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INTERACTIONS BETWEEN COCAINE, TYRAMINE AND NORADRENALINE AT THE NORADRENALINE STORE

BY

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In the cat, the pressor actions of noradrenaline and of adrenaline were generally reduced and that of tyramine was increased during infusions either of noradrenaline or of adrenaline. The increase in the response to tyramine after an infusion of noradrenaline was prevented by cocaine, methyl phenidate and pipradrol. Pipradrol, unlike its isomer azacyclonol, increased the responses to catechol amines but reduced that to tyramine. Cocaine did not prevent the increase in the noradrenaline content of cat kidney and uterus after an infusion of noradrenaline. In the pithed rat, cocaine increased the pressor response to noradrenaline but antagonized that to tyramine. Treatment of the rat with reserpine prevented the effect of cocaine on the response to tyramine but did not modify the potentiation of the response to noradrenaline. Prolonged treatment with cocaine did not lower the tissue noradrenaline levels and did not prevent the noradrenaline depletion by reserpine. It is suggested that interactions between cocaine, tyramine and noradrenaline occur at the point where noradrenaline enters its tissue store.

Cocaine increases pressor and nictitating membrane responses to catechol amines in the cat (Fröhlich & Loewi, 1910; Fleckenstein & Bass, 1953) but depresses responses to tyramine (Tainter & Chang, 1927). The participation of a noradrenaline store in these actions has recently been studied, as Burn & Rand (1958) suggested that the effects of tyramine are mediated by a release of noradrenaline from the walls of arteries. It has been suggested that cocaine may prevent the release of noradrenaline by tyramine (Bejrablaya, Burn & Walker, 1958) and the uptake of noradrenaline by the tissue stores (MacMillan, 1959). However, the output of noradrenaline from the cat spleen during stimulation of the splenic nerves is not reduced in the presence of cocaine (Trendelenburg, 1959). On the other hand the incorporation of [^{3}H]-noradrenaline (Whitby, Hertting & Axelrod, 1960) and the uptake of noradrenaline after an infusion (Muscholl, 1961) are prevented by cocaine.

The present work was undertaken to investigate whether events at the tissue store of noradrenaline play a part in interactions between cocaine, tyramine and noradrenaline.

METHODS

Some cats (1 to 3 kg, of either sex) were made spinal by Dale's method modified as described by Burn (1952), and other cats were anaesthetized with chloralose (60 to 80 mg/kg)

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and sodium pentobarbitone (Nembutal, 6 mg/kg) intravenously. All cats were bilaterally vagotomized. The left common carotid artery was cannulated for recording blood pressure by a mercury manometer. Responses of the right nictitating membrane were sometimes recorded (Burn, 1952) when the preganglionic superior cervical nerve was stimulated using electrodes attached to a Palmer's Student stimulator (1.0 V, 10 shocks/sec for bursts lasting 2 sec, pulse duration 0.3 msec). A femoral vein was cannulated for the administration of single injections. When infusions were given, the other femoral vein was cannulated with polyethylene tubing attached to a Palmer's slow injection pump; this permitted the study of responses to single injections of drugs during an infusion. The influence of cocaine on the increase in noradrenaline content of the uterus and kidney was also studied (Pennefather & Rand, 1960).

Rats (180 to 210 g, Wistar strain) were pithed by the method of Shipley & Tilden (1947), artificially ventilated by a pump and prepared for recording of arterial blood pressure by a procedure similar to that of Brown & Gillespie (1957). Injections were made into the left femoral vein, and blood pressure was recorded from the right common carotid artery using a Condon mercury manometer. Atropine (0.5 mg subcutaneously) was given if the preparation was to be used to assay extracts for noradrenaline.

Extraction of noradrenaline

Plasma samples from the rat or cat were collected into centrifuge tubes containing 0.05 ml. of heparin (5,000 U/ml.). The samples were immediately centrifuged (3,000 rev/min for 15 min) and the plasma was drawn off with a syringe and stored at $-10°$ C.

Cat kidney and uterus, rat heart, spleen, duodenum and uterus were removed and extracted by the method of Burn & Rand (1959). In each experiment the assay was performed on the same or the subsequent day to the extractions, and control experiments showed no loss of activity within 24 hr.

Assay of noradrenaline

Extracts were assayed for activity in terms of noradrenaline on the blood pressure of the atropinized, pithed rat. Repeated doses of the kidney extracts were given until the effects of the slow pressor substance were eliminated (Pennefather & Rand, 1960). Noradrenaline contents of tissues are expressed as ng/g, except for rat spleen, for which the total content is given in ng.

