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The Effects of Substance P on the Preconstricted Pulmonary Vasculature of the Anesthetized Dog (42381)

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Abstract. Substance P is a vasoactive peptide. Nerve fibers containing substance P are present in the media of pulmonary arteries but the physiologic function of substance P in the pulmonary vasculature is unknown. Several doses of substance P were infused intravenously in the anesthetized dog to ascertain its effects on the pulmonary vasculature, both during normoxia and following preconstriction with hypoxia (F_IO_2 0.1) or prostaglandin $F_2\alpha$ ($PGF_2\alpha$ 5 μg/kg/min). Substance P resulted in systemic vasodilatation during normoxia but had minimal effect on the pulmonary vasculature. During hypoxia and $PGF_2\alpha$-induced pulmonary vasoconstriction, substance P significantly lowered pulmonary artery pressure, pulmonary vascular resistance, mean aortic pressure, and total systemic resistance. It had no effect on cardiac output, wedge pressure, and arterial blood gases. To investigate possible mechanisms for substance P-induced vasodilatation, substance P was studied following pretreatment with *N*-acetylcysteine (a radical scavenging agent), methylene blue (an inhibitor of guanylate cyclase), meclofenamate (a cyclooxygenase inhibitor), and atropine (a muscarinic receptor antagonist). None of these agents impaired substance P-induced vasodilatation. Substance P given intravenously is a nonselective vasodilator in the dog but the mechanism of its action remains uncertain.

Substance P is an eleven residue peptide which was first identified in 1931 (1). It causes vasodilation in the coronary (2), systemic (3–5), femoral (4), and visceral beds (4, 6). Substance P, at low doses, causes *in vitro* relaxation of pulmonary artery strips (7), although higher doses are associated with a vasoconstrictor effect. The action of substance P on the preconstricted pulmonary vascular bed has not previously been explored. Substance P-containing nerve fibers are present in the media of pulmonary arteries but the physiologic function of this peptide in the pulmonary vasculature is uncertain (8, 9).

In this study, substance P was infused intravenously in the anesthetized dog to examine its effects on pulmonary hemodynamics. During normoxia, the pulmonary vessels are almost fully dilated and it is difficult to demonstrate additional dilatation in the healthy animal. Consequently, the action of substance P was observed both during normoxia and after preconstriction of the pulmonary vasculature by hypoxia or prostaglandin $F_2\alpha$ ($PGF_2\alpha$). Additional experiments were performed to examine possible mechanisms by which substance P might cause vasodilatation.

Methods. *Dog experiments.* Twenty-two mongrel dogs of either sex (mean weight 23 ± 2 kg) were anesthetized with pentobarbital sodium (30 mg/kg iv), intubated, paralyzed with succinyl choline (2 mg/kg iv), and ventilated with a constant volume respirator (Harvard Apparatus, Model 607, Millis, Mass.). Body temperature was monitored by an esophageal temperature probe (Model 43TA, Yellow Springs Instrument Co., Yellow Springs, Ohio) and was maintained between 37 and 39°C. Polyethylene catheters were placed in the superior vena cava and descending aorta. A Swan–Ganz catheter was advanced into the pulmonary artery. Pulmonary arterial, wedge, and aortic pressures were measured using Statham P23 transducers (Statham Instruments, Oxnard, Calif.) and a Hewlett–Packard 7754B recorder (Hewlett–Packard, Waltham, Mass.). Cardiac output (CO) was determined by the indocyanine green dye method. Pulmonary vascular resistance (PVR) was calculated as mean pulmonary arterial pressure (PAP) minus mean wedge pressure (PW) (mm Hg) divided by cardiac output (liters/min) (PVR = [PAP − PW]/CO). Total systemic resistance (TSR) was calculated as the mean aortic pressure (AO) divided by the cardiac output (TSR = AO/CO). Arterial pH,

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$PaCO_2$, and PaO_2 were measured using an automatic pH and blood gas analyzer (Corning, Model 168). The respirator was adjusted initially to maintain a systemic arterial pH of 7.35–7.45. Ventilation was then maintained constant throughout the experiment. Additional doses of pentobarbital sodium and succinyl choline were given during the protocol to maintain anesthesia and paralysis.

