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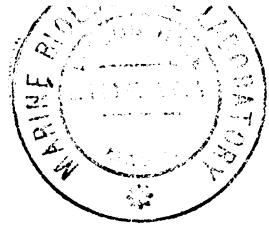

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THE EFFECT OF ADRENOCHROME ON SYMPATHETIC NERVE STIMULATION

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Experiments on spontaneous haemostasis in the rabbit's ear have shown that adrenochrome, which has no vasoconstrictor action of its own (Bacq & Derouaux, unpublished), is, nevertheless, more powerful in shortening the bleeding time than adrenaline (Derouaux, 1941*b*). This could be explained if we assumed that adrenochrome is necessary for the activity of the sympathetic and is the precursor of the transmitter substance of adrenergic nerves. The effect of adrenochrome on the bleeding time could then be explained as an indirect effect on the sympathetic nerve endings (Roskam & Derouaux, 1944).

In fact, Bacq (1933) has shown that adrenochrome restores the effect of accelerans stimulation on the perfused frog heart after prolonged perfusion, when nerve stimulation has become ineffective. No such experiments have yet been performed on warm-blooded animals, but some observations suggest that the effect of adrenaline on sympathetic stimulation may possibly be due to an action of adrenochrome. It has been shown that, after removal of the suprarenals, sympathetic stimulation quickly becomes ineffective (Elliott, 1904), and can then be restored by administration of adrenaline (Burn, 1932; Coombs, 1925; Secker, 1938), or of complete cortico-suprarenal extract (Secker, 1938; Armstrong, Cleghorn, Fowler & McVicar, 1939).

If we assume that the adrenaline injected into the circulation, or released from the suprarenals, is converted in the body to adrenochrome, these observations might suggest that adrenochrome is the precursor of the transmitter substance of adrenergic nerves. We have therefore looked for a possible reactivating effect of adrenochrome on sympathetic activity in the mammal.

METHODS

We perfused rabbits' ears with Locke's solution without interrupting their nerve supply, according to Pissemski's method (1914).

Rabbits weighing 2–2·5 kg., with large ears, were used without anaesthesia.

After isolating the right cervical sympathetic chain, we dissected a short part of the auricular artery on the same side at the base of the ear. The artery was tied with a thread and a very thin cannula was introduced into its peripheral end; the veins were cut at the base of the ear to ensure an easy flow of the perfusion fluid. A Dale-Schuster pump was used to supply the perfusion fluid.

As a rule, the perfusion flow was rather poor at the beginning, but, after 5–10 min., the arteries pulsated regularly and, in most instances, clear perfusing solution dropped from the cut veins.

The drops from the veins were registered automatically on a kymograph with a balance rheograph and an electric signal.

Stimulation of the right sympathetic chain was effected with a transformer, reducing the mains voltage to 3 V., or with an induction coil with a 4 V. accumulator in the primary.

In order to prepare the solution of adrenochrome we added 10 drops of a highly active preparation of the catechol oxidase of the mushroom *Agaricus campestris* to a Locke's solution of pH 7 containing 0·5 μg. adrenaline per c.c. (1/2,000,000). About 3 hr. later, the adrenaline is completely oxidized to adrenochrome.

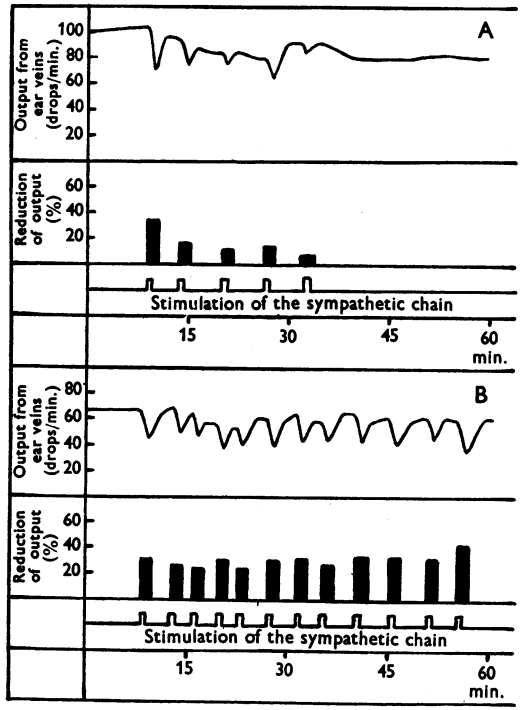

Fig. 1. A, exhaustion of the chemical promediator stock by repeated stimulation of the cervical sympathetic chain, the perfusing solution containing neither adrenochrome nor any substance able to supply it. B, failure to produce exhaustion when perfusing with a solution containing adrenochrome (0·5 μg./c.c.).

