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Dissociation of potentiation of isoprenaline by cocaine from inhibition of uptake in cat spleen

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Summary

1. Cocaine hydrochloride, 10 μg/ml, potentiated isoprenaline and noradrenaline in isolated spleen strips from normal cats and from cats treated with reserpine 24 h previously.

2. Isoprenaline was taken up almost as well as noradrenaline by spleen strips from cats treated with reserpine.

3. Cocaine blocked uptake of noradrenaline but did not reduce uptake of isoprenaline. Drug concentrations used in these studies were the same as in potentiation experiments.

4. It is concluded that inhibition of uptake is not the mechanism by which cocaine potentiates the effect of isoprenaline on the spleen and might be only a contributory factor in the case of noradrenaline potentiation.

Introduction

Various explanations have been put forward from time to time to account for the potentiating effect of cocaine or of chronic denervation on the actions of catecholamines on a variety of tissues innervated by the sympathetic nervous system (see reviews by Furchgott, 1955 ; Trendelenburg, 1963, 1966). In general these have not withstood the test of critical experimentation. The currently popular hypothesis is that the concentration of a catecholamine in the extracellular fluid immediately in contact with the adrenoceptors is normally limited by uptake into the adrenergic nerves, and that cocaine, by blocking this uptake, or chronic denervation, by removing the sites of uptake, increases the concentration near the receptors (Macmillan, 1959). Many observations indicating that inhibition of noradrenaline uptake runs parallel with potentiation fit well with this hypothesis, to the extent that Iversen (1967) has stated that potentiation of catecholamines is a property which all inhibitors of uptake may be expected to share. However, the hypothesis does not account for the supersensitivity to catecholamines produced by cocaine in isolated human placenta (Euler, 1938), a preparation with little or no innervation, for the sites of major catecholamine uptake are in the nerve endings (Hertting, Axelrod, Kopin & Whitby, 1961 ; Strömblad & Nickerson, 1961 ; Hertting & Schiefthaler, 1964 ; Potter, Cooper, Willman & Wolfe, 1965 ; Iversen, 1965). Nor is inhibition of uptake a satisfactory explanation for potentiation of agonists which act on other receptors, for example acetylcholine and histamine (Rosenblueth, 1932), 5-hydroxytryptamine (Innes & Kosterlitz, 1954). Trendelenburg (1963) also has discussed observations which are difficult to reconcile with the uptake hypothesis.

The failure of cocaine to enhance the action of isoprenaline on β-adrenoceptors of various tissues (Roszkowski & Koelle, 1960 ; Stafford, 1963) is in accord with the uptake hypothesis, because uptake of isoprenaline is small (Hertting, 1964 ; Iversen, 1964). Isoprenaline, however, contracts the cat spleen by acting on α-adrenoceptors (Bickerton, 1963). We find that cocaine potentiates this action. This observation, in terms of the uptake hypothesis, is inconsistent with a low uptake of isoprenaline, so we have measured the uptake of isoprenaline by cat spleen and determined the effects of cocaine on it.

Methods

Cats (0·6–1·2 kg) of either sex were killed by a blow on the head. The spleen was removed and placed in cold Krebs-Henseleit solution. Strips 20 mm long and 3 mm wide were cut from the edge of the spleen and placed in individual 10 ml organ baths containing Krebs-Henseleit solution at 38° ±0·2° C and bubbled with a mixture of 95% oxygen and 5% carbon dioxide. Strips were allowed to equilibrate for 1 h before the experiment was started. Isotonic contractions against 1 g tension were recorded on a kymograph at a magnification of ×6. In each experiment to determine the effects of cocaine four strips were taken from a single spleen. Isoprenaline was tested on two strips, noradrenaline on the other two. Cumulative dose-response curves for isoprenaline and noradrenaline were obtained ; each dose was allowed to cause its full effect before the next dose was added. After the strip had fully relaxed, cocaine hydrochloride (10 μg/ml) was added to one of each pair of strips, and the cumulative dose-response curve to isoprenaline or noradrenaline was determined in its presence. The second strip of each pair was used as a control for the effect of time on the response to isoprenaline or noradrenaline. Differences between treatments were analysed from the geometric means of ED50 by analysis of variance and Duncan's multiple range tests (Steel & Torrie, 1960). For experiments in which the effect of cocaine was to be determined on spleen strips depleted of catecholamines, reserpine (1 mg/kg) was injected intraperitoneally 24 h before the spleen was removed.

