*Br. J. Pharmac. Chemother.* (1966), 27, 46–50.

# THE IMMEDIATE SOURCE OF NORADRENALINE RELEASED IN THE HEART BY ACETYL CHOLINE

BY

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(*Received November* 8, 1965)

Acetyl choline produces a sympathomimetic effect upon the atropinized heart. It does this by causing the release of a substance which was early recognized as having the characteristics of a catechol amine (Hoffman, Hoffman, Middleton & Talesnik, 1945) and has since been shown to be noradrenaline (Richardson & Woods, 1959). Sympathetic denervation of the heart and treatment with reserpine reduce or abolish the effect of acetyl choline (Benitez, 1957; Alvarado, Middleton & Beca, 1961). The obvious explanation of these facts is that acetyl choline releases noradrenaline from stores in the postganglionic sympathetic fibres of the heart. But an alternative explanation is possible (Benitez, 1957). The ultimate origin of the noradrenaline released is indeed the sympathetic fibres of the heart, but there is some intermediate storage site other than the nerve fibres which takes up some of the noradrenaline released during normal activity of sympathetic fibres, and later may release it again under the influence of acetyl choline. This site could, for example, be the chromaffin tissue of the heart or the heart muscle cells themselves. It might be a diffuse store so that available histochemical techniques might not detect it.

This paper describes experiments which were designed to distinguish between these possibilities. On the first explanation it should not be possible to restore the sympathomimetic effects of acetyl choline in sympathectomized hearts by the infusion of noradrenaline, whilst on the second it should be possible. Both explanations would be consistent with the restoration of the actions of Ach in reserpinized hearts by infusions of noradrenaline.

## METHODS

The hearts of adult cats of both sexes were used, in the following groups, each of 15 animals:

(*a*) *Normal hearts*—from cats which did not receive any special treatment at all.

(*b*) *Reserpinized hearts*—24 hr before the experiment on the isolated heart the animals were given an intravenous injection of reserpine (1 mg/kg). At the time of the experiment these animals showed lassitude, diarrhoea and relaxation of the nictitating membrane.

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(*c*) *Sympathectomized hearts*—cats were sympathectomized in three stages with a period of seven days between each operation. At the first operation the thoracic sympathetic chain was removed from the stellate ganglion to the fifth thoracic ganglion on one side. At the second, both cervical sympathetic chains were removed from the superior to the middle cervical ganglion. At the third, thoracic sympathectomy was carried out on the second side. The experiment on the isolated heart was carried out 21 days after the first operation and seven days after the last.

Each of these groups of 15 animals was divided in its turn into three subgroups, each of five animals, which received under pentobarbitone anaesthesia (35 mg/kg) intraperitoneal injections as follows. The first subgroup received an injection of 3 ml./kg of Tyrode solution; the second 3 ml./kg of noradrenaline base, 0.1% in water as the bitartrate and the third adrenaline at the same concentration. Thirty min after the intraperitoneal injections, the thorax was opened under artificial respiration and the heart was removed rapidly and set up for perfusion by Langendorff's method at constant pressure with warmed Tyrode solution (NaCl 8.0, $NaHCO_3$ 1.00, KCl 0.20, $CaCl_2$ 0.15, $MgCl_2$ 0.10, $NaH_2PO_4.2H_2O$ 0.50, Glucose 1.00 grams per litre) containing 3 mg/l. of atropine sulphate. The electrocardiogram was recorded with electrodes, one attached to the auricle and the other to the ventricle. The auricular contraction was recorded with an isometric Grass transducer FT-O 2 and the contraction of the ventricle with an isometric Grass FT 10 transducer. Records were made with a Grass P 5 polygraph.

To study the effect of acetyl choline, this was introduced into the aortic cannula in doses of 100 $\mu$g through a tap which allowed the injection of a fixed volume of 0.1 ml. without changing significantly the perfusion pressure. In each experiment, three injections of 100 $\mu$g acetyl choline were given, the first 15 min after setting up the preparation.

