaller dose of L-DOPA given in this group.

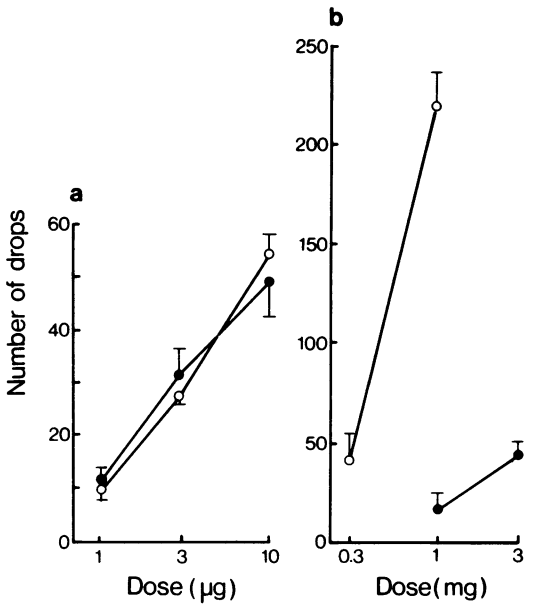

Fig. 4 Dose-response curves for pancreatic secretion in response to intra-arterial injections of (a) dopamine and (b) L-DOPA before (○) and 10-30 min after (●) α-methyldopa (30 mg, i.a.). Each point represents the mean of 5 experiments in 5 dogs and vertical bars indicate the s.e. mean. α-Methyldopa shifted the dose response curve for L-DOPA significantly to the right ($P < 0.01$) but not that for dopamine.

Antagonism between α-methyldopa and L-DOPA

An intra-arterial injection of α-methyldopa (30 mg) did not increase the spontaneous pancreatic secretion. After α-methyldopa (30 mg), the stimulating effect of L-DOPA (1-3 mg) intra-arterially was inhibited but that of dopamine (1-10 μg) was not modified. The inhibitory effect of α-methyldopa lasted for over 4 hours. Dose-response curves obtained from these experiments are shown in Figure 4. The stimulating effect of L-DOPA was significantly depressed by α-methyldopa ($P < 0.01$) but the dose-response curve for dopamine was not modified.

Catecholamine contents in the pancreas

The results are tabulated in Table 1. The amount of dopamine present in the unstimulated pancreas contributed 28.5 ± 1.9% of the total catecholamine present. In addition to dopamine and noradrenaline a trace of adrenaline (about 20 ng/g) could be detected in two control dogs. Secretin-treatment increased significantly the dopamine content ($P < 0.01$) without altering the noradrenaline content. Pretreatment of the dogs with reserpine reduced the noradrenaline content by 96% but did not alter the dopamine content. In the reserpine-treated dogs the spontaneous pancreatic secretion rate was the same as in untreated dogs. The stimulating effect of dopamine on the secretion was also not affected by pretreatment with reserpine. A nearly 6-fold increase in the dopamine content and also an increase (+50%) in the noradrenaline content of the pancreas occurred after L-DOPA administration when the tissue was removed at the height of the response. Even after L-DOPA-stimulated secretion was over, the dopamine value remained increased (see Table 1). Pretreatment with α-methyldopa decreased the noradrenaline content of the pancreas by 73%, dopamine was not significantly changed. The values for dopamine after α-methyldopa are probably slightly overestimated as α-methyldopa treatment can interfere with the estimation method used.

Discussion

In the present experiments the ability of several phenylethylamine derivatives to stimulate exocrine pancreatic secretion was tested. 6-Hydroxydopamine, a metabolite of dopamine in the rat (Senoh, Crevling, Udenfriend & Witkop, 1959) had about one-hundredth of the activity of dopamine on the pancreas of the dog. Epinine when injected intra-arterially into the dog perfused pancreas in doses of 0.3 and 1 mg had a depressant effect on the secretion of pancreatic juice. This is in conflict with results obtained previously by Greengard, Roback & Ivy (1942) who reported that the intravenous injection of epinine stimulated pancreatic secretion. However, after systemic injection the stimulation may be due to an indirect effect or due to contamination of epinine with dopamine. α-Methyldopamine and octopamine were also ineffective.

α-Methyldopa did not affect the response of the pancreas to dopamine. However, it depressed the secretion caused by L-DOPA probably as a consequence of its inhibition of dopa decarboxylase. It could also have acted by blocking the uptake of L-DOPA into the receptor cells as it was reported to inhibit the uptake of L-DOPA into the nerve endings of the isolated vas deferens of the guinea-pig (Thoa, Johnson & Kopin, 1971).

