Effects of Cocaine on Excitation-Contraction Coupling of Aortic Smooth Muscle from the Ferret

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Abstract

The mechanism by which cocaine alters vascular tone is not fully understood. We determined the effects of cocaine on excitation-contraction coupling of isolated ferret aorta. Cocaine in concentrations $\leq 10^{-4}$ M caused a contractile response in a dose-dependent manner. The response of control muscle was significantly larger than that in muscle from ferrets pretreated with reserpine. Cocaine-induced contraction was not affected by endothelial factors, but was significantly inhibited by prazosin 10^{-7} M pretreatment. The intracellular calcium ($[Ca^{++}]_i$), as measured with aequorin, rose in conjunction with cocaine-induced contraction. The degree of contraction generated by 10^{-4} M cocaine decreased after higher concentrations of cocaine $\geq 10^{-3}$ M, while aequorin luminescence remained elevated above the levels before 10^{-6} M cocaine. The dose-response relationships of norepinephrine and sympathetic nerve stimulation were enhanced by 10^{-6} M cocaine in control muscles; this did not occur in muscles from reserpine pretreated ferrets. In conclusion, (*a*) cocaine in concentrations $\leq 10^{-4}$ M caused vascular contraction presumably by its presynaptic action with consequent alpha-1 adrenoceptor activation and consequent $[Ca^{++}]_i$ rise; (*b*) high concentrations of cocaine $\geq 10^{-3}$ M reduced muscle tone by decreasing the Ca^{++} sensitivity of the contractile proteins; and (*c*) supersensitivity to norepinephrine was mediated by cocaine's action on adrenergic nerve endings. (*J. Clin. Invest.* 1991. 87:1322–1328.) Key words: aequorin • intracellular calcium • excitation-contraction coupling • alpha adrenoceptor • vascular smooth muscle

Introduction

In recent years, the incidence of cardiovascular complications associated with cocaine use, including acute myocardial ischemia, have been on the rise in the United States and Europe and have become a serious medical problem (1–8). The pathophysiology of the cardiovascular toxicity after cocaine use is unknown. One of the prevailing hypotheses is that cocaine causes severe vasoconstriction of large conduit vessels with ensuing ischemia of the related organs (2–9); however the vascular actions of cocaine appear to be complex. It is generally accepted that cocaine exerts a marked sympathomimetic activity in part by inhibiting the neuronal uptake$_1$ system (10, 11). This action would be expected to increase the tissue concentration of norepinephrine and result in alpha-adrenergically mediated vasoconstriction (12, 13). On the other hand, cocaine is an effective local anesthetic agent which would be expected to cause a marked relaxation of smooth muscle (9). Thus, the net vascular action of cocaine may be due to either of these two actions alone or in combination. However, the relative contributions of these actions to smooth muscle tone over the effective range of cocaine concentrations have not been systematically described. Moreover, the mechanism by which cocaine alters vascular tone has not been characterized in detail and remains controversial (12–17).

Accordingly, the purpose of this study was to determine the effects of cocaine on contractile or relaxant responses of isolated ferret aortic smooth muscle. We used ferret aorta because intracellular Ca^{++} and contractile responses can be simultaneously measured, the former through use of the bioluminescent Ca^{++} indicator, aequorin (18–21). The specific aims were as follows: first, we examined whether endothelial factors modify vascular responses to cocaine; second, we examined the relative contributions of cocaine's direct (i.e., a local anesthetic effect) and indirect (i.e., an effect on adrenergic nerve endings) actions on smooth muscle tone by comparing the responses of the control muscles with those of muscles from animals that had undergone reserpine pretreatment; third, we examined the mechanism of cocaine-induced contraction by the use of pharmacological receptor antagonists. We also measured intracellular Ca^{++} mobilization in muscles that were loaded intracellularly with aequorin; and fourth, we determined factors by which cocaine potentiates the mechanical responses to exogenous and endogenous norepinephrine.

