W. W. PAWLIK, S. J. KONTUREK, P. GUSTAW, K. CZARNOBILSKI, R. SENDUR, J. JAWOREK, N. YANAIHARA

ROLE OF TACHYKININS IN THE CONTROL OF PANCREATIC SECRETION AND CIRCULATION

Institute of Physiology Academy of Medicine Krakow, Poland
Bioorganic Chemistry Shizuoka College of Pharmacy Shizuoka, Japan

Tachykinins (TK) are family of peptides including substance P (SP), substance K (SK) and neuromedin K (NK) that have been found in the nerves of the gastrointestinal tract and proposed to act as neurotransmitters to affect the motor, secretory and circulatory functions of the gut, but little is known about their action on the pancreas. In this study three series of tests were carried out to determine the action of SP, SK and NK on pancreatic secretion in conscious dogs and amylase release from the dispersed rat pancreatic acini and to correlate the alterations in pancreatic secretory and circulatory effects of TK in anesthetized dogs. SP, SK and NK infused i. v. in graded doses (0.12—1.0 μg/kg per h) in conscious dogs stimulated pancreatic protein outputs reaching, respectively, 38% and 23% of the maximal response to CCK (40 pmol/kg per h). HCO_3^- outputs were also significantly increased but the highest response did not exceed about 5% of secretin (328 pmol/kg per h) maximum. Cholinergic blockade by atropine abolished the pancreatic responses to tachykinins. When added at various concentrations ($10^{-11} - 10^{-7}$ M) to the incubation medium of rat dispersed pancreatic acini, SK, SP and NK increased in concentration-dependent manner the release of amylase from the resting pancreatic acini and augmented the enzyme release induced by CCK-8 and by urecholine. In anesthetized dogs infused with a background dose of secretin (82 pmol/kg per h), addition of SP, SK and NK caused an immediate and dose-dependent increase in the pancreatic blood flow, oxygen consumption and pancreatic secretion accompanied by a dose-dependent decrease in arterial blood pressure. This study shows that TK are potent pancreatic circulatory stimulants and moderate secretagogues both in vivo and in vitro, acting, at least in part, via cholinergic pathway.

INTRODUCTION

Tachykinins (TK) represent a large family of peptides with the common C-terminal aminoacid sequence (Phe-X-Gly-Leu-Met-NH_2) and with the widespread distribution in mammalian tissues (1—4). Initially, substance P (SP) was regarded as the only member of TK known to exist in mammalian brain and the gut (5) especially in the myenteric plexus (6, 7). SP was found to stimulate the contractile activity of the smooth muscle of the gut (4, 8), to induce the hypotension (9) and to stimulate exocrine salivary and pancreatic secretion (10—12).

Recently, two novel TK, substance K (SK) (also called neurokinin alfa and neurokinin A) and neuromedin K (NK) (also called neurokinin beta and neurokinin B) have been isolated from the porcine spinal cord (2, 3). Like SP, the newly discovered TK stimulate contractile activity of smooth muscle of the gut *in vitro* (8) and *in vivo* (13) but little is known about their action on pancreatic secretion and circulation.

The purpose of this study was to determine the effect of SP, SK and NK on exocrine pancreatic secretion in conscious dogs and on amylase release from the dispersed rat pancreatic acini *in vitro* and on the relationship between the pancreatic blood flow and pancreatic secretion in anesthetized dogs.

MATERIAL AND METHODS

Pancreatic secretory studies *in vivo* were carried out on 6 conscious dogs (weight 12—18 kg) prepared with gastric fistulas (GF) and pancreatic fistulas (PF) as previously described (14). The secretory studies started after about 3 mo of recovery from the surgery. The dogs were fasted for about 18 h before each experiment. The GF was opened throughout the experiment to prevent gastric acid from entering the duodenum and releasing duodenal hormones effecting the pancreatic secretion. Secretion from the PF was continously collected in 15-min aliquots, the volume of each sample was recorded and the HCO_3^- and protein concentrations and outputs were measured as described previously (15) and expressed in 15 min outputs. A continuous intravenous infusion of 0.15 M NaCl was delivered by a peristaltic pump by way of a polyethylene catheter inserted into the leg vein. All solutions were made isotonic (300 mOsmol/kg) by adding NaCl as needed. Solutions were infused i. v. at a constant rate of 80 ml/h. After about 30 min of collection of basal pancreatic secretion, SP, SK or NK was added to the i. v. infusion in a constant dose (1 µg/kg per h) or in gradually increasing doses (0.12—1.0 µg/kg per h), each dose being infused for 30 min and then doubled.

For the comparison, the maximal pancreatic protein secretion was elicited in these animals using CCK-8 (400 pmol/kg per h), while maximal HCO_3^- secretion was induced by secretin (328 pmol/kg per h). In separate series of tests a constant dose (1 µg/kg per h) of one of the TK was infused i. v. and when the pancreatic secretory rate reached a well sustained plateau, atropine (10 µg/kg) was injected i. v. in a bolus dose, the examination being continued for next 2 hours.

In tests with i. v. infusion of graded doses of TK, plasma samples were drawn before and 15 min after the administration of each peptide for the determination by specific radioimmunoassays

of pasma gastrin (16, 17), secretin and pancreatic polypeptide (PP) (18) and by bioassay of plasma CCK as described (14).

In vitro studies were carried out on isolated pancreatic acini obtained from male Wistar rats (150—200 g) fasted overnight. Animals were killed, the pancreas was removed and digested by highly purified collagenase (CLSPA 540 U/mg, Cooper Biomedical, Freehold, N. J. USA), according to the method of Amsterdam (19).

Dispersed acini were suspended in the incubation buffer (pH 7.4), containing 24.5 mM Hepes, 98 mM NaCl, 4.0 mM KCl, 11.7 mM K_2PO_4, 1.0 mM $MgCl_2$, 5.0 mM glucose, 1% (wt/vol) essential and nonessential amino acid mixture (SERVA Feinbiochemica, Heidelberg, FRG), 2.0 mM glutamine, 0.2% bovine serum albumin, and 0.01% (wt/vol) trypsin inhibitor.

The acinar suspension was saturated with oxygen, and incubated at 37°C for 30 min in shaking bath in presence of various concentrations (10^{-11}—10^{-7} M) of SK, SP, and NK alone, or in combination with a constant concentration of CCK-8 (10^{-12} M) or urecholine (10^{-6} M) or atropine (10^{-6} M). In control experiments, secretagogue alone was added to the acinar suspension. For comparison, maximal amylase release by CCK-8 (10^{-10} M) was determined in each series of tests. After incubation, the tubes were centrifuged at 1000 g for 5 min, supernatant was separated from the pellet and amylase contents of both fractions were determined separately using the method of Bernfeld (20).

The amylase release was expressed as percent of total amylase content in supernatant and in pellet ($\times 100$) after substraction of basal release for each experiment. Incubations were done in duplicate. Unstimulated amylase release was measured during each tests over whole experiment and presented as control value.

In separate experiments performed on 24 dogs (14—20 kg) anesthetized with chloralose (0.06 g/kg) and methyl carbamate (0.6 g/kg), the effects of SP, SK or NK given in various doses on the pancreatic blood flow and secretion were determined as described (21). For this purpose, the abdomen was opened and the pancreas was gently exposed; an electromagnetic blood flow probe (Scalar, MDL 503, The Netherlands) was placed around the superior pancreatico-duodenal artery and connected to an amplifier for the measurement of the arterial blood flow to the pancreas (SPBF). All vessels arising from the artery that did not perfuse the pancreas were ligated. A side-branch of the central pancreatic vein and femoral artery was cannulated to obtain the blood for measurement of arteriovenous oxygen content difference using a photometric analyzer (A-VOX system) (22). The rate of blood flow through each of the catheters connecting to A-VOX system to the pancreatic vein and femoral artery averaged about 8 ml/min and the catheter volume was about 2.2 ml. The time delay between the blood sampling and the moment of recording of the oxygen content was about 7 s and this delay was ignored.

