Cocaine Blockade of the Acetylcholine Positive Inotropic Response in Ventricular Myocardium (35038)

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Acetylcholine (ACh) in high ("nicotinic") doses produces a catecholamine-dependent positive inotropic response in mammalian ventricular myocardium (1–3). The role of norepinephrine (NE) in mediating this response has been suggested. However, in slowly beating cat papillary muscles (4) and in 4-day-old chick embryo hearts (5) there is a positive inotropic response to ACh which appears to be catecholamine-independent.

Cocaine has two different effects on NE metabolism. First, it produces supersensitivity to NE by blocking its uptake into the adrenergic nerve terminals, and consequently blocking the inactivation of NE (6, 7). Second, cocaine blocks the release of NE by indirect acting amines, *e.g.*, tyramine (8, 9). It was felt that cocaine should be of value in further investigating the role of NE in the positive intropic response of ACh. If NE is released by ACh, then the positive inotropic response mediated by NE might be enhanced by cocaine. However, if ACh releases NE by a similar mechanism to that of tyramine release of NE, then the positive inotropic response might be reduced by cocaine. It is also possible that the measured response would be the result of a combination of the two effects.

Methods. Eighteen adult male cats were anesthetized with sodium thiamylal and the heart was quickly removed and mounted on a cannula. Retrograde perfusion of the aorta was carried out with a modified Krebs–Ringer solution of the following composition. Na^+, 146 m*M*; K^+, 3.6 m*M*; Cl^-, 128 m*M*; Ca^{2+}, 2.5 m*M*; $H_2PO_4^-$, 1.2 m*M*; SO_4^{2-}, 1.2 m*M*; Mg^{2+}, 1.2 m*M*; HCO_3^-, 25 m*M*; and glucose, 5.6 m*M*. The pH was 7.4 and the temperature was 34 ± 0.5°. The solution was oxygenated with 95% O_2±5% CO_2. The fluid was not recirculated.

Mean perfusion pressure was monitored with a Statham P23Db transducer through a side arm on the perfusion cannula. The perfusion pressure was maintained between 25 and 30 mm Hg by adjusting the perfusate flow. Heart rate was maintained constant throughout the experiment at approximately 100 beats/min by electrically pacing the ventricle at voltages slightly above threshold with an American Electronics Laboratory model 104-A stimulator. The ventricular electrode was a fine platinum wire placed at the base of the left ventricle.

Isovolumic left ventricular pressure was monitored using a small latex rubber balloon mounted on an 18-gauge metal cannula and inserted into the left ventricle through a stab wound near the apex. The balloon was secured in the ventricle with a purse string suture. The balloon was filled with a small amount of water (~0.75 ml). It has been shown that, over a wide range, variations in balloon pressure were not a determinant of the magnitude of the responses observed (10). The pressure developed in the balloon was measured with a Statham P23Db transducer. The first derivative of the pressure (dp/dt) was obtained by an active electronic differentiator with a time constant of 1 msec. The differentiator output (dp/dt), the isovolumic left ventricular pressure and the perfusion pressure were recorded on a direct writing oscillograph. Drugs were introduced into the perfusion cannula 10 cm above the coronary ostia. The volumes of the injected drugs were less than 0.5 ml.

Maximum left ventricular isovolumic pressure and dp/dt were used as indices of contractile force. The maximum positive inotropic response of the individual preparations was

TABLE I. Response to Tyramine (10^{-5} g) Before and After Cocaine Pretreatment.

	Pressure[a]	dp/dt[a]
Control	82 ± 7.1[b]	79 ± 5.3
Cocaine 5 × 10^{-6} g/ml	35 ± 6.3	36 ± 4.1

[a] % Maximum response (see text).
[b] SD.

elicited during the experiments by calcium or NE. The response to ACh was expressed as a percentage of the maximum response of the preparation. Groups means were compared with Student's t test and the significance of the differences was expressed as p values.

Three groups of 6 cats each were used. Conditions were identical except in the two experimental groups cocaine hydrochloride in a concentration of 5 × 10^{-8} or 5 × 10^{-6} g/ml was added to the perfusate. Other drugs used were acetylcholine chloride, atropine sulfate, *l*-norepinephrine bitartrate monohydrate, tyramine hydrochloride, lidocaine hydrochloride, and calcium chloride. Solutions were mixed in normal saline, pH adjusted to 6.5 with HCl, and kept iced during the experiment. Doses were expressed as the salt.

Results. Effects of cocaine. When cocaine 5 × 10^{-6} or 5 × 10^{-8} g/ml was in the perfusate, no changes in the indices of contractile force occurred. Cocaine 5 × 10^{-8} g/ml disminished the response of the preparation to tyramine 10^{-5} g, but did not change the NE dose response curve. Cocaine 5 × 10^{-6} g/ml blocked the response of the preparation to tyramine (Table I). This dose has also been shown to cause a shift of the NE dose–response curve to the left in this same system (10).

