Effect of neuropeptide Y on hemodynamics of the rabbit lung

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Lang, Sally A., and Michael B. Maron. Effect of neuropeptide Y on hemodynamics of the rabbit lung. J. Appl. Physiol. 84(2): 618–623, 1998.—We evaluated the effect of neuropeptide Y (NPY) on the hemodynamics of the isolated rabbit lung perfused at constant flow and outflow pressure. Doses of $10^{-8}$ and $10^{-7}$ M NPY increased pulmonary arterial pressure (Ppa) from $11.5 \pm 1.0$ (SE) mmHg to, respectively, $16.4 \pm 1.5$ and $26.0 \pm 3.8$ mmHg (P < 0.05, n = 5 mmHg lungs), with $78 \pm 4\%$ of the increase at $10^{-7}$ M resulting from an increased arterial resistance. At the latter dose, pulmonary capillary pressure increased from $5.8 \pm 0.9$ to $9.4 \pm 1.0$ mmHg (P < 0.05). When administered in the presence of norepinephrine, $10^{-8}$ and $10^{-7}$ M NPY (n = 6) produced extreme increases in Ppa to $66.1 \pm 20.5$ and $114.7 \pm 25.5$ mmHg, respectively, that were due primarily to an increased arterial resistance. To determine the significance of circulating NPY as a pulmonary vasoactive agent, we measured plasma NPY-like immunoreactivity in anesthetized rabbits after massively activating the sympathetic nervous system with veratrine. NPY-like immunoreactivity increased from $74 \pm 10$ to $111 \pm 10$ (SE) pM (P < 0.05). Thus, although NPY is a potent vasoconstrictor in the rabbit lung, it is not likely that plasma NPY concentrations rise sufficiently, even after massive sympathetic nervous system activation, to produce pulmonary vasoconstriction in the intact rabbit.

pulmonary circulation; norepinephrine; veratrine; sympathetic nervous system

NEUROPEPTIDE Y (NPY), a sympathetic cotransmitter, constricts systemic blood vessels (8, 16, 21, 23, 29, 30) and potentiates the vasoconstrictor effects of norepinephrine (NE) and other vasoactive agents (8, 9, 19, 28, 30). These actions are well recognized for the systemic circulation, but the effects of NPY on the pulmonary circuit are not as well understood. Although isolated pig pulmonary arteries (18) and rabbit pulmonary veins (12) contract in response to NPY, the role that such changes in vascular tone might play in altering hemodynamics of the intact lung is not known. Accordingly, in this study, we determined whether NPY increased vascular resistance and altered the arteriovenous resistance distribution in the isolated perfused rabbit lung. We chose the rabbit for this analysis because of previous reports indicating that its isolated pulmonary vessels are responsive to NPY (12, 27).

We found that NPY produced a dose-dependent increase in pulmonary vascular resistance that was characterized by increases in resistance on both the arterial and venous sides of the pulmonary circulation. Accordingly, we conducted two additional sets of experiments to more fully characterize and understand the significance of this response. Because Wahlestadt et al. (27) have reported that NPY and NE reciprocally potentiate the other’s vasoactive effects when administered to isolated rabbit pulmonary arteries, we determined whether NPY produced a greater degree of pulmonary vasoconstriction in the presence of NE. Finally, to determine the physiological relevance of circulating NPY as a pulmonary vasoactive agent, we measured NPY-like immunoreactivity (NPY-LI) in anesthetized rabbits after massively activating the sympathetic nervous system (SNS) with an intracisternal injection of veratrine (24, 25) and compared these concentrations with those shown to produce pulmonary vasoconstriction in the perfused rabbit lung.