The drugs used were (−)-isoprenaline bitartrate dihydrate (Winthrop Laboratories), (−)-noradrenaline bitartrate monohydrate (Calbiochem), cocaine hydrochloride (British Drug Houses), and reserpine (Ciba). Drug concentrations refer to final bath concentrations, expressed as free base for isoprenaline and noradrenaline and as salt for cocaine.

Uptake of isoprenaline or noradrenaline was measured in spleen strips prepared as above but taken from cats depleted of catecholamine stores by reserpine (1 mg/kg) injected intraperitoneally 24 h before the experiment. Noradrenaline content was assayed by the method of Euler & Lishajko (1961). Isoprenaline was assayed by a similar technique with a standard curve for isoprenaline made daily ; in these assays the wavelengths used in adrenaline estimations (435 nm exciting, 540 nm reading) gave the best results. Recoveries of known amounts of isoprenaline and noradrenaline were similar and between 88·6 and 92·2%. With these data, tissue levels of isoprenaline were then determined. If present, adrenaline or noradrenaline interfered with the estimation of isoprenaline, but in spleens from reserpine treated cats the adrenaline and noradrenaline contents were lower than the amounts detectable by the method used (0·002 μg/g tissue) and so did not interfere with the

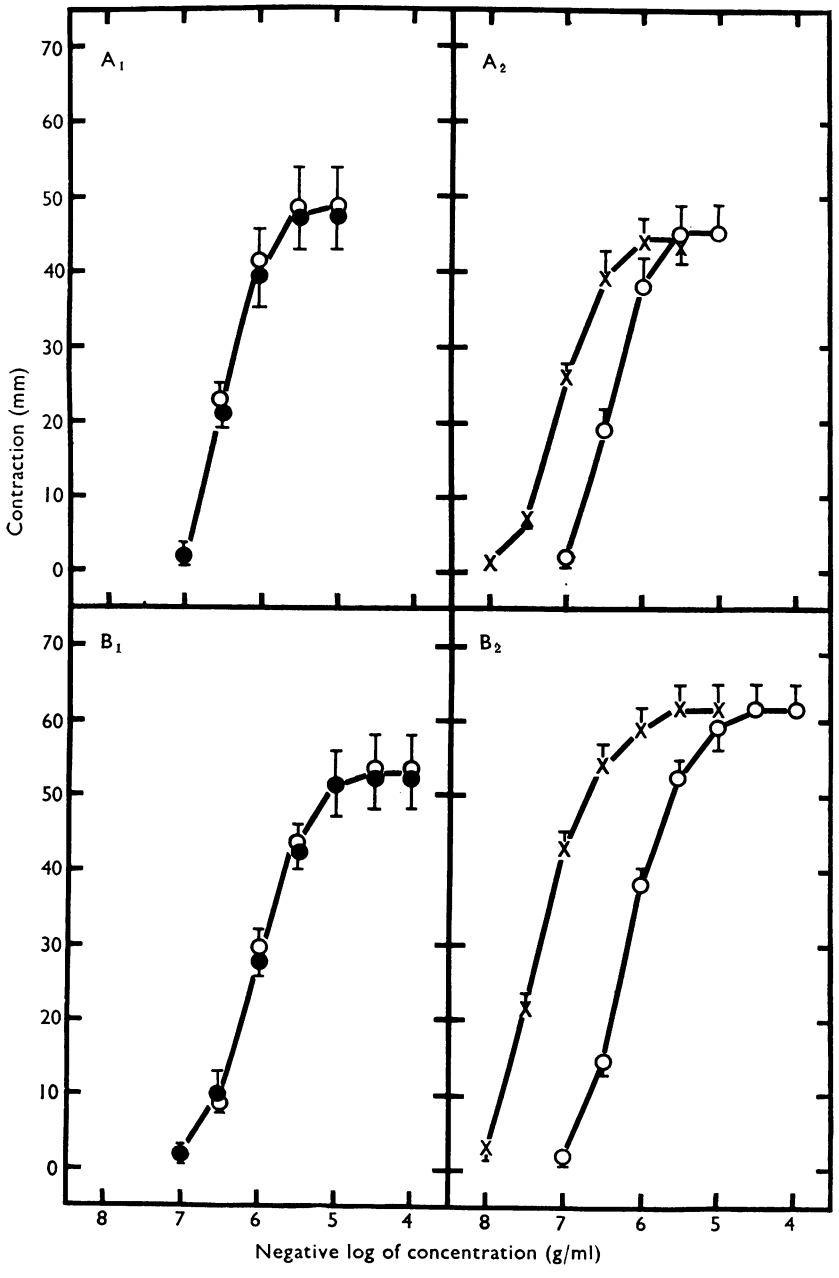

