Life Sciences, Vol. 41, pp. 1585-1590 Pergamon Journals
Printed in the U.S.A.

EFFECT OF SYNTHETIC NEUROMEDIN U-8 AND U-25, NOVEL PEPTIDES IDENTIFIED IN PORCINE SPINAL CORD, ON SPLANCHNIC CIRCULATION IN DOGS

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(Received in final form July 20, 1987)

Summary

Two novel peptides which exert a potent stimulant effect on rat uterus smooth muscle have recently been identified in porcine spinal cord. These peptides designated neuromedin U-8 and U-25 have been reported to exert a hypertensive effect in rats. But further biological activities are not known. In the present study, the effect of these peptides on blood flow in portal vein, superior mesenteric artery and pancreatic tissue and on blood pressure were examined in dogs, utilizing recently developed ultrasonic transit time volume flow meter and laser Doppler flow meter. Neuromedin Us potently reduced blood flow in superior mesenteric artery. The minimum reductions could be observed even at very small doses of neuromedin U-25 (32 fmol/kg) and U-8 (90 fmol /kg), while the maximal reductions of 48.4 and 51.0% were attained at the doses of 320 pmol/kg (U-25) and 900 pmol /kg (U-8), respectively. These peptides also reduced portal vein blood flow, and the maximal reductions of 42.1 and 37.2% were attained at the doses of 32 pmol/kg (U-25) and 90 pmol/kg (U-8), respectively. On the other hand, blood flow in pancreatic tissue increased slightly with the maximal increases of 13.8% at 3.2 pmol/kg (U-25) and 11.8% at 9 pmol/kg (U-8), respectively. The maximal increases of blood pressure were 5.2% at 320 pmol/kg (U-25) and 4.3% at 90 pmol/kg (U-8). Furthermore, neither neuromedin U-25 nor U-8 influenced the axillary artery blood flow, suggesting their selective effect on splanchnic blood flow. Because of the potent and probably selective activity on splanchnic circulation, neuromedin U-25 and U-8 may well be recognized as physiologically significant novel neuropeptides or hormones.

Novel neuropeptides, neuromedin U-8 and U-25, have been identified in porcine spinal cord by a potent contractile activity on rat uterus smooth muscle in vitro, and they have been reported to increase blood pressure in rats (1,2). Their amino acid sequences have been reported to be unique among the brain gut peptides with the two exceptions for the partial C-terminal sequence of Leu-X1-Arg-Pro-Arg-X2-$CONH_2$ found in pancreatic polypeptide (PP) and for

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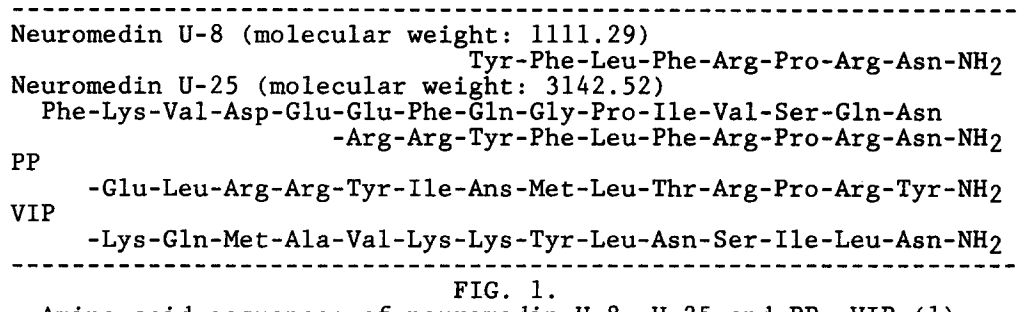

FIG. 1.
Amino acid sequences of neuromedin U-8, U-25 and PP, VIP (1).

the asparaginamide structure found in vasoactive intestinal peptide (VIP) (1) (FIG.1).

However, the details of their hypertensive effect or further biological activities are not known. In the present study, we first investigated the effect of synthetic neuromedin U-8 and U-25 (3) on splanchnic circulation in dogs utilizing newly developed ultrasonic transit time volume flow meter (4) and laser Doppler flow meter (5).

