Roles of Non-cholinergic Intrapancreatic Nerves, Serotonergic Nerves, on Pancreatic Exocrine Secretion in the Isolated Perfused Rat Pancreas

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It has been rereported that axons which display 5-hydroxytryptamine (5-HT) immunoreactivity are abundant in the pancreas and the majority of serotonergic axons terminate within intrapancreatic ganglia, islet and acini. This histological result strongly suggests that intrapancreatic serotonergic nerves could affect to the pancreatic endocrine and exocrine secretion. Thus, this study was aimed to investigate whether intrapancreatic serotonergic nerves could affect pancreatic exocrine secretion and an action mechanism of the intrapancreatic serotonergic nerves. The rats were anesthetized with a single injection of urethane. The median line and the abdominal aorta was carefully dissected and cannulated with PE-50 tubing just above the celiac artery, and then tightly ligated just below the superior mesenteric artery. The pancreatic duct was also cannulated with Tygon microbore tubing. With the addition of serotonin, pancreatic volume flow and amylase output were significantly inhibited electrical field stimulation (EFS). On the other hand, pancreatic volume flow and amylase output were significantly elevated in EFS with the addition of spiperone. EFS application, however, pancreatic volume flow and amylase output had no significant change in cholecystokinin (CCK) alone when serotonin was applied under a 5.6 mM glucose background. Pancreatic volume flow and amylase output under 18 mM glucose background were significantly elevated in CCK plus serotonin than in CCK alone. These data suggest that intrapancreatic serotonergic nerves play an inhibitory role in pancreatic exocrine secretion and an important role in the insulin action or release.

Key Words: Intrapancreatic nerve, Electrical stimulation, 5-hydroxytryptamine, Cholecystokinin, Insulin

INTRODUCTION

Serotonin (5-hydroxytryptamine; 5-HT) is synthesized and released in platelets of enterochromaffin cells of the gastrointestinal mucosa. A small quantity of serotonin exists in the central nervous system [1] and most of serotonin exists in the myenteric plexus of the gastrointestinal system [2]. Although most serotonin exists in the gastrointestinal tract, the roles of serotonin in the gut are still unclear. It is mainly reported that roles of serotonin released from enteric nervous system or enterochromaffin cells in the intestinal mucosa [3]. Thus this study was focused to fined whether intrapancreatic serotonergic nerves could affect pancreatic exocrine secretion in the ex vivo model.

The pancreas is a unique organ which has both endocrine and exocrine functions in the body. Over 20 kinds of diges-tive enzymes, including bicarbonate and water, are secreted from the exocrine part for digestion of foods. Moreover, insulin, glucagon, somatostatin and pancreatic polypeptide are also secreted from the endocrine part. In spite of these dual actions of the pancreas, the effect of serotonin on the pancreas has been controversial. It has been reported that axons which display 5-HT immunoreactivity are abundant in the pancreas and the majority of serotonergic axons terminate within intrapancreatic ganglia, islet, and acini [4]. These histological results strongly suggest that intrapancreatic serotonergic nerves could affect pancreatic endocrine and exocrine secretion.

Pancreatic exocrine secretion is regulated by the autonomic nerve system and gastrointestinal hormones. Recently, it has been reported that pancreatic exocrine secretion is regulated by the interaction of hormones and the neural system [5]. In addition, pancreatic exocrine secretion is affected by pancreatic islet hormones such as insulin and, somatostatin [6]. As seen in the above results and reports, pancreatic exocrine secretion is controled by a complex set of factors. Pancreatic exocrine secretion was enhanced by

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; EFS, electrical field stimulation; CCK, cholecystokinin.

electrical stimulation (EFS) of the vagus nerve, but this enhanced effect was partly blocked by atropine, a muscarinic receptor antagonist [7]. EFS on rat pancreas enhanced pancreatic exocrine secretion by activation of the intrapancreatic nervous system [8,9]. Pre-treatment of atropine partly blocked this effect, but pre-treatment of tetrodotoxin, a nerve blocker, perfectly blocked it [10]. These results suggested that the non-cholinergic nerve system exists in the intrapancreatic nerve system, and it could affect the pancreatic exocrine secretion. There are both peptidergic nerves and cholinergic nerves in the intrapancreatic nerve system [11]. The serotonergic nerve is involved in this peptidergic nerve system [12] and may participate in pancreatic exocrine secretion. Because the axons of serotonergic nerve are distributed close to the non-serotonergic intrapancreatic nerves (mainly cholinergic nerve), the serotonergic nerves can affect pancreatic exocrine secretion indirectly via the affect of the cholinergic nerve. In addition, the serotonergic nerve can affect pancreatic exocrine secretion directly via the affect of the acini and islet cell because the axons of the serotonergic nerves are distributed near acini and islet cells [12].

