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A HYPOTHESIS CONCERNING THE EFFECT OF COCAINE ON THE ACTION OF SYMPATHOMIMETIC AMINES

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There is a store, perhaps in chromaffin tissue, of noradrenaline in tissues with a sympathetic innervation. The store is depleted by treatment with reserpine. Sympathomimetic amines like tyramine act only when this store is present and have no effect when the store is depleted. They also fail to act in the presence of cocaine. Catecholamines like noradrenaline have a much greater action than usual when the store is depleted, and they have a much greater action in the presence of cocaine. It is suggested that cocaine has the effect of blocking release from the store, so that the action of tyramine is abolished and the spontaneous release from the store which is responsible for the normal (low) sensitivity to noradrenaline is stopped. Noradrenaline is taken up into the store in the heart and the vessel wall from the blood, and the disappearance of noradrenaline from the blood is in part due to this uptake and not entirely to destruction. Cocaine may prevent this uptake of noradrenaline by the tissue stores. Experiments on rabbit atria, on the vessels of the rabbit ear and on the heart-lung preparation are described which are consistent with this hypothesis.

An early observation on cocaine was made by Fröhlich and Loewi (1910), who found that it potentiated the action of adrenaline. Tainter and Chang (1927) found that cocaine affected the action of tyramine in the opposite way, reducing or abolishing it. Recently the observation has been made that similar changes occur in animals treated with reserpine; the effects of adrenaline and noradrenaline are potentiated (Bein, Gross, Tripod and Meier, 1953) while the effect of tyramine is abolished (Carlsson, Rosengren, Bertler and Nilsson, 1957). Burn and Rand (1958b) showed that treatment with reserpine removed the store of noradrenaline from the vessel wall and suggested that, while the effects of reserpine were due to the disappearance of the store, cocaine might act by stopping release from the store (Burn and Rand, 1958c); the action of noradrenaline would then be potentiated because more receptors would be free for injected noradrenaline to occupy. Similarly if release from the store was stopped, tyramine would be ineffective. Observations have now been made to test this view of the action of cocaine.

These have been made first on the rate of the isolated atria of the rabbit heart, in which Burn and Rand (1958a) found that when the store of noradrenaline was present the rate was higher. Observations have also been made in the perfused rabbit ear and in the heart-lung preparation of the dog.

METHODS

Rabbit atria were suspended in a 40 ml. bath containing McEwen (1956) solution at 30° aerated with O_2 and 5% CO_2. The upper end of the atria was attached to a spring lever carrying a wire which dipped into a salt solution so that, with every contraction, a circuit containing a post office counter was completed. This recorded atrial beats, which were expressed as beats/minute. When treating rabbits with reserpine, the drug was prepared in a 20% solution of ascorbic acid. Rabbits weighing 2.0 to 2.5 kg. received 3.0 to 5.0 mg. of reserpine intraperitoneally on the first and second day, the amount given on the second day depending on the general condition of the animal. On the third day the rabbit was decapitated, and the atria were removed. Observations were also made by perfusing the vessels of the rabbit ear and recording the outflow with a recorder (Stephenson, 1948). Locke solution was used as the perfusion fluid. It was aerated with O_2 before being placed in the Marriotte bottle. The perfusions were carried out at room temperature. Observations were also made in the heart–lung preparation of the dog. The blood used to fill the reservoir was taken from

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one dog which was anaesthetized with ether; the preparation was then made in a second dog which was first anaesthetized with ether and then with chloralose. The heart rate was counted from an electrocardiogram recorder (Cossor model 1314).

The weights of cocaine refer to the hydrochloride.

Results

Effect of Cocaine on the Atrial Rate.—When normal atria were exposed to cocaine hydrochloride for periods up to 30 min., no change in atrial rate was observed until the concentration was raised to 7.5×10^{-6} or to 10^{-5} g./ml. A decrease in rate was then observed in the presence of cocaine, the rate recovering its former value when the cocaine was removed from the bath. In some experiments (see Fig. 1), the drop in rate in the presence of cocaine became progressively

