Brit. J. Pharmacol. (1961), **16**, 352-359.

EFFECT OF COCAINE AND RELATED DRUGS ON THE UPTAKE OF NORADRENALINE BY HEART AND SPLEEN

BY

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(*Received April* 10, 1961)

Noradrenaline uptake by heart and spleen after intravenous infusion of noradrenaline was measured in the pithed rat. Cocaine, given before the infusion, inhibited the noradrenaline uptake in relation (*a*) to the dose administered and (*b*) to the amount of noradrenaline infused. There was an association between increase in the pressor response to a test dose of noradrenaline and inhibition of the uptake by the heart. Drugs related chemically to cocaine, such as α-cocaine, amethocaine, and atropine, did not alter the noradrenaline uptake or potentiate the blood pressure response to noradrenaline. The noradrenaline uptake by the heart was unchanged after dibenamine, but blocked by the dichloro-analogue of isoprenaline. It was concluded that cocaine specifically prevented the uptake of noradrenaline by tissues, thus increasing the amount of noradrenaline available for combination with adrenergic receptors. The dichloro-analogue of isoprenaline appeared to block both uptake by the heart and the combination with receptors.

Since Fröhlich & Loewi (1910) first described sensitization by cocaine of various organs to adrenaline, different explanations for the potentiating effect of cocaine on responses to catechol amines have been offered. Recently Trendelenburg (1959) reinvestigated the question whether cocaine causes supersensitivity by preventing the destruction of injected noradrenaline. He found that cocaine delayed the inactivation of noradrenaline, and that the elevated plasma concentrations of noradrenaline fully accounted for the increase in blood pressure responses. Trendelenburg (1959) suggested that inactivation may be caused, amongst other means, by a binding process, and such binding by many tissues was demonstrated by Axelrod, Weil-Malherbe & Tomchick (1959) to inactivate circulating adrenaline.

From indirect evidence, Macmillan (1959) concluded that cocaine prevented the uptake of catechol amines by tissue stores, thereby increasing the amount of noradrenaline acting on sympathetic receptors. In a direct test of this hypothesis, Muscholl (1960a) showed that a noradrenaline infusion given to a rat approximately doubled the concentration of noradrenaline in heart and spleen, and that cocaine blocked this uptake. Blocking by cocaine of the accumulation of tritium-labelled noradrenaline in various organs has also been observed by Whitby, Hertting & Axelrod (1960).

The purpose of the present investigation was to investigate the specificity of the effect of cocaine on the uptake of noradrenaline and the quantitative relation between this uptake and the potentiation of the effects of noradrenaline.

METHODS

Male or female rats, weighing 170 to 250 g, were anaesthetized with ether, pithed and maintained by artificial respiration (Muscholl & Vogt, 1957). The blood pressure was recorded from the carotid artery with a Condon-type mercury manometer. A femoral vein was cannulated for injection of drugs. Infusions of noradrenaline dissolved in saline were given into this cannula at a constant rate for a period of 20 min using a motor-driven syringe. After infusions or injections the cannula was rinsed through with saline. The total volume of saline administered in any one experiment never exceeded 3 ml.

Five or twenty min after the end of the infusion of noradrenaline the heart was excised. Atria and large vessels were removed, the myocardium dried on filter paper and weighed. The tissue was homogenized, extracted in acid ethanol, the extract purified and chromatographed on paper. The eluates of the regions containing the noradrenaline and adrenaline were assayed individually on the blood pressure of the pithed rat and on the isolated atropinized rat uterus stimulated with oxytocin, as described by Muscholl (1959).

In some experiments the concentration of noradrenaline in the spleen was estimated by preparing extracts as described for cat heart (Muscholl, 1959).

The following substances were used: (−)-Noradrenaline base dissolved in hydrochloric acid; (−)-adrenaline-(+)-bitartrate (doses and concentrations refer to the bases). Cocaine hydrochloride; amethocaine (Tetracaine) hydrochloride; atropine sulphate; α-cocaine hydrochloride; dibenamine (N,N-dibenzyl-β-chloroethylamine hydrochloride); the dichloro-analogue of isoprenaline, 1-(3′,4′-dichlorophenyl)-2-isopropylaminoethanol hydrochloride (doses refer to the salts).