Pancreatic oxygen consumption (PVO_2) was calculated as the product of the arteriovenous oxygen difference (AVO_2) and the SPBF. The systemic arterial blood pressure (SAP) was also measured via the catheter inserted into the femoral artery. For the secretory studies the minor pancreatic duct was ligated and the major duct was cannulated using 10 cm long polyethylene tube (internal diameter of 0.6 mm) for the continuous collection of the pancreatic juice. The dead space of the catheter was about 28 μl and the related time delay in the flow of pancreatic juice during the secretory response to secretion was only about 10 s. This delay was not considered in the calculation of the secretory outputs. After surgical preparation was completed, the infusion of i. v. secretin (82 pmol/kg per h) was started and about 60 min were allowed for the pancreatic blood flow and exocrine secretion to stabilize and then various doses of SP, SK or NK were injected, the interval between various doses being about 1—2 h to allow the pancreatic blood flow and secretion to return to the basal levels. Throughout the study, the flow rate of the pancreatic juice was monitored at 1 min interwals and the concentration and outputs of HCO_3^- were determined as described (23). Each TK given in graded doses was studied in separate group consisting of 6 dogs. For the comparison of the occurrence of peak pancreatic secretory and blood flow responses,

additional group of 6 dogs was used and each of the TK was injected in a single bolus dose of 1 μg/kg during the infusion of a constant dose of secretin. The arterial blood flow to the pancreas and the HCO_3^- secretory outputs were monitored at 1 min intervals and the occurrence of their peak values were compared.

All TK peptides as well as gastrin, CCK-8 and secretin used in this study were synthesized by one of us (N. Y.) using a solid-phase method in Bioorganic Chemistry of Shizuoka College of Pharmacy (Shizuoka, Japan). PP was obtained as a gift from Dr R. Chance (Lilly, Indianapolis, IN). All peptides were infused in a pure form in the solution containing 1% bovine serum albumin to prevent their degradation during the administration.

Results are expressed as means $\pm$ SEM. Student's t-test or analysis of variance was used to compare the means at the $P < 0.05$ level.

RESULTS

Effects of TK on basal pancreatic secretion and plasma hormone levels in conscious dogs

