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THE INHIBITORY ACTION OF ADRENALINE ON INTESTINAL SMOOTH MUSCLE IN RELATION TO ITS ACTION ON PHOSPHORYLASE ACTIVITY

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Previous work on the electrical changes produced by adrenaline on intestinal smooth muscle has been carried out on preparations from the guinea-pig taenia coli (Bülbring, 1954; Burnstock, 1958). This tissue exhibits rhythmic activity which is the basis of its tone. Adrenaline abolishes this tone because it stops the spontaneous spike discharge. It also depresses the conducted response when the tissue is stimulated electrically and causes an increase of the membrane potential. In the preceding paper (Axelsson & Bülbring, 1961) evidence has been presented indicating that the electrical activity in this tissue may be influenced by the metabolic rate. It was therefore of interest to know whether adrenaline, which is known to increase phosphorylase activity in some types of muscle, had this action also in the smooth muscle of the taenia coli. If so, its effect on electrical activity might be related to its metabolic action.

We therefore carried out experiments in which we measured the effect of adrenaline on phosphorylase activity of taenia coli and, simultaneously, recorded the electrical and mechanical changes which adrenaline produced in another piece of taenia taken from the same animal. We found a good correlation between the biochemical and biophysical changes, suggesting that the latter might be brought about as a consequence of an increased activation of phosphorylase.

Some of the results have been reported to the Physiological Society (Axelsson, Bueding & Bülbring, 1959; Axelsson & Bülbring, 1960).

METHODS

Adult guinea-pigs of both sexes were used. They were killed by stunning and bleeding. The taenia coli was removed by cutting out pieces which were, as far as possible, of equal length *in situ* (25–27 mm). Usually 8–10 pieces could be obtained from one guinea-pig. One end of each piece was fixed to one of a row of prongs at the lower end of a Perspex holder, while the other end was tied to one of a second row of prongs which could slide along the holder. The pieces were thus held parallel and, after immersion in the isolated organ

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bath, all pieces were stretched simultaneously to the same extent (120 % of the original length) by sliding the mobile row of prongs upwards.

At the same time one additional piece of taenia was prepared and inserted into the sucrose-gap apparatus for electrical recording. The method has been described in detail in previous papers (see Bülbring & Burnstock, 1960; Axelsson & Bülbring, 1961). As the preparation in the sucrose gap apparatus was bathed in continuously flowing solution, the fluid in the isolated organ bath containing the remaining pieces was also continuously changed by a similar slow inflow and kept at a constant level by overflow. The composition was the same as that described by Axelsson & Bülbring (1961). The temperature in both apparatus was kept constant at 35° C by the same warm-water circulating system.

The dissection Ringer's solution contained (mM): NaCl 150, KCl 5, $CaCl_2$ 2·5, glucose 11·5. The time required for dissection, weighing and setting up the preparations was on the average 40 min. Another 40 min was allowed for recovery in warm oxygenated Krebs's solution (see Axelsson & Bülbring, 1961) before the experiment was begun. The preparation from which the electrical records were obtained was subjected simultaneously to the same experimental procedures as those applied to the remaining pieces suspended in the isolated organ bath, from which they were removed, one after the other, at certain intervals of time for the determination of phosphorylase activity.

Phosphorylase activity of taenia coli was determined in the following manner. The previously weighed piece of taenia (15–25 mg) was blotted and placed in a container surrounded by ice, cut rapidly to a fine mince, transferred quantitatively into an all-glass micro-homogenizer surrounded by crushed ice and homogenized with 10 volumes of an ice-cold solution containing potassium citrate buffer (pH 6·7; 0·06 M) sodium fluoride (0·05 M) and potassium ethylene diamine tetraacetate (0·005 M): 0·1 ml of this homogenate was added to 0·2 ml. of the reaction mixture. After the addition of the homogenate the final concentrations of the constituents of the assay mixture were: 0·035 m-glucose-1-phosphate, 0·05 g glycogen/100 ml., 0·06 M potassium citrate buffer (pH 6·7), 0·02 M sodium fluoride and 0·005 M ethylene diamine tetraacetate. Immediately following the addition of the taenia homogenate an amount of 0·1 ml. (initial sample) was pipetted into 0·4 ml. of an ice-cold solution of trichloroacetic acid (5 g/100 ml.). The remainder of the assay mixture was incubated at 34° C for 13 min. After this period another 0·1 ml. (final sample) was added to 0·4 ml. of the trichloroacetic acid solution. This final mixture was centrifuged at 3500 *g* for 30 min at 0° C and inorganic phosphate was determined in a sample (0·2 ml.) of the supernatant solution according to the method of Fiske & Subbarow (1925) with the aid of an EEL colorimeter (filter number ORI; final volume, 1·0 ml.). The difference in the concentration of inorganic phosphate between the final and the initial samples was used to calculate the amount of phosphate liberated from glucose-1-phosphate during the period of incubation. No significant amounts of inorganic phosphate were formed under the same conditions when the addition of glycogen to the reaction mixture was omitted. Therefore it was concluded that the production of inorganic phosphate was brought about by the action of phosphorylase. This interpretation was confirmed by the determination of the increase in glycogen produced during incubation; it was found that the increase in glycogen was equimolar, in terms of glucose units, to the amount of inorganic phosphate liberated, according to the reaction, catalysed by phosphorylase:

$$x \text{ glucose-1-phosphate} + n\,G \rightarrow (n+x)G + x \text{ inorganic phosphate},$$

where G represents glucose units of glycogen.

