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THE ACTION OF ADRENALINE ON THE IONIC CONTENT AND ON SODIUM AND POTASSIUM MOVEMENTS IN THE SMOOTH MUSCLE OF THE GUINEA-PIG TAENIA COLI

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The normal effect of adrenaline upon electrical and mechanical activity of the smooth muscle of guinea-pig taenia coli is an arrest of the spontaneous electrical discharge and an associated fall of tension. The cessation of spike activity is almost instantaneous. It is usually followed by hyperpolarization which reaches its maximum within 1 min, but, if the initial membrane potential is high, no hyperpolarization is observed. During prolonged exposure to adrenaline the hyperpolarization is not maintained but gradually passes off or gives way to fluctuations of the membrane potential. On removal of adrenaline a period of depolarization and oscillatory fluctuations of membrane potential follow.

One hypothesis put forward to explain the inhibitory action of adrenaline was the suggestion by Burnstock (1958) that " the increase in membrane potential produced by adrenaline may be due to a transitory shift in the balance of ionic fluxes through the membrane caused by the stimulation of an electrogenic sodium pump."

Bueding & Bülbring (1964) emphasized the coincidence of the inhibitory action of adrenaline with an increased formation of cyclic 3',5'-AMP and a higher tissue concentration of energy rich phosphate compounds (Bueding, Bülbring, Gercken & Kuriyama, 1963). They suggested that the stabilization of the membrane potential was brought about by an increased metabolic energy supply to the membrane, the fixation of calcium being of primary importance.

Another explanation for the change in membrane potential might be that adrenaline increased the membrane permeability to potassium, which would shift the membrane potential towards the potassium equilibrium potential and stop the pacemaker potentials. Previous attempts to demonstrate increased membrane permeability to potassium have failed. Born & Bülbring (1956) showed that adrenaline increased the rate of ^{42}K-uptake (at most by 58%) but the rate of loss was usually unchanged and showed only rarely a slight increase. Similarly, Bauer, Goodford & Hüter (1963) found that adrenaline, in concentrations from 2×10^{-9} to 2×10^{-7}, increased ^{42}K-uptake within 2 min. The effect depended on the dose and was maximally 40%. However, the ^{42}K-loss was not

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consistently changed, nor the internal K-concentration. In contrast, Jenkinson & Morton (1965) recently observed a striking increase of about 100% in the rate of loss of ^{42}K when noradrenaline (3×10^{-7}) was applied to depolarized taenia at 20° C.

In the present experiments the effect of ionic distribution has again been investigated by measuring, in the guinea-pig taenia coli, the effect of adrenaline on the intracellular content of potassium, sodium and chloride, as well as its effect on the uptake and loss of ^{42}K and ^{24}Na.

Some of the results have been reported at the Proceedings of the British Pharmacological Society (Bülbring & Goodford, 1962) and the Proceedings of the Scandinavian Pharmacological Society (1965) (Setekleiv, unpublished).

METHODS

The methods used to study the ionic composition were essentially those of Goodford & Hermansen (1961) and Freeman-Narrod & Goodford (1962). Three or more guinea-pigs were used in each experiment, from each of which six or more pieces of taenia coli 25 mm long were dissected as quickly as possible, weighed to give the fresh weight, and suspended in a modified Krebs solution (mM): Na^+ 137, K^+ 5.9, Mg^{2+} 1.2, Ca^{2+} 2.5, Cl^- 134, $H_2PO^-_4$ 1.2, HCO^-_3 15.5 and dextrose 11.5, equilibrated with 97% O_2/3% CO_2. They were left for at least 1 hr to equilibrate in this solution, were then subjected to conditions specified in the description of results, and were finally reweighed to give the wet weight and analysed. Chloride and ^{36}Cl were measured after dissolving the muscles in alkaline H_2O_2 (Goodford, 1964). [^{35}S]-ethanesulphonate and [^{14}C]-sorbitol were used for the measurement of extracellular space and they were counted by the liquid scintillation method (Goodford & Lüllman, 1962).

For the measurements of rate of efflux of ^{42}K and ^{24}Na the muscles were placed in radioactive solutions to take up tracer, and were then transferred through a series of tubes containing inactive solution so that the washout of tracer could be followed (Goodford, 1965a).

