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Role of neuropeptide Y and its receptor subtypes in neurogenic pulmonary edema

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Abstract

The effect of neuropeptide Y on the number of perivascular carbon deposits, assessed as a measure of lung vascular permeability, was examined in isolated perfused lung preparations of rats. The number of carbon particle deposits after bronchial application of neuropeptide Y was increased in a dose-dependent manner. In the presence of a β-adrenoceptor antagonist, norepinephrine augmented the effects of neuropeptide Y. Peptide YY, an analog of neuropeptide Y, demonstrated a much lower potency for increasing the number of carbon deposits, and neuropeptide Y-(18–36), which elicits a weak antagonist action on the neuropeptide Y Y_3 receptor, significantly decreased the neuropeptide Y-induced increase. Furthermore, examination of the influence of neuropeptide Y-(18–36) pretreatment on fibrin-induced neurogenic pulmonary edema, in rats, revealed a reduction of the protein concentration ratio of tracheal fluid to serum. Therefore, we conclude that neuropeptide Y may elevate vascular permeability in the pulmonary circulation, conceivably through the neuropeptide Y Y_3 receptor, and that neuropeptide Y may in fact play a physiological role even in the in-situ pulmonary circulation.

Keywords: Neuropeptide Y; Pulmonary edema; Sympathetic nervous system; Capillary permeability

1. Introduction

Neuropeptide Y-containing extrinsic and/or intrinsic nerves innervate the blood vessels in the pulmonary circulation and respiratory tract. Kummer et al. (1992) and Lundberg et al. (1982) earlier showed that the sympathetic nerves from the stellate ganglion have neuropeptide Y immunoreactivity. Recently, Sakakibara et al. (1992) showed that electrical stimulation of the postganglionic fibers from the stellate ganglion increases lung vascular permeability in the presence of a vasodilator in rat isolated perfused lung preparations. They suggested that the sympathetic nerves may play a role in directly increasing the vascular permeability in the pulmonary circulation. Therefore, we examined the effects of several neuropeptides, including neuropeptide Y, on pulmonary vascular permeability in isolated perfused rat lungs. Among five neuropeptides tested, neuropeptide Y and neurokinin A dose dependently increased the capillary filtration coefficient value (Hirabayashi et al., 1994).

Neuropeptide Y is a 36-amino acid peptide which binds to multiple receptor subtypes, eliciting physiological responses such as vasoconstriction and presynaptic inhibition of neurotransmitter release (Michel, 1991). In the course of investigating structure-activity relationships for peptide analogs of neuropeptide Y, Wahlestedt et al. (Wahlestedt et al., 1986, 1987) first demonstrated that neuropeptide Y and peptide YY, found mainly in the gut, had a similar potency in enhancing the constriction of the rabbit femoral artery elicited by norepinephrine, whereas neuropeptide Y suppressed the motor response of the rat vas deferens to electrical stimulation to a lesser extent than peptide YY. The neuropeptide Y receptors were tentatively named neuropeptide Y Y_1 receptor for the postjunctional, and neuropeptide Y Y_2 receptor for the prejunctional type. However, in bovine chromaffin cells

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and human neuroblastoma cell lines (Wahlestedt et al., 1992), peptide YY did not specifically bind to cell membranes and was less active than neuropeptide Y: on this basis a neuropeptide Y Y_3 receptor subtype was proposed. Balasubramaniam and Sheriff (1990) had earlier shown that neuropeptide Y-(18–36) inhibited ^{125}I-neuropeptide Y-binding to rat cardiac ventricular membrane, as well as isoproterenol-induced stimulation of adenylate cyclase activity, while Michel (1991) had found neuropeptide Y-(18–36) to elicit a weak antagonist action on neuropeptide Y Y_3 receptors, and partial and full agonist actions at neuropeptide Y Y_1 and Y_2 receptors, respectively.

In neurogenic pulmonary edema, which is sometimes associated with cerebral hemorrhage and epilepsy, the activity of sympathetic nerves is elevated, resulting in an enhancement of transvascular plasma filtration and alveolar flooding. The edema formation is possibly mediated by venoconstriction in the pulmonary circulation, and plasma fluid is extravasated due to the increase in capillary pressure. Furthermore, the edema fluid in the alveolar space contains a high concentration of protein, suggesting an increase in pulmonary vascular permeability (Minnear and Malik, 1982). Therefore, the present study was undertaken to further evaluate the effects of neuropeptide Y on the number of perivascular carbon deposits, assessed as a measure of lung vascular permeability, and to clarify the role of neuropeptide Y in the development of neurogenic pulmonary edema, using a fibrin-induced rat model. In order to determine the neuropeptide Y receptor type involved in the action on the lung vascular permeability, besides neuropeptide Y, peptide YY and neuropeptide Y-(18–36), we also utilized neuropeptide Y-(13–36), which is active at the neuropeptide Y Y_2 receptor (Wahlestedt et al., 1987), and [Leu^{31},Pro^{34}]neuropeptide Y, a selective neuropeptide Y Y_1 receptor agonist (Fuhlendorff et al., 1990).

