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The Effects of Naloxone on Canine Splanchnic Arterial Smooth Muscle (41967)

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Abstract. The pharmacological properties of naloxone on vascular smooth muscle *in vitro* were examined using canine mesenteric arterial segments. Naloxone exerted two different effects on the artery: (A) naloxone at a high concentration (3×10^{-4} *M*) produced a nonspecific vasodilation; and (B) naloxone at lower concentrations (3×10^{-7}, 3×10^{-6}, and 3×10^{-5} *M*) augmented the vasoconstrictor effects of epinephrine and norepinephrine without altering KCl- or serotonin-induced constriction. Naloxone's augmenting effect on epinephrine-induced constriction was dose dependent. Even when the arterial strips were incubated in low calcium (0.8 m*M*) or calcium free Kreb's solution, naloxone (3×10^{-5} *M*) still augmented epinephrine-induced constriction. With respect to naloxone's effect on another α-adrenoreceptor agonist, naloxone (3×10^{-5} *M*) failed to alter phenylephrine-induced constriction. Naloxone's augmenting effect on norepinephrine-induced constriction was abolished when the specimens were incubated with 10^{-5} *M* normetanephrine, while naloxone (3×10^{-5} *M*) still augmented the constriction even when the specimens were incubated with 10^{-5} *M* cocaine. These results suggest that naloxone at lower concentrations may augment the constrictor responses to catecholamines, at least in part, by inhibiting the extraneuronal uptake of those catecholamines.

Since the discovery of enkephalins and β-endorphin, numerous studies have demonstrated that serum β-endorphin and adrenocorticotrophin are synchronously elevated in response to stress (1, 2). Subsequently, the opiate antagonist naloxone has been shown to have beneficial effects in the treatment of endotoxic (3–5) or hypovolemic (6–9) shock by increasing blood pressure, pulse pressure, and cardiac output. However, it is not clear whether these effects are produced directly by altering the peripheral cardiovascular system or altering the central nervous system's regulation of peripheral cardiovascular parameters. If naloxone at therapeutic doses has some direct pharmacological property on vascular smooth muscle, such information may be helpful in elucidating the mechanism of naloxone's beneficial effect in the treatment of shock. But, little is known about the pharmacological properties of naloxone on vascular smooth muscle. Lee and Berkowitz (5) have reported that naloxone at high concentrations (3×10^{-4} to 1×10^{-3} *M*) inhibits the contraction of rat aortic smooth muscle induced by *l*-pentazocine, KCl, and norepinephrine. The effects of naloxone at lower concentrations on vascular smooth muscle have not yet been investigated. It was the purpose of this experiment to investigate the pharmacological properties of naloxone on vascular smooth muscle *in vitro*.

Material and Methods. Adult mongrel dogs of either sex, weighing 12 to 18 kg, were anesthetized with sodium pentobarbital (30 mg/kg) and sacrificed by exsanguination from the femoral artery. The superior mesenteric artery was removed and its distal portion isolated under magnification. It was placed immediately in nutrient Kreb's bicarbonate solution [(m*M*): NaCl 120; KCl 4.5; $CaCl_2$ 2.5; $MgSo_4$ 1.0; $NaHCO_3$ 27.0; KH_2PO_4 1.0; and dextrose 10.0] at 37°C which was gassed with 95% O_2 and 5% CO_2. The pH of the solution ranged from 7.40 to 7.50. The artery was cut into 4-mm-long ring segments which were suspended individually between L-shaped stainless-steel holders in organ baths with a 10-ml working volume. Resting tension was adjusted to 2 g. The preparations were allowed to equilibrate at 37°C for 60 min before use.

Dose–response curves for KCl, serotonin, norepinephrine, epinephrine, and phenylephrine were obtained by cumulative addition of the various agonists. The contractile force

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was recorded isometrically using a Grass FT.03 force-displacement transducer. The transducer signal was amplified and displayed on a Gould 260 multichannel recorder.

It is well known that elevations in extracellular potassium concentration cause depolarization of both adrenergic nerve endings and vascular smooth muscle (10). This results in a contractile response which is partially dependent upon norepinephrine released from adrenergic nerve endings. In order to eliminate the action of norepinephrine released from adrenergic nerve endings, preparations were exposed for 30 min to 10^{-5} *M* guanethidine in the experiment studying the effect of naloxone on the KCl-induced constriction.

