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THE EFFECT OF NIALAMIDE, THYROXINE, RESERPINE AND ADRENALECTOMY ON THE SYMPATHOMIMETIC ACTION OF ACETYLCHOLINE UPON THE HEART

BY

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The sympathomimetic effect of acetylcholine on the atropinized mammalian heart (Hoffman, Hoffman, Middleton & Talesnik, 1945; McDowall, 1946; McNamara, Krop & McKay, 1948) has been attributed to the action of an adrenaline-like substance which is released by acetylcholine in the heart and appears in the coronary effluent concurrently with the increase in rate and amplitude of the cardiac contractions (Hoffman *et al.*, 1945). Recent work (Richardson & Woods, 1959) has shown that, in the rabbit's heart, this substance is noradrenaline. In the experiments reported here, the sympathomimetic effect of acetylcholine was studied in hearts from cats subjected to experimental conditions which are known to alter various factors regulating the cardiac content of catecholamines —that is, treatment with nialamide, an inhibitor of monoamine oxidase (Davey, Farmer & Reinert, 1963), with thyroxine (Raab & Maes, 1947; Goodall, 1951; Hökfelt, 1952) and with reserpine (Bertler, Carlsson & Rosengren, 1956; Lee & Shideman, 1959; Axelrod, Hertting & Potter, 1962). In view of previous results of our group (Benítez, 1957), which show that the sympathomimetic effects of acetylcholine on the heart appear to be diminished by adrenalectomy, the release of adrenaline-like substance in hearts of adrenalectomized cats has also been studied.

METHODS

Adult cats (2–3 kg body weight) were used.

Nialamide pre-treatment

Nialamide in a dose of 10 mg/kg body weight was injected intravenously in 10 cats 2 hr before the experiment on the isolated heart.

Thyroxine pre-treatment

Ten cats were injected subcutaneously with thyroxine daily as follows: (a) for three weeks, 0.1 mg/kg body weight (a moderate loss of weight was observed during this initial period); (b) for five days, 0.5 mg/kg body weight; (c) for a final period of about two weeks, 1 mg/kg body weight. The loss in body weight at the end of this treatment was between 28% and 38%.

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Adrenalectomy

Seven cats were adrenalectomized bilaterally under hydroxydione anaesthesia (100 mg/kg I.V.). During the post-operative period daily subcutaneous injections of prednisone (0.5 mg/kg) and deoxycorticosterone (0.2 mg/kg) were given and 0.9% sodium chloride solution was substituted for drinking water. All animals recovered satisfactorily from the operation. The experiments on the isolated heart were carried out 11–40 days after adrenalectomy.

Reserpine pre-treatment

Ten cats were injected with reserpine, 1 mg/kg, 20–24 hr before the experiment on the isolated heart.

Perfusion of the heart

The hearts were removed under pentobarbitone sodium anaesthesia (30 mg/kg intraperitoneally) and artificial respiration.

The coronary vessels were perfused by a modified Langendorff technique with Tyrode solution at 37° C and equilibrated with 95% oxygen and 5% carbon dioxide. The Tyrode solution had the following composition (mM/l.): Na 149.1, K 2.68, Ca 1.36, Mg 0.49, Cl 141.9, HCO_3 11.9, H_2PO_4 0.32, glucose 5.55. Perfusion pressure was kept constant at 60 mm Hg. The heart was placed in a chamber filled with air at 38° C. Intracoronary injections of acetylcholine, adrenaline, and noradrenaline were made by means of a special tap, without altering the perfusion pressure. All the doses were injected in a volume of 0.1 ml. Intracoronary injections of 0.1 ml. Tyrode solution served as controls and were regularly ineffective.

The hearts were atropinized by adding atropine sulphate (2 mg/l.) to the perfusion fluid.

Ventricular contractions were recorded on a kymograph with a semi-isometric lever attached to the apex of the heart. In all the experiments the diastolic tension was kept at 1 g/ml. of initial cardiac volume.

