Effects of DN-9693, a Synthesized Phosphodiesterase Inhibitor, on Pancreatic Exocrine Secretion in Dogs

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Abstract—The effects of DN-9693, a synthesized phosphodiesterase inhibitor, on the secretion of pancreatic juice were investigated in preparations of the isolated and blood-perfused dog pancreas. DN-9693 injected intraarterially caused a dose-dependent increase in the secretion of pancreatic juice and decrease in the perfusion pressure. The threshold doses to increase the pancreatic secretion and to decrease the perfusion pressure were about 100 µg and 1 µg, respectively. Thus, the secretory response was less effective than the vascular response. The secretory activity of DN-9693 (0.3 mg) was approximately equal to that of 0.03 mg of 3-isobutyl-1-methylxanthine, 0.5 mg of papaverine, 5 mg of theophylline, 0.08 units of secretin and 0.2 units of cholecystokinin. The concentration of bicarbonate in the pancreatic juice induced by DN-9693 was increased, but protein concentration was not. DN-9693-induced pancreatic secretion was not modified by pretreatments with phentolamine, propranolol, atropine, sulpiride and cimetidine. Secretin-induced pancreatic secretion was significantly potentiated by infusion of DN-9693 (10 µg/min), but cholecystokinin-induced one was not. From these results, it is concluded that DN-9693 may produce an increase in pancreatic secretion by acting directly on the pancreatic exocrine gland of the dog, which might be mediated through an increase of intracellular cyclic AMP concentration by inhibiting phosphodiesterase activity.

It was reported that adenosine 3',5'-cyclic monophosphate (cyclic AMP) stimulated the pancreatic secretion of water and electrolytes in cats and dogs (1, 2). Additionally, secretin stimulated the pancreatic secretion concomitant with the increase in the cyclic AMP content in the pancreas (3, 4). Phosphodiesterase inhibitors such as theophylline, papaverine and 3-isobutyl-1-methylxanthine (IBMX) were also shown to stimulate the pancreatic secretion (2, 5, 6). Thus, cyclic AMP may be an intracellular mediator in the process of pancreatic secretion. Along this line, it is of interest to investigate the effect of phosphodiesterase inhibitors on pancreatic secretion. DN-9693 (1,5-dihydro-7-(1-piperidinyl)-imidazo[2,1-b]quinazoline-2(3H)-one dihydrochloride hydrate) is a newly synthesized phosphodiesterase inhibitor and its potency was about 5 times as potent as papaverine in platelets (7). However, there is no available reports on the effect of DN-9693 on pancreatic exocrine secretion. The present experiments were, therefore, undertaken to investigate the effects of DN-9693 on pancreatic secretion in isolated and blood-perfused day pancreas preparations in situ and to compare DN-9693 with secretin and cholecystokinin in the same preparations.

Materials and Methods
Twenty-two mongrel dogs of either sex, weighing from 12–16 kg, were fasted 24 hr and anesthetized with sodium pentobarbital (30 mg/kg, i.v.). During the experimental periods, anesthesia was maintained by additional injection of sodium pentobarbital (5 mg/kg, i.m.) at hourly intervals. The dogs
were tied in a supine position and were respired artificially with room air using a respirator (Harvard Apparatus, model 607). The epigastric abdomen was opened by a midline incision. A polyethylene tube was inserted into the main pancreatic duct, and the drops of pancreatic juice flowing out of the tube were counted with a drop counter. The accessory pancreatic duct was ligated. In order to exclude the effect of gastric juice and bile on pancreatic secretion, the pylorus portion of the stomach was ligated, and bile was drained off through a tube inserted into the bile duct. The pancreas was hemodynamically isolated in situ and perfused at a constant blood flow rate according to the procedure described previously (8). Polyethylene cannulae were inserted into the gastroduodenal and splenic arteries through which the pancreas was perfused with the animal’s own blood conducted from the left femoral artery by means of a peristaltic pump (Harvard Apparatus, model 1210). The systemic blood pressure was monitored by an electromanometer (Nihon Kohden, RP-3) through a catheter inserted into the right femoral artery. A dose of 500 units/kg of sodium heparin was given i.v. at the beginning of perfusion, and a maintenance dose of 200 units/kg was given hourly. In each experiment the speed of the perfusion pump was adjusted initially to give a perfusion pressure approximately equal to the mean systemic blood pressure, and then the speed was maintained throughout the experiment. Under these conditions, the perfusion flow rate was 10±0.8 ml/min (n=22). Drugs were injected into a rubber tube connected to the shank of the arterial cannula leading to the pancreas over 2 sec with a microsyringe. Pancreatic juice was collected for 15 min in the resting state or from the beginning until the end of the secretory response when secretagogues were injected i.a. The volume of pancreatic juice is expressed as an increase from the resting values. Bicarbonate concentration in pancreatic juice was measured by a pH/blood gas analyzer (Corning, model 165/2). Protein concentration in pancreatic juice was measured by the method of Bradford (9), with bovine serum albumin as the standard.