Materials and Methods

Eight mongrel dogs of either sex weighing between 10 to 20 kg were used. After 18 hour fast, each dog was anesthetized by intravenous sodium pentobarbital (25 to 30 mg/kg). A positive pressure mechanical ventilator was applied through an endotracheal tube during the experiment. Left femoral artery was catheterized for monitoring systemic blood pressure by a pressure transducer. Right femoral vein was catheterized for continuous infusion of 0.9 % saline and other agents. Pylorus was ligated and gastric juice was drained extracorporeally. Common bile duct and main pancreatic duct were cannulated while accessory pancreatic duct was ligated. For simultaneous measurement of blood flow in superior mesenteric artery and portal vein, two probes of ultrasonic transit time volume flow meter (TRANSONIC T201, Transonic System Inc. Ithaca NY) (4) were placed to both of them. For monitoring pancreatic capillary blood flow, a probe of laser Doppler flow meter (LD5000, Medpacific Corporation, Seatle WA) (5) was attached to the pancreatic tissue. Additionally, in five dogs pancreatic juice was collected as 10 minute sumples during the experiment, with a background infusion of synthetic secretin (Eisai, Japan) at a dose of 1 U/kg/hr.

After all monitors became stable, bolus injections of synthetic neuromedin U-8 an U-25 resolved in 0.9% saline containing 2% bovine serum albumin (Sigma, U.S.A.) were given via a femoral vein at progressively increasing doses. The doses tested were 0.1, 1, 10, 100 and 1000 ng/kg, which were equivalent to 32, 320 fmol, 3.2, 32 and 320 pmol/kg of neuromedin U-25 and 90, 900 fmol, 9, 90 and 900 pmol/kg of neuromedin U-8, respectively. In addition, by simultaneous measurement of blood flow in right axillary artery and superior mesenteric artery, their responses to 1000 ng/kg of neuromedin Us were observed in two dogs.

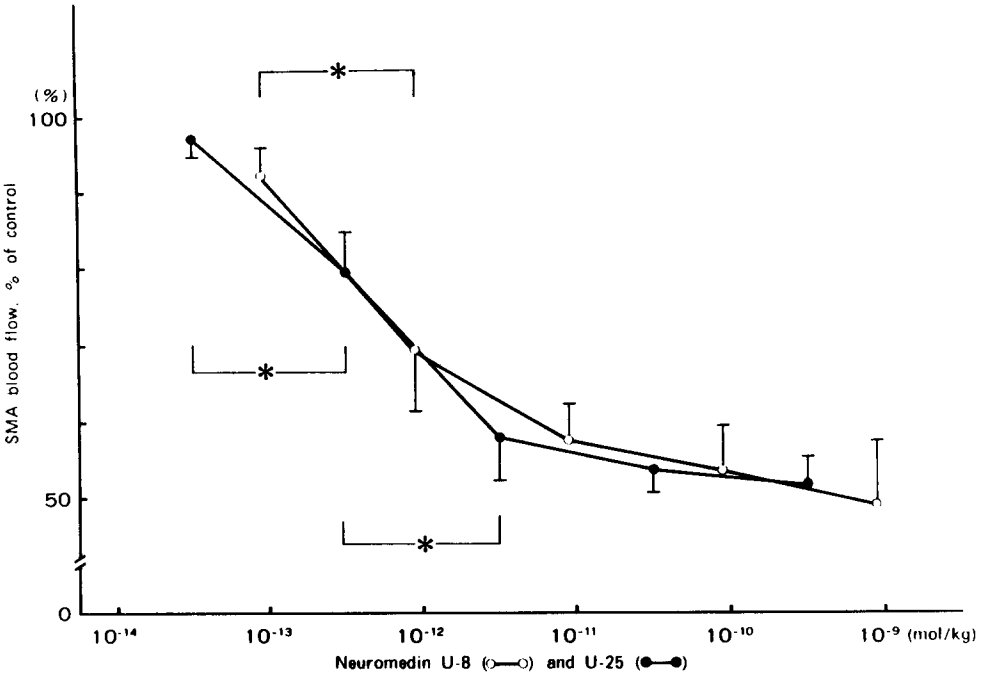

FIG. 2.
Dose-dependent effect of neuromedin U-8 (open circle) and neuromedin U-25 (closed circle) on superior mesenteric artery blood flow. Each circle and bar represents the mean ± SEM, and each * represents significant difference between doses ($p<0.05$).

For statistics, analysis of variance (ANOVA) was used, and differences were recognized as significant when $p<0.05$. Data are expressed as the mean ± SEM.

Results

Basal blood flow in superior mesenteric artery was 5.32 ± 1.05 ml/min/kg. Synthetic neuromedin U-25 and U-8 caused dose-dependent and significant ($p<0.01$) decrease of superior mesenteric artery blood flow, attaining the maximal reductions (48.5 ± 3.6% and 51.0 ± 8.3%) at doses of 320 pmol/kg of U-25 and 900 pmol/kg of U-8, respectively (FIG.2).

