Br. J. Pharmac. Chemother. (1968), **32**, 591–597.

EFFECTS OF COCAINE ON THE RATE OF CONTRACTION TO NORADRENALINE IN THE CAT SPLEEN STRIP: MODE OF ACTION OF COCAINE

BY

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(*Received November* 10, 1967)

The hypothesis widely used to explain the phenomenon of cocaine potentiation of the responses of smooth muscle to noradrenaline is that blockade of the uptake of noradrenaline into nerve terminals by cocaine leads to a greater concentration of noradrenaline in the immediate locale of the receptor (Iversen, 1967). Support for this hypothesis is largely circumstantial, such as the correspondence of uptake of various amines with the degree of potentiation by cocaine (Trendelenburg, 1965), or the route of administration of noradrenaline (de la Lande & Waterson, 1967).

This " blockade-of-uptake " concept has, however, been criticized on many grounds. Maxwell, Daniel, Sheppard & Zimmerman (1962) attack the fundamental assumption: they suggest that, *in vitro* at least, uptake would be insignificant compared with the quantity of agonist available in the medium and that with prolonged exposure it would be likely that the same equilibrium would be reached between bath and receptor whether or not cocaine was present. It was also observed that the dose of cocaine necessary to block uptake was not the same as that necessary to potentiate noradrenaline (Maxwell, Wastila & Eckhardt, 1966). Maxwell *et al.* (1962, 1966) and Bevan & Verity (1967) have suggested that cocaine has a direct action at the adrenergic receptor.

If the contention of Maxwell *et al.* (1962) that uptake could not indefinitely maintain a low concentration of noradrenaline at the receptor is correct, uptake could still affect the rate at which the concentration of agonist at the receptor equilibrates with the bath. In any case the effects of cocaine should be greatest near the beginning of the response, and the initial rate-of-rise of the response should be potentiated more than the final equilibrium contraction. Accordingly the rate of rise and the equilibrium contraction of responses to noradrenaline were measured before and after cocaine. Results indicate that " blockade-of-uptake " cannot completely explain potentiation by cocaine.

METHODS

Strips of spleen capsules were obtained from kittens (less than 2 kg body weight) killed by ether (Innes, 1962). The strips were mounted in 50 ml. baths in a Krebs solution (Na^+, 138.5 mM ; K^+, 4.36 mM ; Ca^{++}, 2.47 mM ; Mg^{++}, 1.16 mM ; Cl, 127.4 mM ; HCO_3, 21.9 mM ; H_2PO_4, 1.16 mM ;

glucose, 49.2 mM) at 37° C and aerated with 95% oxygen and 5% carbon dioxide. Isotonic contractions were recorded on a kymograph using a frontal lever. Isometric contractions were recorded with a strain gauge (Grass) and rectilinear oscillograph (Beckman Dynograph). The strips were at a resting tension of 1.5 g in both cases.

Measurements were made of the equilibrium height of contraction and maximum rate of contraction to various doses of noradrenaline as in Fig. 1. For purposes of comparison, data from each tissue were converted to percent of the greatest response of that tissue (which was not necessarily at the highest dose used). Potentiation of responses is taken as a lateral shift of the dose response curves measured at 50% of maximal response (that is, the ratio of the ED50s). Note that "rate-of-contraction" subsequently refers to the maximum rate of contraction at each dose. This maximum usually occurred very shortly after the start of the response, and was measured as the maximum tangent to the rising slope of the response (inset, Fig. 1).

Significance was tested by t test for paired data.

RESULTS

Isotonic recording

Plotting rate of contraction against dose of noradrenaline (Fig. 1a) gave a sigmoid dose-response curve, as was the dose-response relationship for equilibrium height of contraction (Fig. 1b). The rate of contraction, however, continued to increase at doses which gave maximal equilibrium contractions. The presence of cocaine hydrochloride 1 μg/ml. caused a potentiation of the height of contraction as shown by the shift of the dose-response curve to the left (Fig. 1b). This potentiation was significant ($P<0.05$) at doses of noradrenaline from 0.1 μg/ml. to 1.6 μg/ml. inclusive. The potentiation of ED50 was six-fold, and according to the "blockade-of-uptake" theory, this should represent a six-fold increase in the concentration of noradrenaline in the vicinity of the receptors.