Apomorphine, a drug which was found to act as a dopamine-antagonist on renal vessels (Goldberg & Musgrave, 1971) and the vas deferens (Simon & Van Maanen, 1971) was also able to antagonize

the effect of dopamine on pancreatic secretion but did not influence the response to secretin or pancreozymin.

Our previous and present work thus provides evidence for the existence of a specific dopamine receptor in the pancreas which, when activated, stimulates the secretion of pancreatic juice. As dopamine antagonists do not interfere with the action of secretin or pancreozymin it is unlikely that the action of these two hormones is mediated by dopamine.

However, it is of great interest that the dopamine content of the pancreas increased after pancreatic secretion was stimulated by secretin. A participation of intra-pancreatic dopamine, which might be located at sites to which injected dopamine antagonists cannot penetrate, in the physiological mechanisms which lead to the secretion of pancreatic juice can therefore not be ruled out. Experiments are required in which the turnover rate of L-DOPA to dopamine can be tested in pancreatic tissue in the presence and absence of secretin.

Reserpine had a selective effect on pancreatic noradrenaline, without reducing its dopamine content; this was surprising, especially as Schümann & Heller (1959) found both catecholamines decreased in the pancreas of the sheep after reserpine treatment. However, in ruminants the dopamine in peripheral tissues is mainly located in mast cells (Falck, Nystedt, Rosengren & Stenflo, 1964). Reserpine treatment did not affect spontaneous pancreatic secretion in spite of the nearly complete loss of noradrenaline. Thus, a participation of noradrenergic nerves in exocrine pancreatic secretion is unlikely.

High dopamine content of the pancreas was found after the injection of L-DOPA. Even after its effect on the secretion had ceased, the dopamine values were still four times the normal values. This suggests that some of the surplus dopamine is stored in an 'inactive' state, possibly in zymogen granules.

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References

ALM, P., EHINGER, B. & FALCK, B. (1969). Histochemical studies on the metabolism of L-dopa and some related substances in the exocrine pancreas. *Acta physiol. scand.*, **76**, 106-120.

BAYLISS, W.M. & STARLING, E.H. (1902). The mechanism of pancreatic secretion. *J. Physiol., Lond.*, **28**, 325-353.

FALCK, B., NYSTEDT, T., ROSENGREN, E. & STENFLO, J. (1964). Dopamine and mast cells in ruminants. *Acta pharmacol. (Kbh.)*, **21**, 51-58.

FURUTA, Y., HASHIMOTO, K., IWATSUKI, K. & TAKEUCHI, O. (1973). Effects of enzyme inhibitors of catecholamine metabolism and of haloperidol on the pancreatic secretion induced by L-DOPA and by dopamine in dogs. *Br. J. Pharmac.*, **47**, 77-84.

GOLDBERG, L.I., & MUSGRAVE, G. (1971). Attenuation of dopamine-induced renal vasodilation by bulbocapnine and apomorphine. *Pharmacologist*, **13**, 227.

GREENGARD, H., ROBACK, R.A. & IVY, A.C. (1942). The effect of sympathomimetic amines on pancreatic secretion. *J. Pharmac. exp. Ther.*, **74**, 309-318.

HARPER, A.A. & RAPER, H.S. (1943). Pancreozymin, a stimulant of the secretion of pancreatic enzymes in extracts of the small intestine. *J. Physiol., Lond.*, **102**, 115-125.

HASHIMOTO, K., SATOH, S. & TAKEUCHI, O. (1971). Effect of dopamine on pancreatic secretion in the dog. *Br. J. Pharmac.*, **43**, 739-746.

SCHÜMANN, H.J. & HELLER, I. (1959). Über den Hydroxytyramingehalt der Organe. *Arch. exp. Path. Pharmak.*, **236**, 474-482.

SENOH, S., CREVELING, C.R., UDENFRIEND, S. & WITKOP, B. (1959). Chemical, enzymatic and metabolic studies on the mechanism of oxidation of dopamine. *J. Amer. chem. Soc.*, **81**, 6236-6240.

SIMON, A. & VAN MAANEN, E.F. (1971). Apomorphine and phentolamine antagonism of dopamine and norepinephrine. *Fed. Proc.*, **30**, 624.

TAKEUCHI, O., SATOH, S. & HASHIMOTO, K. (1974). Secretory and vascular responses to various biogenic and foreign substances of the perfused canine pancreas. *Jap. J. Pharmac.*, **24**, 57-73.

THOA, N.B., JOHNSON, D.G. & KOPIN, I.J. (1971). Inhibition of norepinephrine biosynthesis by α-methylamino acids in the guinea-pig vas deferens. *J. Pharmac. exp. Ther.*, **180**, 71-77.

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