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A Comparison of the Effects of Cocaine in Arterial and Venous Smooth Muscle Responses to Vasoactive Stimuli[1] (38030)

S. GREENBERG[2] AND J. P. LONG

Departments of Internal Medicine (Division of Clinical Pharmacology) and Pharmacology, College of Medicine, University of Iowa, Iowa City, Iowa 52242

The observed contractile response, *in vivo* or *in vitro,* of different vascular beds to norepinephrine is dependent on at least four factors which include:

(1) the functional continuity of the cells comprising a particular vascular bed (1–3),
(2) the lability of the intracellular calcium store involved in the contractile process (4–6),
(3) the quantity of exogenously administered norepinephrine employed to initiate the contractile response. High concentrations of norepinephrine will produce membrane depolarization of isolated and intact vascular smooth muscle (7–9). This subject has been excellently reviewed by Holman (2), and
(4) the relative efficiency of the neuronal reuptake mechanism necessary for elimination of transmitter in one vascular bed in relation to another vascular bed (10–15).

Previous studies from this laboratory demonstrated that the mesenteric vein differed from cutaneous arteries and veins (as well as from the mesenteric artery obtained from a corresponding level of the mesenteric vascular tree) in the lability of the calcium exchange and in the inability of norepinephrine to produce a measurable increase in calcium uptake (16). In addition, the circular muscle of small mesenteric veins appears to be less sensitive than the longitudinal muscle to exogenously administered vasoactive stimuli and cocaine (17). It was postulated that circular oriented muscle of mesenteric veins is not as richly innervated with noradrenergic nerves as the longitudinal muscle (17).

The present study was performed to compare the role of neuronal reuptake in the contractile response of small anterior mesenteric veins (with primarily, if not entirely, circular muscle) to exogenously administered norepinephrine with the responses of mesenteric arterial and cutaneous arterial and venous smooth muscle by fluorescence histochemistry and utilization of a dose of cocaine which maximally enhanced the contractile responses to norepinephrine but which did not affect the contractile responses of these smooth muscles to the ionic stimulants potassium or barium chloride. The results suggest that adrenergic neuronal reuptake of catecholamines may play a minor role in termination of the contractile responses of mesenteric veins to norepinephrine. This effect does not appear to be related to the adrenergic innervation of this venous smooth muscle.

Methods. Twenty mongrel dogs of either sex (8–15 kg) were anesthetized with sodium pentobarbital (35 mg/kg, iv). Anterior tibial arteries, mesenteric arteries, anterior mesenteric veins, and dorsal meta-

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[2] Present address: Department of Physiology, University of Michigan, Ann Arbor, Michigan 48104.

tarsal branches of saphenous veins were removed, cut into helical strips, and prepared for superfusion as previously described in detail (16). Each vascular strip was mounted at optimal resting tension, determined by obtaining responses to 9.6 nmoles of epinephrine at various resting tensions until further increments in resting tension produced no further enhancement of the response to this agonist. The arterial strips were allowed to equilibrate 3 hr prior to experimentation. Isometric contractions were measured with Grass FT-03 force-displacement transducers and recorded on a Grass model 5 recorder. The physiologic salt solution (PSS) was maintained at 37.5°, aerated with 95% O_2 and 5% CO_2 (pH 7.4), and contained (millimolar concentration): sodium chloride (113), potassium chloride (3.1), sodium bicarbonate (24.8), calcium chloride (1.53), dextrose (10), magnesium chloride (2.1), monosodium dihydrogen phosphate (1.1), calcium disodiumethylenediaminetetraacetate (0.026), and pyruvic acid (3.2). The flow rate of the superfusate was 3–4 ml/min and remained constant for each vascular strip.

Effect of cocaine on contractile responses of vascular smooth muscle to norepinephrine, barium chloride, and potassium chloride. All experiments were performed after a 3-hr equilibration period. All agonists were administered into the tip of a funnel from which the superfusate flowed (3–4 ml/min) prior to bathing the tissue, in a volume not exceeding 0.1 ml. Cocaine was added to the reservoir containing PSS and was continuously superfused over the vascular strips.