In some experiments ^{42}K-efflux was measured in muscle depolarized with high external potassium. To produce complete depolarization, "K_2SO_4 Ringer" was used of the same composition as that used by Durbin & Jenkinson (1961)—that is, it contained (mM) K_2SO_4 76, $KHCO_3$ 16, $CaCl_2$ 7.5, Na_2SO_4 2.5, $MgCl_2$ 1.15, $NaHPO_4$ 0.5, glucose 6. To produce partial depolarization, "High KCl Ringer" was used in which the K-concentration of the Krebs solution was increased 10 times, to 59 mM, while the Na-concentration was reduced to 84.3 mM.

The late, slow phase of ^{24}Na loss from the taenia was observed in experiments using a continuous flow apparatus (Born & Bülbring, 1956), measuring radioactivity in the washing solution (Persoff, 1960).

Some experiments were carried out under isotonic conditions with a constant load of 1 g applied to the muscle. Others were carried out in isometric conditions, the muscle being fixed at its *in situ* length before dissection in the guinea-pig.

STATISTICAL METHODS

Results were expressed as mean values with the estimated standard error of the mean, and the number of observations in brackets thus: 5.6 ± 0.3 (187). Where more appropriate the number of degrees of freedom were recorded in the brackets, printed in italics. Italics were also used for standard errors calculated by analysis of variance. Means were compared on a probability scale so that smaller values of P corresponded to increasingly significant differences.

RESULTS

The effect of adrenaline on water distribution and ion content

The extracellular space was determined using three different substances (Goodford & Hermansen, 1961; Goodford & Lüllmann, 1962; Goodford & Leach, 1964). Table 1

shows that adrenaline always increased the extracellular space in both isometric and isotonic conditions. A concentration of 10^{-7} adrenaline produced a significant increase already after 30 sec and this effect was maintained for at least 3 min.

In some, but not all experiments, there was also an increase in the ratio of wet weight to fresh weight after adrenaline (Table 2). In these experiments there was no systematic change in calculated cell volume. When, however, the ratio did not increase it was concluded that the cell volume diminished.

TABLE 1

EFFECT OF ADRENALINE ON THE EXTRACELLULAR SPACE OF TAENIA COLI. 35° C. STANDARD SOLUTION

Number of observations in brackets

Solute	Adrenaline concentration (g/ml.)	Duration of exposure (min)	Condition of muscle	Control E.C.S. (g/kg fr. wt.)	Change of E.C.S. with adrenaline (g/kg fr. wt.)	Statistical significance
Inulin	2×10^{-7}	1	Isotonic	351±9 (24)	+17±13 (24)	$0{\cdot}2 > P > 0{\cdot}1$
[^{14}C]-Sorbitol	1×10^{-7}	0·5	Isotonic	399±10 (14)	+35±14 (14)	$0{\cdot}05 > P > 0{\cdot}02$
[^{35}S]-Ethanesulphonate	1×10^{-7}	1	Isotonic	380±8 (20)	+54±15 (10)	$0{\cdot}01 > P > 0{\cdot}001$
[^{35}S]-Ethanesulphonate	1×10^{-7}	3	Isotonic		+47±15 (10)	$0{\cdot}02 > P > 0{\cdot}01$
[^{35}S]-Ethanesulphonate	1×10^{-7}	0·5–3	Isometric	406±9 (24)	+34±14 (42)	$0{\cdot}02 > P > 0{\cdot}01$
			Mean change:		+33±7 (*96*)	$0{\cdot}001 > P$

An increased tissue Na-content was regularly observed and this change was entirely accounted for by the changed extracellular space so that the intracellular Na-concentration was unaltered. On the other hand, no increase of tissue Cl content was observed and, since the extracellular space increased so consistently, this would suggest that the intracellular Cl concentration fell. This is actually shown in Table 2, though it is not significant. The intracellular K concentration also showed a detectable systematic increase of 3 to 5 m-mole/kg which was significant when the results of all experiments were pooled.

TABLE 2

EFFECTS OF 3 MIN EXPOSURE TO ADRENALINE 10^{-7}. ISOMETRIC CONDITIONS. STANDARD SOLUTION. 35° C.