2. Materials and methods

2.1. Animals

Two series of experiments were undertaken, one for elucidating the effects of neuropeptide Y on pulmonary vascular permeability and its receptor subtype, and the other for evaluating the role of neuropeptide Y in the development of neurogenic pulmonary edema. The former was performed with isolated perfused rat lung preparations, and the latter with the so-called fibrin-induced pulmonary edema model, involving separate injections of fibrinogen and thrombin solutions into the cisterna magna of rats. A total of 140 Wistar male rats, weighing 250–300 g, were used, all of which were anesthetized with an intraperitoneal injection of pentobarbital sodium at a dose of 35 mg/kg.

2.2. Isolated lung perfusion preparation

After tracheotomy, we inserted a tracheal tube connected to a large diameter tube. A constant pressure was maintained after deep artificial respiration 3–5 times. Next a mid-thoracotomy was performed, an arterial cannula was inserted into the pulmonary arterial trunk through the right ventricle, and artificial perfusion fluid was gradually introduced. A venous cannula was then inserted into the left auricle through the left ventricle, and perfusion fluid was allowed to flow until the blood was completely replaced by Krebs-Henseleit solution containing 10% of low-molecular-weight dextran, 3% of bovine serum albumin and 10^{-4} M of papaverine. The Krebs-Henseleit solution was composed of NaCl, 118 mM; KCl, 4.7 mM; $CaCl_2 \cdot 2H_2O$, 2.5 mM; $MgSO_4 \cdot 7H_2O$, 1.2 mM; KH_2PO_4, 1.2 mM; $NaHCO_3$, 25 mM; and glucose, 10 mM. The perfusion fluid was aerated with $95\%O_2 + 5\%CO_2$, and the pH value was adjusted to within the range of 7.3–7.5 with hydrochloric acid and sodium bicarbonate solution.

After excision from the body, modified isolated perfused lung preparations were made (Sakakibara et al., 1992). Briefly, the lungs were placed in a moisture chamber, with the temperature maintained at 35°C, and perfused under a Zone III condition (venous pressure > alveolar pressure) with a constant 30 mm H_2O airway pressure. Pulmonary arterial and venous pressures were measured with pressure transducers (Nihon Kohden, LPU-0.1, Tokyo, Japan) positioned at the orifices of the inflow and outflow cannulae, respectively. Pressures were zeroed at the level of the lung hilus, at which level the lungs were hung up with a thread connected to the lever of a force-displacement transducer (Nihon Kohden, TB-611T, Tokyo, Japan). Lung weight was measured with the transducer connected to an amplifier (Nihon Kohden, RP-5, Tokyo, Japan). Perfusion flow was measured by counting the drips from the venous outlet with a tachometer (Nihon Kohden, RT-5, Tokyo, Japan). The drips were collected in a venous reservoir, from which the fluid was pumped up into an arterial reservoir. At the beginning of the experiment, the heights of arterial and venous reservoirs were adjusted and being maintained constant thereafter, so that the perfusion flow was constant between 8 and 12 ml/min. In order to avoid changes in perfusion flow following the administration of drugs into the venous reservoir, papaverine was added in the perfusion fluid as a vascular relaxant. Pulmonary arterial and venous pressure, lung weight and perfusion flow were continuously recorded on a multipurpose polygraph recorder (Nihon Kohden, RM-8, Tokyo, Japan).

2.3. Estimation for vascular permeability

After the lung preparations were made, 90 min were allowed to elapse. This time was necessary to eliminate the effects of other factors such as hemodynamic changes caused by bioactive substances liberated during preparation of the lungs. Thereafter, a carbon solution (C11/1431a; Günther Wagner Pellikan-Werke, Hanover, Germany, 1.2×10^{12} carbon particles/ml) was added to the perfusion fluid (30 μl in 50 ml of perfusion fluid), in order to allow estimation of the degree of vascular permeability of the endothelial wall to carbon particles. A further 30 min were then allowed to elapse, and the carbon particles remaining within the vasculature were washed out with saline. At the end of the perfusion, the lungs were removed and fixed in 10% formalin solution, for subsequent histological examination and counting of retained deposits of carbon particles along the vascular walls and in the alveolar spaces (Sakakibara et al., 1992).

