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THE EFFECT OF COCAINE AND CHRONIC SYMPATHETIC DENERVATION OF THE HEART ON THE CHRONOTROPIC ACTION OF ADRENALINE AND NORADRENALINE

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From observations published during the last year or so, it would appear that post-ganglionic sympathetic denervation usually potentiates the response of effector organs to noradrenaline more than their response to adrenaline. This has been shown for the nictitating membrane of the cat by Bülbring and Burn (1949) and Lockett (1950a), and for the pupil dilator muscle of the cat by Burn and Hutcheon (1949). Lockett (1950b) found that in dogs thoracic and lumbar sympathectomy on the one hand potentiated the pressor response to noradrenaline more than that to adrenaline, but, on the other, increased the chronotropic action of adrenaline more than of noradrenaline.

Although it is known that cocaine sensitizes the nictitating membrane and increases the pressor response not only to adrenaline but also to noradrenaline (cf. Euler, 1950), no quantitative comparisons are available. The chronotropic action of adrenaline appears to be only slightly affected by cocaine. Rosenblueth and Schlossberg (1931) found no effect of cocaine on the response of the denervated cat's heart to adrenaline, while Peralta and Lizarralde (1946) found that there was no effect on the maximal increase in heart rate but that the return to the basal value was retarded. In a brief note, Innes and Kosterlitz (1950) reported that cocaine potentiated the chronotropic action of noradrenaline but not of adrenaline.

METHODS

Operative.—All but five cats were subjected to a preliminary operation under nembutal-ether anaesthesia, in which the right adrenal was removed and the left adrenal medulla denervated by left lumbar sympathectomy and section of the left splanchnic nerves. Usually two to three weeks were allowed for recovery from this operation. Both adrenals of the remaining five cats were removed during the acute experiments.

When the heart was to be deprived of part or the whole of its sympathetic nerve supply before the acute experiment, the following technique was adopted. For chronic removal of the stellate ganglia, both ganglia were removed in a one-stage operation under nembutal-ether anaesthesia by the antero-lateral approach through the first intercostal spaces. Great care had to be taken to avoid causing a pneumothorax by injury to the pleura. For complete chronic sympathetic denervation, the middle cervical ganglia were removed through a midline incision in the neck during the preliminary operation for inactivation of the adrenals. The stellate ganglia and the thoracic ganglia down to T 8 were removed on both sides at a single operation, the stellate ganglia as

already described and the remaining ganglia through the fifth or sixth intercostal spaces. Before closure of the intercostal gap the lungs were maximally inflated in order to reduce the pneumothorax to a minimum. Strict asepsis was observed. Penicillin was injected twice daily for the first two to three post-operative days.

Experimental.—In cats whose hearts had been partly or completely deprived of their sympathetic nerve supply in a preliminary operation, only the vagi remained to be cut in the acute experiment before testing the actions of adrenaline and noradrenaline. For acute denervation, the vagi were cut and then either the middle cervical and stellate ganglia removed or the middle cervical, stellate, and thoracic ganglia down to T 8. The anaesthetics used in the acute experiments were either nembutal (45 mg./kg. body weight), chloralose (0.05 g./kg.)-urethane (0.5 g./kg.), or ether. A few experiments were performed in the spinal preparation one hour after decapitation under ether.

The heart rate was counted on B.P. tracings obtained from the femoral artery with a mercury manometer. The heart rate (beats/min.) during the 30 sec. before the injection of the drugs was taken as the basal heart rate. After the injection, the heart rate was generally counted for 90 sec. and the response given as the mean increase of heart rate (beats/min.) during this period. The responses of the heart to sympathomimetic drugs may differ either in the peak reached, in the duration of the response, or, as is most often found, in both peak and duration. The procedure adopted was considered to be a fairly satisfactory compromise between what was required to characterize the chronotropic responses and the amount of labour involved in counting the heart rate from the B.P. tracings. In a number of experiments the heart rates were counted for 2 min. instead of $1\frac{1}{2}$ min., but this did not lead to a different interpretation of the results. The best method would have been a mechanical counter permitting continuous counting. Attempts have been made by us to use the QRS deflection of the e.c.g. for this purpose. For several reasons, however, the counting from the B.P. tracing proved to be the more reliable of the two methods.

