COMPARATIVE STUDY OF THE SECRETORY RESPONSE TO DOPAMINE AND SEVEN AMINO ACID CONJUGATED DERIVATIVES ON THE BLOOD-PERFUSED CANINE PANCREAS

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Accepted April 10, 1980

Abstract—The secretory responses to seven dopamine amino acid conjugated derivatives were compared with those to dopamine, using blood-perfused canine pancreas preparations. Each of these dopamine derivatives produced a dose dependent increase in the secretion of pancreatic juice. The rank order of the secretory responses and relative potency (dopamine =1) was: N-Ileu-dopamine (0.4)>N-Ala-Glu-dopamine (0.2) > N-γ-Glu-dopamine (0.1)= N-Gly-Pro-dopamine (0.1) > N-Gly-Gly-dopamine (0.01)>N-Gly-Gly-Leu-dopamine (0.005)=N-Gly-Gly-Pro-dopamine (0.005). The duration of action of doses of the derivatives which produced approximately equal secretory responses was about 1.2–2.7 times longer than that of dopamine. Dopamine and N-Ileu-dopamine which has the most potent secretagogue property among seven dopamine derivatives produced a secretin-like secretion of the pancreatic juice containing a high concentration of bicarbonate but had little effect on protein output with lower amylase activity.

Dopamine is a precursor of noradrenaline and has characteristics which form other catecholamines, on systemic blood pressure (1, 2), in the renal or mesenteric vascular bed (1, 3) and in the coronary circulation (4). These effects of dopamine may be the result of actions on specific dopamine receptors (5).

It has been reported that dopamine produced an increase in the secretion of pancreatic juice in dogs but that noradrenaline had no effect on pancreatic secretion (6). As the secretagogue effect of dopamine was antagonized by haloperidol it was suggested that there were specific dopamine receptors in the dog pancreas (7).

Recently, several dopamine derivatives were synthesized and were found to protect the molecule at its metabolically vulnerable sites (8–12). In the present study, secretory responses to dopamine and seven amino acid conjugated derivatives of dopamine were compared, using the blood-perfused dog pancreas preparation. Moreover, we attempted to determine among the seven dopamine derivatives, compositions of the pancreatic juice induced by secretin, dopamine and N-Ileu-dopamine which had the most potent secretagogue properties.

MATERIALS AND METHODS

Ten mongrel dogs of either sex, weighing from 14 to 17 kg, were fasted for 24 hr and anaesthetized with sodium pentobarbital (30 mg/kg, i.v.). During the experimental pro-
cures, anaesthesia was maintained by the additional injection of sodium pentobarbital (5 mg/kg, i.m.) hourly. The dogs were respired artificially with room air using a Harvard respirator (Model 607). The upper abdomen was opened by a midline incision, and 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 gastroduodenal arteries through which the pancreas was perfused with the animal’s own blood conducted from the left femoral artery by means of a Harvard peristaltic pump (Model 1215). The splenic artery was also cannulated and perfused retrogradely with blood from the femoral artery. All experiments were performed under constant perfusion pressure at 100 mmHg. The experimental setup has been previously described in detail (6, 7). A dose of 500 units/kg of sodium heparin was given at the beginning of perfusion and maintenance doses of 2000 units were given hourly.

Drug solutions were injected into a rubber tube connected to the shank of the arterial cannula. The flow of pancreatic juice was measured by a drop counter and the volume of pancreatic juice by a graduated cylinder. The concentration of bicarbonate in mEq/liter was measured by the method of Natelson (13), amylase activity by the method of Caraway (14) and protein concentration by the method of Lowry et al. (15).

Drugs used in this study were dopamine hydrochloride (ICN), N-glycyglycylidopamine hydrochloride (D-1, Eisai Co.), N-glycylprolyldopamine hydrobromide (D-2, Eisai Co.), N-alanylglutamylidopamine methansulphonate (D-3, Eisai Co.), N-glycylglycylidopamine hydrochloride (D-4, Eisai Co.), N-glycylglycyleucydopamine hydrochloride (D-5, Eisai Co.), N-isoleucydopamine hydrochloride (D-6, Abbott), N-(y-glutamyl) dopamine (D-7, Abbott) and secretin (Eisai Co.). All drugs were freshly dissolved in 0.9% w/v NaCl solution (saline) and doses refer to the salt. The volume of drugs injected was 0.01–0.1 ml over a period of 4 sec. For all experimental values, means and standard errors of means (s.e.m.) were calculated.

