The Effect of Cocaine on the Relative Chronotropic Potencies of Sympathomimetics[1] (36778)

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Furchgott (1) detailed the conditions which should be satisfied for an ideal comparison of the relative potencies of directly acting adrenergic agonists such as norepinephrine (NE), epinephrine (Epi) and isoproterenol (Iso). These included the following: (a) The processes other than diffusion which remove any of the agonists from the region of receptors should be blocked. (b) The capacity of other types of receptors in the effector system to be activated by the agonists should be eliminated or made negligible.

In cardiac muscle a principle process for removal of NE and Epi is "uptake" into adrenergic nerve terminals (2). Iso, on the other hand, is not readily taken up into the nerve endings (3). If the uptake process is intact the relative inotropic potencies are Iso $>$ NE $=$ Epi (1, 4, 5). In the presence of cocaine, a drug known to interfere with the neuronal uptake system, the relative inotropic potencies are Iso $\geqq$ NE $>$ Epi (1).

At the time of Furchgott's study there was no evidence for a role for alpha receptors in the response of cardiac muscle to adrenergic agonists, and thus the contribution of alpha receptors was considered to be negligible. Recently, however, Parr and Urquilla (6) have provided evidence that in addition to beta receptors subserving inotropic effects, alpha receptors also play a role in this action. On the other hand, these investigators found no evidence for the role of alpha receptors in the chronotropic response to sympathomimetics. It is possible, therefore, that the role of alpha receptors in inotropic responses may give rise to a different value for relative potencies than when a response is measured in which alpha receptors play no part.

The purpose of this investigation was to examine the relative chronotropic potencies of NE, Epi and Iso, the role of alpha receptors being negligible, in guinea pig atrial preparations in the absence and presence of cocaine.

Materials and Methods. The guinea pigs used in this study were of either sex and ranged in weight from 300 to 600 g. Some animals were injected ip with 1.0 mg/kg of reserpine (Serapsil, Ciba) 24 hr before sacrifice. This dose schedule of reserpine depletes myocardial NE to less than 5% of normal but does not alter the sensitivity of the hearts to stimulants (7, 8).

The animals were killed by a blow on the head, the chest was opened and the right atrium removed according to previously described methods (8, 9). The atria were set up in an isolated organ bath of 30 ml capacity and bathed in a physiological salt solution of the following composition (m*M*): NaCl, 113; KCl, 4.8; $CaCl_2$, 2.5; KH_2SO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25; glucose, 5.5. The solution was continually bubbled with 95% O_2 and 5% CO_2. Bath temperature was 37°. The spontaneous beat of the preparation was recorded with a Grass force-displacement transducer and model 5D polygraph. Two grams of tension were applied to the muscle. Full dose–response curves were obtained by a stepwise increase in the total concentration of drug in the bath. When the response of the pacemaker to the agonist reached a steady level the next dose was given. Only one dose–response curve was obtained with each preparation.

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TABLE I. The Effect of Cocaine (10^{-5} *M*) on the Spontaneous Rate of the Isolated Right Atrium from Untreated and Reserpine-Treated Guinea Pigs.

Pretreatment	Group	Mean rate (beats/min)[a]	*N*[b]
None	Control	198 (±4)	15
	Cocaine	190 (±6)	
Reserpine (1 mg/kg)	Control	189 (±5)	18
	Cocaine	168[c] (±4)	

[a] Standard error of the mean in parentheses.
[b] Number of experiments.
[c] Significantly different from control $p < .01$.

Before the chronotropic response to drugs was tested, the preparations were allowed to equilibrate for 1 hr. In those experiments in which cocaine was used, the drug was added to the bathing solution during the last 20 min of the equilibration period and remained in the solution throughout the construction of the dose–response curve. The concentration of cocaine was 10^{-5} *M*.

Comparisons of the potencies of the three agonists were made at the level of the effective dose producing 50% maximum response (ED_{50}). The potency of NE was set as unity. The relative potencies of the other two agonists were obtained by dividing the geometric mean ED_{50} of NE by the geometric mean ED_{50} of Epi and Iso. Ratios larger than 1 indicate a greater potency while numbers less than 1 indicate lesser potency than NE. The relative potencies are based on the molar concentration of the *l*-isomers of NE and Epi and of racemic Iso. It is likely that if the *l*-isomer of Iso had been used all relative potencies for Iso would have doubled. Differences between values were tested for by means of Student's *t* test. The $p < .05$ level of probability was considered to show a significant difference.

Results. The effect of cocaine on the spontaneous rate of the right atrium is presented in Table I. The rates are mean values recorded just before and 20 min after the addition of cocaine to the bathing solution. In atria obtained from untreated guinea pigs, exposure to cocaine resulted in a slight but transient increase in rate. After 20 min, however, the rate had returned to the pre-cocaine value and was stable. Addition of cocaine to the solution bathing atria obtained from reserpine-treated animals resulted in a negative chronotropic response. After 20 min the rate was reduced by a mean of 21 beats/min. Neither the positive chronotropic nor the negative chronotropic effect of cocaine interfered with the maximal rate the atria could attain in response to the three agonists (Table II).