Each value is the mean and standard error of eight observations

		30 sec	1 min	3 min
$\frac{\text{Wet wt.}}{\text{Fr. wt.}}\times1{,}000$	Adr.	1,053±12	1,032±31	1,021±35
	Control	1,030±30	994±18	1,000±24
E.C.S. (g/kg fr. wt.) measured with [^{35}S]-ethanesulphonate	Adr.	435±16	441±20	445±15
	Control	416±17	411±12	391±19
I.C.S. (g/kg fr. wt.)	Adr.	617±19	591±24	578±30
	Control	606±20	583±17	606±26
$[K]_i$ (m-mole/kg.)	Adr.	128±4	130±2	134±4
	Control	124±6	127±5	129±6
$[Na]_i$ (m-mole/kg.)	Adr.	56±8	61±6	57±4
	Control	61±8	56±6	59±8
$[Cl]_i$ (m-mole/kg.)	Adr.	35±4	40±5	35±5
	Control	45±6	44±5	36±7·6

^{24}Na-efflux

(a) Rapid phase

For these experiments thin pieces of taenia, weighing only 5 mg, were used in order to minimize diffusion effects (Freeman-Narrod & Goodford, 1962). They were suspended isotonically and, after 1 hr equilibration in normal solution, loaded for 15 min in a solution containing ^{24}Na and then washed in inactive solution. Figure 1a shows the rate of ^{24}Na-loss in the presence and absence of 2×10^{-7} adrenaline. At each time of observation the loss was greater from the muscles treated with adrenaline (2×10^{-7}) and the increase, though small, was highly signficant after 1 and 2 min. However, by the third minute the effect was more difficult to measure accurately as a new steady state had been established. Figure 1a shows the same results as in Fig. 1a corrected for ^{24}Na

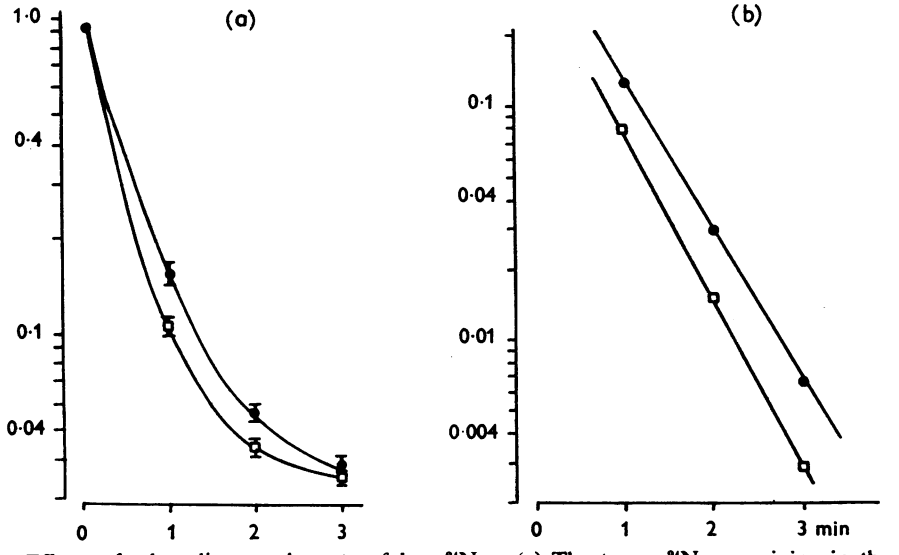

Fig. 1. Effects of adrenaline on the rate of loss ^{24}Na. (a) The tracer ^{24}Na remaining in the taenia suspended isotonically at 35° C after 15 min tracer uptake. Abscissa: duration of immersion in inactive solution, in min. Logarithmic ordinate: the amount of radioactivity in the muscle (counts min $^{-1}$) as a fraction of the initial amount. The standard errors of the means, each of eight observations, are shown. For one series of muscles adrenaline (2×10^{-7}) was added to the inactive washout solution (open squares), and the corresponding rate of loss of tracer was faster than the control (closed circles). (b) The same results corrected for ^{24}Na present in the slow phase (Buck & Goodford, 1966). The adjustment was made by subtracting the same constant (about 0.03) from each observation, giving two straight lines whose slopes measured the rate of loss of tracer. Adrenaline (2×10^{-7}) increased the rate by about 10%. (Symbols as in (*a*).)

present in the slow phase (Buck & Goodford, 1966). The adjustment was made by subtracting the same constant ($\doteqdot 0.03$) from each observation, giving two straight lines whose slopes measured the rate of loss of tracer. Adrenaline (2×10^{-7}) increased the rate by less than 10%. When lower concentrations of adrenaline (2×10^{-9} and 2×10^{-8}) were investigated, paired muscles were used to minimize the errors caused by variation between one guinea-pig and another (Goodford, 1962). It was found that adrenaline again increased the loss of ^{24}Na significantly.