Each lung was embedded in paraffin, sectioned at 10 μm thickness, and stained with eosin. The preparations were examined with a microscope equipped with a video-graphic printer (VP-7000; Fujix Co., Tokyo, Japan) connected to a computer. The carbon particles in a given area of 0.09 mm^2 (0.3×0.3 mm^2) were counted under magnification $\times 400$. Particles constituting ≥ 3 digital dots were considered as carbon particles for counting purposes. Those smaller than or equal to two digital dots were examined each time for confirmation. The counting procedure was repeated in five different areas per section, each near the bronchi (ID: 0.17–0.43 mm), near the pleura (within a distance of 0.43 mm from each), and in intermediate regions. Five continuous sections were used for counting at similar positions and the average value for each region was calculated.

2.4. Experimental protocol

2.4.1. Dose-response relationships

We examined the dose-response relationships of agonists, i.e., neuropeptide Y, peptide YY, neuropeptide Y-(13–36), neuropeptide Y-(18–36), and [Leu^{31}, Pro^{34}]neuropeptide Y, on carbon-particle deposition along the lung vascular wall. For the experiments, 0.2 ml of saline or agent at different concentrations was introduced into the bronchi during spontaneous respiration before the chest was opened. The vascular permeability was estimated as mentioned above, for each dose of agent.

2.4.2. Interaction between neuropeptide Y and other agents

In order to examine a possible interaction between neuropeptide Y and norepinephrine, both of which presumably exist in the sympathetic nerve terminals, we added norepinephrine and propranolol, a β-adrenoceptor-blocking agent, into the perfusion fluid. The effects of neuropeptide Y were then compared between two groups with and without the norepinephrine and propranolol treatments. The following studies were performed in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M in the perfusion fluid.

The effect of neuropeptide Y-(18–36) was examined both in the absence and in the presence of neuropeptide Y. Neuropeptide Y-(18–36) was injected into the femoral vein at a dose of 3 mg/kg before the chest was opened, with subsequent intrabronchial administration of 0.2 ml of saline or neuropeptide Y. Such venous administration was performed in order to prevent the effects of neuropeptide Y in in-situ lungs. After the lung preparations were made, neuropeptide Y-(18–36) was again administered into the perfusion fluid at a concentration of 10^{-6} M. The number of carbon particles with neuropeptide Y-(18–36) and neuropeptide Y treatment were compared to those with neuropeptide Y alone.

Furthermore, to investigate the possible participation of histamine release from mast cells in the accumulation of carbon particles in the extravascular spaces (Grundemar and Håkanson, 1991), rats were pretreated with a histamine-receptor antagonist, mepyramine. Before the intrabronchial administration of 0.2 ml of neuropeptide Y solution, mepyramine was administered at a dose of 1 mg/kg, into the femoral vein, and was added to the perfusion fluid after the lung preparation was made. The number of carbon particle deposits was then compared between neuropeptide Y-treated groups with and without mepyramine.

2.5. Fibrin-induced pulmonary edema

In order to verify a role for neuropeptide Y in the development of neurogenic pulmonary edema, we used a fibrin-induced pulmonary edema model (Ishikawa et al., 1988). Intracisternal administration of fibrinogen and thrombin enhances sympathetic nerve activity, causing alveolar flooding. A tracheal tube was inserted after tracheotomy and catheters were inserted into the right femoral artery and vein for measurement of systemic arterial pressure and for drug injection, respectively. Systemic arterial pressure was measured with a pressure transducer (MPU-0.5; Nihon Kohden Co., Tokyo, Japan), and heart rate was recorded via a tachometer (RT-5, Nihon Kohden Co., Tokyo, Japan) triggered with electrical signals of blood pressure pulse.

After the surgical procedures were completed, rats were treated with 0.2 ml of neuropeptide Y-(18–36) 10^{-6} M, injected into the bronchi during inspiration, and also 1 mg/kg of neuropeptide Y-(18–36) i.v. The other rats were treated with saline instead of neu-

ropeptide Y-(18–36). Then animals were fixed in a prone position with a stereotaxic instrument. Respiratory movement was monitored with a strain-gauge transducer (TB-611T, Nihon Kohden Co., Tokyo, Japan), the lever of which was connected to the dorsal thoracic skin with a thread. Room temperature was maintained at approximately 25°C. Bilateral vagotomy was performed at the midcervical position 15 min before the experiment. Fibrinogen and thrombin were dissolved in saline at concentrations of 100 mg/ml and 200 U/ml (pH 6.5), respectively. These solutions, 0.05 ml each, were successively injected into the cisterna magna of the rats after removal of 0.1 ml of cerebrospinal fluid. It took only 30 min to perform these procedures after the induction of anesthesia.