l-Adrenaline (B.D.H.) was dissolved in 0.01 N-HCl and *l*-noradrenaline D-bitartrate monohydrate (Sterling-Winthrop Institute) in water, both in a concentration of 1 mg. base/ml. These stock solutions were kept at $-5°$ C. The final dilutions were made immediately before the experiment with Ringer-Locke solution containing ascorbic acid (1 mg./ml.). The drugs were injected into the femoral vein by means of a recalibrated tuberculin syringe. The volumes injected varied between 0.1 and 0.4 ml., and the time taken for the injection was always 15 sec. The quantities injected are given as the free bases.

Care was taken to maintain the temperature of the cats as constant as possible. The variations did not exceed 1° C. and were usually of the order of 0.5° C. Within these limits, no correlation was found between changes in body temperature, basal heart rates, and chronotropic responses to adrenaline and noradrenaline.

The results were examined statistically by Mr. M. H. Quenouille and Mr. F. H. C. Marriott, who used the methods of R. A. Fisher (Quenouille, 1950). The significance of a difference is expressed as the probability P of an observed difference occurring at random in a homogeneous population.

RESULTS

1. *The chronotropic actions of* l*-adrenaline and* l*-noradrenaline on hearts with intact sympathetic nerve supply*

It is a well-known fact that, in the intact animal, the chronotropic actions of adrenaline and noradrenaline are modified reflexly by the changes in arterial B.P., resulting with noradrenaline in a considerable bradycardia. For this reason an attempt was made to study the chronotropic actions of adrenaline and noradrenaline

after section of the vagus and sinus nerves. It was found, however, that with the sympathetic tone unrestrained by the buffer nerves the basal heart rate was very high (280 beats/min.) and small doses of adrenaline and noradrenaline had no or little effect. After removal of both stellate ganglia, the basal heart rate dropped to a mean of 183 beats/min. and measurable chronotropic responses were obtained.

It thus became obvious that the vasomotor centre had to be excluded, which was done by testing the chronotropic responses in the decapitate preparation. In each of four cats the responses to noradrenaline were smaller than to adrenaline (0.8–1.6μg.). Severing the preganglionic fibres to the stellate ganglia caused little change, as also did removal of the stellate ganglia. In the latter case, the mean increase after 1.6 μg. of either drug was greater by four beats/min. than before or after section of the preganglionic fibres; this difference was statistically significant ($P<0.05$). The responses to adrenaline remained greater than those to noradrenaline. There was a tendency for the basal heart rate to be lower after removal of the stellate ganglia (177 beats/min.) than before and after section of the preganglionic fibres to the ganglia (199 and 190 beats/min. respectively), but this effect was very much smaller than that described above for the anaesthetized cat with buffer nerves cut.

2. *The effects of cocaine and chronic sympathetic denervation on the chronotropic action of* l-*adrenaline and* l-*noradrenaline*

Results were obtained on 19 cats with acute removal of the stellate and middle cervical ganglia, 5 cats with complete acute denervation of the heart, 10 cats with chronic removal of the stellate ganglia, and 2 cats with complete chronic sympathetic denervation. In all cats the vagi were divided acutely.

Before the administration of cocaine, the mean basal heart rate was 164 in the cats with acute removal of the stellate ganglia, 165 in those with complete acute denervation, and 173 in those with chronic removal of the stellate ganglia or chronic complete sympathetic denervation. These differences were not significant. The change in basal heart rate after 8 mg. cocaine hydrochloride/kg. body weight was +18 in the experiments with acutely denervated hearts and −1 in those with chronically denervated hearts. Thus, cocaine significantly increased the heart rate in cats with acutely denervated hearts, 18 ± 4 ($P<0.001$); the difference between the cocaine effects on the acutely and chronically denervated hearts was also significant, 19 ± 8 ($P<0.05$).