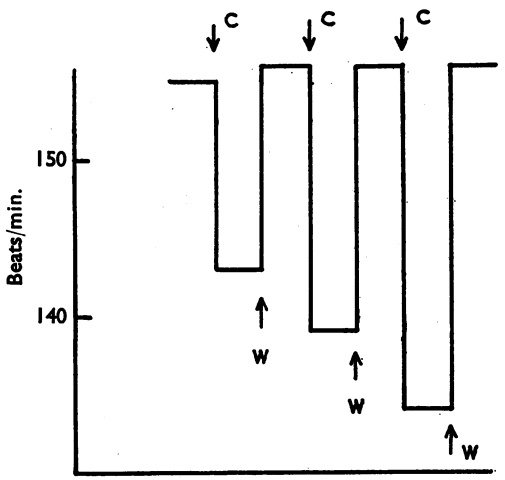

FIG. 1.—Atria from normal rabbit. Ordinates are the atrial rate. At C cocaine (10^{-5} g./ml.) was added to the bath. This slowed the rate in the next 30 min. as shown. When cocaine was removed at W the rate returned to the initial level in about 5 min.

greater with successive applications of cocaine. The results in different atria are shown in Table I in which the decrease recorded for each preparation was observed three or four times.

TABLE I

EFFECT OF COCAINE HYDROCHLORIDE ON RATE OF NORMAL ATRIA AFTER APPLICATION FOR 30 MIN. Cocaine concentration 10^{-5} g./ml. Values are given as beats/min.

Expt. No.	Decrease of Rate in Successive Trials
1	10, 8, 8
2	12, 16, 18, 18
3	6, 13, 10, 11
4	9, 12, 11, 6
5	12, 17, 22
6	11, 22, 35

Effect of Cocaine on Atria from Rabbits Treated with Reserpine.—When the atria were removed from rabbits treated with reserpine, their rate of beating was lower than that of atria from normal rabbits, the mean rate being 115/min. compared with 130/min. When the atria were exposed to cocaine for 30 min., the effect was not the same in all preparations. In 4 in which the spontaneous rate was low, namely 114/min., and in which reserpine appeared to have exerted a full effect, the rate was depressed in the presence of cocaine, but was not restored when the cocaine was removed. An example of such a preparation is given in Fig. 2. In one other preparation, there was a partial restoration on washing out the cocaine, but, as the experiment progressed, the restoration was less and less complete. Finally in 2 in which the spontaneous rate was high, namely 140/min., the rate was depressed in the presence of cocaine, and was restored when it was washed out as in normal atria.

Potentiation of Adrenaline by Cocaine.—Evidence that cocaine potentiates the effect of adrenaline on the isolated heart of the cat was obtained by Burn and Tainter (1931), but there is no previous evidence that cocaine potentiates the action of adrenaline on the atria. Adrenaline in a concentration of 10^{-7} g./ml. caused a rise of rate of 10 to 30 beats/min. when allowed to act for 10 min., and this effect was found to be increased in the presence of cocaine hydrochloride 10^{-6} g./ml. An example of an experiment is given in Table II. When an observation

TABLE II

INCREASE OF ATRIAL RATE BY ADRENALINE AND ITS POTENTIATION WHEN COCAINE WAS ADDED 10 MIN. PREVIOUSLY

The values are increase in beats/min. observed consecutively in one experiment. Adrenaline concentration, 10^{-7} g./ml.; cocaine concentration, 10^{-6} g./ml.

Adrenaline	30	
Adrenaline + cocaine		43
Adrenaline	11	
"	10	
Adrenaline + cocaine		50
Adrenaline	31	
Adrenaline + cocaine		35
Adrenaline	14	
Adrenaline + cocaine		46
	19	43

was to be made in the presence of cocaine, it was first added to the bath and time was allowed to ensure that it did not modify the rate before adrenaline was added. Table II shows that in the presence of cocaine the mean increase in rate caused by adrenaline was more than twice that in the absence of cocaine. The mean effects in three experiments are given in Table III.