The mean ED_{50} of the three sympathomimetics in the absence and presence of cocaine are presented in Table II. In atria from both untreated and reserpine-treated animals when neuronal uptake is inhibited by cocaine the sensitivity to NE and Epi is increased substantially whereas the sensitivity to Iso is not significantly altered. The increase in sensitivity to NE and Epi produced by cocaine is more pronounced in atria from reserpine-treated animals than in atria from untreated animals.

The relative potencies of the three agonists are presented in Table III. When neuronal uptake is inhibited by cocaine the order of potency changes from Iso > Epi $\geqq$ NE to Iso $\geqq$ NE > Epi. The same order of potency occurs in atria from untreated and reserpine-treated animals.

Discussion. The results reported here are qualitatively similar to those reported by Furchgott (1) concerning the relative potencies of NE, Epi and Iso on cardiac beta adrenergic receptors and further emphasize the influence of neuronal uptake on potency. When uptake is eliminated (*i.e.*, in the presence of cocaine) the relative potencies change from Iso > Epi $\geqq$ NE to Iso $\geqq$ NE > Epi. The quantitative differences between this study and that of Furchgott are probably due to inherent differences between the processes which lead to an inotropic or a chronotropic response. One such difference is that inotropic responses are subserved by both alpha and beta receptors whereas only beta receptors are involved in the chronotropic response (6).

Trendelenburg (10) has demonstrated that the addition of cocaine to guinea pig right

TABLE II. The Effect of Cocaine (10^{-5} *M*) on the Sensitivity to NE, Epi and Iso in Atria Obtained from Untreated and Reserpine-Treated Guinea Pigs.

Pretreatment	Group	*N*[a]	Mean rate (beats/min)		Geometric mean ED_{50} ($\times 10^{-10}$ *M*)	Ratio (control/ cocaine)
			Initial	Maximal		
None	NE, control	5	203 (± 9)	328 (± 3)	1660 (592–4649)[c]	16
	NE, cocaine	5	197 (± 7)	326 (± 6)	102[b] (38–271)	
	Epi, control	5	199 (± 7)	324 (± 6)	838 (467–1505)	4
	Epi, cocaine	6	185 (± 9)	317 (± 5)	210[b] (134–320)	
	Iso, control	5	191 (± 4)	330 (± 7)	8 (3.5–18.2)	0.3
	Iso, cocaine	4	196 (±13)	329 (± 7)	26 (11.4–59.5)	
Reserpine	NE, control	10	186 (± 7)	329 (± 7)	714 (252–2026)	150
	NE, cocaine	9	168[b] (± 7)	312 (±15)	4.8[b] (.37–62)	
	Epi, control	5	187 (± 9)	325 (± 8)	745 (179–3098)	10
	Epi, cocaine	5	168[b] (± 7)	325 (± 2)	75[b] (31–182)	
	Iso, control	4	186 (±13)	311 (±16)	5.9 (.42–82)	1.6
	Iso, cocaine	4	166[b] (± 7)	305 (± 7)	3.7 (.48–28)	

[a] Number of experiments.
[b] Significantly different from own control, $p \leq .05$.
[c] 95% confidence intervals.

atria results in a positive chronotropic response. Since the positive chronotropic response is absent in atria obtained from reserpine-treated animals, he attributed this action of cocaine to release of endogenous norepinephrine. The present experiments confirm those observations (Table I). Such an effect of cocaine could distort the dose–response relationship of an adrenergic agonist which might result in an erroneous estimate of relative potency. This appears to be true in part since the change in relative potencies induced by cocaine differs quantitatively after reserpine treatment. In atria from untreated animals there is only a twofold difference in potency between NE and Epi in the presence of cocaine. After reserpine pretreatment, however, the potencies of NE and Epi differ by 15-fold when uptake is blocked by cocaine. The release of endogenous NE by cocaine thus appears to influence the potency of exogenous amines. It is concluded, therefore, that the most accurate determination of chronotropic potencies is obtained in the presence of cocaine and in the absence of endogenous NE.

Summary. It is concluded that the relative chronotropic potencies of NE, Epi and Iso are similar to the relative inotropic potencies reported previously. If neuronal uptake is

TABLE III. The Effect of Cocaine (10^{-5} *M*) on the Relative Potencies of NE, Epi and Iso in Atria Obtained from Untreated and Reserpine-Treated Guinea Pigs.

Pretreatment	Group	Potency relative to NE		
		NE	Epi	Iso
None	Control	1	2	208[a]
	Cocaine	1	0.5[a]	4
Reserpine	Control	1	0.96	120[a]
	Cocaine	1	0.07[a]	1.3

[a] Significantly different from NE, $p < .05$.

functional the relative potencies are Iso > Epi ≧ NE. When neuronal uptake is excluded as a means of removing agonists from the vicinity of receptors (*i.e.*, in the presence of cocaine) the potencies become Iso ≧ NE > Epi. This change in the order of relative potencies is due to the fact that in the presence of cocaine the ED_{50} values of NE and Epi are reduced while the ED_{50} of Iso remains the same. The order of potency of these three adrenergic agonists is not changed by endogenous norepinephrine depletion induced by reserpine. The sensitization of atria to NE and Epi by cocaine is, however, substantially greater after reserpine.

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