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EFFECTS OF CHANGES IN IONIC ENVIRONMENT ON THE ACTION OF ACETYLCHOLINE AND ADRENALINE ON THE SMOOTH MUSCLE CELLS OF GUINEA-PIG TAENIA COLI

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The action of acetylcholine and adrenaline on single cell activity of the taenia coli has been described by Bülbring (1955, 1957): Acetylcholine depolarizes the membrane, increases the spike frequency and prolongs the spike duration. Adrenaline slows or abolishes the spike discharge, reduces the duration of the action potential and causes a rise of membrane potential. These observations were confirmed and expanded by Burnstock (1958*a*, *b*) using the sucrose-gap method. He discussed the action of acetylcholine in relation to the movement of Na^+ and K^+ ions across the membrane and compared it with its action on the end-plate region of skeletal muscle. He also speculated that the increase of membrane potential produced by adrenaline might be due to the stimulation of an electrogenic sodium pump, and the inhibition of spike activity to an inhibition of the carrier mechanism for sodium or some other ion. Bülbring & Burnstock (1960), using the sucrose-gap method, investigated the conditions causing tachyphylaxis and potentiation of the effects of acetylcholine, histamine and 5-hydroxytryptamine on the membrane activity of taenia coli. They interpreted these two phenomena in relation to the rates of active ion transport and utilization of metabolic energy. Axelsson, Bueding & Bülbring (1961) observed the effect of adrenaline using both biochemical and electrophysiological methods. They came to the conclusion that the hyperpolarization and stabilization of the membrane by adrenaline had a causal relationship to the increase of phosphorylase activity (see also Bülbring, 1960).*

The effects of acetylcholine and adrenaline on single cell activity in taenia coli are similar to those described in uterine muscle (Marshall, 1959; Kuriyama, 1961) and to those of parasympathetic and sympathetic nerve stimulation in rabbit colon (Gillespie, 1961*a*, *b*).

The work to be described here was undertaken to find out whether the

* See Note added in proof p. 74.

action of acetylcholine and adrenaline could be related to specific changes in ionic permeability of the smooth muscle cell membrane. The results indicate a non-selective increase in membrane permeability by acetylcholine, while adrenaline may act more specifically on Na conductance and, by increasing metabolic energy supply, stimulate active ion transport.

Some of the results have been reported at a meeting of the British Pharmacological society (Bülbring & Kuriyama, 1962) and at the Symposium on Vascular Smooth Muscle (Bülbring, 1962).

METHODS

Experimental apparatus and procedure were the same as those described by Bülbring (1954, 1955, 1957), Holman (1958), Kuriyama (1963), Bülbring & Kuriyama (1963). Unless stated otherwise acetylcholine and adrenaline were injected into the bathing solution. The organ bath had a capacity of 3 ml. the solution flowed continuously at the rate of 2–3 ml./min, thus the drug concentrations given in the results, unless otherwise stated, are those at the moment of injection, and they were then progressively diluted until washed out.

The normal Krebs's solution used in all experiments contained (mM): Na 137·4, K 5·9, Mg 1·2, Ca 2·5, Cl 134, H_2PO_4 1·2, HCO_3 15·5, glucose 11·5, and was aerated with 3 % CO_2 + 97 % O_2. Solutions containing abnormal ion concentrations were prepared as described in the preceding papers (Kuriyama, 1963; Bülbring & Kuriyama, 1963). Acetylcholine chloride and adrenaline base (Burroughs Wellcome and Co.) were used; concentrations are weight per volume.