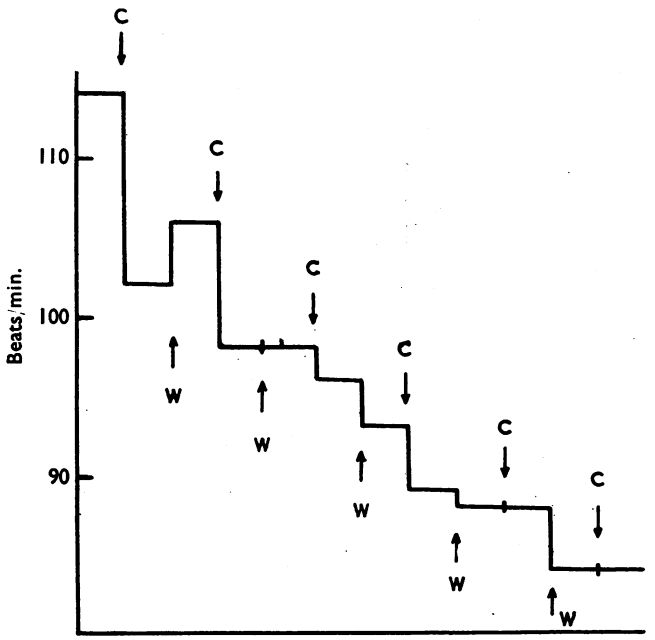

FIG. 2.—Atria from a rabbit treated with reserpine. Ordinates are the atrial rate. Note that, although the addition of cocaine (C) slowed the rate, removal of the cocaine (W) did not restore the rate except partly after the first addition.

Potentiation of Noradrenaline by Cocaine.—In other atria the action of noradrenaline was examined. Whereas adrenaline was tested in a concentration of 10^{-7} g./ml., noradrenaline was applied in half this concentration, and it appeared to cause a greater rise in the rate. The action of noradrenaline was clearly potentiated by cocaine as the mean results of three experiments in Table III show.

TABLE III

MEAN RESULTS SHOWING INCREASES IN ATRIAL RATE CAUSED BY ADRENALINE AND BY NORADRENALINE AND THE AUGMENTED INCREASES IN THE PRESENCE OF COCAINE

Concentrations: adrenaline, 10^{-7} g./ml.; noradrenaline, 5×10^{-8} g./ml.; cocaine, 10^{-6} g./ml. The rates are given as beats/min.

Expt. No.	Adrenaline	Adrenaline and Cocaine	Potentiation
1	19	43	2·3
2	60	73	1·2
3	19	35	1·8
Mean	33	50	1·8
	Noradrenaline	Noradrenaline and Cocaine	
4	41	76	1·85
5	27	75	2·8
6	87	104	1·2
Mean	52	85	1·95

Potentiation in Atria from Rabbits Treated with Reserpine.—The effect of adrenaline alone was greater in these atria. Thus the mean increase in rate caused by adrenaline (Table III, Expt. nos. 1, 2, and 3) was 33 beats/min., while the mean increase in rate caused by the same concentration of adrenaline in atria from rabbits treated with reserpine (Table IV) was 62 beats/min. Table IV also shows that the potentiation of the effect of adrenaline by cocaine was almost absent in three of the atria, and small in the fourth.

Similar experiments were carried out with noradrenaline on atria from rabbits treated with reserpine. Noradrenaline had more effect in increasing the rate of these atria (mean increase 69 beats/min.) than it had in increasing the rate of normal atria (mean increase 52). As with adrenaline, the potentiation of the effect of noradrenaline by cocaine was much less than in the normal atria, the mean effect of cocaine in 4 out of 5 experiments being very small.

Potentiation by Cocaine in Vessels of Rabbit Ear.—Experiments were carried out to observe whether cocaine potentiated the constrictor action of noradrenaline and of adrenaline in the vessels of the perfused rabbit ear. It was found that cocaine regularly caused potentiation, as shown in Fig. 3 in which 0.002 μg. of noradrenaline was

TABLE IV

MEAN RESULTS SHOWING INCREASES IN ATRIAL RATE CAUSED BY ADRENALINE AND BY NORADRENALINE IN ATRIA FROM RESERPINE-TREATED RABBITS AND THE EFFECT OF COCAINE

Concentrations: adrenaline, 10^{-7} g./ml.; noradrenaline, 5×10^{-8} g./ml.; cocaine, 10^{-6} g./ml. The rates are given as beats/min.

