PHYSIOLOGY

ANALYSIS OF THE MECHANISMS OF ACTION OF ADRENALIN AND THE INFLUENCE OF THE SYMPATHETIC NERVE UPON THE HEART

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In the action of adrenalin upon an isolated heart the short duration of the observed effect attracts attention. This fact has been explained by the majority of observers as being due to a rapid inactivation of the adrenalin; some authors talk of this being a "habituation" of the tissue or of the adrenalin receptors [1, 2, 7, 8].

Since up to now there has been no unanimity of opinion as to this fleeting adrenalin effect, we investigated this question experimentally. We started from the suppositions of Kh. S. Koshtoyants concerning the influence of the so-called "mediators of the nerve stimulus" as occurring through their inclusion in the chemical dynamics of the effector organs and tissues, including also in this process the products of the metabolic exchange of these "mediators". [3].

EXPERIMENTAL

The experiments performed on frog hearts isolated by the method of Straub, had the aim of elucidating whether the action of adrenalin ceases because of its destruction. Adrenalin was added to the cannula in such quantities that its concentration in the perfusate was $1:10^{-6}$. After the cessation of the positive inotropic cardiac reaction the perfusate with adrenalin was replaced for 10 minutes with Ringer solution, which was in turn replaced by the perfusate containing adrenalin that had been previously drawn from the cannula. This alternation of mixtures from the cannula was done several times.

It developed that the one and the same amount of adrenalin in the course of three hours caused an inotropic positive effect on the isolated heart 16 times each time, be it noted, the effect disappearing and reappearing after the alternate introduction into the heart of Ringer solution and then the adrenalin perfusate, (Fig. 1A). Calculations showed that even on the 16th time there was a 40% active adrenalin response. These results testify that, in spite of the cessation of the adrenalin effect, enough adrenalin remains to evoke a new cardiac response.

In the next experimental series we used "constant pressure" perfusion of the heart. In this case the heart is under the constant influence of a fresh adrenalin solution, always coming under a constant pressure. The heart was perfused for 12-14 minutes by an adrenalin solution of 1.10^{-6}. As long as the adrenalin perfusion was continued, the amplitude of cardiac contractions was increased, i.e., the inotropic influence of adrenalin remained positive (Fig. 1B). When, however, adrenalin was made to act on a heart isolated by the Straub method, the inotropic adrenalin effect lasted only 2-4 minutes, after which the amplitude of contractions diminished back to the base line. These experiments render improbable the supposition that adrenalin diminishes cardiac sensitivity to adrenergic substances and causes "habituation". If this were really so, then the results of "constant pressure" perfusion experiments and perfusions of hearts isolated by the method of Straub would be the same.

On the basis of the described series of experiments we can conclude that the short duration of adrenalin activity upon the isolated frog heart is not connected with its destruction in the working heart and that adrenalin itself does not diminish the specific cardiac sensitivity to it.

In their structure the sympathins resemble adrenalin and, according to A.M. Utevsky [5], are really a mixture of adrenalin and the products of its metabolism. The mechanisms of action by the sympathetic nerves and action by adrenalin have much in common, and therefore, we performed experiments studying the effects produced by stimulation of the sympathetic nerve.

These experiments were done on frog hearts with an intact sympathetic innervation both by the "constant pressure" perfusion and the method of Straub. To obtain the sympathetic effect the sympathetic chain was stimulated between the second and third ganglia using current from an induction coil.

In hearts being perfused by the method of Straub a positive inotropic response to sympathetic nerve stimulation lasts 1-1.5 minutes, after which the amplitude of cardiac contractions returns to base. The length of the sympathetic response is not altered even when the nerve is stimulated for more than 0.5 minutes. With "constant pressure" perfusion of the heart the diminution of the inotropic response appears much later so that stimulation of the nerve for 3-5 minutes does not cause the sympathetic effect to disappear entirely (Fig. 2). Repeated stimulation of the sympathetic nerve in hearts perfused by the method of Straub leads to a rapid disappearance of the positive inotropic reaction (Fig. 3). Thus, at the fifth nerve stimulation (at four minute intervals) in the course of 50 seconds the height of the inotropic reaction falls to 10% of the original. In the same experimental conditions the hearts being perfused by the constant pressure method at the fifth stimulus retain 90% of the original. Increasing the time interval between stimuli retards the diminution of the inotropic reaction on the heart. Thus in hearts perfused by the Straub method the fifth sympathetic nerve stimulation (interval between stimuli—10 minutes) equals 45-50% of the base effect.