METHODS

Isolated perfused rabbit lung preparation. Fourteen New Zealand White rabbits [3.0 ± 0.4 (SD) kg] were anesthetized with an intramuscular injection of a mixture of xylazine (5.2 mg/kg; Butler, Columbus, OH), chlorpromazine HCI (2.2 mg/kg, Rugby Laboratories, Rockville Center, NY), and ketamine HCl (26.1 mg/kg, Fort Dodge Laboratories, Fort Dodge, IA). The esophagus was ligated and cut at the level of the fifth cervical vertebra to prevent aspiration of stomach contents into the lungs. Heparin sodium (5,000 U; US Amersham Life Sciences, Arlington Heights, IL) and dextran (20 ml; Abbott Laboratories, North Chicago, IL) were administered through a polyethylene catheter placed in the right carotid artery. Approximately 100 ml of arterial blood were drawn, and the chest was opened. A cannula was inserted into the main pulmonary artery, an opening was made in the left atrial appendage, and perfusion of the lungs (at ~25–50 ml/min) was begun immediately (Masterflex pump, Cole-Parmer Instruments, Niles, IL) with an artificial perfusate containing (in mM) 89.0 sodium chloride, 5.0 potassium chloride, 2.0 calcium chloride, 1.0 magnesium sulfate, 24.0 sodium bicarbonate, 1.0 sodium phosphate, 20.0 sodium acetate, 1.0 dextrose, and 6 g/dl bovine serum albumin. The first 150–200 ml of perfusate were used to flush the blood from the pulmonary vessels and discarded. The left atrium and trachea were then cannulated, and the lungs were removed from the animal. As recirculation of the perfusate was begun, the collected blood (~100 ml) was mixed with the perfusate (~50 ml) to produce an approximate circulating volume of 150 ml having an average hematocrit of 16.0 ± 1.6 (SD%). Erythrocytes were included in the perfusate because of observations suggesting that erythrocytes may help to maintain normal vascular permeability in isolated lungs (22). The perfusate was pumped from a reservoir, through a heat exchanger (to maintain blood temperature at 37°C), into the pulmonary artery, out the left atrium, and back into the reservoir. All tubing was Tygon (Cole-Parmer, Niles, IL). The lungs were covered with plastic wrap to prevent drying.

Pulmonary capillary pressure (Ppc) was determined by using the double-occlusion technique of Dawson et al. (5). Baseline pulmonary arterial pressure (Ppa) and Ppc were $12.7 \pm 2.6$ and $6.3 \pm 2.0$ mmHg, respectively. Pulmonary venous pressure (Ppv; 2.5 ± 1.0 mmHg) was set by adjusting the height of the reservoir. The average flow rate was $193 \pm$
32 ml/min. Because flow remained constant throughout each experiment, the arterial pressure gradient (Ppa – Ppc) and venous pressure gradient (Ppc – Ppv) represent estimates of arterial and venous resistance, respectively.

The lungs were ventilated with a gas mixture of 15% O2-5% CO2-80% N2 by using a Harvard large-animal ventilator that had been modified to inflate the lungs to a constant end-inspiratory pressure (10.8 ± 1.8 SD mmHg). This was accomplished by placing a water overflow system in the inspiratory line to vent any excess tidal volume delivered by the ventilator to the atmosphere. End-expiratory pressure (0.6 ± 0.4 mmHg) was also set by a water-overflow system. Ventilatory frequency was 20–24 breaths/min. Control blood gas values were PO2 = 118 ± 21 (SD) Torr, PCO2 = 41 ± 4 Torr, and pH = 7.399 (range, 7.341–7.46). Effect of NPY on pulmonary hemodynamics. In six lungs, porcine NPY (Bachem California, Torrence, CA) was administered in increasing doses to produce calculated initial perfusate NPY concentrations ranging from 10−10 to 10−7 M. Ppa and Ppc were determined under control conditions and after NPY administration. Successive NPY doses were administered at intervals averaging 13 ± 3 (SD) min. At NPY doses that significantly increased Ppa (10−9 and 10−7 M), Ppa did not recover and remained elevated during this interval. Accordingly, the 10−7 M dose was administered at a time when Ppa was still elevated from the 10−8 M dose. In two of these lungs, papaverine HCl (30 mg; Eli Lilly, Indianapolis, IN), a smooth muscle relaxant, was administered after the highest dose of NPY to determine whether the increase in Ppa was actively mediated. In two additional lungs, equivalent volumes of saline were administered (at 10 ± 2 min intervals) to serve as time controls.