Under the assay conditions described above, enzymic activity was proportional to the concentration of the homogenate and to the time of incubation over a period of 15 min. Phosphorylase activity was expressed in micromoles of inorganic phosphate liberated per minute by one gram of smooth muscle (wet weight).

In contrast to the phosphorylase of liver and of skeletal muscle the optimal priming concentration of glycogen for taenia coli phosphorylase was found to be extremely low.

Therefore it would appear that phosphorylase of smooth muscle might not be identical with the enzymes which catalyse the same reaction in skeletal muscle and liver.

Glycogen and glucose-1-phosphate were purified according to the procedure of Sutherland & Wosilait (1956). The stock solution of glucose-1-phosphate was prepared by adjusting the pH to 6·7 with HCl (2N). Glass-distilled water was used throughout.

While no attempt was made to determine the minimal concentrations of glucose-1-phosphate and of glycogen required for maximal phosphorylase activity, it was found that a molarity of 0·035 for glucose-1-phosphate, a concentration of 0·05 % for glycogen and a pH of 6·7 were optimal for enzymic activity. The composition of the reaction mixture used for the assay of phosphorylase activity of taenia coli was based on these observations, in order to ensure that enzymic activity was measured under optimal conditions. It should be noted that adenosine-5-monophosphate (AMP) in molar concentrations varying between 1×10^{-4} and 1×10^{-2} did not stimulate phosphorylase activity of taenia coli. Similarly, no activation was observed on addition of sulphydryl compounds (e.g. cysteine, glutathione).

In some experiments the glycogen concentrations of pieces of taenia coli (15–25 mg) were determined according to the method of Hassid & Abraham (1957). The precipitated glycogen was dissolved in 0·5 ml. of water, and the colorimetric determination was carried out on a mixture of a portion of 0·4 ml. and of 0·8 ml. of the anthrone reagent. The optical density was determined in the EEL colorimeter (filter number ORI).

RESULTS

The effect of adrenaline in normal solution

Adrenaline in concentrations of $1–2 \times 10^{-7}$ was found to increase phosphorylase activity in all experiments. The maximum effect was usually seen within 2 min. In the parallel strip, from which electrical records were obtained, adrenaline caused usually hyperpolarization (17 out of 20 observations), and this occurred, without exception, at the time at which the increase of phosphorylase activity in the parallel strip of tissue was observed.

Figure 1 shows a typical experiment. While adrenaline abolished the spontaneous spike activity, and caused an increase of the membrane potential of almost 20 mV, the phosphorylase activity, measured at the same time in another piece of taenia, increased rapidly to 126 %. It then declined again, as measured subsequently in further pieces, returning to the original value after 18 min exposure to adrenaline. The hyperpolarization persisted in this experiment for 10 min, but then the membrane potential also returned to the initial level, although adrenaline was still present. When it was washed out the membrane depolarized, and there followed a period during which spikes were discharged at a very high frequency; at this time phosphorylase activity in the parallel piece was low. After 15 min the initial conditions were restored. In normal conditions the phosphorylase activity of different pieces of taenia removed from the organ bath after various periods up to 2 hr did not differ by more than $\pm$ 5 %.

Table 1 gives 20 observations made in 13 different experiments of phos-

phorylase activity before and after the administration of adrenaline. They are arranged according to the time which elapsed between the setting up of the preparation and the determination of phosphorylase. It will be seen that, on the average, the phosphorylase activity was somewhat higher during the first hour than during the second and third hour. Accordingly, the percentage increase produced by adrenaline was less at first than later in the experiment. However, the mean values reached at any time in the presence of adrenaline did not differ by more than 2% between the two groups.

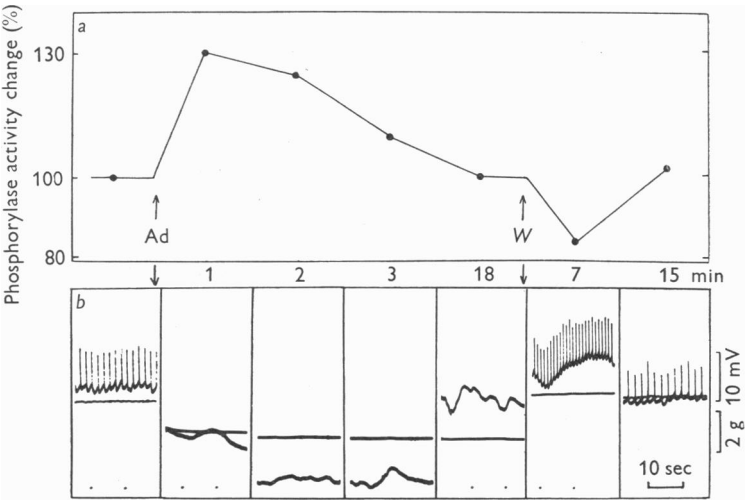

Fig. 1. The effect of adrenaline 2×10^{-7} on (*a*) phosphorylase activity and on (*b*) the electrical activity, membrane potential and tension. The records were taken at the same time as the phosphorylase activity was measured.