Tension. It had already been observed that the rate of loss of tracer sodium from unstretched muscles was slower than from the controls (Freeman-Narrod & Goodford, 1962). We now found that adrenaline had no effect on the rate of loss of ^{24}Na from unstretched taenia, but the absence of effect might have been due to diffusion delays since the muscles were a little shorter and thicker.

Potassium-free solution. Pieces of taenia were allowed to equilibrate as usual in normal solution for 1 hr at 35° C, and were then transferred to ^{24}Na-solution in order to load up with tracer. After 6 min, when all the rapidly exchanging sodium would already have exchanged, the muscles were transferred to another radioactive solution of identical specific activity but containing no potassium. This might inhibit sodium extrusion if it were coupled to the uptake of potassium, and 9 min later, after a total loading time of 15 min, the pieces were re-transferred to an inactive solution still without potassium. They were finally removed for analysis after another minute.

The potassium content of these muscles was significantly lower (79.2 ± 1.6 (6) m-mole/kg fresh weight) than normal 92.0 ± 1.3 (8), and potassium was probably still being lost into the extracellular space. The extracellular solution would therefore still

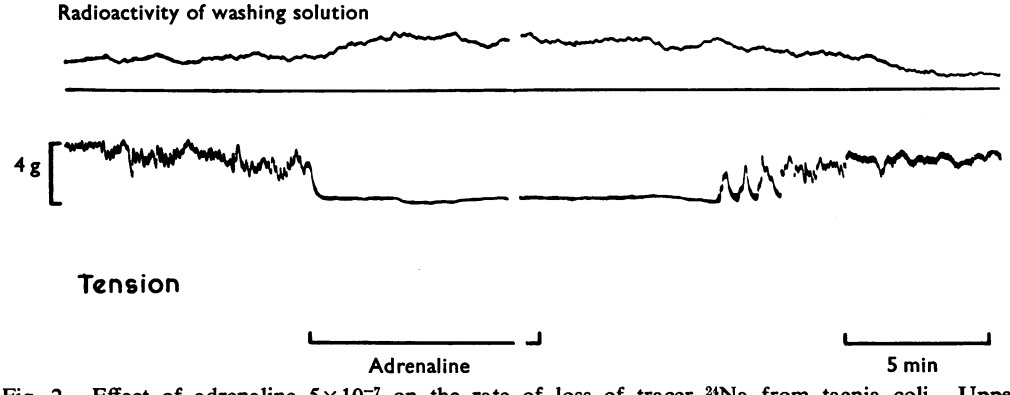

Fig. 2. Effect of adrenaline 5×10^{-7} on the rate of loss of tracer ^{24}Na from taenia coli. Upper tracing: radioactivity in washing solution flowing continuously past the muscle. Straight line: background radioactivity. Lower tracing. isometric tension developed simultaneously by the muscle. Observations were made at 35° C and began about 1 hr after the taenia had been transferred to an inactive solution following 3 hr tracer uptake. When adrenaline 5×10^{-7} was added to the superfusion fluid, the tension fell and the rate of ^{24}Na washout increased by nearly 50%. The tracing is interrupted for 4 min and starts again just before adrenaline is washed out.

contain some potassium (Harris & Burn, 1949), and perhaps for this reason the loss of ^{24}Na in these experiments was almost identical with the loss in normal solution. Alternatively, the effect of adrenaline might be on a Na-channel which is not coupled to K.

(b) Slow phase

Another series of experiments was designed to investigate the action of adrenaline on the slow phase of Na-efflux. A muscle was fixed isometrically in a small chamber through which solution flowed continuously. It was first loaded with ^{24}Na using a closed

circulation for several hours. The flow system was then switched to an open circuit supplied by a reservoir containing inactive solution. The efflux was followed by recording the radioactivity of the effluent continuously and simultaneously with the muscle tension. When the rapid phase of ^{24}Na-loss had passed and the slow phase had been in progress for over 1 hr, adrenaline 5×10^{-7} was added to the perfusing solution. There was a rapid fall of tension (Fig. 2), and the loss of radioactive sodium from the muscle started to increase until it exceeded the control rate by about 50%. This effect was reproducible over several hours of ^{24}Na-efflux from the slow phase. It was repeated in three different preparations.