Detection of adrenaline-like substance

The presence of an adrenaline-like substance in the effluent from the coronary vessels was detected by its action on the rectal caecum of the fowl, using the technique described in previous papers (Hoffman *et al.*, 1945 ; Middleton, Middleton and Tohā, 1949). The coronary effluent was allowed to drip directly upon the caecal preparation as it flowed from the heart. Since atropine was present in the coronary effluent, it served to suppress the caecal response to any of the injected acetylcholine coming from the heart.

The " adrenaline equivalent " of the coronary effluent was estimated by comparing the diminution of caecal tonus elicited by the effluent, with that induced by known concentrations of adrenaline. Caecal tonus was recorded isotonically with a gravity level.

The drugs used in these experiments were the following: acetylcholine hydrochloride, atropine sulphate, ergotamine tartrate, l-thyroxine sodium pentahydrate, deoxycorticosterone acetate, pentobarbitone sodium, hydroxydione, l-noradrenaline bitartrate, crystalline adrenaline, nialamide, reserpine and prednisone.

RESULTS

Effect of nialamide and thyroxine pre-treatment

After pre-treatment with nialamide or thyroxine, the cardiostimulating effect of the intracoronary injection of submaximal doses of acetylcholine, as well as the concomitant release of an adrenaline-like substance, were greater than those observed in hearts from untreated animals (Table 1).

TABLE 1

AVERAGE PERCENT INCREASE OF PEAK VENTRICULAR TENSION AND AVERAGE CONCENTRATION OF ADRENALINE-LIKE SUBSTANCE IN CORONARY EFFLUENT AFTER THE INTRACORONARY INJECTION OF ACETYLCHOLINE

*$P<0.01$, significant difference from normal hearts

Pre-treatment	Hearts (no.)	Dose of acetylcholine (μg)	Increase in peak ventricular tension (%)	"Adrenaline equivalent" (ng/ml.)
Untreated	13	50	85	1·00
Nialamid	10	50	162*	2·40*
Thyroxine	7	50	173*	4·10*
Adrenalectomy	7	50	50*	0·31*
Reserpine	7	50	0*	0·00*
Reserpine	7	100	0*	0·00*

The experiment shown in Fig. 1 illustrates a result in an untreated heart. Fifty micrograms of acetylcholine, injected in the coronary arteries, induced a marked positive inotropic effect. Soon afterwards, the rectal caecum, which was continuously irrigated with the coronary effluent dripping from the heart, showed a diminution of its tonus approximately equal to the relaxation induced by a concentration of adrenaline of 1 ng/ml.

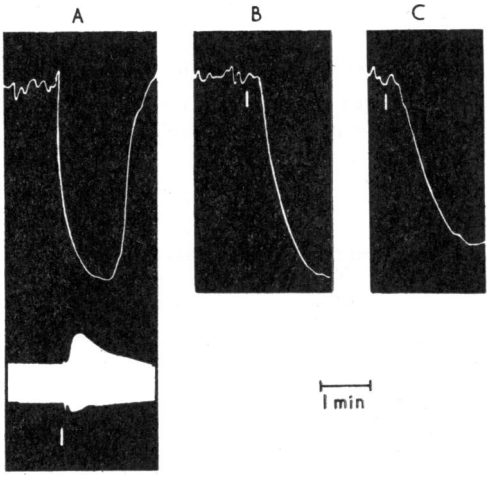

Fig. 1. Atropinized normal heart. Upper record: tonus of rectal caecum irrigated by coronary effluent; lower record; ventricular contractions. Marks indicate the intracoronary injection of acetylcholine or the irrigation of the caecum with adrenaline. (A) Acetylcholine (50 μg; (B) irrigation of caecum with adrenaline 1 ng/ml.; (C) irrigation with adrenaline 0.8 ng/ml.

Figure 2 shows an experiment on a heart from a nialamide pre-treated animal. At (A) the intracoronary injection of 50 μg acetylcholine induced a more intensive positive inotropic effect and a greater release of adrenaline-like substance (equivalent to a concentration of about 5 ng/ml. adrenaline) than did the same dose in the normal heart of Fig. 1.