Thus, this study was aimed to investigate whether intrapancreatic serotonergic nerves could affect pancreatic exocrine secretion and an action mechanism of the intrapancreatic serotonergic nerves.

METHODS

Experimental animals

Sprague-Dawley rats were used in the experiment which succeeded in experimental animal center of Hallym University. The environment of breeding room was maintained at condition that temperature was $23\pm2^{o}C$ and relative humidity was $55\pm10\%$. Artificial lighting maintained 12 hours per day. The rats were anesthetized with a single intraperitoneal injection of 20% urethane (Sigma, USA) at a dose of 0.7 ml/100 g of body weight. The rats were sacrificed by an intravenous overdose injection of urethane after isolation of the pancreas. Food was forbidden from rats for 24 h before the experiment, but they were allowed to drink water freely.

Preparation of the isolated perfused pancreas

The isolated pancreas was prepared according to a method described previously [10]. The rats were anesthetized with a single injection of urethane. The median line and the abdominal aorta was carefully dissected and cannulated with PE-50 tubing (I.D. 0.58 mm, O.D. 0.97 mm; Clay Adams, USA) just above the celiac artery, and then tightly ligated just below the superior mesenteric artery. The pancreatic duct was also cannulated with Tygon microbore tubing (I.D. 1.27 mm, O.D. 2.28 mm; Fisher Scientific Co, USA) to drain the perfusate. To prevent a duodenal secretion inflow each beginning and ending of duodenum was cannulated with plastic tubing.

The pancreas was perfused with modified Krebs-Henseleit solution (pH 7.4, 305 mosmol/kg water) through the celiac and superior mesenteric arteries at a flow rate of 1.2 ml/min using a minipuls pump (Gilson, France). The perfusate contained 5.6 mM glucose (Sigma, USA), 0.1% bovine serum albumin (Sigma, USA) and 3% Dextran T-70

(Sigma, USA), and was continuously oxygenated with 95% O_2 and 5% CO_2. Krebs-Henseleit solution basically consists of NaCl 191.5 mM, $NaHCO_3$ 25 mM, KCl 5.6 mM, $MgCl_2$ 1 mM, NaH_2PO_4 1.15 mM and $CaCl_2$ 2.5 mM.

The pancreas was isolated with the duodenum but separated from neighboring organs and tissues. The pancreas was placed in temperature-controlled experimental chamber at $37^{o}C$, which was also continuously supplied with Krebs-Henseleit solution at a flow rate of 0.35 ml/min and oxygenated. After equilibration period of 30 min, pancreatic juice was collected through the experiment every 15-min.

Effects of EFS on pancreatic exocrine secretion

To investigate effects of intrapancreatic neuronal activation, electrical field stimulation (EFS) was carried out with applying biphasic square waves (parameters: 12 V, 2 ms and 8 Hz for 45 min) to the isolated pancreas through a pair of coiled platinum electrodes immersed in the experimental chamber 5 cm apart [10]. Pancreatic juice was collected in every 15-min period.

Effects of CCK on pancreatic exocrine secretion

To confirm pancreatic exocrine secretion stimulated by CCK-8 in the isolated perfused rat pancreas, CCK-8 (Squibb, USA) at a concentration of 5 pmol added to the perfusate contained 5.6 mM glucose for 60 minutes. In order to find effects of endogenous insulin on pancreatic exocrine secretion, a concentration of glucose in the perfusate was elevated from 5.6 mM to 18 mM before 45 min administration of CCK-8.