Temperature change

Na-efflux from taenia coli increases over 100 times when the temperature is raised from 4° C to 35° C (Buck & Goodford, 1966). At 25° C the tissue is still capable of increasing the Na-efflux. An effect of adrenaline on the sodium efflux might therefore be detected if adrenaline is applied when the tissue is warmed to this intermediate temperature.

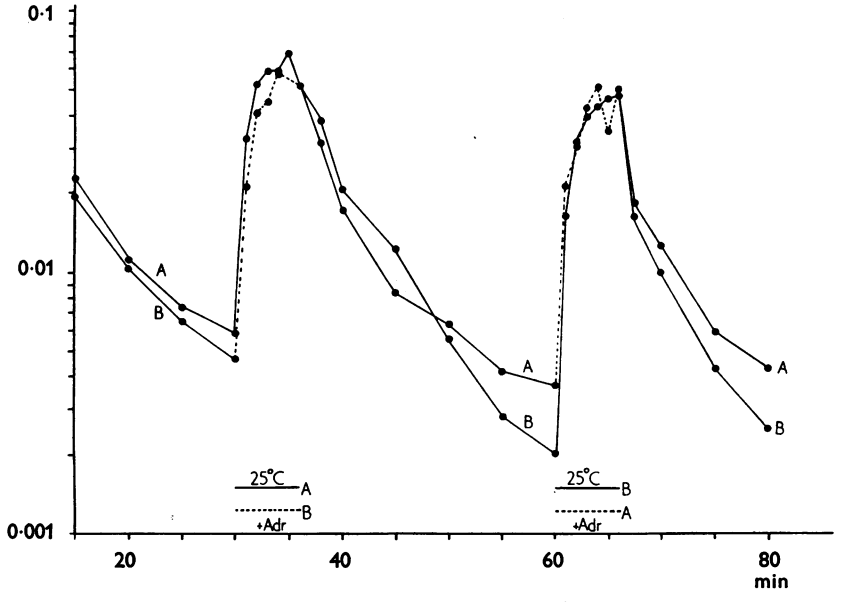

Fig. 3. The effect of raising the temperature from 4° C to 25° C on ^{24}Na-efflux: fraction of ^{24}Na lost min^{-1}. Abscissa: washout time in min. A: the temperature was raised for six min, first in the absence of adrenaline, cooled again and, 30 min later, warmed in the presence of adrenaline 10^{-7}. The muscle was then reloaded and the experiment was repeated; B: in the reversed order. For further details see text.

Pieces of taenia were immersed isometrically in ^{24}Na-solution at 35° C, and slowly cooled to 4° C, when they took up sodium. They were then transferred to inactive solution in the cold and left for ½ hr so that extracellular tracer diffused away from the tissue. The temperature was then raised to 25° C for six min and the rate of loss of ^{24}Na increased (Fig. 3A) until the muscle was cooled again to 4° C. The warming

period trial was repeated half an hour later in the presence of adrenaline (10^{-7}) and cooled once more. Subsequently, the tissue was transferred to the radioactive solution and loaded again with ^{24}Na. The efflux experiment was repeated, but now the adrenaline was applied during the first warming period (Fig. 3B). As can be seen from Fig. 3, adrenaline had no effect on the loss of ^{24}Na under these conditions. The same result was obtained in three other pieces of taenia.

^{42}K-uptake

The uptake of radioactive potassium by the taenia was measured in isotonic conditions in the presence and absence of adrenaline (10^{-7}), and confirmed the observations in isometric conditions by Born & Bülbring (1956) and those in isotonic conditions of Hüter, Bauer & Goodford (1963). The rate of uptake in isotonic experiments was