RESULTS

The effect of acetylcholine in normal ionic environment and in different external K^+ concentrations

In the experiment illustrated in Fig. 1 acetylcholine was added to the bath to produce a concentration of 10^{-6} which was maintained for 10 sec by stopping the flow of solution, and then progressively washed out. The changes in membrane potential, spike frequency, spike height and spike duration are shown at two different film speeds. Acetylcholine 10^{-6} depolarized the muscle membrane by a maximum of 18 mV. The time taken to maximum depolarization was 10–20 sec. The overshoot potential was usually slightly decreased (on the average by 4 mV) during the peak of depolarization. This did not occur in the experiment shown in Fig. 1, but is very marked in Fig. 6. The maximum rates of rise and fall were decreased (mean values from 8·6 to 1·6 V/sec and from 7·4 to 1·3 V/sec, respectively). As the acetylcholine was washed away the membrane repolarized, the size of the action potential increased again, but the rate of fall remained slow, so that the negative after-potential was very prolonged. There followed a phase, lasting several minutes, during which the membrane often became hyperpolarized beyond the initial level, and spike activity ceased. The membrane potential and spike frequency then

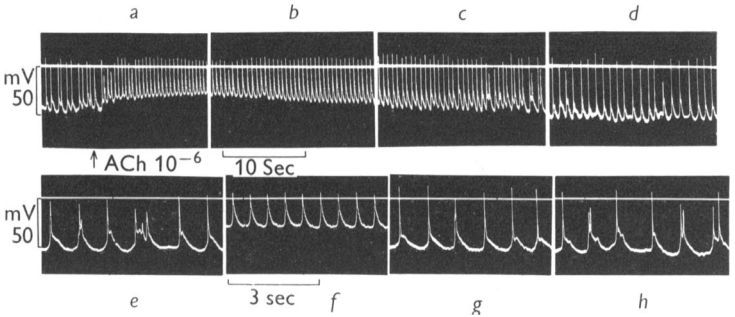

Fig. 1. The effect of acetylcholine on the membrane potential and electrical activity. Top row: slow film speed, *a*, start of exposure to ACh; *b*, end of exposure to ACh; *c*, 70 sec after removing ACh; *d*, 2 min later. Bottom row: fast film speed, *e*, before ACh, *f*, at peak, *g*, 80 sec after removing ACh, *h*, 2 min later.

TABLE 1. Effect of acetylcholine (10^{-6} g/ml.)

Cell	Membrane potential (mV)		Maximum overshoot potential (mV)		Time to peak of depolarization (sec)	Duration of effect (sec)	After-hyperpolarization beyond the initial level (mV)	Duration of hyperpolarization (sec)
	Before ACh	Depolarization caused by ACh	Before ACh	At peak of depolarization				
1	60	18	6	4	12	210	3	30
2	53	15	2	0	18	180	6	240
3	48	13	8	4	20	260	6	240
4	36	3	3	2	4	50	15	600

fluctuated before normal activity was resumed (cf. Bülbring & Burnstock, 1960).

In general, the effect of acetylcholine depended on the conditions prevailing before treatment. Table 1 gives the effect of acetylcholine 10^{-6} in four different cells of the same tissue. The degree of depolarization of the membrane was greater, the higher the initial level of the membrane potential. Conversely, during recovery, the hyperpolarization of the membrane beyond the initial level was greater, the lower the initial level of the membrane potential had been. Figure 2 shows that acetylcholine produced no depolarization when, in normal solution, the membrane potential was initially as low as 30 mV. On the other hand, when the membrane was depolarized to this level by increasing the external potassium concentration, acetylcholine produced still further depolarization. It became ineffective when the potential had fallen to about 15 mV. At that level acetylcholine sometimes caused a slight increase in membrane potential (cf. Burnstock, 1958*a*). A similar effect was observed when acetylcholine was applied simultaneously with excess K^+, i.e. when it was already present in the solution containing a high K^+ concentration. The effect of 12 mM-K^+ was then accelerated and potentiated, but that of 59 mM not, the depolarization being, within a few millivolts, the same as without acetylcholine.

Another phenomenon was observed in excess potassium. Figure 3 shows the effect of acetylcholine (10^{-6}) on the membrane and on the tension in 18 mM-K^+. Excess potassium had increased the frequency of spike discharge (*a*) and acetylcholine caused a small further acceleration associated with an increase in tension (*b*). A second exposure to acetylcholine (*c*), however, slightly slowed the spike frequency but nevertheless caused a rise in tension. This observation may be related to the effects described by Evans, Schild & Thesleff (1958) and by Edman & Schild (1961*a*, *b*) on the completely depolarized membrane suggesting a change in calcium permeability.