TABLE 1. Phosphorylase activity during the first, second and third hour after setting up the preparation, before and after adrenaline (Ad)

First hour. Phosphorylase activity (μmoleP/g)			Second and third hours. Phosphorylase activity (μmoleP/g)		
Control	After Ad	%	Control	After Ad	%
4·30	5·32	126	5·07	8·42	166
5·18	5·10	98	4·83	6·29	130
4·58	5·73	125	4·40	5·20	118
4·70	4·58	98	5·10	6·04	119
5·93	7·48	126	3·96	6·30	159
5·25	6·45	123	3·32	4·66	140
4·62	5·64	122	4·42	6·26	142
5·23	7·12	137	4·02	6·30	157
4·45	6·27	141	4·55	5·48	120
4·23	5·96	141	4·98	5·80	139
Mean 4·85	5·98	120	4·56	6·08	140

In two experiments in which adrenaline was applied repeatedly the control phosphorylase values declined in the course of the experiment ($2\frac{1}{2}$ hr) to 85 % of the first determination. In the beginning of these experiments the exceptional observations occurred, when adrenaline, although stopping the spike activity, caused depolarization. At the same time it was found to have no effect on phosphorylase activity in the parallel pieces (98 % of the controls). Later, in the course of the same experiments, when control values were lower, adrenaline increased the phosphorylase activity as it did in all other experiments.

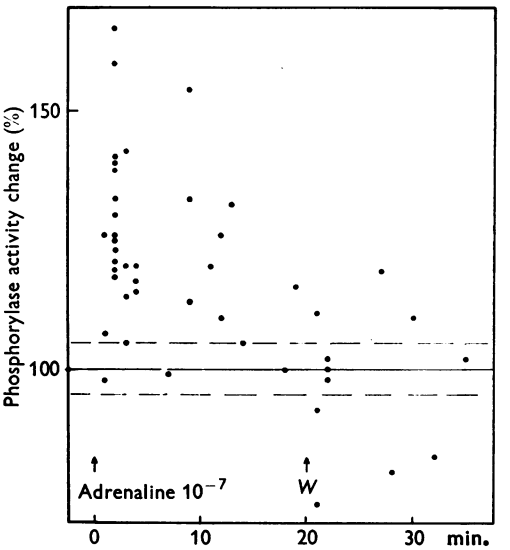

Fig. 2. The percentage change in phosphorylase activity during the exposure to adrenaline (1 and 2×10^{-7}) and after its removal (*W*).

The time course of the change in phosphorylase activity produced during an exposure to adrenaline, which lasted up to 20 min, was followed in several experiments. Figure 2 shows a diagram in which all the determinations made in the presence, and also after the removal, of adrenaline (1 or 2×10^{-7}) are presented as percentage of the first control in each experiment plotted against time. It is seen that during the exposure to adrenaline there was a steep initial rise in phosphorylase activity and the large majority of the results showed an increase above the normal range of variation (± 5 %); none were below. After the removal of adrenaline there were four observations below the normal range, four within, and three above. During this period the membrane was usually depolarized or underwent violent fluctuations. The observations were, however, too few to

allow a definite conclusion that there was a strict correlation as suggested by experiments like that shown in Fig. 1.

When lower concentrations of adrenaline were given the peak increase in phosphorylase activity was reached later. Thus, with 1 or 2×10^{-7} adrenaline the peak occurred after 2 min, with 4×10^{-8} it occurred after 7 min and with 1×10^{-8} it occurred after 12 min.

The effect of adrenaline in glucose-free solution

The results obtained with adrenaline in normal solution were consistent with the view that adrenaline might produce its biophysical effects indirectly, by increasing the rate of energy supply from glycogen breakdown. We therefore tried to deplete the glycogen store by exposure to glucose free solution.

The glycogen content of taenia coli varied greatly from one experiment to another, ranging from 0·044 to 0·128 % wet weight with a mean of 0·073 % ± 0·004 (S.E. of mean; 20 observations). Control pieces taken from the same animal, however, did not differ by more than 10 %.

TABLE 2. Glycogen content of taenia coli (% wet weight)

Hours after exposure to glucose-free medium ...	0 (30 min in normal medium)	1	2	4
Expt. 1	0·128	0·044	0·019	0·022
2	0·080	0·048	0·025	0·022
3	0·077	0·045	0·037	0·019
4	0·068	0·043	0·025	0·021

If pieces of taenia were exposed to dissecting Ringer's solution (room temperature, not oxygenated) for 3 hr they lost 50 % of their glycogen content. The glycogen loss during the average dissection period of about 40 min was not determined, but after 30 min suspension in oxygenated Krebs's solution at 37° C the normal glycogen content was present. It remained for 3 hr in normal conditions in two experiments, in another it fell to 70 % of the initial value.

Exposure to glucose-free solution led to a progressive reduction in glycogen content. The results of four experiments are shown in Table 2. Within 1 hr the glycogen content fell to 50 %, within $2\frac{1}{2}$ hr to 30 %, but after 4 hr it was still 25 % of the normal control. Thus, within the period of our experiments the removal of glucose from the medium severely diminished the glycogen store, but did not deplete it completely.

The biophysical effects produced by the removal of glucose have been described in detail in the previous paper (Axelsson & Bülbring, 1961). We found no consistent effect on the membrane potential. In four out of

seven experiments the first change after the removal of glucose was an increase in membrane potential; this was followed by depolarization. In two experiments there was little change, in one experiment we observed a progressive depolarization.

During the first stage of glucose depletion the phosphorylase activity remained either unchanged or was higher than the normal control. In this condition adrenaline had little effect or actually caused a decrease. Such a depression of phosphorylase activity by adrenaline was never seen in normal solution. The results of three experiments are shown in Table 3. During the second and third hour of exposure to glucose-free solution phosphorylase activity declined progressively and, when it had become very low, adrenaline caused a large increase bringing it back to the normal range.