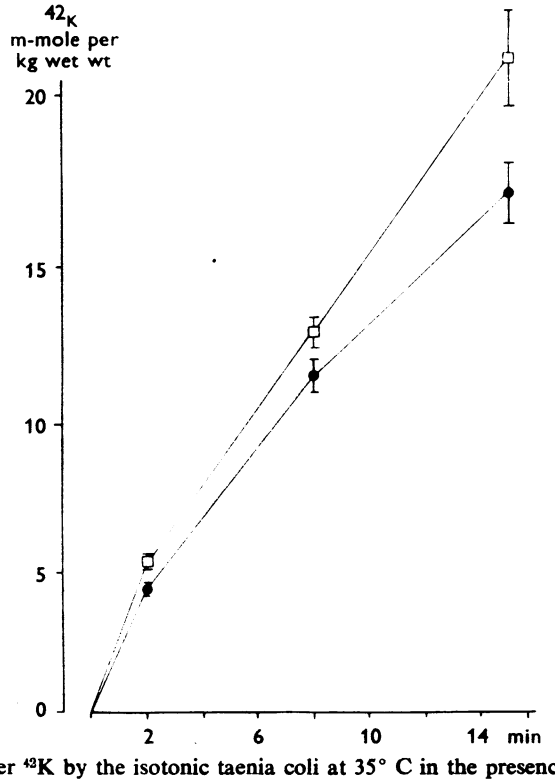

Fig. 4. Uptake of tracer ^{42}K by the isotonic taenia coli at 35° C in the presence (□) and absence (●) of adrenaline (10^{-7}). Abscissa: duration of immersion in radioactive potassium solution. Increased logarithmic ordinate: amount of tracer taken up by the muscle. The S.E. of each value (the mean of six observations) is shown.

increased by adrenaline so that the appropriate semi-logarithmic plot was a straight line (Fig. 4) instead of the curve normally found. It has been suggested that the curvature normally observed is due to an inhomogeneous distribution of potassium in the tissue (Goodford & Hermansen, 1961 ; Bauer *et al.*, 1963 ; Buck & Goodford, 1966), and the present observations might indicate that more of the tissue potassium is exchangeable

in the presence of adrenaline than under control conditions. They might alternatively be due to a changed ratio of cell volume to cell surface area, as has previously been observed by Freeman-Narrod & Goodford (1962).

^{42}K-loss

The rate of loss of potassium from the taenia was measured under isometric conditions in 19 experiments (Fig. 5). In eight experiments adrenaline increased the rate of ^{42}K-loss.

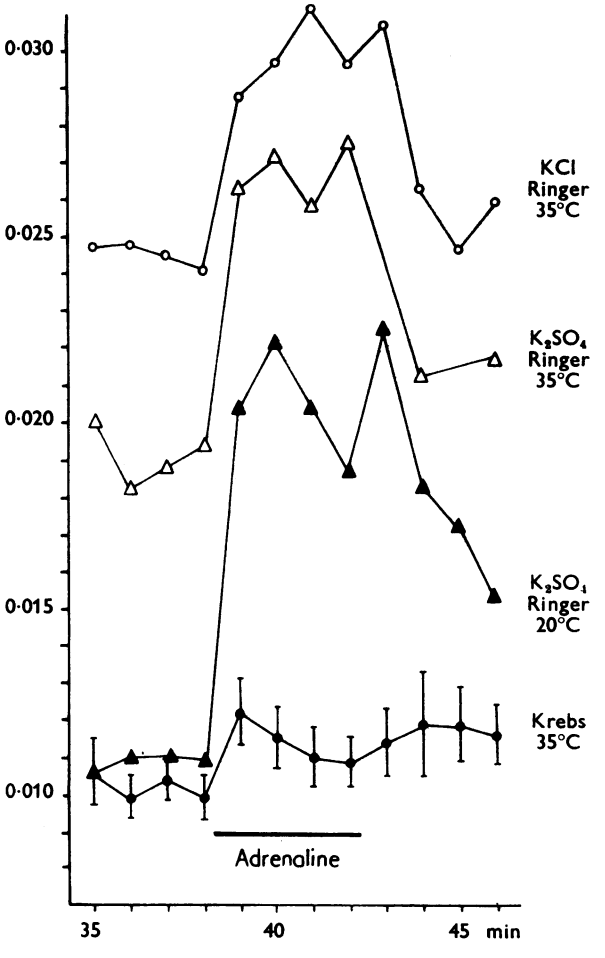

Fig. 5. The effect of adrenaline on ^{42}K-loss. Ordinate: fraction of ^{42}K lost min^{-1}. Abscissa: washout time in min. ● in Krebs solution at 35° C; ▲ in K_2 SO_4 Ringer at 20° C; △ in K_2 SO_4 Ringer at 35° C; ○ in high KCl Ringer at 35° C. For description see text.