The presence of acetylcholine 10^{-6} in a solution containing low or zero K^+ prevented the hyperpolarization caused by K^+ deficiency. On the other hand, if acetylcholine was applied to the preparation after it had been exposed to K^+-free solution for 30 min, the K^+ deficiency blocked the action of acetylcholine. Such an experiment is shown in Fig. 4. While spontaneous spike activity was still present, acetylcholine depolarized the membrane transiently and accelerated the spike frequency, but there was no increase in tension (Fig. 4*b*). Subsequently the membrane repolarized and spikes slowed and stopped 15 min later. When the membrane activity had ceased, acetylcholine did not induce spike discharges but the membrane potential was at first still depolarized by a few millivolts. When

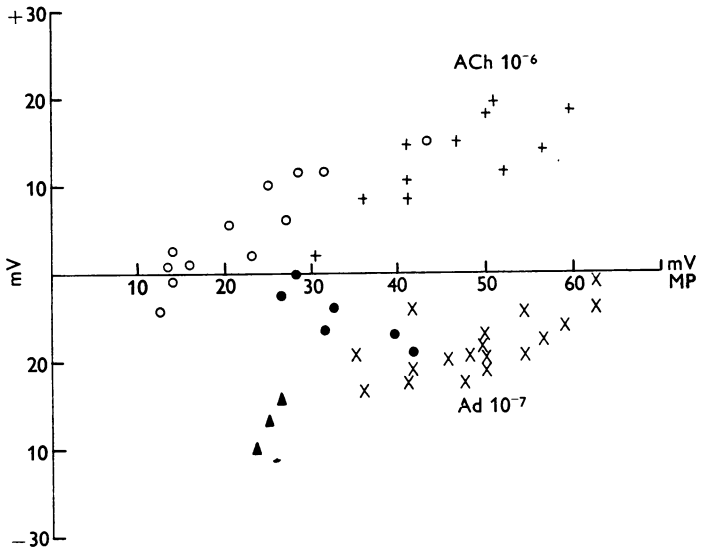

Fig. 2. The relation between the initial membrane potential (abscissa) and the depolarization (ordinate, upwards) caused by acetylcholine in normal Krebs's solution (+) and when the membrane was depolarized by excess K^+ (○); and the hyperpolarization (ordinate downwards) caused by adrenaline in normal solution (×), in excess K^+ (●) and in the presence of acetylcholine (▲). This figure includes only the values obtained when the micro-electrode remained in the same cell for a period outlasting the peak of the drug effect.

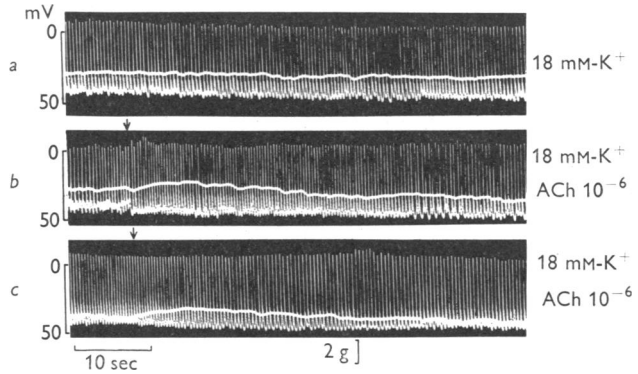

Fig. 3. *a*, Membrane activity and tension after 18 min exposure to excess K^+ (18 mM); *b*, the effect of acetylcholine (10^{-6}) 2 min after *a*; *c*, acetylcholine effect 3 min after *b*.

the muscle had been exposed to K^+ free solution for 30 min, acetylcholine caused no change in membrane potential (Fig. 4*c*).