RESULTS

Intra-arterial injections of secretin (0.03–0.3 units), dopamine (1–10 μg) or one of amino acid conjugated derivatives (3–1000 μg) produced a prompt increase in the rate of the

![Fig. 1. Typical secretory responses of the canine pancreas to secretin, dopamine, N-Gly-Pro-dopamine (D-2), N-Gly-Gly-Pro-dopamine (D-4) and N-Ileu-dopamine (D-6) injected intra-arterially.](image-url)
secretion of the pancreatic juice which was dose-dependent. The secretory effects by these compounds continued for 2–20 min. Successive injections were given as the response to each preceding injection wore off completely; there was no evidence of tachyphylaxis. As demonstrated in Fig. 1, a drop of pancreatic juice was produced at about a 1.5 min intervals

| Compounds                | No. of exp | Pancreatic juice (μl) mean ± s.e.m. | Duration (sec) mean ± s.e.m. |
|--------------------------|------------|------------------------------------|-----------------------------|
| Secretin (unit)          |            |                                    |                             |
| 0.03                     | 10         | 150 47                             | 151 16                      |
| 0.1                      | 10         | 437 76                             | 345 35                      |
| 0.3                      | 10         | 1117 196                           | 643 46                      |
| Dopamine (μg)            |            |                                    |                             |
| 1.0                      | 10         | 237 53                             | 177 12                      |
| 3.0                      | 10         | 442 62                             | 250 21                      |
| 10.0                     | 10         | 987 172                            | 426 28                      |
| N-Gly-Gly-dopamine (μg)  |            |                                    |                             |
| 100                      | 6          | 108 30                             | 181 21                      |
| 300                      | 6          | 332 45                             | 238 32                      |
| 1000                     | 5          | 829 91                             | 456 39                      |
| N-Gly-Pro-dopamine (μg)  |            |                                    |                             |
| 30                       | 5          | 192 21                             | 158 18                      |
| 100                      | 5          | 322 47                             | 263 27                      |
| 300                      | 5          | 885 111                            | 416 33                      |
| N-Ala-Glu-dopamine (μg)  |            |                                    |                             |
| 10                       | 6          | 244 30                             | 384 11                      |
| 30                       | 6          | 424 41                             | 661 19                      |
| 100                      | 6          | 1024 164                           | 936 26                      |
| N-Gly-Gly-Pro-dopamine (μg) |      |                                    |                             |
| 100                      | 3          | 30 4                               | 40 7                        |
| 300                      | 4          | 85 7                               | 153 12                      |
| 1000                     | 4          | 292 18                             | 300 23                      |
| N-Gly-Gly-Leu-dopamine (μg) |        |                                    |                             |
| 100                      | 3          | 100 20                             | 95 8                        |
| 300                      | 4          | 240 28                             | 175 10                      |
| 1000                     | 4          | 370 32                             | 402 25                      |
| N-Ileu-dopamine (μg)     |            |                                    |                             |
| 3                        | 6          | 217 33                             | 290 18                      |
| 10                       | 6          | 397 42                             | 662 22                      |
| 30                       | 6          | 922 96                             | 1240 36                     |
| N-γ-Glu-dopamine (μg)    |            |                                    |                             |
| 30                       | 5          | 109 22                             | 284 13                      |
| 100                      | 5          | 345 51                             | 651 24                      |
| 300                      | 5          | 760 105                            | 983 45                      |
during the resting state of the perfused pancreas. Figure 1 shows typical secretory responses to secretin, dopamine, N-Gly-Pro-dopamine (D-2), N-Gly-Gly-Pro-dopamine (D-4) and N-Ileu-dopamine (D-6). Other dopamine derivatives also produced the secretory effects in the same manner, although their potencies and durations were different. The results are summarized in Table 1. The secretory activity of dopamine (3 \( \mu g \)) was approximately equivalent to that of secretin (0.1 unit) but the duration of action was shorter.