The increase was usually most pronounced during the first minute after the adrenaline application and varied from 20 to 165% of the control period before adrenaline was applied (4 min). In five experiments no change was observed, while in the remaining six experiments a small reduction of the rate of ^{42}K-efflux, ranging from 15 to 28%, was

found during the first 2 or 3 min. An increased rate of loss of ^{42}K usually followed after the adrenaline had been washed out lasting several minutes.

The inconsistent effect of adrenaline on ^{42}K-loss may be interpreted as the resultant effect of two or more opposing mechanisms. For instance, the increased permeability to potassium would raise the membrane potential. The high membrane potential would reduce the effect of increased K-permeability on the rate of loss of ^{42}K. Furthermore, the cessation of the spontaneous spike discharge produced by adrenaline might cause a decrease in potassium efflux, thus masking an increased K-permeability. We therefore studied the effect of adrenaline on muscles which were rendered quiescent by exposure to hypertonic solution (Tomita, 1966). In four out of five experiments it was found, however, that in this quiescent tissue adrenaline also caused a small increase in ^{42}K-loss, ranging from 12 to 31% (mean 25%). It is therefore likely that the hyperpolarization by adrenaline which is still observed in hypertonic solution (unpublished observation) is a factor reducing the K-efflux, rather than the stoppage of discharge.

Jenkinson & Morton (1965) reported an increase in the rate of potassium loss after application of noradrenaline 3×10^{-7} in guinea-pig taenia coli, depolarized with K_2SO_4 Ringer, at 20° C. We consistently obtained the same result. In four experiments adrenaline 3×10^{-7} caused a large increase in the rate of ^{42}K-efflux at 20° C (Fig. 5). At 35° C the initial ^{42}K-efflux was much higher than that in K_2SO_4 Ringer at 20° C and that at 35° C in Krebs solution. The increase caused by adrenaline was less pronounced at the higher temperature (Fig. 5). In 12 experiments the external potassium concentration was increased only 10 times (59 mM) by replacing sodium chloride of the Krebs solution with potassium chloride. The initial rate of ^{42}K-efflux was still higher and the effect of adrenaline was smaller than in K_2SO_4 Ringer (Fig. 5) at the same temperature.

DISCUSSION

The present experiments were carried out, firstly, to investigate the effects of adrenaline upon the ionic distribution in the smooth muscle of the guinea-pig taenia coli. Secondly, experiments were designed to ascertain if any changes of ion movement caused by adrenaline could be shown to be effects upon membrane permeability or upon the active transport of ions.

No great change in the intracellular concentration of potassium, sodium or chloride was found in spite of the striking effect of adrenaline on the electrical activity and the membrane potential. A similarly constant ionic distribution was found in the uterus before, during and after pregnancy in cat, rat and guinea-pig, in spite of the big change in the membrane potential (Casteels & Kuriyama, 1965), and this was explained by a change in membrane permeability. Adrenaline may act in a similar way, but its effects in many experiments were so small, and often so inconsistent, that each experiment had to be repeated many times before the results could be accepted with confidence. It became increasingly necessary to consider whether artifacts could be responsible for each particular observation. For example, it was observed that the rate of ^{24}Na loss from the taenia under isotonic conditions, was increased by adrenaline. But this small effect might have been due to the increased extracellular space, or to a change in the ratio of cell volume to surface area, or to slight movements of the tissue when it relaxed.

In order to test the action of adrenaline on the coupled Na-K pump, it was applied to the taenia at the moment when the temperature was raised from 4° to 25° C. According to Buck & Goodford (1966), this increase of temperature stimulates the active extrusion of Na and the uptake of K, but not maximally, since a further increase would be possible if the temperature were raised still higher. It was found that adrenaline had no effect under these conditions, and it must be concluded that it has no effect on active ion transport during recovery from cooling.

