Effect of Reserpine on Pancreatic Exocrine Secretion Induced by Mesencephalic Reticular Stimulation in Rats

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= 국문초록 =

흰쥐에서 Reserpine이 중뇌망상체의 자극으로 유발된 췌장의 외분비 기능에 미치는 영향

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최근에 마취한 흰쥐에서 중뇌망상체를 전기적으로 자극하면 췌장의 외분비 기능이 증가하며 이러한 결과는 망상체의 자극으로 인하여 교감신경계의 활성도가 상승하기 때문이라는 보고가 있다. 한편 교감신경계의 활성도가 상승할 경우 교감신경계의 전달 물질인 catecholamine이 교감신경 종말 뿐만 아니라 부신수질에서도 유리된다고 알려져 있다. 그러므로 본 연구에서는 중뇌망상체의 자극으로 인하여 췌장의 외분비 기능이 증가함에 있어 교감신경계가 중요한 역할을 담당하는지를 확인하고, 이때 부신수질이 관여하는가를 알아보고자 하였다. 마취한 흰쥐에게 atropine (1 mg/kg) 또는 reserpine (5 mg/kg)을 투여하거나 또는 부신을 적출한 다음 중뇌망상체를 전기 자극하면서 췌장액을 채취하였다. 사용한 전기자극의 매개변수는 1.3 V, 40 Hz, 2 msec이었다. atropine과 reserpine을 투여하면 마취한 흰쥐의 자발적 췌장액 분비량과 단백질 분비량은 모두 유의하게 감소하였으나 부신을 제거하면 췌장액 분비량에는 이렇다할 변동이 없는 반면에 단백질 분비량은 유의하게 감소하였다. 중뇌망상체를 전기자극하면 췌장액 분비량과 단백질 분비량 모두가 유의하게 증가하였다. 이러한 망상체의 자극효과는 atropine 전처치에 의하여 이렇다할 영향을 받지 않았으나 reserpine 전처치에 의하여 소실되었다. 그러나 부신을 적출하면 망상체 자극에 의한 췌장액 분비량의 증가는 유지되는 반면에 단백질 분비량의 증가는 소실되었다. 한편 미주신경을 절단한 흰쥐에서 중뇌망상체를 자극하는 동안에 경동맥의 수축기 및 이완기 혈압이 상승하였는데 이러한 망상체의 자극효과도 reserpine의 투여에 의하여 유의하게 감소되었다.

본 실험의 결과를 종합하여 보면 마취한 흰쥐에서 중뇌망상체의 자극은 교감신경계를 활성화시켜 췌장액 분비량과 단백질 분비량에 촉진적인 영향을 미치며, 이때 활성화된 교감신경계는 부분적으로 부신을 경유하게 췌장의 단백질 분비에 촉진적인 영향을 미치는 것으로 생각된다.

Key Words: Pancreatic secretion, Reticular formation, Brain stimulation, Reserpine, Adrenalectomy, Rat

INTRODUCTION

It has been recently reported that electrical stimulation of the reticular formation of the mesencephalon results in an increase in pancreatic exocrine secretion of anesthetized rats (Park et al., 1986). However, the neural mechanism of the reticular stimulation, which exerts a stimulatory effect on the pancreatic exocrine secretion is, so far, unclear. It has been observed that a β-adrenoceptor antagonist inhibits the increase in the pancreatic exocrine secre-