Graded doses of SP, SK and NK evoked a dose-dependent increase in basal pancreatic HCO_3^- and protein secretion reaching the highest level at dose of 0.5 μg/kg per h i. v. Further increase in the dosage of the compounds did not result in any significant rise in the HCO_3^- and protein outputs. The mean highest protein response to SP, SK and NK reached respectively, 38%, 30% and 23% of the maximal response to CCK-8 (400 pmol/kg per h) (Fig. 1). The
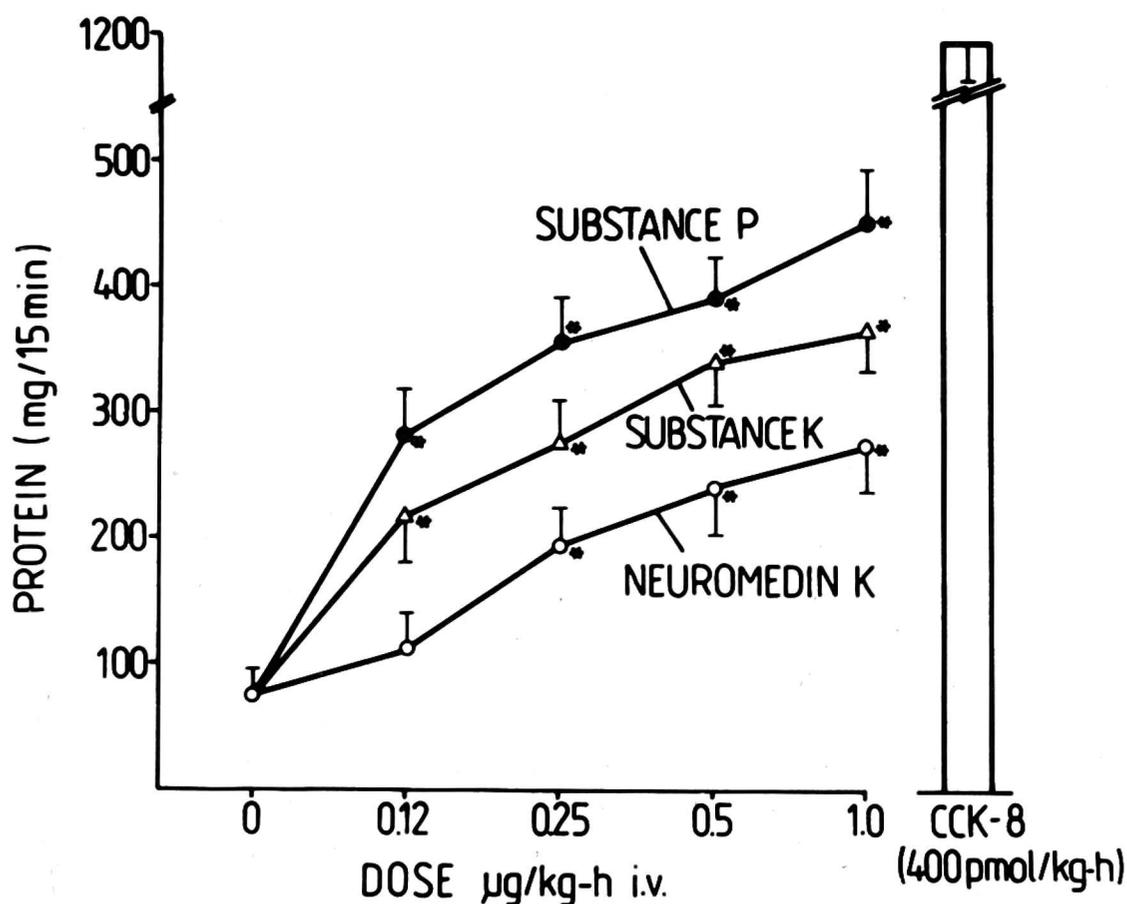
Fig. 1. Effect of graded doses of SP, SK and NK (0.12—1.0 μg/kg per h) on basal pancreatic protein secretion. Column represents maximal protein response to CCK-8 (400 pmol/kg per h). Asterisk indicates significant (p < 0.05) increase above basal value. In this figure and subsequent figures, each line is mean $\pm$ SEM of 6 experiments on 6 dogs.
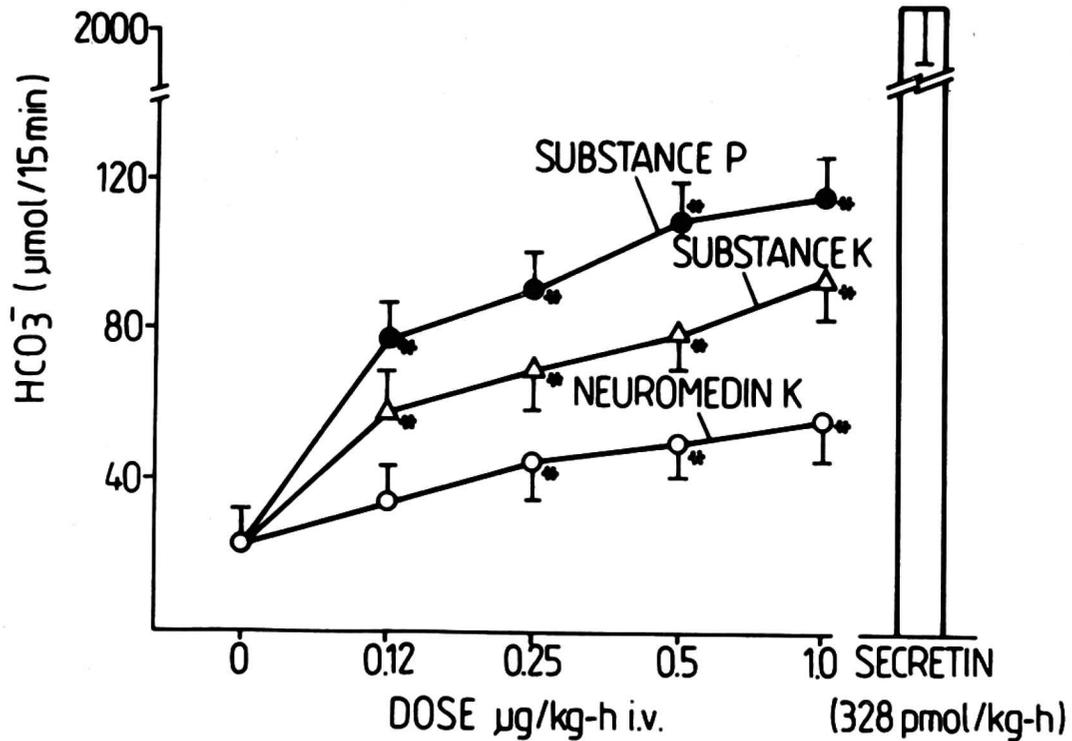
Fig. 2. Effect of graded doses of SP, SK and NK (0.12—1.0 μg/kg per h) on basal pancreatic HCO_3^- secretion. Column represents maximal bicarbonate response to secretin (328 pmol/kg per h). Asterisks indicate significant (p < 0.05) increase above basal value.
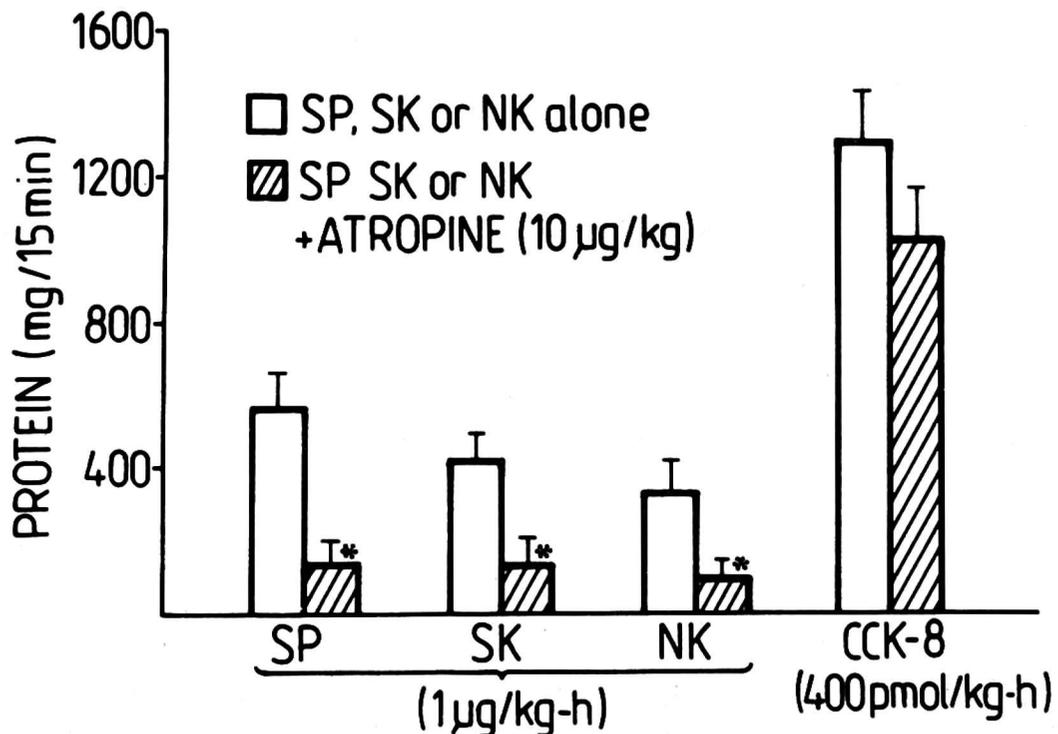

Fig. 3. Pancreatic protein response to one dose (1.0 μg/kg per h) of SP, SK and NK and to CCK-8 (400 pmol/kg per h) before and after administration of atropine (10 μg/kg). Asterisk indicates significant (p < 0.05) decrease below control response.

HCO_3^- output was also significantly increased by each of TK used but peak response did not exceed 5% of secretin (328 pmol/kg per h) maximum *(Fig. 2)*. Pancreatic HCO_3^- and protein secretion in respose to SP, SK and NK, but not to CCK-8, was abolished by the administration of atropine (10 µg/kg) *(Fig. 3)*.

The plasma gastrin, CCK and secretin responses were not influenced by SP, SK or NK but the PP response showed a small but significant increase at the highest dose of the TK *(Table 1)*.

Table 1. Effects of substance P, substance K and neuromedin K at the highest dose (1.0 µg/kg per h) on plasma levels of gastrin, CCK, secretin and PP. Means $\pm$ SEM of 6 tests on 6 dogs.

	Gastrin (pM)	CCK (pM)	Secretin (pM)	PP (pM)
Basal	28 ± 4	0.7 ± 0.3	1.3 ± 0.4	17 ± 3
Substance P	27 ± 3	0.6 ± 0.3	1.2 ± 0.3	39 ± 4*
Substance K	29 ± 3	0.7 ± 0.4	1.4 ± 0.4	42 ± 6*
Neuromedin K	30 ± 4	0.9 ± 0.3	1.3 ± 0.3	27 ± 4*

* Significant ($p < 0.05$) increase above the basal value

Effects of TK on amylase release from pancreatic acini under basal conditions and following secretory stimulation

Basal amylase release from the rat pancreatic acini averaged $3.0 \pm 4.0\%$ of the total. The presence of SK, SP and NK in the incubation medium resulted in the concentration-dependent stimulation of amylase release, reaching the maximal level at 10^{-8} M of SK and SP and at 10^{-7} M of NK. Further increase in the concentration of the TK did not result in any significant rise in enzyme secretion *(Table 2)*.

When graded concentrations of SK or SP (10^{-11}—10^{-6} M) were combined with a constant, submaximal concentration of urecholine (10^{-6} M), the amylase release showed a significant increase over the level obtained with urecholine alone ($11.82 \pm 1.6\%$) and reached peak at 10^{-9} M of these TK. The combination of urecholine with NK did not result in any significant increment in amylase release as compared to urecholine alone.