Methods

Tissue preparation. The thoracic aorta was removed from adult male ferrets under chloroform anesthesia. Circular strips (3 × 5–6 mm) were prepared from the vessel according to techniques previously described (18–21), preserving perivascular tissues at room temperature in a dissecting dish filled with oxygenated PSS of the following composition (mM): 120 NaCl, 5.9 KCl, 11.5 dextrose, 25 NaHCO$_3$, 1.2 MgCl$_2$, 1.2 NaH$_2$PO$_4$, and 2.5 CaCl$_2$. Unless otherwise noted, the endothelium was removed by rubbing the luminal surface of the aorta. The strips were mounted in organ baths filled with 50 ml of PSS gassed with 95% O$_2$-5% CO$_2$ (pH 7.4 at 37.5°C). One end of each preparation was fixed by a clamp and the other end was attached to a force transducer (Statham UC-2; Gould Inc., Cleveland, OH). Isometric contraction along the circular direction was recorded on a chart strip recorder (Gould Inc.; model 2200S). After equilibration for 60 min, muscles were repeatedly contracted with 60 mM KCl and then washed with PSS at 30-min intervals. Before each contraction, the strips were progressively stretched and the process was continued until contractile re-

sponses reached a maximal value (aequorin luminescence [L_{max}]).[1] The KCl solution was prepared by an equimolar replacement of sodium chloride with KCl. After obtaining L_{max}, KCl-induced tension was allowed to return to basal levels before experimental protocols were initiated.

Reserpine pretreatment. Reserpine is known to deplete adrenergic nerve endings of their catecholamine content in various organs (22). Thus, reserpine (5 mg/kg, i.m.) was administered to some animals for one day before killing. Short term treatment with single doses of reserpine has been shown to produce functionally complete depletion of catecholamines from the adrenergic nerve endings of ferrets (24) and other mammals within 24 h, although supersensitivity to norepinephrine does not become manifest for several days (23–27). As shown in the results, we found that the contractile response of muscles from reserpine pretreated ferrets to tyramine, which evokes a release of catecholamine from adrenergic nerve endings, was markedly decreased compared with muscles from control animals, while responses to norepinephrine and high KCl solution were not changed. These results suggest that in tissues from ferrets that have undergone short term reserpine pretreatment, catecholamine content of the perivascular nerve endings was significantly depleted while function of the postsynaptic effector sites, including alpha-adrenoceptors, may not have changed.

Transmural nerve stimulation. In selected tissues, transmural nerve stimulation was performed to evoke endogenous norepinephrine release from perivascular nerve endings. Pulses of 30 V and 0.5 ms duration at variable frequencies of 0.1, 1.0, 4.0, and 8 Hz were delivered through two platinum-iridium electrodes (1 mm in diameter) that were located in parallel with both sides of the entire length of the muscle strip. Trains of stimuli at a given frequency were continued until contractile responses reached a maximal level, and a frequency-response relation was determined.

We found in preliminary studies that contractions produced by the electrical transmural stimulation at 4 Hz were inhibited by nearly 100% after 0.1 μg/ml tetrodotoxin, and were inhibited by more than 50% after 10^{-7} M prazosin. Therefore, these results indicate that the transmural stimulation had resulted primarily in activation of adrenergic nerve endings rather than direct stimulation of smooth muscles.

Aequorin loading and intracellular Ca^{++} measurements. Aequorin was loaded intracellularly by a chemical procedure described previously (see references 18 and 19 for details). In prior studies, this procedure did not alter contractile responses to phenylephrine or high KCl solution (20, 21). Aequorin luminescence was monitored using a light-tight enclosure for the organ bath and photomultiplier tube (9635QA; Thorn-EMI Inc. Clinton, CT). Anodal current output from the tube (in nA) and isometric force were simultaneously recorded on a pen recorder and stored on magnetic tape with a data recorder (model 3964A; Hewlett-Packard Co., Palo Alto, CA). To estimate intracellular Ca^{++} ([Ca^{++}]$_i$) levels, aequorin luminescence was calibrated by the method of Allen and Blinks (28). At the end of each experiment, distilled water containing 10 mM $CaCl_2$ and 2% Triton X-100 was added to the organ bath to lyse the muscle membranes, and the light signal was recorded. L_{max} for each muscle was then calculated by multiplying the integrated light signal by the peak to integral ratio (2.5 at 37.5°C). After subtraction of dark current, aequorin luminescence (L) at experimental points of interest was expressed as the ratio of L/L_{max}. The ratio was then converted to [Ca^{++}]$_i$ using a standard calibration curve for log L/L_{max} assuming an intracellular [Mg^{++}] of 0.5 mM (for details see reference 28).

Drugs and chemicals. Cocaine HCl (Mallinckrodt Inc., St. Louis, MO), prazosin, atropine, norepinephrine, and tyramine (Sigma Chemical Co., St. Louis, MO) were used in these experiments.