Drugs. Substance P was made up as a stock solution in acetic acid using polyethylene containers. Contact with glass or polystyrene was avoided. Substance P is strongly adsorbed by these materials and once adsorbed is readily oxidized and destroyed (10). Stock solution was frozen and individual aliquots were unthawed each day immediately prior to use. Due to the similarity of substance P's index of refraction to that of saline, careful examination of the solution with a magnifying glass was required to confirm complete dissolution (10).

Experimental design: Part 1. Following an initial equilibration period of 1 hr, eight dogs were exposed to a 15-min hypoxic challenge (F_IO_2 0.1) (Fig. 1). Hemodynamics and arterial blood gases were measured prior to the hypoxic period as well as at 8 and 15 min of hypoxia. During second and third hypoxic challenges, substance P was infused throughout the last 7 min of hypoxia (0.1 and 0.2 μg/kg/min, respectively). After a recontrol period under normoxic conditions, prostaglandin $F_2\alpha$ (5 μg/kg/min) was infused intravenously for 4 min. Hemodynamics and blood gases were measured at the end of the infusion. Following a further recontrol period, substance P (0.2 μg/kg/min) was given intravenously for 10 min. Six minutes into the infusion, a second 4-min prostaglandin $F_2\alpha$ challenge (5 μg/kg/min) was given. Hemodynamics and blood gases were measured before, and at the end of, the prostaglandin $F_2\alpha$.

Part 2. In an additional eight dogs, the hemodynamic responses to hypoxia were recorded, with and without a low-dose infusion of substance P (0.0125–0.025 μg/kg/min; Fig. 1). If pulmonary vasodilatation was not achieved at the first dose, the second dose was used here and during the subsequent interventions. After a recontrol period, *N*-acetylcysteine (1 ml/min, 20% solution) was infused iv for 30 min. An hypoxic challenge was superimposed on the second 15 min of the infusion and substance P was given intravenously (0.0125–0.025 μg/kg/min) over the last 7 min of hypoxia. Hemodynamics and arterial blood gases were measured 15, 23, and 30 min after the start of the *N*-acetylcysteine infusion.

Following a recontrol period, an identical

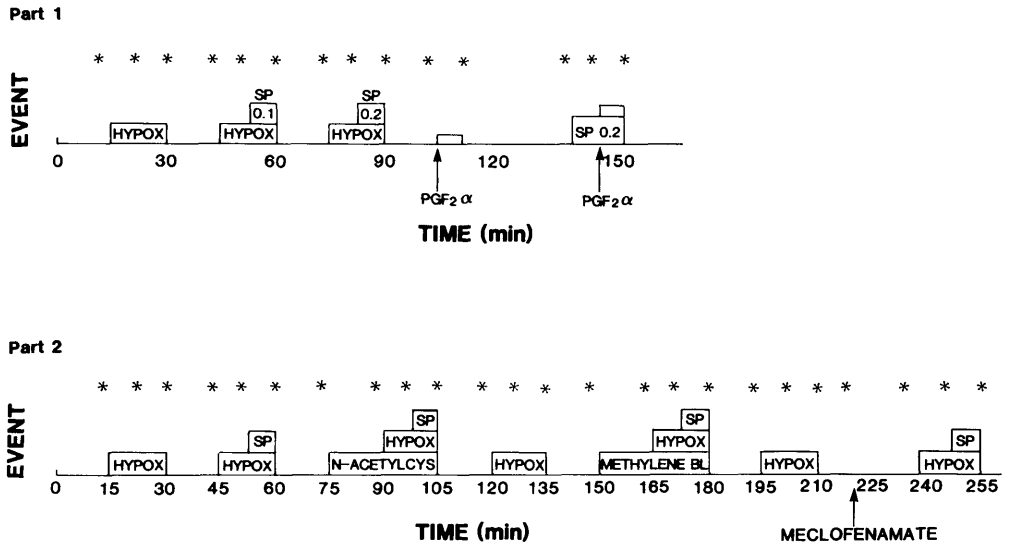

FIG. 1. Protocol for administration of substance P. *Measurement of hemodynamics and arterial blood gases; $PGF_2\alpha$, infusion of prostaglandin $F_2\alpha$ (5 μg/kg/min × 4 min); hypoxia, ventilation with F_IO_2 0.1 × 15 min; S.P., period of infusion of substance P.

cycle was begun substituting methylene blue (1.0 mg/kg/min for 30 min; dissolved in 0.9% saline) for *N*-acetylcysteine (Fig. 1). After a further control period, meclofenamate (4 mg/kg) was given as an intravenous bolus. Twenty minutes later an hypoxic challenge was started and an infusion of substance P was given during the last 7 min of hypoxia as before. Hemodynamics and arterial blood gases were measured 20, 28, and 35 min after administration of meclofenamate. Because of its long half-life, meclofenamate was given last in the protocol.