Expt. No.	Adrenaline	Adrenaline and Cocaine	Potentiation
7	66	73	1·1
8	68	97	1·4
9	43	43	1·0
10	71	75	1·0
Mean	62	72	1·1
	Noradrenaline	Noradrenaline and Cocaine	
11	50	86	1·7
12	77·5	94	1·2
13	85	93	1·1
14	85	79	0·93
15	46	58	1·2
Mean	69	82	1·2

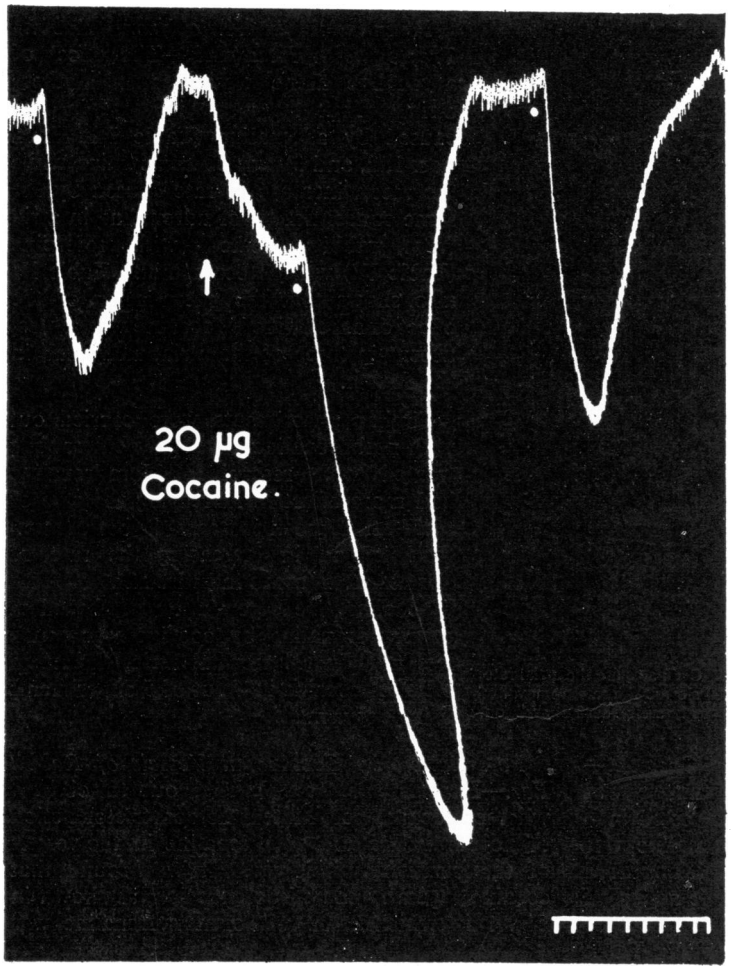

FIG. 3.—Record of venous outflow from rabbit ear perfused with Locke solution. At the white dots an injection of 0·002 µg. of noradrenaline was made into the artery cannula. Before the second of these injections 20 µg. cocaine was injected at arrow. This increased the effect of noradrenaline. Time, min.

injected three times. The second injection was preceded by the injection of 20 µg. of cocaine hydrochloride, and noradrenaline then caused much greater vasoconstriction. Similar results were obtained with adrenaline.

Observations in the Heart-Lung Preparation.— Tyramine causes an increase in rate in the heart-lung preparation and Bejrablaya, Burn and Walker (1958) have already shown that in the presence of cocaine the effect was greatly reduced. Since they showed that tyramine had almost no effect on the rate in a preparation made from a dog previously given reserpine, they concluded that tyramine must act by liberating noradrenaline. The effect of giving cocaine has now been tested, not before the injection of tyramine, but after the injection when the rise in rate had reached its peak. Fig. 4 shows the increase in rate in one experiment in which 1 mg. of tyramine hydrochloride was injected into the superior vena cava; the increase lasted for 25 min. The injection of tyramine was then repeated, and 1 min. later 20 mg. of cocaine was injected. The rate fell rapidly and was back to the initial level in 8 min.

Cocaine and Reserpine. — An exhaustive study of the action of reserpine in the heart-lung preparation has been made by Krayer and Fuentes (1958) and Paasonen and Krayer (1958), who have followed the rise in heart rate and the diminution in the amount of noradrenaline in the cardiac tissue. Some experiments have been made to see how the effect of reserpine on the heart rate was modified when cocaine was given beforehand and when it was given during the action of reserpine. Fig. 5 shows the rise in heart rate in an experiment (**A**) in which 1 mg. of reserpine was added to the venous reservoir, no cocaine being given, and also the rise in an experiment (**B**) when 20 mg. of cocaine was added to the venous reservoir 25 min. before the addition of 1 mg. of reserpine. In this experiment it seemed clear that the presence of cocaine almost abolished the rise in rate which reserpine would have caused. The effect of cocaine in other experiments was less, as shown in Table V,

TABLE V

EFFECT OF 1 MG. RESERPINE ON THE ATRIAL RATE WHEN ADDED TO RESERVOIR IN HEART-LUNG PREPARATION IN THE DOG. COCAINE (20 MG.) WAS ADDED 15–20 MIN. PREVIOUSLY IN THE LAST THREE EXPERIMENTS

Rates are given as beats/min.

