J. Physiol. (1946) 105, 38–44 612.173.1

THE PRESENCE OF A SYMPATHOMIMETIC SUBSTANCE IN EXTRACTS OF MAMMALIAN HEART

By U. S. v. EULER, *From the Physiology Department, Karolinska Institutet, Stockholm*

(*Received* 31 *December* 1945)

In a previous communication from this laboratory it has been shown that extracts of various mammalian organs, except placenta, contain a sympathomimetic substance, resembling adrenaline in its action on the blood pressure (Euler, 1945). The spleen was found to be especially rich in this substance, the extracts producing a pressor effect equivalent to about 10 μg. adrenaline/g. fresh tissue. A closer study of the purified substance derived from spleen extracts revealed distinct differences between the active substance and adrenaline (Euler, 1946). The spleen extract, though about as active as adrenaline on heart and blood pressure, in amounts which give the same ferric chloride colour reaction, was distinctly less active on the isolated non-pregnant cat's uterus, the isolated intestine and in its pupil-dilating action. Furthermore, the active substance did not give the typical fluorescence reaction as described by Gaddum & Schild (1934) for adrenaline. Another difference was found in the action on the blood pressure after big doses of ergotamine or ergotoxine. Instead of giving the reversed action like adrenaline, the active substance still produced a rise in blood pressure, though the action was always substantially reduced. The actions of the substance were, in each type of test, roughly in agreement with those of the adrenaline-isomer *dl*-3 : 4-dihydroxy-*nor*-ephedrine, which, again, closely resembles *nor*-adrenaline or non-methylated adrenaline (Schaumann, 1931). It should be remembered that, as early as 1910, Barger & Dale postulated that the sympathetic effector substance would be different from adrenaline and more like a catechol compound with a non-methylated amino-group in the side chain. The results of Cannon & Rosenblueth (1937), concerning the release of sympathomimetic substances in vivo on nerve stimulation (sympathin), have strongly supported this view.

As to the occurrence of adrenaline-like compounds in the heart, Loewi (1936) has reported that extracts of mammalian hearts (rabbit) contain a small amount of a sympathomimetic substance giving the fluorescence reaction of adrenaline. Later Shaw (1938) found in the same organ small amounts of a

substance giving the arseno-molybdate colour reaction equivalent to 0·015–0·04 μg. adrenaline/g. The specific increase in colour after addition of alkali was only moderate, however, indicating that the colour reaction could only partly be due to adrenaline. With a modification of Shaw's technique Raab (1943) found higher amounts of colour-giving substances in heart extracts from man and rat (0·7–1 μg./g.) though only a small fraction—as in Shaw's experiments—seemed to be adrenaline itself.

Using biological test methods, Cannon & Lissák (1939) observed that extracts of cat's heart contained some sympathomimetic compound in amounts corresponding to 0·5–0·8 μg. adrenaline per g. of fresh tissue as judged by the pressor action on the cat. The active substance was believed to be identical with adrenaline which was assumed to have been liberated from the adrenergic fibres of the organ. That such fibres may set free an adrenaline-like substance was originally shown by Gaddum & Khayyal (1936).

Quite recently, Hoffman, Hoffman, Middleton & Talesnik (1945) have reported that acetylcholine liberates an adrenaline-like body into the perfusion fluid of an isolated, atropinized heart. This observation is in accord with the results of McDowall (1944) who found that minute doses of acetylcholine stimulate the heart and sensitize it to adrenaline.

Summarizing the evidence, it may be stated that mammalian heart extracts contain an adrenaline-like substance in amounts up to 1 μg./g. Judging from Shaw's colour reaction only part of this is adrenaline itself.

By using the method of purification of organ extracts, described in earlier communications, it was hoped to be able to decide whether the active substance found in the heart conformed with adrenaline or whether it could be distinguished from it by biological or chemical methods.

METHODS

Extracts were made of heart muscle from cattle, horse and cat in the following way. The fresh organs were minced and extracted with 2 vol. of ethanol, to which was added 0·2 c.c. 10 N-sulphuric acid per kg. The filtrate was concentrated in vacuo to a small volume; the lipids being removed with ether. An extract of this type (crude extract) was used in the experiment illustrated in Fig. 1. This extract could be purified by extraction with ether containing some 5% brain-lipids or pure lecithine. The active substance taken up by the lipid-ether solution was again extracted by shaking with a small volume of 5% sodium sulphate. After precipitation of the sodium sulphate with 3 vol. of ethanol, filtering, and evaporation of the ethanol, the extract still contained a small amount of depressor material of choline ester type. This could be removed by treatment with fuller's earth in a congo-neutral solution.