Incubation of the dispersed acini in presence of a constant concentration of CCK-8 (10^{-12} M) caused submaximal stimulation of amylase release. When increasing concentrations of SK or SP (10^{-11}—10^{-7} M) were added to the incubation medium containing CCK-8 (at 10^{-8} M), the amylase release ahowed further increase over the level obtained with CCK-8 alone ($10.8 \pm 0.9\%$) reaching peak at 10^{-9} M of TK. Again, the addition of NK to the urecholine did not result in any significant increase in amylase release as compared to CCK-8 alone.

Atropine added to the incubation at a concentration of 10^{-6} M did not affect significantly the amylase release from the resting acini but when added at

Table 2 Amylase release from the pancreatic acini in response to SP. SK and NK added concentrations either alone or in combination with CCK-8 (10^{-12} M), urecholine (10^{-6} M) or atropine (10^{-6} M). Means $\pm$SEM of 6 separate experiments performed in duplicates.

	TK concentration (M)				
	10^{-11} M	10^{-10} M	10^{-9} M	10^{-8} M	10^{-7} M
SK	$\pm$5.8	$\pm$7.5	11.2	12.2	12.1
	$\pm$0.4	$\pm$0.5	+0.9	$\pm$1.4	$\pm$0.8
SP	$\pm$4.4	$\pm$6.5	08.4	10.2	09.4
	$\pm$0.6	$\pm$0.4	$\pm$0.8	$\pm$0.9	$\pm$1.0
NK	$\pm$4.0	$\pm$4.7*	6.3*	6.4*	7.1*
	$\pm$0.3	$\pm$0.4	$\pm$0.4	$\pm$0.7	$\pm$1.0
URECHOLINE+SK	14.0	14.8	16.8+	16.7+	16.2+
	$\pm$0.6	$\pm$0.7	$\pm$0.4	$\pm$0.8	$\pm$0.4
URECHOLINE+SP	13.2	13.2	14.6+	14.5+	14.0
	$\pm$1.2	$\pm$0.8	$\pm$1.2	$\pm$1.3	$\pm$1.0
URECHOLINE+NK	11.6**	12.2**	12.6**	12.0**	12.8**
	$\pm$0.4	$\pm$1.0	$\pm$0.8	$\pm$0.7	$\pm$1.2
CCK-8+SK	14.1	15.4	16.5	15.2	16.0+
	$\pm$1.2	$\pm$1.4	$\pm$0.8	$\pm$0.6	$\pm$0.6
CCK-8+SP	13.0	14.4	14.8	14.8+	14.7+
	$\pm$0.8	$\pm$0.7	$\pm$1.0	$\pm$0.8	$\pm$0.6
CCK-8+NK	11.2	11.3	12.3	12.1	12.4
	$\pm$1.0	$\pm$1.2	$\pm$0.8	$\pm$0.8	$\pm$1.0
ATROPINE+SK	4.7	6.8	10.4	11.8	10.4
	$\pm$0.6	$\pm$1.2	$\pm$1.6	$\pm$1.2	$\pm$1.0
ATROPINE+SP	4.3	6.1	7.6	9.2	8.8
	$\pm$0.4	$\pm$0.5	$\pm$1.2	$\pm$0.8	$\pm$0.9
ATROPINE+NK	4.2	4.1	6.4	6.8	7.1
	$\pm$0.5	$\pm$0.4	$\pm$0.6	$\pm$0.4	$\pm$0.8

*Significant ($p<0.05$) decrease below the value obtained with SP

**Significant ($p<0.05$) decrease below the value obtained with CCK-8+SK or urecholine+SK

+Significant ($p<0.05$) increase above the value obtained with urecholine or CCK-8 combined with SK or SP added at 10^{-11}M.

the same concentration to gradually increasing concentrations of SK, it failed to affect the increase in amylase release *(Table 2)*. Also atropine added in gradually increasing concentrations (10^{-20}—10^{-5} M) to the constant concentration of SK (10^{-8} M) did not affect the amylase response to this TK and these rersults have not been included.

Effects of TK on pancreatic blood flow, oxygen consumption and secretion.

In tests with anesthetized dogs, the control blood flow in the superior pancreatico-duodenal artery (SPBF) in the resting and secretin-stimulated
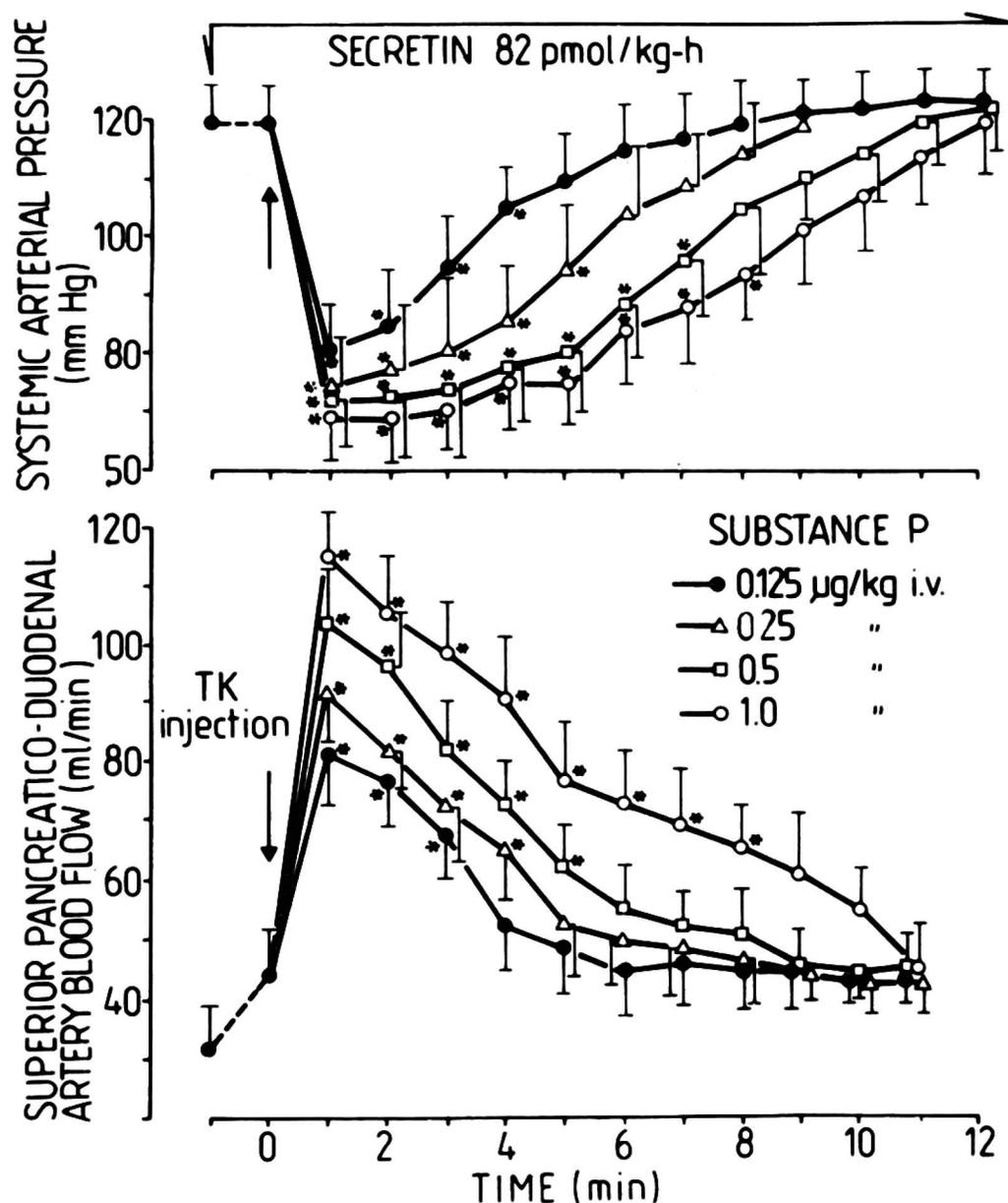
Fig. 4. Effects of substance P (SP) injected intravenously in graded doses on systemic arterial pressure and pancreatic blood flow (SPBF) in anesthetized dogs. Data are means ± SEM of 6 tests on 6 dogs. Asterisks indicate sugnificant change from control values obtained with secretin alone.

pancreas averaged 34 ± 3 and 43 ± 8 ml/min, respectively. The increase in the pancreatic blood flow after secretin was accompanied by a rise in oxygen consumption from the resting value od 1.82 ± 0.15 ml/min to 2.27 ± 0.20 ml/min. Mean systemic arterial pressure was 127 ± 12 mm Hg and did not change significantly after secretin alone.