RESULTS

Action of cocaine on the uptake of noradrenaline by heart and spleen. The concentration of noradrenaline in the heart of 9 normal rats was found to be 0.59 ± 0.02 μg/g (mean ± s.e. of the mean). This agrees well with the value of 0.61 μg/g reported previously by Muscholl (1959). Likewise, the concentration of adrenaline in the normal hearts (46 ± 5 ng/g) fell within the range found by Muscholl (1959).

TABLE 1

CONTENT OF NORADRENALINE AND ADRENALINE IN THE HEART AND OF NORADRENALINE IN THE SPLEEN OF THE PITHED RAT AFTER INFUSION

Concentrations in μg or ng/g fresh tissue, mean ± s.e. of the mean. Number of estimations in brackets. * Individual figures. Rats killed 5 min after cessation of intravenous infusion of saline or 30 min after intravenous administration of the drug. Dichloroisoprenaline was injected into tail vein

			Heart		Spleen
Treatment	Drug	Dose mg/kg	Noradrenaline μg/g	Adrenaline ng/g	Noradrenaline μg/g
—	Untreated controls	—	0·59 ± 0·02 (9)	46 ± 5 (5)	0·19 ± 0·04 (6)
Pithed	Saline infusion, 3 ml./20 min	—	0·58 ± 0·04 (4)	38 ± 10 (4)	0·19 ± 0·07 (3)
Pithed	Cocaine	10	0·61*; 0·60 (2)	40; 21 (2)	0·09; 0·17 (2)
Pithed	Dibenamine	25–50	0·55 ± 0·02 (3)	—	—
—	Dichloroisoprenaline	30	0·60 ± 0·01 (3)	—	—

The concentration of noradrenaline in the spleen and the concentrations of the catechol amines in the heart were not altered by the pithing procedure used (Table 1). Control values given in Table 2 represent the means of untreated rats and of pithed control animals infused with saline.

In hearts excised 5 min after the end of an infusion of 20 μg of noradrenaline administered during a period of 20 min, the concentration of noradrenaline was 1.05 ± 0.04 μg/g. This corresponded to a net uptake of 0.46 μg/g or 78% of the control value. The adrenaline concentration of the heart was not affected by the noradrenaline infusion (Table 2), but the noradrenaline concentration of the spleen had risen by 0.15 μg/g (79% increase).

TABLE 2

EFFECT OF COCAINE ON THE NORADRENALINE UPTAKE BY THE HEART AND BY THE SPLEEN AFTER AN INFUSION OF NORADRENALINE

Concentrations in μg or ng/g fresh tissue, mean$\pm$s.e. of the mean. Number of estimations in brackets. Organs were removed 5 min after end of infusion

Dose of cocaine mg/kg	μg of noradrenaline infused in 20 min	Heart		Spleen
		Noradrenaline μg/g	Adrenaline ng/g	Noradrenaline μg/g
—	—	0·59$\pm$0·02 (13)	42$\pm$5 (9)	0·19$\pm$0·03 (9)
—	20	1·05$\pm$0·04 (21)	36$\pm$9 (10)	0·34$\pm$0·02 (10)
10	20	0·85$\pm$0·03 (4)	32$\pm$5 (4)	0·26$\pm$0·04 (4)
20	20	0·74$\pm$0·08 (8)	35$\pm$8 (6)	0·22$\pm$0·02 (8)
—	5	0·85$\pm$0·03 (10)	—	—
2·5	5	0·62$\pm$0·03 (5)	—	—

In experiments in which the heart was excised 20 min after the infusion terminated, the concentration of noradrenaline was still high, being 1.06 ± 0.08 μg/g ($n = 5$).