In contrast to the effect of cocaine on the height of contraction, cocaine did not cause potentiation of the rate of contraction (Fig. 1a), and actually reduced the maximal rate of contraction produced by supramaximal doses. This reduction appeared to be the result of repeated prolonged exposure to high doses of noradrenaline, and occurred after the same total number of exposures to supramaximal doses even in the absence of cocaine. Results were obtained serially from low dose to high.

Isometric recording

Because the shortening of the muscles might interfere with the rate of shortening, the same experiments were carried out using an isometric recording technique. This procedure had the added advantage that the response time was much less, allowing shorter exposure of the tissue to noradrenaline, thus minimizing the possibility of a nonspecific desensitization limiting the contraction or rate-of-contraction and reducing the cocaine effects as may have occurred with the isotonic recording.

Results similar to those with isotonic recording were obtained with isometric recording. Cocaine caused a large potentiation (6.5-fold ratio of ED50s) of the equilibrium contraction (Fig. 2b) but no potentiation of the contraction rate (Fig. 2a) (1.3-fold ratio of ED50s which was not significant in this dose range). While there was a significant three-fold potentiation for the ED20, the equilibrium contraction was potentiated eight-fold in the same dose range (4–16 μg/ml.).

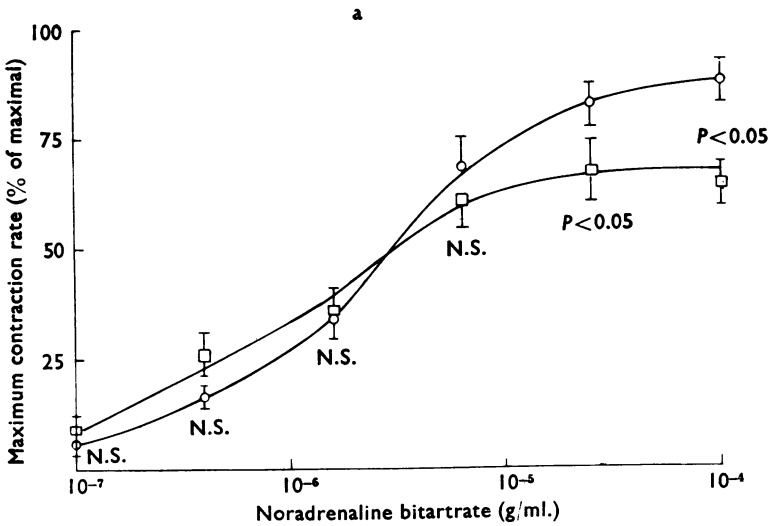

FIG. 1a

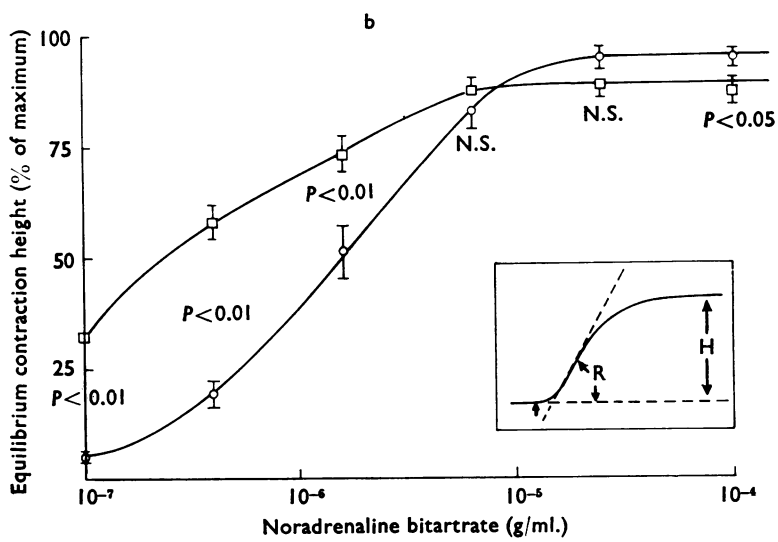

FIG. 1b

Fig. 1. Effect of cocaine on maximum rate (a) and equilibrium height (b) of isotonic contractions of kitten spleen to noradrenaline. Before (○) and after (□) cocaine sulphate 1 μg/ml. Bars represent standard errors ($n=10$) calculated on non-paired basis. Probabilities were obtained by t test for paired data. N.S.=not significant ($P>0.05$). Inset: Drawing of response showing where measurements were made. The maximum rate of responding was measured as the maximum tangent (R) to the rise of the response: H is the equilibrium height measurement.

