J. Physiol. (1961), **156**, *pp.* 344–356
With 10 *text-figures*
Printed in Great Britain

METABOLIC FACTORS AFFECTING THE ELECTRICAL ACTIVITY OF INTESTINAL SMOOTH MUSCLE

BY J. AXELSSON AND EDITH BÜLBRING
From the Department of Pharmacology, University of Oxford

(*Received* 9 *December* 1960)

In a recent investigation it was found that the abolition of spontaneous spike discharges and the hyperpolarization, which adrenaline caused in intestinal smooth muscle, occurred simultaneously with an increase of phosphorylase activity (Axelsson, Bueding & Bülbring, 1959). The hyperpolarizing effect of adrenaline was found to be decreased or abolished when the muscle was depleted of glycogen by exposure to glucose-free medium.

This suggested that the effect of adrenaline on the electrical activity of smooth muscle was to some extent brought about by an influence on metabolic rate. It was therefore interesting to see if other conditions, in which a direct influence on metabolic rate could be expected, would affect the electrical activity in a similar way.

Two preliminary reports of such experiments have already been given (Axelsson & Bülbring, 1960*a*, *b*).

METHODS

All experiments were performed on the guinea-pig's taenia coli. Simultaneous records of membrane potential, action potentials and tension were obtained by the sucrose-gap method (Stämpfli, 1954; Burnstock & Straub, 1958).

Some modifications of the apparatus were made to improve temperature control and effect quick changes of temperature. Otherwise the apparatus was the same as that described by Burnstock (1958) and Bülbring & Burnstock (1960).

The composition of the bathing solution was (mM): Na^+ 137·47, Ca^{2+} 2·49, K^+ 5·93, Mg^{2+} 1·19, Cl^- 134·11, HCO_3^- 15·48, $H_2PO_4^-$ 1·19, glucose 11·50. This solution was saturated with a gas mixture of 97 % O_2 and 3 % CO_2. Its pH was 7·4–7·5. In those experiments in which glucose was withdrawn, the composition of the solution was otherwise unchanged; or, in a few experiments, the glucose was replaced by an equivalent amount of sucrose. In some experiments the NaCl was replaced by an equivalent amount of LiCl; the composition of the solution was otherwise unchanged. The temperature was usually 37° C; it could be changed by more than 10° C in one minute.

The metabolic inhibitors used were: Mono-iodoacetic acid (IAA) in concentrations from 10^{-6} to 10^{-5} and sodium azide (NaN_3) in the same concentrations.

The tension was recorded with a mechano-electric transducer valve (RCA 5734) mounted as described by Bülbring (1955). The initial tension of the muscle was adjusted to 0·5–1 g.

RESULTS

The effect of temperature changes on the spontaneous electrical activity of the smooth muscle and on the rate at which it can be electrically driven

A rise in temperature (from a range of 23–27° C to 33–37° C) temporarily slowed the frequency of spontaneous spike discharge, which in some experiments stopped for 1–2 min. Usually this effect was accompanied by a rise in membrane potential. This response to raising the temperature was seen best when the preparation had been kept from the beginning of the experiment slightly above room temperature (23–27° C) and showed relatively slow spontaneous activity.

Figure 1 shows a typical temperature effect on a muscle at very low initial tension. The rise in temperature caused immediate cessation of electrical activity, and hyperpolarization. When spike activity started again at the higher temperature it was much faster than before.

In Fig. 2 a typical effect of lowering the temperature is seen. On cooling, the activity became faster and there was a considerable depolarization. It should be noted that at 33° C the membrane potential between spikes was fairly stable. As soon as cooling started each spike was followed by a hyperpolarization and, as the frequency increased, there was a slow membrane depolarization between spikes, so that at 27° C the potential oscillated and spikes occurred on top of each wave. As the temperature declined further the frequency of discharge diminished accordingly. Later, when the temperature was allowed to reach the initial height, the membrane potential rose again and it also became stable between spikes.

When the initial tension was very high, as in Fig. 3, only a very short-lasting inhibition was seen when the temperature was raised; one spike dropped out. The higher temperature caused no secondary increase in frequency. However, each individual tension response was stronger, and the rate of relaxation became faster.

A transient inhibition during warming, varying in degree, was seen in every experiment, each including a number of observations. In some experiments the muscle was subjected to electrical stimulation. It was found to be more easily driven when moderately cooled. Conversely, warming always caused some spikes to fall out and sometimes made the preparation inexcitable for a short time.