Part 3. The effects of atropine on substance P-induced vasodilatation were examined in six additional dogs. An initial 15-min hypoxic challenge was followed by a 15-min normoxic period and the dog was reexposed to hypoxia. Substance P (0.025 μg/kg/min iv) was infused during the final 7 min of hypoxia as in part 2. Hemodynamics and arterial blood gases were again measured immediately prior to the hypoxic challenge as well as after 8 and 15 min of hypoxia. In addition, hemodynamics were measured 30 sec after the onset of substance P infusion. Next, the protocol was repeated with the administration of atropine (1 mg iv bolus) 1 min before the infusion of substance P. Acetylcholine (500 μg) was infused at the end of the hypoxic period during which atropine was administered, to assess the adequacy of the muscarinic blockade.

Statistical analysis. Values were expressed as the means and standard errors of the mean. Differences within groups were assessed initially by analysis of variance on repeated measures. Post hoc comparisons were made using Tukey's test. Results were considered statistically significant when P values were <0.05. Comparisons among three groups were made using analysis of variance, together with Tukey's test. For assessment of effect of a drug on normoxic hemodynamics within a group, the unpaired Student t test (two-tailed) was used.

Results. *Part (i). Effects of Substance P (0.1–0.2 μg/kg/min) on hemodynamics during normoxia, hypoxia, and the infusion of $PGF_2\alpha$: Normoxia.* During normoxia, substance P decreased mean aortic pressure (AOP) and total systemic resistance (TSR), while increasing cardiac output ($P < .05$ for each; Table 1). Pulmonary artery pressure was not affected but there was a consistent small fall in pulmonary vascular resistance (PVR).

Hypoxia. During hypoxia, both doses of substance P significantly lowered AOP and TSR compared to hypoxic control levels (Table 1). PAP and PVR fell significantly at the higher dose (0.2 μg/kg/min) (Fig. 2). Wedge pressure (WP) and cardiac output were unaffected by substance P during hypoxia (Table 1).

Prostaglandin $F_2\alpha$. During $PGF_2\alpha$-induced vasoconstriction, the higher dose of substance P decreased PAP, AOP, and TSR (Table 1). CO and PW remained unchanged. Substance P had no significant effect on arterial blood gases at either dose.

Part (ii). Effects of prior treatment with N-acetylcysteine (NAC), methylene blue (MB) and meclofenamate on the hemodynamic response to substance P (0.0125–0.025 μg/kg/min): Normoxia. NAC significantly elevated PAP and CO, while TSR declined (Table 2). MB increased AOP, PAP, and PVR. Meclofenamate caused a small elevation in AOP, though this was of questionable physiologic significance. None of the three drugs significantly affected arterial blood gases, cardiac output, or wedge pressure during normoxia.

Hypoxia. Low dose substance P (0.0125–0.025 μg/kg/min) significantly decreased hypoxic pulmonary artery pressure and pulmonary vascular resistance (Fig. 3; Table 2). Aortic pressure and total systemic resistance were both decreased. Cardiac output, pulmonary capillary wedge pressure, and arterial blood gases were unaffected by low-dose substance P during hypoxia. NAC, MB, and meclofenamate did not significantly affect arterial blood gases, CO, PAP, PVR, or WP during hypoxia (Table 2), nor did they alter the effects of substance P on these variables. The fall in AOP (Δ) induced by substance P was augmented by all three agents (Fig. 4). The fall in TSR (Δ) was augmented by MB and meclofenamate.