Data analysis. Results are reported as mean±SEM. Unless otherwise noted, smooth muscle responses to agonists are expressed as a percentage of maximal tension evoked by 60 mM KCl. The EC_{50} (effec-

tive concentration of agonist required to cause 50% of the maximal contractile response to the agonist) was calculated using a computerized nonlinear equation method (29). Statistical analysis of data was performed by Student's t test for paired or unpaired observations. When more than three data points or dose-response relationships were compared, analysis of variance and multiple comparison tests were used to identify differences among groups. A probability of less than 5% was considered statistically significant.

Results

Effects of endothelial cells. Contractile responses to cocaine were examined in muscles with ($n = 8$) or without endothelial cells ($n = 8$). The functional integrity of the endothelium was tested by the presence of endothelium-dependent relaxation to carbachol in a concentration of 10^{-7} M in muscles precontracted with norepinephrine 10^{-7} M; 62±8% relaxation and 10±4% contraction occurred in muscles with and without endothelium, respectively. As shown in Table I, a contractile response to cocaine in muscles with intact endothelium did not differ from those without endothelium. Therefore, in the following experiments, muscles without endothelial cells were used.

Effects of reserpine pretreatment. To examine the degree to which cocaine's indirect actions on adrenergic nerve terminals contributes to changes in smooth muscle tone, the effects of cocaine on control muscles were compared with those in muscles from animals pretreated for 24 h with reserpine (Fig. 1, Table I). There was no statistically significant difference in rest-

Table I. Smooth Muscle Tone in Control Muscles with or without Endothelial Cells and Muscles from Reserpine Pretreated Ferrets

	Control muscles		Reserpine pretreated muscles
	E (+)	E (−)	
Resting tension (*g*)	2.1±0.2	1.9±0.1	2.1±0.1
(*g/mm²*)	3.6±0.4	3.4±0.3	3.8±0.4
	($n = 8$)	($n = 18$)	($n = 18$)
Contraction by 60 mM			
KCl (*g*)	3.1±0.4	3.3±0.2	3.6±0.4
(*g/mm²*)	4.2±0.5	4.4±0.5	5.5±0.8
	($n = 8$)	($n = 18$)	($n = 18$)
Contraction induced by tyramine			
10^{-5} M (percentage of 60	55±5	64±5	9±3*
mM KCl contraction)	($n = 6$)	($n = 7$)	($n = 8$)
Contraction induced by cocaine (percentage of 60 mM KCl contraction)			
10^{-5} M cocaine	33±4	38±6	6±1*
10^{-4} M cocaine	60±4	63±8	16±4*
10^{-3} M cocaine	51±6	55±9	6±4*
3×10^{-3} M cocaine	14±6	21±11	3±6
	($n = 8$)	($n = 8$)	($n = 10$)

Data are presented as mean±SEM and were obtained from at least six animals in each group. Numbers in parentheses are the numbers of tissues tested. The force (F) per cross-sectional area (CSA) was calculated as follows: F/CSA = tension × tissue density (1.05) × optimal length/tissue weight. E (+), endothelial cells were preserved; E (−), endothelial cells were abraded. * $P < 0.01$ versus the control group.

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1. *Abbreviations used in this paper:* [Ca^{++}]$_i$, intracellular calcium; EC, effective concentration; L, aequorin luminescence.
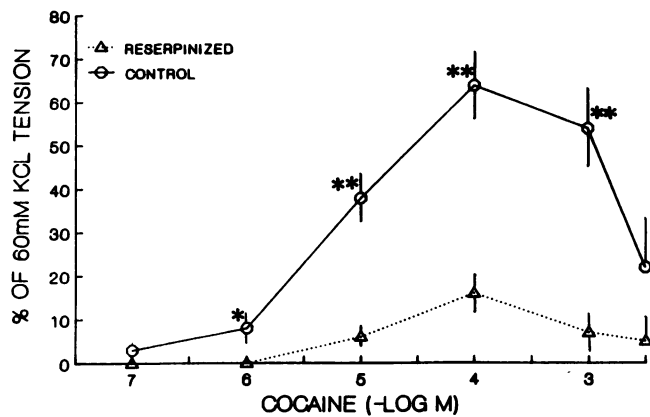
Figure 1. Dose-response relationship in control muscles (*open circles*) and muscles from ferrets retreated with reserpine (*open triangles*). Cocaine in concentrations from 10^{-7} M to 10^{-4} M caused dose-dependent contraction and cocaine in concentrations $\geq 10^{-3}$ M produced relaxation. The constrictive responses of control muscles were significantly larger than those of muscles from reserpinized animals. (*$P < 0.05$ and **$P < 0.01$ vs. reserpine pretreatment; values, mean±SE).