Basal blood flow in portal vein was 10.39 ± 2.48 ml/min/kg. Synthetic neuromedin U-25 and U-8 caused dose-dependent and significant ($p<0.01$) decrease of portal vein also, attaining the maximal reductions (42.1 ± 9.0% and 37.2 ± 5.4%) at doses of 32 pmol/kg and 90 pmol/kg, respectively (FIG.3).

In response to 32 pmol/kg of neuromedin U-25, blood flows in superior mesenteric artery and portal vein began to fall at 15 seconds and reached to the maximal reduction at 30 seconds, then gradually returning to the preinjection levels at about 10 minutes

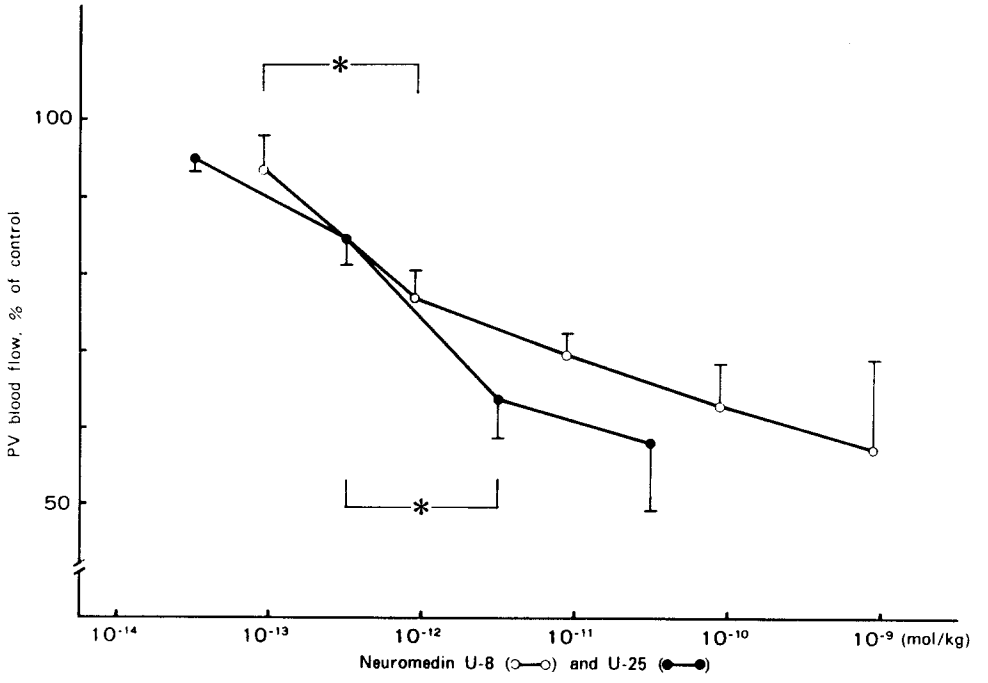

FIG. 3
Dose-dependent effect of neuromedin U-8 (open circle) and U-25 (closed circle) on portal vein blood flow. Each circle and bar represents the mean ± SEM, and each * represents significant difference between doses ($p<0.05$).

after administration. Neuromedin U-8 altered these blood flows just like neuromedin U-25 till the maximal reduction, but the blood flows returned to the preinjection levels at 3 to 4 minutes after administration of 90 pmol/kg of neuromedin U-8 (FIG.4). The increasing doses prolonged duration of reduction in a dose-related manner.

Axillary artery blood flow was not influenced by neuromedin U-25 (32 pmol/kg) or U-8 (90 pmol/kg), while superior mesenteric artery blood flow measured simultaneously was obviously reduced.

In response to neuromedin U-25, pancreatic tissue blood flow showed slight increases of 2.3 ± 1.0% (32 fmol/kg), 8.0 ± 3.0% (320 fmol/kg), 13.8 ± 3.9% (3.2 pmol/kg), 7.9 ± 2.4% (32 pmol/kg) and 5.6 ± 1.1% (320 pmol/kg) respectively. Neuromedin U-8 (90, 900 fmol/kg, 9, 90 and 900 pmol/kg) also slightly increased pancreatic blood flow (0.7 ± 2.0%, 7.6 ± 2.1%, 11.8 ± 4.4%, 11.5 ± 3.2% and 11.2 ± 5.0%), respectively. However, these responses were not recognized as significant by ANOVA.