NPY-NE interactions. In six lungs, designated as NE + NPY, we determined the effects of an infusion of NE bitartrate (Sigma Chemical, St. Louis, MO; 140 pg base/min) on the NPY dose-Ppa relationship. The NE infusion was started 30 min before administration of the lowest dose of NPY and continued while the dose-response curve was being determined. In one lung, papaverine was administered after the 10−6 M dose. In one experiment, perfusate samples (3 ml) were collected under baseline conditions, and at 30 and 60 min during NE infusion, for the determination of perfusate NE concentration by high-performance liquid chromatography as previously described (15). Plasma NPY-LI after massive SNS activation. Five rabbits (2.9 ± 0.2 kg) were anesthetized with thiamylal sodium (18 mg/kg; Biotal, Boehringer Ingeldheim Animal Health, St. Joseph, MO) in an ear vein. A tracheal cannula was inserted, and the lungs were mechanically ventilated with a gas mixture of 40% O2-60% N2. A polypropylene catheter was placed in the right carotid artery to administration of additional anesthetic (α-chloralose, 50 mg/kg, Sigma Chemical) and for measurement of arterial pressure. Control blood gas values were PO2 = 157 ± 45 Torr, PCO2 = 31 ± 11 Torr, and pH = 7.366 (range, 7.310–7.425). After the animal had stabilized, 0.4 ml of veratrine (800 µg/ml; Sigma Chemical) was injected into the oesophagus magna to massively activate the SNS. Veratrine was used in these experiments because it produces an extreme degree of centrally mediated SNS activation that is characterized in the rabbit by the development of large increases in blood pressure and plasma NE concentration (24, 25). For determination of plasma NPY-LI, arterial blood samples (3 ml) were drawn under baseline conditions, when arterial pressure had reached its highest value after veratrine administration (5–10 min after injection) and at 30-min intervals for 120 min. One animal died 90 min after veratrine administration. NPY-LI was determined by radioimmunoassay (Peninsula Laboratories, Belmont, CA), as previously described (14), with antiserum raised against porcine NPY. The antiserum had 100% cross-reactivity with porcine NPY and <0.1% cross-reactivity with other peptides of similar structure. The assay detection limit was 10 pg/tube. Extraction recoveries from rabbit plasma spiked with either 125I-labeled NPY or porcine NPY were 70–80%. Statistical analysis. The data were analyzed by using a one-way repeated-measures analysis of variance, followed by the use of a Student-Newman-Keuls test to determine significant differences from control values.

RESULTS

Figure 1 is a representative pressure tracing showing the increase in Ppa produced by 10−7 M NPY. Figure 1 also shows that the administration of papaverine resulted in an initial rapid fall in Ppa, which was followed by a more gradual reduction. The dose-response relationship between the calculated perfusate NPY concentration and Ppa for five of the six lungs administered NPY is shown in Fig. 2. (The sixth lung was not included in the mean, because an extreme degree of pulmonary hypertension developed after the 10−7 M dose that was not characteristic of the response observed in the remaining lungs in the group. In this lung, Ppa increased to 115 mmHg, and edema fluid appeared in the airway.) In the remaining five lungs, significant increases in Ppa (P < 0.05) occurred after the 10−6 M (44 ± 14%) and 10−5 M (136 ± 48%) doses. No edema fluid was observed in these lungs. In the two lungs in which papaverine was administered after the 10−5 M NPY dose, Ppa fell from 40.5 to 9.0 mmHg (Ppa before any NPY administration was 9.5 mmHg) and from 23.5 to 12.0 mmHg (baseline Ppa was 11.2 mmHg; Fig. 1), respectively. Saline administration produced no increases in Ppa in the two lungs in which saline rather than NPY was administered.

Changes in the pulmonary vascular resistance distribution after NPY administration are shown in Fig. 3. The highest NPY dose (10−7 M) significantly increased both the arterial and venous pressure gradients of the
lung, with 78 ± 4% of the increased resistance occurring on the arterial side. As a result of the increase in venous tone, Ppc increased from 5.8 ± 0.9 to 9.4 ± 1.0 mmHg (73 ± 30%, P < 0.05) at the highest NPY dose (Fig. 2).