Drugs used in this study were DN-9693 (kindly donated by Daiichi Seiyaku, Co., Ltd., Tokyo, Japan), secretin (Eisai, 1 μg=150 CHR units), cholecystokinin (Eisai, 1 μg=150 CHR units), phentolamine mesylate (Nihon Ciba-Geigy), propranolol hydrochloride (Sigma), atropine sulphate (Torii), sulpiride hydrochloride (Fujisawa) and cimetidine (Fujisawa). Secretin and cholecystokinin were dissolved in 0.15 M NaCl to which bovine serum albumin had been added, to give a 0.1% solution, and other drugs were dissolved in 0.15 M NaCl.

Statistical analysis was carried out by means of the Student’s paired and unpaired t-test.

Results

Effects of close-arterial injection of DN-9693, secretin and cholecystokinin on the secretion of pancreatic juice: A flow of pancreatic juice observed in the resting state in all preparations, the rate being 8.2±0.7 μl/min (mean±S.E.M., n=14).

Typical secretory responses to DN-9693, secretin and cholecystokinin are illustrated in Fig. 1. When DN-9693 (1–1000 μg) was in-
jected, the secretion of pancreatic juice was increased within 1 min, reached a maximum 2 min later, and then gradually returned to the resting level. The threshold dose of DN-9693 for increasing secretion was about 0.1 mg. The responses were dose-dependent and no tachyphylaxis occurred. Secretin and cholecystokinin increased a relatively short-lasting pancreatic secretion. These actions were rapid in onset, reached a maximum within 1–2 min and subsided after 5–7 min. Dose-response curves for DN-9693, secretin and cholecystokinin are shown in Fig. 2 in combination with data of other phosphodiesterase inhibitors, ISMX, papaverine and theophylline, cited from our previous reports (5, 6). The secretory activity of DN-9693 (0.3 mg) was approximately equal to that of 0.03 mg of IBMX, 0.5 mg of papaverine, 5 mg of theophylline, 0.08 units of secretin or 0.2 units of cholecystokinin.

I.a. injection of DN-9693 produced a dose-dependent decrease in the perfusion pressure. The maximum decreases in perfusion pressure were 1±0.5, 4±1, 8±2, 14±2, 32±5, 40±5 and 41±4 mmHg when DN-9693 at

![Secretory responses to 3-isobutyl-1-methylxanthine (IBMX), DN-9693 (DN), papaverine (PA), theophylline (TH), secretin (S) and cholecystokinin (CCK) injected intraarterially. All points indicate mean values and vertical bars standard errors (n=6). The values of IBMX, papaverine and theophylline were cited from previous reports of our laboratories (5, 6).](image)

**Table 1.** Effects of DN-9693, secretin and cholecystokinin on the bicarbonate and protein secretion in pancreatic juice

| Secretagogues       | Bicarbonate concentration (mmol/ml) | Protein concentration (mg/ml) |
|---------------------|-------------------------------------|------------------------------|
| Resting state (n=14)| 21.2±4.6                            | 18.4±2.1                     |
| DN-9693 (μg, n=6)   | 32.3±2.8                            | 13.2±3.1                     |
| 100                 | 54.6±4.1*                           | 22.3±2.7                     |
| 300                 | 63.6±4.9*                           | 18.3±3.5                     |
| Secretin (units, n=6)|                                    |                              |
| 0.03                | NT                                  | 20.2±3.4                     |
| 0.1                 | 37.5±4.5*                           | 18.3±2.1                     |
| 0.3                 | 66.7±6.8*                           | 13.3±1.9                     |
| Cholecystokinin (units, n=6) |      | 36.4±5.7*                     |
| 0.1                 | NT                                  | 58.5±6.1*                    |
| 0.3                 | 25.1±7.5                            | 91.2±14.0*                   |
| 1.0                 | 28.7±7.1                            |                              |

Each value is the mean±standard error. Pancreatic juice was collected for 15 min in the resting state or from the beginning until the end of the secretory response when secretagogues were injected intraarterially. *Statistically different from resting values, P<0.05. NT, not tested.
doses of 1, 3, 10, 30, 100, 300 and 1000 µg were injected, respectively (n=6).

Effects of several blocking agents on DN-9693-induced pancreatic secretion: Blocking agents were injected intraarterially 2 min before the injection of DN-9693. The doses administered were enough to block the actions of their respective agonist (5, 6); 100 µg of phentolamine, 300 µg of propranolol, 100 µg of atropine, 1 mg of sulpiride and 1 mg of cimetidine in each of 3–4 experiments. None of these agents appreciably altered the secretory responses to DN-9693 (0.3 and 1 mg).