The cats, used for the blood-pressure tests, were anaesthetized with chloralose and usually given 0·1 mg. ergotamine tartrate (Gynergen) per kg.i.v. and 8–10 mg. cocaine hydrochloride per kg.i.m. ½ hr. before the commencement of the testing in order to increase the pressor responses.

The crude extracts were used whenever the total pressor activity per g. of heart was determined. When the properties of the active substance were studied in greater detail, the purified extracts were used.

The blood-pressure actions were compared with *l*-adrenaline and *dl*-3 : 4-dihydroxy-*nor*-ephedrine.

Tests were also made on the isolated non-pregnant uterus of the cat. The usefulness of this test object, originally pointed out by Barger & Dale (1910), was borne out by our experiments. The fluorescence reaction was carried out according to the method described by Gaddum & Schild (1934).

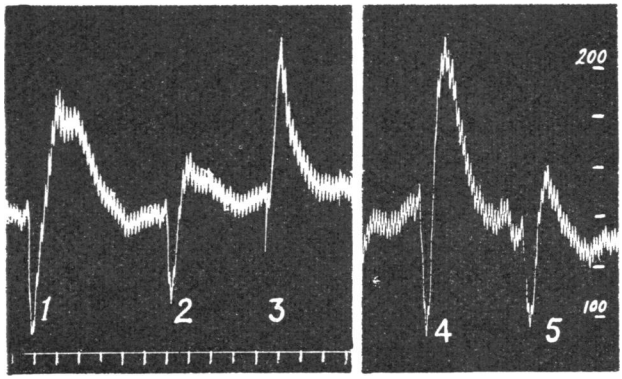

Fig. 1. Cat. Chloralose. Blood-pressure records. Crude extracts of cattle heart. (1) 0·3 g. (2) 0·06 g. (3) 1 μg. adrenaline. (Cocaine 10 mg./kg.) (4) 0·06 g. (effect equivalent to about 0·5 μg. adrenaline). (5) 0·06 g. extract treated with N/10-NaOH at 100° C. for 10 min. Time ½ min.

RESULTS

Blood pressure

The crude extracts of fresh heart muscle (whole heart) regularly caused a rise in blood pressure preceded by a short fall (Fig. 1). The effect in most experiments corresponded to some 5 μg. adrenaline per g. heart. When purified extracts were used, the depressor action almost completely disappeared and the pure pressor action was left (Figs. 2, 3). The blood-pressure response of the active extracts generally showed a typical difference from adrenaline, the initial rise in pressure with the former being definitely quicker. The active substance in this respect behaved more like *dl*-3 : 4-dihydroxy-*nor*-ephedrine (Fig. 3). After cocaine the effect of the heart extracts was increased in about the same proportion as adrenaline or *dl*-3 : 4-dihydroxy-*nor*-ephedrine. After doses of ergotamine or dihydro-ergotamine, sufficient to reverse the action of adrenaline (Rothlin, 1944), the pressor action of the heart extracts, like that of *dl*-3 : 4-dihydroxy-*nor*-ephedrine, was still present, though considerably decreased as shown in Fig. 3. The similarity between the two substances is

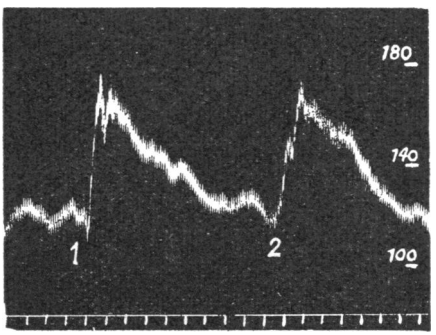

Fig. 2. Cat. Chloralose. Blood-pressure records. (1) 0·1 c.c. lipid-ether extract of cattle-heart extract. (2) 1 μg. adrenaline. Time ½ min.

quite obvious and suggests that adrenaline, if present at all, takes but an insignificant part in the action.