Drugs

The drugs used, with their standard doses, were as follows: noradrenaline (2 μg in the cat; 10 ng in the rat), tyramine (200 μg in the cat; 5 μg in the rat); other drugs used in the cat experiments were adrenaline (5 μg), α-methyl noradrenaline (Corbasil, 5 μg), ephedrine (500 μg), cocaine (4 mg/kg), methyl phenidate (4 mg/kg), pipradrol (0.5 mg/kg) and azacyclonol (2 to 32 mg/kg). All these drugs were given intravenously. Some rats received reserpine (5 mg/kg) intraperitoneally 24 hr before each experiment. In the experiments where standard doses of amines were given during intravenous infusions of noradrenaline or of adrenaline, the rates of infusion ranged between 2.75 and 44 μg/min (100 μg/ml. in solution). In these experiments the initial pressor response to the infusion was allowed to subside until the blood pressure was steady either at the level observed before the infusion or at a slightly higher level. The standard doses of the amines were then injected during the infusion, usually about 15 to 30 min after its start. In some experiments the blood pressures of anaesthetized cats given the highest rate of infusion (44 μg/min) did not return to the starting level and the effects of the single doses were then masked.

RESULTS

Effects of noradrenaline infusions on responses to single standard doses of noradrenaline and adrenaline in the cat. Percentage changes in the pressor activity of the single injections of noradrenaline are shown in Fig. 1. The lowest rate of

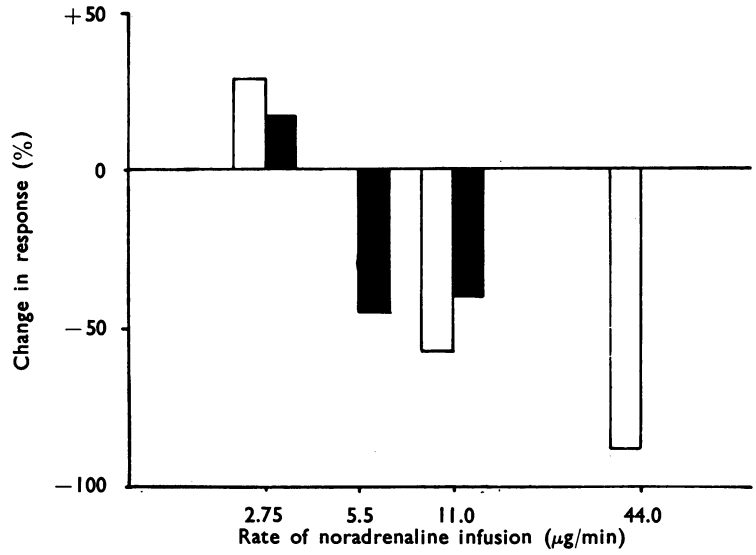

Fig. 1. Effect of rate of infusion of noradrenaline on the pressor response to a single injection of noradrenaline (2 μg) in cats. The response is expressed as a percentage change in the response to the same dose before the infusions. The mean effects are shown for five spinal cats (open columns) and for five anaesthetized cats (closed columns). The higher rates of infusion depressed the responses to noradrenaline.

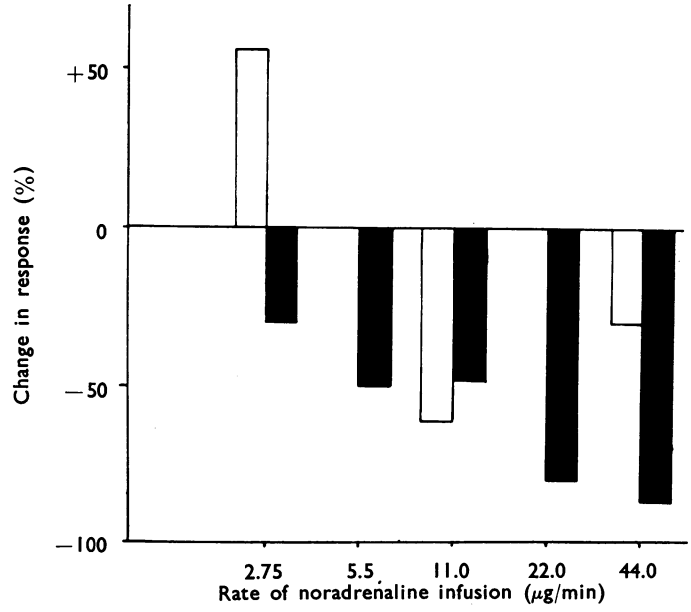

Fig. 2. Effect of rate of infusion of noradrenaline on the pressor response to a single injection of adrenaline (5 μg) in cats. The response is expressed as a percentage change in the response to the same dose before the infusions. The mean effects are shown for five spinal cats (open columns) and for five anaesthetized cats (closed columns). The higher rates of infusion depressed the responses to adrenaline.

