THE STIMULATING ACTION OF ACTH AND RELATED POLYPEPTIDES ON THE SPONTANEOUS RHYTHM OF ISOLATED HEART MUSCLE CELLS IN VITRO

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(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 6, No. 11, pp. 18-23, November, 1963
Original article submitted April 10, 1963)

In addition to its action on the adrenal cortex, ACTH influences metabolism and the function of many other tissues. Besides exerting extra-renal effects it influences the frequency of the heartbeat, as shown by Kraier et al. [9] on a dog heart-lung preparation. α-MSH (a hypophyseal hormone stimulating melanocytes) exerted a similar action on the isolated dog heart [9].

In the course of our researches on the spontaneous rhythmic activity of isolated check embryo heart cells it occurred to us to find whether these cells, which may in one sense be regarded as a model of the rhythmically beating heart, would react to the above-named hypophyseal hormones in the same way as does the intact organ of the adult dog. Also, a heart cell cultured in a polysynthetic medium is a more reliable object for a study of the accelerating influence of ACTH and MSH and probably of other cardiac stimulants than is the adult dog heart supplied with blood.

EXPERIMENTAL METHOD

The ventricles of chick embryos aged 12 or 13 days were separated into distinct cells by means of combined enzymatic and mechanical treatment. The cells were cultured in polished concave object glasses at 37° in a semi-synthetic nutritive medium containing as its natural component 10% of calf serum (medium M19). The action of ACTH and other polypeptides was tested by a method described previously [15]. The rate of beating of every 20th cell was measured at 37°; the results were expressed as the mean value and the standard deviation. If nothing occurred to prevent it the pulse count was continued for 20 min, starting at the 5th min after the last change of medium. For continuous observation on a single cell before and after treatment with hormones the cells were cultured as a separate experiment in a perfusion chamber of capacity 0.4 ml and prepared from perspex; itwas supplied with a glass window.

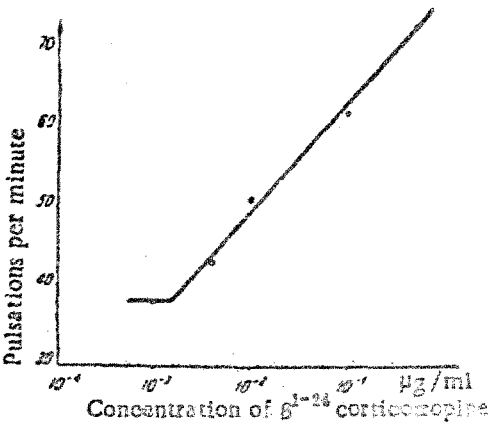

Fig. 1. Relationship of the action of corticotropin β^{1-24} to the contraction rate of a one-day culture of isolated chick embryo heart cells to concentration. Ordinate: increments of the contraction rate made 5-10 min after the addition of corticotropin.

In a separate set of experiments we used the Langendorf preparations of spontaneously beating hearts of adult rats perfused in a closed system with 80 ml of Tyrode's solution containing glucose; it was used either without ACTH, or with it at 30°. As in the previous experiments [17] we measured the amount of glucogen phosphorylase in the hearts (the a-form and the total enzyme). Subsequently we obtained the microsome fraction from the cerebral cortex of adult guinea pigs and measured their adenosine triphosphatase (AT-ase)activity in the presence of ACTH, and without it [13].

We used the following hypophyseal hormones and synthetic polypeptides: [illegible] in a concentration of 2 units/ml; perfusion of [illegible] with the pig hypophysis having a corticotropic activity of 24 U/mg (Dresden drug works); corticotropin

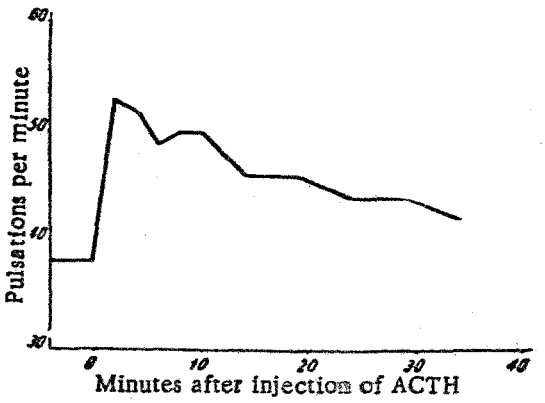

Fig. 2. Variation with time of the action of corticotropin β^{1-24} (0.02 μg/ml) on the pulsation frequency of a culture of isolated chick embryo heart cells. The curve is drawn through the mean values of the pulse frequencies of three cells of a 2-day culture kept in a perfusion chamber of capacity 0.4 ml. Rate of perfusion 3 ml per min.

tropin A_1 from the pig hypothesis with a steroid activity of approximately 70 ME/mg [1], and corticotropin A_1 treated with periodate and boron hydride (both were given to us by Dr. Dixon, Cambridge [2]); synthetic corticotropin β^{1-24} (Ciba AG, Basel [7]); α-MSH (tridecapeptide) (Professor Hofmann, Pittsburg [6]); synthetic oxytocin (Park Davis, Ann Arbor); Synthetic lysinvasopressin (Dr. Wigershausen, Berlin-Buch). Next we tested synthetic angiotensin (Ciba AG, Basel), synthetic bradykinin (FEB Berlin-Chemi, Berlin-Grunau), dichloroisoproteronol (DCJ, Ely Lille Laboratory, Indianapolis), Yeratramine (Professor Kraier, Boston).*