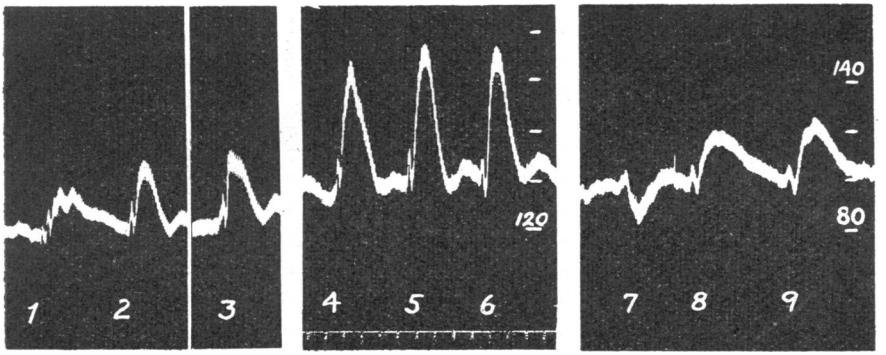

Fig. 3. Cat. Chloralose. Blood-pressure records. (1) 1 μg. adrenaline. (2) 1 μg. *dl*-3 : 4-dihydroxy-*nor*-ephedrine. (3) 0·1 c.c. lipid-ether extract of cattle heart, treated with fuller's earth. (Cocaine 8 mg./kg.) (4) 1 μg. adrenaline. (5) 1 μg. *dl*-3 : 4-dihydroxy-*nor*-ephedrine. (6) 0·1 c.c. lipid-ether extract of cattle heart, treated with Fuller's earth. (Dihydro-ergotamine 0·3 mg./kg.) (7) 3 μg. adrenaline. (8) 3 μg. *dl*-3 : 4-dihydroxy-*nor*-ephedrine. (9) 0·3 c.c. lipid-ether extract of cattle heart, treated with fuller's earth (same extract as in (3) and (6)). Time ½ min.

Isolated non-pregnant cat's uterus

Though the experiments on the cat's blood pressure after ergotamine in high doses or dihydro-ergotamine in doses of 0·2–0·3 mg./kg. seem to show conclusively that the active substance bears a much closer resemblance to catechol

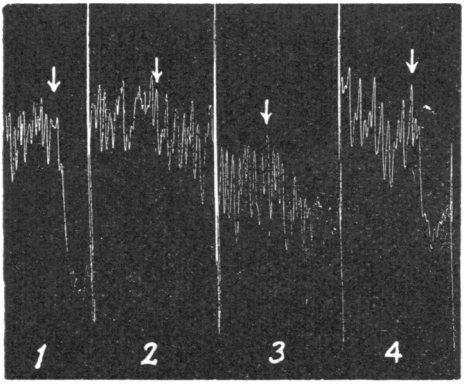

Fig. 4. Cat. Isolated non-pregnant uterus. Volume of bath 30 c.c. (1) 1 μg. adrenaline. (2) 1 μg. *dl*-3 : 4-dihydroxy-*nor*-ephedrine. (3) 0·2 c.c. lipid-ether extract of cattle heart, treated with fuller's earth (same extract as in Fig. 3). (4) 1 μg. adrenaline.

compounds without a methyl group linked to the amino-nitrogen than to adrenaline, it seemed desirable to test the action on the non-pregnant cat's uterus. As shown in Fig. 4, the heart extract had a much weaker inhibitory

action than adrenaline, even in a dose which had twice the pressor action of the given amount of adrenaline. The heart extract thus compares much better with *dl*-3 : 4-dihydroxy-*nor*-ephedrine, the action of which is also shown in Fig. 4. Addition of a small amount of adrenaline to the heart extract, before adding it to the uterus bath, promptly caused an inhibitory action, demonstrating that any adrenaline which might have been present in the extract would have been effective.

Fluorescence and colour reactions

Purified extracts were matched with adrenaline solutions so as to give the same pressor actions. When tested for the fluorescence reaction, the adrenaline solution (10 μg./c.c.) gave the characteristic strong fluorescence, whereas the heart extract did not give any definite reaction. The heart extracts showed a weak fluorescence before the addition of alkali, which disappeared when alkali was added. It may be concluded that the amount of adrenaline present in the extracts must have been small as compared with the other compound.

The colour reaction with phosphotungstic acid by the method of Folin, Cannon & Denis (1912) was found, in the purified extracts, to be of the same order as that in an equipressor solution of adrenaline. The same applied to the catechol reaction with ferric chloride. Both these methods, especially the latter, which was more reliable, could therefore be used with a reasonable degree of safety in order to evaluate the amount of active substance in the purified extracts.