The contractile response to 40 m*M* KCl was first obtained on each ring segment then the preparations were repeatedly washed. The contractions induced by 40 m*M* KCl were taken as 100%; mean absolute values were 9.25 ± 0.41 g (n = 23). Contractile activities of the other agonists are expressed as a percentage of the contraction elicited by this standard dose of 40 m*M* KCl.

The contractile activity of each agonist was measured several times until the response became stable. After the response became stable, a control response was obtained. Then, the effects of naloxone on the constrictor response were examined.

In studies examining the effects of naloxone on constrictions of mesenteric artery produced by several agonists, preparations were exposed for 2 min to naloxone (3×10^{-7}, 3×10^{-6}, 3×10^{-5}, or 3×10^{-4} *M*) before addition of agonists.

Following administration of agonists to specimens incubated with naloxone, all specimens were washed about five times with nutrient medium and again treated with the agonists alone. It was certified that measured contractility returned to values obtained with the agonists alone before the naloxone treatment.

The present results demonstrated that naloxone at concentrations of 3×10^{-7}, 3×10^{-6}, and 3×10^{-5} *M* augments epinephrine or norepinephrine-induced constrictions of canine mesenteric arteries. Three other experiments, therefore, were performed in order to investigate the mechanism by which naloxone augments epinephrine-induced constriction. In the first study designed to evaluate the influence of external calcium on naloxone's ability to augment epinephrine-induced constriction, nutrient Kreb's solutions containing various concentrations of calcium (2.5, 0.8, and 0 m*M*) were used. Arterial segments were incubated in the particular Ca^{2+} solution for 30 min before beginning the experiment. The preparations were exposed for 5 min to 3×10^{-5} *M* naloxone.

In a second experiment, the effect of naloxone on constrictions produced by an α-adrenoreceptor stimulating agent was examined. Phenylephrine was used as an α_1-adrenoreceptor agonist (11).

In a third experiment, the possibility that naloxone may augment the constrictor responses to catecholamines by inhibiting neuronal or extraneuronal uptake of catecholamines was tested. Preparations were incubated for 15 min with 10^{-5} *M* cocaine or 10^{-5} *M* normetanephrine before addition of norepinephrine.

Statistical analysis of the comparisons of control and treatment responses was done at a specific naloxone concentration, and at each specific agonist concentration using a paired t test. For graphic presentation, the control values were averaged together. Computation of dose–response curves and ED_{50} values was done using probit analysis with the SAS (Statistical Analysis System) computer program. Values were considered to be significantly different at $P < 0.05$.

Drugs and solutions used in this study were 5-hydroxytryptamine, (−)-epinephrine (+)-bitartrate, (±)arterenol hydrochloride, L-phenylephrine hydrochloride, and normetanephrine hydrochloride (Sigma Chemical Co.); naloxone (DuPont Pharmaceuticals, Lot 82-037); cocaine hydrochloride (Mallinckrodt Co.); and guanethidine sulfate (Ciba Geigy). Norepinephrine or epinephrine was dissolved using 50 m*M* phosphate buffer solution (pH 7.4) which contains 0.1% ascorbate. All other drugs were dissolved in saline.

Results. Naloxone itself (3×10^{-7}, 3×10^{-6}, 3×10^{-5}, 3×10^{-4} *M*) did not induce a contraction or relaxation of the

arterial rings in the absence of any other agonist.

Naloxone's effect on KCl- or serotonin-induced contraction. The addition of KCl (20 to 80 m*M*) produced a concentration-dependent contraction of canine mesenteric arteries (Fig. 1). Naloxone (3×10^{-4} *M*) inhibited the constriction induced by 40, 60, or 80 m*M* KCl, while naloxone at lower concentrations (3×10^{-5}, 3×10^{-6}, and 3×10^{-7} *M*) failed to alter KCl-induced constriction (Fig. 1). Serotonin at concentrations of 10^{-8} to 10^{-5} *M* contracted the vessels in a concentration-dependent manner. Naloxone (3×10^{-4} *M*) suppressed serotonin-induced constriction of mesenteric artery at serotonin concentrations of 10^{-6} and 10^{-5} *M* by 13.5 ± 2.3% (mean ± SE) and 13.2 ± 2.2%, respectively, while it slightly augmented the constriction induced by 10^{-7} *M* serotonin (n = 7). Naloxone at lower concentrations (3×10^{-5}, 3×10^{-6}, and 3×10^{-7} *M*) failed to alter constriction induced by serotonin at all concentrations tested (n = 7).