A single i. v. bolus injection of SP, SK or NK in various doses ($0.125-1.0 \mu g/kg$) resulted in an almost immediate and dose-dependent drop in the mean systemic arterial pressure and a rise in the SPBF *(Figs. 4; 5, 6)*. The
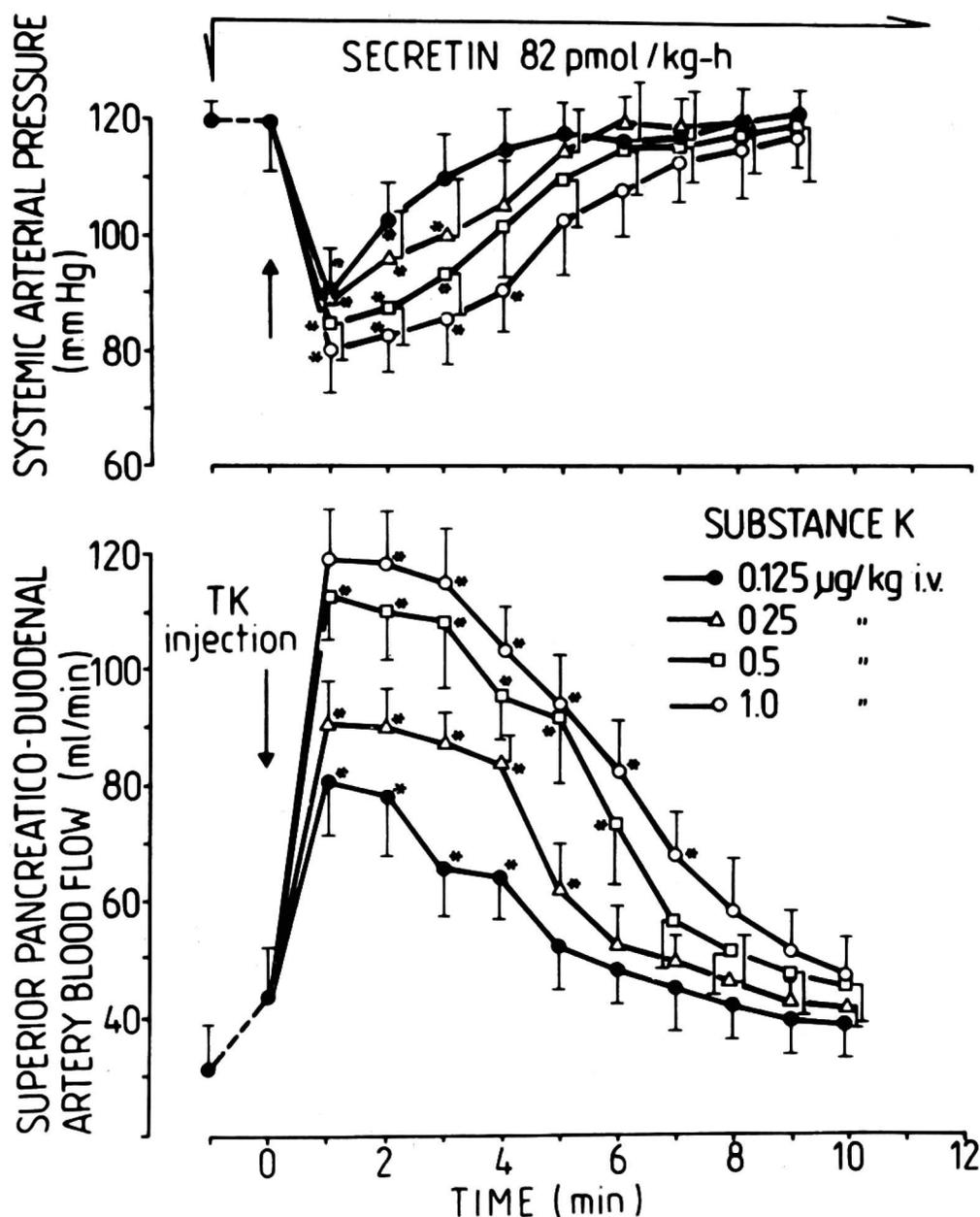
Fig. 5. Effects of substance K (SK) injected intravenously in graded dose on systemic arterial pressure and pancreatic blood flow (SPBF) in anesthetized dogs. Data are means ±SEM of 6 tests on 6 dogs.

peak changes of both parameters with all TK used were observed during the first minute after their administration and reached similar values at a given dose of tested TK, SP, SK and NK in the highest dose (1 μg/kg) increased SPBF by about 160, 173 and 40%, respectively. Systemic arterial pressure and pancreatic blood flow approximated within 8—10 min to the control values.

The response to i. v. injection of the peptides included a significant dose-dependent increase in pancreatic oxygen consumption. This parameter reached a peak value at a highest dose (1.0 μg/kg) of SP, SK and NK and was increased by 35%, 28% and 13%, respectively *Fig. 7).*