The degree of development of pulmonary edema was estimated by the appearance of edema froth from the tracheal tube. We estimated edema as being present when froth appeared within 15 min or without compression of lungs after excision, and otherwise as absent, even when froth appeared with compression. In all control rats, edema fluid appeared in the tracheal tube without compression of lungs within 5–7 min after injection of fibrinogen and thrombin. Fluid was collected in plastic tubes and stored, as well as blood serum, for later analysis of protein concentration, using Biuret's method. The ratio of protein concentration in tracheal fluid to that in serum was calculated and compared between neuropeptide Y-(18–36)-pretreated and untreated rats.

2.6. Statistics and chemicals

Differences between means were examined for significance by analysis of variance. Statistical significance was evaluated by Scheffé's method, at a level of 0.05. Values were expressed as means and their standard errors.

A neuropeptide Y analog, neuropeptide Y-(18–36), was synthesized with a Peptide Synthesizer (431A, Applied Biosystems, Foster City, CA, USA), separated and purified by HPLC (130A, Applied Biosystems, Foster City, CA, USA). Fast reversed-phase chromatography was performed using acetonitrile for elution with porous silica-prepacked columns (Aquapore RP-300 C-18 and Prep-10 C-18, Applied Biosystems, Foster City, CA, USA). Final samples contained 97.5% of neuropeptide Y-(18–36), the sequence of which was confirmed with a sequencer (477A, Applied Biosystems, Foster City, CA, USA).

Norepinephrine bitartrate and papaverine hydrochloride were purchased from Tokyo Kasei Kogyo Co. (Tokyo, Japan). Neuropeptide Y was purchased from the Peptide Institute (Osaka, Japan). Propranolol (Inderal) was purchased from Sumitomo Kagaku Co. (Osaka, Japan). Mepyramine maleate, neuropeptide Y-(13–36), [Leu^{31},Pro^{34}]neuropeptide Y, and peptide YY were purchased from Sigma Chemical Co. (St. Louis, USA). Fibrinogen was purchased from Midori Juji Co. (Osaka, Japan) and thrombin from Mochida Pharmaceutical Co. (Tokyo, Japan).

3. Results

3.1. Effects of neuropeptide Y on number of carbon particle deposits

Carbon deposits near bronchi are shown in Fig. 1 (A) and (B), obtained in the absence and presence of neuropeptide Y 10^{-10} M, respectively. Obviously the number of carbon deposits in the alveolar wall in the presence of neuropeptide Y was greater than that in its absence. It was also noted that the alveolar wall was thicker after neuropeptide Y.

In control left lungs treated with saline, the number of extravascular carbon particles deposited along the vasculature was different among the three regions investigated. In general, the number of carbon deposits

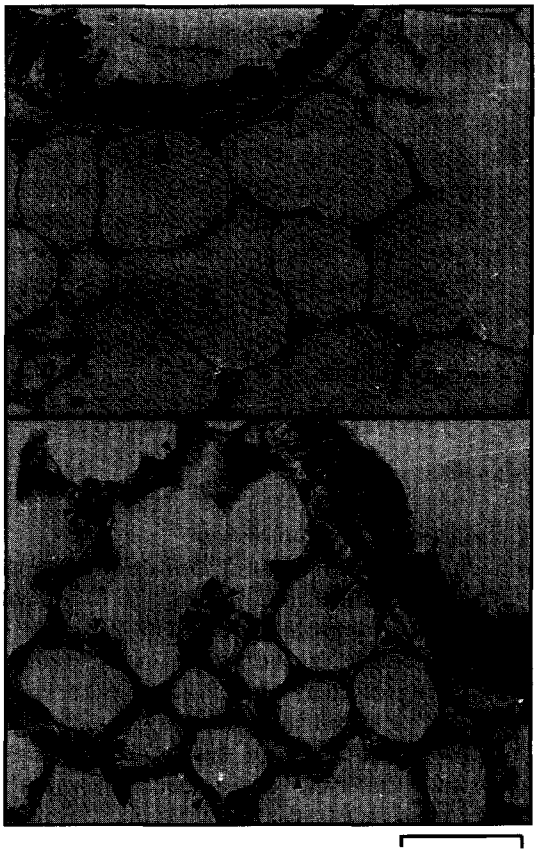

Fig. 1. Photomicrographs of rat lungs after administration of carbon solution. Large arrowheads indicate bronchi and small arrowheads carbon deposits. The number of carbon particles and alveolar wall thickness obtained in the presence of neuropeptide Y 10^{-10} M (B) were greater than those in its absence (A). Horizontal bar below the photograph indicates a scale of 50 μm.