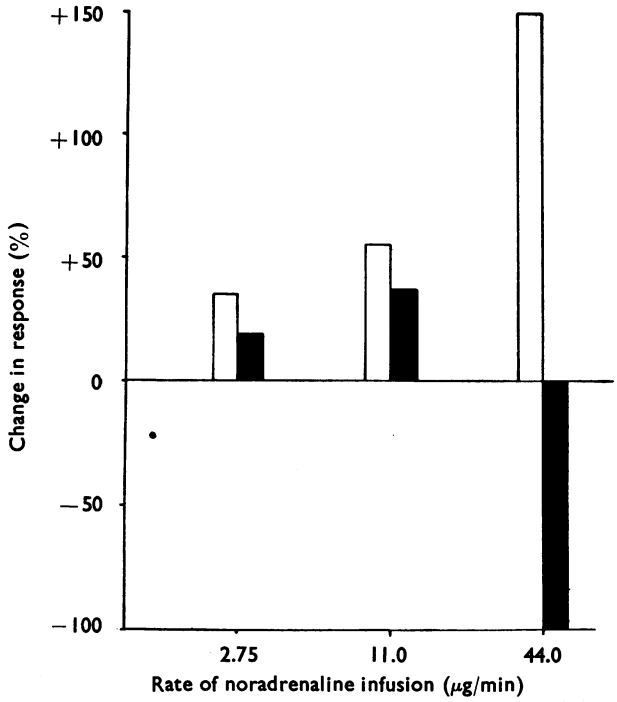

Fig. 3. Effect of rate of infusion of noradrenaline on the pressor response to a single injection of tyramine (200 μg) in cats. The response is expressed as a percentage change in the response to the same dose before the infusions. The mean effects are shown for five spinal cats (open columns) and for five anaesthetized cats (closed columns). In cats of the spinal group, an increase in the rate of noradrenaline infusion caused a progressive increase in the response to tyramine.

noradrenaline infusion increased the pressor response to the single injection of noradrenaline, but higher rates depressed the response.

In spinal cats the pressor response to the single injections of adrenaline was also increased during the lowest rate of noradrenaline infusion, but in the anaesthetized cats this response was decreased (Fig. 2). Higher rates of noradrenaline infusion depressed the adrenaline responses in cats of both groups.

Effects of noradrenaline infusions on responses to single standard doses of tyramine in the cat. In spinal cats there was a progressive increase in the response to tyramine as the rate of noradrenaline infusion increased (Fig. 3). In the anaesthetized cats responses to tyramine increased at the lower rates of infusion, but the pressor effect of the infusion of 44 μg/min of noradrenaline was so intense that the action of tyramine was completely masked.

Effects of adrenaline infusions on responses to single standard doses of noradrenaline, adrenaline and tyramine in the cat. The effects of adrenaline infusions on responses to the sympathomimetic amines were similar to, but smaller than, those produced by infusions of noradrenaline. Increasing the rate of infusion decreased progressively the pressor responses to noradrenaline, but increased the