A backgroung infusion of secretin (82 pmol/kg per h) caused by itself only a small increase in the pancreatic volume flow (about 180±38 μl/min) and in HCO_3^- outputs (about 9±2 μmol/min). The combination of TK at a dose of 1 μg/kg with a background stimulation with secretin resulted in a marked
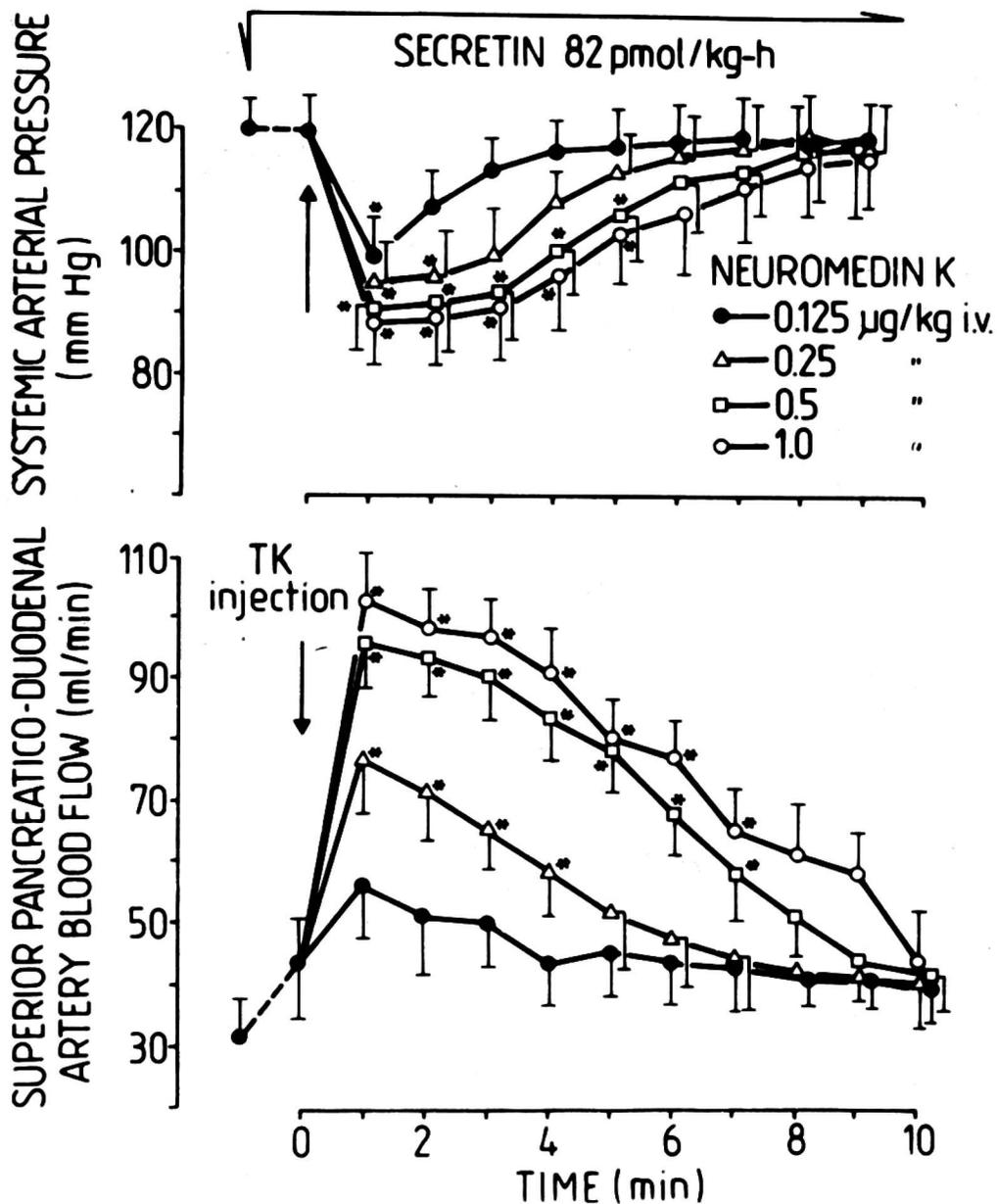
Fig. 6. Effects of neuromedin K (NK) injected intravenously in graded doses on systemic arterial pressure and pancreatic blood flow (SPBF) in anesthetized dogs. Data are means ± SEM of 6 tests in 6 dogs.

increase in both the volume flow and the HCO_3^- secretion. The HCO_3^- secretory response in tests with SP, SK and NK started to increase in the first minute after the TK administration to reach the peak in the second minute. The peak secretory output returned to the control level within 8 min. The peak secretory responses achieved with all three TK used were similar *(Fig. 8)*. The increase in the pancreatic blood flow in these tests was almost immediate and it reached the peak value within the first min after the TK administration.

DISCUSSION

This study compared the pancreatic secretory and circulatory effects of three tachykinins detected in mammalian neural tissues incuding SP, SK and NK showing high degree of sequence homology at their C-terminal portion. All
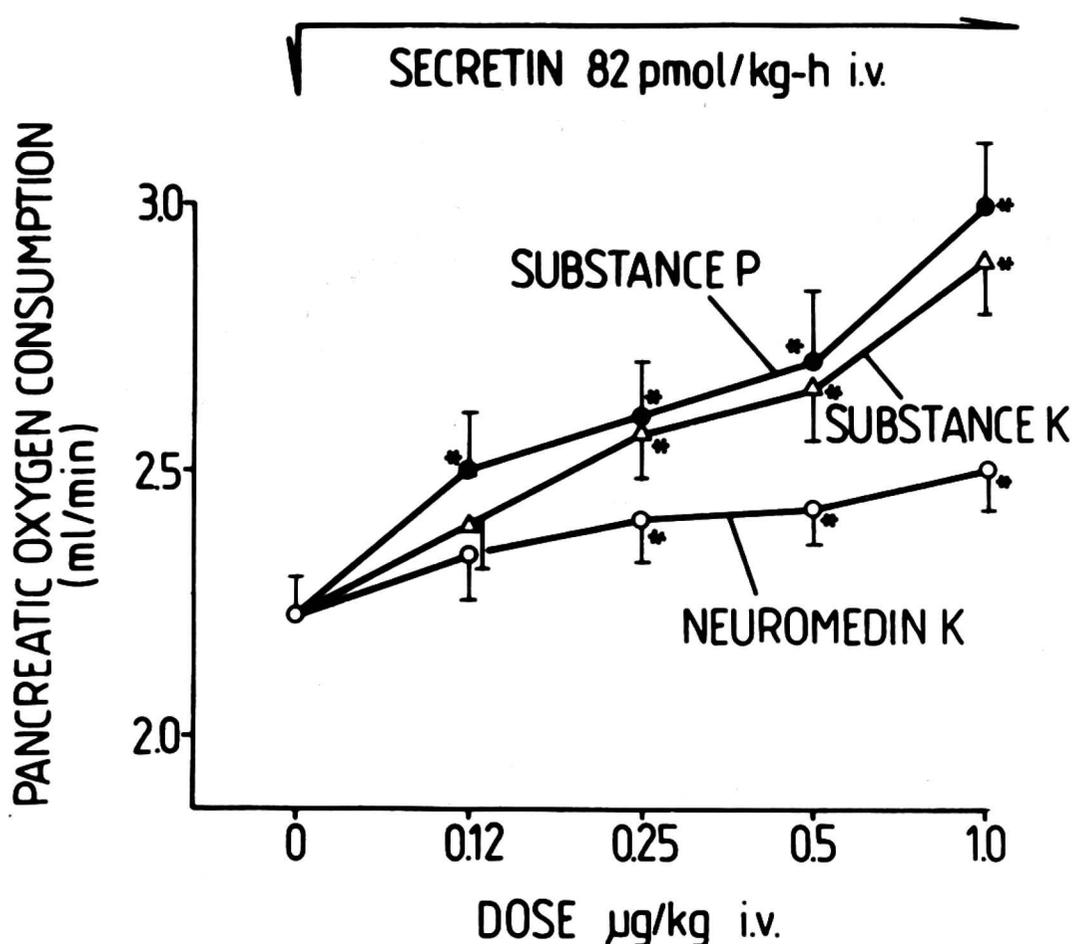
Fig. 7. Effects of tachykinins injected intravenously in graded doses on pancreatic oxygen consumption in anesthetized dogs. Data are means $\pm$ SEM of 6 tests on 6 dogs. Asterisks indicate significant (p < 0.05) increase above control value obtained with secretin alone.