Part (iii). Effects of prior treatment with atropine on the hemodynamic response to substance P (0.025 μg/kg/min). Substance P caused systemic and pulmonary vasodilatation as reported in parts (i) and (ii). Thirty seconds after beginning the infusion of substance P during hypoxia the aortic pressure, total systemic resistance, and pulmonary vascular re-

TABLE I. HEMODYNAMIC EFFECTS OF SUBSTANCE P DURING HYPOXIA AND PROSTAGLANDIN $F_2\alpha$-INDUCED VASOCONSTRICTION

	Ao (mm Hg)	Pa (mm Hg)	PW (mm Hg)	CO (liters/min)	TSR (mm Hg/ liters/min)	PVR (mm Hg/ liters/min)
Normoxia	148 ± 5	15 ± 1	6 ± 1	3.8 ± 0.4	43 ± 5	2.5 ± 0.3
8 min hypoxia	176 ± 8	32 ± 3	7 ± 1	4.5 ± 0.4	41 ± 3	5.7 ± 0.5
15 min hypoxia	165 ± 7	27 ± 1	6 ± 1	3.8 ± 0.4	46 ± 4	5.6 ± 0.6
Normoxia	140 ± 5	14 ± 1	5 ± 1	3.8 ± 0.4	39 ± 3	2.4 ± 0.3
8 min hypoxia	171 ± 7	29 ± 2	7 ± 1	3.8 ± 0.3	46 ± 4	5.9 ± 0.4
15 min hypoxia ± substance P (0.1 μg/kg/min)	141 ± 9*	26 ± 2	6 ± 1	4.2 ± 0.3	34 ± 2*	4.8 ± 0.7
Normoxia	143 ± 5	15 ± 1	6 ± 1	3.2 ± 0.3	47 ± 4	2.9 ± 0.3
8 min hypoxia	169 ± 5	29 ± 1	6 ± 1	3.4 ± 0.2	51 ± 3	7.0 ± 0.5
15 min hypoxia ± substance P (0.2 μg/kg/min)	128 ± 9*	23 ± 1*	6 ± 1	4.0 ± 0.2	32 ± 2*	4.3 ± 0.4*
Normoxia	137 ± 6	15 ± 1	5 ± 1	3.2 ± 0.3	45 ± 5	3.0 ± 0.4
Normoxia + $PGF_2\alpha$	144 ± 6	28 ± 2	6 ± 1	3.3 ± 0.3	46 ± 4	7.2 ± 1.0
Normoxia	134 ± 6	14 ± 1	5 ± 1	2.8 ± 0.2	50 ± 4	3.4 ± 0.3
Normoxia + substance P (0.2 μg/kg/min)	91 ± 4***	14 ± 1	5 ± 1	3.3 ± 0.2***	28 ± 2***	3.0 ± 0.4
Normoxia + substance P (0.2 μg/kg/min) + $PGF_2\alpha$	106 ± 5**	22 ± 2**	4 ± 1	3.1 ± 0.2	35 ± 2**	6.0 ± 1.0

Note. All values are given as means ± standard error of the mean. *$P < .05$ for the difference between the 8- and 15-min values during the initial hypoxic challenge compared to the difference between the 8- and 15-min hypoxic values in subsequent hypoxic challenges. **$P < 0.05$ for difference between the value during prostaglandin $F_2\alpha$ infusion compared with the value during simultaneous infusion of prostaglandin $F_2\alpha$ and substance P (0.2 μg/kg/min). ***$P < 0.05$ for difference between the value during normoxia prior to substance P infusion and the value obtained after infusion of substance P. Ao, mean aortic pressure; Pa, mean pulmonary pressure; PW, pulmonary wedge pressure; CO, cardiac output; TSR, total systemic resistance; PVR, pulmonary vascular resistance. $N = 8$.

sistance fell compared to the preinfusion values (−45 ± 12 mm Hg, −33 ± 9 mm Hg/liter/min), −1.3 ± 0.3 mm Hg/liter/min, respectively) ($P < 0.05$ for each). The cardiac output increased transiently, but significantly, with infusion of substance P (+0.6 ± 0.2 mm Hg/liter/min) ($P < 0.05$). At the end of the infusion, the aortic pressure, total systemic resistance, and pulmonary vascular resistance were all decreased compared to the preinfusion hypoxic values ($P < 0.05$ for each) (Table 3). The initial rise in cardiac output following substance P had diminished by the end of the infusion. The effects of substance P on the pulmonary and systemic vasculature during hypoxia were not affected by atropine. The aortic pressure, total systemic resistance, and pulmonary vascular resistance again fell acutely (−46 ± 7 mm Hg, −28 ± 5 mm Hg/liter/min and −2.0 ± 0.6 mm Hg/liter/min), while the cardiac output rose (+0.5 ± 0.2 liter/min). At the end of the substance P infusion the vasodilatation had again moderated, though the aortic pressure, total systemic resistance, and pulmonary vascular resistance were still depressed compared to the preinfusion hypoxic value for each (Table 3). The infusion of acetylcholine at the end of each hypoxic period in which atropine had been given was not associated with any hemodynamic changes, while infusion of acetylcholine during hypoxia to dogs prior to administration of atropine caused falls in aortic pressure, total systemic resistance, and pulmonary vascular