As a result of the above, it did not appear that the vanishing cardiac reaction to adrenalin was connected with destruction of the adrenalin. A similar deduction can be made from the experiments with the sympathetic nerve. The cause of cessation of response to sympathetic nerve stimulation cannot be destruction of the active sympathins, as in the opposite case-long continued stimulation-the effect would persist as long as there was stimulation. It might also be supposed that there is a fatiguing of the synapses to ganglionic stimulation. This, it appears, is confirmed in the experiments in which the diminution is retarded by 10 minute intervals between stimuli as compared to 4 minute intervals. However, if it was merely a question of synaptic fatigue this would be the same with the constant pressure perfusions. But it has been shown that in these latter preparations the diminution is only 10% against 90% in the hearts prepared by the method of Straub. The "habituation," advanced by some authors, of the muscle tissues or receptors to sympathin and adrenalin can be excluded as a reason, since in our constant pressure perfusion experiments adrenalin remained in constant constant pressure perfusion experiments adrenalin remained in constant contact with heart tissues for up to 14 minutes. This did not produce "habituation."

All this gives us reason to think that the diminished reaction of the heart of a frog to adrenalin and to sympathetic nerve stimulation , is bound up with the formation of special products of adrenalin metabolism. In favor of such a supposition are experiments in the course of which, the "constant pressure" perfusion maintains the heart in prolonged contact with adrenalin, and the inotropic response is maintained, apparently for the reason that the products of adrenalin metabolism are being constantly washed out. These metabolic products are unstable and change into other products, not possessing the quality of depressing the cardiac response to the adrenalin-sympathin system. This would explain the fact that sympathetic nerve stimulation every 4 minutes diminishes the inotropic response faster than stimulation at 10 minute intervals, for in the greater interval of time there is more opportunity for the depressing substances to change into inactive products. In the heart perfused by the Straub method, the action of adrenalin or the constant stimulation of the sympathetic nerve causes these products to appear in the perfusate, as long as there exist in the perfusate sympathin and adrenalin. With prolonged sympathetic nerve stimulation, in spite of constant pressure perfusion, there is still some diminution of the inotropic response. These results do not contradict the above expressed theory, as the formation of sympathin upon sympathetic nerve stimulation occurs in the whole thickness of the heart muscle, only part of which is rinsed out by the Ringer solution, so that the metabolic products remaining can exert some depressing effect.

In the experiments conducted it has been established that with repeated use the activity of adrenalin gradually diminishes. This is, probably, connected with the physiological action of adrenalin, as analogous conditions in vitro show no difference between this adrenalin and adrenalin prepared freshly. There is an opinion that the adrenalin effect is bound up with its oxidation [4]. It is quite conceivable that it is these very