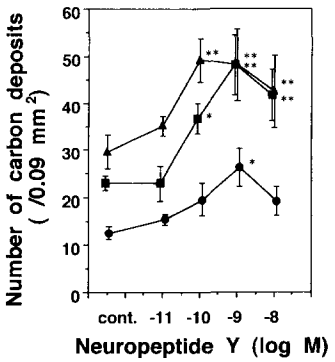

Fig. 2. Effects of neuropeptide Y on number of carbon particle deposits in an 0.09 mm^2 area in three different regions of left lungs, i.e., near bronchi, near pleura, and the intermediate region. Neuropeptide Y was administered into the bronchi during spontaneous respiration. Closed triangles, squares and circles, respectively, indicate the results for near the bronchi, in the intermediate region between the bronchi and pleura, and near the pleura. Neuropeptide Y caused a dose-dependent increase in the number of carbon deposits in all regions, this being most marked in the intermediate region. Six rats were used for the control, and for each concentration of neuropeptide Y. Asterisks, * and **, indicate statistical significance at the levels of 0.05 and 0.01, respectively.

was greater near the bronchi than near the pleura, and in between in the intermediate region. The average number of carbon deposits in left lungs was 29.6 ± 3.7 near the bronchi, 23.1 ± 1.4 in the intermediate region, and 12.6 ± 1.4 near the pleura ($n = 6$). Neuropeptide Y administered into the bronchi caused a dose-dependent increase in the number in all regions, as shown in Fig. 2, this being most marked in the intermediate region. Similar results were obtained in the right lungs. With 10^{-8} M of neuropeptide Y, no further increment, but rather a decline, was obtained, and in such lungs extremely severe alveolar flooding was observed.

3.2. Effects of norepinephrine and propranolol on neuropeptide Y-induced increase in number of carbon particle deposits

The results for the effects of norepinephrine 10^{-6} M and propranolol 10^{-7} M in the perfusion fluid on the neuropeptide Y-induced increase in the number of perivascular carbon particles are shown in Fig. 3, with significant elevation observed in all lung regions after treatment with neuropeptide Y 10^{-10} M ($P < 0.05$). In contrast, the combination of norepinephrine and propranolol slightly, but not significantly, decreased the number of carbon deposits in the absence of neuropeptide Y. The enhanced responses to neuropeptide Y depended upon the concentration of either neuropeptide Y or norepinephrine in the presence of 10^{-7} M of propranolol (data not shown).

3.3. Effects of peptide YY, neuropeptide Y-(13–36), neuropeptide Y-(18–36), and [Leu^{31},Pro^{34}]neuropeptide Y on number of perivascular carbon deposits

Peptide YY significantly increased the number of carbon particle deposits concentration dependently ($P < 0.05$) (Fig. 4) as compared to saline in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M, but peptide YY hardly affected it in their absence. It was noted that the potency of peptide YY for increasing the number of carbon deposits was much lower than that of neuropeptide Y obtained in the presence of norepinephrine and propranolol. Neither neuropeptide Y-(13–36), [Leu^{31},Pro^{34}]neuropeptide Y nor neuropeptide Y-(18–36), up to a concentration of 10^{-6} M, affected the number of carbon particles in lungs, even in the presence of norepinephrine and propranolol (data not shown).

3.4. Effects of neuropeptide Y-(18–36) or mepyramine on neuropeptide Y-induced increase in perivascular carbon deposits

Pretreatment with neuropeptide Y-(18–36) inhibited the responses to neuropeptide Y in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M. Neu-

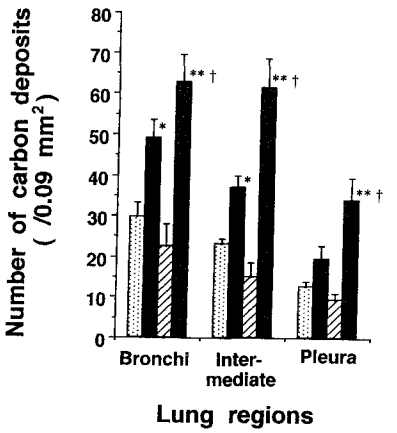

Fig. 3. Effects of norepinephrine and propranolol on the increase in a number of carbon particle deposits in response to neuropeptide Y. Neuropeptide Y 10^{-10} M or saline was administered into the bronchi. Carbon particle deposits were counted in three different regions in left lungs, i.e., near the bronchi, in the intermediate region between the bronchi and pleura and near the pleura. Two columns (1 and 3) indicate the number of carbon deposits obtained with saline (control), and the other two columns (2 and 4) indicate the number of carbon deposits obtained with neuropeptide Y, in the absence and presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M, respectively. The number of animals used in the absence of norepinephrine and propranolol was six and that in their presence was four. Asterisks, * and **, indicate statistical significance at the levels of 0.05 and 0.01, respectively, compared with neuropeptide Y-untreated group. Symbol † indicates statistical significance at the level of 0.05, compared with neuropeptide Y-treated group in the absence of norepinephrine and propranolol.