A qualitative analysis of the effects of cocaine and of chronic sympathetic denervation on the chronotropic actions of adrenaline and noradrenaline is given in Tables I and II. Each cat received 2 to 3 different doses (0.4–2 μg.) of the drugs before and after the intramuscular injection of cocaine hydrochloride (8 mg./kg.). In cats with acutely denervated hearts adrenaline was generally more effective than noradrenaline, and in cats with chronically denervated hearts the reverse holds. After cocaine was given to cats with acutely denervated hearts, noradrenaline was more effective than adrenaline. The effect of cocaine on the response to adrenaline in cats with acutely denervated hearts was not consistent, while in those with chronically denervated hearts a depression occurred significantly more often than a potentiation ($P<0.05$). With noradrenaline, given to cats with acutely denervated hearts, potentiation by cocaine was preponderant ($P<0.001$), whereas in the animals with chronically denervated hearts no consistent effect could be observed.

TABLE I

Comparison of the chronotropic responses, before and after cocaine, of cats with acute or chronic removal of the stellate ganglia or complete sympathetic denervation of the heart. The vagi were cut acutely*

Treatment	Number of cats in which the chronotropic responses were :	
	Adrenaline> noradrenaline	Noradrenaline> adrenaline
Acute denervation:		
Before cocaine	13	3
After cocaine	0	16
Chronic denervation:		
Before cocaine	1	11
After cocaine	0	12

* The results of 8 exps. in which the doses of adrenaline and noradrenaline were not identical have been omitted from this table.

TABLE II

Effect of cocaine on the chronotropic responses to adrenaline and noradrenaline of cats with acute or chronic removal of the stellate ganglia or complete sympathetic denervation of the heart. The vagi were cut acutely. Figures indicate the number of cats in each category

Effect of cocaine	Adrenaline		Noradrenaline	
	Acutely Denervated hearts	Chronically Denervated hearts	Acutely Denervated hearts	Chronically Denervated hearts
Potentiation	3	1	21	5
Depression	10	8	2	5
Doubtful*	11	3	1	2

* A mean difference of less than ± 3 beats/min. was considered doubtful.

The anaesthetic appeared to have had little or no influence, since similar results were obtained in cats anaesthetized with nembutal or chloralose-urethane and in the non-anaesthetized decapitate preparation. Further, differences in basal heart rate had no obvious effects on the chronotropic responses.

The examination of the dose-response relationship showed that the logarithms of the increases in heart rate varied linearly with the logarithms of the doses of adrenaline and noradrenaline. The regression coefficients (Table III) for log increase in heart rate on log dose varied only slightly between the different groups, adrenaline before and after cocaine, and noradrenaline before and after cocaine. The geometric means of the responses confirmed that the chronotropic action of noradrenaline was increased by cocaine in the acutely but not in the chronically denervated cats and, also, that chronic sympathetic denervation had a potentiating effect. The chronotropic responses to adrenaline were not affected by the different operative treatments.

It appeared to be of interest to examine the effect of cocaine on the chronotropic response of hearts with intact sympathetic innervation. In 4 decapitate cats, cocaine hydrochloride (8 mg./kg.) potentiated the response to noradrenaline very

TABLE III

EFFECT OF DIFFERENT OPERATIVE TREATMENTS ON CHRONOTROPIC RESPONSES

Operative treatment	Geometric means of increases in heart rate (beats/min.) calculated for			
	1 μg. *l*-adrenaline		1 μg. *l*-noradrenaline	
	Before Cocaine	After Cocaine	Before Cocaine	After Cocaine
Acute removal of stellate and middle cervical ganglia and section of vagi (19 cats)	21	17	13	30
Acute denervation of heart (5 cats)	21	20	16	37
Removal of stellate ganglia at least 7 days previously and acute section of vagi (10 cats)	19	12	27	29
Sympathetic denervation of heart 8–9 days previously and acute section of vagi (2 cats)	25	19	60	60
Regression coefficients with S.E. (log increase in heart rate on log dose)	0.69 ±0.05	0.82 ±0.07	0.76 ±0.07	0.59 ±0.04