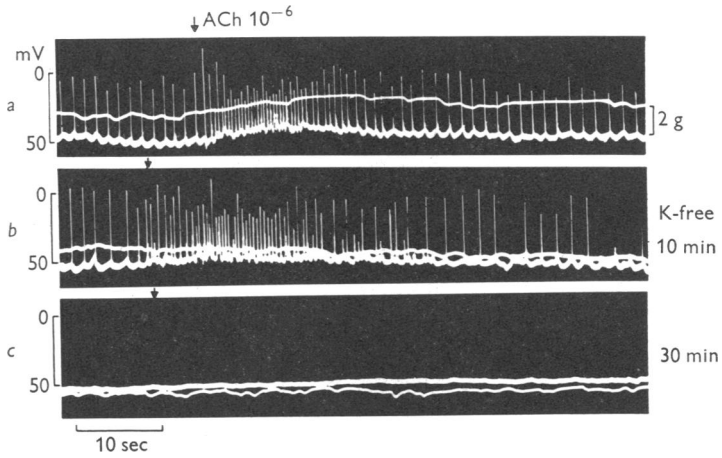

Fig. 4. The effect of removing external potassium on the action of acetylcholine on membrane potential and tension. *a*, control response to ACh in normal solution; *b*, after 10 min and *c*, after 30 min exposure to K^+ free solution.

The effect of acetylcholine in sodium deficiency and sodium excess

When 50 % of the NaCl was replaced with LiCl or Tris Cl the membrane activity scarcely changed, as described in the preceding paper (Bülbring & Kuriyama, 1963). In LiCl (50 % replacement of NaCl) acetylcholine increased the spike frequency, but the depolarization was smaller than in normal solution because, after 1 hr in the LiCl, the membrane potential had already fallen. Moreover, there was no increase in tension. This effect was entirely different from that observed in the Tris-Cl solution (50 % replacement of NaCl), in which acetylcholine depolarized and accelerated spike discharge as in normal solution and the tension development followed these changes closely.

In sodium-free (Tris-Cl solution) acetylcholine 10^{-6} depolarized the membrane but it did not increase the spike frequency. Later, when membrane activity had completely ceased after more than 1 hr exposure to sodium-free solution, acetylcholine still depolarized the membrane.

In a solution containing excess Na^+ (204 mM-Na^+, other ions unchanged as the solution was prepared with sodium ethanesulphonate), the membrane was slightly depolarized and developed large local potentials triggering multiple spike discharges (cf. Bülbring & Kuriyama, 1963). Excess Na^+ greatly increased the action of acetylcholine, which caused a greater depolarization of the membrane and a greater acceleration of spike

frequency and its effect was prolonged 4–5 times, persisting for several minutes (Fig. 5). Also the after-hyperpolarization during the recovery period was accentuated, reaching 6–10 mV, and the suppression of spike discharge continued for several minutes.

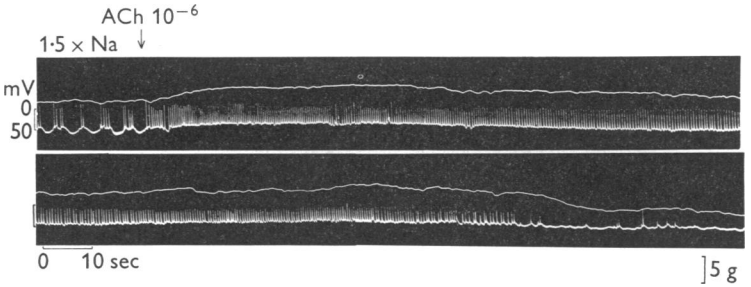

Fig. 5. The action of acetylcholine in the presence of excess sodium (204 mM). Continuous record.

Effect of acetylcholine in calcium-free solution and in the presence of excess calcium (7·5 mM)

In the absence of Ca^{2+} acetylcholine was ineffective. Only during the initial transient excitatory phase, which is often seen on removal of Ca^{2+}, was the effect of acetylcholine additive. But when muscle activity had ceased in Ca^{2+}-free solution, acetylcholine neither depolarized the membrane nor produced spikes.

In excess Ca^{2+} the action of acetylcholine was enhanced. It depolarized the membrane more than in standard Krebs's solution (mean value of 13 mV) and also caused a greater acceleration of the spike discharge (from 1·1 to 2·1/sec in normal Ca^{2+} and from 0·7 to 2·8/sec in excess Ca^{2+}). The potentiation in excess calcium was similar to that observed in excess sodium.