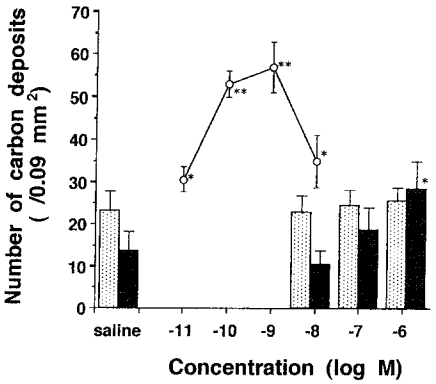

Fig. 4. Effects of neuropeptide Y and peptide YY on the number of carbon deposits in an 0.09 mm^2 area in the intermediate region of perfused left lungs. Columns (1, 2) indicate the results obtained with peptide YY in the absence and presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M, respectively. The numbers of animals were six for saline (control), and four for each concentration of neuropeptide Y and peptide YY. Open circles indicate the results obtained for neuropeptide Y in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M. The number of animals was four for each concentration of neuropeptide Y. Asterisks * and ** indicate statistical significance compared with saline group (control), at the levels of 0.05 and 0.01, respectively.

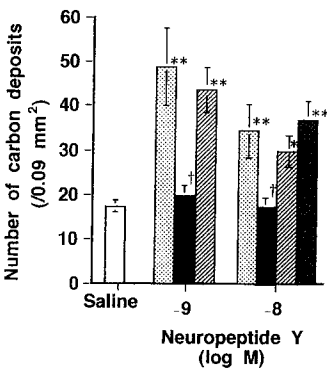

Fig. 5. Effects of mepyramine and neuropeptide Y-(18–36) on the number of carbon deposits in an 0.09 mm^2 area in the intermediate region of perfused lungs. The experiments were performed in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M in the perfusion fluid. The numbers of experiments performed were four for saline (open column), for neuropeptide Y (stippled columns), and for neuropeptide Y under pretreatment with mepyramine 10^{-6} M (hatched columns) and 10^{-5} M (thick hatched columns), and five for neuropeptide Y under pretreatment with neuropeptide Y-(18–36) 10^{-6} M (black column). Asterisks * and ** indicate statistical significance at $P < 0.05$ and $P < 0.01$, respectively, compared with saline, and † at $P < 0.05$, compared with neuropeptide Y in the absence of neuropeptide Y-(18–36).

ropeptide Y-(18–36) significantly decreased the average number of carbon particle deposits in a 0.09 mm^2 area in the intermediate region of left lungs, as shown in Fig. 5, from 49.9 ± 9.0 ($n = 4$) to 20.2 ± 2.5 ($n = 5$) in response to 10^{-9} M of neuropeptide Y ($P < 0.05$), and from 34.6 ± 4.9 ($n = 4$) to 17.8 ± 3.0 ($n = 5$) in response to 10^{-8} M of neuropeptide Y ($P < 0.05$).

The number of carbon particles retained in 0.09 mm^2 areas of the intermediate region of left lung tissue, in response to neuropeptide Y in the presence of norepinephrine 10^{-6} M and propranolol 10^{-7} M, was examined in the presence of the histamine-receptor antagonist, mepyramine. As shown in Fig. 5, neither 10^{-6} M nor 10^{-5} M of mepyramine affected the number of particles, which was still significantly greater than that obtained with saline ($P < 0.05$).