A consistent effect of adrenaline was to increase the ^{24}Na efflux by 50% during the slow phase of exchange. This result may be related to Bueding & Bülbring's (1964) suggestion that the fixation of calcium at the cell membrane may be of primary importance for the inhibitory action of adrenaline. There is evidence that K^+, Na^+ and Ca^{2+} compete for superficial anionic sites in the guinea-pig taenia coli (Goodford, 1965b, 1966) and these sites may be in the cell membrane as Wilbrandt & Koller (1948) have suggested for the heart muscle. Adrenaline could therefore have dislodged sodium by favouring the fixation of calcium at the anionic membrane site. This would produce hyperpolarization by reducing the sodium permeability of the membrane. An alternative interpretation of the result would be that adrenaline stimulated an active extrusion of sodium which might be electrogenic. In view of the increased ^{24}Na efflux, a slight loss of sodium and a secondary small gain of potassium may occur, and a detectable increase of $[K]_i$ is in fact shown by the results in Table 2.

The clearest and most reproducible observations were upon the uptake of tracer potassium which was invariably raised by 2×10^{-7} adrenaline. Hüter *et al.* (1963) have found that doses down to 2×10^{-9} were effective. However, these authors also found that the potassium content was not consistently influenced and, like Born & Bülbring (1956), saw no clear effects on ^{42}K-loss.

In the present experiments the ^{42}K-loss was increased by adrenaline in eight out of 19 experiments in normal solution, but in six experiments a slight decrease of potassium efflux was seen. If the muscle was depolarized with K_2SO_4 Ringer, the ^{42}K-loss was always increased by adrenaline. This observation confirms that of Jenkinson & Morton (1965), who used noradrenaline, and who explained the result by an increased permeability of the membrane to potassium. When the muscle was only partly depolarized with KCl, adrenaline only slightly increased K-efflux. It is known (Bülbring & Kuriyama, 1963) that adrenaline fails to cause hyperpolarization when the external K-concentration exceeds 30 mM, whereas in normal solution it may increase the membrane potential by as much as 20 mV. This hyperpolarization would oppose the loss of K from the cell and might therefore mask the change in K-permeability, if this is studied by measuring ^{42}K-loss. Adrenaline does not always cause hyperpolarization even in normal conditions, and this may account for the variability of our results. In six experiments the efflux of K was actually reduced by adrenaline suggesting that the hyperpolarization, which we assume was responsible for this reduction, could not solely be due to an increased K-permeability. It must, at least partly, be due to other factors, and this conclusion is supported by the observation that $[K]_i$ increased slightly during the application of adrenaline.

Our experimental results are consistent with the view that one action of adrenaline is to increase the potassium permeability of the cell membrane. However, the hyperpolarization in normal solution decreases the outward movement of potassium. There-

fore the increased K-permeability, if measured by the rate of loss of ^{42}K, is not always detectable. Another action of adrenaline, possibly on the sodium channel, contributes to the hyperpolarization and in this way also reduces the rate of loss of ^{42}K. Further experiments on the role of calcium, on the sequestration of ions in the tissue, and on the possibility of an electrogenic sodium extrusion are required.

SUMMARY

1. In the guinea-pig taenia coli the wet weight, inulin space, [^{35}S]-ethanesulphonate space and [^{14}C]-sorbitol space, the sodium, potassium, and chloride contents, and ^{24}Na and ^{42}K exchange have been measured in the presence and absence of adrenaline.

2. Adrenaline (2×10^{-8} or 2×10^{-7}) increased the extracellular space and the total sodium content of the tissue. The intracellular ion contents and the intracellular ion concentrations did not change significantly.

3. The rate of loss of tracer ^{24}Na, during the initial rapid phase, was slightly increased by adrenaline (<10%). The rate of loss of tracer ^{24}Na, during the slow phase of exchange, was increased by 50%.

4. Adrenaline increased the rate of uptake of ^{42}K.

5. Adrenaline also increased the rate of loss of ^{42}K in muscle which was completely depolarized by K_2SO_4 Ringer. This effect was less in muscle partly depolarized by KCl. In normal solution the effect was smaller and inconsistent.

6. The observations indicate that adrenaline increases the potassium permeability of the membrane, but that the hyperpolarization, observed in normal conditions, reduces the outward movement of potassium. Hence the increased loss of tracer ^{42}K is not always detectable. It is suggested that part of the hyperpolarization is brought about by another action of adrenaline—for example, the fixation of Ca at the membrane—affecting the sodium channel only. Furthermore, it is possible that adrenaline stimulates an active electrogenic sodium extrusion.

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