Partial dose–response curves were obtained for norepinephrine (0.6–0.9 nmoles), $BaCl_2$ (24–96 μmoles), and KCl (50–200 μmoles) prior to, during, and 30 min after superfusion with cocaine. Each vascular strip was challenged with each of the 3 doses of all agonists. The doses of agonist used produced approximately 20, 50, and 80% of the maximal contractile response of the tissue for the agonist. The maximal contractile response to norepinephrine was always greater in each of the muscles than the maximal contractile response to either KCl or $BaCl_2$ (11). Each tissue was challenged with increasing doses of norepinephrine or KCl every 15 min. Twenty minutes elapsed between succeeding doses of $BaCl_2$. This procedure resulted in reproducible contractile responses to each of the agonists and a return to baseline tension after each dose of agonist prior to obtaining a response to succeeding doses. The responses of the vascular strips to the agonists were stable and were independent of the order of agonist administration (16).

Catecholamine fluorescence histochemistry. Portions of arteries and veins were frozen in isopentane cooled in liquid nitrogen, dried at 5×10^{-5} mm Hg at −35° over P_2O_5 for 8 days, brought to room temperature and exposed to paraformaldehyde (stored at a relative humidity of 18%) at 80° in an oven for 1 hr. The tissues were imbedded in paraffin and sections 8 μm in thickness were cut on a Leitz rotary microtome (E. Leitz, Inc., New York, NY), mounted on glass slides with Leitz nonfluorescent immersion oil, and examined in a Leitz microfluorometer as previously described (18).

Drugs and chemicals. L-Norepinephrine bitartrate was obtained from Levophed, Sterling–Winthrop Research Institute, New York, NY, $BaCl_2$ and KCl from Baker Chemicals and cocaine from University of Iowa Hospitals.

Statistical evaluation of data. Dose-effect data were analyzed by an analysis of variance randomized complete block factorial design. The means were analyzed for significance by the Duncan's New Multiple Range Test or by Tukey's procedure. A P value of 0.05 or less ($P < 0.05$) was chosen for statistical significance (19, 20).

Results. Effect of potassium, barium, and norepinephrine on vascular smooth muscle. Each of the vascular strips developed tension when challenged with potassium (KCl), barium ($BaCl_2$), or norepinephrine or tyramine (Table I). The maximal contractile response of arteries to KCl and $BaCl_2$ and tyramine was significantly less than the contractile response to norepinephrine. The contractile responses to these agonists were stable and reproducible for

TABLE I. Effect of Cocaine (2.33×10^{-6} *M*) on the Contractile Response of Superfused Helical Strips of Vascular Smooth Muscle to Tyramine.[a]

		Contractile response to tyramine (mg ± SE)			
		Control		Cocaine	
Preparation	*N*	0.172	17.2	0.172	17.2
Mesenteric vein	4	8 ± 7	33 ± 14	0 ± 0*	0 ± 0*
Mesenteric artery	4	15 ± 7	40 ± 17	0 ± 0*	0 ± 0*
Saphenous vein	4	347 ± 39	1176 ± 297	127 ± 64	421 ± 75*
Tibial artery	4	70 ± 24	329 ± 78	0 ± 0*	21 ± 10*

[a] The mean values are the contractile responses of 4 vascular strips to 0.172 and 17.2 μmoles of tyramine before and during superfusion with 2.33×10^{-6} *M* cocaine.

* Significantly differs ($P < 0.05$) from control values with Student's paired *t* test (20).

at least 10 hr (16). The magnitude of response to each of the agonists tested were independent of the sequence of administration. These results were described previously in detail (16).