3.5. Effects of neuropeptide Y-(18–36) on the development of fibrin-induced pulmonary edema

Intracisternal injections of fibrinogen and thrombin increased the systemic arterial pressure and heart rate, with irregular respiratory movement such as Cheyne-Stoke's respiration. In all control animals, edema froth appeared in the tracheal tube less than 7 min after the injections of fibrinogen and thrombin. Pretreatment with neuropeptide Y-(18–36) caused an initial decrease in the systemic arterial pressure, which then gradually increased to level off within 10 min at the original blood pressure. The incidence of pulmonary edema slightly declined from 100% to 88%, and the ratio of lung weight to body weight (lung-body index) was hardly altered, i.e., 8.4 ± 0.4 in neuropeptide Y-(18–36)-pretreated rats ($n = 8$), only slightly higher than the 7.5 ± 0.4 control value ($n = 6$). The time required for appearance of edema froth in the tracheal tube was prolonged. The edema froth appeared within 10 min in two out of eight animals and did not appear within 15 min in the other animals. After the chest was opened, tracheal fluid was obtained from each animal. As shown in Table 1, the concentration of protein in serum and tracheal fluid in saline-treated rats was 5.6 ± 0.4 g/dl and 4.3 ± 0.4 g/dl ($n = 6$), respectively. The protein concentration ratio of tracheal fluid to serum in the

Table 1
Effects of neuropeptide Y-(18–36) on protein filtration across the vascular wall in the development of fibrin-induced pulmonary edema

	n	Serum protein concentration (S) (g/dl)	Tracheal fluid protein concentration (F) (g/dl)	Ratio of protein concentrations (F/S) %
Saline	6	5.6 ± 0.4	4.3 ± 0.4	76.9 ± 4.9
Neuropeptide Y-(18–36)	8	5.2 ± 0.2	3.1 ± 0.4	58.9 ± 7.1 [a]

[a] Statistical significance at the level of 0.01, compared with saline.

neuropeptide Y-(18–36)-pretreated group was 58.9 ± 7.1% ($n = 8$), significantly lower than that in the saline-treated group (76.9 ± 4.9%, $n = 6$) ($P < 0.01$).

4. Discussion

The results of the present study show that neuropeptide Y may have a role in increasing lung vascular permeability, possibly by enhancing plasma extravasation in the pulmonary circulation in the case of neurogenic pulmonary edema. In an in vivo study with the fibrin-induced pulmonary edema model (Ishikawa et al., 1988), neuropeptide Y-(18–36) prolonged the time required for edema froth to appear in the tracheal tube, reduced the incidence of severe pulmonary edema from 100% to 83%, and decreased the froth to serum protein ratio. Since neuropeptide Y-(18–36) is an analog of neuropeptide Y, acting as a receptor antagonist, it is likely that neuropeptide Y, which is endogenously released from sympathetic nerve terminals, plays a role in the development of neurogenic pulmonary edema. The inhibitory effects of neuropeptide Y-(18–36) on the development of fibrin-induced pulmonary edema may be ascribed to a blockage of the neuropeptide Y-induced increase in lung vascular permeability, as shown in the in vitro study in terms of the number of carbon deposits in perfused lung preparations.

Fibrin-induced pulmonary edema is a neurogenic pulmonary edema, which is usually associated with enhanced sympathetic nerve activity, pulmonary vascular hypertension and an increase in lung capillary permeability. The mechanism of its development, however, has not yet been elucidated, especially for protein-rich edema froth, i.e., increased vascular permeability. Since neither norepinephrine nor epinephrine infusion increased the lung vascular permeability, Van der Zee et al. (1980) postulated that the increase in vascular permeability might be due to the release of unknown substances. Some humoral factors, such as O_2 metabolites and neutrophil elastase, have been shown to increase vascular permeability (Baird et al., 1986), and therefore we used artificial perfusion fluid instead of blood in the present study.

Sakakibara et al. (1992) showed that even in the presence of an α-adrenoceptor-blocking agent or after treatment with reserpine, a depleter of catecholamines from sympathetic nerve terminals, electrical stimulation of sympathetic nerves still enhances vascular permeability. Therefore they suggested that the elevated activity of sympathetic nerves may increase pulmonary vascular permeability, through the action of neurotransmitters other than catecholamines. Neuropeptide Y has been shown to exist in sympathetic nerve terminals (Kummer et al., 1992; Lundberg et al., 1982) and may be released with norepinephrine when sympathetic nerves are activated. In immunohistochemical studies, Sheppard et al. (1984) and Kummer et al. (1992) showed that neuropeptide Y-containing nerves are distributed in the brain, generally in accordance with norepinephrine-synthesizing enzyme (tyrosine hydroxylase) immunoreactivity. They also showed that such nerve fibers may exist in the perivascular sympathetic nerves in lungs or bronchi.