three TK tested exhibited a week stimulation of pancreatic HCO_3^- secretion and moderate stimulatory influence on protein secretion. All three TK also caused an immediate and dramatic increase in the pancreatic blood flow accompanied by a significant rise in pancreatic oxygen consumption that preceded by about 1 min the increase in the exocrine pancreatic secretion. The stimulation of basal pancreatic secretion by SP has been reported previously (10, 11) and this has been atributed to the action of SP directly on the pancreatic secretory cells. This is supported by the finding that a radioactively labelled derivative of SP is specifically bound to dispersed pancreatic acinar cells and SP caused a partial stimulation of calcium outflux, cellular accumulation of cGMP and the release of amylase from acinar cells of guinea pigs (24, 25). Our results obtained from the rat dispersed pancreatic acini confirm that SP is a direct stimulant of amylase secretion by acinar cells. SP-induced acinar stimulation has an additive effect on amylase release by cholinergic agonist (urecholine) and by CCK but was reduced in part by
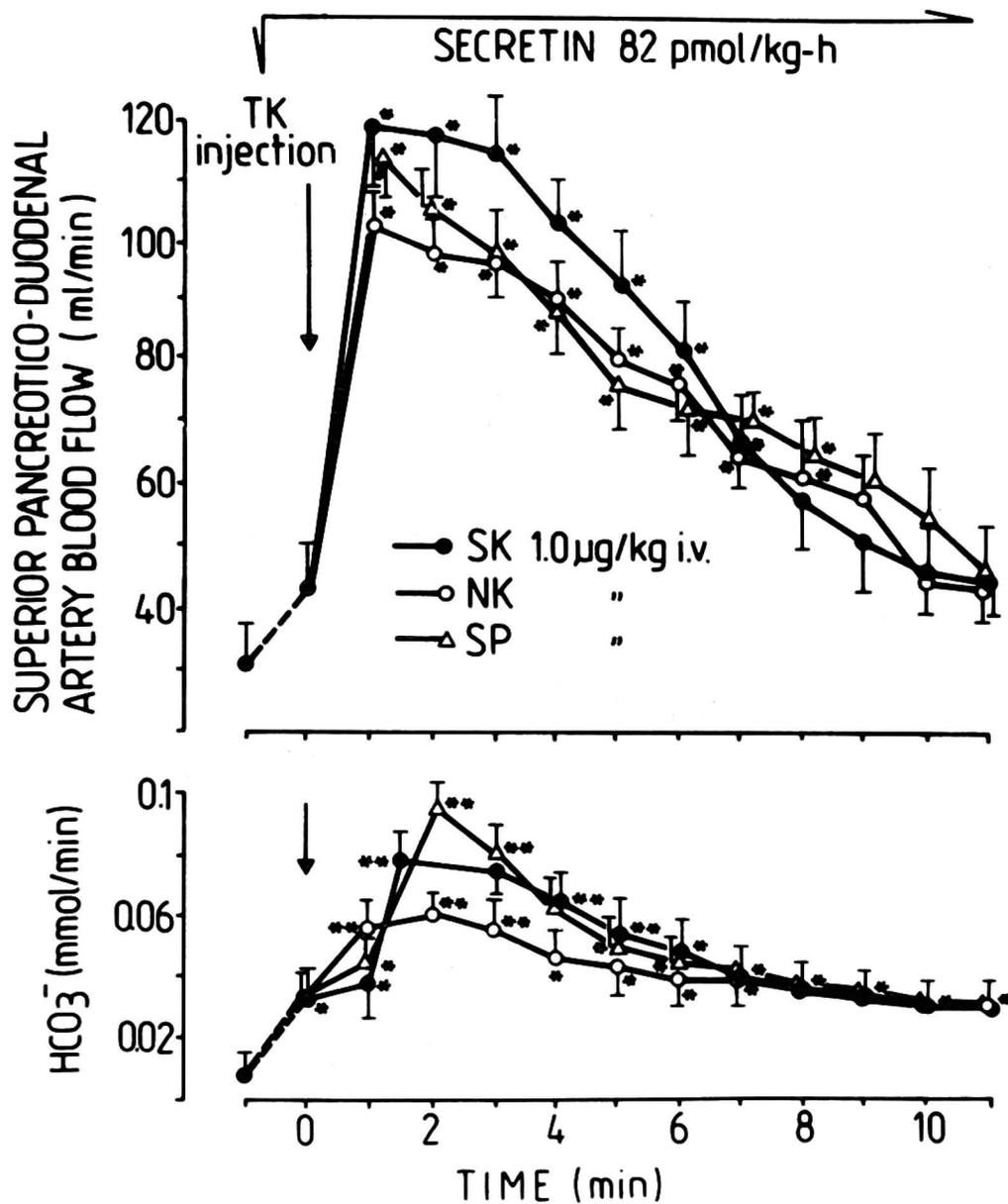
54

Fig. 8. Changes in the blood flow in the superior pancreatico-duodenal artery and in secretin-induced pancreatic bicarbonate outputs after i. v. injection of SP, SK and NK in one dose (1.0 μg/kg). Single asterisk indicates significant (p<0.05) increase above the value obtained with secretin alone. Data are means ±SEM of 6 tests in dogs.

atropine suggesting an existence of the interaction between secretagogues whose actions on acinar cells involves muscarinic, CCK and TK receptors. This study shows that the novel TK found in mammalian tissues such as SK and NK also exhibit the pancreatic secretagogue effects both *in vivo* and *in vitro*. These TK differ, however, in their potency of the stimulation of pancreatic secretion, the rank order of potency in the induction of protein secretion *in vivo* from the pancreatic fistula being SP> SK > NK and in amylase release from *in vitro* isolated pancreatic acini being SK> SP> NK. Similar relative potency of TK for stimulating amylase secretion, mobilizating cellular calcium and inhibiting the binding of ^{125}I-physalemin was reported in isolated guinea pig acinar cells (25, 26). The existence of the subtypes of specific high affinity receptors for TK has also been confirmed in the smooth muscle cells of the guinea pig ileum but the order of potency of these TK was different from that the stimulation of pancreatic secretion (27).

It is worth emphasizing that the blockade of cholinergic receptors by atropine had no influence on the action of TK on the isolated smooth muscle cells of the gut (27) but eliminated the stimulation of intestinal motility by SP *in vivo* (28). Similarly, stropine failed in this study to affect the TK-induced amylase release from *in vitro* isolated pancreatic acini but significantly suppressed the stimulatory action of TK on pancreatic protein secretion *in vivo*. These results suggest that TK *in vivo* may interact with receptors of cholinergic neurons in the pancreas as has been proposed for other tissues (29).

The observed stimulation of pancreatic protein secretion *in vivo* could be partly attributed to the action of TK directly on the pancreatic acinar cells but the fact that TK also enhanced pancreatic HCO_3^- secretion suggest that some other mechanisms may also be involved. This is why we measured also plasma levels of gut hormones such as secretin, gastrin and CCK to explain this phenomenon but neither of these peptides was affected by any of TK used. The rise in the plasma levels of PP could reflect the well known phenomenon that any type of pancreatic secretory stimulation is accompanied by the increased release of PP, whose role in this stimulation remains unknown (14, 30).

Although TK are known to affect peripheral circulation (1) and that the changes in the pancreatic blood flow are known to play an important role in the control of pancreatic secretion (31) no detailed informations are available regarding the relation between the pancreatic secretory and circulatory effects of TK. This study shows that TK are potent vasoactive agents as suggested previously (32). The question remains whether TK-induced increase in pancreatic secretion results in the secondary rise in the pancreatic blood flow or whether the increase in the blood supply to the pancreas is the primary effect of TK that in turn contributes to the enhancement of pancreatic secretory activity. This study demonstrates that all three TK tested caused an immediate and marked rise in the pancreatic blood flow and the systemic hypotention probably caused by an extensive vasodilation in the mesenteric circulation. We also found that TK evoked a significant increase in pancreatic oxygen consumption that could be due either to a direct metabolic stimulation or to an increase in the blood flow *per se* (33). Our finding that the peak increase in the pancreatic blood flow appeared about 1 min before the increase in the secretory stimulation could be interpreted that the pancreatic secretion following TK might be related, at least in part, to the increase in the pancreatic circulation as described for other vasoactive peptides (31).