RESULTS

The chemical promediator of the sympathetic nervous system

Exhaustion of chemical promediator stock by repeated stimulation. Pissemski's perfusion of the ear enabled us to show the disappearance of the response to the sympathetic after repeated stimulation (Fig. 1 A). At the start of the

experiment, stimulation of the cervical sympathetic for 45–60 sec. caused a striking decrease in the number of drops. The vaso-motor response became progressively less, until it finally disappeared after 5–10 periods of stimulation and sometimes even earlier.

We have assumed that each such series of repeated stimuli transforms some of the chemical promediator into the mediator proper. As the stimuli were repeated, the stock of chemical promediator was used up and less mediator was discharged.

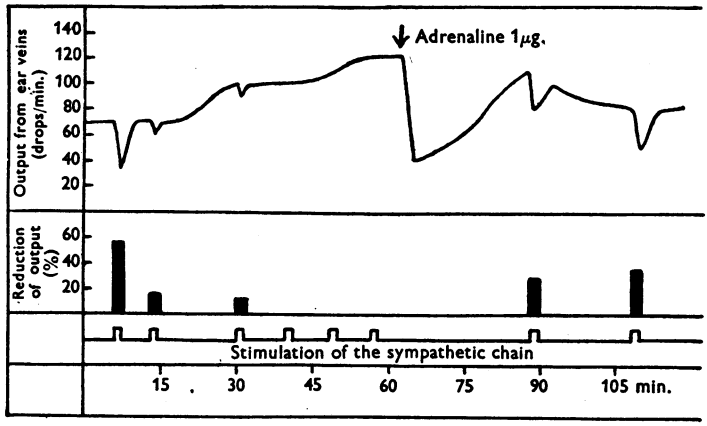

Fig. 2. Restoration of sympathetic excitability by adding adrenaline (1 μg.) to the perfusion fluid.

Restoration of sympathetic excitability by adding adrenaline to the perfusion fluid. After complete disappearance of the response we injected 1 μg. adrenaline into the perfusion fluid. After a strong vaso-constriction lasting about 10 min., stimulation of the sympathetic chain again caused a decreased flow in the perfused ear (Fig. 2).

We have assumed that the renewed response to the sympathetic is due to the transformation of the injected adrenaline into adrenochrome (or some closely related oxidation product of adrenaline), and a consequent reconstitution of the stock of chemical promediator.

Inexhaustibility of the promediator by perfusion with a solution containing adrenochrome. Adrenochrome (0·5 μg./c.c.) was added to the perfusion fluid before we tried to exhaust the stock of chemical promediator by repeated stimulation of the sympathetic chain after perfusion for 30 min.

Under these conditions we could not obtain any decrease of the vaso-motor response (Fig. 1 B).

Increase of the vaso-motor response by addition of adrenochrome to the perfusion fluid. In some experiments vascular anastomosis prevented pure Locke solution from perfusing the ear: a small quantity of blood was mixed with the perfusion fluid.

In these, as in the preceding experiments, we could not obtain a disappearance of the vaso-motor response after repeated stimulation, probably because the circulating blood carries oxidized adrenaline to the extremities of the sympathetic system. Nevertheless, in one of our experiments, we noticed during the stimulation an average reduction of 29% in the perfusion flow through the ear after 18 periods of stimulation in 45 min. After adding 0·5 μg./c.c. adrenochrome to the solution, the reduction increased to 43%. Thus, adrenochrome increased by some 50% the vaso-motor effect of the sympathetic stimulation (Fig. 3).