Amylase assay

To investigate pancreatic digestive enzyme secretion, activity of representative enzyme α-amylase was measured. α-amylase activity in the pancreatic juice was determined by method of Rick and Stegbauer [13]. In brief, 1 ml starch solution (1%, W/V) is put in 1 ml phosphate buffer (10 mM KH_2PO_4, 10 mM Na_2HPO_4, 10 mM NaCl, pH 6.9) and mixed with $1,000\sim3,000$ times diluted pancreatic juice (20 μl). Then it reacted in $37^{o}C$ incubator for 10 minutes. One % starch solution was heated in $100^{o}C$ for 20 minutes to melt and centrifuged in $4^{o}C$ at a speed of 3,000 rpm for 20 minutes then the supernatant was used. After reaction 2 ml dinitrosalicylic acid solution (1% 3,5 -dinitrosalycylic acid, 30% potassium sodium tartrate, 400 mM NaOH) was put in it and heated in $100^{o}C$ for 5 minutes to appear color of maltose, the reactive product. The reactive tube was cooling in room temperature for 30 minutes then measured a degree of extinction at 546 nm by spectrophotometer (Kontron, Swiss). Amylase activity 1 unit decided amount of amylase which produce 1 μm maltose for 1 minute with above condition and converted each 15-min (amylase output; U/15 min). Pancreatic juice was collected in polyethylene tube which has volume of 2.4 μl/cm then measured length to determine volume flow. All reagents were bought from Sigma (USA).

Statistical analysis

All data are expressed as mean±SE. Student's t test or paired t test was used for statistical analysis of the data. The difference was considered significant when the p value

was < 0.05.

RESULTS

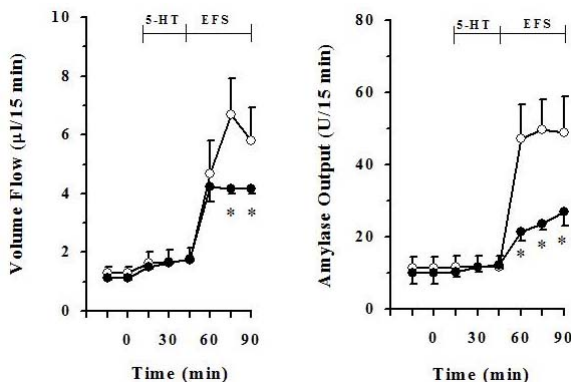## *Effects of serotonin on EFS-induced pancreatic exocrine secretion*

To investigate effects of serotonin (5-HT) on EFS-induced pancreatic exocrine secretion, serotonin (Sigma, USA) at a concentration of $2 \mu M$ was perfused with the perfusate containing 5.6 mM glucose from 45 min earlier EFS applied to the end of the experiment, during total 90 min.

When EFS was applied to the pancreas as shown Fig. 1, pancreatic flow rate and amylase output were increased. Pancreatic flow rate was elevated from $1.29 \pm 0.20 \mu l/15$ min in basal to $6.69 \pm 1.23 \mu l/15$ min in peak level. Amylase output was also elevated from $11.47 \pm 2.91 \mu l/15$ min to $49.71 \pm 8.24 \mu l/15$ min in peak level. When serotonin was added to the perfusate, however, the flow rate and amylase output were markedly decreased. Pancreatic flow rate was decreased from $6.69 \pm 1.23 \mu l/15$ min to $4.02 \pm 0.31 \mu l/15$ min in peak level. Amylase output was also decreased from $49.71 \pm 8.24 \mu l/15$ min to $21.58 \pm 2.92 \mu l/15$ min.

Effects of serotonin antagonist on EFS-induced pancreatic exocrine secretion

To investigate effects of serotonin antagonist on EFS-induced pancreatic exocrine secretion, spiperone hydrochloride (Tocris, USA), 5-HT2A serotonin antagonist, at a concentration of $5 \mu M$ was perfused with the perfusate containing 5.6 mM glucose from 45 min earlier EFS applied to the end of the experiment.

When EFS applied to the pancreas as shown Fig. 2, pancreatic flow rate and amylase output were increased. Pancreatic flow rate was elevated from $1.18 \pm 0.09 \mu l/15$ min to $5.55 \pm 0.92 \mu l/15$ min in peak level. Amylase output was also elevated from $9.86 \pm 2.88 \mu l/15$ min to $45.74 \pm 9.42 \mu l/15$ min in peak level. Pancreatic flow rate of EFS plus spiperone, serotonin antagonist, was elevated from $1.24 \pm 0.20 \mu l/15$ min to $9.22 \pm 0.57 \mu l/15$ min in peak level. Amylase output of EFS plus spiperone was elevated from $17.56 \pm 2.81 \mu l/15$ min to $205.42 \pm 28.60 \mu l/15$ min in peak level. Pancreatic vol-

ume flow was significantly elevated in EFS plus spiperone from after 30 min EFS applied to the end of this experiment than in EFS alone ($p < 0.05$). Pancreatic amylase output was significantly elevated in EFS plus spiperone than EFS alone during EFS applied 45 min ($p < 0.05$).