Effect of bilateral adrenalectomy and reserpine pre-treatment

As shown in Table 1, the positive inotropic effect of submaximal doses of acetylcholine and the concurrent liberation of an adrenaline-like substance were substantially

diminished by adrenalectomy. Reserpine in the doses used resulted in the complete abolition of the cardio-stimulating effect of 50 μg acetylcholine. Larger doses of acetylcholine of 100 μg or more were also ineffective.

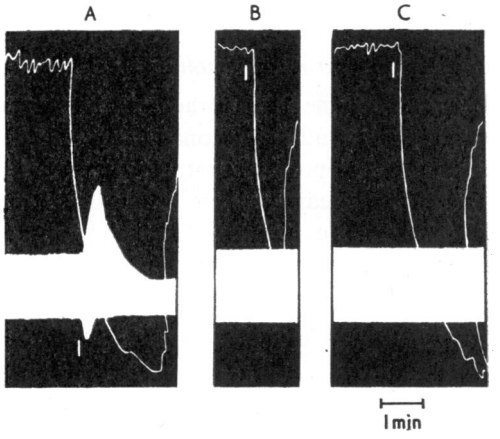

Fig. 2. Atropinized nialamide heart. Upper record: tonus of rectal caecum irrigated by coronary effluent; lower record; ventricular contractions. Marks indicate the intracoronary injection of acetylcholine or irrigation of the caecum with adrenaline. (A) Acetylcholine 50 μg; (B) irrigation of caecum with adrenaline 1 ng/ml.; (C) irrigation with adrenaline 5 ng/ml.

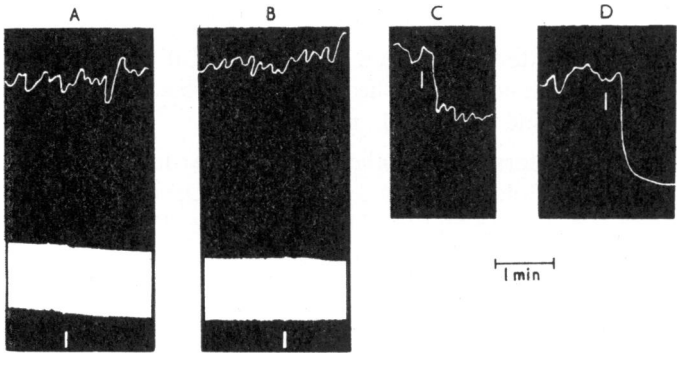

Fig. 3. Atropinized reserpine hearts. Upper record: tonus of rectal caecum irrigated by coronary effluent; lower record: ventricular contractions. Marks indicate the intracoronary injection of acetylcholine, or the irrigation of the caecum with adrenaline. (A) Acetylcholine 20 μg; (B) acetylcholine 50 μg; (C) irrigation of the caecum with adrenaline 0.5 ng/ml.; (D) irrigation with adrenaline 1 ng/ml.

The record in Fig. 3 shows a typical experiment in a heart from a reserpine pre-treated cat. 20 μg and 50 μg acetylcholine (at (A) and (B) respectively) did not modify either the ventricular contractions or caecal tonus. The caecum was, however, highly sensitive to adrenaline as shown by the pronounced relaxation of the organ induced by concentrations of adrenaline as low as 0.5 ng/ml. (at (C)).

In all the various groups of hearts, irrigation of the rectal caecum of the fowl with ergotamine tartrate in concentration of 10 mg/l. abolished the relaxing effects of the coronary effluent from hearts under the cardiostimulating action of acetylcholine. This fact supports the belief that the substance present in the perfusate is adrenaline-like in nature.

Cardiac response to adrenaline and to noradrenaline

The cardiac reactivity to adrenaline and noradrenaline was studied after nialamide, or reserpine pre-treatment, or after adrenalectomy. The results obtained rule out the possibility that the differences in the positive inotropic effects of acetylcholine are due to changes in the reactivity of the heart to the adrenaline-like substance released by acetylcholine, since in all these various groups of hearts the reactivity to adrenaline and to noradrenaline was unchanged compared with that of hearts from untreated cats.