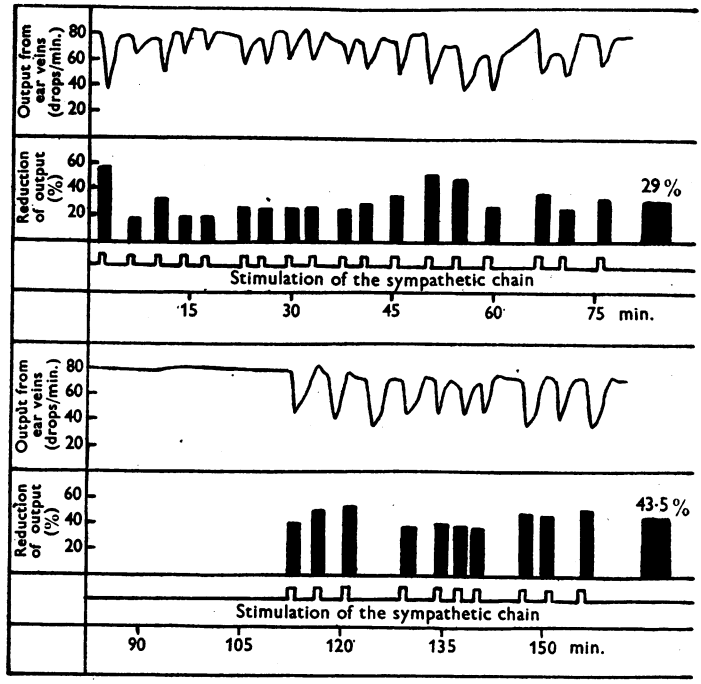

Fig. 3. Perfusion contaminated with blood. Increase of the vaso-motor sympathetic response by addition of adrenochrome (0·5 μg.) to the perfusion fluid.

Negative results with monoxime and monosemicarbazone of adrenochrome. When the response to sympathetic stimulation had completely disappeared, we tried to restore it by reconstituting the chemical promediator stock.

For this purpose we used two stable derivatives of adrenochrome, the monoxime (Green & Richter, 1937; Veer, 1942) and the monosemicarbazone (Braconier, Le Bihan & Beaudet, 1943) of adrenochrome. These substances are without any sympathomimetic effect (Bacq & Derouaux, unpublished). Both reduce the bleeding time in rabbits after rather a long period (Derouaux, 1943).

Addition of 0·5 μg. monoxime or monosemicarbazone of adrenochrome per c.c. to the perfusion fluid did not restore the response to the sympathetic.

Adrenochrome is the chemical promediator of the post-ganglionic fibres. As was to be expected, it is impossible to restore the normal effect of sympathetic stimulation by addition of monoxime or monosemicarbazone of adrenochrome, because of the stability of these substances in vitro and the latent period of their haemostatic action.

Since adrenochrome reduces the bleeding time without any delay, we were induced to suppose that this substance, or a very closely related component, is acting as a chemical promediator.

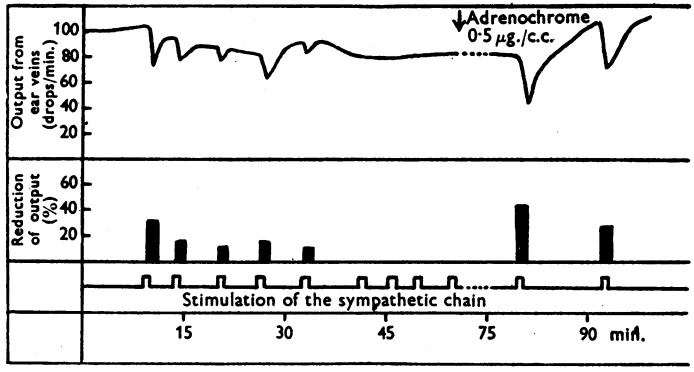

Fig. 4. Restoration of effect of sympathetic stimulation by adding adrenochrome to the perfusion fluid.

In order to prove this, we exhausted the response of a perfused ear to the sympathetic by repeated stimulation. Then we tried to revive it by addition of adrenochrome to the perfusing solution in a concentration of 0·5 μg./c.c. This did not alter the perfusion flow, but restored the response to the sympathetic, exhausted by repeated stimulation. Before perfusing the ear with adrenochrome, stimulation of the cervical sympathetic chain did not reduce the venous outflow; afterwards the same stimulation reduced it greatly (Fig. 4).

DISCUSSION

The site of origin of the chemical adrenergic mediator and the conditions of its discharge

The rabbit's ear perfused with Locke solution progressively loses its response to sympathetic stimulation. It can be restored by adding adrenochrome in a concentration of 0·5 μg./c.c. to the perfusing fluid. In the same concentration, monoxime and monosemicarbazone of adrenochrome are inactive. These facts lead to the conclusion that the discharge of the chemical adrenergic mediator by stimulation of the post-ganglionic fibres cannot take place unless there is a promediator present at their peripheral ends. The complex constitutional formula of adrenaline also suggests the existence of such a promediator, for

there is no glandular structure that is capable of synthesizing the β (3-4-dioxyphenyl)-ethanol-methylamine from simpler elements at the ends of the sympathetic nerves.

Our experiments clearly indicate the presence of such a chemical promediator akin to adrenaline (cf. Bacq, 1935) and strongly suggest that the promediator is adrenochrome, or a closely related compound.

The experiments also have a bearing on another problem, namely, whether sympathin is discharged by the ends of the post-ganglionic fibres or by the cells which they supply.