With adrenaline, whether before or after cocaine, the effects of different operative treatments were not significantly different. With noradrenaline, before cocaine, the responses were significantly lower in the acutely operated than in the chronically operated animals ($P<0.01$). With noradrenaline after cocaine, there was no difference between the acutely and chronically operated cats; complete chronic sympathetic denervation led to greater responses than chronic removal of the stellate ganglia ($P<0.05$).

markedly (+52, 35, 18, 55 beats/min.), while, with the exception of one cat, the response to adrenaline was practically unaffected (+4, 2, 2, 24 beats/min.).

Since the rise of arterial B.P. was much greater after noradrenaline than after adrenaline, it was necessary to test the possible effects of variations in arterial B.P. on chronotropic responses. For this reason, an experiment was designed in which the chronotropic responses and the changes in arterial B.P. were expected not to run parallel (Fig. 1). Cocaine potentiated the chronotropic responses to noradrenaline; these responses were not further influenced by tetraethylammonium chloride or by dibenamine. On the other hand, the rise in arterial B.P. after injection of noradrenaline was potentiated by both cocaine and tetraethylammonium chloride and then almost completely abolished by dibenamine. It is further to be noted that the basal level of B.P. was very much lower after dibenamine than in the early stages of the experiment, and thus changes in basal B.P. appear to have very little influence on the chronotropic responses.

Discussion

The main conclusions which may be derived from the reported results are that cocaine and chronic postganglionic sympathetic denervation potentiate the chronotropic action of noradrenaline but not that of adrenaline.

If a similar effect did obtain for adrenaline, it was too small to be demonstrable with the methods employed by us. There is general agreement that cocaine does not increase the peak of cardiac acceleration reached after adrenaline injection (Rosenblueth and Schlossberg, 1931; Peralta and Lizarralde, 1946; present results). As to the prolongation of the adrenaline action by cocaine reported by Peralta and

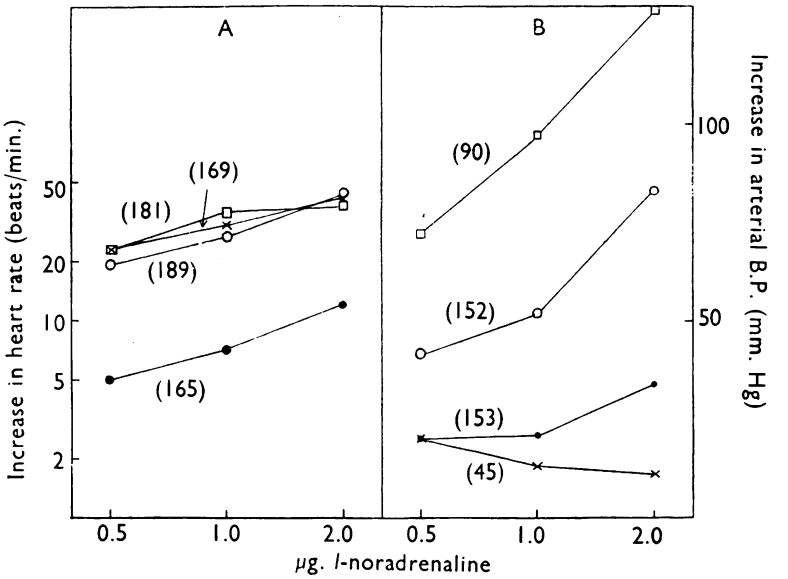

FIG. 1.—Cat, anaesthetized with nembutal (45 mg./kg.), both adrenals, stellate and middle cervical ganglia acutely removed. Increase in (A) heart rate and (B) arterial B.P. during first 1½ min. after injection of *l*-noradrenaline; before cocaine ●——●; after cocaine hydrochloride (8 mg./kg.) c——c; cocaine followed by tetraethylammonium chloride (30 mg./kg.) □——□; and dibenamine (15 mg./kg.) ×——×. The mean values of basal heart rate and arterial B.P. are given in parentheses.