FIG. 1. Potentiating effect of cocaine hydrochloride (10 μg/ml) on isoprenaline and noradrenaline effects in spleen strips from normal cats. Dose-response curves to isoprenaline (A_1, A_2) and noradrenaline (B_1, B_2). A_2 and B_2: ○, without cocaine; ×, with cocaine hydrochloride 10 μg/ml. A_1 and B_1: ○, without cocaine; ●, time control without cocaine, 90 to 100 min after first dose-response curve.

estimations of isoprenaline. The data were analysed by Student's t test for unpaired data.

Results

Potentiation of isoprenaline and noradrenaline by cocaine

Cumulative dose-response curves to isoprenaline and noradrenaline before and after cocaine hydrochloride (10 μg/ml) were compared in five experiments on spleen strips from normal cats and six experiments on strips from cats given reserpine (1 mg/kg intraperitoneally) 24 h before the experiment. Four strips from the same spleen were used in each experiment; isoprenaline and noradrenaline were each tested on two strips, one strip before and after cocaine, the second strip as a time control without cocaine. In both series of experiments cocaine potentiated both isoprenaline and noradrenaline, while the time control dose-response curves were unchanged (Figs. 1 and 2). Potentiation of isoprenaline was significantly less than of noradrenaline ($P<0{\cdot}001$). Statistical analysis of the results is shown in Tables 1 and 2.

Uptake of isoprenaline by cat spleen

Strips of spleen from cats given reserpine (1 mg/kg intraperitoneally) 24 h before experiment were exposed to isoprenaline (100 or 1 μg/ml) for 5 min and then washed until the strips had relaxed. Other strips from the same animal were not exposed to isoprenaline and served as controls. All strips were then taken from the organ