It should be remembered that a change in the effective driving rate might be the consequence not only of a change in excitability but equally of a change in conductivity. In using the sucrose-gap method the failure to record a spike in response to a supra-maximal stimulus might be due to blocked or impaired conduction in the piece of muscle between the stimulating and recording electrodes. This objection might therefore be

valid for all experiments where electrical stimulation was used. Similarly, when no spontaneous activity was recorded, it would only indicate that no spontaneous firing was taking place at the point of recording, but block of conduction might obscure activity elsewhere.

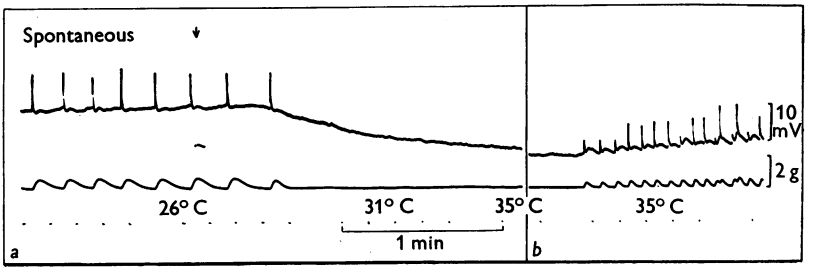

Fig. 1. Guinea-pig taenia coli. Upper record, membrane potential and electrical activity; lower record, tension. (*a*) A rise in temperature (starting at arrow) from 26 to 35° C stopped spontaneous activity and increased the membrane potential: (*b*) 2 min later spontaneous discharge returns at a faster rate in spite of the higher membrane potential.

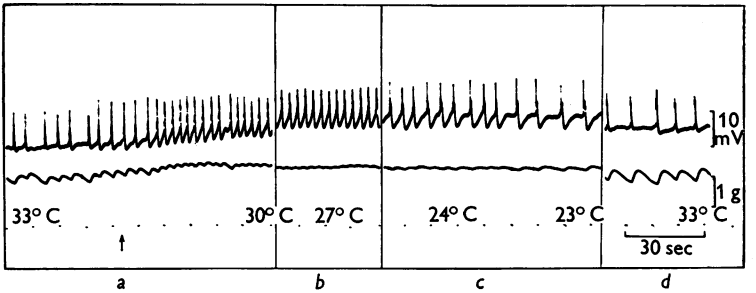

Fig. 2. The effect of cooling (starting at arrow). Records as in Fig. 1. 40 sec interval between (*a*) and (*b*); 20 sec between (*b*) and (*c*); 5 min between (*c*) and (*d*). For description see text.

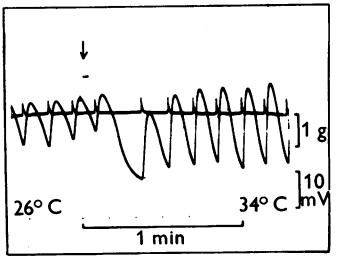

Fig. 3. The effect of warming (starting at arrow). Records as in Fig. 1.

The effect of removing and returning the external glucose

The procedure of removing and returning the external glucose could be repeated many times and at short intervals. A great number of observations could therefore be made in each experiment. Removal of glucose gradually abolished the muscle tension (Feldberg & Solandt, 1942). This was due to a failure of the spikes to evoke a tension response. Indeed, the removal of glucose from the medium usually increased the spontaneous electrical activity of the muscle. Moreover, while the range of frequencies at which the preparation could be driven in normal solution was narrow and very close to the frequency of spontaneous discharge, the driving range widened in the absence of glucose, increasing particularly towards the higher frequencies. But as the spontaneous membrane activity and its electrical excitability increased, the tension declined.

Figure 4 shows a typical effect of withdrawal of external glucose. In normal solution (*a*) the muscle responded to only every other of a series of stimuli applied at a frequency of 36/min. The spontaneous discharge was 9/min. Fifteen minutes after the removal of glucose (*b*) the muscle responded with an action potential to every stimulus (frequency, strength and duration of stimuli unchanged) and the frequency of spontaneous discharge was now 33 spikes/min. In spite of the increased rate of spike discharge the tension was now very low.