Lizarralde (1946), these authors used considerably larger doses of adrenaline than were employed in the present investigation. It is possible that a slight prolongation remained unnoticed by us, as we counted the heart rate for only 90 sec. after injection of adrenaline. With regard to the effects of chronic denervation, Burrett (1940–1) denervated the hearts of cats and followed up for each individual animal the maximal increase in heart rate after injection of adrenaline at varying intervals after denervation. The increase in sensitivity found by this author was not detectable by us. This may be due to differences in technique: at the operation for denervation Burrett not only removed the sympathetic nerve supply to the heart but also cut the vagal fibres, whereas we divided the vagi immediately before testing the chronotropic responses. Further, we inactivated the adrenals, while Burrett did not. Finally, it was inherent in the design of our experiments that we had to compare the effects of acute and chronic denervation in different cats, which made the evaluation of small effects impossible.

Lockett (1950b) found that in bitches chronic thoracic and lumbar sympathectomy increased the chronotropic action of adrenaline more than that of noradrenaline. The tests were made while the dogs were asleep and had been given atropine. Although the experimental procedure differed thus in several ways from ours, the possibility of a species difference will have to be kept in mind.

No detailed examination has so far been made to determine the relationship between duration of postganglionic sympathetic denervation and potentiation of the

chronotropic response to noradrenaline. The fact that in a small number of cats with acute removal of the stellate ganglia (three experiments) the responses to noradrenaline were fully potentiated before the administration of cocaine, i.e., within one or two hours after denervation, seems to suggest that the process of potentiation may be very rapid.

From the evidence so far available, it is difficult to offer an explanation for the rise in basal heart rate which is found when cocaine is given to cats with acutely denervated hearts, but which is absent in cats with chronically denervated hearts. The phenomenon may possible be due to a sensitization by cocaine of the acutely denervated heart to the small quantities of noradrenaline present in the circulating blood.

The analysis of the phenomena described is still incomplete, and an attempt to formulate a hypothesis would be premature. There is, however, one fact which will have to be embodied in any such hypothesis, viz. that, with or without previous interruption of the sympathetic nerve supply, cocaine instantaneously causes a potentiation of the chronotropic action of noradrenaline similar to that found after chronic sympathetic denervation. It is of further interest to note that the enhancement of the chronotropic responses to adrenaline and noradrenaline appears in principle to obey the same laws as found in the sensitization of the pupil and the nictitating membrane to these drugs (Bülbring and Burn, 1949 ; Burn and Hutcheon, 1949 ; Lockett, 1950a).

Summary

1. In the spinal cat, either before or after acute section of the preganglionic fibres to the stellate ganglia or acute removal of the ganglia, the cardio-accelerator response to *l*-adrenaline was usually greater than to *l*-noradrenaline (0.4–2.0 μg.).

2. No significant differences were found in the basal heart rates of cats with acute or chronic removal of the stellate ganglia or chronic sympathetic denervation. In cats with acutely denervated hearts, cocaine hydrochloride (8 mg./kg.) raised the basal heart rate.

3. In cats with acutely denervated hearts, adrenaline generally had a greater cardio-accelerator effect than noradrenaline, while in cats with chronic sympathetic denervation of the heart noradrenaline was always more effective than adrenaline. Cocaine potentiated the chronotropic response to noradrenaline in cats with acutely denervated hearts. Cocaine had no significant effect on the response to noradrenaline in cats with chronic sympathetic denervation of the heart and to adrenaline in cats with acutely denervated hearts, while it depressed the adrenaline response in cats with chronic sympathetic denervation of the heart. No consistent difference was found between cats with complete sympathetic denervation of the heart and those with removal of the stellate ganglia only.

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