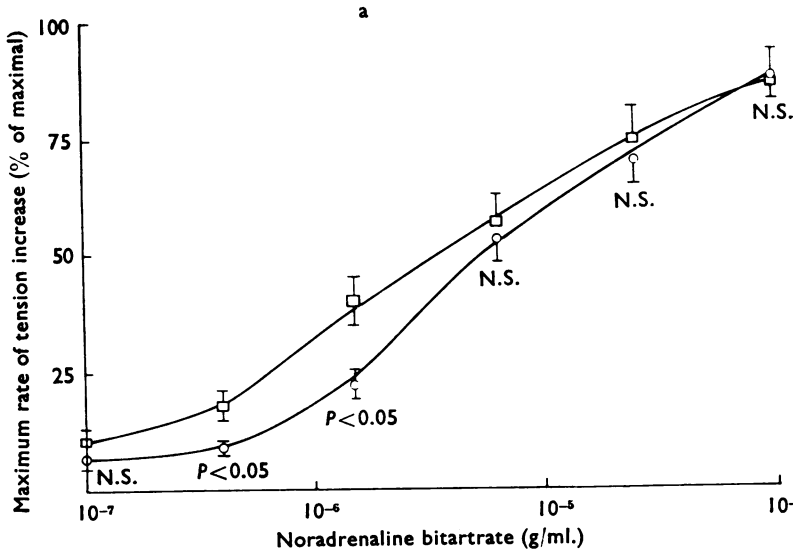

FIG. 2a

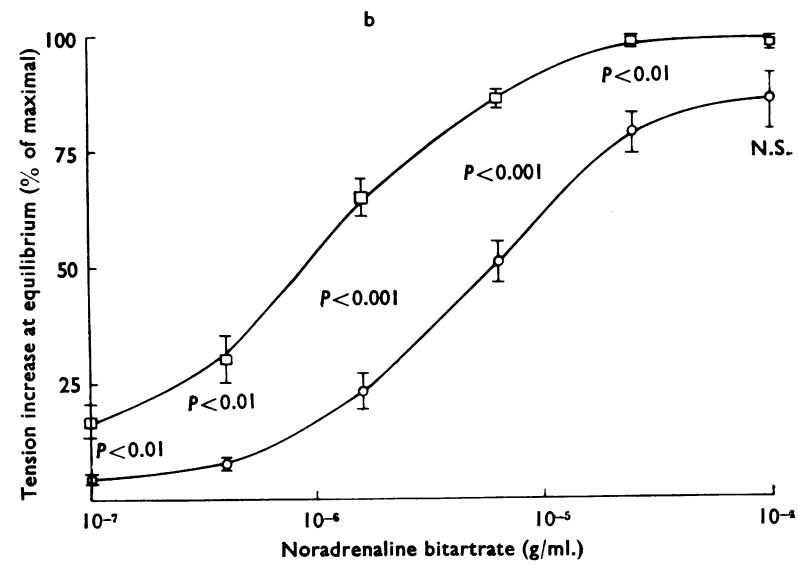

FIG. 2b

Fig. 2. Effect of cocaine on maximum rate (a) and equilibrium tension (b) of isometric contractions of kitten spleen capsule to noradrenaline. As for Fig. 1, except $n=9$.

There was no depression of contraction rate or equilibrium in the isometric experiments at the high doses.

Dose-effect of cocaine

Several experiments were performed to discover effects on the response to the ED50 dose of noradrenaline (about 6 μg/ml.) of varying the dose of cocaine from 0.01 to 100 μg/ml. With either method of recording, increasing the cocaine concentration caused successive increase in the contraction equilibrium with little change in rate of contraction. Thus results in the previous experiments did not depend on the dose of cocaine used.