TABLE 3. Phosphorylase activity (μmoleP/g) in normal and in glucose-free solution before and after adrenaline

	Normal solution		Glucose-free solution			
Expt. No.	Control	After adrenaline	Time of exposure (min)	Control	After adrenaline	Change (%)
1	—	—	18	4·53	3·65	67
			45	4·70	5·08	108
			90	5·53	6·01	109
			115	3·28	5·73	175
2	4·40	5·20	67	6·48	5·20	80
3	5·25	6·45	180	3·42	5·18	152

The biophysical effects produced by adrenaline in the absence of glucose from the medium were complex, and three aspects will have to be considered, the membrane potential, the spike activity, and the tension.

Prolonged exposure to glucose-free medium gradually abolished the hyperpolarizing action of adrenaline which, in the end, often caused depolarization instead. The gradual change is shown in Fig. 3. It may be noted that the depolarization after 4 hr exposure to glucose-free medium occurred from a very similar level of membrane potential (see reference line at beginning of each record) as the hyperpolarization produced by adrenaline after only 1 hr exposure. However, while the spikes were initially (*a*) abolished throughout the whole period of exposure to adrenaline, they returned in (*b*) and (*c*) while adrenaline was still present (not shown in the figure).

In the absence of glucose the change in the effect of adrenaline on spontaneous spike activity was indeed more consistent than the change in the effect on the membrane potential. Figures 4, 5 and 6 are all taken from the same experiment in order to show the sequence of events. In normal solution (Fig. 4*a*) adrenaline caused the usual hyperpolarization

and abolished the spikes, which remained absent throughout. After washing out (Fig. 4*b*) they returned at an increased rate and consequently the tension rose to a level higher than that seen initially. After 70 min exposure to glucose-free solution (Fig. 4*c*) the spikes remained absent for only 4 min; they can be seen to occur before adrenaline was washed out. The tension which had been low as a result of the removal of glucose was at first increased by the addition of adrenaline. When the spikes stopped (note the longer latency for the cessation to occur) the tension was of

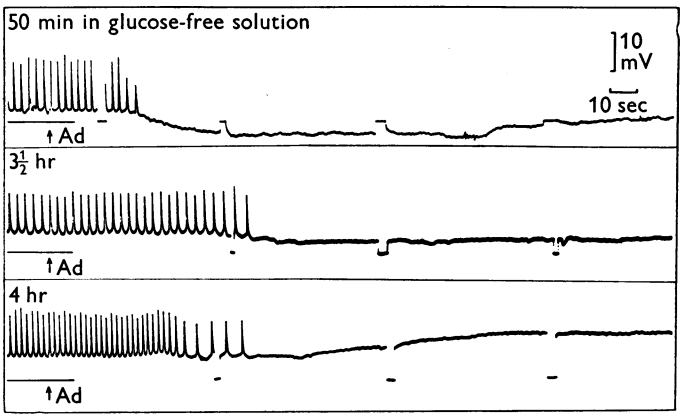

Fig. 3. The gradual change of the effect of adrenaline 10^{-7} on the membrane potential during prolonged exposure to glucose-free solution. (The records in this and subsequent figures are periodically interrupted to check possible drift.)

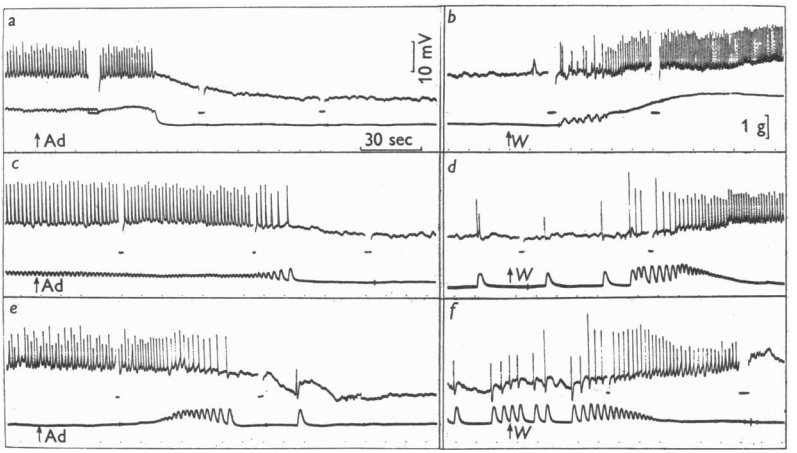

Fig. 4. The effect of adrenaline 10^{-7} on membrane potential and electrical activity (upper record) and on tension (lower record) in normal solution (*a*). After 10 min exposure, at *W*, the adrenaline was washed out (*b*). Adrenaline was given again 70 min (*c* and *d*), and 1 hr 50 min after removal of glucose (*e* and *f*). For description see text.

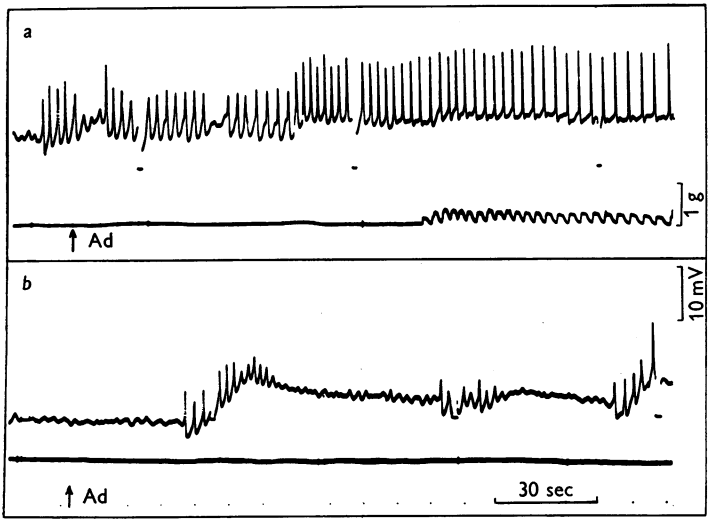

Fig. 5. Continuation of the same experiment as that shown in Fig. 4. (*a*) The effect of adrenaline in the absence of glucose for 2 hr 40 min; (*b*) after 15 min in normal solution, following 4 hr 30 min exposure to glucose-free solution.