During superfusion with cocaine (2.3×10^{-6} *M*), the contractile responses of venous and arterial smooth muscle to potassium chloride and barium chloride were not significantly different from control values

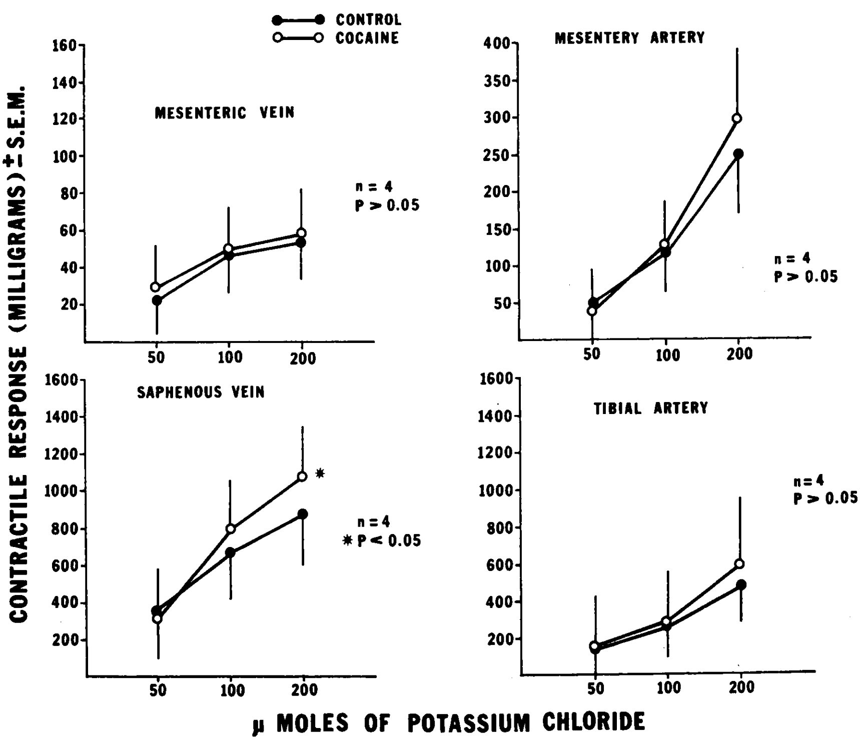

FIG. 1. Effect of cocaine (2.3×10^{-6} *M*) on contractile responses of superfused helical strips of vascular smooth muscle to potassium chloride. The ordinate represents the change in tension (in milligrams) after the administration to the preparation of the agonists before and 30 min after addition of cocaine to the superfusate. Abscissa: concentration of agonist. An asterisk denotes that the responses significantly differ from control values ($P < 0.05$).

