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β-ADRENOCEPTOR STIMULATION OF EXOCRINE SECRETION FROM THE RAT PANCREAS

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1 Effects of catecholamines given intravenously on exocrine secretion from the pancreas were investigated in anaesthetized rats. The flow rate of pancreatic juice under resting conditions was 11.1 ± 3.2 μl per hour in 100 animals.

2 Dopamine (0.3–3 mg/kg) and isoprenaline (1–10 μg/kg) induced almost the same increase in the pancreatic secretion, so that dopamine was 300 times less potent than isoprenaline. The relative potency of the two amines for stimulation of pancreatic secretion was equivalent to that for β-stimulation of the contractile force of the left ventricle *in vivo*.

3 Propranolol (0.5 mg/kg) antagonized completely the dopamine- and isoprenaline-induced stimulation of the pancreatic secretion.

4 Haloperidol (10 mg/kg) failed to suppress the secretory effect of dopamine on the exocrine pancreas but abolished the dopamine-induced hypotension.

5 The dopamine-induced secretion was not modified by atropine (3 mg/kg), phenoxybenzamine (3 mg/kg), vagotomy or pithing.

6 Adrenaline and noradrenaline (10 μg/kg) induced secretion after phenoxybenzamine treatment (3 mg/kg).

7 It is suggested that the rat pancreas has a stimulatory β-adrenoceptor mechanism of exocrine secretion.

Introduction

Since the hormonal regulation of the exocrine secretion from the pancreas was established by the discoveries of secretin (Bayliss & Starling, 1902) and pancreozymin (Harper & Raper, 1943), autonomic nerve activities, especially cholinergic mechanisms (Dean & Matthews, 1972; Matthews & Petersen, 1973) have frequently been discussed in conjunction with the hormonal regulation, but there is little evidence in the literature to indicate that secretion of pancreatic juice is due to stimulation of adrenoceptors. Greengard, Roback & Ivy (1942) examined the actions of a large number of sympathomimetic amines administered intravenously on exocrine secretion from the canine pancreas and observed an inhibitory effect with almost all sympathomimetic amines except epinine, DOPA and dopamine which showed a definite secretory activity. Hashimoto, Satoh & Takeuchi (1971) confirmed an induction of a profuse flow of juice by dopamine given intra-arterially to the isolated blood perfused canine pancreas and they concluded that the canine pancreas has a specific dopamine receptor, stimulation of which induces a potent secretin-like response (Furuta, Iwatsuki, Takeuchi & Hashimoto, 1972; Furuta, Hashimoto, Iwatsuki & Takeuchi, 1973).

The present study was undertaken to examine the effects of these sympathomimetic amines on the exocrine secretion from the rat pancreas.

Methods

Male Wistar rats weighing from 280 to 350 g were fasted overnight before experiments but allowed to drink water *ad libitum*. The animals were anaesthetized with urethane (0.6 g/kg, i.p. and 0.6 g/kg, i.m.). The abdomen was opened through a midline incision and the pylorus was ligated. The proximal end of the common bile duct was cannulated with polyethylene tubing (PE 10, Clay Adams) and the bile was drained out. The distal end of the duct was cannulated with polyethylene tubing (o.d. 1.0 mm) close to the duodenal orifice, and the free end of the cannula was connected to a calibrated polyethylene tube (2 μl/cm

length) in order to measure the rate of flow of pancreatic juice. The incised abdomen was covered with a vinyl sheet and the animals were warmed under an electric lamp. The pancreatic secretion was measured at 10 or 30 min intervals. When the rate of secretion under resting conditions became constant approximately one hour after surgical procedure, the effects of drugs were tested. An injection of drug solution, 10 or 30 μl, was followed by injection of 0.2 ml of 0.9% w/v NaCl solution (saline). Drug effects on secretion are expressed in volume (μl) of pancreatic juice. The carotid artery blood pressure was measured by a pressure transducer (Nihon Kohden MPU-0.5) and the heart rate by a tachometer (Nihon Kohden RT-5) triggered by the pulse wave of blood pressure. Pithed rats were prepared as follows: under ether anaesthesia animals were tracheotomized and artificially respired with a respiration pump (Harvard, Model 680). After transection of the spinal cord at C1 with suprasegmental destruction, the spinal cord was destroyed by a stiff wire. Finally, the contractile force of the ventricle was measured in order to compare potencies of catecholamines for β-adrenoceptor stimulation between the pancreatic secretion and myocardial contraction in the rat. The technique was as follows: the animals were artificially respired and the chest was opened by a midline incision and a Walton-Brodie strain gauge arch was sutured to the left ventricle.