Relative potencies of dopamine and the derivatives on the pancreatic secretion and the duration of action are compared in Table 2. As shown in Table 2, dopamine was the most

### Table 2. Comparison of dopamine and amino acid conjugated derivatives for secretory responses and the durations on the blood-perfused dog pancreas. Relative activity is the reciprocal of the dose producing a response matching that to 1–3 \( \mu g \) of dopamine

| Compounds                  | Relative activity | Volume of pancreatic juice | Duration |
|---------------------------|-------------------|----------------------------|----------|
| Dopamine                  | 1                 |                            | 1        |
| N-Ileu-dopamine (D-6)     | 0.4               | 2.70                       |
| N-Ala-Glu-dopamine (D-3)  | 0.2               | 2.54                       |
| N-\( \gamma \)-Glu-dopamine (D-7) | 0.1           | 1.86                       |
| N-Gly-Pro-dopamine (D-2)  | 0.1               | 1.60                       |
| N-Gly-Gly-dopamine (D-1)  | 0.01              | 1.48                       |
| N-Gly-Gly-Leu-dopamine (D-5) | 0.005           | 1.20                       |
| N-Gly-Gly-Pro-dopamine (D-4) | 0.005           | 1.40                       |

### Table 3. Concentration of bicarbonate, protein and amylase in the pancreatic juice

| Compounds                  | Bicarbonate (mEq/liter) | Protein (mg/ml) | Amylase (unit/ml) |
|---------------------------|-------------------------|-----------------|-------------------|
| Resting state             | 16.2 \( \pm \) 3.6      | 55.6 \( \pm \) 4.5 | 1620 \( \pm \) 52.3 |
| Secretin (unit)           |                          |                 |                   |
| 0.03                      | 27.4 \( \pm \) 4.2      | 19.1 \( \pm \) 1.2 | 495 \( \pm \) 32.0 |
| 0.1                       | 44.9 \( \pm \) 3.7      | 20.3 \( \pm \) 1.7 | 310 \( \pm \) 13.5 |
| 0.3                       | 75.3 \( \pm \) 5.8      | 17.6 \( \pm \) 3.5 | 213 \( \pm \) 12.8 |
| Dopamine (\( \mu g \))    |                          |                 |                   |
| 1                         | 21.6 \( \pm \) 7.6      | 21.1 \( \pm \) 7.1 | 450 \( \pm \) 52.5 |
| 3                         | 39.5 \( \pm \) 6.5      | 18.5 \( \pm \) 4.9 | 224 \( \pm \) 12.4 |
| 10                        | 60.1 \( \pm \) 7.6      | 17.7 \( \pm \) 6.1 | 207 \( \pm \) 9.8  |
| N-Ileu-dopamine (\( \mu g \)) |                  |                 |                   |
| 3                         | 19.5 \( \pm \) 1.2      | 9.8 \( \pm \) 0.7  | 325 \( \pm \) 11.7 |
| 10                        | 32.8 \( \pm \) 3.8      | 7.4 \( \pm \) 0.6  | 250 \( \pm \) 7.8  |
| 30                        | 51.6 \( \pm \) 4.4      | 6.2 \( \pm \) 0.5  | 190 \( \pm \) 4.3  |

Results are expressed as mean \( \pm \) standard error of 5 experiments. Pancreatic juice was collected for 15 min in the resting state and from the beginning until the end of the secretory response when each compound was injected intra-arterially. The amylase unit is defined as the amount of enzyme that will hydrolyse 10 mg of starch in 30 min at 37°C.
potent among the seven derivatives in producing the pancreatic juice. The rank order of the potency to secrete the pancreatic juice was dopamine > N-Ileu-dopamine > N-Ala-Glu-dopamine > N-γ-Glu-dopamine = N-Gly-Pro-dopamine > N-Gly-Gly-dopamine > N-Gly-Gly-Leu-dopamine = N-Gly-Gly-Pro-dopamine. On the other hand, the duration of action of the derivatives was 1.2–2.7 times longer than that of dopamine. The rank order of the duration was the same as the potency of the secretory response in seven dopamine derivatives.