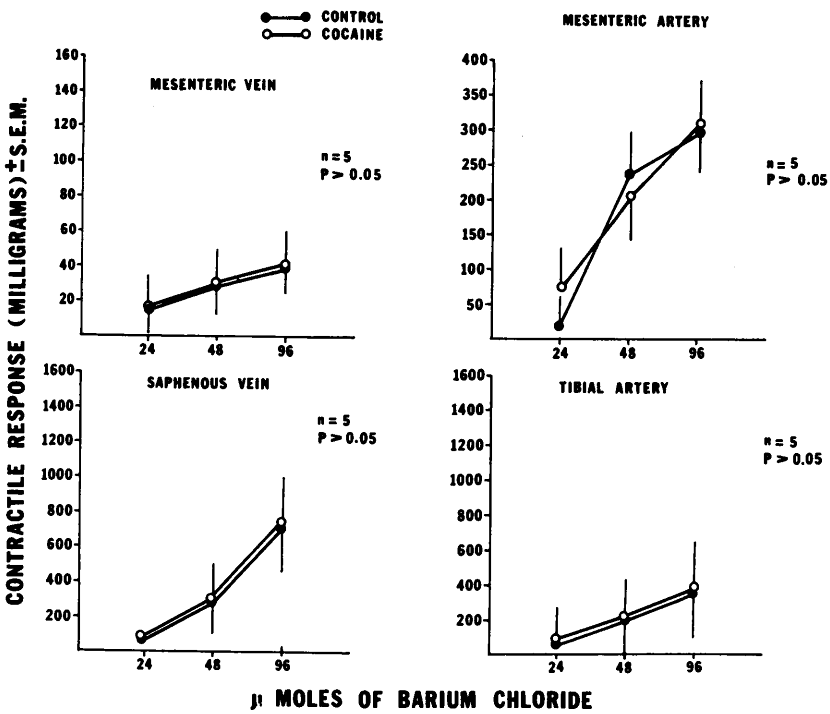

FIG. 2. Effect of cocaine ($2.3 \times 10^{-6} M$) on contractile response of superfused helical strips of vascular smooth muscle to barium chloride. (Legend same as Fig. 1.)

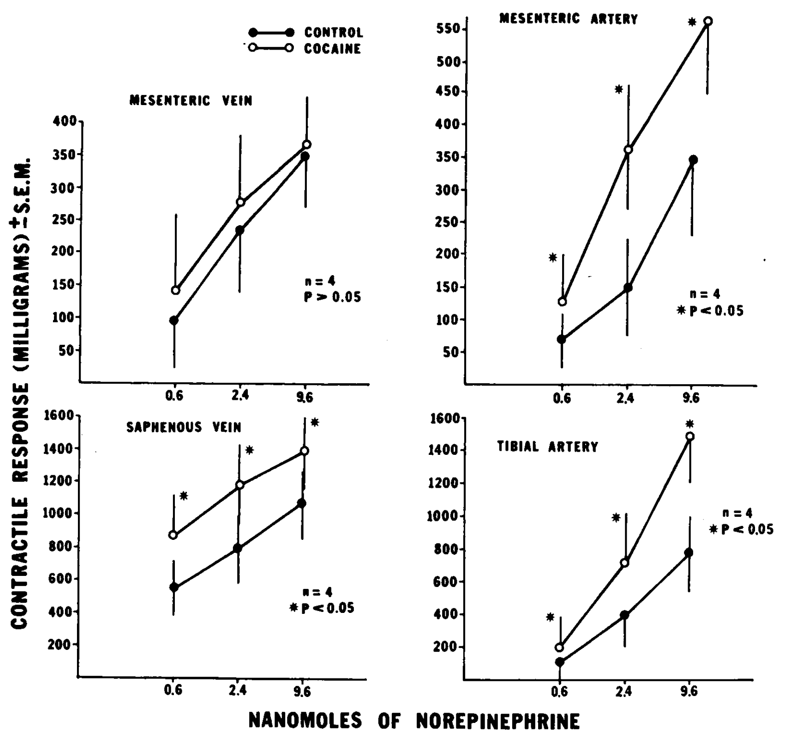

FIG. 3. Effect of cocaine ($2.3 \times 10^{-6} M$) on the contractile responses of vascular smooth muscle to norepinephrine. (Legend same as Fig. 1.)

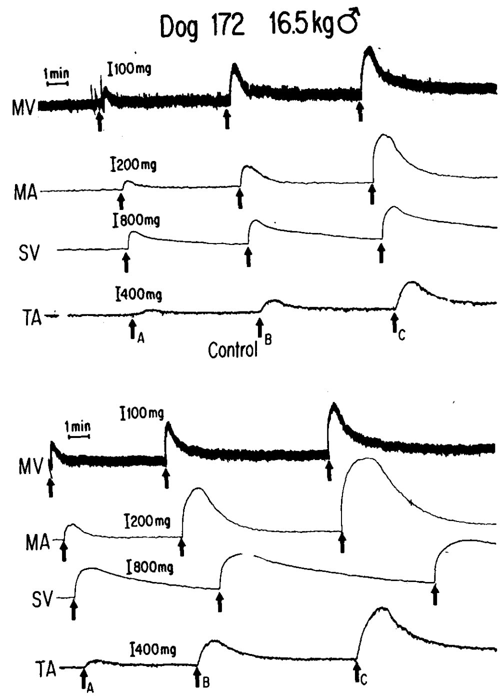

FIG. 4. A typical record illustrating the effect of cocaine (2.3×10^{-6} M). On contractile responses of superfused anterior mesenteric veins (MV), mesenteric arteries (MA), dorsal metatarsal branches of saphenous veins (SV), and anterior tibial arteries (TA) to norepinephrine (0.6, 2.4, and 9.6 nmoles, administered at arrows). Cocaine was in contact with vascular strips for 30 min before reevaluation of responses to norepinephrine.

