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Potentiation by cocaine and 3,3-di(*p*-aminophenyl)-propylamine (TK 174) of the effect of isoprenaline and noradrenaline on isolated strips of cat spleen

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Contractions of cat isolated spleen strips by noradrenaline and isoprenaline are brought about by interaction with α-adrenergic receptors. The effect of isoprenaline cannot be diminished by *in vivo* reserpine pretreatment. The effects of both noradrenaline and isoprenaline are potentiated by cocaine and by a new diphenylpropylamine derivative TK 174 [3,3-di(*p*-aminophenyl)propylamine]. Noradrenaline is potentiated more by TK 174 than by cocaine while the reverse is seen with isoprenaline. These results suggest that beside the inhibition of noradrenaline uptake another factor may be involved in the mechanism of action of cocaine, and to a lesser extent of TK 174.

PREVIOUSLY Leszkovsky, Tardos & others (1966, 1967a) reported that the peripheral effects of noradrenaline are much increased by the diphenylpropylamine derivative 3,3-di(*p*-aminophenyl)propylamine (TK 174). They have also suggested (Leszkovszky, Tardos & others, 1967b) that the mechanism of action of this substance is similar to that of cocaine.

Further experiments on isolated organs have been made to test this hypothesis. Isoprenaline was included because its inactivation in the organism differs from that of noradrenaline (Hertting, 1964). The isolated spleen strip was chosen because it is contracted by isoprenaline as well as by noradrenaline.

Experimental

METHODS

Cats weighing 2·6 to 3·7 kg were splenectomized under chloralose-urethane anesthesia. Some experiments were made on spleens from cats pretreated with reserpine (2 mg/kg of crystalline reserpine dissolved in 20% ascorbic acid) intraperitoneally, 24 hr before surgery.

The excised spleens were either used immediately or stored in Locke solution at 4° for 24 hr. Like Bickerton, Rockhold & Micalizzi (1962) and de Geus, Bernards & Verduyn (1956), we found the sensitivity to sympathomimetic amines of spleens so stored not to be diminished.

Strips—1 cm wide and 2 cm long—were cut from the spleens and attached under 1·5 g tension to an isotonic lever (magnification of 1 : 20). The 30 ml organ bath was vigorously gassed with pure oxygen and maintained at 38·5°. The bathing fluid had the following composition (%): NaCl 0·75, KCl 0·042, $CaCl_2$ 0·024, $NaHCO_3$ 0·024, NaH_2PO_4 0·014, glucose 0·1. The experiment was begun after an equilibration period of about 40 min. Noradrenaline and isoprenaline were used in constant concentrations of $1{\cdot}48 \times 10^{-6}$M and $2{\cdot}018 \times 10^{-4}$M, respectively. Cocaine and TK 174 were added to the organ bath 5 min before the amine. As

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the contraction of splenic smooth muscle is rather slow, a contraction was recorded until it reached a maximum, then the preparation was washed. The vertical heights of contractions measured on the kymographs were evaluated; mean values with their standard errors are listed in the results.

The drugs used were: (—)-noradrenaline bitartrate, (±)-isoprenaline hydrochloride, Dibenamine hydrochloride, propranolol hydrochloride, cocaine hydrochloride, reserpine and TK 174 (molecular weight: 241·32).

Results

Noradrenaline, $1{\cdot}48 \times 10^{-6}$M, induced contractions of 20 to 30 mm which were halved by 3·30 to $6{\cdot}60 \times 10^{-7}$M Dibenamine.

Increasing equimolar concentrations of cocaine and TK 174 were tested. High concentrations ($1–2 \times 10^{-4}$M) of both produced contractions of spleen strips. This phenomenon, however, was not observed with the lower concentrations used to potentiate catecholamine effects. Between two concentrations, noradrenaline was added to the bath at least twice to detect accidental changes in the sensitivity of the organ. Contractions recorded in the presence of cocaine or TK 174 were compared with those immediately preceding them. Table 1 shows the heights of contractions recorded both before and after the addition of the test substance, as well as the difference between them in both absolute (mm) and relative (percentage) terms.