In all cases the percentage changes in the maximum systolic tension of the auricle and of the ventricle and in the heart rate were measured between the period just before the injection of acetyl choline and the time of its maximal effect. The frequency or the systolic tension immediately before the injection was assigned a value of 100%. For statistical treatment these percentages were converted to their logarithm to the base 10. An analysis of variance (F test) was carried out to determine whether the responses of the various subgroups were homogeneous or heterogeneous. Then the test of Duncan was used to determine which pairs of subgroups were significantly different from one another. Only the effect of the first dose of acetyl choline was considered.

The drugs used in these experiments were the following: acetyl choline hydrochloride, atropine sulphate, reserpine, pentobarbitone sodium expressed as salt, L-noradrenaline bitratrate, and crystalline adrenaline.

## RESULTS

Typical records are shown in Fig. 1. The F test and the test of Duncan were applied to the values of the systolic tension of the auricle and ventricle and the heart rate. Table 1 shows the means of the various subgroups and Table 2 the results of the test of Duncan.

The results for the ventricle were most clear cut and will be considered first. Sympathectomy reduced greatly and reserpine practically abolished the sympathomimetic action of acetyl choline on ventricular contraction. Pretreatment with noradrenaline restored the effect of acetyl choline on reserpinized hearts to a level which was not significantly different from that on normal hearts, but it failed to restore the effect of acetyl choline on sympathectomized hearts. Adrenaline had no significant effects.

The results for the auricle were less striking. While reserpine abolished the effects of acetyl choline on heart rate and auricular contraction, again practically completely, sympathectomy reduced these effects by only about one third. The sympathomimetic actions of Ach on normal and on sympathectomized hearts were not significantly altered

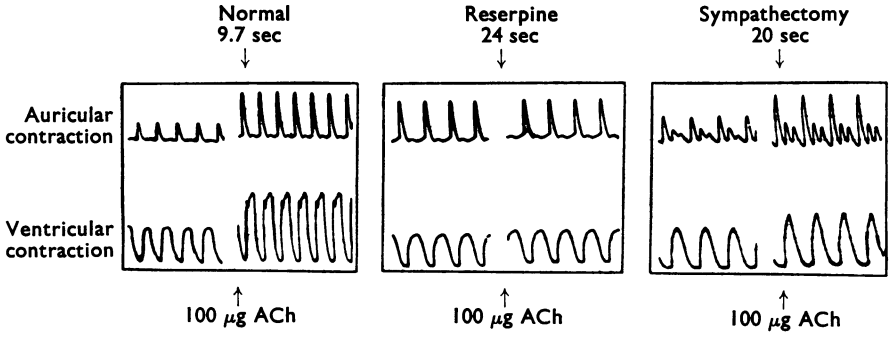


Fig. 1. The effect of 100 μg acetyl choline upon the isolated atropinized heart of the cat. The lower arrow separates the control period from the period of the maximal effect of the drug. At the upper arrow is shown the interval between the two pieces of record. Each animal had received a control intraperitoneal injection of 3 ml. of Tyrode solution.

TABLE 1

THE EFFECTS OF A DOSE OF Ach OF 100 μg UPON THE FREQUENCY OF BEATING AND THE MAXIMUM TENSION OF THE AURICLE AND VENTRICLE OF ATROPINIZED HEARTS OF NORMAL, RESERPINIZED AND SYMPATHECTOMIZED CATS WHICH HAD RECEIVED VARIOUS OTHER TREATMENTS SHORTLY BEFORE THE EXPERIMENT