A dose of 10 or 20 mg/kg of cocaine given 5 to 15 min before the noradrenaline infusion reduced significantly the net uptake of noradrenaline by heart and spleen measured 5 min after the end of the infusion (Table 2). Again, the adrenaline

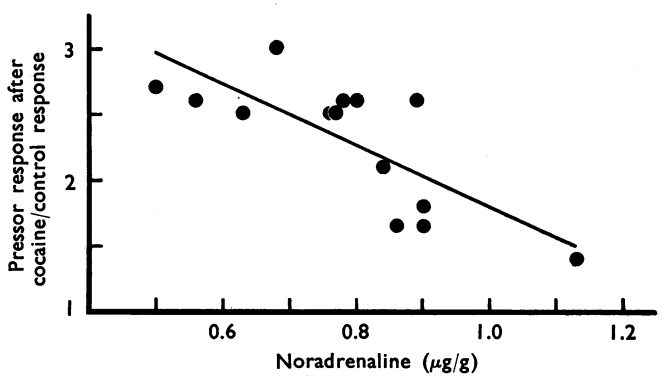

Fig. 1. Potentiation of the pressor response to noradrenaline by cocaine plotted against the noradrenaline concentration in the rat heart after a noradrenaline infusion. Each point represents an estimation on a pithed rat injected with cocaine (10 to 20 mg/kg) and given a noradrenaline infusion of 20 μg in 20 min. Ordinate: Ratio of the pressor response to a test dose of noradrenaline (2 to 5 ng) after cocaine/control response. Abscissa: Noradrenaline concentration of the heart measured 5 to 20 min after terminating infusion. The calculated regression is significant at the 1% level.

concentration of the heart was unchanged. Concentrations of noradrenaline measured 20 min after terminating the infusion (0.74 ± 0.05 μg/g, $n=6$) were also lowered by the previous injection of 10 mg/kg of cocaine.

In 14 out of 18 experiments the pressor response to a small test dose of noradrenaline was recorded before and after cocaine (10 to 20 mg/kg) had been injected. Subsequently, 20 μg of noradrenaline was infused. The pressor response to noradrenaline after cocaine varied inversely to its concentration measured in the heart after the infusion, and this relation could be expressed as a regression (Fig. 1). Since increases of concentrations of noradrenaline in the hearts above control values (0.59 μg/g) gave an estimate of the uptake by the heart, the results in Fig. 1 show that, with increasing depression of the uptake of noradrenaline by cocaine, there was increasing sensitization of the pressor response to noradrenaline.

Control experiments were made to eliminate the possibility that cocaine acted by reducing the concentration of noradrenaline in the heart *per se* rather than by preventing the uptake of exogenous noradrenaline. Table 1 shows that cocaine did not alter the concentrations of catechol amines in heart and spleen significantly. In two other experiments the amount of noradrenaline accumulated by the heart during a noradrenaline infusion of 20 μg did not decrease after the subsequent injection of 10 or 20 mg/kg of cocaine; the concentrations of noradrenaline in the hearts were 1.04 and 1.05 μg/g, respectively, and thus did not differ from the mean of 1.05 μg/g for rats receiving a noradrenaline infusion without pretreatment with cocaine.

Injection of a small dose (2.5 mg/kg) of cocaine had no effect on the uptake of noradrenaline by the heart if 20 μg of noradrenaline was infused, but completely blocked the uptake of noradrenaline when only 5 μg was infused (Table 2). The results of experiments using doses of 0.15 to 2.5 mg/kg of cocaine and infusions

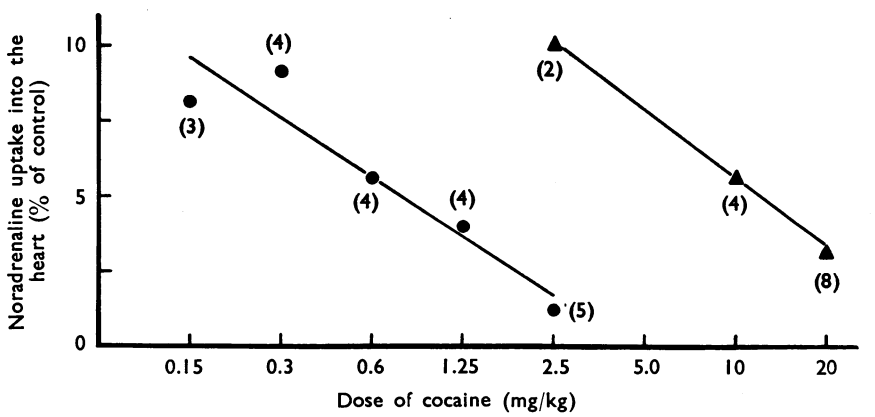

Fig. 2. Dose of cocaine plotted against % inhibition of noradrenaline uptake by the heart. Hearts removed 5 min after terminating noradrenaline infusion of 5 μg (●) or 20 μg (▲) per rat given in 20 min. Number of estimations in brackets. Noradrenaline uptake calculated as % of control values from Table 2 (for 5 μg infusions 0%=0.59 and 100%=0.85 μg/g; for 20 μg infusions 0%=0.59 and 100%=1.05 μg/g).