REFERENCES

1. Erspamer V. The tachykinin peptide family. *Trends Neurosci* 1981; 4: 267—269.
2. Kanagawa K, Minamino N, Fucuda A, Matsuo H. Neuromedin K: a novel mammalian tachykinin indentified in porcine spinal cord. *Biochem Biophys Res Commun* 1983; 14: 533—540.

3. Kimura S, Okada M, Sugita Y, Kanazawa I, Munekata E. Novel neuropeptides, neurokinin alpha and beta isolated from porcine spinal cord. *Proc Jap Acad. Sci.* 1983; B 59: 101—104.
4. Hunter JC, Maggio JE. Pharmacological characterization of a novel tachykinin, isolated from mammalian spinal cord. *Eur J Pharmacol* 1984; 97: 159—160.
5. Euler VS, Gaggum JH. An unidentified depressor substance in certain tissue extracts. *J Physiol London* 1931; 72: 74—84.
6. Kuwahara A, Ishikawa T, Mikamis S, Yanaihara N. Distribution of neurons containing immunoreactivity for gastrin-releasing peptide (GRP), substance P and vasoactive intestinal polypeptide (VIP) in the rat gastric wall. *Biomed Res* 1983; 4: 473—478.
7. Schultzberg M, Hokfelt T, Nilsson G, et al. Distribution of peptide and catecholamine-containing neurons in the gastrointestinal tract of rat and guinea-pig: immunohistochemical studies with antisera to substance P, vasoactive intestinal polypeptide, enkephalins, somatostatin, gastrin, cholecystokinin, neurotensin and dopamine beta-hydrolase. *Neuroscience* 1980; 5: 689—744.
8. Kimura S, Goto K, Ogawa T, Sugita Y, Kanazawa I. Pharmacological characterization of novel mammalian tachykinins, neurokinin alfa and neurokinin beta. *Neurosci Res* 1984; 2: 97—104.
9. Pernow B. Substance P. *Pharmacol Rev* 1983; 35: 85—141.
10. Thulin L, Holm I. Effect of substance P on the flow of hepatic bile and pancreatic juice. In: Substance P, New York, Raven, 1977; pp. 247—251.
11. Konturek SJ, Jaworek J, Tasler J, Cieszkowski M, Pawlik W. Effect of substance P and its C-terminal hexapeptide on gastric and pancreatic secretion in the dog. *Am J Physiol* 1981; 241, G74—G81.
12. Gardner JD, Jensen RT. Secretagogue receptors on pancreatic acinar cells. Physiology of the Gastrointestinal Tract. Ed. by L. R. Johnson Raven Press, New York 1987, pp. 1109—1127.
13. Kuwahara A, Yanaihara N. Action of the newly discovered mammalian tachykinins, substance K, neuromedin K, on gastroduodenal motility of anesthetized dogs. *Reg Pept* 1987; 17: 221—228
14. Konturek SJ, Konturek P, Bielański W, Szewczyk K. CCK receptors in release of pancreatic polypeptide (PP) in dogs. *Dig Dis Sci* 1989; 34: 849—856.
15. Konturek SJ, Jaworek J. Cieszkowski M, Pawlik W, Kania J, Bloom SR. Comparison of effects of neurotensin and fat on pancreatic stimulation in dogs. *Am J Physiol* 1983; 244: G590—G598.
16. Konturek SJ, Tasler J, Cieszkowski M, Coy DH Schally AV. Effect of growth hormone release-inhibiting hormone on gastric secretion, mucosal blood flow and serum gastrin. *Gastroenterology* 1976; 70: 737—741.
17. Konturek SJ, Tasler J, Bilski J, Konturek J, Bielański W. Studies on the localization of secretin release from canine intestine. *Digestion* 1986; 34: 207—215.
18. Konturek SJ, Swierczek S, Kwiecień N, et al. Gastric secretory and plasma hormonal reponses to sham-feeding of varying duration in patients with gastroduodenal ulcer. *Gut* 1981; 22: 1003—1010.
19. Amsterdam A, Solomon TE, Jamieson JD. Sequential dissociation of exocrine pancreas into lobules, acini and individual cells. *Methods Cell Biol* 1978; 20: 362—376.
20. Bernfeld P. Amylases alpha and beta. In Methods in Enzymology (Academic, New York) 1955; pp. 139—148.
21. Konturek SJ, Yanaihara N, Pawlik W, Jaworek J, Szewczyk K. Comparison of helodermin, VIP and PHI in pancreatic secretion and blood flow in dogs, *Reg Pept* 1989; 24: 155—166.
22. Shepherd AP, Burgar CG. A solid-state arteriovenous oxygen difference analyzer for flowing whole blood. *Am J Physiol* 1977; 232: H437—H440.

23. Domschke S, Konturek SJ, Domschke W, et al. Cyclic-AMP and pancreatic bicarbonate secretion in response to secretin in dogs. *Proc Soc Exp Biol Med* 1975; 150: 773—779.

24. Sjodin L, Conlon TP, Gustavson C, Uddholm K. Interaction of substance P with dispersed pancreatic acinar cells from the guinea pig. Stimulation of calcium outflux, accumulation of cyclic GMP and amylase release. *Acta Physiol Scand* 1980; 109: 107—110.

25. Jensen RT, Gardner JD. Interaction of physalaemin, substance P and eledoisin with specific membrane receptors on pancreatic acinar cells. *Proc Natl Acad Sci USA,* 1979; 76: 5679—5683.

26. Uhlemann ER, Rottman AJ, Gardner JD. Actions of peptides isolated from amphibian skin on amylase from dispersed pancreatic acini. *Am J Physiol* 1979; 236: E571—E576.

27. Sonquet JC, Grider JR, Bitar KN, Makhlouf GM. Receptors for mammalian tachykinins on isolated smooth muscle cells. *Am J Physiol* 1985; 248: G533—G538.

28. Thor P, Sendur R. Konturek SJ. Influence of substance P on myoelectrical activity of the small bowel. *Am J Physiol* 1982; 243: G493—G496.

29. Holtzer P, Lembeck F. Neurally mediated contraction of ileal longitudinal muscle by substance P. *Neurosci Lett* 1980; 17: 101—105.

30. Beglinger C, Taylor IL, Grossman MI, Solomon TE. Pancreatic polypeptide release: Role of stimulants of exocrine pancreatic secretion in dogs. *Gastroenterology.* 1984; 87: 530—536.

31. Kvietys PR, Mc Lendon IM, Bulkley GB, Perry MA, Granger CN. Pancreatic circulation: intrinsic regulation. *Am J Physiol* 1982; 242: G596—G602.

32. Chahl LA, Walker SB. Responses of the rat cardiovascular system to substance P, neurotensin and bombesin. *Life Sci* 1981; 29: 2009—2015.

33. Beijer HJM, Haas AHJ, Charbon GA. Pancreatic O_2 consumption and CO_2 output during secretin-induced exocrine secetion from the panreas in the anesthetized dog. *Pflugers Arch* 1984; 400: 318—323.

Author's address: W. W. Pawlik, Institute of Physiology Medical Academy, ul. Grzegórzecka 16, 31-531 Kraków, Poland.