The following drugs were used: secretin (Boots), acetylcholine chloride (Daiichi), dopamine hydrochloride (Tokyo Kasei), L-β-3,4-dihydroxyphenylalanine (L-DOPA, Nippon, Kayaku), adrenaline hydrochloride (Daiichi), noradrenaline hydrochloride (Sankyo), isoprenaline hydrochloride (Nikken Kagaku), propranolol hydrochloride (Sumitomo Kagaku), phenoxybenzamine hydrochloride (Tokyo Kasei), benserazide hydrochloride (Ro 04-4602, Hoffmann-La Roche), atropine sulphate (Boehringer) and haloperidol (Dainippon Seiyaku). All drugs were dissolved in, and diluted with, saline to give the required final concentrations. One microgram of natural secretin (GIH Laboratory, Karolinska Institute) corresponds to 34 units of secretin (Boots) (Vagne, Stening, Brooks & Grossman, 1968; Takeuchi, Satoh & Hashimoto, 1974). All doses were expressed in terms of the salts except haloperidol and secretin.

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

Results

Exocrine secretion from the pancreas under resting conditions

A sparse flow of pancreatic juice was observed under resting conditions, the mean rate being 11.1 $\pm$ 3.2 μl per hour in 100 rats. The flow rate was almost constant in each case during a period of experiment of more than 5 hours.

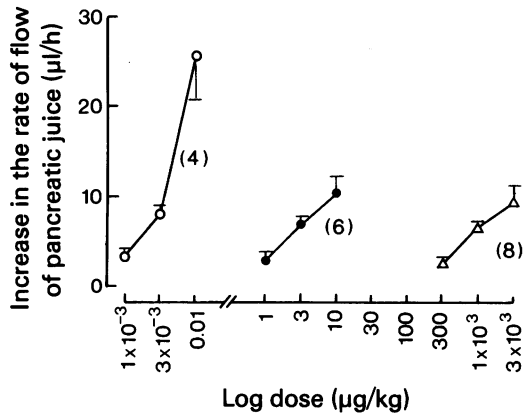

Figure 1 Dose-response curves for the increase in pancreatic secretion induced by secretin (○), isoprenaline (●) and dopamine (△) in anaesthetized rats. Vertical bars represent s.e. means and the figures in parentheses represents the number of experiments.

Effects of secretin

Secretin (1–10 ng/kg) greatly increased the secretion of pancreatic juice in a dose-dependent manner as shown in Figure 1. Neither the systemic blood pressure nor the heart rate were modified, even with the highest dose of secretin tested.

Effects of acetylcholine and catecholamines

Acetylcholine (0.1–1 mg/kg) increased the secretion of pancreatic juice, while it induced profound cardiovascular effects. A maximum secretory response was observed at a dose of 0.3 mg/kg and a further increase in dose decreased the volume of pancreatic juice as shown in Table 1.

Dopamine (0.3–3 mg/kg) caused an increase in pancreatic secretion in a dose-dependent manner although the potency was approximately 100,000 times less than that of secretin as shown in Figure 1. The dopamine-induced increase in secretion reached a maximum 10 min after the injection and returned to the initial level within 60 minutes. A rapid elevation of blood pressure followed by a long-lasting fall and a reflex bradycardia followed by a long-lasting tachycardia were induced but these responses disappeared within 15 to 20 minutes.

Noradrenaline or adrenaline (1–10 μg/kg) had little effect on the secretion of pancreatic juice, whereas isoprenaline (1–10 μg/kg) increased the exocrine se-

cretion from the pancreas in a dose-dependent manner (Table 1). Isoprenaline was approximately 300 times more potent than dopamine as shown in Figure 1. The effect of isoprenaline on pancreatic secretion lasted for approximately one hour. Noradrenaline and adrenaline induced a marked elevation of blood pressure and a reflex vagal stimulation, whilst isoprenaline caused hypotension and tachycardia which lasted for 10 to 30 minutes.

Effect of dopamine in vagotomized and pithed rats

Bilateral vagotomy at the cervical level did not affect the secretion of pancreatic juice under resting conditions or the dopamine-induced secretion, even though the reflex bradycardia was absent. The pancreatic secretion did not differ in pithed animals from the control values, not only under resting conditions but also during the stimulatory response to dopamine as shown in Table 1.

Antagonism of atropine, haloperidol, phenoxybenzamine and propranolol to acetylcholine- and catecholamine-induced secretion

Results are shown in Table 1. Atropine (3 mg/kg) completely blocked the secretory effect induced by acetylcholine but not that induced by dopamine. The reflex vagal stimulation induced by dopamine, i.e., bradycardia was completely abolished by atropine.