Response to ACh. ACh (5 × 10^{-5} and 10^{-4} g) produced an initial negative and then a large positive inotropic response in this preparation as previously reported (1). This positive inotropic response was reduced 40% by cocaine (5 × 10^{-8} g/ml) (Table II), and blocked by cocaine 5 × 10^{-6} g/ml (Table II and Fig. 1). The magnitude of the positive response to large doses of ACh decreased with subsequent doses of ACh, therefore cocaine was initially added to the perfusate and the data represent the response to the first dose of ACh given. The diminished positive response to subsequent doses of ACh could be blocked if the preparation was pretreated with atropine 8 × 10^{-6} g. Table I shows the increased positive inotropic responses in the atropine pretreated preparations and the decreases in the positive responses produced by cocaine.

Late positive inotropic response. Figure 2 demonstrates that although the large initial positive inotropic response to ACh 5 × 10^{-5} g is blocked by cocaine, there is a smaller but definite positive inotropic response which occurred in each of the 12 cocaine pretreated preparations. This late positive inotropic response occurred after 60 to 90 sec as opposed to the positive inotropic response which occurred in the control preparations in 5 to 10 sec. The late response was absent in the 6 preparations not pretreated with cocaine. It should also be noted that two hearts which

TABLE II. Response to Acetylcholine (10^{-4} g) Before and After Cocaine Pretreatment.

	Pressure[a]		dp/dt[a]	
Control	38. ± 16[b]		45. ± 12	
Cocaine 5 × 10^{-8} g/ml	16. ± 6	$p \leq 0.10$	18. ± 8	$p \leq 0.01$
5 × 10^{-6} g/ml	0.04 ±	$p \leq 0.001$	0.04 ±	$p \leq 0.001$
After atropine				
Control	72 ± 10		76 ± 12	
Cocaine 5 × 10^{-8} g/ml	58 ± 7	$p \leq 0.01$	56 ± 8	$p \leq 0.01$
5 × 10^{-6} g/ml	15 ± 4	$p \leq 0.001$	19 ± 5	$p \leq 0.001$

[a] % Maximum response (see text).
[b] SD.

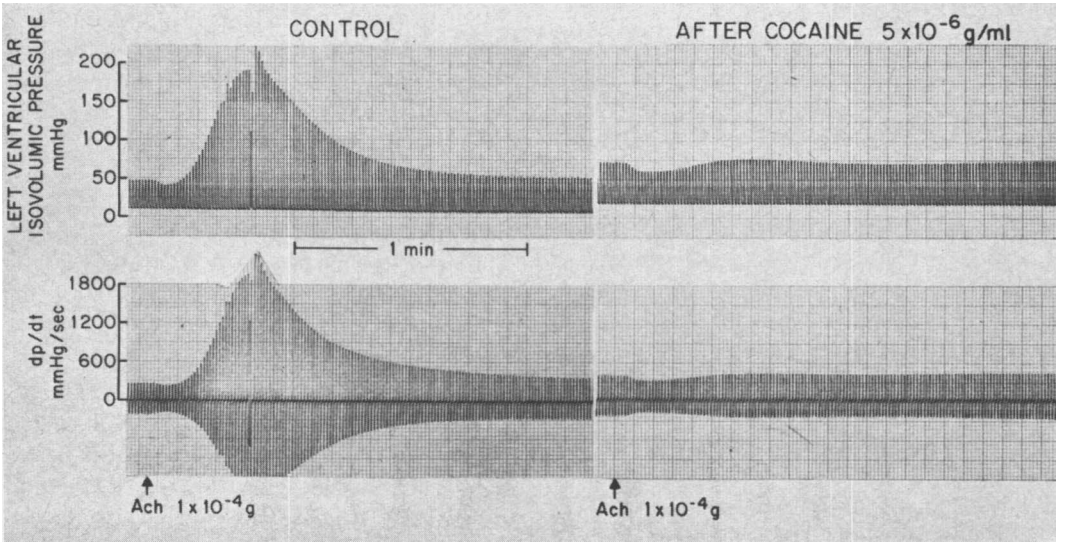

FIG. 1. The effect of cocaine 5×10^{-6} g/ml in the perfusate on the positive inotropic response to acetylcholine 10^{-4} g. The initial negative response to acetylcholine is enhanced by the cocaine pretreatment.

had been depleted of catecholamines by chronic cardiac denervation showed no late positive response either before or after cocaine administration.

Anesthetic properties of cocaine. Lidocaine 10^{-5} and 10^{-4} g/ml, used in these experiments as a model of a local anesthetic, was ineffective in blocking the positive response to ACh.

Discussion. The positive inotropic response of the isolated cat heart to ACh is diminished by cocaine in a dose-dependent manner. Cocaine blocks the release of norepinephrine by indirect acting (tyramine-like) sympathomimetic amines (8, 9). The positive inotropic response to ACh in this preparation has been shown to be dependent on intact catecholamine stores (1). Furthermore it is blocked by curare (1). It appears, therefore, that ACh stimulates nicotinic receptors which releases norepinephrine in a manner similar to that of tyramine. This sequence seems to be the mechanism responsible for the positive inotropic response to doses of ACh in the range of 10^{-5} to 10^{-4} g in this preparation. Lee and Shideman (5) demonstrated that cocaine 3.33×10^{-5} g/ml blocked the positive inotropic action of a ganglionic stimulator, tetraethylammonium, in atropine-pretreated cat papillary muscles. This appears to be similar to the cocaine blockade of the positive response to ACh.