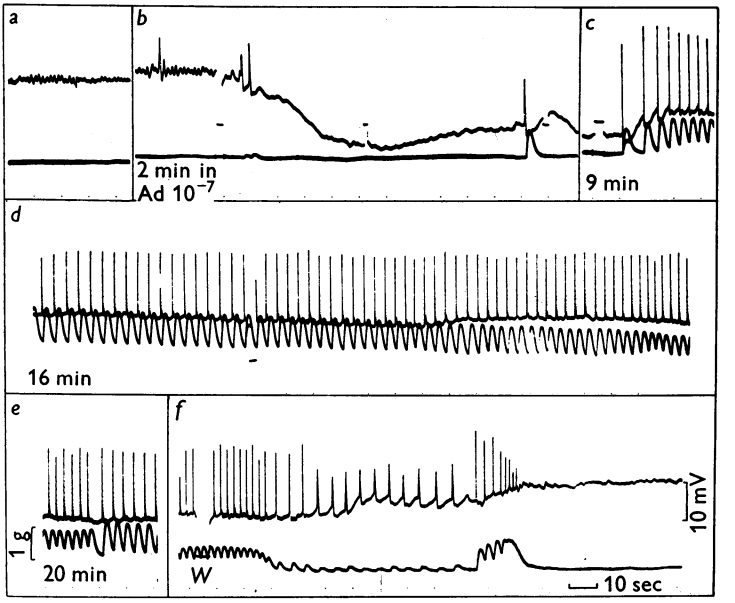

Fig. 6. Continuation of the same experiment as that of Figs. 4 and 5. The preparation had been exposed to glucose-free solution for 4 hr 30 min. Readmitting glucose for 1 hr produced no recovery (*a*). The effect of adrenaline 10^{-7} is shown in *b*, *c*, *d* and *e*, and of its removal (*W*) in *f*. For description see text.

course abolished, but as soon as spikes returned the tension response was large. After washing out the adrenaline (Fig. 4*d*) there was the usual increased rate of spike discharge but in the absence of adrenaline the tension declined and soon disappeared. After 1 hr 50 min exposure to glucose-free solution (Fig. 4*e*) the spikes, discharged at a rapid rate, failed to produce tension. Adrenaline stopped the spikes only for 90 sec. Its first effect was to restore the tension response which remained strong throughout the exposure and disappeared one minute after the removal of adrenaline (Fig. 4*f*).

When the preparation had been exposed to glucose-free solution for 2 hr 40 min it was still spontaneously active but there was no tension response to the spikes (Fig. 5*a*). At this stage adrenaline caused a depolarization and increased the rate of spike discharge. After exposure to glucose-free solution for $4\frac{1}{2}$ hr the normal amount of glucose was added to the solution. This produced no recovery in a preparation in which all spontaneous activity had ceased. The addition of adrenaline 15 min later (Fig. 5*b*) caused a depolarization, and initiated spikes which produced, however, no tension. Continued exposure to normal solution failed to bring about a recovery (Fig. 6*a*). The membrane potential oscillated slightly, there was no tension. In the presence of adrenaline (given 45 min after the dose shown in Fig. 5*b*) a few spikes were observed (Fig. 6*b*), producing a trace of tension, but they stopped immediately as the membrane hyperpolarized by 20 mV. When this hyperpolarization passed off, spikes were initiated. They produced a strong tension response and they continued (*c*, *d*, *e*) as long as adrenaline was present, for 26 min. At this stage removal of adrenaline (*f*) abolished the spikes, and consequently also the tension within 2 min.

This experiment has been described in such detail because it not only showed the variety and complexity of the effects produced by adrenaline in glucose-free solution but also the gradual change from an inhibitory to a stimulant action.

Anaerobic conditions and the effect of insulin

In an attempt to increase the rate and the extent of the glycogen depletion some preparations were exposed to glucose-free solution gassed with N_2 and CO_2. However, we found that anaerobic conditions did not reduce the glycogen content below 20 % after 1 hr. Moreover, prolonged exposure to glucose-free solution in anaerobic conditions appeared to produce irreversible changes, as the tissue only regained 50 % of the original glycogen 2 hr after readmission of O_2 and the normal concentration of glucose. Increasing the glucose concentration to 5 times normal and the addition of insulin 4 u./l. had no effect on glycogen restoration. On the

other hand, if the absence of glucose and oxygen did not exceed 20 min, the tissue regained up to 80 % of its original glycogen content within 60 min. In a single experiment a high glucose concentration of the medium supplemented by insulin increased glycogen resynthesis above the initial content.