tion induced by the reticular stimulation but the vagotomy does not exert any influence on it (Park et al., 1986). It has been also reported that the blood pressure of the carotid artery rises during the reticular stimulation in vagotomized rats (Lee & Choi, 1987). The observations lead to hypothesize that the reticular stimulation may excite the sympathetic nervous system, which results in the increase in the pancreatic exocrine secretion. It has been well documented in rats that noradrenaline, a neurotransmitter of the sympathetic nervous system, stimulates the pancreatic exocrine secretion while propranolol, a β-adrenoceptor antagonist, reduces not only the spontaneous pancreatic secretion but also pancreatic secretion induced by noradrenaline (Furuta et al., 1978; Lingard & Young, 1983; Pearson et al., 1984; Shin et al., 1986; Lee & Choi, 1987). In the mean time, it has been wellknown that the sympathetic excitation results in release of noradrenaline not only from the sympathetic nerve endings but also from the adrenal medulla (Hadley, 1984). However, it is not known, at the present time, whether the adrenal medulla is involved in the action of the reticular stimulation which increases the pancreatic exocrine secretion.

Thus, the present investigation was undertaken to elucidate 1) whether the sympathetic nervous system plays an important role and 2) whether the adrenal medulla is involved in the action of the reticular stimulation which facilitates the pancreatic exocrine secretion in anesthetized rats.

METHODS

Materials

Forty-nine male albino rats of the Sprague-Dawley strain (weighing 300-350 g) were used for the present investigation. For the purpose of food control, the rats were kept in this laboratory for at least 2 weeks before use. Atropine (Sigma, USA) in a dose of 1 mg/kg was administered through a jugular vein 30 min prior to the electrical stimulation of the reticular formation. Reserpine (Sigma, USA) in a dose of 5 mg/kg was injected subcutaneously 3 days before the experiment. The adrenal glands were bilaterally removed 7 days before the experiment. The adrenalectomized rats were supplied with 0.9% NaCl solution instead of tab mater.

Collection of pancreatic secretion

The rats were anesthetized by intraperitoneal injection of urethane (Sigma, USA) in a dose of 1 g/kg after 24h fasting but water *ad libitum*. The pancreatic duct was connulated at the duodenal end with a polyethylene tubing (i.d., 0.25 mm; o.d., 0.76 mm, Fisher Co., USA) while bile juice was diverted into the jejunum. The gastroduodenal junction was tightly ligated to prevent passage of gastric acid into the duodenum. A jugular vein was cannulated with a saline-filled tubing for the future injection of atropine or 0.9% NaCl solution. 10 min-samples of the pancreatic secretion were sequentially collected for determination of the secretory volume and protein output. The protein output was calculated from the secretory volume and the protein concentration in the 10 min-sample. The protein concentration was measured by spectrophotometry (DU-8B, Beckman, USA) of the samples diluted 1:100 or 1:200 in 0.04 M Tris buffer (pH 7.8) at a 280 nm wave length. Bovine serum albumin (Sigma, USA) was used as the standard.

Measurement of arterial blood pressure

For the purpose of investigating an influence of the reticular stimulation on the sympathetic activity, blood pressure of the carotid artery in rats with or without reserpine was monitored on a Dynograph (model #R612, Sensormedics, USA) during the whole experimental period. The bilateral vagus nerves of the rat were resected to eliminate a possible influence of the vagus nerve on the arterial blood pressure.

Electrical stimulation of reticular formation

After surgery for collection of pancreatic secretion, the rat was mounted in a stereotaxic apparatus (model #1404, David-Kopf Ins., USA). A pair of coaxial electrodes (NEX-100, Rhodes Med. Ins., USA) were bilaterally inserted in the reticular formation of the mesencephalon with the guide of the brain atlas (König & Klippel, 1974). The tips of the electrodes were aimed to place at 1.3 mm rostral to the obex, 1.6 mm lateral to the midline and 1.4 mm below the dorsal surface of the brain. When the volume of pancreatic juice secreted in 10 min was consistent, the reticular formation was electrically stimulated for 10 min using a stimulator (model #200, Bioscience, England). The stimulus parameter was 1.3 V, 40 Hz, 2 msec width, which was verified by an oscilloscope (model #1980B, Hewlett-Packard Co., USA). The 10 min-samples of the pancreatic secretion were sequentially collected during the whole experimental period. At the end of the experiment, the rat was perfused with 10% formaline solution through the heart. The brain was removed and stored in the fixing solution. The exact location of the electrode tips was examined on photographes which were taken of serial forzen sections of the brain at 70 μm and enlarged at X5 (Kim et al., 1976).