Effects of serotonin antagonist with atropine on EFS-induced pancreatic exocrine secretion

To investigate effects of serotonin on EFS-induced pancreatic exocrine secretion with atropine, atropine at a concentration of $2 \mu M$ was perfused with the perfusate containing 5.6 mM glucose with or without spiperone hydrochloride.

Pancreatic flow rate of EFS plus atropine was from $0.76 \pm 0.09 \mu l/15$ min elevated to $2.99 \pm 0.51 \mu l/15$ min in peak level. Amylase output of EFS plus atropine was also elevated from $8.08 \pm 1.78 \mu l/15$ min to $23.66 \pm 8.37 \mu l/15$ min

Fig. 2. Effect of spiperone hydrochloride (5-HT antagonist, $5 \mu M$) on electrical field stimulation (EFS, 12 V, 2 ms, 8 Hz)-induced pancreatic volume flow and amylase output. Each points ($\bigcirc$, EFS alone; $\bullet$, EFS+5-HT antagonist) represent the mean±S.E. of 6~7 experiments. *The value is significantly ($p < 0.05$) different from that of EFS alone.
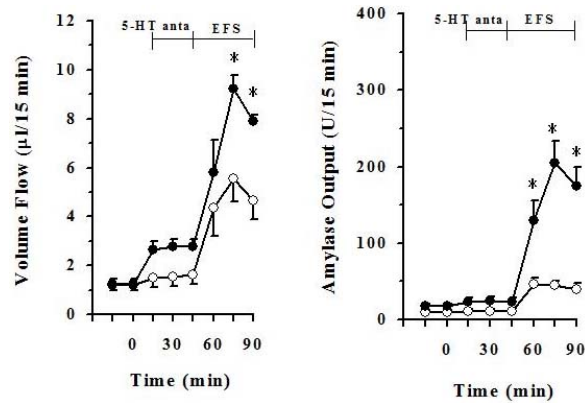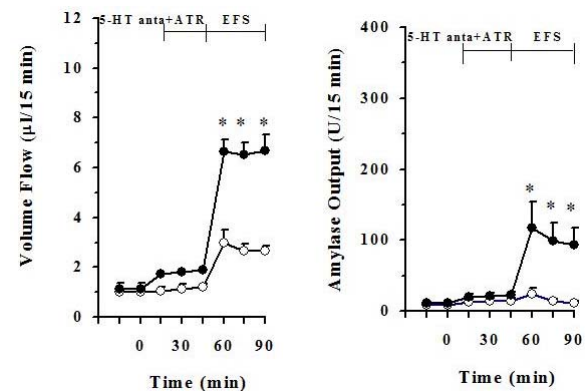
Fig. 1. Effect of serotonin (5-HT, $2 \mu M$) on electrical field stimulation (EFS, 12 V, 2 ms, 8 Hz)-induced pancreatic volume flow and amylase output. Each points ($\bigcirc$, EFS alone; $\bullet$, EFS+5-HT) represent the mean±S.E. of 7 experiments. *The value is significantly ($p < 0.05$) different from that of EFS alone.

Fig. 3. Effect of spiperone hydrochloride (5-HT antagonist, $5 \mu M$) with atropine (ATR, $2 \mu M$) on electrical field stimulation (EFS, 12 V, 2 ms, 8 Hz)-induced pancreatic volume flow and amylase output. Each points ($\bigcirc$, EFS+ATR; $\bullet$, EFS+ATR+5-HT antagonist) represent the mean±S.E. of 6~7 experiments. *The value is significantly ($p < 0.05$) different from that of EFS plus atropine.
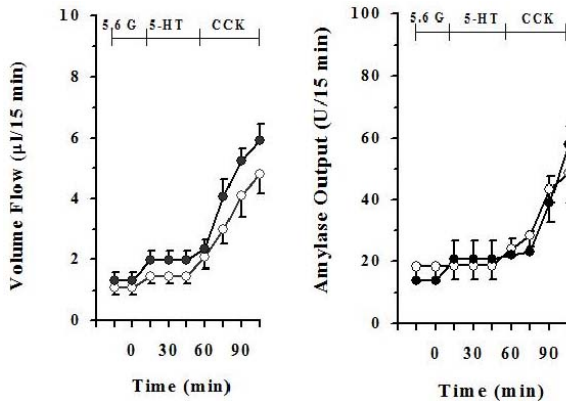
Fig. 4. Effect of serotonin (5-HT, 2μM) on cholecystokinin (CCK, 5 pmol)-induced pancreatic volume flow and amylase output under 5.6 mM glucose background. Each points ($\bigcirc$, CCK alone; $\bullet$, CCK+ 5-HT) represent the mean±S.E. of 6 experiments. *The value is significantly (p < 0.05) different from that of CCK alone.