EXPERIMENTAL RESULTS

Relationship of Dose to Effect. As can be seen from Fig. 1 the threshold concentration of polypeptide required to increase the beat frequency of a chick embryo heart cell lies between 0.003 and 0.004 μg/ml. Between this concentration and the concentration of 0.5 μg/ml the curve is linear on a semilogarithmic plot. It was not useful to continue the measurements to concentrations of above 0.5 μg/ml, because at this strength the contractions become irregular. We would like to draw attention to the fact that in Fig. 1 the values do not indicate the maximum increase of pulse frequency for a given concentration of corticotropin, but the mean value for the period 5-25 min after the polypeptide had been added (Fig. 2). Because of the variability of the response of the canine heart to ACTH it is interesting to note that the increase of frequency attained at different concentrations of corticotropin β^{1-24} were reliably repeated from one culture to another.

Like corticotropin β^{1-24} synthetic A-MSH increases the pulsation frequency of heart cells, but to a smaller extent (Table 1).

As Table 1 shows, the effect of ACTH and MSH is completely specific. Thus, up to a concentration of 1 μg per ml the polypeptide hormones of the posterior hypophyseal – oxytocin and vasopressin exert either a very weak stimulant action on the heart, or none at all (see Table 2). Bradykinin is also without any such effect. Angiotensin increases the pulsation rate of cultured heart cells at concentrations above 0.25 μg/ml.

A highly purified preparation of natural corticotropin A_1 causes a considerable increase in the pulsation rate of the heart when given in a concentration of 0.01 μg/ml (see Table 2). Corticotropin A_1 previously treated with periodate or boron hydride [2] exerted no effect on a second culture (No. 218-3) of the same hearts made at the same time. In a subsequent experiment (culture No. 288, see Table 2) the hormone treated with 0.1 μg/ml periodate and boron hydride caused a considerable increase in pulsation frequency, and some effect was shown at a concentration of 0.04 μg/ml. Native corticotropin A_1 in the same set of experiments exerted a stronger action. The increased

TABLE 1. Influence of α-NSH and Other Biologically Active Polypeptides on the Spontaneous Contraction Rate of a 1-Day Culture of Isolated Chick Embryo Ventricle Cells

Culture	Polypeptide	Pulsation per minute at a polypeptide concentration (μg/ml) of:					
		0	0.01	0.04	0.1	0.25	1
145	α-MSH	53±7	53±6	62±9	71±7	–	–
56	Oxytocin	53±9	–	–	–	–	55±7
276-1	Vasopressin	51±5	–	–	–	–	53±5
276-2	Bradykinin	53±5	–	–	–	–	53±5
295	Angiotensin	51±3	–	–	53±6	57±6	70±7

* I wish to convey my deep thanks to these colleagues and firms for their kindness in making available the preparations.

TABLE 2. Influence of Corticotropin A_1 and Corticotropin A_1 Treated with Periodate and Boron Hydride on a 1-Day Culture of Isolated Check Embryo Heart Cells

Culture	Polypeptide	Concentration (mμg per ml)	Pulsation rate (per min)		P
			without polypeptide	with polypeptide	
218-1	Corticotropin A_1	0.01	50±9	59±10	< 0.01
218-2	" "	0.1	47±6	58±7	< 0.01
218-3		0.1	53±6	53±5	1
288-1	Corticotropin A_1	0.04	48±3	60±5	< 0.01
288-2	" "	0.1	50±5	66±9	< 0.01
288-3		0.04	50±3	55±5	< 0.01
288-4		0.1	50±4	61±5	< 0.01

frequency of pulsation evoked by the periodate-boron hydride-corticotropin A_1 (0.1 μg/ml) in this experiment corresponded quantitatively to the increase achieved with a corticotropin A_1 concentration of 0.04 μg/ml. This and subsequent experiments with corticotropin A_1 showed that the stimulant action exerted by this hormone preparation on the rhythmically beating chick embryo heart muscle cells was reduced by 50-90% after treatment with periodate and boron hydride. Boright et al. [1] have shown that this treatment reduces the adrenocorticotropic action of the hormone by 99%, leaves unchanged the hypoglycemic action, and reduces the lipolytic influence on isolated fatty tissues of the adult rat by approximately 90%.

In molar concentrations corticotropin A_1, consisting of a chain of 39 aminoacids, exerted approximately the same stimulant action as did corticotropin β^{1-24}. A preparation of ACTH (obtained from Dresden), whose steroidogenic activity was only one-third of that of corticotropin A_1, exerted the same cardiac stimulant action (see Table 3). This result may be attributed to the fact that this preparation contained, in addition to ACTH, compounds which appeared also to be polypeptides, and which resembled ACTH in the strength of their cardiac stimulant effect independently of the presence or absence of a weak steroidogenic action.