Analysis of data

For the purpose of evaluating the effect of the reticular stimulation on the pancreatic exocrine secretion, net changes of the pancreatic secretory volume (μl/10 min) and protein output (μg/10 min) after the reticular stimulation were calculated from the corresponding values before the reticular stimulation. All data were expressed as M.S.E. The paired or non-paired test and the Wilcoxon matched-pairs signed-ranks test were used for statistical analysis of data. *P* values less than 0.05 were considered significant.

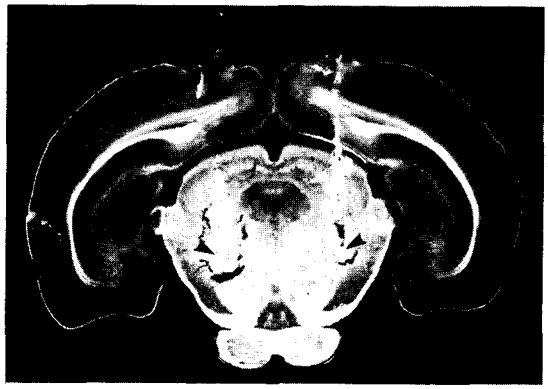

Fig. 1. A coronal section of a rat brain at the level of the superior colliculus showing the exact location of electrodes. Arrows indicate the tip of the electrodes.

RESULTS

Histological findings

As shown in Fig. 1, it was confirmed from photographes that the tips of the stimulating electrodes were bilaterally placed in the reticular formation of the mesencephalon at the level of the superior colliculus. Results obtained from rats in which the electrode tips were not placed in the aimed location were excluded from data.

Pancreatic secretion in response to reticular stimulation

In control rats which received 0.9% NaCl solution intravenously only, the spontaneous secretory volume and protein output of the pancreas were 9.14±1.09 μl/10 min and 424.07±48.46 μg/10 min respectively under the urethane anesthesia. The effects of atropine, reserpine and adrenalectomy on the spontaneous exocrine secretion of the pancreas are shown in Fig. 2 and 3. After 20 min of the atropine administration, the secretory volume and protein output were 6.85±1.25 μl/10 min and 277.25±37.44 μg/10 min respectively, which were signif-