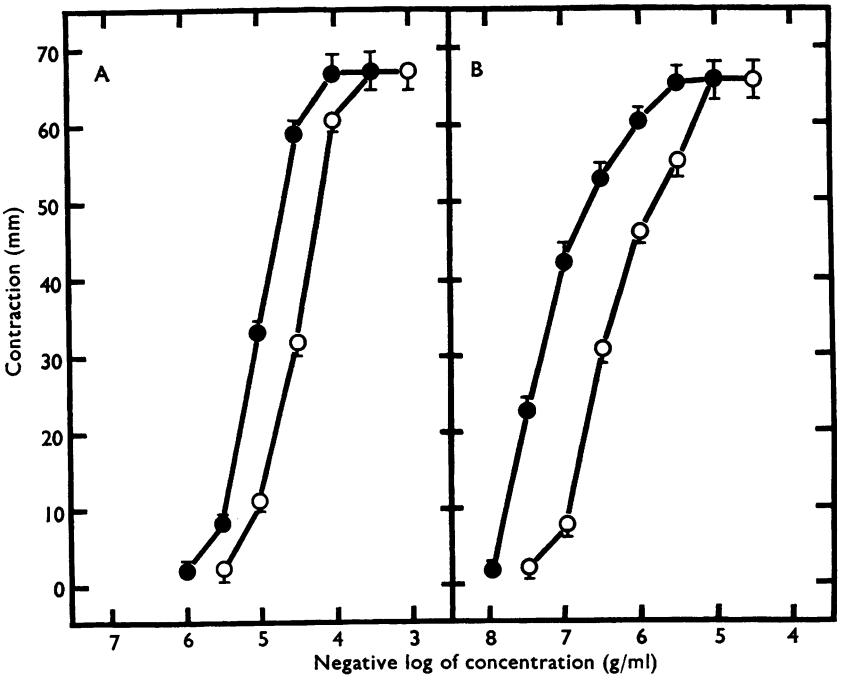

FIG. 2. Potentiating effect of cocaine hydrochloride (10 μg/ml) on isoprenaline and noradrenaline effects in spleen strips from cats given reserpine, 1 mg/kg intraperitoneally 24 h before experiment. Dose-response curves to isoprenaline, A, and noradrenaline, B. ○, without cocaine; ●, with cocaine.

baths ; the control tissues were assayed for total catecholamines, and treated tissues for isoprenaline. Isoprenaline was taken up by spleen in each of thirteen experiments. The isoprenaline contents were $2{\cdot}19 \pm 0{\cdot}28$ μg/g after loading with 100 μg/ml, and $0{\cdot}32 \pm 0{\cdot}056$ μg/g after loading with 1 μg/ml. Total catecholamine content of unloaded strips was less than 0·002 μg/g.

Effect of cocaine on uptake of isoprenaline and noradrenaline

The effects of cocaine on uptake of isoprenaline and noradrenaline were tested in eighteen experiments. For each experiment, five strips from the same spleen were used. In all cases the cats were given reserpine (1 mg/kg intraperitoneally) 24 h before the experiment. Two strips were exposed to a loading concentration of isoprenaline, in one strip in the presence of cocaine hydrochloride (10 μg/ml) applied 5 min before isoprenaline, in the second without cocaine. Two strips were similarly exposed to noradrenaline with or without cocaine. The fifth strip was exposed to cocaine only, and served as a control. Loading concentrations of isoprenaline or noradrenaline were 0·1, 1 and 10 μg/ml. Strips were exposed to the catecholamines for 5 min, washed till the contraction had disappeared, and then

TABLE 1. *Potentiation of isoprenaline and noradrenaline by cocaine hydrochloride* (10 μg/ml)

Drug	Treatment	Mean ED50 (μg/ml)	95% confidence limits Lower	Upper	Significance
Isoprenaline	Control	36·6	33·6	40·0	N.S.
	Time—Control	39·5	36·2	43·1	
Noradrenaline	Control	0·99	0·86	1·14	N.S.
	Time—Control	1·02	0·89	1·17	
Isoprenaline	Control	41·0	33·6	50·1	$P<0{\cdot}001$
	Cocaine	8·7	7·1	10·7	
Noradrenaline	Control	0·84	0·62	1·12	$P<0{\cdot}001$
	Cocaine	0·07	0·05	0·09	

TABLE 2. *Potentiation of isoprenaline and noradrenaline by cocaine hydrochloride* (10 μg/ml) *after reserpine*

Drug	Treatment	Mean ED50 (μg/ml)	95% confidence limits Lower	Upper	Significance
Isoprenaline	Control	28·8	26·7	31·0	$P<0{\cdot}001$
	Cocaine	9·7	9·0	10·4	
Noradrenaline	Control	0·57	0·50	0·58	$P<0{\cdot}001$
	Cocaine	0·10	0·09	0·11	

TABLE 3. *Effect of cocaine hydrochloride* (10 μg/ml) *on uptake of isoprenaline and noradrenaline in spleen strips from reserpine-treated cats*