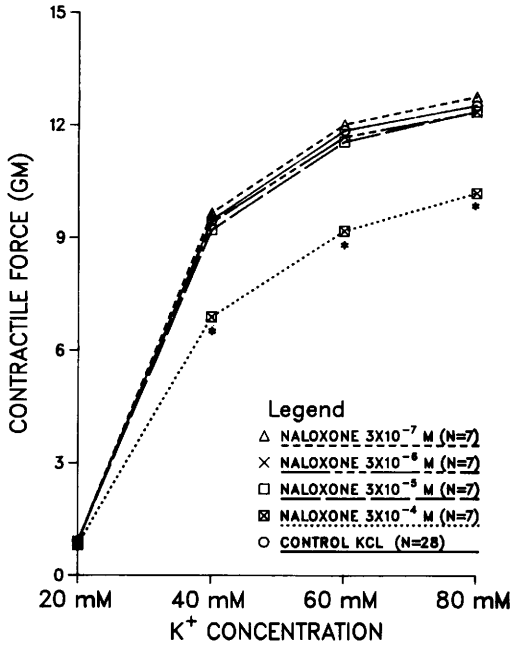

FIG. 1. The effects of naloxone treatment on the vasoconstrictor responses of canine mesenteric arteries induced by potassium chloride. Statistical analysis of the comparisons of control and treatment responses was done using a paired t test since the data were matched by preparation. For graphic presentation, the control values were averaged together. The amount of contractile force produced by KCl-induced depolarization is expressed in grams. Results are expressed as means. *$P < 0.05$. n = Number of arterial specimens examined.

Naloxone's effect on epinephrine or norepinephrine-induced constriction. Epinephrine or norepinephrine at concentrations of 10^{-7} to 3×10^{-5} *M* contracted the vessels in a concentration-dependent manner. As shown in Table 1, naloxone at concentrations of 3×10^{-7} *M* augmented the constriction induced by 10^{-6} and 3×10^{-6} *M* epinephrine. Naloxone at 3×10^{-6} *M* augmented the epinephrine-induced constriction at epinephrine concentrations of 10^{-7} to 10^{-5} *M*; naloxone at 3×10^{-5} *M* also augmented the constriction at epinephrine concentrations of 10^{-7} to 3×10^{-6} *M*. As an overall effect, naloxone (3×10^{-7}, 3×10^{-6}, and 3×10^{-5} *M*) treatment shifted the ED_{50} of epinephrine to lower values. At 3×10^{-4} *M*, naloxone augmented the constriction produced by 10^{-7} to 10^{-6} *M* epinephrine, though it suppressed the constriction at higher concentrations (10^{-5} and 3×10^{-5} *M*) of epinephrine.

With respect to norepinephrine-induced constriction, 3×10^{-5} *M* naloxone shifted the ED_{50} of norepinephrine to lower value (Table III), while 3×10^{-6} and 3×10^{-7} *M* naloxone failed to shift the ED_{50}.

Influence of external calcium on naloxone's ability to augment epinephrine-induced constriction. Naloxone (3×10^{-5} *M*) augmented epinephrine-induced constriction, even when the arterial strips were incubated in low calcium (0.8 m*M*) or calcium free Kreb's solution (Table II).

Naloxone's effect on another α-adrenoreceptor agonist. The α_1-adrenoreceptor stimulating agent phenylephrine (11) produced a dose–response curve similar to norepinephrine. The effect of naloxone (3×10^{-5} *M*) on phenylephrine-induced constriction was then tested comparing the ED_{50} values of phenylephrine in the control and naloxone treated groups. No significant difference was observed between the two groups (n = 10).