Initial Rate	Maximum Rate	Maximum Increase in Rate	Total Area of Increase Determined Graphically
156	203	47	446
137	181	44	324
126	162	36	160
169	202	33	202
169	173	4	57

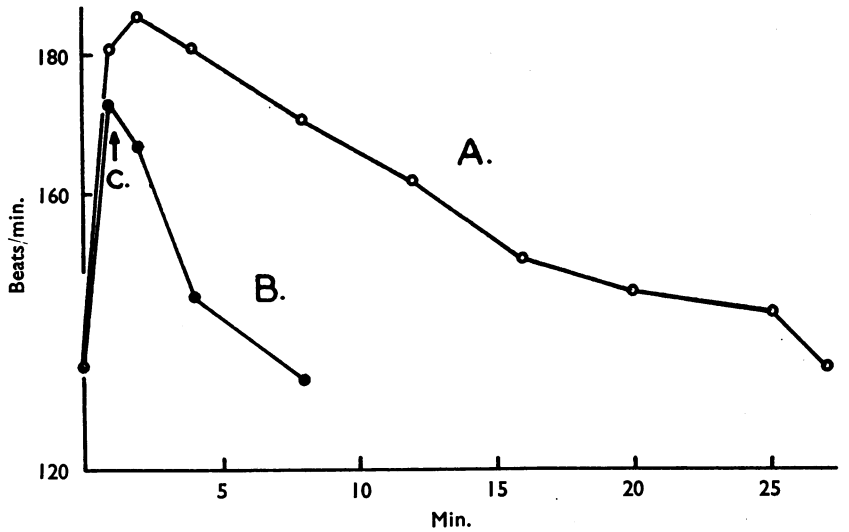

FIG. 4.—Records of heart rate taken from dog heart-lung preparation. Ordinates: beats/min. Abscissae: time in min. A shows effect of injecting 1 mg. of tyramine. B shows the effect of repeating this injection, but adding 20 mg. of cocaine (C at arrow) to the reservoir 1 min. later.

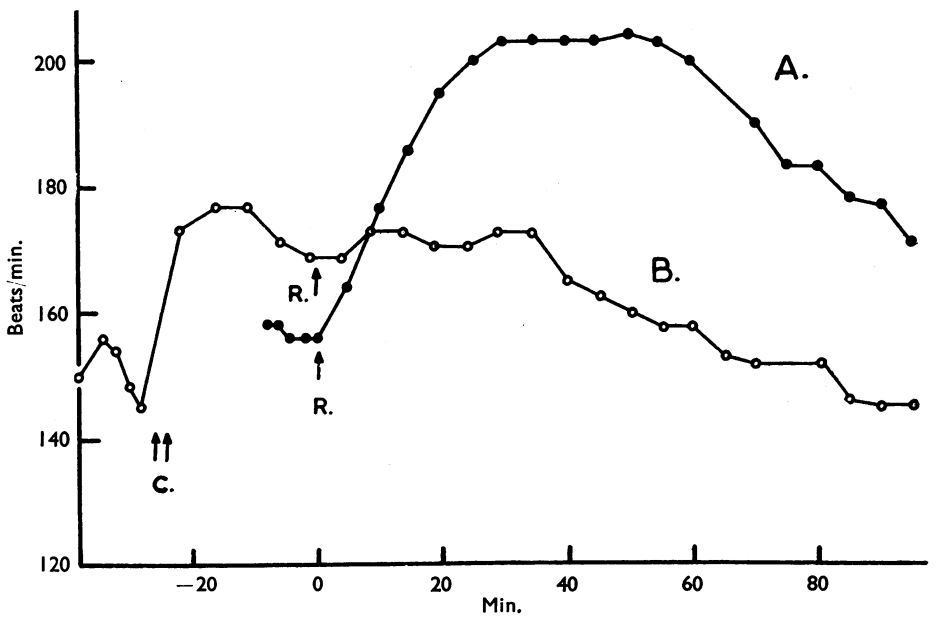

FIG. 5.—Records of heart rate from a dog heart-lung preparation. A shows effect of adding 1 mg. of reserpine (R) to the reservoir. B, taken in another experiment, shows the effect of adding 10 mg. of cocaine at each arrow (C) to the reservoir, and then of adding 1 mg. of reserpine (R) to the reservoir.

but it appeared to reduce the rise in rate both in height and duration. The effect on the rate was recorded not only as the maximum increase, but as the total area of increase obtained by plotting the rate on squared paper.