Systemic blood pressure measured at femoral artery seemed to by increased by the doses of neuromedin U-25 and U-8. However, the maximal increases observed were only 5.2 ± 0.4% at a dose of

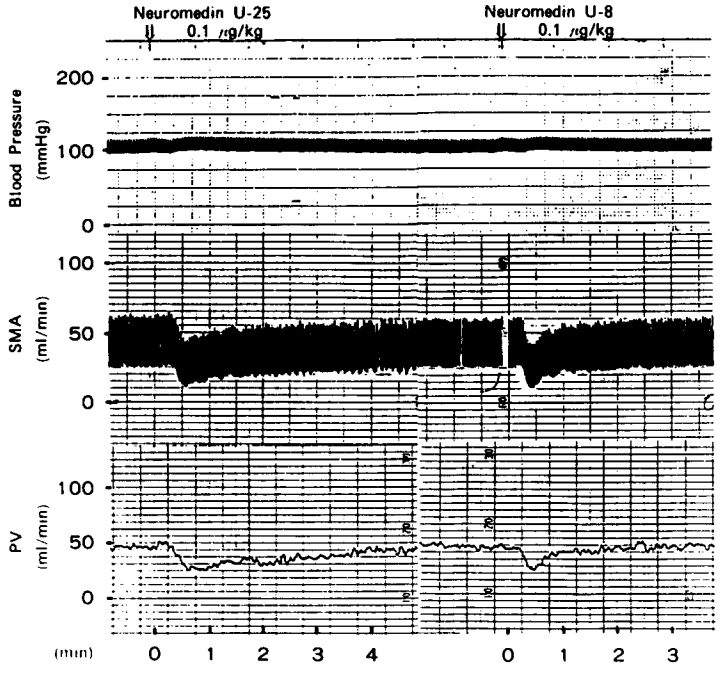

FIG. 4.
Responses of blood pressure (upper), superior mesenteric artery blood flow (middle) and portal vein blood flow (lower) to neuromedin U-25 (32 nmol/kg, left) and neuromedin U-8 (90 nmol/kg, right), by simultaneous measurement.

320 pmol/kg of neuromedin U-25 and 4.3 ± 1.3% at a dose of 90 pmol/kg of neuromedin U-8. These hypertensive effects were not recognized as significant by ANOVA.

Basal volume and protein output of pancreatic juice were 2.1 ± 0.8 ml/10min and 4.3 ± 1.3 mg/10min, respectively. Neuromedin Us caused no significant changes on exocrine pancreatic secretion.

Discussion

The present study first investigated the effect of synthetic neuromedin U-25 and U-8 on splanchnic circulation and exocrine pancreas, and demonstrated that these peptides potently reduced the blood flow in superior mesenteric artery and portal vein without influencing axillary artery blood flow, whereas these peptides retained only a slight hypertensive effect. In addition, pancreatic tissue blood flow showed a slight increase in response to neuromedin Us in spite of the prominent decrease of superior mesenteric artery and portal vein blood flow. These observations lead us to speculate that neuromedin U-25 and U-8 might exert specific effect on intestinal and/or some other splanchnic blood flow.

Concerning potency of these peptides, the hypertensive effect

of neuromedin U-25 has been reported to be three times more potent than U-8 in rats (1). From this study in dogs, neuromedin U-25 seems to be more potent than U-8 at relatively greater doses (FIG. 2 and 3), but the differences cannot be recognized to be significant.

Very recently, Domin et al. have reported that neuromedin U like immunoreactivity is detected in porcine, rat, guinea pig and human central nervous system and ileum by a specific radioimmunoassay (6). According to this report, the amount of neuromedin U like immunoreactivity in ileum is comparable to that in central nervous system, and only a single molecular form of neuromedin U like immunoreactivity which is similar to neuromedin U-25, is detected in porcine, rat and human tissue extract.

Among brain gut peptides, pancreatic polypeptide(PP), peptide YY and neuropeptide Y (NPY) are known to induce vasoconstriction (7). We examined synthetic PP (8) and synthetic NPY (9) by the same measurement system as the present study (unpublished data). And we found that the reducing effect of neuromedin U-25 and U-8 on intestinal blood flow are more potent than PP and NPY. In addition, PP and NPY but not neuromedin U-25 and U-8, decrease pancreatic blood flow. So, neuromedin U-25 and U-8 retain a unique effect among brain gut peptides which have ever been known.

In conclusion, this study first demonstrates that neuromedin U-25 and U-8 induce prominent decrease of blood flow in superior mesenteric artery and portal vein, and slight increase of systemic blood pressure and pancreatic tissue blood flow. These novel peptides may be considered to retain some physiological significance as neural, paracrine or hormonal modulator.

Acknowledgement

This study was supported by a grant from the Ministry of Education, Japan (#A6144060).

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