Time Course of the Action. Kraier et al. [9] report that the cardiac stimulant actions of ACTH and MSH on the isolated canine heart begins to decrease after a few minutes. As can be seen from Fig. 2, the duration of the action of corticotropin β^{1-24} on the pulsation of the culture of embryo heart cells is also relatively weak. The pulsation frequency reaches a maximum value immediately after contact with a medium containing corticotropin, and has a tendency to fall to the original level within 1 hour. Similar effects were obtained also after the addition of natural ACTH and α-MSH.

TABLE 3. Elimination of the Accelerator Action of ACTH on Spontaneously Pulsating Isolated Chick Embryo Heart Cells (by use of veratramine and DCI)

Culture	Growth in days	Preparation added	Pulsations per min
73-2-a†	2		39±8
73-2-b	2	ACTH*	66±8
73-2-c	2	ACTH + veratrimine . .	40±8
73-3-a	2	"	36±7
73-3-b	2	Veratramine	36±6
73-3-c	2	ACTH + veratramine . .	38±5
52-6-a	3	"	36±7
52-6-b	3	ACTH	71±11
52-6-c	3	"	35±6
52-6-d	3	DCI	36±7
52-6-e		ACTH	34±7

The Influence of Veratramine and DCI on the Action of ACTH. It has been shown [9] that veratramine, which is the antagonist of the accelerating influence of sympathomimetic amines [8], suppresses the accelerating influence of ACTH on an adult canine heart-lung preparation. The dose of the alkaloid used by Kraier et al. was 0.8 ml/liter. As can be seen from Table 3, almost exactly the same concentration of veratramine (1 μg/ml of medium) is also effective in blocking the

manifested by a combination of ACTH and ACI was maintained even when media containing ACTH but no ACI were used. As can be seen from Table 3 the effect of ACTH in increasing the pulsation frequency may be eliminated by washing with control medium.

It is known that ACI is an inhibitor of β-adrenergic receptors of the heart [10]. It also blocks the accelerator action of adrenaline in chick embryo heart cultures [14]. From the results of Table 3 we may conclude that suppression of the accelerator influence of ACTH on pulsation frequency of isolated heart cells is brought about by a catechol amine system. This conclusion, however, is at variance wi th the fact that Krayer and his coworkers [9] found no appreciable reduction of the action of ACTH and MSH on the heart rate of a canine heart-lung preparation previously treated with reserpine. They maintain that the action of the hypophyseal hormones which they observed cannot be brought about by the catechol amines contained in the heart, most of which are washed out of the tissue. Apparently the suppression of the stimulant action of ACTH mediated by DCI is a nonspecific effect occurring independently of β-adrenergic block.

The Tests of the Action of ACTH on Phosphorylase Activity of Cardiac Muscle and of Microsomal ATP-ase. Sympathetic amines increase the pulsation frequency both of the intact heart and of cultured cells, and they activate glycogen phosphorylase (transformation of phosphorylase A into phosphorylase B in the heart muscle [5]). ACTH also activates phosphorylase not only in the adrenals [4] but also in fatty tissue [12]. An analysis of these facts in relation to the results of Table 3 led us to study the influence of ACTH on the phosphorylase of cardiac muscles. For this purpose we carried out experiments on 4 Langendorf rat hearts. The injection of ACTH (Dresden Pharmaceutical Works) in a final concentration of 0.1 μg/ml caused the contraction frequency to rise from 168 to 198 per min. For the determination of phosphorylase, the heart was frozen at the moment of maximum contraction rate. Analysis showed that the proportion of phosphorylase A in the total enzyme was 10%, and the amount was not increased by the injection of ACTH. This result, however, does not exclude the possibility that ACTH activates phosphorylase selectively in the regions of the heart which initiate the contractions.

Recently we have reported [13] that the principal polypeptide–protamine, used in concentration which accelerate the pulsation rate of chick embryo heart cells greatly increases ATP-ase activity in the microsomal fraction of the adult guinea pig cerebral cortex; this result appears to be due to the mechanism of active transport of cations. It, therefore, seemed interesting to test the influence on cerebral microsomes of one of the hypophyseal polypeptides which accelerate the pulsation rate. Experiments carried out on this fraction of tissue homogenate showed that ACTH (Dresden Pharmaceutical Works) used in concentrations from 0.1 to 40 μg/ml exerts no influence on the rate of breakdown of ATP by the cerebral microsomes; and this is true whether or not sodium and potassium ions are present. The same thing may be said of the breakdwon of ATP by the isolated cell membranes of the adrenal cortex [11]. Apparently the stimulant action of ACTH depends upon a mechanism different from that of protamine, whose action is related to its properties as a polyvalent cation [13].

SUMMARY

ACTH and α-MSH increase the rate of spontaneous pulsation of single isolated myoblasts cultured from the chick embryo heart. For synthetic β^{1-24} corticotropin the threshold concentration for this effect lies between .003 and .004 μg/ml of medium. The accelerator action of ACTH on cardiac cells is reduced when the ACTH is treated with periodate and boron hydride, and is totally inhibited by treatment of the cells with veratramine or dichloroisoproteronol.

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