An Increase of Tissue Cyclic AMP Level by Adenosine in the Dog Pancreas without Stimulation of Exocrine Secretion

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Abstract—In the vascularly isolated and self-hemoperfused dog pancreas, secretin (0.025 clinical units) and adenosine (1.0 mg) administered intra-arterially (i.a.) increased tissue cyclic AMP level by about 46% and 37%, respectively. Although the increases in the nucleotide were not statistically different, only secretin stimulated exocrine secretion. The increase in cyclic AMP induced by adenosine was significantly reversed by pretreatment with theophylline (0.3 mg, i.a.). These results suggest that the effect of adenosine on cyclic AMP formation occurs mainly in the non-exocrine system of the dog pancreas through A2/Ra-receptors.

Accumulated evidence indicates that secretion of water and electrolytes from pancreatic exocrine glands is mediated intracellularly, at least in part, through adenosine 3',5'-cyclic monophosphate (cyclic AMP) (1-4). On the other hand, Burnstock (5) postulated that purine-related compounds act on two types of receptors: P1- and P2-receptors, the P1-receptor being involved in the activation or inhibition of adenylate cyclase. Along this line, it seems worthwhile to investigate the effect of adenosine on tissue cyclic nucleotide concentration in the pancreas. In the present study, we compared secretin and adenosine in the stimulation of exocrine secretion and in the formation of tissue cyclic AMP in the vascularly isolated and self-hemoperfused dog pancreas (6).

Forty-seven adult mongrel dogs of either sex, weighing from 7 to 12 kg, were used. They were fasted for 24 hr and anesthetized with 30 mg/kg of sodium pentobarbital given i.v. During the experimental periods, anesthesia was maintained by additional injections of sodium pentobarbital (5 mg/kg, i.m.). An endotracheal tube was inserted, and animals were ventilated artificially with room air (Harvard Apparatus, Model 607). The supra-umbilical abdomen was opened by a midline incision. Polyethylene cannulae were inserted into the pancreaticoduodenal and splenic arteries, and the pancreas was perfused with the animals' own blood pumped from the right femoral artery (Harvard Apparatus, Model 1210). A dose of 500 USP units/kg of sodium heparin was given i.v. at the beginning of the perfusion and maintenance doses of 200 USP units/kg were given hourly. All experiments were performed at constant perfusion flow rates (16.3±0.9 ml/min, mean±S.E., n=6) which were determined at the beginning of the experiments under constant perfusion pressure (100 mmHg) and were kept constant during the experiments. Details of the vascularly isolated and self-hemoperfused preparation were described in a previous paper (6). The blood flow to the pancreas was measured by an electromagnetic flow-meter (Nihon Kohden, Model MF-25), and the perfusion pressure was determined by a transducer (Nihon Kohden, Model RP-3). The rate of pancreatic secretion was monitored with a drop counter attached to the tip of the pancreatic cannula, and the secretory volume was measured with a graduated cylinder. The drugs used in this study were secretin (1.0 mg=3.75x10^4 clinical units, Boots), adenosine (Wako Pure Chemical Industries), papaverine hydrochloride (Dainippon Seiyaku) and theophyl-

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line (Sigma). Secretin was dissolved in saline to which bovine serum albumin had been added to give a 0.1% solution (7), and other drugs were dissolved in saline. All drugs except for papaverine were injected into Y-shaped rubber tubing connected to the two arterial cannulae over 4 sec (i.a.). Papaverine was infused into the same rubber tubing by means of a Harvard infusion pump (Model, 901D) at a rate of 0.5 mg/min.

Before and 3 min after a single injection of secretin (0.025 units, i.a.) or adenosine (1.0 mg, i.a.), or 4 min after theophylline (0.3 mg, i.a.), a small piece of the pancreatic tissue was quickly excised from an arbitrary part of the pancreas and blotted with a filter paper, and then it was immediately frozen in liquid nitrogen. Frozen sections were lyophilized under a vacuum overnight. Lyophilized sections, after being weighed (about 50 mg), were homogenized in 4 ml of 6% trichloroacetic acid by an ultra-disperser (Yamato Scientific Co., Model LK-21). The homogenate was centrifuged at 3000×g for 15 min. The supernatant was washed 3 times with 6 ml of water-saturated ether in order to remove trichloroacetic acid. After succinylation of nucleotides in the supernatant, the cyclic AMP and guanosine 3',5'-cyclic monophosphate (cyclic GMP) concentrations were determined by radioimmunoassay using 125I-RIA kits (Yamasa Shoyu Co.). Statistical analyses were performed by means of paired and unpaired t-tests, and a P value of less than 0.05 was considered as statistically significant. The present study was performed from June, 1985 to February, 1986, and sampling of the pancreatic tissue for the measurement of cyclic nucleotides was carried out during the summer.