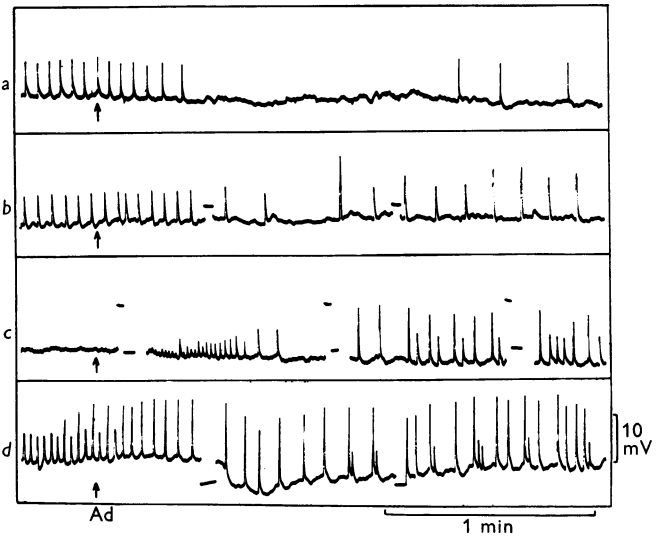

Fig. 7. The effect of adrenaline 10^{-7} on electrical activity (*a*) in normal solution; (*b*) 21 min after removal of glucose and oxygen; (*c*) 45 min after readmission of oxygen, glucose concentration 5 times normal + insulin 4 u./l.; (*d*) 15 min after (*c*).

The biophysical action of adrenaline was changed by the removal of glucose in the same way in aerobic and anaerobic conditions, i.e. the inhibitory effect was gradually abolished. In the experiment shown in Fig. 7 adrenaline caused the usual inhibition (*a*) in normal solution. After 20 min depletion (*b*) the inhibition was less. Oxygen was readmitted. Glucose in 5 times the normal concentration and insulin 4 u./l. were given at the same time. The membrane became hyperpolarized and all activity stopped (*c*). Adrenaline now led to a reappearance of spikes at a high frequency. After recovery had proceeded for 1 hr in the presence of high glucose and insulin, the usual inhibitory effect of adrenaline was slowly restored.

The effect of temperature

We also attempted to reduce the metabolic effect of adrenaline by reducing the temperature. When we measured the effect of temperature on phosphorylase activity, we found that at 20° C it was still 50 % of that at

34° C, and by plotting the results shown in Table 4 it could be determined that phosphorylase activity would stop at only about 4° C.

One preparation was cooled from 37 to 5° C. At 15° C all spontaneous activity had stopped and there was a slight depolarization. At 5° C adrenaline was given, but it had no effect whatsoever on the membrane

TABLE 4. The effect of temperature on phosphorylase activity.
Activity at 34° C = 100

Temperature during assay (° C)	Percentage activity
5	8·5
10	16
15	29
17	43
19	51
21	56
23	63

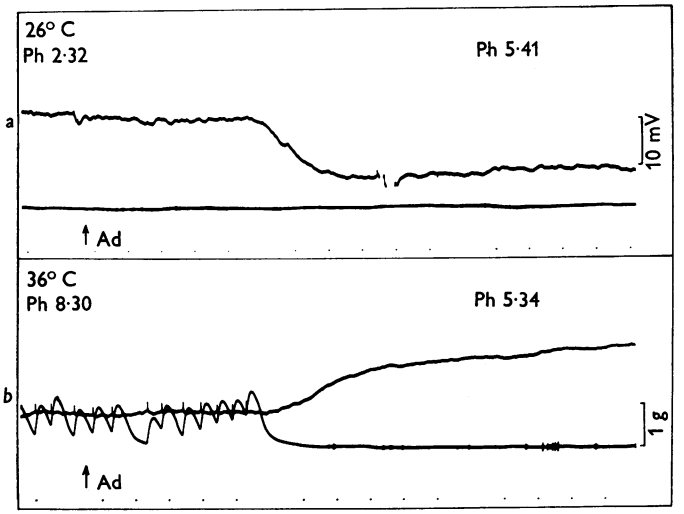

Fig. 8. The effect of adrenaline on phosphorylase activity (Ph) and on the membrane potential, (*a*) at 26° C; and (*b*) at 36° C, when the initial membrane potential was 30 mV higher than that in (*a*). Time marker 10 sec. For further description see text.

potential. Another preparation was only cooled to 30° C. At this temperature adrenaline caused slow oscillations of the membrane potential, 6 mV in amplitude at a frequency of 2–3/min.

A third preparation was set up at 26° C and kept at this temperature without an initial period at 37° C. It showed no spike activity. When adrenaline was given at this temperature it caused a large hyperpolarization, as is shown in Fig. 8*a*. At the same time it increased phosphorylase

activity from 2·32 to 5·41 μmoleP/g. Having been at 26° C for 2 hr, the preparation was warmed to 36° C. There was a 30 mV increase in the membrane potential, and after 14 min spikes appeared. Phosphorylase activity rose to 8·30 μmoleP/g (Fig. 8*b*). Adrenaline now caused depolarization though it stopped the spike activity. At the same time it reduced phosphorylase activity to 5·34 μmoleP/g.

Metabolic inhibitors

A few experiments were carried out to investigate the effect of metabolic inhibitors on the action of adrenaline. We used low concentrations to allow the effect of adrenaline on electrical activity to be observed. We found that in the presence of azide the hyperpolarizing action of adrenaline was reduced, but it continued to stop spontaneous spike discharge. When azide had acted for longer periods and had abolished spontaneous activity adrenaline produced no effect.