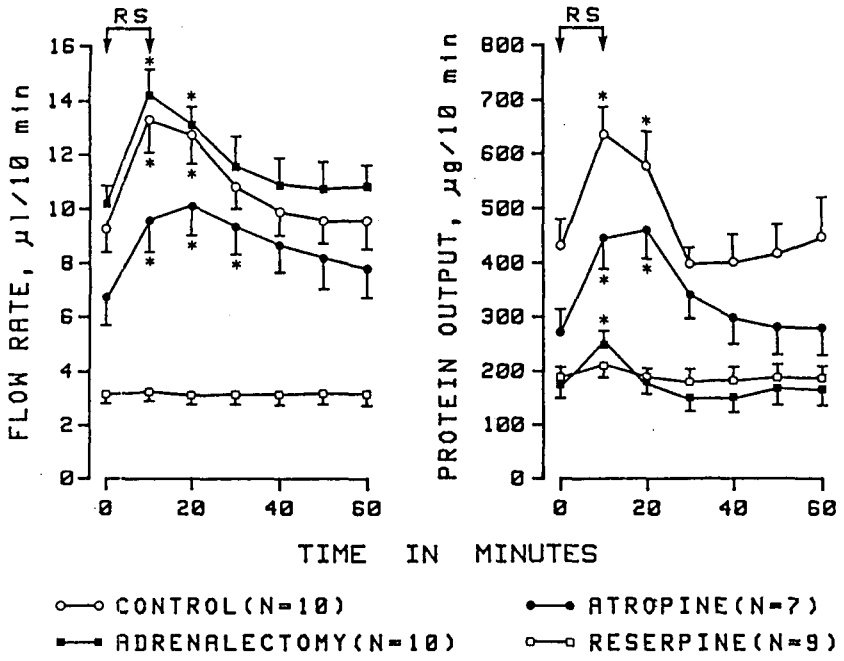

Fig. 2. Pancreatic flow rate and protein output in response to the reticular stimulation (RS) in anesthetized rats with treatment of atropine, adrenalectomy or reserpine. Each point represents MSE. The asterisk indicates the value is significantly different from the corresponding value before the reticular stimulation.

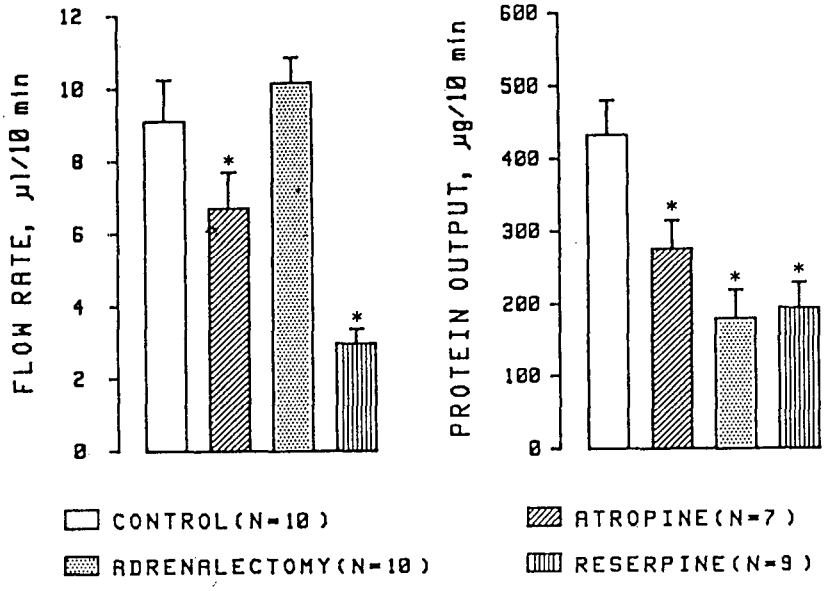

Fig. 3. Spontaneous pancreatic flow rate and protein output in response to atropine, adrenalectomy or reserpine in anesthetized rats. Each bar respresnts MSE. The asterisk indicates the value is significantly different from that of the control rats.