Effects of DN-9693, secretin and cholecystokinin on the bicarbonate and protein concentration in the pancreatic juice: Summarized data are shown in Table 1. DN-9693 and secretin produced a dose-dependent increase in the bicarbonate concentration, while cholecystokinin did not affect it. As for the protein concentration in the pancreatic juice, cholecystokinin significantly increased it, but DN-9693 and secretin did not affect it when the dose was increased up to 3–10 times with respect to the initial values.

Effects of infusion of DN-9693 on the secretin- and cholecystokinin-induced pancreatic secretion: Figure 3 shows the pancreatic secretion induced by secretin (0.1 units) and cholecystokinin (0.3 units) before and during the infusion of DN-9693 (10 µg/min). There was no increase in pancreatic secretion when a relatively small dose of DN-9693 (10 µg/min) alone was infused. However, the secretin-induced secretion was potentiated during the simultaneous infusion of DN-9693.

![Fig. 3](image)

**Fig. 3.** Effects of i.a. infusion of 10 µg/min of DN-9693 on the secretin- and cholecystokinin-induced pancreatic secretion. S: secretin, 0.1 units; CCK: cholecystokinin, 0.3 units. Statistically different from the control, P<0.05.

On the other hand, cholecystokinin-induced secretion was not modified by the infusion of DN-9693.

**Discussion**

It is accepted 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, cholecystokinin and cholinergic drugs increase cyclic GMP concentrations in the pancreas associated with protein secretion in isolated guinea pig pancreas slices (10, 11) and in the isolated dog pancreas (12).

It has been reported that many of the phosphodiesterase inhibitors inhibit not only cyclic AMP hydrolysis but also cyclic GMP hydrolysis (13, 14). DN-9693, a newly synthesized phosphodiesterase inhibitor, has been shown to inhibit selectively AMP phosphodiesterase in platelets (7). The present experiments show that DN-9693 causes a dose-dependent decrease in perfusion pressure from 1 µg and stimulates the pancreatic exocrine secretion from 100 µg. Since in the present experiments, pancreatic arterial beds were perfused at constant flow rate, decreases in perfusion pressure reflect directly increases in arterial vasodilation. Thus, vasodilation induced by DN-9693 is about 100 times more sensitive than DN-9693-stimulated pancreatic secretion. It was reported that the threshold doses to cause vasodilation in the mesenteric, femoral and renal arterial beds were 1–3 nmol (0.1–0.3 µg) (15). Pancreatic juice induced by DN-9693 was rich in bicarbonate rather than protein, suggesting that the action of DN-9693 resembled that of secretin but not that of cholecystokinin. These results may be explained by assuming that DN-9693 is a more specific cyclic AMP phosphodiesterase inhibitor than a cyclic GMP phosphodiesterase inhibitor (7).

The secretory potency of DN-9693 and other phosphodiesterase inhibitors cited (5, 6) may be arranged in the following order: IBMX > DN-9693 > papaverine > theophylline (Fig. 2). As a cyclic AMP phosphodiesterase inhibitor, the potency of each compound was reported to be as follows: DN-9693 is about 5 times as potent as papaverine in rat platelets.
papaverine is about 11 times as potent as theophylline in rat cerebrum (16); and IBMX has been reported to be about 15 times more potent than theophylline in guinea pig heart (17). Although these investigations were made on different preparations, the ranking order of inhibitory potency of these compounds may be: DN-9693>IBMX>papaverine>theophylline. This order and the order of their secretory potencies are about the same, confirming that cyclic AMP is an important intracellular mediator of fluid secretion. The reversed order of DN-9693 and IBMX may be explained by 1) DN-9693 has been shown to inhibit selectively cyclic AMP phosphodiesterase in platelets but weakly in the heart (7). On the other hand, IBMX has non-selective inhibitory effects on both cyclic AMP and cyclic GMP phosphodiesterase in platelets and in the heart (18). 2) The direct actions of phosphodiesterase inhibitors are dependent on the potency to inhibit phosphodiesterase activity in a cell-free preparation or on the ease with which the cell membrane is penetrated (19). That is, DN-9693 may be a more potent phosphodiesterase inhibitor than IBMX, but may penetrate the cell membrane of the pancreas with more difficulty than IBMX.

It was reasonable that infusion of DN-9693 potentiated secretin-induced secretion but not the cholecystokinin-induced secretion, since secretin caused the pancreatic secretion by increasing cyclic AMP in the pancreas (1). Similar results were obtained that papaverine potentiated responses to secretin (5).

The DN-9693-induced secretion was not modified by adrenergic, cholinergic, dopaminergic and histaminergic antagonists, indicating that DN-9693 may act directly on the pancreatic exocrine system without implicating these receptors.

From these observations, it is concluded that DN-9693 may produce an increase in pancreatic secretion by acting directly on the exocrine pancreatic gland of the dog, which might be mediated through the increase of intracellular cyclic AMP concentration by inhibiting phosphodiesterase activity.

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