DISCUSSION

Assuming that the uptake theory of cocaine potentiation is correct, one would, because the rate of rise of the response shows a normal dose-response relationship, predict that the rate of contraction at any submaximal dose of noradrenaline would be potentiated by cocaine, at least to the same degree as the final (equilibrium) height of contraction. At any time after the addition of noradrenaline a greater amount of noradrenaline should have reached the receptors, and hence a greater force and a greater rate of contraction should be developed, when cocaine has blocked the uptake. This potentiation should be particularly marked near the beginning of the response, which is where the measurements of the (maximum) contraction rate were made. If cocaine potentiates the equilibrium six-fold by blocking uptake, then in the absence of cocaine five-sixths of the noradrenaline diffusing to the locale of the receptor must be removed by uptake. Near the beginning of the response the uptake capacity would be very great compared with the amount of noradrenaline at the receptor. Thus the potentiation by cocaine should actually be greatest near the beginning of the response. However, this predicted potentiation of the rate of contraction did not occur.

There are other serious deficits in the blockade-of-uptake hypothesis. The concept assumes that the sites of uptake are located close enough to the receptors that uptake can materially affect the concentration of agonist at the receptors, and prevent equilibration with the bathing medium. For this to be so, both sites would have to be located together in a region of limited access for noradrenaline. This does not seem to be the case in most smooth muscles where receptors are not all located in close proximity to adrenergic synapses—and the spleen capsule in particular would seem to be not directly innervated, but responses to sympathetic nerve stimulation result from noradrenaline diffusing away from adrenergic innervation of splenic blood vessels (Haefely, Hürliman & Thoenen, 1964). If the amount of noradrenaline in the bath is inexhaustible compared with the amount taken up into nerve terminals, then at equilibrium the concentration of noradrenaline at receptors not in close proximity to nerve terminals should reach the same concentration as that of the bathing medium. This should not be affected by cocaine. Thus the same equilibrium contraction should occur regardless of cocaine—which is obviously not the case.

An alternative explanation is that cocaine also acts near the receptor to alter drug-receptor combination as originally proposed by Maxwell, Plummer, Daniel, Schneider &

Povalski (1958). The term " allosteric " might be appropriate to describe the deformation of the receptor by cocaine. The inhibition of uptake may be coincidental and unrelated to potentiation of noradrenaline or, at most, only partly responsible for potentiation.

In describing this effect in terms of " occupancy " receptor kinetics it is immediately apparent that potentiation could not be the result of an increase in receptor efficacy (intrinsic activity), because such a change should also cause an increased rate of rise of the response. An increase in affinity might explain the potentiation of equilibrium without potentiation of contraction rate, particularly if the increased affinity was achieved by a decreased dissociation ; however, in cases of relatively free access of drug to receptor an increase in affinity might well result in more rapid occupancy of receptors and hence a greater rate of contraction.

Paton's (1961) " rate " hypothesis of receptor activation allows a simpler solution. Although the spleen capsule does not respond with the peak phase predicted by the rate-theory it is reasonable to assume that (1) the rate of contraction is mechanically limited so that the fade sets in before the peak is reached, and (2) the maximum contraction rate is the result of the same force development as is responsible for the peak in othr tissues. This force is proportional to " k_1x " where " k_1 " is the association rate constant for the drug-receptor complex and " x " is the drug concentration. I suggest that the cocaine-induced receptor deformation may cause an increased " offset " (k_2) of the drug from the receptor, thus increasing receptor availability at equilibrium. The equilibrium (proportional to $\frac{k_2 x}{x + k_2/k_1}$) would be expected to increase, but the rate of rise (proportional to k_1x) should not.

Thus while blockade-of-uptake is probably sufficient to explain potentiation of noradrenaline *in vivo*, where uptake could materially reduce circulating levels of noradrenaline, it does not seem to be sufficient for potentiation *in vitro*. It seems more likely that the main mode of potentiation by cocaine is a direct effect on the adrenergic receptor to allow increased utilization of receptors.

SUMMARY

1. According to the " blockade-of-uptake " concept, cocaine should potentiate the rate at which equilibrium contraction is reached more than the final equilibrium, *in vitro*.

2. Cocaine did not potentiate contraction rate although the final contraction height was potentiated.

3. It is suggested that " blockade-of-uptake " is insufficient to explain potentiation by cocaine *in vitro*.

4. Cocaine may deform adrenergic receptors to alter receptor kinetics, to allow increased receptor utilization.

The author is grateful to Professor C. W. Nash for many helpful discussions, to Miss Joyce Miyashita, Miss Pat Moore and Miss Gail Mydansky for conducting a preliminary study, and to Mr. John Lejderman for his technical assistance. This work was supported by the Alberta Heart Foundation.

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