Adenosine at doses up to 1.0 mg, i.a., did not affect the resting rate of pancreatic secretion (refs. 8, 9 and Fig. 1A). In 4 experiments, the effect of adenosine on papaverine (0.5 mg/min, i.a.)-stimulated exocrine secretion was investigated. The resting rate of pancreatic secretion was 101.7±28.4 µl/15 min (mean±S.E., n=4). During a background infusion of papaverine (0.5 mg/min, i.a.), the secretory rate increased to 548.5±30.8 µl/15 min (mean±S.E., n=4). In this condition (100%/3 min), a single injection of adenosine (1.0 mg, i.a.) caused a slight but appreciable increase in the secretory rate (135.8±7.6%/initial 3 min, mean±S.E., n=4, P<0.05), as shown in Fig. 1B.

Three min after adenosine (1.0 mg, i.a.), tissue cyclic AMP level increased significantly by about 37%. The effect of adenosine, however, was significantly inhibited by theophylline (0.3 mg, i.a.) administered 1 min before adenosine. Theophylline (0.3 mg, i.a.) itself did not affect cyclic AMP level of the tissue (Fig. 2) and secretin (0.025 units, i.a.).
stimulated exocrine secretion (8). The summarized data are shown in Fig. 2. With regard to cyclic GMP, the values after saline, theophylline and adenosine were 103.2±4.3, 103.8±5.3 and 110.7±5.3% (mean±S.E., n=6), respectively. Thus, adenosine did not affect the tissue cyclic GMP level. Three min after secretin (0.025 units, i.a.), the tissue cyclic AMP increased significantly (Fig. 2), but the value of tissue cyclic GMP was not affected (-1.7±4.4%, mean±S.E., n=6, P>0.05). Control, absolute values after saline injection for cyclic AMP and cyclic GMP were 3.1±0.2 pmol/mg dry weight and 75.2±3.2 fmol/mg dry weight (mean±S.E., n=6), respectively.

In the present study, adenosine increased tissue cyclic AMP level, and the effect was significantly inhibited by theophylline. Theophylline is a competitive inhibitor of the P1-receptors (5). Since 0.3 mg of theophylline, i.a. did not increase tissue cyclic AMP level as shown in Fig. 2, the inhibitory concentration of theophylline for the pancreatic phosphodiesterase seems to be higher than the concentration used here: in this study, 0.3 mg of theophylline, i.a., resulted in a transient peak concentration of 1.54 mM, roughly calculated from the mean perfusion flow rate and the rate of drug administration. Thus we concluded that there are adenosine A2/R1-receptors, the subtype of the P1-receptors which activate adenylate cyclase (10, 11), in the dog pancreas.

Since indomethacin and meclofenamate, potent cyclooxygenase inhibitors, did not affect dopamine- or secretin-stimulated hydro-mineral secretion in the dog (12) and since exogenously applied prostaglandin (PG) E2, PG12, PGD2 and thromboxane B2 did not influence secretin- or dopamine-stimulated, or basal exocrine secretion (12, 13), the P2-receptors, if there were any in the dog pancreas, seem to have no significant role in pancreatic exocrine secretion.

Cyclic AMP has been thought to be an important intra-cellular mediator of hydro-mineral secretion from pancreatic exocrine glands, especially the ductular system (1–4). In this study, secretin (0.025 units, i.a.) and adenosine (1.0 mg, i.a.) increased tissue cyclic AMP by about 46% and 37%, respectively. Although the difference of the increase of cyclic AMP is not significant (P>0.05), only secretin stimulated pancreatic exocrine secretion (refs. 8, 9 and Fig. 1A). The discrepancy may be explained as follows: The adenosine effect on cyclic AMP formation only partially occurred in the exocrine system. In other words, the net increase of cyclic AMP by adenosine seems to reflect mainly the response of non-exocrine tissues of the pancreas.

Papaverine is a potent phosphodiesterase inhibitor and not a xanthine derivative, and it can stimulate hydromineral exocrine secretion in the dog pancreas (3). In addition, papaverine was shown to stimulate cyclic AMP formation in the pancreatic tissue of the dog without affecting the tissue cyclic GMP (3). Since adenosine enhanced papaverine-stimulated exocrine secretion (Fig. 1B), it is probable that adenosine acted on the A2/R1-receptors in the ductular cells to promote the formation of cyclic AMP to a subthreshold level.

It is concluded that adenosine acts on pancreatic exocrine glands, especially the ductular system, to promote cyclic AMP formation to a subthreshold level for triggering exocrine secretion and that the net increase in the tissue cyclic AMP induced by adenosine seems to reflect mainly the response of non-exocrine tissues of the pancreas, since adenosine increased tissue cyclic AMP level to a similar extent to secretin, but did not stimulate pancreatic exocrine secretion.

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