When glucose was returned to the solution a varying degree of inhibition was observed in every experiment. This inhibition was usually of long duration. The records shown in Fig. 5 are taken from the same experiment as those in Fig. 4, at a later stage after glucose had been removed repeatedly for several periods of about 15 min. The typical effect of reintroducing glucose is seen. The spontaneous spike frequency in glucose-free medium (*a*) was 24/min. The preparation could be driven up to the rate of 42/min. The frequency of 20/min was too slow, the muscle fired spontaneously between the stimuli, and there seemed to be no correlation between the stimili and the action potentials. The presence of glucose for only 4 min (*b*) slowed spontaneous activity down to 6/min and the driving rate was also greatly reduced. At a frequency of 32/min only every second or third stimulus evoked a spike, but the tension was already greatly improved. After 18 min in glucose-containing solution (*c*) the preparation could still only be driven at 3/min. After 30 min in glucose-containing solution (*d*) the maximal driving rate had risen to 20/min.

In many experiments the effect of reintroducing the glucose was best seen in the beginning, after the first long withdrawal of the external glucose. Sometimes after repeated withdrawal of glucose the effect of reintroducing it was small and short-lasting as far as the spontaneous

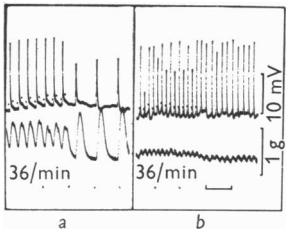

Fig. 4. The effect of removing the glucose from the bathing solution: *a* normal; *b* glucose-free. Records as in Fig. 1. Note the increase in driving rate and in spontaneous activity after the removal of glucose. Time marker, 10 sec. Further description see text.

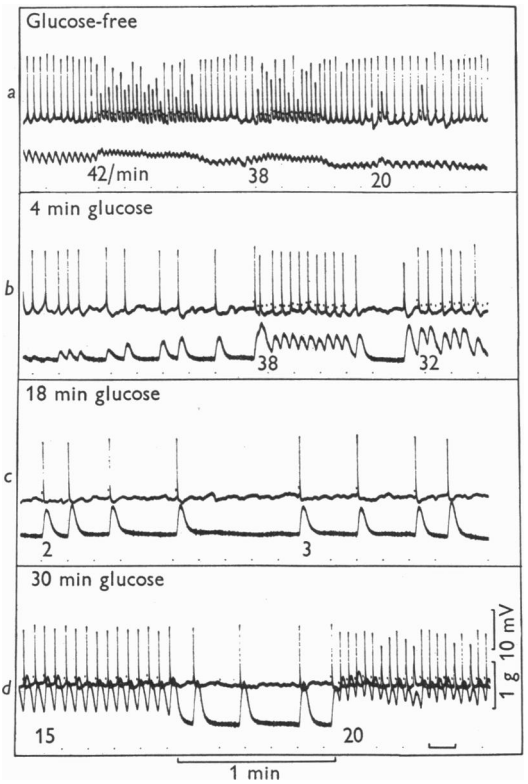

Fig. 5. The effect of returning the glucose after exposure to glucose-free solution. Records as in Fig. 1. Figures give driving rate per minute. Time marker, 10 sec. For description see text.

activity was concerned, but it was more clearly seen on the driving rate. Figure 6 shows an example of this kind. The upper record (*a*) was taken from a muscle in glucose-free solution easily driven at a frequency of 34/min. After the reintroduction of glucose the preparation soon responded with a spike only to every other stimulus. It should be noted, however, that there was at first a tension response to each stimulus. This observation indicated that the failure to record a spike was here due to a

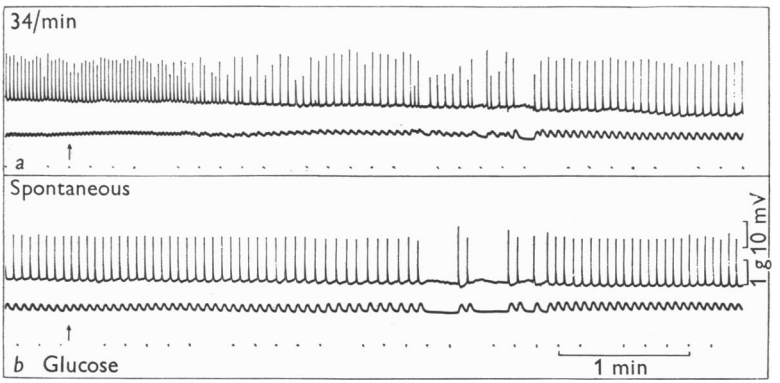

Fig. 6. The effect of returning glucose (at arrow) after repeated exposure to glucose-free medium, totalling 45 min in (*a*) and 85 min in (*b*). The depressed excitability is seen in the driven preparation in (*a*), a more transient effect on spontaneous activity in (*b*).

failure in conduction. The lower record (*b*) shows the effect of the third application of glucose, after 20 min withdrawal, on the spontaneous activity of the same muscle. There was only a very short-lasting inhibition. In both records the electrical activity was slowed while the tension increased.