Effect of acetylcholine in chloride-deficient solution

The effect of replacing chloride with ethanesulphonate has been described in a preceding paper (Kuriyama, 1963). During the initial excitatory stage acetylcholine increased the spike frequency and depolarized the membrane further, but tension development was smaller than in Krebs's solution. During the later quiescent phase of chloride deficiency acetylcholine suddenly evoked spike activity in groups of rapid bursts, and the membrane became depolarized. There was, however, no increase in tension (Fig. 6). This ability of acetylcholine to evoke spikes after activity had ceased in Cl^--deficient solution contrasts with its inability to do so in the absence of K^+, Na^+ or Ca^{2+}.

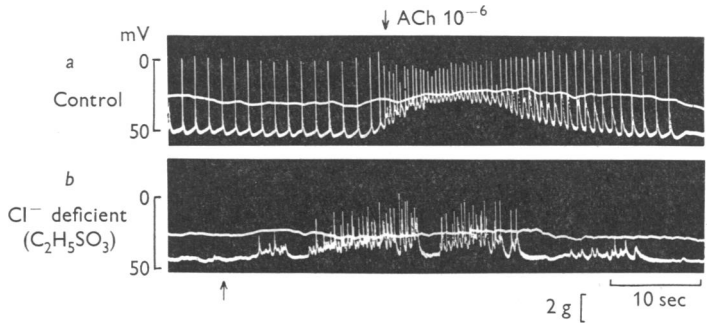

Fig. 6. The effect of chloride deficiency on the action of acetylcholine. *a*, control; *b*, after 35 min exposure to a solution in which NaCl was replaced by $NaC_2H_5SO_3$.

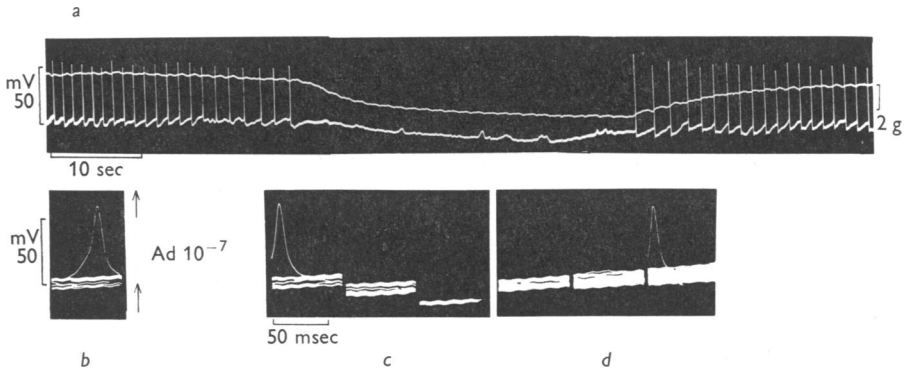

Fig. 7. *a*, The action of adrenaline on membrane potential, electrical activity and tension; *b*, sweep, showing a spike in normal solution; *c*, after application of adrenaline, showing the last spike before activity ceased; *d*, showing the first spike at return of activity.

The effect of adrenaline

Adrenaline abolished spike activity usually without any preceding change of spike height. This block was not caused by the hyperpolarization of the membrane, but usually preceded it or occurred without hyperpolarization (Burnstock, 1958*b*). The degree of hyperpolarization depended on the height of the membrane potential prevailing at the moment of administration of adrenaline. This relation is shown in Fig. 2. The effect of adrenaline (10^{-7}) on the membrane activity and tension development is illustrated in Fig. 7. During the stage of slowed spike frequency the rates of rise and fall of the action potential were increased from 7·8 to 9·2 V/sec and from 7·2 to 7·9 V/sec, respectively, and the half-duration of the spike was decreased from 9 to 7·8 msec. When the spikes reappeared after washing out, the first action potentials were very large.

The maximum rates of rise and fall were increased to 9·6 V/sec and to 8·4 V/sec, respectively, and the half duration of the action potential decreased to 6·3 msec. The membrane then depolarized to a lower level than before and the spike frequency was increased.