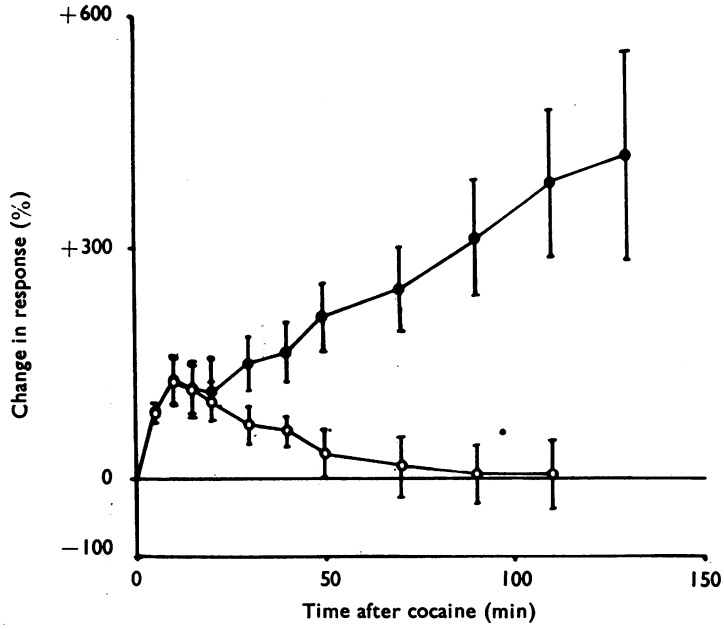

Fig. 4. The mean percentage changes in pressor responses to noradrenaline after a single dose of cocaine (4 mg/kg) in four untreated (●——●) and in four reserpine-treated (○——○) pithed rats. The upright bars represent the standard errors of the means. The initial increase in the response to noradrenaline after cocaine was still present after treatment with reserpine.

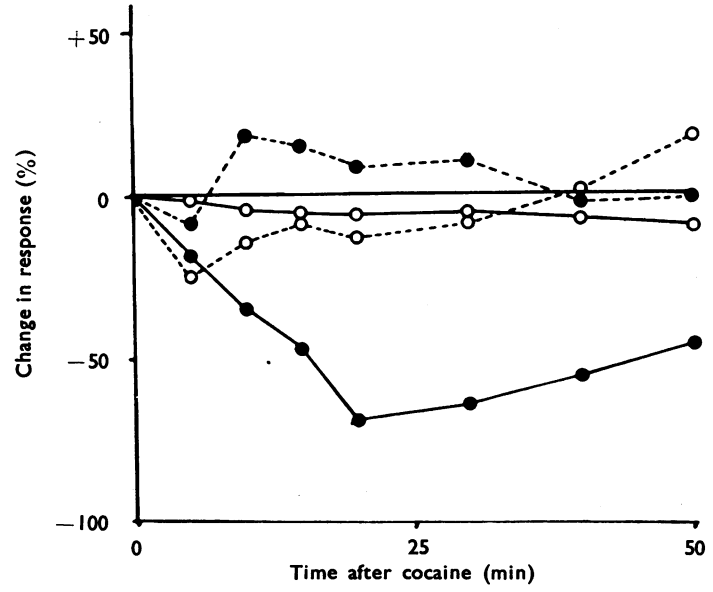

Fig. 5. Effect of cocaine on pressor responses to tyramine in control (solid lines) and reserpine-treated (broken lines) pithed rats. Open circles, responses to tyramine after cocaine (2 mg/kg); closed circles, after cocaine (4 mg/kg). The reduction in response to tyramine by cocaine (4 mg/kg) was abolished by treatment with reserpine.

responses to tyramine. Adrenaline infused at the lower rates slightly increased the pressor responses to adrenaline, but at the highest rate the response was depressed.

Effect of cocaine, methyl phenidate and pipradrol on responses to sympathomimetic amines. Cocaine, methyl phenidate and pipradrol each increased both pressor and nictitating membrane responses of the spinal cat to the standard doses of noradrenaline, adrenaline and α-methyl noradrenaline but reduced the responses to tyramine and to ephedrine. The response of the nictitating membrane to preganglionic nerve stimulation was also increased. In contrast, azacyclonol in doses up to 32 mg/kg did not modify any of these responses.

Interactions between cocaine, noradrenaline and tyramine were studied in more detail using the blood pressure of the pithed rat. When cocaine (2 or 4 mg/kg) was injected during a series of injections of standard doses of noradrenaline, the initial subsequent response to noradrenaline was always increased. This initial increase in response was followed by a slight decline, and then a marked secondary increase (Fig. 4). In rats previously treated with reserpine, the initial increase in response to noradrenaline after cocaine was still present, but the secondary increase was abolished.

When cocaine (4 mg/kg) was injected into pithed rats during a series of injections of standard doses of tyramine, the responses to subsequent doses of tyramine were reduced (Fig. 5). A smaller dose of cocaine (2 mg/kg) did not reduce the responses to tyramine. After reserpine the reduction in responses to tyramine by cocaine (4 mg/kg) was abolished (Fig. 5).