Influence of inhibitors of neuronal or extraneuronal uptake of catecholamines on naloxone's ability to augment norepinephrine-induced constriction. As shown in Table III, naloxone (3×10^{-5} *M*) shifted the ED_{50} value of norepinephrine to a lower value. Cocaine

TABLE I. THE VASOCONTRACTILE RESPONSES OF CANINE MESENTERIC ARTERIES INDUCED BY EPINEPHRINE WITH AND WITHOUT NALOXONE TREATMENT

Treatment	n	% Constriction to 40 mM KCl					ED_{50} value ($\times 10^{-6}$ M)	Shift of ED_{50} value ($\times 10^{-6}$ M)	t test
		10^{-7} M	3×10^{-7} M	10^{-6} M	3×10^{-6} M	10^{-5} M	3×10^{-5} M			
Control	6	4.6 ± 0.8	22.5 ± 4.2	91.5 ± 11.0	140.5 ± 10.8	168.3 ± 8.4	176.6 ± 7.5	1.18 ± 0.16		
Naloxone (3×10^{-7} M)	6	4.6 ± 1.2	22.7 ± 5.7	100.5 ± 12.7*	148.7 ± 9.6*	170.6 ± 7.7	177.3 ± 7.1	1.03 ± 0.12	−0.15 ± 0.05	$P < 0.05$
Control	6	3.3 ± 1.1	15.5 ± 4.4	85.6 ± 13.2	140.0 ± 10.7	166.5 ± 8.4	176.3 ± 7.5	1.34 ± 0.18		
Naloxone (3×10^{-6} M)	6	6.4 ± 2.2*	35.3 ± 8.5*	121.6 ± 10.2*	152.2 ± 7.6*	172.8 ± 6.7*	176.8 ± 6.9	0.75 ± 0.08	−0.59 ± 0.11	$P < 0.05$
Control	6	3.6 ± 1.2	16.1 ± 4.9	86.5 ± 12.6	139.4 ± 9.7	163.3 ± 7.8	172.0 ± 7.4	1.29 ± 0.18		
Naloxone (3×10^{-5} M)	6	10.3 ± 3.8*	56.9 ± 9.6*	128.0 ± 7.2*	156.9 ± 5.2*	168.9 ± 5.3	172.2 ± 5.4	0.57 ± 0.05	−0.72 ± 0.14	$P < 0.05$
Control	6	3.1 ± 0.9	16.9 ± 6.1	83.4 ± 9.5	136.2 ± 6.3	159.0 ± 4.3	168.0 ± 4.3	1.20 ± 0.13		
Naloxone (3×10^{-4} M)	6	9.3 ± 2.3*	47.0 ± 5.7*	111.4 ± 5.6*	139.2 ± 4.7	149.9 ± 4.5**	154.2 ± 4.4**	0.88 ± 0.14	−0.32 ± 0.20	NS

Note. The vasocontractile response to epinephrine is expressed as a percentage of the contraction elicited by a standard dose of 40 mM KCl. Results are expressed as means ± SEM. n = Number of arterial specimens examined.
* Augmentation, $P < 0.05$.
** Inhibition, $P < 0.05$.

(10^{-5} M) also shifted the ED_{50} to a lower value. Even when preparations were incubated with cocaine before addition of naloxone, the ED_{50} value of norepinephrine was shifted to a lower value by naloxone (3×10^{-5} M) though the amount of the shift was decreased in the presence of cocaine. On the other hand, naloxone (3×10^{-5} M) failed to alter the ED_{50} value when the preparations were incubated with normetanephrine (10^{-5} M) before addition of naloxone ($n = 9$).

Discussion. The pharmacological effects of naloxone on vascular smooth muscle have not previously been investigated in detail. The results of this experiment revealed that naloxone at high concentration (3×10^{-4} M) suppressed the contractions induced by KCl (40, 60, and 80 mM), serotonin (10^{-6} and 10^{-5} M), epinephrine (10^{-5} and 3×10^{-5} M), and norepinephrine (3×10^{-6}, 10^{-5}, 3×10^{-5} M). These results appear to be consistent with the report by Lee and Berkowitz (5) that naloxone at high concentrations (3×10^{-4} to 1×10^{-3} M) reduced rat aortic contractions produced by *l*-pentazosine, KCl, and norepinephrine. Together, these findings suggest that high concentrations of naloxone exert a nonspecific or possibly a local anesthetic-like vasodilating effect. In addition, Lee and Berkowitz (5) demonstrated that the vasodilating effect of naloxone was dependent on calcium concentration in the medium. This dependence was not tested in our experiment.

The results of this study also demonstrate that low concentrations (3×10^{-6} and 3×10^{-5} M) of naloxone augment the contractile responses of canine mesenteric artery to epinephrine or norepinephrine without altering KCl- or serotonin-induced constriction. In order to elucidate the mechanism of this specific augmenting effect of naloxone, the influence of calcium in nutrient medium on the augmentation ability of naloxone was evaluated using Kreb's solution which contains various concentrations of calcium. Since naloxone (3×10^{-5} M) in Kreb's solution containing decreased calcium shifted the ED_{50} value of epinephrine to a lower value, it appears that naloxone's augmenting effect on epinephrine-induced constriction of canine mesenteric artery is not related to the concentration of external calcium.