Concentrations of bicarbonate, protein and amylase in the pancreatic juice which was collected for 15 min in the resting state and in the juice induced by secretin, dopamine and one of the most potent amino acid derivatives, N-Ileu-dopamine, were determined. The data are summarized in Table 3. In the resting state, bicarbonate, protein and amylase concentrations were 16.2 mEq/liter, 55.6 mg/ml and 1620 units/ml, respectively. The composition of the pancreatic juice induced by N-Ileu-dopamine was quite similar to that of dopamine and secretin, causing an increase in the volume of juice with higher concentration of bicarbonate and little effect in the protein output with lower amylase activity.

Each amino acid itself (1 mg, i.a.) had no effect on the secretion.

DISCUSSION

The secretory effects of dopamine in dogs have been well documented as follows. Dopamine stimulated the secretion of pancreatic juice with a high content of bicarbonate while the protein content was low. On the other hand, noradrenaline and adrenaline did not induce the pancreatic secretion as previously reported (6, 16, 17). This response to dopamine was similar to that of secretin, and the site of action of dopaminergic receptors at the ductural cells was suggested (16, 18). L-dopa, a precursor of dopamine, also stimulated the secretion of pancreatic juice, that was blocked by a dopa decarboxylase inhibitor (7). The effect of either L-dopa or dopamine was significantly attenuated by haloperidol, a dopamine receptor antagonist (7, 17). These observations strongly suggested that there were specific receptors to secrete the pancreatic juice by dopamine in the canine pancreas.

In the present study, the effects of dopamine and seven amino acid conjugated derivatives of dopamine were compared, with respect to secretion of pancreatic juice, on the blood-perfused dog pancreas. Each of these dopamine derivatives caused a prompt and profuse increase in the secretion of pancreatic juice as did dopamine. The most potent one of seven dopamine derivatives tested was N-Ileu-dopamine, although it was 2.5 times less potent than dopamine. It was 4 times more potent than the other derivative with a single amino acid (N-γ-Glu-dopamine). Next in order of potency were the conjugates with dipeptides. The least potent were the conjugates with tripeptides. Although dopamine was the most potent in producing the secretory effects, the duration of action was the shortest of all the dopamine derivatives. The canine pancreas was also observed to be secreting the enzyme rich juice even in the resting state (18).

Since amino acid itself had no effect on the secretion of the pancreatic juice and the pancreas is the second richest organ in aminoacylaryl amidase activity after the kidney (19), free dopamine seems to cause the pharmacological responses. Therefore, the mode of
action of dopamine derivatives as well as dopamine on the pancreas was quite similar to that of secretin, as reported previously (7). Slow hydrolysis by the enzyme (8), appears to be responsible for the weak action of the secretion and long duration by dopamine derivatives.

McDonald and Goldberg (1) reported that dopamine produced a dilatation of the renal vascular bed, and they suggested dopamine for clinical application. In fact, dopamine had been used in the treatment of shock, congestive heart failure, cirrhosis oliguric renal failure and drug intoxication (5). However, the interaction of dopamine with α-adrenergic receptors can lead to undesirable side effects (20). Recently, N-Ileu-dopamine (8) and γ-Glu-dopamine (21) have been reported to have a specific and prolonged renal effect and to increase the renal blood flow. In the endocrine system of the pancreas, dopamine stimulated glucagon release and inhibited insulin release, and then produced hyperglycemia (22). But γ-glu-dopamine was less effective in causing hyperglycemia (23). Therefore, dopamine aminoacyl derivatives may be drugs of choice where greater renal perfusion is desired without untoward effects.

Acknowledgements: We are grateful to Eisai Pharmaceutical Co. Ltd., Tokyo, Japan for their supply of dopamine derivatives, and to Mrs. Yumiko Itoh for preparing the manuscript.

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