($P > 0.05$) (Figs. 1 and 2). In contrast to these findings, cocaine enhanced the contractile responses of cutaneous arterial and venous smooth muscle and mesenteric arterial smooth muscle to norepinephrine (Figs. 3 and 4). The dose-response curves to norepinephrine were shifted to the left in a parallel fashion (the P value of the F-ratio for the cocaine–norepinephrine interaction terms was greater than 0.2). Cocaine did not significantly affect the contractile responses of mesenteric venous smooth muscle to norepinephrine (Figs. 3 and 4). Higher concentrations of cocaine produced nonspecific enhancement of the contractile responses of vascular smooth muscle to each of the agonists and this effect will be reported on in a separate communication (Cheng and Long, this laboratory, manuscript in preparation).

Table I summarizes the effect of cocaine (2.3×10^{-6} M) on the contractile responses of the four types of vascular smooth muscles to tyramine. It is obvious from these findings that the responses to this indirectly acting amine were reduced significantly in all vascular strips.

Fluorescence microscopy. The appearance of a section of innervated arterial and venous smooth muscle from the mesenteric and cutaneous vascular beds of the same dog is presented in Fig. 5. All four vascular strips were treated simultaneously by the fluorescence histochemical method outlined in *Methods*. The nonspecific fluorescence of the elastic laminae is quite clear in Figs. 5-1 and 5-3. The catecholamine fluorescence of the mesenteric artery, tibial artery, and dorsal metatarsal veins is predominantly in the adventitial-medial border. The dorsal metatarsal vein has some catecholamine fluorescence in the medial layer of smooth muscle. The mesenteric vein has a diffuse and sparse catecholamine fluorescence (Fig. 5-2).

Discussion. The results of the present experiments demonstrate that a concentration of cocaine producing maximal potentiation of contractile responses to norepinephrine with minimal effects on the responses of vascular smooth muscle to the ionic stimuli barium chloride and potassium chloride fails to enhance the contractile responses of mesenteric veins to norepinephrine yet abolishes the contractile responses of this vascular strip to tyramine. The mesenteric vein, in contrast to mesenteric arteries and cutaneous arteries and veins, has a sparse, diffuse adrenergic innervation occurring through the medial muscle layer.

The contractile response of isolated vascular smooth muscle to tyramine is mediated primarily by release of catecholamines from the adrenergic nerve terminals innervating the vasculature (21, 24). Since tyramine must be taken up into the adrenergic nerves to release norepinephrine, the contractile response to tyramine is reduced when the neuronal catecholamine reuptake mechanism, utilized by tyramine to enter the nerve terminal, is inhibited by cocaine (25, 26). Conversely, since the