No significant changes in Ppa were observed after NE administration (Fig. 4). In the presence of NE, however, NPY administration produced extreme increases in Ppa at the 10^{-8} and 10^{-7} M doses (Fig. 4). In three of these lungs, pulmonary edema developed after the 10^{-8} M dose, so no additional NPY was administered. In one of these lungs, we administered papaverine after the 10^{-8} M dose, and Ppa fell from 54 to 12.2 mmHg (baseline Ppa was 12.9 mmHg). Edema was observed in the remaining three lungs after the highest (10^{-7} M) dose of NPY. Figure 5 shows that an increase in the pulmonary arterial pressure gradient was primarily responsible for the increased Ppa produced by NPY + NE. In the experiments in which perfusate NE was measured during the NE infusion, NE was 9,260 pg/ml after 30 min of infusion (the time corresponding to the moment when we began to administer NPY to the lung) and was 19,201 pg/ml at 60 min (after the highest dose of NPY).

The baseline plasma NPY-LI [74 ± 10 (SE) pM] observed in the intact rabbits was similar to that reported by others (13). Veratrine administration transiently increased arterial pressure by 155 ± 28% (Fig. 6). By 30–60 min after veratrine administration, NPY-LI was increased an average 57% [111 ± 11 (SE) pM] over baseline values (Fig. 6). After this time, NPY-LI slowly decreased but remained elevated for the remainder of the experiment.
We found that NPY at concentrations of $10^{-8}$ and $10^{-7}$ M increased $P_{pa}$ in the isolated perfused rabbit lung (Figs. 1 and 2). Because the lungs were perfused at constant flow and outflow pressure, the increase in $P_{pa}$ represented an increase in vascular resistance. The ability of papaverine, a smooth muscle relaxant, to reduce the increased pressure indicated that the increase in vascular resistance was actively mediated (Fig. 1). Lower concentrations of NPY had no significant effect on pulmonary hemodynamics (Fig. 2). In contrast to our results, Delaunois et al. (6) observed no increases in vascular tone at a perfusate NPY concentration of $10^{-8}$ M in the isolated buffer-perfused rabbit lung. The reason for this disparity is not clear but may relate to technical differences.

At the $10^{-7}$ M NPY concentration, both pulmonary arterial and venous resistances were significantly increased (Fig. 3). The increase in venous resistance is consistent with observations of Kinsey and Russell (12), who found that NPY caused isolated rabbit pulmonary veins to constrict. In contrast, Wahlestedt et al. (27) found that the rabbit pulmonary artery either contracted weakly or not at all, even after being exposed to micromolar NPY concentrations. The reason for the differences between the intact lung and isolated pulmonary artery response to NPY is unknown but could relate to the possibility that the increase in arterial resistance observed in the intact lung might have been produced by constriction of more distal portions of the pulmonary artery or by arterioles that were not evaluated in the isolated vessel study of Wahlestedt et al. (27). This possibility is suggested by observations of Madden et al. (17), who found that isolated small (<300 µm diameter) feline pulmonary arteries constricted in response to hypoxia, whereas larger (>500 µm) arteries did not exhibit significant hypoxic constriction.

Although $10^{-7}$ M NPY produced both arterial and venous constriction in the perfused rabbit lung, the preponderant effect was on the arterial side of the circulation. This change in resistance distribution could have been the result of a heterogeneous distribution of NPY receptors in the arterial and venous sides of the pulmonary circulation, possible differences in the transmission process between receptor and vascular smooth muscle, or differences in the amount of and/or the ability of the vascular smooth muscle of the pulmonary arteries and veins to increase the resistance of their respective vascular segments. Although there appears to be no available information regarding the first two possibilities, there is evidence to support the third. In this regard, the rabbit pulmonary vein contains less vascular smooth muscle than the pulmonary artery (2), and 80% of the increase in vascular resistance that occurs in perfused rabbit lungs after KCl administration has been reported to be located in the large and small pulmonary arteries (3). In the latter study, the degree of vasoconstriction produced by KCl was considered to be indicative of the quantity of functional smooth muscle. Although the increase in venous resistance was relatively smaller than the increase in arterial resistance, the increase in venous tone resulted in a 73% increase in capillary pressure at the $10^{-7}$ M NPY dose (Fig. 4).