The figures given are the arithmetic means of the percentages of the control level that the frequency or tension reached at the time of the maximal effect of Ach

| Group | Sub-group | No. | Control frequency, beats/min | Frequency, % of control | Auricular tension, % of control | Ventricular tension, % of control |
|---|---|---|---|---|---|---|
| Normal | Tyrode | 1 | 156 | 118·6 | 245·2 | 205·6 |
| | Noradrenaline | 2 | 167 | 117·2 | 355·8 | 254·6 |
| | Adrenaline | 3 | 144 | 109·2 | 194·4 | 189·0 |
| Reserpine | Tyrode | 4 | 143 | 97·3 | 96·4 | 105·0 |
| | Noradrenaline | 5 | 168 | 104·7 | 159·0 | 192·1 |
| | Adrenaline | 6 | 150 | 99·7 | 92·3 | 119·2 |
| Sympathectomy | Tyrode | 7 | 148 | 108·4 | 199·4 | 128·4 |
| | Noradrenaline | 8 | 142 | 101·6 | 185·2 | 108·4 |
| | Adrenaline | 9 | 154 | 100·6 | 128·6 | 131·1 |

TABLE 2

RESULTS OF THE TEST OF DUNCAN, PERFORMED UPON THE RESULTS SUMMARIZED IN TABLE 1

The numbers 1–9 refer to the sub-groups of Table 1. A cross indicates that there is a significant difference at 5% between the two sub-groups to which the cross corresponds

Frequency

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ■ | | | x | | x | | x | x |
| 2 | | ■ | | x | | x | | x | x |
| 3 | | | ■ | x | | | | | |
| 4 | x | x | x | ■ | | | | | |
| 5 | | | | | ■ | | | | |
| 6 | x | x | | | | ■ | | | |
| 7 | | | | | | | ■ | | |
| 8 | x | x | | | | | | ■ | |
| 9 | x | x | | | | | | | ■ |

Auricular tension

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ■ | | | x | x | x | | | |
| 2 | | ■ | x | x | x | x | x | x | x |
| 3 | | x | ■ | x | | x | | | |
| 4 | x | x | x | ■ | x | | x | x | x |
| 5 | x | x | | x | ■ | x | | | |
| 6 | x | x | x | | x | ■ | x | x | x |
| 7 | | x | | x | | x | ■ | | |
| 8 | | x | | x | | x | | ■ | |
| 9 | | x | | x | | x | | | ■ |

Ventricular tension

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | ■ | | | x | | x | x | x | x |
| 2 | | ■ | | x | | x | x | x | x |
| 3 | | | ■ | x | | x | x | x | x |
| 4 | x | x | x | ■ | x | | | | |
| 5 | | | | x | ■ | x | x | x | x |
| 6 | x | x | x | | x | ■ | | | |
| 7 | x | x | x | | x | | ■ | | |
| 8 | x | x | x | | x | | | ■ | |
| 9 | x | x | x | | x | | | | ■ |

by pretreatment with noradrenaline or with adrenaline. In reserpinized hearts adrenaline had no significant effect but noradrenaline increased the effect of acetyl choline on auricular contraction and on heart rate, though the latter increase was not significant.

To summarize the results for the auricle and ventricle: that component of the sympathomimetic effect of acetyl choline which had been abolished by sympathectomy was not restored by pretreatment with noradrenaline whilst that which had been abolished by reserpine was substantially restored with noradrenaline.

Changes in the sensitivity of the heart to catechol amines might have been produced by the denervations or by the injections of drugs carried out before acetyl choline was administered and so might have contributed to the results obtained. Similar observations to those already described for acetyl choline were therefore carried out to test this point.

A dose of adrenaline or noradrenaline of 1 $\mu$g was used because it gave an effect upon the heart similar to that of 100 $\mu$g of acetyl choline, the dose used in the first group of experiments. Effects on heart rate and on auricular and ventricular contraction were again measured and the number of animals in each subgroup was again five.

In the first set of observations, no significant difference was found between the responses to noradrenaline of normal, reserpinized and sympathectomized hearts nor did pretreatment with noradrenaline establish any difference. In the second set of observations it was found that the response of normal hearts to adrenaline and to noradrenaline was not significantly affected by pretreatment with noradrenaline or adrenaline except in one case. Pretreatment with noradrenaline did reduce significantly the effects of adrenaline on the heart rate.