The effect of metabolic inhibitors

Metabolic inhibitors have been found to cause an initial stimulation (Born & Bülbring, 1955), but Bülbring & Lüllman (1957) found that iodoacetate was an exception as, in a concentration of 10^{-3} M, it caused no increase of electrical activity. We have used fifty times lower concentrations and observed that, like dinitrophenol (DNP) and azide, iodoacetate increased the rate of spontaneous spike discharge. In one experiment it was also found to increase the driving rate of the muscle from 14 to 30/min. The effect of IAA was not easily reversible and reproducible within the same experiment.

The effect of a metabolic inhibitor on the usual effect produced by temperature change

IAA, in the concentration of 5×10^{-6} g/ml., was found to abolish the usual inhibitory effect of a sudden rise in temperature on the electrical activity of the taenia coli. Figure 7 shows that the rise in temperature, which in normal conditions (*a*) stopped the spike activity, in the presence

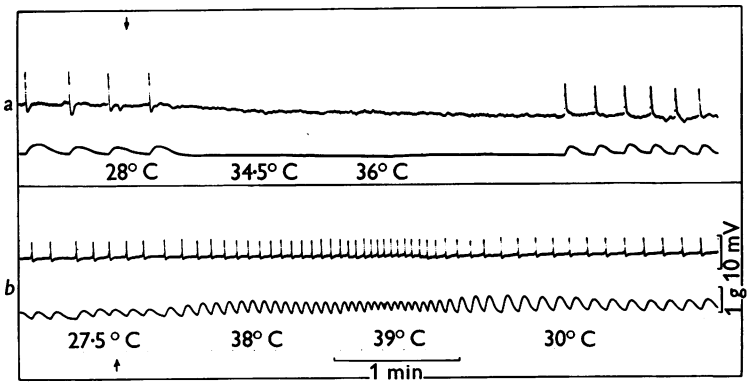

Fig. 7. The abolition of the effect of raising the temperature by iodacetate: (*a*) normal solution; (*b*) after 27 min in IAA. Note transient inhibition of electrical activity and hyperpolarization in (*a*) and immediate acceleration of spike discharge in (*b*). Records as in Fig. 1.

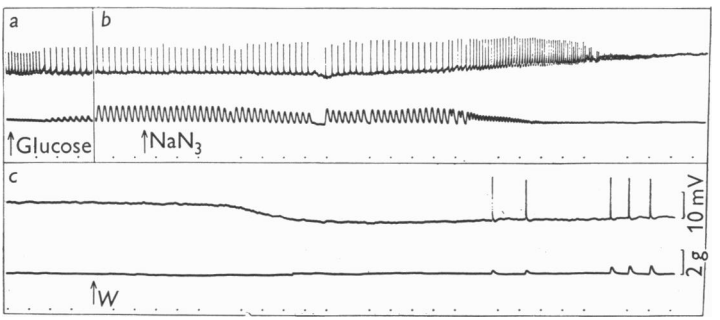

Fig. 8. The effect of returning glucose after prolonged exposure to glucose-free solution (*a*); the abolition of this effect by azide, given at arrow, (*b*); and the increase of membrane polarization (*c*) after removal of azide (*W*). Records as in Fig. 1. Time marker, 10 sec.

of IAA (*b*) only increased the spike frequency. The records also show the acceleration of spontaneous activity by IAA. When lower concentrations of IAA (2×10^{-6}) were used, its effect was sometimes reversible, but it was difficult to determine a suitable concentration. Either the muscle activity

was irreversibly blocked after a very short period of increased activity, or it was not affected at all. Thus there were only a few successful experiments in that the temperature effect could be re-tested after recovery.