Fig. 5. Effect of serotonin (5-HT, 2μM) on cholecystokinin (CCK, 5 pmol)-induced pancreatic volume flow and amylase output under 18 mM glucose background. Each points ($\bigcirc$, CCK alone; $\bullet$, CCK+ 5-HT) represent the mean±S.E. of 6 experiments. *The value is significantly (p < 0.05) different from that of CCK alone.

in peak level. Pancreatic flow rate of EFS plus spiperone and atropine was elevated from $1.11 \pm 0.25 \mu$l/15 min to $6.67 \pm 0.65 \mu$l/15 min in peak level. Amylase output of EFS plus spiperone and atropine was also elevated from $10.74 \pm 2.35 \mu$l/15 min to $117.36 \pm 36.35 \mu$l/15 min in peak level (Fig. 3). Pancreatic volume flow and amylase output were significantly higher in EFS plus spiperone and atropine than in EFS plus atropine during EFS applied 45 min (p < 0.05).

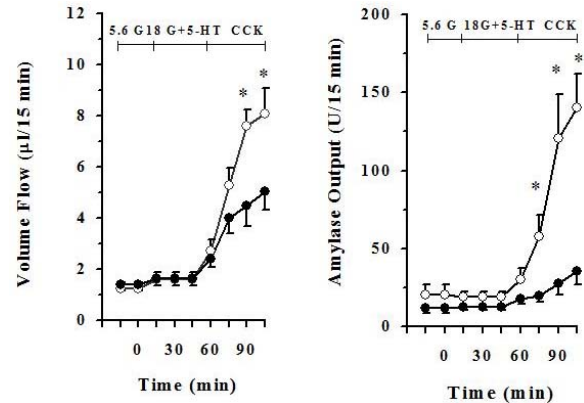### Effects of serotonin on CCK-induced pancreatic exocrine secretion under 5.6 mM glucose background

To investigate effects of serotonin on CCK-induced pancreatic exocrine secretion, serotonin at a concentration of 2μM was perfused with the perfusate containing 5.6 mM glucose during 105 min from 45 min before administration of CCK-8 to the end of this experiment.

Administration of CCK to the perfusate stimulated the pancreatic flow rate and amylase output. Pancreatic flow rate was elevated from $1.08 \pm 0.24 \mu$l/15 min to $4.80 \pm 0.62 \mu$l/15 min in peak level. Amylase output was also elevated from $18.25 \pm 4.58 \mu$l/15 min to $48.49 \pm 9.51 \mu$l/15 min in peak level. Pancreatic volume flow of CCK plus serotonin was elevated from $1.34 \pm 0.26 \mu$l/15 min to $5.92 \pm 0.54 \mu$l/15 min in peak level. Amylase output of CCK plus serotonin was also elevated from $14.09 \pm 4.53 \mu$l/15 min to $57.78 \pm 6.06 \mu$l/15 min in peak level. Pancreatic volume flow and amylase output had no significance between in CCK alone and in CCK plus serotonin under the 5.6 mM glucose background (Fig. 4).

Effects of serotonin on CCK-induced pancreatic exocrine secretion under 18 mM glucose background

To investigate effects of serotonin on CCK-induced pancreatic exocrine secretion under 18 mM glucose background, serotonin at a concentration of 2μM was perfused with the perfusate containing 18 mM glucose during 105 min from 45 min before administration of CCK-8 to the end of this experiment.

When CCK administrated to the perfusate under 18 mM glucose background as shown Fig. 5, pancreatic flow rate

and amylase output were increased. Pancreatic flow rate was elevated from $1.25 \pm 0.22 \mu$l/15 min to $8.10 \pm 0.98 \mu$l/15 in peak level. Amylase output was also elevated from $20.39 \pm 6.22 \mu$l/15 min to $140.07 \pm 21.91 \mu$l/15 min in peak level. These values represent that endogenous insulin potentiated the action of CCK in pancreatic flow rate and amylase output (Fig. 4, 5).