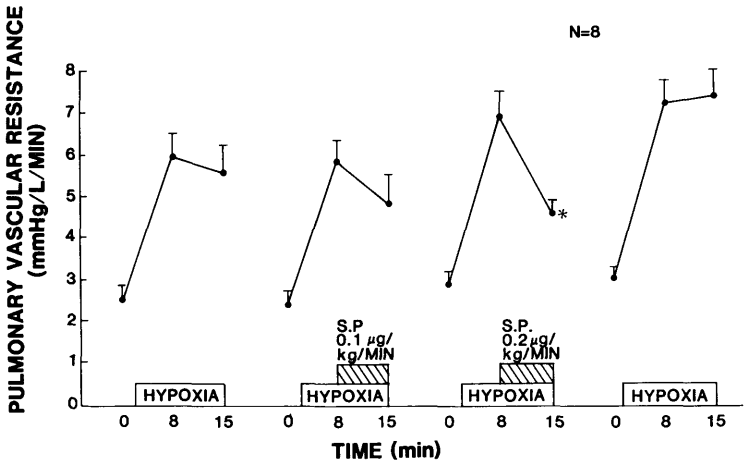

FIG. 2. Infusion of substance P reduces pulmonary vascular resistance during hypoxia. *$P < .05$ for difference between 8- and 15-min hypoxic values; hypoxia, period of ventilation with F_IO_2 0.1 (15 min); S.P., period of infusion of substance P.

resistance (-50 ± 15 mm Hg, -37 ± 7 mm Hg/liter/min, and -3.1 ± 0.5 mm Hg/liter/min, respectively) ($P < 0.05$ for each).

Discussion. The infusion of exogenous substance P caused marked systemic vasodilatation during normoxia, while there was only an insignificant decrease in pulmonary vascular resistance. During hypoxia, substance P produced both systemic and pulmonary vasodilatation. Aortic and pulmonary arterial pressures fell while cardiac output was unchanged. The physiologic significance of this vasodilatation is uncertain. However, it has been suggested that substance P may play an autoregulatory role in the control of jejunal blood flow (6). Vagal stimulation causes degranulation of enterochromaffin cells of the jejunum and can increase the release of substance P into the lumen of the intestine 20-fold. Substance P is present in nerve terminals in the media of pulmonary arteries and veins of the guinea pig (8, 9), and it is possible that it could play a local autoregulatory function in the pulmonary vascular bed.

Substance P binds to carboxyl terminal-specific receptors in the dog carotid artery (11), but the mechanism by which this binding causes relaxation is unknown. In particular, the relaxation induced by substance P is not diminished by pretreatment with atropine, naloxone, and α or β blockers (11). Similarly, pretreatment of human subjects with indomethacin does not reduce the systemic hypotension which follows the intravenous administration of substance P (3). In the anesthetized dog, adrenergic, cholinergic, and histamine blockers fail to prevent the fall in systemic pressure caused by substance P (12). Indomethacin and meclofenamate partially reduce the systemic hypotension in the dog, suggesting that cyclooxygenase products may contribute to the vasodepressor effects of substance P in this species (12).

The present studies examined mechanisms by which substance P might initiate vasodilatation in the pulmonary vasculature. In addition to the inhibition of cyclooxygenase by meclofenamate, the effects of inhibiting guanylate cyclase and of scavenging oxygen radicals were examined. The infusion of meclofenamate, in a dose which inhibits prostaglandin synthesis in the dog (13, 14), did not alter the pulmonary vasodilatation caused by substance P, suggesting that cyclooxygenase products are not involved. The fall in systemic pressure and resistance induced by substance P showed a small increase after meclofenamate. Worthen *et al.* have described dose-dependent pulmonary vasoconstriction and systemic vasodilatation in the anesthetized normoxic rabbit (15). This pulmonary vasoconstriction was abolished by meclofenamate. These authors also noted pulmonary vasodilatation, rather than constriction, following administration of

TABLE II. HEMODYNAMIC EFFECTS OF SUBSTANCE P ON HYPOXIC VASOCONSTRICTION FOLLOWING PRETREATMENT WITH *N*-ACETYLCYSTEINE, METHYLENE BLUE, OR MECLOFENAMATE