The effect of metabolic inhibitors on the effect of returning the glucose after a period of depletion

Metabolic inhibitors also abolished the inhibitory action of restoring the normal glucose concentration after a period of depletion. The record in Fig. 8*a* is taken from a preparation which in the absence of glucose could be driven at a frequency of 44/min. When glucose was added (*a*) the preparation failed to respond and a spike was only evoked by every other stimulus. The addition of NaN_3 in (*b*) abolished the effect of glucose and the preparation became more excitable again. It then became depolarized to such an extent that stimulation became ineffective. When NaN_3 was washed out (*c*) at *W*, glucose being present throughout, the membrane repolarized and spike activity reappeared.

The effect of removal of glucose on changes in electrical activity normally produced by temperature changes

In these experiments the temperature effect was first recorded in normal conditions. Then the glucose was withdrawn from the solution. During the gradual depletion the effect of raising the temperature was tried repeatedly and it was found that its inhibitory effect became gradually weaker until it was fully abolished. The abolition usually coincided with the point when the tension response was nearly abolished, which occurred after a period varying from $\frac{1}{2}$ to 2 hr. The glucose was then returned to the bathing solution and, as soon as the first inhibitory effect of the glucose had passed off, the effect of raising the temperature was tested again. It then produced its usual inhibitory action.

In Fig. 9 the inhibitory action of a rise in temperature in normal solution is not shown. After 80 min in glucose-free solution (*a*) the rise in temperature had still some inhibitory effect. After 2 hr in glucose-free solution (*b*) the tension response was almost abolished. In spite of this, and in spite of the temperature being lower than in (*a*), the spontaneous activity was faster. A rise in temperature had now no inhibitory effect, it only accelerated the spike frequency.

The effect of substituting Li for Na on the usual effect of glucose and of raising the temperature

In two experiments all the NaCl of the test solution was replaced by LiCl, Na being present only as $NaHCO_3$ in its normal concentration. The

effect of removing the glucose and its replacement was tested, both on spontaneous activity and driving rate.

The results are shown in Fig. 10. In (*a*) spontaneous activity is seen first, then the spikes increased in amplitude when they were evoked by electrical stimulation (33/min). This muscle had been in glucose-free

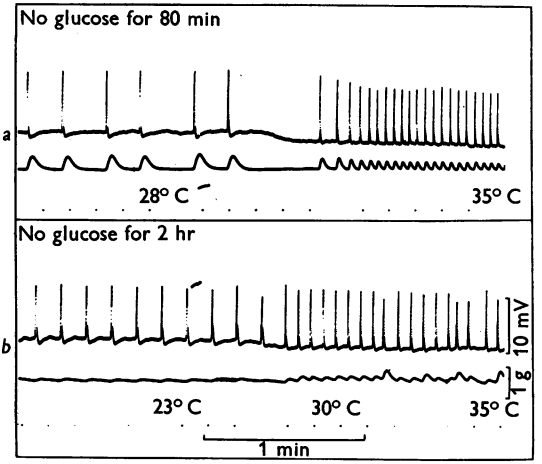

Fig. 9. The inhibitory action on raising the temperature (*a*) being gradually abolished by prolonged exposure to glucose-free solution (*b*). Records as in Fig. 1.

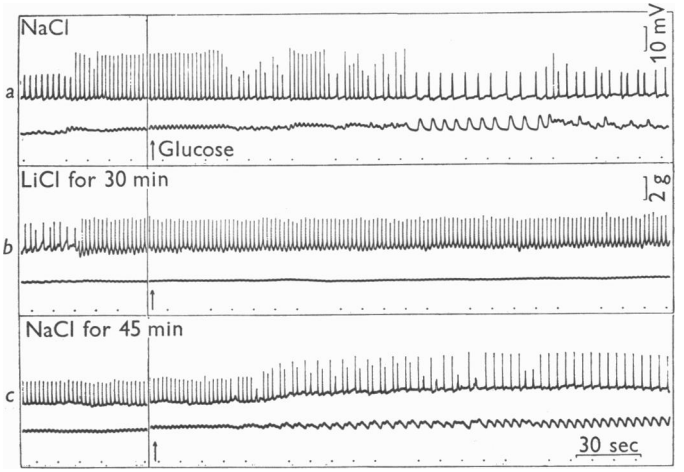

Fig. 10. In each record the muscle had been exposed to glucose-free solution for 30 min. Substitution of NaCl by LiCl (*b*) abolished the inhibition caused by returning glucose (at arrow) to the medium containing NaCl (*a*) and (*c*). In (*a*) and (*b*) a short period of spontaneous activity is shown before electrical stimulation began. In (*c*) the preparation was electrically stimulated throughout. Records as in Fig. 1.

solution for 30 min and the usual inhibitory effect of glucose was seen. In (*b*) the same procedure was repeated, but in a solution which contained Na in only 11% of the normal concentration. Glucose now had no effect. In (*c*) after the normal Na concentration had been restored glucose regained its inhibitory action.