ing tension or in the amplitude of contraction produced by 60 mM KCl in these two groups. Contractions of muscles from reserpine pretreated ferrets in response to 10^{-5} M tyramines were significantly smaller than those from control animals (Table I), indicating that the catecholamine content had been significantly depleted in tissues from the reserpine pretreated animals. As shown in Fig. 1, cumulative doses of cocaine produced a dual action on tension development in control muscles: dose-dependent contraction at concentrations from 10^{-7} to 10^{-4} M followed by a marked relaxation at higher concentrations $\geq 10^{-3}$ M. The contractile response of muscles from reserpine pretreated animals was markedly decreased compared with that of control muscles.

Effects of prazosin and atropine on cocaine-induced contraction. Due to the slow and incomplete washout of high cocaine concentrations $\geq 10^{-3}$ M, it was difficult to obtain complete dose-response curves in experiments designed to investigate the mechanism of cocaine's action. For this reason, results shown in Table II are derived from preparations in which cocaine concentrations $\leq 10^{-4}$ M were tested before and after vehicle, 10^{-7} M prazosin (eight muscles from six ferrets) or 10^{-7} M atropine (seven from six ferrets). Preliminary experiments in ferret aorta confirmed that prazosin and atropine in these concentrations inhibited 10^{-7} M norepinephrine and 10^{-7} M carbachol-induced contractions by more than 50%. Table II shows that cocaine-induced contraction was significantly inhibited by prazosin while the contractile response was not affected by vehicle or atropine.

Relationship between intracellular Ca^{++} and muscle tone. The $[Ca^{++}]_i$ change during cocaine-induced contraction was examined (Fig. 2, Table II). A typical recording of tension and $[Ca^{++}]_i$ with aequorin after the addition of 10^{-4} M cocaine is shown in Fig. 2. Cocaine 10^{-4} M caused a sustained contraction that reached a plateau in 16±3 min and $[Ca^{++}]_i$ rose slowly in conjunction with tension development. The amplitude of the contraction induced by 10^{-4} M cocaine in aequorin loaded preparations was 55±6% of that produced by 60 mM KCl ($n = 7$), the extent of which was comparable with that in control

muscles that had not undergone the aequorin loading procedure (63±8%). Estimated intracellular Ca^{++} levels rose from 200±10 nM under control conditions to 220±5 nM ($n = 7$; $P < 0.05$ vs. control) after cocaine. Experiments with two successive administrations of cocaine 10^{-4} M at an interval of 60 min produced the same effects on the tension and light responses ($n = 6$). As shown in Table III, both the cocaine-induced contraction and the increase in aequorin luminescence were significantly inhibited after treatment with prazosin 10^{-7} M ($n = 7$).

The mechanism of cocaine-induced vasorelaxation was investigated, as shown in Fig. 3. Increasing concentrations of cocaine to 10^{-3} M and 3×10^{-3} M resulted in a marked vasorelaxation, while aequorin luminescence levels remained elevated around the level induced by 10^{-4} M cocaine (Table IV). Beta-adrenoceptor blockade with propranolol 10^{-7} M that was administered 2 to 3 min before cocaine 10^{-3} M did not affect the relaxation induced by the high concentrations of cocaine. In preliminary studies, we found that cocaine in concentrations $\geq 10^{-3}$ M inhibited the aequorin luminescence reaction in vitro. In addition, it is known that the cell membrane is permeable to local anesthetic agents like cocaine (30). For these reasons, quantitative analysis of $[Ca^{++}]_i$ was not performed in experiments where cocaine concentrations $\geq 10^{-3}$ M were used, since this might lead to significant underestimation of the actual $[Ca^{++}]_i$ levels. It is safe to say, however, that $[Ca^{++}]_i$ after such high concentrations of cocaine increased above the level produced by 10^{-4} M cocaine.