DISCUSSION

The present results confirm the findings of previous investigators (Loewi, 1936; Cannon & Lissák, 1939) that heart extracts contain a substance in some ways resembling adrenaline. On the other hand, the results presented here do not support the opinion of these authors that the active substance is identical with adrenaline. Even if it be admitted that small amounts of adrenaline might have been present in the extracts, which appears probable since chromophile cell groups have been found around the heart, the present results clearly show that their significance must have been moderate. The biological effects as well as the chemical tests seem to leave no doubt that the substance responsible for the sympathomimetic actions in the heart extracts is different from adrenaline, and is, instead, intimately related to some substance resembling *nor*-adrenaline.

The criticism may be advanced that the purification method by means of lipid-ether selectively favours the extraction of a non-methylated compound. The fact that added adrenaline is extracted in about the same proportion as the active substance argues against this assumption. The comparatively large sympathomimetic activity actually found makes it improbable that greater amounts of adrenaline could be present in addition.

Cannon & Lissák suggested that the active substance was adrenaline because the extracts (1) strongly dilated the pupil, (2) inhibited the uterus, and (3) caused a fall of blood pressure after ergotoxine. It is probable that a quantitative analysis with purified extracts would have revealed certain differences from adrenaline. It should be borne in mind that the non-methylated compound of the type of *nor*-adrenaline is not entirely devoid of inhibitor action, although this effect is less prominent than its augmentor action. A reversed effect after ergotamine not infrequently occurred in our experiments, too, with less purified extracts, but this effect disappeared on further purification—notably after treatment with fuller's earth—without any significant loss of pressor activity before ergotamine. The reversed action is very likely due to the presence of vaso-dilator substances exerting their action more conspicuously after partial inhibition of the pressor action.

On the other hand, it appears that the activity found in our heart extracts agrees well with that of the sympathin (E) released on stimulation of the cardio-accelerator nerves in Cannon & Rosenblueth's experiments.

SUMMARY

1. Extracts of heart from cattle, horse, and cat contain a sympathomimetic substance resembling adrenaline in its action on heart and blood pressure. The active substance differs, however, from adrenaline in certain respects.

2. The action of the active substance is, like adrenaline, enhanced by cocaine, but is not as readily inhibited by ergotamine.

3. The inhibitory effect of the active substance on the non-pregnant uterus of the cat is much weaker than that of adrenaline in an equipressor dose.

4. The active substance gives the colour reactions with phosphotungstic acid and with ferric chloride about as strongly as adrenaline but not the fluorescence reaction.

5. The amount of the active substance corresponds to about 5 μg./g. of fresh tissue of adrenaline in its pressor action.

6. The similarities between the active substance and catechol-ethanol-amine on the one hand, and the postulated sympathin (E) on the other is noted, and its possible relation to these is discussed.

This work has been supported by a grant from Astra Ltd., Södertälje, Sweden, which is gratefully acknowledged.

Dihydro-ergotamine, which was used in some experiments, was kindly supplied by Messrs Sandoz, A. G., Basel.

REFERENCES

Barger, G. & Dale, H. H. (1910). *J. Physiol.* **41**, 19.

Cannon, W. B. & Rosenblueth, A. (1937). *Autonomic Neuro-Effector Systems.* New York: MacMillan.

Cannon, W. B. & Lissák, K. (1939). *Amer. J. Physiol.* **125**, 765.

Euler, U. S. v. (1945). *Nature, Lond.*, **156**, 19.

Euler, U. S. v. (1946). *Acta physiol. Scand.* (in the Press).

Folin, O., Cannon, W. B. & Denis, W. (1912). *J. biol. Chem.* **13**, 477.

Gaddum, J. H. & Khayyal, M. A. (1936). Cited in Gaddum, J. H., *Gefässerweiternde Stoffe der Gewebe.* Leipzig: Thieme.

Gaddum, J. H. & Schild, H. (1934). *J. Physiol.* **80**, 9*P*.

Hoffman, Hoffman, Middleton & Talesnik (1945). *Amer. J. Physiol.* **144**, 189. Cited by McDowall, R. J. S., *J. Physiol.* 1946, **104**, 392.

Loewi, O. (1936). *Pflüg. Arch. ges. Physiol.* **237**, 504.

McDowall, R. J. S. (1944). *J. Physiol.* **103**, 33*P*.

Raab, W. (1943). *Biochem. J.* **37**, 470.

Rothlin, E. (1944). *Helv. physiol. pharmacol. Acta*, **2**, C 48.

Schaumann, O. (1931). *Arch. exp. Path. Pharmak.* **160**, 127.

Shaw, F. H. (1938). *J. Physiol.* **32**, 19.