Haloperidol (10 mg/kg) did not modify the pancreatic secretion under resting conditions and failed to diminish the secretagogue effect of dopamine. A long-lasting fall in the systemic blood pressure induced by dopamine was greatly attenuated.

Phenoxybenzamine (3 mg/kg) did not modify the pancreatic secretion either under resting conditions or following the stimulatory response to dopamine. However, the same dose unveiled the stimulatory effects on pancreatic secretion of noradrenaline and adrenaline. Hypertension and reflex bradycardia induced by dopamine, noradrenaline and adrenaline were suppressed to a large extent by phenoxybenzamine.

Propranolol (0.5 mg/kg) significantly reduced pancreatic secretion under resting conditions and depressed completely the stimulatory responses to both dopamine and isoprenaline, while the tachycardia and hypotension caused by dopamine or isoprenaline were only partly attenuated by this dose of propranolol.

Effect of L-DOPA

L-DOPA (20 mg/kg) caused an apparent increase in pancreatic secretion. The response reached a maximum 20 to 30 min after the injection of L-DOPA and lasted for more than 2 h (Figure 2). Blood pressure and heart rate were scarcely changed by L-DOPA injection.

A dopa decarboxylase inhibitor, benserazide (3 mg/kg) clearly diminished the secretagogue effect of L-DOPA as shown in Figure 2, but did not affect the pancreatic secretion under resting conditions; 8.7 ± 1.0 and 9.8 ± 1.0 μl per hour, ($n = 6$), before and after treatment respectively.

Comparison of inotropic potency between dopamine and isoprenaline

Intravenous injection of 3 to 30 μg/kg of dopamine had a positive inotropic effect on the rat left ventricle which was dose-dependent. The inotropic potency of dopamine was approximately 300 times less than that of isoprenaline (Figure 3), which was almost the same potency ratio as that for the secretagague effect of these two amines.

Discussion

In the present study, intravenous administration of secretin (0.003 to 0.01 μg/kg:1 μg = 34 units) caused a profuse flow of pancreatic juice in the rat, whilst almost the same dose-response relation was observed with 0.1 to 0.3 units/kg given intravenously in the dog in a previous study (Hashimoto *et al.*, 1971). Furthermore, a stimulatory effect of acetylcholine, though not so prominent, was completely antagonized by atropine in both rat and dog. From these results the authors concluded that common mechanisms were responsible in both species for the stimulatory effects of secretin and acetylcholine on exocrine secretion from the pancreas.

In contrast, the effects of catecholamines on pancreatic secretion were different in rat and dog, not only in the doses that were effective but also in the pattern of responses. In this study dopamine (1 to 3 mg/kg i.v.) caused a marked increase in secretion in the rat, while a similar effect was observed with 10 to 30 μg/kg of dopamine given intravenously in the dog in the previous studies referred to above. Thus, dopamine is 100 times more potent in the dog than in the rat. Furthermore the secretagogue effect of dopamine in the rat was completely antagonized by propranolol but not by haloperidol, while in the dog it was completely blocked by haloperidol but not by propranolol (Hashimoto *et al.*, 1971; Furuta *et al.*, 1973). A definite increase in pancreatic secretion in the rat was induced by isoprenaline which was 300 times more potent than dopamine and was completely blocked by propranolol. This difference in potency for the pancreatic secretion was almost equivalent to that of β-activity on the contractile force

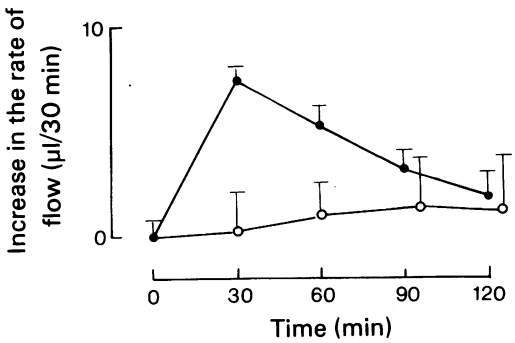

Figure 2 Effect of L-DOPA (20 mg/kg) on pancreatic secretion before (●) and after (○) benserazide (3 mg/kg) in anaesthetized rats. Each value is the mean of six experiments and vertical bars indicate s.e. means.