	Ao (mm Hg)	Pa (mm Hg)	PW (mm Hg)	CO (liters/min)	TSR (mm Hg/ liters/min)	PVR (mm Hg/ liters/min)
Normoxia	148 ± 5	12 ± 1	4 ± 1	3.2 ± 0.3	51 ± 6	2.8 ± 0.4
8 min hypoxia	168 ± 5	25 ± 2	3 ± 1	3.6 ± 0.4	50 ± 6	6.6 ± 0.8
15 min hypoxia	163 ± 5	24 ± 1	3 ± 1	3.6 ± 0.3	49 ± 5	6.4 ± 0.4
Normoxia	144 ± 5	12 ± 1	3 ± 1	3.2 ± 0.3	50 ± 7	2.9 ± 0.4
8 min hypoxia	164 ± 5	25 ± 2	3 ± 1	3.2 ± 0.3	54 ± 5	7.0 ± 0.8
15 min hypoxia + substance P	135 ± 8*	20 ± 2*	3 ± 1	3.7 ± 0.7	39 ± 4*	4.8 ± 0.5*
Normoxia	144 ± 4	10 ± 1	3 ± 1	2.4 ± 0.2	62 ± 5	3.1 ± 0.4
N-Acetylcysteine + Normoxia	146 ± 4	13 ± 1**	3 ± 1	3.3 ± 0.3**	47 ± 5**	3.2 ± 0.4
N-Acetylcysteine + 8 min hypoxia	170 ± 5	29 ± 3	4 ± 1	4.1 ± 0.3	44 ± 4	6.6 ± 0.8
N-Acetylcysteine + 15 min hypoxia + substance P	123 ± 8*,***	22 ± 3*	3 ± 1	4.3 ± 0.4	30 ± 3*	4.7 ± 0.5*
Normoxia	145 ± 6	12 ± 1	3 ± 1	2.6 ± 0.2	59 ± 5	3.6 ± 0.4
Methylene blue + normoxia	153 ± 5*	15 ± 1**	3 ± 1	2.6 ± 0.2	62 ± 5	4.9 ± 0.5**
Methylene blue + 8 min hypoxia	175 ± 5	28 ± 3	3 ± 1	3.1 ± 0.3	58 ± 5	8.0 ± 0.7
Methylene blue + 15 min hypoxia + substance P	128 ± 6*,***	22 ± 2*,***	3 ± 1	3.4 ± 0.3	39 ± 3*,***	6.1 ± 0.7*
Normoxia	149 ± 5	12 ± 1	3 ± 1	2.4 ± 0.2	66 ± 6	4.0 ± 0.6
Meclofenamate + normoxia	155 ± 5*	13 ± 1	3 ± 1	2.3 ± 0.2	71 ± 6	4.0 ± 0.6
Meclofenamate + 8 min hypoxia	171 ± 5	26 ± 2	3 ± 1	2.7 ± 0.3	67 ± 7	9.0 ± 0.8
Meclofenamate + 15 min hypoxia + substance P	120 ± 9*,***	20 ± 2*	3 ± 1	3.1 ± 0.3	42 ± 5*,***	6.1 ± 0.9*

Note. *$P < .05$ for difference in values obtained at 8 and 15 min of hypoxia, before and after infusion of substance P. **$P < .05$ for difference between value before and after infusion of a drug (*N*-acetylcysteine, methylene blue, meclofenamate) during normoxia. ***$P < .05$ for the difference between the 8- and 15-min hypoxic values obtained with substance P compared to the difference between the 8- and 15-min hypoxic values obtained with coadministration of substance P and another drug. Ao, mean aortic pressure; Pa, mean pulmonary artery pressure pulmonary wedge; PW, pressure; CO, cardiac output; TSR total systemic resistance; PVR, pulmonary vascular resistance. $N = 8$.