Effects of cocaine on norepinephrine responses. The dose-response relationship to norepinephrine was obtained in eight muscles from six control ferrets (Fig. 4, left) and six from six ferrets that had undergone reserpine pretreatment (Fig. 4, right). In control muscles, the level of contraction induced by norepinephrine in concentrations from 10^{-8} M to 10^{-6} M was enhanced after 10^{-6} M cocaine; $-\log (EC_{50})$ was significantly ($P < 0.01$) increased from 7.3±0.2 to 7.8±0.4 by 10^{-6} M cocaine. This low concentration of cocaine alone produced less than 10% of the 60-mM KCl contraction (Fig. 1). Therefore, the dose-response curve of norepinephrine was shifted to the left in a parallel manner by the synergistic interaction between cocaine and norepinephrine. No such supersensitivity to nor-

Table II. Effects of Prazosin and Atropine Pretreatments on Cocaine-induced Contraction

	Concentrations of cocaine (M) (percentage of 60 mM KCl contraction)			
	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Prazosin pretreatment ($n = 8$)				
Before	4±1	6±2	39±6	68±6
After prazosin 10^{-7} M	0	0	7±2*	18±3*
Atropine pretreatment ($n = 7$)				
Before	4±0	8±2	35±6	68±6
After atropine 10^{-7} M	2±0	4±1	31±6	62±4
No pretreatment ($n = 6$)				
Before	2±1	4±1	44±6	72±5
After	1±1	2±1	40±5	66±6

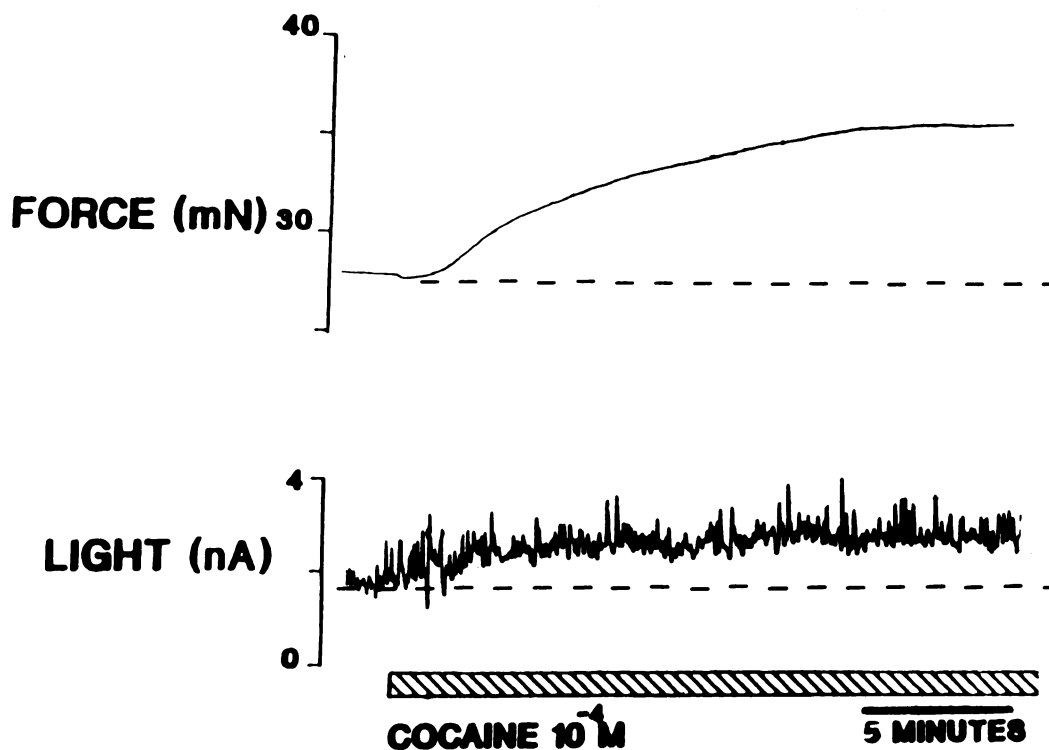Data are presented as mean±SEM. * $P < 0.01$ vs. before pretreatment.

Figure 2. A representative tracing of force development (upper) and aequorin luminescence (lower noisy trace) after addition of cocaine 10^{-4} M in aequorin loaded preparation. Note that, in response to cocaine, the light level increased in conjunction with force (see Table III for summary data).

epinephrine was observed in muscles from reserpine pretreated ferrets.

We also studied the frequency-response relationship of transmural nerve stimulation. As presented in Table V, the contractile force of eight muscles from six control ferrets in response to transmural nerve stimulation increased in a frequency-dependent manner. Contractions in response to 0.1, 1, and 4 Hz stimulation frequencies were significantly potentiated after treatment with 10^{-6} M cocaine, while those in response to an 8 Hz stimulation frequency were not significantly increased. In six muscles from six reserpine pretreated ferrets, responses to transmural nerve stimulation were not significantly changed after 10^{-6} cocaine.