of 5 μg of noradrenaline are summarized in Fig. 2. It can be calculated from the regression lines that 0.8 mg/kg of cocaine caused 50% inhibition of uptake when 5 μg noradrenaline was infused, whereas 11.6 mg/kg of cocaine was required to produce the same percentage inhibition with infusion of 20 μg of noradrenaline.

Since there was little variation in the heart weights between the experimental groups, changes in concentrations also represented changes in total content of catechol amines.

Drugs chemically related to cocaine. These effects of cocaine might be related to its local anaesthetic action. Amethocaine, which has about 4 times the local anaesthetic activity of cocaine, did not increase the blood pressure response to noradrenaline in the pithed rat, nor did it inhibit the uptake of noradrenaline by the heart (Table 3).

Atropine, which is structurally related to cocaine, had no sensitizing effect on the noradrenaline pressor response and neither did it alter the uptake of noradrenaline (Table 3).

TABLE 3

EFFECT OF DRUGS ON THE NORADRENALINE UPTAKE INTO THE HEART AFTER AN INFUSION OF NORADRENALINE

Mean of noradrenaline concentration in μg/g$\pm$s.e. of the mean. Number of estimations in brackets. Hearts were removed 5 min after cessation of infusion of 20 μg of noradrenaline in 20 min

Drug given before noradrenaline infusion	mg/kg	Heart noradrenaline μg/g
No drug	—	1·05$\pm$0·04 (21)
Amethocaine	5–10	1·24$\pm$0·10 (3)
Atropine	20	1·02$\pm$0·04 (3)
α-Cocaine	20	1·04$\pm$0·05 (5)
Dibenamine	25–50	0·91$\pm$0·10 (5)
Dichloroisoprenaline	30	0·64$\pm$0·05 (5)

Foster, Ing & Varagić (1955) have shown that α-cocaine, a structural isomer of cocaine, has some local anaesthetic properties, inhibits monoamine oxidase to the same extent as cocaine but lacks the potentiating action on the catechol amines in the spinal cat. In 5 rats, α-cocaine had no action on the blood pressure response to noradrenaline or on the uptake of noradrenaline by the heart (Table 3).

Adrenaline-blocking agents. According to Brown & Gillespie (1957), blocking of the adrenaline receptors increased the noradrenaline output of the spleen, presumably by preventing the combination of noradrenaline with the receptors. In the present study two blocking agents were tested. Dibenamine (25 to 50 mg/kg), which blocks only α-receptors (Ahlquist, 1958), and the dichloro-analogue of isoprenaline (30 mg/kg), which blocks only β-receptors (Powell & Slater, 1958 ; Moran & Perkins, 1958). By themselves neither modified the concentration of noradrenaline in the heart (Table 1). In the pithed rat the maximum pressor effect of noradrenaline infused at a rate of 20 μg/20 min was 88$\pm$4 mm Hg, and it occurred within the first few minutes of starting the infusion. Dibenamine, injected before

the noradrenaline infusion, abolished completely the initial pressor effect of noradrenaline. However, during the course of the infusion the blood pressure rose steadily, reaching a maximum at the end of the infusion. Nevertheless, this rise of blood pressure (24 ± 4 mm Hg) was significantly lower than the maximum rise in control animals not given dibenamine. The uptake of noradrenaline by the heart was not significantly affected by doses of dibenamine which effectively blocked the α-receptors (Table 3).

The dichloro-analogue of isoprenaline, injected before the noradrenaline infusion, significantly increased the maximum blood pressure rise caused by noradrenaline (114 ± 4 mm Hg). The uptake of noradrenaline by the heart was completely blocked by the analogue (Table 3).