low doses of substance P when the pulmonary vasculature was preconstricted. The pulmonary vasoconstriction they found may have been due to differences of species and dose, the use of a bolus injection in the rabbit as opposed to infusion in the dog, and the administration of substance P during normoxia in the rabbit instead of hypoxia in the dog. However, when substance P was infused in the dog during normoxia in the current experiments, no change in pulmonary arterial pressure or resistance occurred. A previous study found substance P to reduce total pulmonary vascular resistance during normoxia, although this was prior to the era of synthetic substance P (2). Grunstein *et al.* observed that the substance P action on the airways involves peripheral cholinergic action (16). Subsequently they studied the effects of atropine on the pulmonary vasoconstrictor effect of substance P in rabbits. They found that atropine (2 mg/kg iv) enhanced pulmonary vasoconstriction. This dose is 40 times the dose needed to completely inhibit the effects of acetylcholine on the pulmonary vasculature in the present study. The current study, as well as two

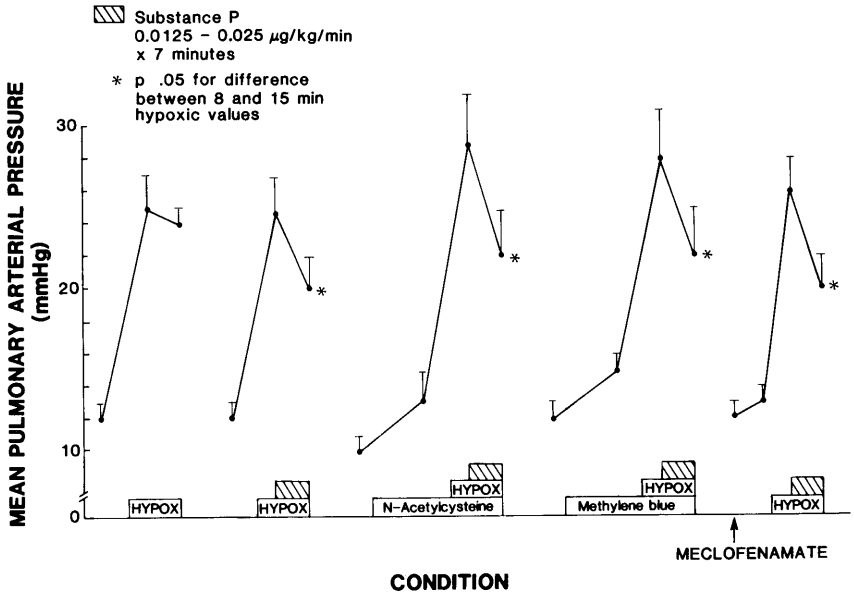

FIG. 3. Reduction of mean pulmonary arterial pressure during hypoxia (F_IO_2 0.1) by intravenous infusion of substance P is not blocked by *N*-acetylcysteine, methylene blue, or meclofenamate.

previous reports (2, 11), show no inhibition by atropine of the systemic vasodilatation caused by substance P. In addition, atropine had no effect on the pulmonary vasodilatation induced by substance P and consequently it seems unlikely that muscarinic receptors are involved.

Substance P can cause marked vasodilatation *in vitro* by an endothelial-dependent mechanism (17). Endothelium-dependent va-

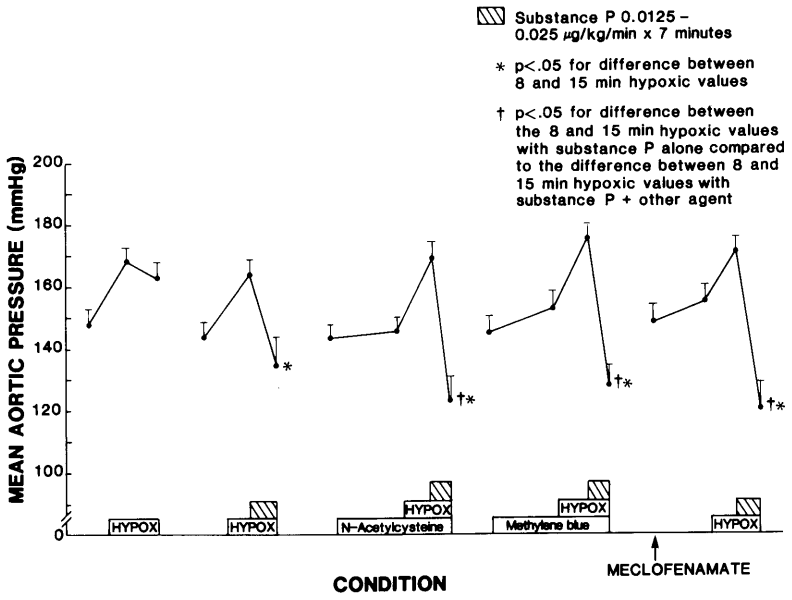

FIG. 4. Reduction of mean aortic pressure by intravenous infusion of substance P during hypoxia is not prevented by *N*-acetylcysteine, methylene blue, or meclofenamate.

