Effects of Synthesized Phosphodiesterase Inhibitors, DM 9278 and HWA 285, on Pancreatic Exocrine Secretion of the Dog

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Abstract—The effects of synthesized phosphodiesterase inhibitors, DM 9278 and HWA 285, on pancreatic exocrine secretion were investigated in isolated and blood-perfused canine pancreas. Close-arterial injections of DM 9278 (10–300 μg) and HWA 285 (300–3000 μg) caused dose-dependent increases in the flow rate of pancreatic juice and perfusion blood flow. Bicarbonate concentration in the pancreatic juice stimulated by DM 9278 (300 μg) or HWA 285 (3000 μg) was significantly higher than that in the resting pancreatic juice, although neither of the compounds affected protein concentrations in the pancreatic juice. In the secretory volume, 100 μg of DM 9278 corresponded roughly to 1000 μg of HWA 285, 0.1 units of secretin or 0.3 units of pancreozymin. These secretory and vascular effects were not modified by pretreatment with atropine or sulpiride. This study suggests that both DM 9278 and HWA 285 act directly on ductular cells of the pancreas and induce secretion of water and electrolytes.

In pancreatic exocrine secretion, it is well established that adenosine 3’,5’-cyclic monophosphate (cyclic AMP) is a mediator of secretin-induced fluid secretion (1, 2). Additionally, guanosine 3’,5’-cyclic monophosphate (cyclic GMP) seems to be a possible intracellular mediator of enzyme secretion (3–5). Along this line, it is of interest to investigate the effects of phosphodiesterase (PDE) inhibitors on the pancreatic exocrine secretion. In fact, several PDE inhibitors such as theophylline, amino-phylline, papaverine and nicardipine were shown to stimulate pancreatic exocrine secretion (6–8).

In the present study, we employed two of the newly synthesized PDE inhibitors: one was HWA 285, a xanthine derivative (9, 10), and the other was DM 9278, an imidazoquinazolinone derivative (11). The effects of the two compounds on isolated, blood-perfused canine pancreas were investigated.

Materials and Methods

Sixteen adult mongrel dogs of either sex, weighing 17.1 kg on the average, were used. They were fasted for 24 hours before experiments and anesthetized with sodium pentobarbital (30 mg/kg, i.v.). The supranavel abdomen was opened by a midline incision. A polyethylene tube was inserted into the main pancreatic duct for collection of the pancreatic juice. The accessory pancreatic duct was ligated. Polyethylene cannulae were inserted into the pancreaticoduodenal and splenic arteries through which the pancreas was perfused with the animal’s own blood conducted from the right femoral artery by means of a Harvard peristaltic pump (Model 1210). All experiments were performed under constant perfusion pressure at 100 mmHg. The experimental setup has been previously described in detail (12, 13). A dose of 500 units/kg of sodium heparin was given i.v. at the beginning of perfusion and maintenance doses of 2000 units per dog.
were given hourly. The rate of pancreatic secretion was measured with a drop counter, and the volume of pancreatic juice was measured with a graduated cylinder. The concentration of protein was determined by the method of Lowry et al. (14), with bovine serum albumin as the standard. The concentration of bicarbonate was measured by a blood-gas analyzer (Corning, Model 165/2) which requires a minimal sample volume of 175 μl. Drugs used in this study were 1, 2, 3, 4, 8, 9, 10, 12-octahydro-[2, 1-b]-pyrido-[2, 3-f]-quinazolin-9-one (DM 9278; synthesized by Daiichi Seiyaku, Tokyo, Japan; mol. wt. 242.28; Fig. 1), 1-(5-oxohexyl)-3-methyl-7-propylxanthine (HWA 285; synthesized by Höchst A.G., Werk Albert, Wiesbaden; mol. wt. 306.36; Fig. 1), secretin (Eisai), pancreozymin (Eisai), atropine sulphate (Torii) and sulpiride (Fujisawa). Secretin was dissolved in 0.9% saline solution to which bovine serum albumin had been added, to give a 0.1% solution (15), and other drugs in 0.9% saline solution. Drugs were injected into a rubber tubing connected to the shanks of arterial cannulae over a 4 sec period. The doses of secretin and pancreozymin are indicated in Crick, Harper and Raper units (16). Statistical analysis was carried out by means of Student's t-test. A P value less than 0.05 was considered statistically significant.