In the present study, norepinephrine in the presence of propranolol, a β-adrenoceptor-blocking agent, enhanced the effects of neuropeptide Y on the number of carbon particles deposited. Hashiba et al. (1989) previously showed that the incidence of fibrin-induced pulmonary edema in rats treated with reserpine was greatly increased by pretreatment with norepinephrine and propranolol. Subsequently, Sakakibara et al. (1992) showed that the effect of electrical stimulation of postganglionic nerves from the stellate ganglion in lungs pretreated with reserpine was greatly enhanced by norepinephrine in the presence of propranolol, suggesting that some endogenous substance, whose effect on lung vascular permeability is enhanced by α-adrenoceptor activation, may have been released from the sympathetic nerve terminals. This result is similar to our findings with exogenous administration of neuropeptide Y. With respect to an interaction between norepinephrine and neuropeptide Y, Grundemar and Högestätt (1992) showed that while neuropeptide Y has a weak vasoconstricting action on isolated vascular smooth muscles, it is a powerful pressor agent in the systemic circulation. They explained this discrepancy by hypothesizing that α-adrenoceptor stimulation induces a heightened responsiveness to neuropeptide Y which is lacking in isolated blood vessels. Furthermore, Wahlestedt et al. (1985) and Hieble et al. (1989) showed that neuropeptide Y, released with norepinephrine upon electrical nerve stimulation, enhances norepinephrine-evoked vasoconstriction, as evidenced by a leftward shift of the concentration-response curve with unchanged maximum response in endothelium-intact vascular strips. Thus, it may be possible that the α-adrenoceptor plays a role in enhancing the increase in vascular permeability caused by neuropeptide Y, and that such an interaction strongly mediates the development of neurogenic pulmonary edema.

Papaverine, a vasodilator, was used to inhibit changes in capillary pressure which might occur with vaso- and/or veno-constriction caused by neuropeptide Y and other endogenous substances, and the pulmonary arterial, venous and capillary pressures were hardly changed throughout our perfusion experiments. Lang et al. (1992) also reported that intravenous administration of neuropeptide Y had little effect on pulmonary blood pressure. Thus the increase in pulmonary vascular permeability may not be ascribed to any change in lung capillary pressure. Furthermore, while neuropeptide Y induces a release of histamine

from mast cells (Grundemar and Håkanson, 1991), the present study showed that the histamine-receptor antagonist, mepyramine, did not block the increase in the vascular permeability induced by intrabronchial administration of neuropeptide Y. We conclude that neuropeptide Y itself may increase the lung vascular permeability, the action being direct on vascular endothelial cells and not secondary to release of histamine.

At least three types of neuropeptide Y receptors have been thoroughly characterized, neuropeptide Y Y_1, Y_2 and Y_3 receptors. Neuropeptide Y-(18–36) may be a partial agonist for the neuropeptide Y Y_1 receptor, a full agonist for the neuropeptide Y Y_2 receptor, and a pure antagonist for the putative neuropeptide Y Y_3 receptor system (Wahlestedt et al., 1986, 1987, 1992; Michel et al., 1990). Peptide YY is a full agonist at neuropeptide Y Y_1 and Y_2 receptors, but has little affinity for the neuropeptide Y Y_3 receptor subtype (Hieble et al., 1989). In the present study, comparison of the numbers of extra-vascular carbon particles indicated peptide YY 10^{-6} M to be much less effective than 10^{-9} M of neuropeptide Y. Furthermore, neither neuropeptide Y-(13–36) nor [Leu^{31},Pro^{34}]neuropeptide Y, neuropeptide Y Y_1 and Y_2 receptor agonists respectively, affected the number of carbon particles in lungs, and also pretreatment with neuropeptide Y-(18–36) inhibited the effects of peptide YY (unpublished observation). Thus, it is conceivable that peptide YY may have only a partial action on lung vascular permeability, possibly via the neuropeptide Y Y_3 receptor. Moreover, the pretreatment with neuropeptide Y-(18–36) markedly inhibited the effects of neuropeptide Y, from which we can conclude that neuropeptide Y exerts its major influence on lung vascular permeability through the neuropeptide Y Y_3 receptor of rat pulmonary endothelial cell membranes. This is the first finding of such a role for a neuropeptide Y Y_3 receptor, to our knowledge.

In conclusion, neuropeptide Y is a candidate for the factor(s) that mediate the increase in pulmonary vascular permeability in neurogenic pulmonary edema. Norepinephrine appears to enhance this effect of neuropeptide Y through α-adrenoceptors on the endothelial cell membrane, and therefore sympathetic nerve activation, which evokes the release of both norepinephrine and neuropeptide Y, might be expected to increase plasma extravasation under conditions of elevated capillary pressure, resulting in the development of edema. The increase in capillary pressure can be reduced by vasodilators, whereas the elevated vascular permeability can be diminished by a neuropeptide Y Y_3 receptor antagonist, e.g., neuropeptide Y-(18–36). Thus, treatment of patients with both types of agents in combination may inhibit the development of neurogenic pulmonary edema more efficiently than administration of either agent alone.

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