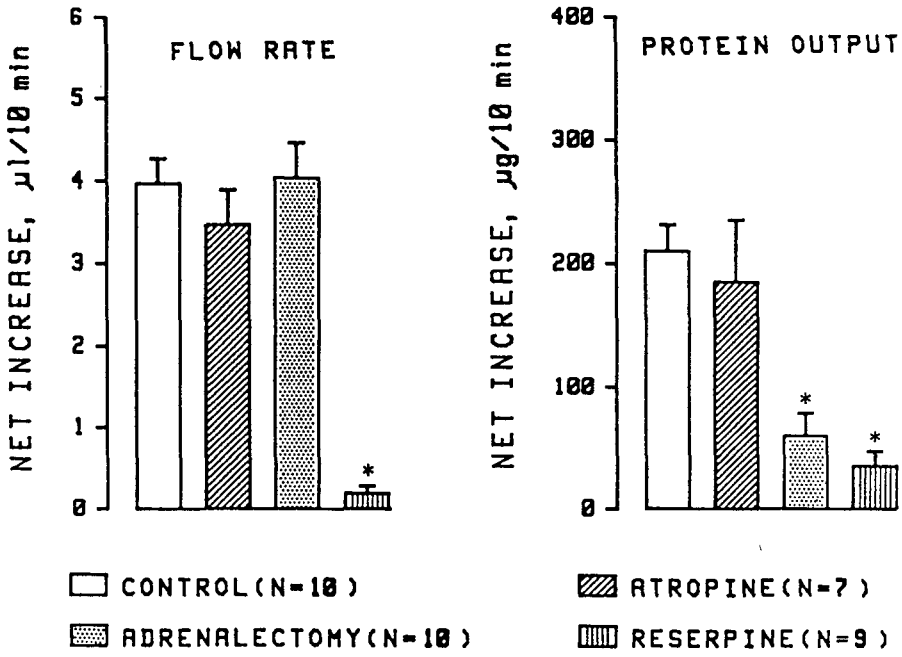

Fig. 4. Net increases in the pancreatic flow rate and protein output in response to the reticular stimulation in anesthetized rats with treatment of atropine, adrenalectomy or reserpine. Each bar represents MSE. The asterisk indicates the value is significantly different from that of the control rats.

icantly ($p<0.05$) lower than the corresponding value of the control rats. In the reserpine-treated rats, the secretory volume and protein output were 3.11 ± 0.31 μl/10 min and 200.74 ± 20.49 μg/10 min respectively, which were significantly ($p<0.005$) lower than the corresponding value of the control rats. In the adrenalectomized rats, the secretory volume was 10.18 ± 0.68 μl/10 min, which was not different from that of the control rats while the protein output was 180.51 ± 39.64 μg/10 min, which was significantly ($p<0.005$) lower than that of the control rats.

As shown in Fig. 2 and 4, electrical stimulation of the mesencephalic reticular formation resulted in remarkable increase in pancreatic exocrine secretion. In the control rats, the secretory volume and protein output of the pancreas during the reticular stimulation were 13.11 ± 1.34 μl/10 min and 636.59 ± 50.73 μg/10 min respectively, which were significantly ($p<0.005$) higher than the corresponding value before the reticular stimulation. The net increases in the secretory volume and protein output were 3.98 ± 0.31 μl/10 min and 212.52 ± 27.04 μg/10 min respectively. The effect of the reticular stimulation on the secretory volume and protein output was completely blocked by reserpine while they were not affected by the atropine-treatment. However, the adrenalectomy blocked the increase in the protein output only. As shown in Fig. 4, the net increases in the secretory volume and protein output induced by the reticular stimulation in the atropine-treated rats were 3.28 ± 0.66 μl/10 min and 180.74 ± 60.97 μg/10 min respectively, which were not different from the corresponding value of the control rats. In the reserpine-treated rats, the net increases in the secretory volume and protein output induced by the reticular stimulation were 0.15 ± 0.11 μl/10 min and 32.29 ± 9.71 μg/10 min respectively, which were significantly ($p<0.005$) lower than the corresponding value of the control