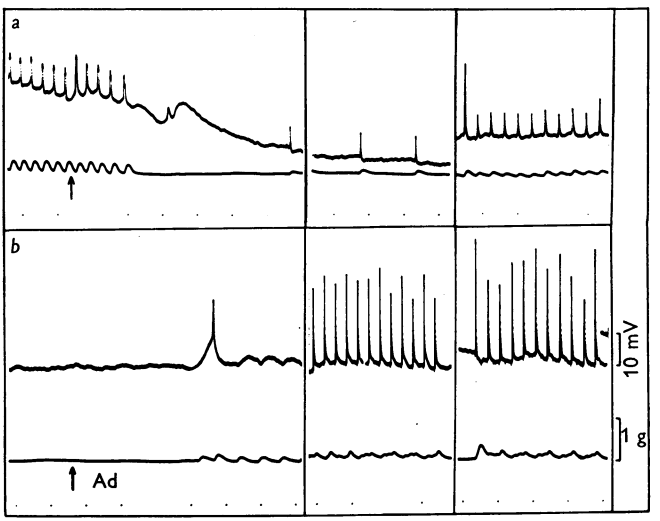

Fig. 9. The effect of adrenaline 5×10^{-7} on membrane potential, electrical activity and tension (*a*) in the presence of iodoacetate 10^{-5} for 38 min, (*b*) for 90 min. The second record in each row was taken 2 min, the third 5 min, after giving adrenaline (see text). Time marker 10 sec.

On the other hand, iodoacetic acid (IAA) 10^{-5} reduced not only the hyperpolarizing action of adrenaline but also its action on spontaneous spike activity and on the response to electrical stimulation. When IAA had abolished all spontaneous activity the effect of adrenaline was reversed. It now caused depolarization and initiated spikes (see Fig. 9).

Replacement of sodium

The experiments so far were consistent with the view that the activation of phosphorylase by adrenaline might increase the rate of energy supply for processes stabilizing the membrane, e.g. some of the energy might be utilized for the active extrusion of sodium. We therefore studied in three experiments the action of adrenaline in the absence of sodium from the medium. NaCl was replaced by LiCl and $NaHCO_3$ by $KHCO_3$ and a corresponding amount of KCl was withdrawn. The effects produced by substituting

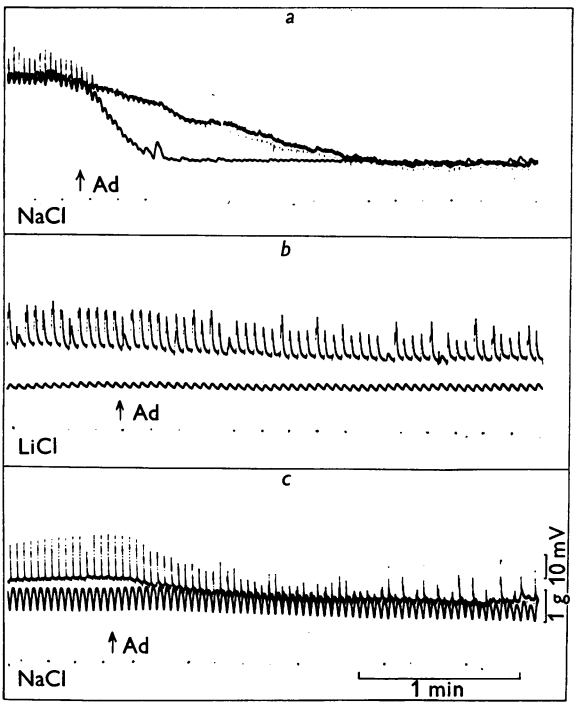

Fig. 10. The effect of adrenaline 5×10^{-7} on a preparation driven electrically (*a*) in normal solution; (*b*) 17 min after replacing NaCl by LiCl; (*c*) 85 min after replacing LiCl by NaCl.

Na with Li have been described elsewhere (Axelsson & Bülbring, 1959; Axelsson, 1960). Its main effect is to cause a decline of the tension response though it does not impair spike activity for several hours. Figure 10 shows records from an experiment in which the preparation was electrically driven. In normal solution (*a*) adrenaline abolished the conducted response to electrical stimulation, the tension fell to zero and the membrane was hyperpolarized. Substitution of NaCl by LiCl abolished the action of adrenaline almost completely (*b*), and even after 85 min in normal solution the adrenaline effect showed only a partial recovery (*c*).

DISCUSSION

The taenia coli of the guinea-pig, which was the intestinal smooth-muscle preparation used for the experiments, is a tissue possessing a high degree of spontaneous activity. Its membrane potential is very unstable and in normal conditions it probably never reaches a true resting value. Adrenaline relaxes this muscle because it stops the spontaneous spike discharge. It also causes an increase of the membrane potential, which may rise by as much as 20 mV.

We found that adrenaline increased phosphorylase activity in the smooth muscle of the taenia coli. When the biochemical effect was measured in parallel strips from the same taenia as that in which the biophysical changes were recorded, the increase in phosphorylase activity was seen at the same time as that at which the hyperpolarization was observed.

It may be assumed that the activation of phosphorylase in smooth muscle by adrenaline is brought about by a mechanism similar to that discovered by Sutherland & Rall (1958) in liver, i.e. by an increase in the formation of cyclic adenosine-3′,5′-phosphoric acid (3′,5′-AMP). This compound exerts a stimulating effect on the enzymic synthesis of active phosphorylase in liver (Rall, Sutherland & Berthet, 1957), as well as in skeletal and heart muscle (Rall & Sutherland, 1958; Krebs, Graves & Fisher, 1959). If the production of glucose-1-phosphate in taenia coli were limiting the rate of carbohydrate metabolism, activation of phosphorylase would increase the supply of metabolic energy.