DISCUSSION

The results reported here show that cocaine blocked the noradrenaline uptake from the circulating blood by heart and spleen without affecting the endogenous concentrations of noradrenaline and adrenaline in these organs. Muscholl (1960b) found reserpine blocked the uptake of noradrenaline by the heart, but, in contrast to cocaine, it decreased the endogenous concentration of noradrenaline. There may be, therefore, a difference in the mechanisms by which cocaine and reserpine prevent the uptake by tissues of noradrenaline from the circulating blood.

The present results are in agreement with the work of Whitby *et al.* (1960), who found that an injection of 5 μg/kg of cocaine depressed the uptake of tritium-labelled noradrenaline by the adrenal glands, heart and spleen of the cat. There was a corresponding elevation of blood concentrations of noradrenaline within the first 3 min following the injection, as noted by Trendelenburg (1959).

The inhibition of the uptake of noradrenaline was related to the potentiation of the responses to noradrenaline observed after cocaine (Fig. 1). Moreover, with increasing doses of noradrenaline, much larger doses of cocaine were required to inhibit uptake (Fig. 2). Finally, the specificity of the action of cocaine was demonstrated. Of the four chemically related drugs, cocaine, atropine, amethocaine, and α-cocaine, only cocaine inhibited the noradrenaline uptake by the heart and caused supersensitivity towards noradrenaline.

Doses as small as 0.2 mg/kg of cocaine are known to potentiate the actions of noradrenaline on the nictitating membrane and spleen of the spinal cat (Trendelenburg, 1959), but, in the present experiments, doses of 0.15 and 0.3 mg/kg did not significantly reduce the uptake of noradrenaline by the heart (Fig. 2). This apparant lack of correlation between the effects of cocaine on the uptake and the potentiation of noradrenaline may be explained by the following considerations. With infusions of 20 and 5 μg of noradrenaline, the doses of cocaine needed to produce a 50% inhibition of the uptake of noradrenaline were 11.6 and 0.8 mg/kg, respectively Therefore relatively much smaller doses of cocaine are required to block the uptake of somewhat smaller amounts of infused noradrenaline. The doses of noradrenaline injected by Trendelenburg (1959) were smaller (3 to 16 μg/kg) than the lowest dose infused in the present experiments (5 μg per rat being about 25 μg/kg).

Although the action of cocaine on the noradrenaline uptake was specific in the sense that some closely related drugs did not possess such an effect, blocking of the noradrenaline uptake is also known to occur with reserpine and was seen with the dichloro-analogue of isoprenaline. The latter, like cocaine, did not affect the endogenous concentrations of noradrenaline in the heart, but the type of block exerted by these two drugs was quite dissimilar. It might be assumed that cocaine, apart from blocking the sites which take up noradrenaline from the blood, does not block the adrenaline receptors of the heart, since it does not block but potentiates noradrenaline effects on atria (Macmillan, 1959). The isoprenaline analogue, on the other hand, exerts a specific block of the adrenaline receptors of the heart (Moran & Perkins, 1958). Both agents reduce the uptake of noradrenaline by the heart to the same extent if employed in a sufficient dose. This indicates that block of adrenaline receptors by the dichloro-analogue of isoprenaline was not reflected in a greater inhibition of the noradrenaline uptake. Possibly the noradrenaline which combined with adrenergic receptors was metabolized and therefore could not be detected by the method used, whereas the noradrenaline taken up by the tissue stores was protected and could be estimated after extracting the whole heart. It is interesting to note that dibenamine, which does not block the receptors responsible for increase in rate and force of contraction of the heart (Nickerson, 1949), did not prevent noradrenaline from being held by the tissues.

From the present experiments the following conclusions may be drawn: Injected noradrenaline not only combines with adrenergic receptors but is stored in considerable concentrations at other sites. Cocaine prevents the uptake into these sites, thereby increasing the amount of noradrenaline available for combination with the adrenaline receptors. The dichloro-analogue of isoprenaline blocks *both* the uptake of noradrenaline into the stores and the combination with the adrenaline receptors.

This work was supported by a grant from the Deutsche Forschungsgemeinschaft. My thanks are due to Dr. H. R. Ing for a sample of α-cocaine and to Miss Gisela Ibeling for technical assistance.

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