One similar experiment was done in which the effect of raising the temperature was tested in low sodium. It was also found to be reduced in lithium solution.

DISCUSSION

A rapid increase in temperature from 23–27° C to 33–37° C was found to cause a transient depression or abolition of spontaneous electrical activity. It was also found to depress the conducted response to electrical stimulation. This observation was consistent with the hypothesis that the inhibition was somehow exerted via increased metabolic rate, which would be expected to rise when the temperature was raised by 10° C. Two attempts to test this hypothesis have been described. First, if the temperature effect was due to a change in metabolic rate, it should not be seen in the presence of metabolic inhibitors. Secondly, it would be expected to be abolished when the preparation was depleted of glycogen. It was found that in both conditions a rise in temperature had no inhibitory effect.

It was also found that moderate cooling caused a transient increase in spontaneous frequency and driving rate in many experiments. If this was due to a decreased metabolic rate, metabolic inhibitors would be expected to produce the same effect. This is what was observed.

On the basis of the same hypothesis it could be expected that removal of glucose as external energy source, and depletion of muscle glycogen, would accelerate the spontaneous spike discharge and facilitate electrical stimulation. This was observed in all experiments. Moreover, when the external source of energy was restored, an immediate inhibition of both the spontaneous spike discharge and of the driving rate was seen. This inhibition, produced by returning the glucose to the medium, was abolished by metabolic inhibitors.

The changes in electrical activity induced by the experimental conditions described here could thus all be explained as being secondary to changes in metabolic rate. This interpretation is supported by an observation shown in Fig. 8 of the following paper (Axelsson, Bueding & Bülbring, 1961) in which phosphorylase activity was increased 3–4 times when the temperature was raised from 26 to 36° C. Usually, when the preparation was warmed, we observed an increase in membrane polarization which coincided with and outlasted the cessation of electrical activity. The close correlation between the recorded polarization and spike activity indicated a true hyperpolarization.

It was known (Feldberg & Solandt, 1942) that removal of glucose abolished smooth-muscle tone. In the present work it was found that this was due, not to a cessation of electrical activity, but to the failure of the action potentials to evoke a tension response. When the external energy source was restored by returning the glucose to the medium the tension response was immediately increased. The increase in the force of the mechanical response coincided with the depression of the membrane excitability, providing further support for the assumption that the membrane stabilization was brought about by an increased rate of energy supply. It should be noted that the effect of returning the glucose could be easily reproduced in the same preparation once it was sufficiently depleted of glycogen.

The potency of several substances, as substitutes for glucose as external source of energy after glucose depletion, has been tested in a separate investigation (Axelsson, Bülbring & Krebs, unpublished). The relative abilities to reduce spontaneous activity and electrical excitability (driving rate) and to restore the tension response were used as criteria. This investigation showed that sodium acetate and sodium acetoacetate could be utilized after glucose depletion. Like glucose they temporarily suppressed electrical activity. Evidence that these substances provided energy was seen in the fact that their application also restored the tension response.

The similarity between the effects of returning the glucose and of raising the temperature is striking. It is tempting to think that the cause for the latter is essentially the same, i.e. a rise in the rate of energy supply. The effect of returning the glucose is usually of long duration. The temperature effect is always short-lasting, as the muscle accommodates to the higher rate of metabolism, and the final result is an increased frequency. This would be expected at higher temperature, rather than the initial change in the opposite direction.

The question remained how an increased rate of energy supply caused the membrane stabilization. If some of the energy were utilized for the active extrusion of sodium, it would become of interest to see what would happen in a sodium free solution. We did a few experiments in which the Na was substituted by Li, making the assumption that Li was not as readily pumped out as Na (Keynes & Swan, 1959). We found that the effects of both glucose and temperature were now much less and sometimes absent.