Discussion

This study was performed because the mechanism of action of cocaine has not been fully delineated in vascular smooth mus-

Table III. Relationship of $[Ca^{++}]_i$ and Force during Cocaine-induced Contraction in the Presence and Absence of Prazosin (n = 7)

	Cocaine 10^{-4} M		Cocaine 10^{-4} M after pretreatment with prazosin 10^{-7} M	
	Before	After	Before	After
Force (g/mm^2)	3.2±0.6	5.1±1.4‡	3.0±0.6	3.3±0.8
Light (nA)	1.5±0.3	2.6±0.4*	1.6±0.2	1.7±0.3
Estimated $[Ca^{++}]_i$ (nM)	200±10	220±5*	190±10	200±10

Data are presented as mean±SEM. * $P < 0.05$, ‡ $P < 0.01$ vs. before cocaine.

cle. The major findings of this study are as follows: first, cocaine-induced alterations in vascular tone do not differ in muscles with or without endothelial cells; second, the contractile response of control muscles to cocaine in concentrations $\leq 10^{-4}$ M was significantly larger than that of muscles from reserpine pretreated ferrets in which the catecholamine content of the adrenergic nerve endings was depleted. The cocaine-induced contraction and the consequent $[Ca^{++}]_i$ rise were inhibited after prazosin pretreatment; third, relaxation produced by more than 10^{-3} M cocaine was not associated with a decrease in $[Ca^{++}]_i$; and fourth, cocaine-induced supersensitivity to exogenous and endogenous norepinephrine did not occur in muscles from animals receiving short term pretreatment with reserpine.

It is well recognized that the endothelium plays an important role in the regulation of vascular smooth muscle tone (31). This study, however, indicates that the cocaine-induced contraction was not affected by the presence or absence of the endothelium, indicating that cocaine-induced contraction is not dependent upon the endothelial factor(s). This result is in agreement with a preliminary study in rabbit aortic smooth muscle (32).

Results of this study are compatible with studies in isolated rat tail arteries by Webb et al. (12, 13), which indicated that contraction induced by cocaine 10^{-4} M was blocked after pretreatment with an alpha adrenergic antagonist, phentolamine, 10^{-5} M, and after chemical denervation with 6-hydroxydopamine. Analysis of contractile responses of control muscles and muscles from reserpine pretreated animals (Fig. 1, Table I) suggests that the contractile effects of cocaine in concentrations $\leq 10^{-4}$ M may be due to its action on adrenergic nerve endings, whereas the relaxant effects of higher concentrations may be due to its direct actions on smooth muscle. The cocaine-induced contraction was inhibited by prazosin, but not by atropine pretreatment. Therefore, it is reasonable to propose that
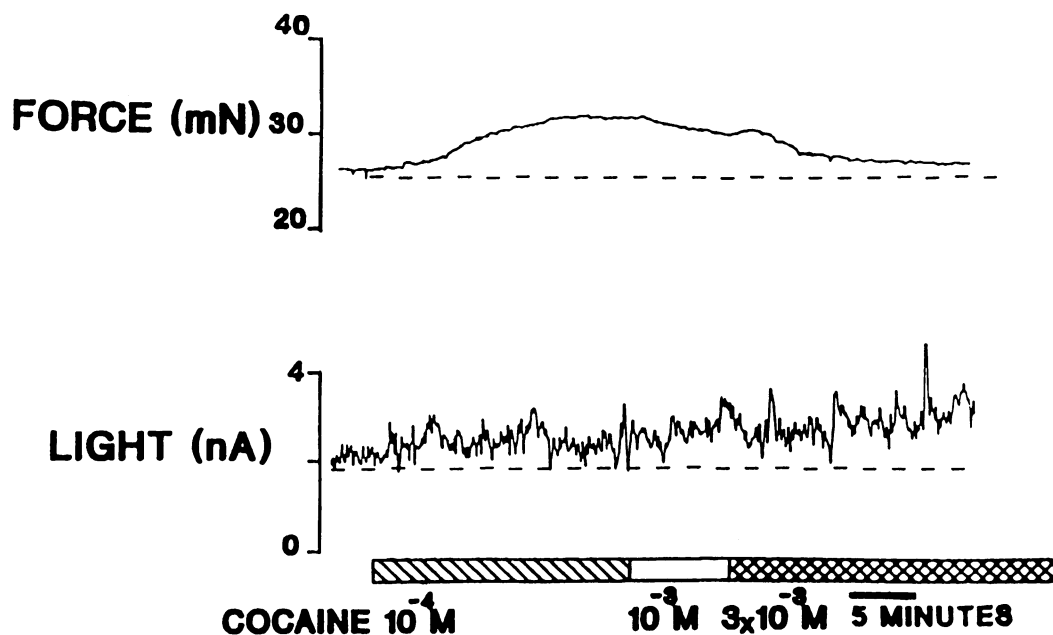
Mechanism of Cocaine-induced Vascular Contraction **1325**