Pancreatic volume flow of CCK plus serotonin under 18 mM glucose background was elevated from $1.42 \pm 0.25 \mu$l/15 min to $5.04 \pm 0.73 \mu$l/15 in peak level. Amylase output of CCK plus serotonin under 18 mM glucose background was also elevated from $11.72 \pm 3.48 \mu$l/15 min to $35.73 \pm 8.57 \mu$l/15 min in peak level. As shown in Fig. 5, these values of pancreatic volume flow and amylase output under 18 mM glucose background were significantly inhibited by administration of serotonin (p < 0.05).

DISCUSSION

In the present study, EFS-induced pancreatic exocrine secretion in the isolated perfused rat pancreas was inhibited by serotonin, including volume flow and amylase output. Spiperone hydrochloride, a serotonin antagonist, significantly enhanced pancreatic secretion of both fluid and amylase. The previous report states that 5-HT3 receptor antagonists significantly increased pancreatic fluid and protein outputs [14]. These results indicate that serotonin is involved in regulation of pancreatic secretion. EFS-induced pancreatic exocrine secretion was completely blocked by tetrodotoxin and partially inhibited by atropine [10]. Similarly, spiperone hydrochloride-induced pancreatic exocrine secretion was partially inhibited by atropine in this study. These results agree with previous reports that 5-HT1 receptor agonist produced a dose-related inhibition of pancreatic exocrine secretion through a modulation of the vagal cholinergic pathway [15]. On the other hand, it is reported that 5-HT2 and 5-HT3 receptor antagonists inhibited pancreatic exocrine secretion [16]. These results are in opposition to our present study. The reason is that they put emphasis on pancreatic exocrine secretion of fluid thus investigated acid-induced pancreatic secretion. However, our

study put emphasis on pancreatic exocrine secretion of fluid and amylase output, therefore investigating EFS-induced pancreatic exocrine secretion.

This present study shows that pancreatic exocrine secretion in the isolated perfused rat pancreas had no significant difference between CCK alone administration and CCK plus serotonin administration when perfused with glucose at a dose of 5.6 mM. Interestingly, pancreatic exocrine secretion significantly inhibited by administration of serotonin when perfused with a high concentration of glucose, at a dose of 18 mM, prompted endogenous insulin secretion. These data suggest that action of serotonin on pancreatic exocrine secretion is dependent on insulin. This is in agreement with the finding that the neural 5-HT1 receptor was involved in the control of endocrine pancreatic function [17]. The pancreas has a portal system called the 'insulo-acinar axis' between the endocrine part and the exocrine part. In a rat, about 6% of the total pancreatic blood flow passes through this insulo-acinar axis [18]. For this insulio-acinar axis, pancreatic islet hormones can affect pancreatic exocrine secretion. Insulin enhanced the action of CCK [19,20] and the interaction of CCK and secretin enhanced pancreatic enzyme secretion mediated by insulin [21].

An action of serotonin on the insulin release or insulin action is still inconsistent. Both stimulatory [22,23] and inhibitory effects have been published. The inhibitory reports used an in vitro perfused islet cell model, so this different method made different results. As an *ex vivo* model, the isolated perfused rat pancreas model was used in this study. Isolated perfused rat pancreas model could eliminate effects of all external nerves and other gastrointestinal hormones perfectly. It is quite effective to know the role of a single nerve fiber or hormone. The pancreas can maintain its exocrine secretion with perfect isolation of external nerves as intrapancreatic nerves continue basal activity [24]. Thus an isolated perfused rat model is the proper model to study intrapancreatic nerves.

The controversial results might be based on the fact that serotonin plays a important role in insulin release. The present study also shows serotonin has a relationship with insulin. By using the isolated perfused rat pancreas model, all other effects can be eliminated. Therefore this study suggests serotonin has an inhibitory role in insulin release. On the contrary, 5-HT elicited marked increases in insulin secretion from normal pancreas but had an inhibitory effect on insulin secretion from diabetic pancreatic tissues [25]. This report shows another possibility that serotonin can have both an inhibitory and stimulatory effect on insulin release.

These data suggest that intrapancreatic serotonergic nerves play an inhibitory role in pancreatic exocrine secretion and an important role in the insulin action or release. To elucidate these problem more clearly, a dose-dependent experiment of serotonin will be carried out. In addition, other types of serotonergic antagonist will be administered to the pancreas to clarify detailed serotonin action on pancreatic exocrine secretion. Moreover, this present study did not measure insulin volume in the perfusate. To measure accurate insulin volume will help to elucidate the insulin- serotonin relationship. Further study is necessary to study a insulin release for serotonin action mechanism.

ACKNOWLEDGEMENTS

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