Results

Effects of close-arterial DM 9278 and HWA 285 on the secretion of pancreatic juice and pancreatic blood flow: A flow of pancreatic juice was observed in the resting state, the rate being 48.2±0.7 μl/10 min (mean±S.E.M. of 7 experiments). The pancreatic perfusion flow was 18.5±2.2 ml/min (mean±S.E.M. of 9 experiments).

Typical secretory and vascular responses to DM 9278, HWA 285 and secretin are illustrated in Fig. 2. Within a minute or so after an injection of each compound, a flow of pancreatic juice began to increase and reached maximum in two or three minutes. Then it gradually returned to the resting level. Secretory responses to DM 9278, HWA 285, secretin and pancreozymin injected close-arterially are expressed in Fig. 3, in combination with the data for nicardipine, papaverine and aminophylline cited from a previous report by Iwatsuki et al. (8) who performed studies on the same preparation as employed here. Both DM 9278 and HWA 285 dose-dependently increased the secretory volume of pancreatic juice. These secretagogues may be arranged in the following order of secretory potency: nicardipine>DM 9278>papaverine>HWA 285>aminophylline. Even on a molar basis, this order was unchanged. Dose-dependent increases in perfusion blood flow induced by DM 9278 and HWA 285 are presented in Fig. 4. At 300 μg, increases in perfusion flow induced by the two compounds were almost the same, while secretory response to DM 9278 was far greater than that to HWA 285 as shown in Fig. 3. On the contrary to the conspicuous vascular effect of DM 9278 or HWA 285, secretin caused a very small vascular response (Fig. 2).

Absence of blocking effect of atropine and sulpiride on the secretory and vascular responses to DM 9278 and HWA 285: Blocking agents were given close-arterially 2 min prior to the injections of DM 9278 or
HWA 285. The doses administered were 200 μg of atropine and 1000 μg of sulpiride in each of the 4 experiments. Both of these blocking agents did not appreciably alter the

Fig. 2. Typical secretory and vascular responses to DM 9278 (100 μg), HWA 285 (1000 μg) and secretin (0.1 units) from an isolated and blood-perfused canine pancreas. Drugs were injected close-arterially. PF indicates the rate of perfusion blood flow.

Fig. 3. Secretory responses to nicardipine (a), DM 9278 (b), papaverine (c), HWA 285 (d), aminophylline (e), secretin (f) and pancreozymin (g) injected close-arterially. All points indicate mean values and vertical bars standard error of the mean from 6 experiments. a, c and e were cited from a previous report by Iwatsuki et al. (8) and are expressed in broken lines. Pancreatic juice was collected from the beginning until the end of the secretory response.
secretory or vascular responses to DM 9278 and HWA 285. However, atropine (200 μg, i.a.) inhibited the secretory and vascular responses to acetylcholine, and sulpiride (1000 μg, i.a.) inhibited those to dopamine.

Effects of DM 9278, HWA 285, secretin and pancreozymin on the bicarbonate and protein concentration in the pancreatic juice: Summarized data are shown in Table 1. DM 9278, HWA 285 and secretin did not affect the protein concentration significantly, while pancreozymin significantly and dose-dependently increased it. As for bicarbonate concentrations in the pancreatic juice, DM 9278, HWA 285 and secretin at the largest doses used significantly increased it, but pancreozymin did not affect it. Composition of the pancreatic juice stimulated by DM 9278 or HWA 285, therefore, resembled to that stimulated by secretin.