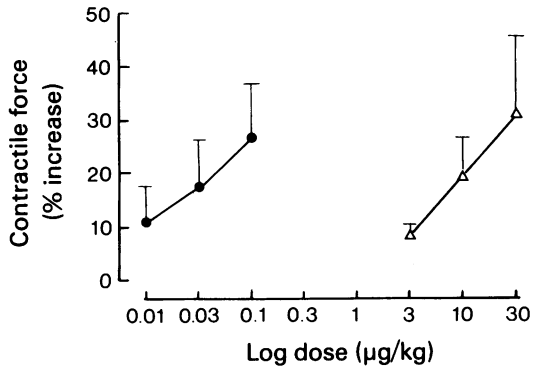

Figure 3 Dose-response curves for percentage increase in the contractile force of the left ventricle *in vivo* with isoprenaline (●) and dopamine (△) in anaesthetized rats. Each value is the mean of four experiments and vertical bars represent s.e. means.

Table 1 Effects of intravenous administration of secretagogues on the rat exocrine pancreas

	Pancreatic secretion (μl/h) before and after treatment						
Drugs	*Before treatment*	*After atropine*	*After haloperidol*	*After phenoxybenzamine*	*After propanolol*	*After vagotomy*	*After pithing*
Acetylcholine							
Control	8.0 ± 1.1	7.6 ± 1.2					
0.1 mg/kg	10.7 ± 1.6						
0.3 mg/kg	16.1 ± 2.8*	6.2 ± 0.7					
1 mg/kg	14.7 ± 2.0*						
Dopamine							
Control	9.5 ± 0.8	10.2 ± 1.2	10.2 ± 2.0	9.7 ± 1.7	5.3 ± 0.7*	10.1 ± 2.4	11.6 ± 0.8
0.3 mg/kg	12.2 ± 0.6*			13.4 ± 0.8		11.2 ± 1.0	
1 mg/kg	15.8 ± 1.1*	15.0 ± 1.5*	17.6 ± 2.1*	16.0 ± 2.3*	2.9 ± 0.3	15.6 ± 1.7*	18.5 ± 2.7*
3 mg/kg	18.5 ± 2.3*					17.9 ± 1.8*	
Isoprenaline							
Control	10.1 ± 1.0				6.7 ± 0.6*		
1 μg/kg	13.0 ± 0.9						
3 μg/kg	17.1 ± 0.9*				6.0 ± 1.4		
10 μg/kg	20.3 ± 1.2*						
Noradrenaline							
Control	10.5 ± 1.5			9.3 ± 0.7			
1 μg/kg	10.0 ± 1.3			11.2 ± 1.1			
3 μg/kg	11.1 ± 1.3			12.7 ± 1.1			
10 μg/kg	12.8 ± 0.7			15.1 ± 1.5*			
Adrenaline							
Control	8.2 ± 0.9			7.7 ± 1.5			
1 μg/kg	7.6 ± 2.1						
3 μg/kg	9.5 ± 1.6						
10 μg/kg	10.6 ± 1.7			16.1 ± 2.1*			

Values represent the mean ± s.e. mean of experimental results obtained from six animals.
* $P < 0.05$.

of the left ventricle. In contrast, isoprenaline was ineffective in stimulating pancreatic secretion in the dog. From these results, the dopaminergic mechanism of exocrine secretion from the pancreas, which was shown to exist in the dog, is excluded in the rat, in which species β-adrenoceptor mechanism has been demonstrated in the present study.

Noradrenaline or adrenaline (3 to 10 μg/kg) given intravenously did not increase the exocrine secretion from the pancreas in the rat and decreased secretion in the dog; phenoxybenzamine treatment unveiled their secretagogue effects in the rat. In the dog, no stimulator-effect was observed even after phenoxybenzamine treatment. These results are consistent with a β-adrenoceptor stimulatory mechanism in the rat but a dopaminergic mechanism in the dog. Concerning α-adrenoceptor inhibition of the pancreatic exocrine secretion in the dog, Greengard *et al.* (1942) ascribed the reduction of secretion to the secondary effect of vasoconstriction, while Barlow, Greenwell, Harper & Scratcherd (1971) reported a direct inhibitory effect of catecholamines on the secretory cells of the pancreas. In the isolated, blood-perfused canine pancreas, there was no clear relation between vascular and secretory responses (Takeuchi *et al.*, 1974). Although it is not easy to reach a definite conclusion on this relation, the α-adrenoceptor inhibitory mechanism seems to be more dominant than the β-adrenoceptor stimulatory mechanism in the rat.

L-DOPA induced a definite increase in pancreatic secretion after a certain delay in the response in rat and dog, which was completely antagonized by dopa decarboxylase inhibitors (Takeuchi *et al.*, 1971; Furuta *et al.*, 1973; Furuta, Hashimoto, Ishii & Iwatsuki, 1974). These observations suggest that the effect of L-DOPA is due to its conversion to dopamine in the exocrine cells of the pancreas (Alm, Ehinger & Falck, 1969).

The results indicate a definite species difference in the sympathetic control of pancreatic secretion at least between dog and rat.

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