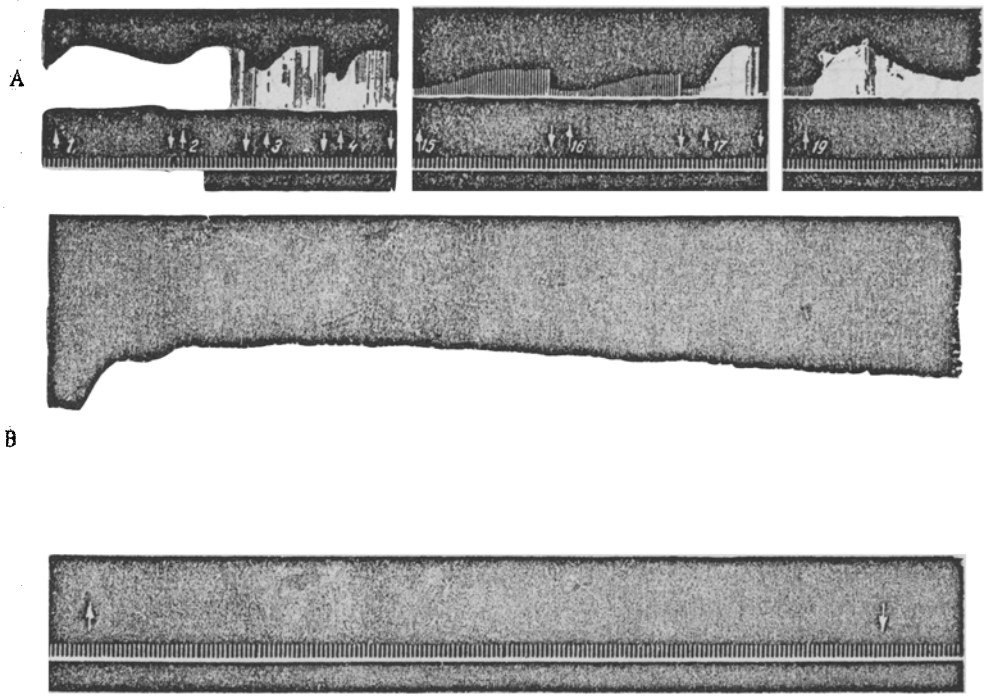

Fig. 1. Reaction of a heart isolated by the method of Straub with repeated introduction of adrenalin perfusates (A), with constant pressure perfusion of physiologic solution with adrenalin (B). Significance of tracings (from above down): amplitude of cardiac contractions; — beginning of perfusion; introduction — 1-16 — of perfusate with adrenalin, 17 — control adrenalin solution, 19 — freshly made adrenalin solution; — stopping perfusion. Time interval marks (5 sec.).

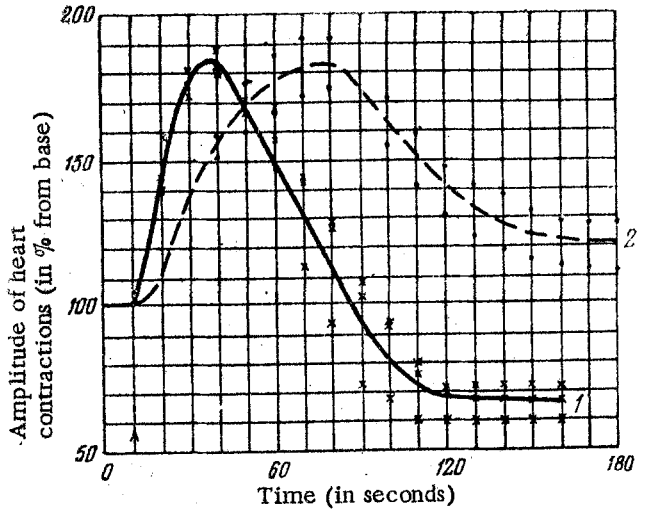

Fig. 2. Inotropic reaction of the heart of a frog to sympathetic nerve stimulation: 1) perfusion by the Straub method, 2) with constant pressure perfusion, x) separate experiments; beginning of sympathetic nerve stimulation.

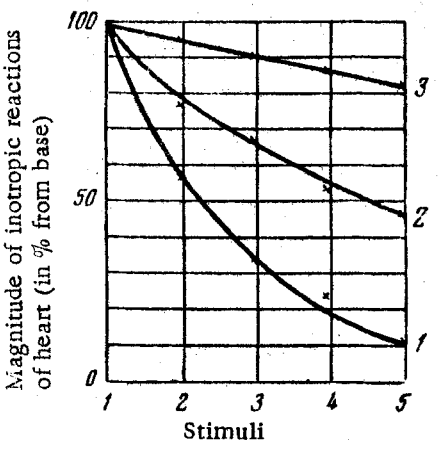

Fig. 3. Positive intropic reaction in the frog heart to repeated sympathetic nerve stimulation: 1) perfused by Straub method, 4 min. between stimuli: 2) same with 10 min. intervals: 3) Constant pressure perfusion, time interval between stimuli 4 minutes.

oxidative substances that retard the adrenalin effect. These products may be, apparently, the products of "quinoid" oxidation of adrenalin, to which points the work of A.M. Utevsky [6], in which he established the appearance of adrenalin and products of its "quinoid" oxidation in the perfusate of a working heart after sympathetic nerve stimulation.

From all these considerations we can conclude that the short duration of the adrenalin effect and the effect rising from sympathetic nerve stimulation are connected with formation of some products of adrenalin metabolism, which hinder the specific manifestations of the workings of the sympathetic-adrenalin system.

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