In the experiment shown in Fig. 8 the muscle was exposed to four different concentrations of adrenaline for 5 sec each time by stopping the flow of solution for this period. The duration of the block of spike generation was proportional to the adrenaline concentration (4 sec by 10^{-8}, 12 sec by 5×10^{-8}, 21 sec by 10^{-7} and 32 sec by 5×10^{-7}). The four cells had similar membrane potentials and the hyperpolarization was very small.

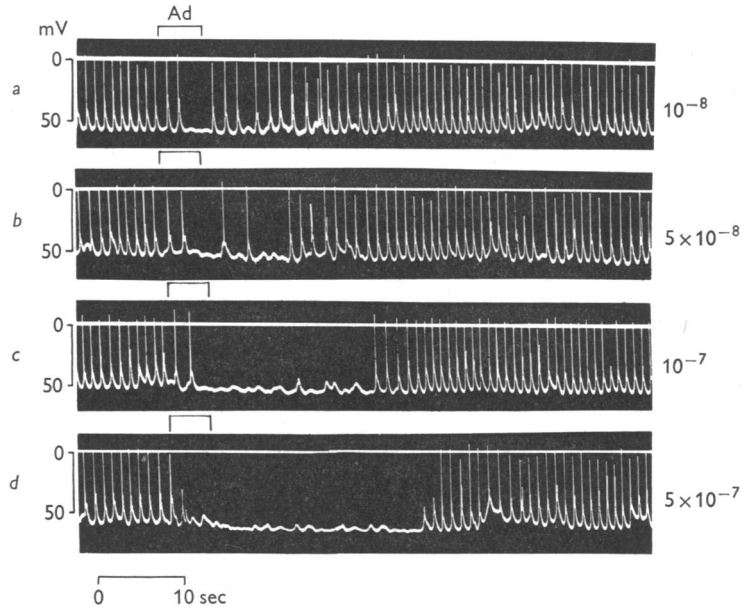

Fig. 8. The effects of four different adrenaline concentrations when the initial membrane potential was high, causing little or no hyperpolarization but stopping spike discharge for periods proportional to the adrenaline concentration, *a*, 10^{-8}; *b*, 5×10^{-8}; *c*, 10^{-7}; *d*, 5×10^{-7}.

The effect of adrenaline in K^+ excess and K^+ deficiency

When the membrane was depolarized by acetylcholine to about 25 mV, the hyperpolarization caused by adrenaline was greatly increased, following closely the relation normally observed between the adrenaline effect and the initial membrane potential, as shown in Fig. 2. In contrast, when the membrane was depolarized by excess K^+ to about 25 mV, no hyperpolarization was produced by adrenaline. Figure 9 shows an experiment in which adrenaline was applied in two different concentrations to a muscle

which had been depolarized previously by 18 mM-KCl; it blocked spike generation and the membrane was hyperpolarized. However, when the muscle had been depolarized previously by 24 mM-KCl, block of spike discharges, but no change in membrane potential was observed.