TABLE 1. POTENTIATION OF THE EFFECT OF NORADRENALINE ($1{\cdot}48 \times 10^{-6}$M) ON ISOLATED STRIPS OF CAT SPLEEN

Substance	Conc. M	No. of exps	Height of contractions* before the addition of the substance to be tested: before (mm)	Height of contractions* before the addition of the substance to be tested: after (mm)	Increase due to the substance tested in: absolute (mm)	Increase due to the substance tested in: relative (%)
					terms	
Cocaine hydrochloride	$8{\cdot}8 \times 10^{-7}$	5	22·8 ± 2·9	33·6 ± 5·6	10·4 ± 5·0	47
	$4{\cdot}4 \times 10^{-6}$	5	26·8 ± 4·4	47·8 ± 7·7	21·0 ± 6·6	78
	$2{\cdot}1 \times 10^{-5}$	5	35·8 ± 5·5	65·6 ± 13·2	29·8 ± 9·4	84
TK 174	$1{\cdot}7 \times 10^{-7}$	6	30·7 ± 4·5	39·2 ± 4·9	8·5 ± 2·9	28
	$8{\cdot}3 \times 10^{-7}$	6	26·4 ± 5·1	47·4 ± 6·6	20·1 ± 6·4	79
	$4{\cdot}2 \times 10^{-6}$	6	36·2 ± 11·8	65·8 ± 14·3	29·7 ± 4·8	82
	$2{\cdot}1 \times 10^{-5}$	5	36·4 ± 10·2	73·8 ± 15·0	37·4 ± 6·2	103

* Mean values ± s.e.

It can be seen from Table 1 that the effect of noradrenaline is enhanced by both cocaine and TK 174. The potency of TK 174 is approximately five times that of cocaine.

Isoprenaline, $2{\cdot}018 \times 10^{-4}$M also produced contractions of 20–30 mm. These were fully abolished by Dibenamine ($3{\cdot}30$ to $6{\cdot}60 \times 10^{-7}$M) but were left unchanged by propranolol ($0{\cdot}676$ to $1{\cdot}69 \times 10^{-5}$M). They were not inhibited by reserpine pretreatment; in 9 strips taken from spleens of 5 reserpinized cats, $2{\cdot}018 \times 10^{-4}$M isoprenaline produced contractions of 33·9 ± 9·2 mm. This mean value is higher than any of those observed on non-pretreated cat spleens (see Table 2, column 4) but it does not differ significantly from the larger of these values.

The potentiating effect on isoprenaline contractions of cocaine and TK 174 was examined as in the experiments with noradrenaline. Detailed results are in Table 2.

TABLE 2. POTENTIATION OF THE EFFECT OF ISOPRENALINE ($2{\cdot}018 \times 10^{-4}$M) ON ISOLATED STRIPS OF CAT SPLEEN

Substance	Conc. M	No. of exps	Height of contractions* before the addition of the substance to be tested: before (mm)	Height of contractions* before the addition of the substance to be tested: after (mm)	Increase due to the substance tested in: absolute (mm)	Increase due to the substance tested in: relative (%)
Cocaine hydrochloride	$1{\cdot}8 \times 10^{-7}$	8	$30{\cdot}3 \pm 4{\cdot}3$	$37{\cdot}0 \pm 5{\cdot}1$	$6{\cdot}7 \pm 3{\cdot}3$	22
	$8{\cdot}8 \times 10^{-7}$	9	$26{\cdot}7 \pm 4{\cdot}2$	$31{\cdot}7 \pm 3{\cdot}3$	$5{\cdot}0 \pm 1{\cdot}6$	18
	$4{\cdot}4 \times 10^{-6}$	8	$24{\cdot}8 \pm 3{\cdot}1$	$35{\cdot}0 \pm 3{\cdot}1$	$10{\cdot}1 \pm 1{\cdot}3$	41
	$2{\cdot}1 \times 10^{-5}$	13	$32{\cdot}9 \pm 5{\cdot}1$	$52{\cdot}0 \pm 4{\cdot}6$	$19{\cdot}1 \pm 5{\cdot}8$	58
TK 174	$8{\cdot}3 \times 10^{-7}$	9	$16{\cdot}7 \pm 4{\cdot}4$	$19{\cdot}8 \pm 3{\cdot}8$	$3{\cdot}1 \pm 1{\cdot}5$	18
	$4{\cdot}2 \times 10^{-6}$	14	$30{\cdot}7 \pm 6{\cdot}5$	$29{\cdot}9 \pm 5{\cdot}7$	$-0{\cdot}8 \pm 6{\cdot}7$	-3
	$2{\cdot}1 \times 10^{-5}$	12	$29{\cdot}1 \pm 4{\cdot}1$	$39{\cdot}3 \pm 5{\cdot}4$	$10{\cdot}2 \pm 2{\cdot}9$	45