Either thesis has supporters of note: the first, Parker (1932), Bacq (1935) and Dale (1935); the second, Cannon (1933), though his arguments are less strong. Supposing the ends of the nervous fibres enter the smooth muscle cells, Cannon thinks that 'a secretion from these minute twigs could not directly enter the blood and thus influence distant organs, but would first mingle with the fluid of the cell. In that case the escaping humoral agent would certainly be in part of muscular origin.' Comparative physiology (Parker, 1932) and embryology (Bacq, 1935) would be in favour of the nervous origin of the chemical adrenergic mediator for the ganglionic sympathetic cells and the chromaffin cells of mammals have the same embryological origin. The same fundamental function may therefore be attributed to both: namely, the discharge of adrenaline when stimulated by the pre-ganglionic fibre. Finally, Dale judiciously remarks that 'the permanent association of a particular neurone with one kind of transmission would be more easily interpreted if the transmitting mechanism were a part of the nerve ending'.

The arguments for the sympathetic origin of the adrenergic chemical mediator are certainly attractive, but they are not supported by the data we have collected. We have shown that the sympathetic excitability of a definite organ, namely the ear, depends on the presence in this organ of a stock of adrenochrome, acting as an adrenergic chemical promediator. Furthermore, the fact has previously been established that an injection of adrenochrome shortens the bleeding time for several hours (Derouaux, 1941 *a*, *b*). It is, on the contrary, unable to modify the arrest of an active haemorrhage (Roskam & Derouaux, 1944).

Several of our previous findings (Derouaux & Roskam, 1937; Roskam, 1938; Derouaux, 1941 *a–d*, 1942 *a*, *b*) suggest that spontaneous haemostasis is under the direct control of the local sympathetic nervous system, as is also the reaction of blood vessels to pricking or to cutting (Magnus, 1923, 1924; Herzog, 1925; von Bernuth, 1925; Heimberger, 1925; Leschke & Wittkower, 1926; Macfarlane, 1941; Chen & Tsai, 1947; Hugues, 1947) which are certainly factors affecting bleeding time.

These facts agree with our hypothesis: the mechanical stimulation produced by cutting skin, perivascular tissues and blood vessels, has a local vaso-

constrictor effect due to a local discharge of adrenergic chemical mediator or sympathin. At the peripheral extremities of the sympathetic nervous system, there is a stock of adrenergic chemical promediator. The larger this supply the greater the discharge of sympathin. Previous injection of adrenaline, of sympathomimetic amines or of adrenochrome increases the supply of promediator and hence the vasoconstrictor response to any stimulus.

Derouaux (1941*b*) observed, however, that in a rabbit whose ears were denervated 4 weeks previously, an injection of 1 μg. adrenaline retains its haemostatic action, and this lasts, as in a normal animal, for 15, 30 and 60 min. As previously stated, this action is certainly due to an oxidation product of adrenaline, adrenochrome or a closely related derivative. Since, 4 weeks after the extirpation of the superior cervical ganglion, the post-ganglionic fibres have undoubtedly degenerated, it must be concluded that the discharge of sympathin can occur without any participation of the sympathetic nervous system.

There appear to be two possible explanations: either a tissue between the nerve fibre and the peripheral cell and not degenerating after nerve section is responsible for the phenomena we have studied; or the peripheral cell, supplied with a post-ganglionic fibre, is able to store the chemical promediator and then to turn it into sympathin without the help of the sympathetic system. We consider the second hypothesis the more likely and of some importance from a theoretical point of view. We believe that the conversion of the inactive chemical promediator into active sympathin takes place in the cells supplied with post-ganglionic fibres, usually following a stimulation of the latter. It could, however, be the result of a direct stimulation of the peripheral cell itself.

SUMMARY

1. Observations on spontaneous haemostasis raised the question whether adrenochrome or a closely related compound is the promediator of the chemical transmission of the adrenergic nervous impulses. In order to solve that problem, ears of rabbits were perfused with Locke solution without interrupting their nervous supply.

2. Under these conditions the vascular effect of sympathetic stimulation was progressively abolished, but was restored by the addition of adrenaline or adrenochrome to the perfusion fluid.

3. The response was maintained if the perfusion fluid contained adrenochrome.

4. Stable derivatives of adrenochrome did not restore the effect of sympathetic stimulation.

5. These experiments suggest that the adrenergic chemical promediator is adrenochrome or a closely related compound.

6. Since spontaneous haemostasis is governed by the sympathetic system,

and the haemostatic action of adrenaline, due to adrenochrome, persists in a rabbit whose ears were denervated 4 weeks before the experiment, it must be concluded that the storage of adrenochrome (or of the closely related compound acting as adrenergic promediator) and its conversion into sympathin may occur in the cells supplied by post-ganglionic fibres.

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