Effect of noradrenaline infusions on the pressor response to tyramine in spinal cats after cocaine, methyl phenidate or pipradrol. The increase in response to

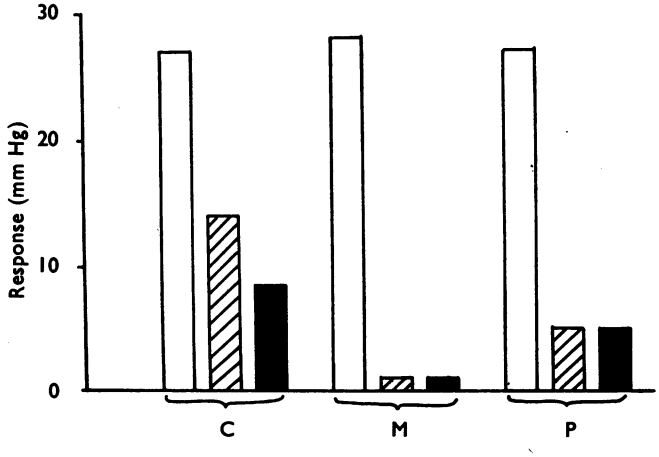

Fig. 6. Effect of infusions of noradrenaline (1 mg over 40 min) on the pressor responses to tyramine (200 μg) in spinal cats, after treatment with cocaine (C, 4 mg/kg), methyl phenidate (M, 4 mg/kg) or pipradrol (P, 0.5 mg/kg). Each drug was injected intravenously 20 min before the infusion of noradrenaline. Open columns, responses before treatment; hatched columns, after treatment; filled columns, after treatment and after infusion of noradrenaline. The increase in response to tyramine after an infusion of noradrenaline was prevented by cocaine, methyl phenidate and pipradrol.

tyramine after an infusion of noradrenaline (1 mg over 40 min) was invariably prevented by cocaine, methyl phenidate or pipradrol. Each drug was tested in three cats, and Fig. 6 shows the pressor responses to tyramine observed in one experiment from each group of cats. In contrast, after reserpine, the response to tyramine is increased by an infusion of noradrenaline (Burn & Rand, 1958).