Loading drug	Cocaine hydrochloride	Tissue content (μg/g tissue) Loading concentration 0·1 μg/ml	1 μg/ml	10 μg/ml	0
Noradrenaline	0	$0{\cdot}35 \pm 0{\cdot}03$	$0{\cdot}56 \pm 0{\cdot}07$	$1{\cdot}40 \pm 0{\cdot}10$	
Noradrenaline	10 μg/ml	$0{\cdot}06 \pm 0{\cdot}02$	$0{\cdot}17 \pm 0{\cdot}05$	$0{\cdot}54 \pm 0{\cdot}26$	$<0{\cdot}002$
		$P<0{\cdot}001$	$P<0{\cdot}005$	$P<0{\cdot}001$	
Isoprenaline	0	$0{\cdot}25 \pm 0{\cdot}03$	$0{\cdot}32 \pm 0{\cdot}06$	$0{\cdot}96 \pm 0{\cdot}02$	
Isoprenaline	10 μg/ml	$0{\cdot}25 \pm 0{\cdot}04$	$0{\cdot}31 \pm 0{\cdot}07$	$0{\cdot}95 \pm 0{\cdot}03$	
		N.S.	N.S.	N.S.	

assayed for isoprenaline and noradrenaline content. Cocaine significantly reduced the uptake of noradrenaline but did not alter the uptake of isoprenaline (Table 3).

Discussion

The results obtained on strips of the spleen do not support the hypothesis that cocaine generally causes supersensitivity by inhibiting the uptake of catecholamines. Cocaine potentiated both noradrenaline and isoprenaline but inhibited uptake of noradrenaline only. After cocaine the dose-response curves for isoprenaline and noradrenaline were both shifted to the left without an increase in the maximum contraction. Our results with isoprenaline are in agreement with those of Leszkovszky & Tardos (1968), who showed potentiation by cocaine of a single selected dose of isoprenaline.

Uptake of noradrenaline and isoprenaline was tested on strips from cats treated with reserpine in order to deplete noradrenaline stores. Thus uptake of the catecholamines could be estimated without the interference of a large amount of noradrenaline in the control tissues. This procedure should not affect the validity of the test of the uptake hypothesis, since cocaine potentiated noradrenaline and isoprenaline in strips from cats treated with reserpine nearly as effectively as in strips from normal cats. In addition, for the tests of uptake we selected loading concentrations of noradrenaline and isoprenaline and an exposure time similar to the concentrations and exposure time used in the tests of potentiation. We did not expect the spleen to take up substantial amounts of isoprenaline from the lower loading concentrations, for other tissues do not readily take up isoprenaline (Iversen, 1964). However, isoprenaline was taken up almost as well as noradrenaline. Cocaine had no effect on the uptake of isoprenaline, but strikingly inhibited uptake of noradrenaline. Potentiation of isoprenaline cannot therefore be attributed to inhibition of uptake, and another mechanism of potentiation must be sought. The potentiation of noradrenaline was greater than of isoprenaline, however, so it is possible that inhibition of uptake may have an additional effect in the case of noradrenaline. Our results agree with the conclusion of various workers that inhibition of uptake cannot fully account for the development of supersensitivity (Maxwell, Wastila & Eckhardt, 1966 ; Bevan & Verity, 1967 ; Kalsner & Nickerson, 1969 ; Varma & McCullough, 1969).

Uptake of catecholamines in the spleen has not yet been as thoroughly studied as has uptake in the rat heart. Rat heart has two uptake mechanisms, $Uptake_1$, responsible for uptake at low loading concentrations, and $Uptake_2$, responsible for uptake at higher loading concentrations (Callingham & Burgen, 1966 ; Iversen, 1967). Isoprenaline is not taken up significantly by $Uptake_1$ but is taken up by $Uptake_2$, while cocaine inhibits $Uptake_1$ but not $Uptake_2$. Our results suggest that the uptake mechanisms in the cat spleen and the rat heart are different since the concentrations of isoprenaline we have used in the spleen overlap the effective concentrations for $Uptake_1$ and $Uptake_2$, and uptake is not inhibited by cocaine. On the other hand, uptake of noradrenaline over the whole range of concentrations studied is inhibited by cocaine.

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