Previous observations, especially those reported in the preceding paper (Axelsson & Bülbring, 1961), indicate that the metabolic rate may be an important factor influencing membrane excitability and controlling membrane polarization in smooth muscle. Our present results suggest that the increased supply of energy in the presence of adrenaline is utilized by processes in the cell membrane requiring energy.

Evidence for an accelerated rate of active Na extrusion in the presence of adrenaline has recently been obtained (E. Bülbring & P. J. Goodford, unpublished observations), when it was found that the rate of loss of ^{24}Na from taenia coli was approximately doubled by adrenaline, while the rate of uptake was reduced. Furthermore, we observed that adrenaline failed to produce its usual stabilizing effect when the Na in the medium was replaced by Li, which, as was shown by Keynes & Swan (1959) for frog skeletal muscle, is extruded much more slowly than Na.

An increased supply of energy by adrenaline activating phosphorylase should be prevented if the tissue was depleted of glycogen. It proved to be impossible to deplete the glycogen store completely. Even after 4 hr exposure to glucose-free solution there remained 20–25 % of the original

glycogen concentration in the muscle. This low level was reached in a shorter time, but it was not reduced further, in anaerobic conditions. It may be that the residual material is not identical with glycogen, but that it is another polysaccharide which cannot serve as a source of metabolic energy. If this were so, the failure of adrenaline to stabilize the membrane would be explained. In all experiments the action of adrenaline was profoundly altered, and in some reversed, when the preparation was exposed for many hours to glucose-free solution.

We may assume that, as long as there is glycogen available which can be utilized, adrenaline will accelerate its break-down. However, the resulting energy will be distributed between the contractile mechanism and the cell membrane. During the first stages of glucose depletion (see Fig. 4) adrenaline always produced hyperpolarization after a variable latency and to a variable degree. Its capacity to prevent spike discharge was diminished, but the tension could still be restored. When the glycogen store was presumably very low, and as is apparent from the figures shown in Table 3, the resulting portion was less easily broken down, adrenaline had a depolarizing action accelerating spike discharge (see Fig. 5). The available energy was first employed for the restoration of tension and only after a long latency it appeared to be available for the stabilization of the membrane. After still further depletion this metabolic action completely disappeared.

The striking effect of adrenaline during the period of recovery, when the preparation depended on adrenaline for a normal rhythmic activity, raises the question whether it might also exert an action on glucose uptake and utilization.

Of the metabolic inhibitors tested, iodoacetate appeared to be the most effective in abolishing the stabilizing effect of adrenaline on the cell membrane. Dinitrophenol (Burnstock, 1958) and azide only partially antagonized adrenaline.

A reversal of the action of adrenaline was observed in several conditions. After prolonged exposure to glucose-free solution and also in the presence of IAA adrenaline caused depolarization and initiated spikes. This result could be interpreted as a stimulant action—presumably by some change in membrane permeability—which was unmasked when the metabolic action had been abolished.

A few observations were recorded when adrenaline caused depolarization, but nevertheless stopped all spike activity. They occurred twice at an early stage in normal solution, twice at an early stage of exposure to glucose-free solution, and finally twice when, after exposure to low temperature, the preparation was warmed to 37° C. We have evidence that in these various conditions phosphorylase activity may be relatively high. In

those experiments in which we measured the effect of adrenaline on phosphorylase activity in parallel with observing its depolarizing action we found that adrenaline produced either no change or diminished phosphorylase activity. These results cannot as yet be explained and further experiments are required.

The remarkable coincidence between the hyperpolarization and the activation of phosphorylase in normal conditions supports the assumption of a causal relation. On the other hand, in some tissues 3′,5′-AMP and adrenaline increase the rates of other reactions concerned with the utilization of carbohydrate and of lipids (Cahill, Leboeuf & Flinn, 1960; Mansour & Menard, 1960; Rizak, 1960; Vaughan, 1960); therefore, biochemical mechanisms other than activation of phosphorylase may be associated with the hyperpolarizing action of adrenaline on taenia coli. Finally, the possibility cannot be excluded that 3′,5′-AMP may induce hyperpolarization by a direct effect on the membrane.

SUMMARY

1. Isolated guinea-pig taenia coli was used for recording biophysical changes from one strip and measuring phosphorylase activity in other strips subjected to the same experimental conditions.

2. In normal solution adrenaline stopped spontaneous spike activity, abolished the conducted response to electrical stimulation and caused hyperpolarization. At the same time adrenaline increased phosphorylase activity.

3. In glucose-free solution, as the preparation was gradually depleted of glycogen the stabilizing effect of adrenaline on the cell membrane and the hyperpolarization became gradually less, until, after prolonged exposure to glucose-free medium, the effect of adrenaline was reversed. It caused depolarization and initiated spikes.

4. A similar stimulant action was observed in the presence of iodoacetic acid which abolished the inhibitory action of adrenaline.

5. Cooling to 5° C abolished the hyperpolarizing action of adrenaline. When the preparation was warmed again to 37° C and phosphorylase activity was relatively high, adrenaline diminished it and caused depolarization.

6. The stabilizing effect on the cell membrane normally produced by adrenaline was not seen when sodium in the solution was substituted by lithium.

7. The results are consistent with the view that adrenaline has a dual action. It appears to have a direct depolarizing effect on the membrane which, however, is normally masked by its more powerful inhibitory action.

The experiments suggest that adrenaline exerts its inhibitory effect by increasing the rate of energy for active stabilizing processes at the cell membrane.

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