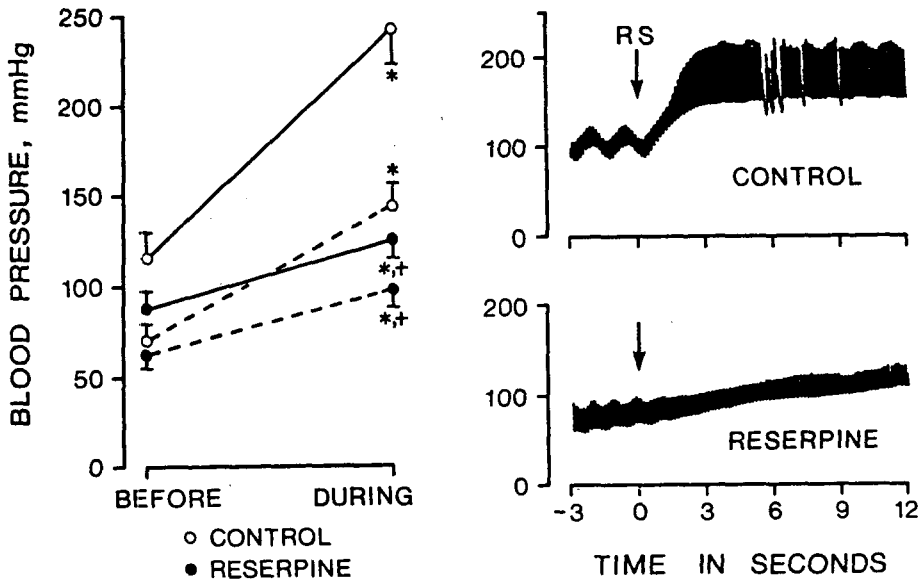

Fig. 5. Effect of the reticular stimulation (RS) on arterial blood pressure in anesthetized rats with or without reserpine. Vagotomy was performed in all rats 30 min before RS. The solid line indicates the systolic blood pressure while the dashed line indicates the diastolic blood pressure. The asterisk represents the value is significantly different from the corresponding value before RS. The cross indicates the value is significantly different from the corresponding value of control rats.

rats. In the adrenalectomized rats, the net increase in the secretory volume was $4.08\pm0.54\ \mu l/10$ min which was not different from that of the control rats while the net increase in the protein output was $62.60\pm21.12\ \mu g/10$ min which was significantly ($p<0.005$) lower than that of the control rats.

Arterial blood pressure in response to reticular stimulation

As shown in Fig. 5, the reticular stimulation resulted in definite rise of blood pressure of the carotid artery in the anesthetized rats. In the control rats systolic blood pressure rose from 116.17 ± 8.05 mmHg at the basal state to 245.67 ± 25.23 mmHg during the reticular stimulation while diastolic blood pressure also rose from 70.17 ± 4.23 mmHg to 143.83 ± 12.18 mmHg. The rises of the blood pressures were statistically significant ($p<0.005$). On the other hand, the increases in the blood pressures induced by the reticular stimulation in the reserpine-treated rats were less prominent. The systolic and diastolic blood pressures of the reserpine-treated rats were 86.61 ± 6.11 and 63.36 ± 8.84 mmHg respectively before the reticular stimulation, which was significantly ($p<0.005$) lower than the corresponding value of the control rats. The systolic and diastolic blood pressures also rose significantly ($p<0.005$) to 122.89 ± 7.84 and 98.17 ± 7.07 mmHg respectively during the reticular stimulation. However, the net changes of the systolic and diastolic blood pressures in the reserpine-treated rats, 36.57 ± 8.36 and 36.81 ± 7.13 mmHg respectively, were significantly ($p<0.01$) lower than the corresponding value of the control rats, 72.83 ± 9.76 and 70.50 ± 12.30 mmHg respectively.

DISCUSSION

The present investigation clearly shows that the

electrical stimulation of the reticular formation in the mesencephalon increases the secretory volume and protein output of the pancreas in anesthetized rats. The results of the present investigation together with previous reports (Park et al., 1986; Lee & Choi, 1987) provide a strong evidence that the reticular formation plays an important role in the pancreatic exocrine secretion. The reticular formation, thus, seems to contain a regulatory mechanism of gastrointestinal function. It has been already reported that when the decerebration was performed at the mid-collicular level the contractility of the antrum increased (Babkin & Kite, 1950) and the plasma concentration of gastrin rose in cats (Limbardi et al., 1981). The hypothalamus in dogs (Gilsdorf et al., 1966) and amygdaloid complex in rats (Mine et al., 1985) have been also reported to exert a regulatory influence of the pancreatic exocrine secretion. These findings seem to suggest that the reticular formation as well as the hypothalamus and amygdaloid complex could be an important brain structure which participates in the central neural mechanism of the cephalic phase of the pancreatic exocrine secretion (Preshaw et al., 1966; Sarles et al., 1968; Novis et al., 1971; Anagnostides et al., 1984).