DISCUSSION

The results described in this paper support the assumption that the sympathomimetic effect of acetylcholine on the atropinized mammalian heart is dependent on the release of pharmacologically active catecholamine-like substance(s) from the heart. Evidence obtained by others could provide an explanation of the changes in the release of adrenaline-like substance observed in our experiments.

Thus, the augmented sympathomimetic effect of acetylcholine on our hyperthyroid hearts, could be correlated with inhibition of catechol-O-methyl-tranferase (D'Iorio & Leduc, 1960; D'Iorio & Mavdrides, 1963) and/or the increase in cardiac catecholamine content (Raab & Maes, 1947; Goodall, 1951; Hökfelt, 1952) induced by thyroxine. Earlier work of this laboratory (Hoffman, Hoffman & Talesnik, 1947) demonstrated an increased sensitivity of the hyperthyroid heart of the cat to adrenaline; this has now been confirmed for both adrenaline and noradrenaline.

Monoaminoxidase inhibitors, on the other hand, appear to diminish the catecholamine content of the cat's heart (Goldberg & Shideman, 1962; Davey *et al.*, 1963). It should be pointed out, however, that according to existing evidence (Davey *et al.*, 1963) monoaminoxidase inhibitors not only inhibit monoaminoxidase, but have several other effects. In the cat they block the re-entry of released catecholamines into the storage site(s) present in nerve endings, a fact that could explain the increased output of adrenaline-like substance in our nialamide hearts and the greater sympathomimetic effect of acetylcholine.

Reserpine, in turn, has been shown to reduce the cardiac catecholamine content of the cat (Lee & Shideman, 1959), rabbit (Bertler *et al.*, 1956) and rat (Axelrod *et al.*, 1962).

According to several authors, bilateral adrenalectomy does not alter the cardiac catecholamine content either in the cat (Raab & Maes, 1947) or in the rat (Hökfelt, 1952). Benítez (1957), however, observed that the sympathomimetic effect of acetylcholine is diminished in adrenalectomized cats. Our finding that adrenalectomy results in a decreased release of adrenaline-like substance from the isolated cat's heart could explain Benítez's observation. A change of the stability of bound catecholamines induced by adrenalectomy, a hypothesis that requires further experimental exploration, could provide a basis to explain the fact that in spite of an unchanged catecholamine content, the

sympathomimetic cardiac effects of acetylcholine are diminished. However, it is possible that the treatment we used to compensate for the excision of the adrenal cortex contributed to our results. We have not yet tested this possible explanation of our observations by administering our supportive therapy without excising the adrenals.

The small difference in peak ventricular tension increase in nialamide hearts as compared with hyperthyroid hearts contrasts with the fact that the release of adrenaline-like substance is almost twice as great in the hyperthyroid hearts. This might be explained on the assumption that the reactivity—that is, the response of hearts of both groups to equal concentrations of adrenaline-like substance—is similar for both groups of hearts, and that in both groups the concentrations of substance released are sufficient to induce a maximal increase of peak ventricular tension.

SUMMARY

1. The sympathomimetic action (increase in strength of ventricular contractions and release of adrenaline-like substance) of acetylcholine was studied in the atropinized, isolated hearts of cats pre-treated with nialamide, with thyroxine, or with reserpine, and of adrenalectomized cats.

2. It was found that the effect of 50 μg acetylcholine on ventricular contractions is significantly augmented in nialamide and hyperthyroid hearts, and significantly diminished in adrenalectomy hearts, as compared with untreated hearts. In reserpine hearts, doses of up to 100 μg acetylcholine were ineffective.

3. With the possible exception of nialamide as compared with hyperthyroid hearts a correlation was observed between the intensity of the effects of acetylcholine and the release of adrenaline-like substance from the heart.

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