* Mean values ± s.e.

It is clear from these data that the effect of isoprenaline on the cat isolated spleen strip is potentiated by both cocaine and TK 174. The latter is effective only at the higher ($2{\cdot}1 \times 10^{-5}$M) concentration while the former is active at the concentration 5 times lower.

Discussion

Isolated spleen strips are contracted by both noradrenaline and isoprenaline. This effect is due to interaction with α-adrenergic receptors; it can be blocked by low concentrations of Dibenamine. The effect of isoprenaline could not be blocked by propanolol. We found isoprenaline to have about one hundredth of the potency of noradrenaline, as did Bickerton (1963) and Kizaki & Abiko (1966) in experiments made on the spleens of various species.

The effect of isoprenaline is due to its direct action on the receptors, as it is not diminished by a reserpine pretreatment sufficient to deplete catecholamines from cat spleen (Thoenen, Tranzer & others, 1966). The same action has recently been shown for another α-adrenergic effect of isoprenaline by Gay, Rand & Wilson (1967).

Cocaine increased the effect of noradrenaline in accordance with the literature on its potentiation of various sympathetic effects. The new diphenylpropylamine derivative TK 174 also increased this noradrenaline effect. TK 174 has been found to be more potent than cocaine in potentiating the effect of noradrenaline on the guinea-pig isolated vas deferens (Leszkovszky & others, 1967b). Now it has been shown to be more potent on the isolated spleen. Thus, it may be supposed that TK 174 inhibits uptake of noradrenaline into nerve terminals more actively than cocaine. Our results suggest, however, that another factor may play a role in the effect of cocaine. We found that the effect of isoprenaline is also potentiated by cocaine. According to most investigators, the effects of isoprenaline are not increased by cocaine (Smith, 1963; Stafford, 1963;

Andén, Corrodi & others, 1964; Hardman, Meyer & Clark, 1965; Trendelenburg, 1966; Bhagat, Bovell & Robinson, 1967). Thus, our observation seems to be contrary to most of the published data. A result similar to ours has recently been published by Gay & others (1967) who found in experiments on isolated perfused ear arteries of the rabbit that the vasoconstrictor action of isoprenaline was much increased by cocaine. It seems worthwhile to note that in those reports which failed to demonstrate any potentiation by cocaine of isoprenaline effects, various β-adrenergic actions of isoprenaline were being examined. Like us, Gay & others (1967) found it was an α-adrenergic effect, resulting in smooth muscle contraction, that was potentiated by cocaine.

Although the effect of isoprenaline was potentiated less by TK 174 than by cocaine, for noradrenaline their potency ratio was reversed. This shows that besides the block of noradrenaline uptake there must be some other factor in the mechanism of action which is not equally strong for both substances.

The possibility of a direct muscular action of cocaine is suggested by the recent work of Bevan & Verity (1967) who stated that cocaine increased the maximum response of both normal and nerve-free (denervated) strips of rabbit aorta to noradrenaline.

This other action of TK 174 (possibly direct muscular) is weaker than that of cocaine. Considering this fact together with the lack of any central nervous excitatory activity of TK 174 (Leszkovszky & others, 1967b), we suggest that TK 174 can advantageously be used as a pharmacological tool for studying supersensitivity to sympathomimetic amines and related questions.

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