Figure 3. A representative tracing of force (upper) and aequorin luminescence (lower noisy trace) during cumulative administration of cocaine from 10^{-4} M to 3×10^{-3} M. Administration of 10^{-3} M and 3×10^{-3} M cocaine resulted in vasorelaxation, while the light signal remained elevated (see Table IV for summary data).

cocaine in concentrations $\leq 10^{-4}$ M caused a contractile response by its presynaptic effects and subsequent alpha-1 adrenoceptor stimulation. We speculate that endogenous norepinephrine released from adrenergic nerve endings played a major role in producing the cocaine-induced contraction, because it has been demonstrated that cocaine increases norepinephrine overflow from perfused vascular tissues and inhibits reuptake into the adrenergic nerve endings (10, 11). Some studies, however, suggest that cocaine-induced contraction can be produced by other mechanisms: (a) cocaine increases influx of calcium across the sarcolemma by changing membrane permeability (33, 34); and (b) cocaine produces responses by augmenting postsynaptic alpha-adrenoceptor function (16, 35, 36). Thus, we studied $[Ca^{++}]_i$ mobilization during cocaine-induced contraction as well as the effects of the drug on norepinephrine's dose-response relationships.

$[Ca^{++}]_i$ handling during cocaine-induced changes in smooth muscle tone has not been reported to date. In this study, cocaine-induced contraction developed in parallel with the rise in $[Ca^{++}]_i$, which was blocked by prazosin pretreat-

ment. Thus, it appears reasonable to conclude that the $[Ca^{++}]_i$ mobilization during the cocaine-induced contraction is mediated by alpha-1 adrenoceptors rather than by nonspecific diffusion of the Ca^{++} into the cytoplasm. We have previously reported that alpha-1 adrenoceptor activation produced a transient increase in the $[Ca^{++}]_i$ level during force development followed by a lower plateau level during the period of force maintenance in ferret vascular smooth muscle (18, 19, 37). The absence of the initial Ca^{++} transient during cocaine-induced contraction may be explained as follows: first, the intensity of alpha adrenoceptor activation exerted by cocaine was less than maximal, because the amplitude of the cocaine-induced contraction was $\sim 50\%$ of the response to 10^{-5} M norepinephrine in our study. Ruzycky and Morgan (37) have recently shown that the transient $[Ca^{++}]_i$ spike associated with phenylephrine-induced contractions was most pronounced at the maximally effective concentrations of this agonist; and second, local anesthetic agents like cocaine are known to affect $[Ca^{++}]_i$ handling by the sarcolemma (38, 39) and sarcoplasmic reticulum (40, 41); these actions might modify $[Ca^{++}]_i$.

The mechanism by which cocaine decreases tension from the previous elevated level (i.e., relaxation) has not been extensively studied. According to experiments in other organs, cellular actions of local anesthetic agents like cocaine involve (a) reduction of the rate of rise and amplitude of the action poten-
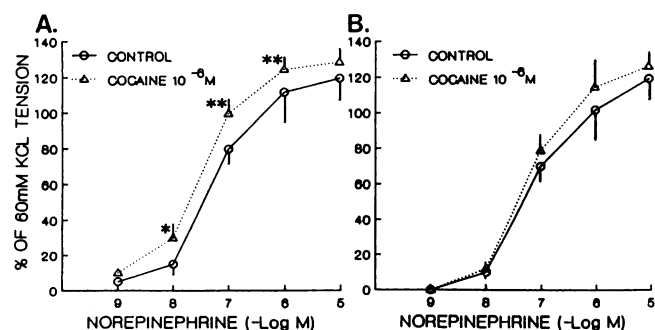

Figure 4. Norepinephrine dose-response relationship before (open circles-solid line) and after cocaine 10^{-6} M (open triangles-dashed line) in control muscles (A: left) versus muscles from animals pretreated with reserpine (B: right). *$P < 0.05$ and **$P < 0.01$ vs. values before cocaine).