In the present study, the effect of the reticular stimulation on the pancreatic exocrine secretion is not affected by atropine but it is definitely abolished by reserpine. Since reserpine is a wellknown sympathetic blocking agent which deplets stores of catecholamines in the nervous system, this result strongly suggests that the reticular stimulation exerts a facilitatory influence on the pancreatic exocrine secretion through the sympathetic nervous system instead of the parasympathetic nervous system. Similar results have been observed in anesthetized rats (Park et al., 1986; Lee & Choi, 1987), in which the effect of the reticular stimulation is not changed by cervical vagotomy but completely abolishey by propranolol, a β-adrenoceptor antagonist. The sympathetic activity appears to be increased by the reticular stimulation in rats. Systolic and diastolic blood pressures of the carotid artery in vagotmoized rats are markedly elevated during the reticular stimulation and the elevation of the blood pressure is inhibited by reserpine in the present study. The elevation of the blood pressure during the reticular stimulation has been also reported in cats (Wang & Ranson, 1939). It is difficult to explain from the present investigation how the reticular stimulation increases the sympathetic activity. However, it has been already observed that the reticular formation contains nerve fiber tracts that run from the limbic system named "visceral brain" to the lower autonomic centers in the brain stem and spinal cord (Brodal, 1958; Saper et al., 1976). Thus, it is inferred that the electrical stimulation applied on the reticular formation may result in excitation of the nerve fiber tracts.

It is possible that catecholamines acting on the adrenoceptors in the pancreas could be released not only from the sympathetic nerve endings but also from the adrenal medulla when the sympathetic nervous system is excited (Hadley, 1984). The adrenalectomy, in the present study, did not block the increase in the pancreatic secretory volume but definitely reduced the increase in the protein output, which where induced by the reticular stimulation. These results suggest that the facilitatory influence of the reticular stimulation on the pancreatic protein secretion could be partially mediated through the adrenal medulla in rats.

The spontaneous pancreatic secretion including water and enzymes appears to be under the facilitatory control of the cholinergic activity as well as the sympathetic activity in rats. Atropine in the present investigation and bilateral vagotomy in the previous reports (Park et al., 1986; Lee & Choi, 1987) decrease the spontaneous exocrine secretion of the pancreas in anesthetized rats. Reserpine, a sympathetic blocking agent depleting stores of catecholamines in the nervous system and adrenal medulla (Weiner, 1985), reduces the spontaneous

pancreatic secretion of anesthetized rats in the present study. It has been also found in anesthetized rats that the spontaneous pancreatic secretion is stimulated by phentolamine, a α-adrenoceptor antagonist while it is inhibited by propranolol, a β-adrenoceptor antagonist (Furuta et al., 1978; Shin et al., 1987; Lee & Choi, 1987). It has been documented that noradrenaline induces the spontaneous pancreatic secretion which, is blocked by proparanolol (Lee & Choi, 1987). Thus, it seems to be apparent that the sympathetic neurotransmitter increases the spontaneous pancreatic secretion by acting on the β-adrenoceptor in the rat pancreas. However, there seems to be species difference in action of the sympathetic nervous system on the pancreatic exocrine secretion. It has been demonstrated that the β-adrenoceptor in the pancreas of cats (Harper & Vass, 1941; Elisha et al., 1984) and dogs (Rudick et al., 1973; Kelly et al., 1977) exerts an inhibitory influence on the pancreatic exocrine secretion. The adrenal medulla seems to be also involved in the sympathetic control of the pancreatic enzyme secretion. Adrenalectomy in the present observation decreases the spontaneous protein secretion of the pancreas.

It is concluded from the present investigation that the electrical stimulation of the reticular formation in the mesencephalon results in increase in the pancreatic exocrine secretion through the sympathetic nervous system, and that the facilitatory influence of the sympathetic nervous system on the pancreatic protein secretion is partially mediated through the adrenal medulla.

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