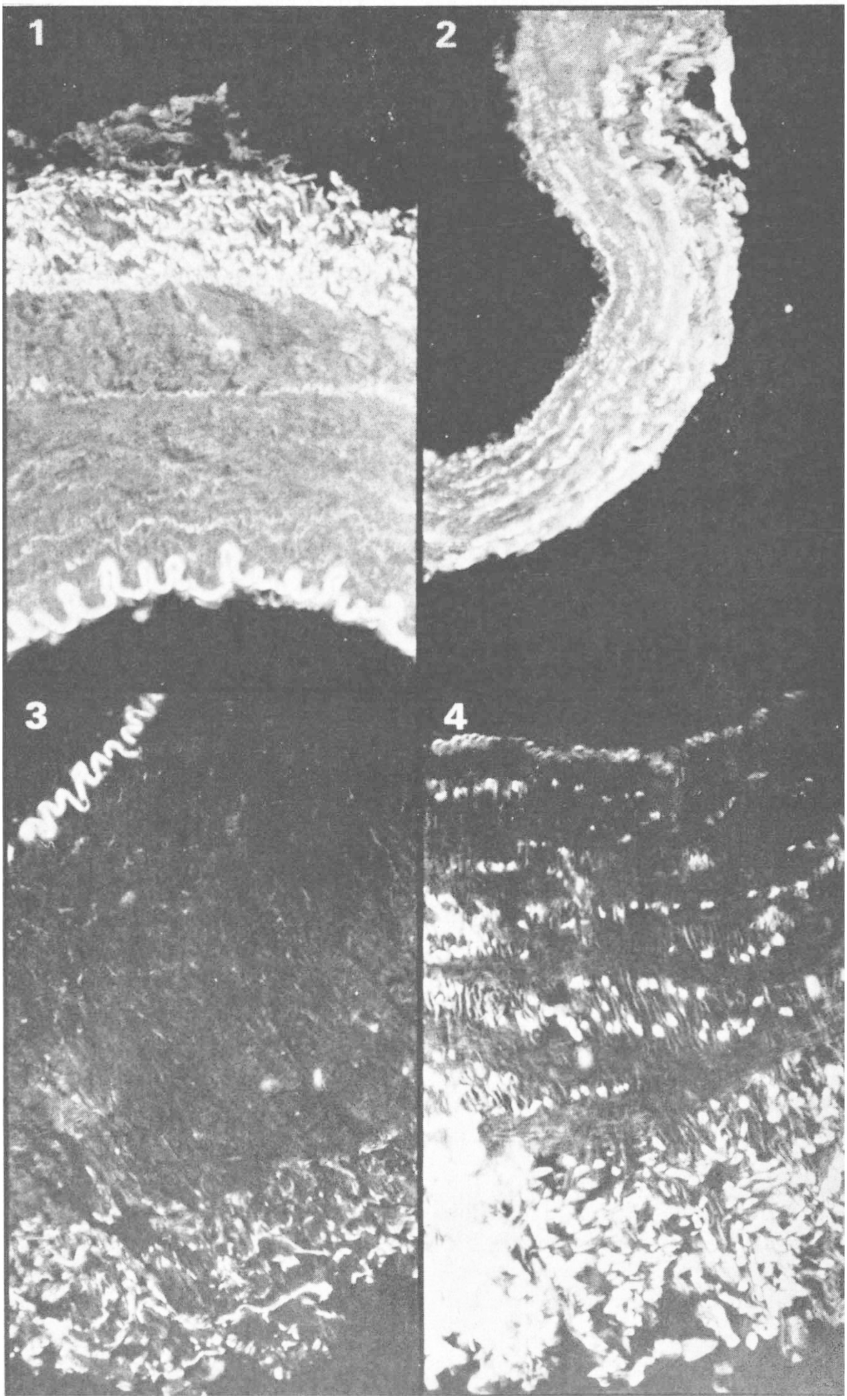

FIG. 5. Fluorescence micrographs of canine vascular smooth muscle in cross-section. Notice the uniformly distributed sparse fluorescence of panel 2 and the fluorescence primarily localized to the adventitial medial border of panels 1, 3, and 4. In addition, note well the wall thickness of the corresponding mesenteric and cutaneous vessels taken at the same point along