When reserpine was given first, and the addition of cocaine was made 44 min. later, the cocaine caused a large rise in rate as shown in Fig. 6. Further additions of cocaine were made as shown in Fig. 6, but it is not clear whether they had any action.

DISCUSSION

The observations may now be considered in the light of the hypothesis concerning the action of cocaine which was mentioned in the introduction. Burn and Rand (1958a) found that normal atria isolated in the organ bath contracted at a mean rate of 146/min., while atria from rabbits treated with reserpine contracted at a mean rate of 112/min. They concluded that the noradrenaline present in the normal atria was responsible for the higher rate and that there must always be a small

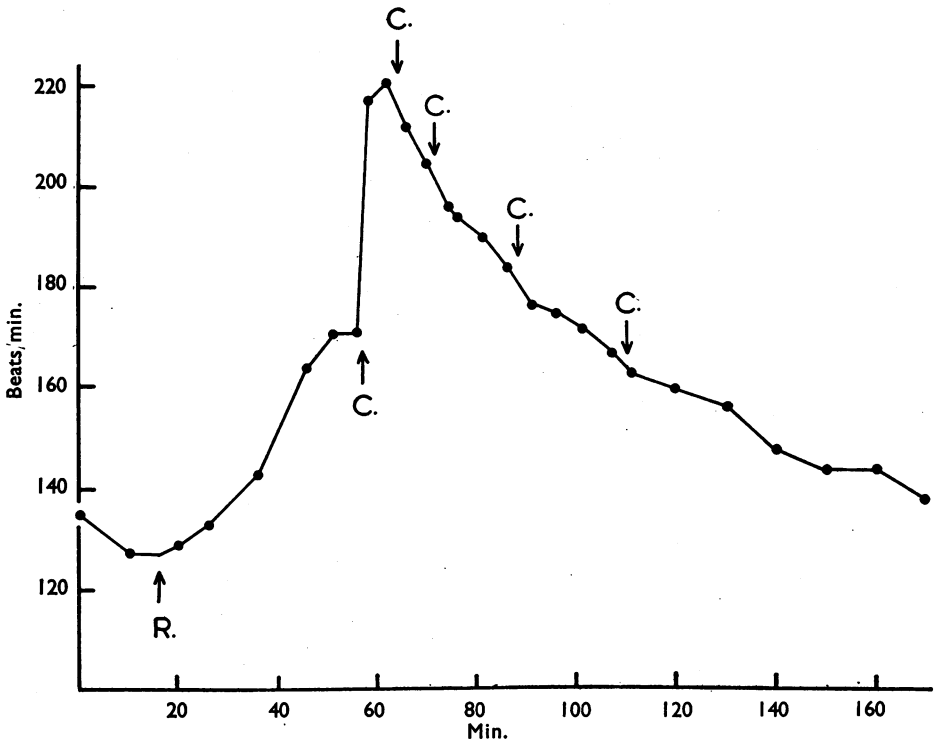

FIG. 6.—Record of heart rate from a dog heart-lung preparation. At R, 1 mg. of reserpine was injected into the superior vena cava. At C, 10 mg. of cocaine was injected. Note the increase in rate caused by the first injection of cocaine.

discharge of noradrenaline from the store. The hypothesis suggested that cocaine stopped this discharge, and in the present experiments it was found that cocaine reduced the rate of beating sometimes by as much as 35 beats/min., the rate returning to the previous level on washing out the cocaine. Furthermore, this action of cocaine was not observed in the majority of atria from rabbits treated with reserpine. In such atria there was no store of noradrenaline. When cocaine was added to the bath there was some fall in rate, but, on washing out the cocaine, the rate did not return to the initial level; the rate fell progressively with further additions of cocaine. There is no evidence to explain this progressive fall, but it may have been due to some other depressant action made evident in atria treated with reserpine, or even to a spontaneous fall.