After atropine pretreatment, the positve inotropic response to ACh is approximately doubled. This heightened response is still attenuated by cocaine. The enhanced response is probably due to atropine blockade of the muscarinic receptors which, when stimulated, produce a negative inotropic response. Initial negative inotropic responses have been described earlier (1) and are evident in Fig. 1. The negative response is enhanced when the positive inotropic response is blocked by cocaine (Fig. 1).

The late positive inotropic response occurred in all cocaine-pretreated preparations but in none of the control preparations. The late response was probably due to the fact that, despite the cocaine, some NE was released following ACh administration. As the muscarinic effect (the negative inotropic effect of ACh) begins to wane, the positive inotropic effect of the released NE becomes unmasked, as cocaine has also blocked the inactivation of NE. It has previously been shown that catecholamine-depleted hearts do not manifest a positive inotropic response to ACh (1). The hypothesis that the late posi-

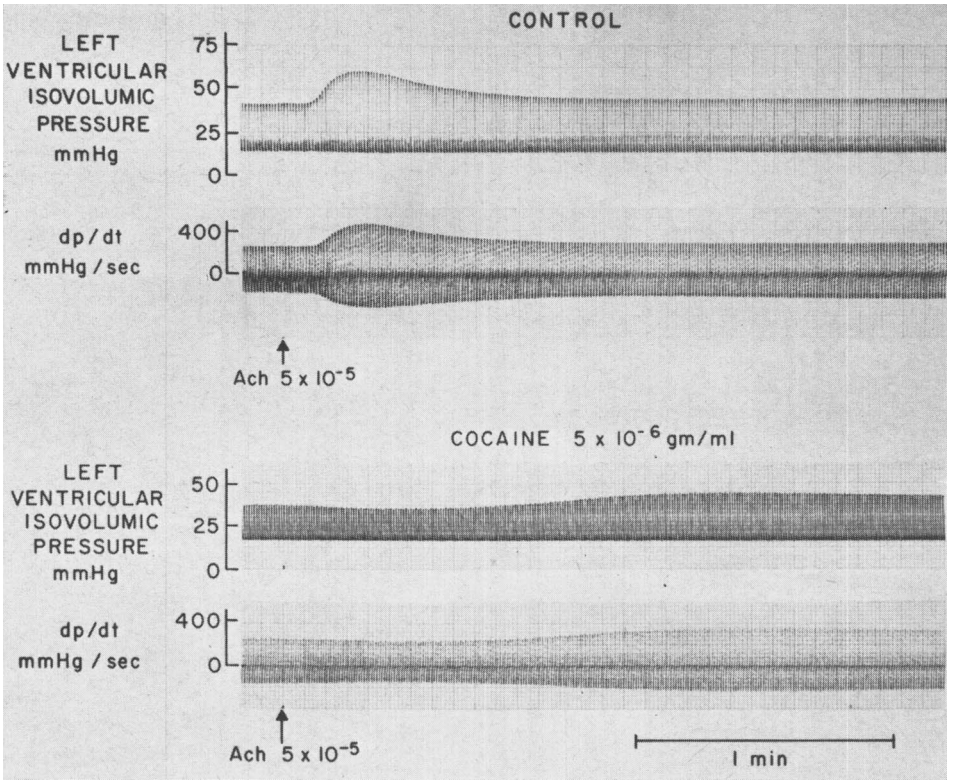

FIG. 2. A late positive inotropic response in the cocaine pretreated preparations, not seen in the control preparations, is seen here 60 to 90 sec after the injection of ACh (see text for discussion).

tive response to ACh is dependent upon catecholamines is borne out by the fact that it could not be elicited in the denervated heart either before or after cocaine. Hollenberg *et al.* (11), using a different preparation, have reported positive inotropic responses in ventricular myocardium after infusions of ACh were ended. These responses are probably the effects of catecholamines which become manifest as the negative effects of ACh diminish.

Cocaine has local anesthetic properties and to demonstrate that its blockade of ACh was not a nonspecific effect, lidocaine was used in this preparation instead of cocaine. Lidocaine does not block the positive inotropic response of the preparation to ACh 10^{-4}.

The blockade by cocaine of the positive inotropic response to ACh in the isolated cat heart preparation is further evidence that, in this preparation, the response is mediated by the release of norepinephrine.

Summary. In isolated cat heart preparations, ACh in doses of 10^{-4} g elicits a positive inotropic response. This response is blocked by cocaine. It appears that the blockade of the response by cocaine is similar to cocaine blockade of the response to indirect acting amines, *e.g.*, tyramine. This is further evidence that the positive inotropic effect of ACh in ventricular myocardium is mediated by NE release.

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