It is, however, likely that the relationship between the sources of energy or the metabolic rate and the excitability or conductivity of the smooth muscle is much more complex than through a direct stimulation or inhibition of sodium extrusion. Other factors such as interference with the

free Ca concentration as a consequence of increased metabolic rate, or primary changes in passive Na or K permeability, are alternative explanations. All these actions, together with changes in active Na transport, might play a role in determining the final results. The experimental facts presented here can, in view of the method, be subjected to many interpretations but, recognizing the indirectness of the evidence, we have come to the conclusion that the metabolic rate influences the electrical activity and excitability in intestinal smooth muscle.

SUMMARY

1. Isolated smooth muscle preparations from guinea-pig taenia coli were used for measurements of tension with simultaneous records of membrane potential and action potentials obtained with the sucrose-gap method.

2. Two types of experiments affecting the metabolic rate and the energy supply were carried out: (*a*) changing the temperature, (*b*) removing and returning the glucose in the bathing fluid.

3. A rapid rise of temperature caused a transient inhibition of spontaneous spike discharge and depressed electrical excitability. Moderate cooling increased the spontaneous spike frequency and facilitated electrical stimulation.

4. Similarly, withdrawal of glucose increased the rate at which a preparation could be electrically stimulated and sometimes also the frequency of spontaneous spike discharge. Returning the glucose to the medium caused a transient depression of the spontaneous electrical activity and of the driving rate.

5. In the absence of glucose the action potentials, which continued to be discharged for several hours, failed to evoke a tension response. This was restored by returning the glucose to the medium at the same time at which electrical activity was slowed.

6. The effects of both glucose and temperature were abolished by metabolic inhibitors. The effects of temperature were also abolished after prolonged exposure to glucose-free medium.

7. The inhibitory effects of both temperature and glucose on the electrical activity of the muscle were greatly diminished when Na was substituted by Li in the bathing fluid.

8. The influence of metabolic rate on the electrical activity of this smooth muscle is discussed.

One of the authors (J. A.) wishes to express his gratitude for a grant received from the Icelandic Scientific Research Fund.

REFERENCES

AXELSSON, J., BUEDING, E. & BÜLBRING, E. (1959). The action of adrenaline on phosphorylase activity and membrane potential of smooth muscle. *J. Physiol.* **148**, 62–63*P*.

AXELSSON, J., BUEDING, E. & BÜLBRING, E. (1961). The inhibitory action of adrenaline on intestinal smooth muscle in relation to its action on phosphorylase activity. *J. Physiol.* **156**, 357–374.

AXELSSON, J. & BÜLBRING, E. (1960*a*). The effect of removal of glucose from the medium on the electrical excitability of smooth muscle. *J. Physiol.* **153**, 8*P*.

AXELSSON, J. & BÜLBRING, E. (1960*b*). The metabolic basis for the inhibitory action of adrenaline on intestinal smooth muscle. *J. Physiol.* **153**, 30*P*.

BORN, G. V. R. & BÜLBRING, E. (1955). The effect of 2:4-Dinitrophenol (DNP) on the smooth muscle of the guinea-pig's taenia coli. *J. Physiol.* **127**, 626–635.

BÜLBRING, E. (1955). Correlation between membrane potential, spike discharge and tension in smooth muscle. *J. Physiol.* **128**, 200–221.

BÜLBRING, E. & BURNSTOCK, G. (1960). Membrane potential changes associated with tachyphylaxis and potentiation of the response to stimulating drugs in smooth muscle. *Brit. J. Pharmacol.* **15**, 611–624.

BÜLBRING, E. & LÜLLMANN, H. (1957). The effect of metabolic inhibitors on the electrical and mechanical activity of the smooth muscle of the guinea-pig's taenia coli. *J. Physiol.* **136**, 310–323.

BURNSTOCK, G. (1958). The effects of acetylcholine on membrane potential, spike frequency, conduction velocity and excitability in the taenia coli of the guinea-pig. *J. Physiol.* **143**, 165–182.

BURNSTOCK, G. & STRAUB, R. W. (1958). A method for studying the effects of ions and drugs on the resting and action potentials in smooth muscle with external electrodes. *J. Physiol.* **140**, 156–167.

FELDBERG, W. & SOLANDT, O. M. (1942). The effect of drugs, sugar and allied substances on the isolated small intestine of the rabbit. *J. Physiol.* **101**, 137–171.

KEYNES, R. D. & SWAN, R. C. (1959). The permeability of frog muscle fibres to lithium ions. *J. Physiol.* **147**, 626–638.

STÄMPFLI, R. (1954). A new method for measuring membrane potentials with external electrodes. *Experientia*, **10**, 508–509.