The increase in atrial rate caused by adding adrenaline or noradrenaline to the bath was greater in the presence of cocaine, the effect of cocaine being almost to double the increase. This action of cocaine can be interpreted as indicating that cocaine not only prevents a discharge of noradrenaline from the store but that it also prevents the uptake of noradrenaline by the store. Evidence that cardiac tissue takes up noradrenaline was described by Raab and Gigee (1955), but it depended on the administration of amounts of noradrenaline or of adrenaline which were far beyond the physiological range. They injected 10 mg./kg. intraperitoneally, and this for a dog of 10 kg. would be a dose of 100 mg. They observed, for example, that after the injection, noradrenaline rose from 0.202 μg./g. heart muscle to 1.16 μg./g., and, when a mixture of noradrenaline and adrenaline was injected, to a value of 2.4 μg./g. Later Euler (1956) gave noradrenaline by intravenous infusion as well as by intraperitoneal injection in the cat. He infused amounts up to 7.8 μg./kg./min. for 30 min. and injected mixtures of noradrenaline and adrenaline up to 2 mg./kg. into the peritoneal cavity. He found that these amounts did not significantly alter the catechol amine content of the heart, spleen, liver, kidney or skeletal muscle, when prepared 5 to 28 min. after the end of the infusion.

Evidence of a different kind that noradrenaline was taken up from the blood by artery walls, the nictitating membrane and the spleen has been obtained by Burn and Rand (1958c), and that it was taken up by the heart has been obtained by Bejrablaya *et al.* (1958). These workers found that the action of tyramine was absent when the

animal had been treated with reserpine. Since this treatment was shown to discharge the store of noradrenaline in the blood vessels (Burn and Rand, 1958b), the spleen (Burn and Rand, 1959) and the heart (Bertler, Carlsson and Rosengren, 1956), it was concluded that tyramine acted by liberating noradrenaline. It was found that an infusion of noradrenaline into the spinal cat restored the action of tyramine on the blood pressure, the nictitating membrane, and the spleen, and a similar infusion into the heart-lung preparation increased the duration of the action of tyramine on the heart rate.

Recently Gillespie and Mackenna (1959) have obtained evidence that noradrenaline and adrenaline can be taken up by a tissue in an isolated organ bath. They have made observations by stimulating the lumbar colonic nerves and have recorded relaxation of the colon. When the preparation was made from an animal previously given reserpine, they found that sympathetic stimulation caused contraction and not relaxation, but that after the addition of noradrenaline or of adrenaline to the bath, then after 15 min. or more stimulation once more caused relaxation.

These results suggest that when noradrenaline is put into the blood stream or into an isolated organ bath, some of it acts on sympathetic receptors producing its usual effect, while some of it is taken up by the store. If cocaine prevents the uptake by the store, then in the presence of cocaine the whole amount will act on the sympathetic receptors. The observations of Trendelenburg (1959) support this. He has studied the rate of disappearance of noradrenaline from the blood of the spinal cat after the injection of 25 μg./kg. and found that it was greatly reduced in the presence of cocaine, and that the effect on the blood pressure was correspondingly increased.

The potentiation by cocaine of the action of noradrenaline and of adrenaline in atria, and also in the perfused rabbit ear, may therefore be due to cocaine blocking the uptake by the store. The greater effect of noradrenaline and adrenaline in atria from rabbits treated with reserpine and the much smaller potentiating action of cocaine may be due to the action of reserpine which, having depleted the store, presumably by competing for and occupying the storage receptors, prevents relatively small concentrations of noradrenaline from displacing it from these receptors.

The evidence obtained from the experiments in the heart-lung preparation indicated that cocaine was able to cut short the release of noradrenaline from the store by the action of tyramine. There was also evidence that it could reduce the effect of reserpine, provided that the cocaine was given in large doses beforehand. If, however, reserpine was given first, the effect of cocaine was to potentiate the action of the noradrenaline which the reserpine was releasing. If the hypothesis put forward is correct, it is possible that some of the noradrenaline released by reserpine was being taken up again by the store, and that cocaine prevented this uptake.

In conclusion it is scarcely necessary to say that we are dealing here with new conceptions. The evidence for the existence of a store possibly present in chromaffin tissue, from which noradrenaline can be discharged and by which it can be taken up, is already considerable. But the mechanisms involved are unknown and hypotheses concerning them can only be tentative.

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