TABLE III. ATROPINE DOES NOT ALTER THE EFFECTS OF SUBSTANCE P ON THE SYSTEMIC OR PULMONARY VASCULATURE DURING HYPOXIA

	Aortic pressure Δ(8–15 min) (mm Hg)	Pulmonary artery pressure Δ(8–15 min) (mm Hg)	Cardiac output Δ(8–15 min) (liters/min)	Pulmonary vascular resistance Δ(8–15 min) (mm Hg/liter/min)	Total systemic resistance Δ(8–15 min) (mm Hg/liter/min)
Hypoxic control	−4 ± 3	+1 ± 1	+0.1 ± 0.1	+0.4 ± 0.3	−2 ± 1
Hypoxia + substance P (0.025 μg/kg/min)	−24 ± 7*	−3 ± 1	+0.1 ± 0.2	−1.2 ± 0.4*	−10 ± 5*
Hypoxia + substance P (0.025 μ/kg/min) + Atropine (1 mg)	−21 ± 6*	−1 ± 1	+0.2 ± 0.1	−1.6 ± 0.4*	−13 ± 4*

Note. All values are expressed as the means ± standard error of the mean. Δ(8–15 min)—Values are obtained by subtracting the value measured after 15 min of hypoxia from that measured after 8 min of hypoxia. *$P < 0.05$ value differs from hypoxic control value.

sodilatation is associated with an increase in cyclic GMP in the vascular smooth muscle (18). Methylene blue is an inhibitor of guanylate cyclase and causes an increase in the tone of vascular strips *in vitro* (19). In the current study, methylene blue produced an increase in both pulmonary artery pressure and resistance under normoxic conditions, an observation which raises the possibility that cyclic GMP levels are important in the maintenance of the low resistance of the pulmonary vasculature. However, because methylene blue has a number of actions, such as the interruption of electron transport (20) and the oxidation of NADPH (21), other mechanisms are also possible. Although this dose of methylene blue had hemodynamic effects, it did not reduce the pulmonary vasodilatation induced by substance P.

In vitro studies of methylene blue have demonstrated inhibition of endothelium-dependent relaxation and elevation of vascular smooth muscle cyclic GMP at doses of 10–100 μ*M* (22, 23). The inhibition was effective within 5–10 min of incubation (22, 23). The dogs received approximately 500 mg methylene blue (molecular weight 374) over 23 min prior to the infusion of substance P. The dye enters cells rapidly and consequently the concentration of methylene blue within the vasculature should have been sufficient to inhibit guanylate cyclase. Attempts to increase the dose of methylene blue were associated with severe pulmonary hypertension and systemic hypotension. As tissue levels of cyclic GMP were not measured, a role for cyclic GMP in transducing the effects of substance P cannot be entirely excluded.

It has been suggested that the mediator of endothelial-dependent vascular relaxation may be a free radical intermediate and the relaxation induced by acetylcholine has been inhibited by radical scavengers (18). *N*-acetylcysteine is a sulfhydryl containing compound, which has radical scavenging properties (24). The infusion of *N*-acetylcysteine in the dog did not alter the effect of substance P on the pulmonary vasculature. However, it was vasoactive in that it caused systemic vasodilatation and an increase in cardiac output prior to substance P infusion. Additional experiments using other scavengers would be required to exclude the possibility that radicals are involved.

These studies indicate that substance P is a nonspecific vasodilator in that it reduces both pulmonary and systemic vascular resistance. In fact, it appears to have a greater effect on the systemic circulation. Although substance P has been shown to reduce systemic arterial pressure in the anesthetized dog in a dose-dependent manner (4), this relationship was not demonstrated in these experiments, possibly because different batches of substance P were used in parts (i) and (ii) of the protocol. The mechanism of substance P-induced vasodilatation does not seem to involve the products of cyclooxygenase metabolism. Although

methylene blue itself increased pulmonary arterial pressure and resistance, it did not reduce either the pulmonary or systemic vasodilatation caused by substance P. While substance P is known to stimulate endothelial-dependent vasodilatation, associated with an increase in cyclic GMP, in these studies, methylene blue, an inhibitor of guanylate cyclase, failed to change the vascular activity of substance P.

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