NPY and NE have been found to potentiate one another’s vasoconstrictor effects in many vascular beds (7, 9, 27, 30). In the isolated rabbit pulmonary artery, Wahlestadt et al. (27) observed that NE and NPY reciprocally potentiated the degree of constriction caused by the other vasoactive agonist. The intact rabbit lung appears to behave in a similar fashion. The administration of NPY in the presence of NE produced severe pulmonary hypertension (Fig. 4) that resulted primarily from profound increases in arterial resistance (Fig. 5). In one NPY + NE experiment in which papaverine was administered, $P_{pa}$ returned to baseline values, indicating that the extreme increases in $P_{pa}$ were actively mediated and not a consequence of the edema. Although these data demonstrate that pulmonary vasoconstriction can produce extreme increases in $P_{pa}$ in the isolated pump-perfused rabbit lung, it is not likely that such high pressures would develop in the intact rabbit, because right heart failure would likely intervene.

In contrast to the above results in the isolated rabbit lung, NPY (in the same range of doses) does not produce pulmonary vasoconstriction in either the intact anesthe-
tized dog or cat or in isolated perfused lungs from these species (4, 14, 20). Additionally, the infusion of NE into the blood perfusing the isolated canine left lower lung lobe does not alter the NPY dose-Ppa relationship (14). These observations suggest that species differences may exist in the capacity of the pulmonary vasculature to respond to NPY.

All six lung preparations that received NE and NPY and the one lung that developed severe pulmonary hypertension after the $10^{-7}$ M dose of NPY became edematous. These observations suggest that the edema was related to the extreme degree of pulmonary hypertension that developed. The basis for a hydrostatic explanation for the edema is not readily apparent, however, because only relatively small increases in Ppc occurred after NPY administration. Because fluid has been found to also leave the pulmonary vasculature from extraalveolar vessels upstream of (as well as downstream from) the capillaries, it is possible that the edema resulted from fluid filtration occurring from sites upstream of the increased arterial resistance (1). Additionally, extremely high pressures in the pulmonary arteries might have been transmitted to some pulmonary capillaries if the pattern of vasoconstriction was not uniform throughout the lung. Alternatively, it is possible that an increase in vascular permeability might have played a role in producing the edema. In isolated perfused rat lungs, $10^{-7}$ M NPY has been found to increase the capillary filtration coefficient (11), and lower concentrations ($10^{-10}$ M) have been observed to increase the leakage of carbon particles from the vasculature (10). In the rabbit lung, $10^{-8}$ M NPY has been found to have no effect on the capillary filtration coefficient, but higher NPY concentrations were not evaluated (6).

Although massive sympathetic activation produced by the intracisternal administration of veratrine significantly increased plasma NPY-LI in intact rabbits in our study, the magnitude of the increase (57%) was relatively modest (to $10^{-10}$ M, Fig. 6) and was significantly less than the increase we previously observed in dogs (431%) after veratrine administration (14). Thus, in the rabbit, massive SNS activation produces increases in circulating NPY-LI that are two orders of magnitude less than that required to produce a significant degree of pulmonary vasoconstriction in the isolated perfused rabbit lung. Moreover, in the presence of plasma NE concentrations of the same order of magnitude as those observed after massive SNS activation in the rabbit (24), the observed increases in NPY-LI would be expected to produce only minimal increases in Ppa (Fig. 4). These data suggest that circulating NPY is not likely to produce pulmonary vasoconstriction after SNS activation, even in the presence of concentrations of circulating NE that may occur under these conditions. This conclusion is predicated, however, on the assumption that the sensitivity of the pulmonary vasculature to NPY is similar in the isolated perfused and in situ rabbit lung. Although we do not know whether the isolated perfusion condition alters pulmonary vasoactivity from that which would be observed in the intact rabbit, the artificial perfusion conditions should be kept in mind when considering these results. Finally, it is conceivable that neurally released NPY might increase pulmonary vascular tone if NPY and NE concentrations are higher at neuronal release sites (8, 26).

In summary, we have shown that NPY produces pulmonary vasoconstriction in the isolated perfused rabbit lung that results from increases in both arterial and venous resistance. Although the increase in arterial resistance was larger than that occurring on the venous side, the observed degree of pulmonary vasoconstriction was sufficient to increase Ppc. The administration of NPY in the presence of NE potentiated the vasoconstriction caused by NPY, and at higher NPY concentrations resulted in severe pulmonary hypertension and edema.

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