Table IV. Relationship of Force and Aequorin Luminescence after Cumulative Addition of Cocaine (n = 7)

| | | Cocaine (M) | | |
	Control	10^{-4}	10^{-3}	3×10^{-3}
Force (g/mm^2)	3.0±0.5	5.2±1.3*	4.8±1.2*	3.4±0.6
Light (nA)	1.8±0.4	2.7±0.2*	2.6±0.2*	2.8±0.3*

Data are presented as mean±SEM. * $P < 0.01$ vs. control condition.

Table V. Effects of Electrical Transmural Stimulation on Contractile Responses of Muscles from Control Ferrets and from Reserpine Pretreated Ferrets

	Frequency (Hz) of Stimulation (percentage of 60 mM KCl contraction)			
	0.1	1.0	4.0	8.0
Muscles from control ferrets ($n = 8$)				
Before cocaine	8±2	38±6	68±8	74±8
After cocaine	16±2*	78±10‡	80±14‡	82±12
Muscles from reserpine pretreated ferrets ($n = 6$)				
Before cocaine	0±1	8±3	18±6	15±6
After cocaine	0	6±1	24±4	22±5

Data are presented as mean±SEM. * $P < 0.05$, ‡ $P < 0.01$ vs. before cocaine.

tial with no appreciable change in resting membrane potentials (38, 39, 42); (*b*) reduction in ion influx across the sarcolemma (38, 39); and (*c*) decrease in Ca^{++} release and/or extrusion by the sarcoplasmic reticulum (40, 43, 44). If a decrease in tension induced by high concentrations of cocaine is due to any of these effects, $[Ca^{++}]_i$ will decline in parallel with tension. In this study, however, $[Ca^{++}]_i$ remained elevated despite a decrease in tension. Therefore, it is clear that the mechanism of cocaine-induced decrease in tension cannot be explained by any of the possible mechanisms mentioned above. Alternatively, it appears reasonable to propose that the Ca^{++} sensitivity of the contractile proteins or myofilaments decreased after cocaine. Since preliminary studies indicated that cocaine in concentrations ≥ 10^{-3} M suppresses the Ca^{++}-aequorin luminescence relationship, it was difficult to pursue the subcellular mechanism of the $[Ca^{++}]_i$ changes during the cocaine-induced tension decrease. Although Kuriyama and Suyama (16) reported that the Ca^{++}-force relationship was unchanged by cocaine 10^{-4} M in saponin skinned guinea pig mesentery artery, the concentrations employed by these investigators were lower than those causing vasorelaxation in this study. A recent study from our laboratory has shown that negative inotropic actions of cocaine in myocardial tissues correlate with $[Ca^{++}]_i$ levels (45). Thus, the mechanisms by which high concentrations of cocaine reduces contractile force may differ between cardiac and smooth muscles.

Provided that cocaine-induced supersensitivity to norepinephrine is due solely to cocaine's action on adrenergic nerve endings, it will be lost when the amount of catecholamine in the nerve endings is decreased. This study shows that muscles from ferrets that have undergone reserpine pretreatment failed to respond to tyramine (Table I), and that cocaine did not induce supersensitivity to norepinephrine or adrenergic nerve stimulation (Fig. 4) in muscles from ferrets that underwent reserpine pretreatment. Therefore, we may conclude that cocaine-induced supersensitivity to exogenous and endogenous norepinephrine was presumably produced by its action on the adrenergic nerve endings. These results may confirm a study by Webb et al. (13) that cocaine-induced supersensitivity to norepinephrine did not occur after pretreatment with 6-hydroxydopamine.

These findings are strengthened by clinical reports that human coronary arteries respond to alpha adrenoceptor stimulation by constriction (46, 47). Moreover, cocaine concentrations that caused contraction of aortic smooth muscles of the ferret (10^{-6} M to 10^{-4} M) were fairly comparable with the plasma concentrations reported in cocaine abusers and in cocaine-related deaths. Plasma cocaine levels in those patients is reported to be 0.2–100 μg/ml (6×10^{-7} to 3×10^{-4} M) (48–50). However, there are obvious difficulties in comparing the results of our study with clinical reports of cocaine-induced cardiovascular events, because there appear to be marked differences among vascular beds of various species in regard to their responsiveness to cocaine (5). This is due to differences in receptor types and density among vascular tissues from different species. Despite these limitations, we believe that this study sheds light on the mechanisms underlying cocaine-induced changes in vascular tone and provides experimental clues that at least partially elucidate the pathophysiology of cocaine-related cardiovascular complications.

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