TABLE II. THE INFLUENCE OF CALCIUM ION ON NALOXONE (3×10^{-5} *M*)-INDUCED POTENTIATION OF THE VASOCONTRACTION BY EPINEPHRINE

Concentration of Ca^{2+} (m*M*)	Treatment	*n*	ED_{50} value ($\times 10^{-6}$ *M*)	Shift of ED_{50} value ($\times 10^{-6}$ *M*)	*t* test
2.5	Control	15	1.02 ± 0.07		
	Naloxone	15	0.63 ± 0.05	−0.39 ± 0.05	$P < 0.001$
0.8	Control	10	0.94 ± 0.02		
	Naloxone	10	0.49 ± 0.02	−0.45 ± 0.02	$P < 0.001$
0	Control	8	1.38 ± 0.09		
	Naloxone	8	0.73 ± 0.08	−0.65 ± 0.03	$P < 0.001$

Note. Values are expressed as means ± SEM. *n* = Number of arterial specimens examined.

The possibility that naloxone may act directly on α-adrenoreceptors was also examined. Although naloxone (3×10^{-5} *M*) augmented epinephrine and norepinephrine-induced constriction, it failed to alter phenylephrine-induced constriction. Therefore, it seems unlikely that naloxone exerts its augmenting effect on epinephrine or norepinephrine-induced constriction simply by direct stimulation of α_1-adrenoreceptors in the mesenteric artery.

Another possibility that naloxone may act on catecholamine disposition mechanisms was also examined. The augmenting effect of naloxone on norepinephrine-induced constriction was abolished when the preparations were incubated with normetanephrine before addition of naloxone, while naloxone still augmented the constriction even when the preparations were incubated with cocaine. Therefore, naloxone at lower concentrations may augment the constrictor responses to norepinephrine or epinephrine, at least in part, by inhibiting the extraneuronal rather than neuronal uptake of those catecholamines. In order to clarify this point, further studies will be necessary.

The effects of naloxone at lower concentrations on epinephrine or norepinephrine-induced constriction are quite different when comparing cerebral artery to mesenteric artery. In canine basilar artery, naloxone (3×10^{-7} to 3×10^{-5} *M*) selectively inhibited epinephrine or norepinephrine-induced constriction (12). In monkey cerebral artery, naloxone failed to alter the constrictor responses to norepinephrine (13). The mechanism of this difference in naloxone actions on epinephrine or norepinephrine-induced constriction of cerebral versus splanchnic artery is still unknown and requires further study.

The beneficial effects of naloxone in endotoxic (3–5) or hypovolemic (6–8) shock are well known. However, naloxone's precise mechanisms of action remain controversial. Holaday and Faden (4) have suggested that the beneficial effect of naloxone in shock

TABLE III. THE INFLUENCE OF COCAINE ON NALOXONE-INDUCED POTENTIATION OF THE VASOCONTRACTION BY NOREPINEPHRINE

Treatment	*n*	ED_{50} Value ($\times 10^{-6}$ *M*)	% of the shift of ED_{50} value	*t* test
Control (NE)	9	1.98 ± 0.17		
Naloxone (3×10^{-5} *M*) + NE	9	1.52 ± 0.14	23.26 ± 2.18	$P < 0.001$
Cocaine (10^{-5} *M*) + NE	9	0.58 ± 0.04		
Cocaine (10^{-5} *M*) + naloxone (3×10^{-5} *M*) + NE	9	0.47 ± 0.03	18.55 ± 1.50	$P < 0.001$

Note. Values are expressed as means ± SEM. *n* = Number of arterial specimens examined. NE = norepinephrine.

may be mediated through specific antagonism of the hypotensive action of pituitary endorphins.

It has also been suggested that naloxone exerts its protective effects in hemorrhagic shock through positive inotropic effects on papillary muscle as well as an improved metabolic state, including inhibition of proteolysis and stabilization of lysosomal membranes (14). Alternatively, or in conjunction, our data suggest that naloxone may increase blood pressure and peripheral vascular resistance by augmenting the constrictor responses of splanchnic arteries induced by endogenous catecholamines.

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