**Table 1.** Effects of close-arterial injections of DM 9278, HWA 285, secretin and pancreozymin on the protein and bicarbonate concentration in pancreatic juice

| Compounds          | Protein concentration (mg/ml) | HCO₃⁻ concentration (mmol/liter) |
|---------------------|--------------------------------|----------------------------------|
| Resting state (n=5) | 31.7± 2.8                      | 18.7± 3.0                        |
| DM 9278 (μg, n=6)   |                                |                                  |
| 10                  | 39.1± 8.6                      | NT                               |
| 30                  | 41.6± 9.0                      | NT                               |
| 100                 | 33.4± 8.7                      | NT                               |
| 300                 | 38.2± 7.7                      | 89.8±18.6*                      |
| HWA 285 (μg, n=6)   |                                |                                  |
| 300                 | 30.2± 4.7                      | NT                               |
| 1000                | 39.0± 4.8                      | NT                               |
| 3000                | 38.6± 4.2                      | 94.1± 7.9***                    |
| Secretin (units, n=6) |                                |                                  |
| 0.03                | 32.6± 4.3                      | NT                               |
| 0.1                 | 24.6± 3.3                      | NT                               |
| 0.3                 | 26.0± 5.3                      | 74.2± 5.5***                    |
| Pancreozymin (units, n=6) |                        |                                  |
| 0.1                 | 37.8± 3.8                      | NT                               |
| 0.3                 | 61.8± 12.9*                    | NT                               |
| 1.0                 | 77.5± 8.0***                   | 22.8± 3.8                       |

Each value is the mean±standard error. Pancreatic juice was collected from the beginning until the end of the secretory response. Asterisks indicate statistical significance at *P<0.05, **P<0.01 and ***P<0.001, when compared with resting values. NT, not tested.

**Discussion**

Evidence has accumulated that fluid secretion of the pancreas is largely a function of secretin whose effect is mediated intracellularly, at least in part, through cyclic AMP (1, 2). With regard to cyclic GMP, pancreozymin and cholinergic drugs increase cyclic GMP concentrations in the pancreas associated with amylase secretion in isolated guinea-pig pancreas slices (4, 16). Moreover, exogenously applied cyclic GMP or 8-bromo cyclic GMP increased enzyme secretion in
dispersed acini from the guinea-pig pancreas (18) and the perfused dog pancreas (5).

It has been reported that many of the synthesized PDE inhibitors inhibit not only cyclic AMP hydrolysis but also cyclic GMP hydrolysis (19, 20). In the present study, two of the newly synthesized PDE inhibitors were shown to have a secretory property, and the pancreatic juice induced by them was rich in bicarbonate rather than protein. That is, both DM 9278 and HWA 285, like secretin, elicited fluid secretion, suggesting cyclic AMP increase in the pancreatic tissue rather than cyclic GMP increase. These results may be explained by assuming that both of the compounds are more specific to cyclic AMP PDE than cyclic GMP PDE so protein secretion which occurred to some extent was masked by the dominant fluid secretion.

DM 9278- or HWA 285-induced vasodilation may not contribute to its secretory action, because vasodilators such as isoproterenol or bradykinin never increased the rate of pancreatic secretion as reported before (6). Moreover, the resting secretion of the pancreatic juice is unchanged by a transient increase in the perfusion flow rate (7).

Since the secretory and vascular responses to DM 9278 or HWA 285 were not modified by pretreatment with atropine and sulpiride, neither a cholinergic nor a dopaminergic effect is involved in the responses (21–23).

As a cyclic AMP PDE inhibitor, the potency of each compound, employed or cited here, was reported as follows: Nicardipine is 3 to 4 times as potent as papaverine (24) and about 100 times as potent as aminophylline (25). HWA 285 is 3 to 9 times as potent as theophylline (10). DM 9278 is 14 to 56 times as potent as papaverine (S. Chiba et al., unpublished data). Papaverine is about 11 times as potent as theophylline (26). Although these investigations were made on different preparations, the ranking order of inhibitory potencies of these compounds for PDEs may be: DM 9278 > nicardipine > papaverine > HWA 285 > aminophylline. This order and the order of their secretory potencies are almost the same, confirming that cyclic AMP is an important intracellular mediator of fluid secretion. The reversed order of nicardipine and DM 9278 may be explained in the same way as Endoh et al. (27) did: The direct actions of PDE inhibitors are dependent 1) on the potency to inhibit PDE activity in the cell-free preparation and 2) on the ease with which the cell membrane is penetrated. That is, nicardipine is a less potent PDE inhibitor than DM 9278, but may penetrate the cell membrane more easily than DM 9278.

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