contractile response to exogenously administered catecholamines is, to varying degrees, terminated by the reuptake of norepinephrine into the adrenergic nerve terminals, inhibition of the catecholamine reuptake mechanism would be expected to enhance the contractile responses to norepinephrine. Superfusion of vascular smooth muscle with cocaine inhibited the pressor response of the cutaneous vasculature and mesenteric arteries to tyramine and enhanced the contractile responses to norepinephrine. Furthermore, the contractile responses to the ionic smooth muscle stimulants barium chloride and potassium chloride were essentially unchanged from control values during superfusion with cocaine. These data justify the conclusion that these effects were mediated by cocaine-induced inhibition of the neuronal reuptake mechanism for catecholamines. The contractile responses of the mesenteric vein to tyramine were small in magnitude yet susceptible to inhibition by cocaine. The contractile responses of mesenteric veins to norepinephrine were not susceptible to inhibition by concentrations of cocaine which enhanced the contractile responses of the other arteries and veins to norepinephrine. These findings suggest that the adrenergic neuronal reuptake of tyramine accounts for the contractile response to this indirectly acting amine yet adrenergic neuronal reuptake appears to play a minimal role in terminating the response of mesenteric veins to exogenously administered (and perhaps neuronally released) norepinephrine.

Zimmerman and coworkers (13, 14) demonstrated that the endogenous catecholamine content of canine cutaneous veins was approximately 50% of the catecholamine content of cutaneous arterial smooth muscle. Similar findings were demonstrated for mesenteric veins and arteries (27, 28). Both the mesenteric and cutaneous veins have a diffuse innervation when compared with their corresponding arteries (Fig. 5). The cutaneous veins have a greater rate of catecholamine uptake than the cutaneous arteries (13, 14). To our knowledge a comparison of the rates of mesenteric arterial and venous catecholamine reuptake has not been published.

It is possible, therefore, that the mesenteric veins may also have a faster rate of neuronal reuptake of catecholamines than mesenteric arteries since they are less sensitive to a given frequency of nerve stimulation than corresponding cutaneous veins which would be predicted if the neuronal reuptake mechanism was very efficient and only small quantities of norepinephrine "escaped" reuptake to activate the postjunctional smooth receptors. However, if this were the situation, it would also be anticipated that the contractile responses to norepinephrine would be greatly enhanced by cocaine since the effective concentration of norepinephrine at the alpha adrenergic receptor should be increased after blockade of the amine reuptake pump mechanism (10, 13, 14, 29). However, cocaine did not affect the contractile response to norepinephrine. These findings indirectly suggest, as first proposed by Zimmerman and coworkers for vascular smooth muscle (13, 14), that differences may exist in the arterial and venous membrane catecholamine transport systems. Alternatively, if the distance of the synaptic cleft between the adrenergic varicosity and the mesenteric venous smooth muscle cell is large, then the effects of adrenergic neuronal reuptake blockade by cocaine would also be very minimal (30). Further biochemical and ultrastructural studies are necessary to elucidate the mechanism of the relative resistance of the circular muscle of canine mesenteric veins to the actions of cocaine.

Summary. Cocaine ($2.3 \times 10^{-6}\,M$) enhanced the contractile responses of superfused mesenteric arteries, and cutaneous arteries and veins to norepinephrine. This effect was probably related to inhibition of the neuronal catecholamine reuptake mech-

the vascular tree. The mesentric artery (1) wall thickness is much greater than the mesenteric vein (2). The tibial artery (3) and dorsal metatarsal vein (4) have similar thickness. The wall-to-lumen ratio of these latter vessels are similar *in vivo*. Magnification ×120.

anism and not to nonspecific supersensitivity since the contractile responses to tyramine were significantly reduced but the contractile responses to barium and potassium chlorides essentially unchanged. The adrenergic nerves of these blood vessels were confined to a dense innervation in the region of the adventitial medial border with some nerves apparently present in the outer portion of the media (dorsal metatarsal vein). In contrast to these findings, the anterior mesenteric veins responded only with a small response to tyramine, which was inhibited by cocaine. However, the contractile responses to norepinephrine were not enhanced 30 min after the addition of cocaine to the superfusate. The anterior mesenteric vein displayed a sparse and diffuse adrenergic innervation. It was concluded that the inability of cocaine to enhance the contractile responses of mesenteric veins to norepinephrine resulted from the sparse distribution of the nerve endings in this vein and perhaps from a difference in the neuronal membrane adrenergic amine reuptake mechanism.

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