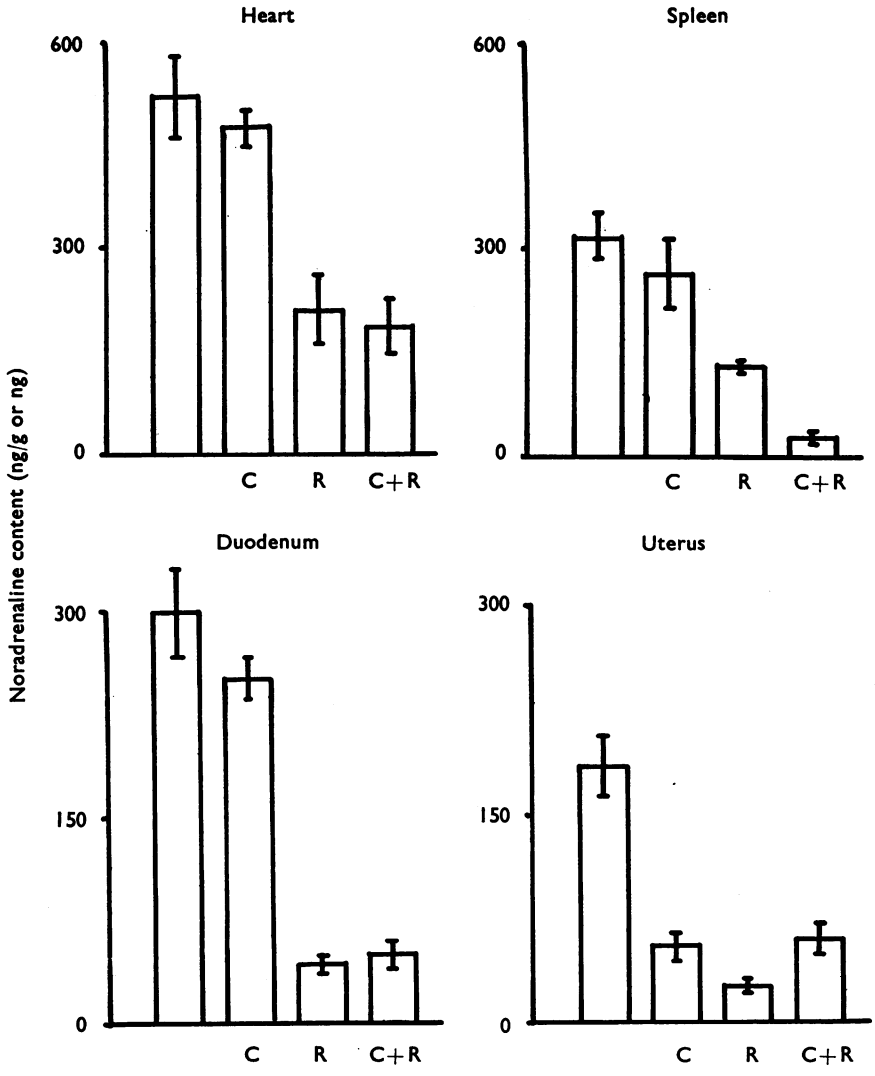

Fig. 7. Effect of cocaine (C, 8 mg/kg/day for 8 days), reserpine (R, 5 mg/kg for 1 day), or both cocaine and reserpine (C+R) on the noradrenaline content of rat heart, uterus, duodenum (expressed as ng/g) and spleen (expressed as total ng). The left-hand columns show the noradrenaline content in organs from untreated rats. After the last dose had been given the rats were pithed on the following day and organs taken 1 hr later. The upright bars represent the standard errors of the means. Treatment with cocaine did not significantly lower noradrenaline levels except in the uterus. The effect of reserpine was not prevented by cocaine.

Effects of cocaine and reserpine on the noradrenaline content of rat tissues. The mean noradrenaline levels of organs taken from rats 1 hr after pithing are shown in Fig. 7. Each group contained ten rats. Rats of one group were injected intraperitoneally with cocaine (8 mg/kg) for 8 days and pithed on the ninth day ; only in the uterus was the noradrenaline depleted. A single dose of reserpine (5 mg/kg) given the previous day lowered the noradrenaline concentration in all four tissues. When reserpine was injected 5 min after the eighth dose of cocaine, and the rats pithed on the following day, the depletion of noradrenaline in the heart and duodenum was not altered, that in the uterus was slightly less, and that in the spleen was greater.

Effect of cocaine on the uptake of noradrenaline by cat tissues. With five spinal cats, the effect of cocaine on noradrenaline uptake by the kidney and by the uterus was studied. The noradrenaline content of organs taken 20 min after the end of the infusion of noradrenaline (1 mg over 40 min) was compared with that of control organs taken before the infusion (Fig. 8). Cocaine (8 mg/kg) was injected intravenously in four divided doses (two at about 20 min before the infusion of noradrenaline, one 10 min before, and one just after the infusion had begun). In both the kidney and in the uterus the uptake of noradrenaline still occurred in the presence of cocaine. This lack of effect of cocaine may be the result of it competing with the large amount (1 mg) of noradrenaline infused, thereby displacing the cocaine from its site of action.