Thus, as the sympathomimetic effects of acetyl choline are brought about by the release of noradrenaline, all the effects upon its action which we have described can be attributed to changes in the amount of noradrenaline released rather than to changes in the sensitivity of the heart to noradrenaline.

## DISCUSSION

Our results confirm those of Benitez (1957) and Alvarado, Middleton & Beca (1961) in that they show that the stimulating effect of acetyl choline on the ventricle of the isolated heart is reduced by depletion of the catechol amines of the heart either by sympathectomy or by treatment with reserpine. We have extended observations to the auricle and have found a quantitative difference between it and the ventricle. Reserpine practically abolishes the effect of acetyl choline on both the auricle and the ventricle whilst sympathectomy reduces much more strikingly the effect of acetyl choline on the ventricle. This may be because there had been some reinnervation of the heart or because the sympathectomy was not complete. A more likely explanation is that there are some structures within the heart other than the postganglionic sympathetic fibres of cells in the paravertebral ganglia, which form noradrenaline and release it in response to acetyl choline, and that in the auricle these structures contribute a much larger fraction of the noradrenaline released by acetyl choline than they do in the ventricle. These structures could well be nerve fibres which arise from sympathetic ganglion cell bodies which are distal to the excised ganglia and are situated either upon sympathetic nerve trunks or within the heart itself.

Noradrenaline restored the sympathomimetic effect of acetyl choline when this had been reduced by reserpine but did not restore it when it had been reduced by sympathectomy. Noradrenaline was effective in restoring the sympathomimetic effect of Ach only when the cardiac sympathetic nerves were intact anatomically. For acetyl choline to have its full effect, the sympathetic postganglionic fibres must be intact and must contain noradrenaline. Acetyl choline exerts its effect in part in the auricle and nearly completely in the ventricle by releasing noradrenaline from stores within the postganglionic sympathetic fibres of cells in the paravertebral ganglia. Our results provide no support for a view that there is some intermediate store which takes up noradrenaline liberated from postganglionic sympathetic fibres and releases it under the influence of acetyl choline.

These results were all obtained on hearts which were perfused with a solution containing atropine so that the muscarinic effects of acetyl choline were abolished. Elsewhere we have shown (Viveros, 1965) that acetyl choline may have a sympathomimetic effect on the ventricle in the absence of atropine. Acetyl choline has only a sympathomimetic effect on the ventricle if care is taken to stop it having any effect on the rate of beating of the heart, either by producing complete heart block surgically, or by pacing the ventricle electrically or by applying the acetyl choline to the ventricle alone through the appropriate coronary artery. It appears then that atropine, in the present experiments, served merely to reveal the sympathomimetic effects of acetyl choline. It was not necessary for them to take place.

SUMMARY

1. The sympathomimetic action of acetyl choline upon the heart is reduced by sympathectomy and by reserpine.

2. Treatment with noradrenaline restores the sympathomimetic action of Ach when it has been reduced by reserpine but not when it has been reduced by sympathectomy.

3. Acetyl choline produces its sympathomimetic effect upon the ventricle of the heart of the cat by releasing noradrenaline from stores in postganglionic sympathetic fibres. It has not been possible to show that the ventricle contains a further store, outside the postganglionic sympathetic fibres, which can take up noradrenaline and release it under the influence of acetyl choline.

This research was supported by the Faculty of Medicine of the University of Chile (Grant 60–26) and by the Rockefeller Foundation (Grant 63015) under a joint programme. The authors wish to thank Dr Vincente Astudillo for statistical advice, Sr Erasmo Madrid for technical assistance and Productos Quimicos Ciba Ltda, Instituto Bioquimico Beta and Laboratorios Silesia for the gift of drugs.

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