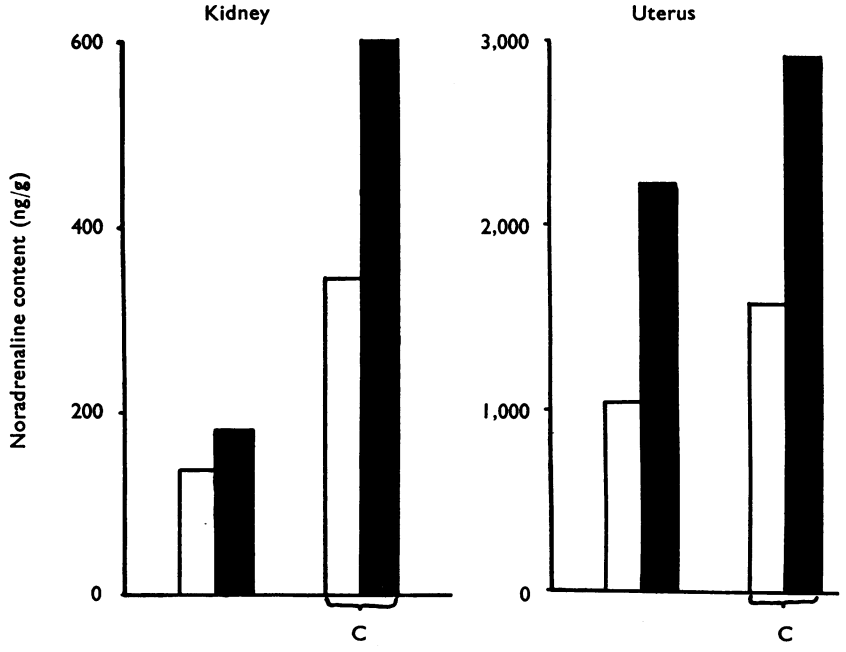

Fig. 8. Effect of cocaine (C, 8 mg/kg intravenously in four divided doses) on the increase in noradrenaline content (ng/g) of the cat kidney and cat uterus after an infusion of noradrenaline (1 mg over 40 min). Open columns, before noradrenaline; closed columns, after noradrenaline. Noradrenaline uptake was not prevented by the cocaine.

DISCUSSION

Infusions of noradrenaline and adrenaline

In the spinal cat high rates of infusion (2.75 μg/min and above) either of noradrenaline or of adrenaline increased the pressor response to a single dose of tyramine. Although tyramine is thought to act by releasing noradrenaline from a store (Burn & Rand, 1958), the response to a single dose of noradrenaline was decreased during the high rates of infusion (5.5 μg/min and above). Nasmyth (1962) has suggested that the action of tyramine may be modified by variations in the concentration of noradrenaline in the extracellular fluid, while Muscholl (1960) found that in the rat treated with reserpine noradrenaline increased the pressor responses to tyramine without replenishing the noradrenaline in the tissue stores. The present results show that as the rates of infusion of noradrenaline or of adrenaline were increased so the pressor response to tyramine was further increased but that to noradrenaline was further decreased. It is possible, therefore, that tyramine enters the tissue store by the same route as noradrenaline and thereby impedes its entry; when tyramine is present, a greater proportion of the circulating catechol amine may be diverted from the store and more may reach the receptor.

Actions of cocaine

In the spinal cat, cocaine prevented the potentiation of the pressor response to tyramine by an infusion of noradrenaline. The depleting drug reserpine, however, does not prevent this potentiation (Burn & Rand, 1958). In the pithed rat, prolonged treatment with cocaine did not impede the access of reserpine to the tissue store and generally did not itself deplete the tissue noradrenaline. These findings indicate that cocaine may be acting at the site where tyramine and noradrenaline interact.

In the rat, cocaine did not antagonize pressor responses to tyramine after treatment with reserpine, yet the potentiation of noradrenaline was unchanged. After reserpine the residual action of tyramine may be exerted directly on the receptor, and any interaction between cocaine and tyramine elsewhere may not further reduce the response to tyramine. Although cocaine prevents the uptake of noradrenaline by rat heart and spleen (Muscholl, 1961) it was not possible to demonstrate this for cat kidney and uterus by the method of Pennefather & Rand (1960).

Actions of methyl phenidate and pipradrol

In the cat, methyl phenidate and pipradrol had actions similar to those of cocaine in that they increased the pressor and nictitating membrane responses to catechol amines, yet reduced the responses to tyramine and ephedrine. Azacyclonol, an isomer of pipradrol, was inactive. Methyl phenidate and pipradrol, like cocaine, also prevented the potentiation of the responses to tyramine by an infusion of noradrenaline. In one respect, molecules of cocaine, methyl phenidate and pipradrol are similar to those of noradrenaline, for they contain nitrogen and oxygen atoms which can come together by free rotation. Interactions between these drugs may depend on this chemical similarity, for the inactive substances α-cocaine (Varagić, 1957) and azacyclonol do not have this stereochemical feature.

Mechanism of interaction

These results are consistent with the hypothesis that tyramine and noradrenaline enter the tissue store by a common pathway which is competed for by cocaine, methyl phenidate and pipradrol. When tyramine enters the tissue store before releasing noradrenaline, it may impede the entry of circulating noradrenaline. When there is more circulating noradrenaline, then the proportion of amine reaching the receptor may be increased. In a similar way, cocaine may increase responses to noradrenaline by diverting part of the injected amine from the store onto the receptor. In addition, cocaine may prevent tyramine from entering the store and releasing noradrenaline and so antagonize its effect. Thus, all drugs acting at the point where noradrenaline enters its tissue store may have effects which form part of a continuous series. Hence, noradrenaline passes rapidly through into the store, tyramine passes less quickly and thus may impede the entry of circulating noradrenaline for a time, while cocaine, methyl phenidate and pipradrol do not pass through into the store and may remain attached to the site impeding the access of both noradrenaline and tyramine.

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