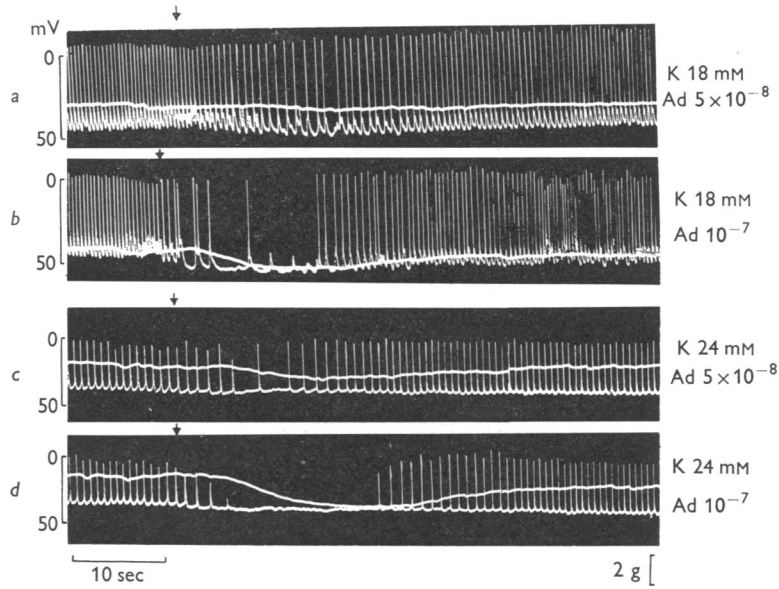

Fig. 9. The effect of excess potassium on the action of adrenaline 5×10^{-8} and 10^{-7}. *a*, after 13 min and *b*, after 16 min exposure to 18 mM-K^+; *c*, after 26 min and *d*, after 29 min in 24 mM-K^+.

In the absence of K^+ the effect of adrenaline was unchanged. Not only during the initial excitatory phase in K^+-free solution was spike discharge stopped and the membrane hyperpolarized, but also during the late quiescent phase was the hyperpolarization of the membrane by adrenaline frequently more than in normal solution. Thus, the mean values of five experiments were as follows: Initial membrane potential in Krebs's solution 47 mV, in the presence of adrenaline 62 mV. Initial membrane potential in the absence of K^+ 59 mV, in the presence of adrenaline 67 mV.

The effect of adrenaline in Na^+ excess and Na^+ deficient solution

Though the membrane was depolarized by excess Na^+ (204 mM), the hyperpolarization due to adrenaline was less than in normal Na^+. The block of spike activity was either absent or short-lasting. The local potential, which was very large in excess Na^+, was reduced by the addition of adrenaline.

Reduction of the external Na^+ concentration to 50 % had no effect on the action of adrenaline, but in sodium-free (Tris-Cl) solution the action of adrenaline was very much reduced or abolished. In some experiments adrenaline neither blocked the spike discharge nor hyperpolarized the membrane (Fig. 10), while in others the membrane potential was slightly increased without block of spike generation.

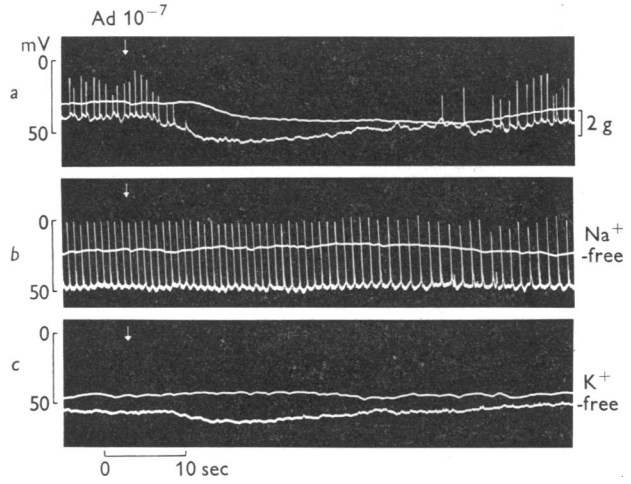

Fig. 10. The effect of adrenaline: *a*, in normal solution; *b*, after 20 min in Na^+-free solution; *c*, after 30 min in K^+-free solution.

The effect of adrenaline in Ca^{2+} excess and Ca^{2+}-free solution

In the presence of excess calcium (7·5 mM) the blocking effect of adrenaline was potentiated, the duration of the inhibition being much longer than in standard Krebs's solution. The membrane potential was raised only slightly or remained unchanged (Fig. 11*b*) (maximum hyperpolarization 6 mV, mean value 4 mV).

In the absence of Ca^{2+} the effect of adrenaline was abolished. During the short initial excitatory phase caused by Ca^{2+}-free solution adrenaline blocked the spike generation and the membrane was hyperpolarized. However, after 30 min exposure adrenaline caused no hyperpolarization and sometimes slightly depolarized the membrane (Fig. 11*c*).

The effect of adrenaline in Cl^--deficient solution

Like acetylcholine, adrenaline was still effective in a solution deficient in Cl^-.

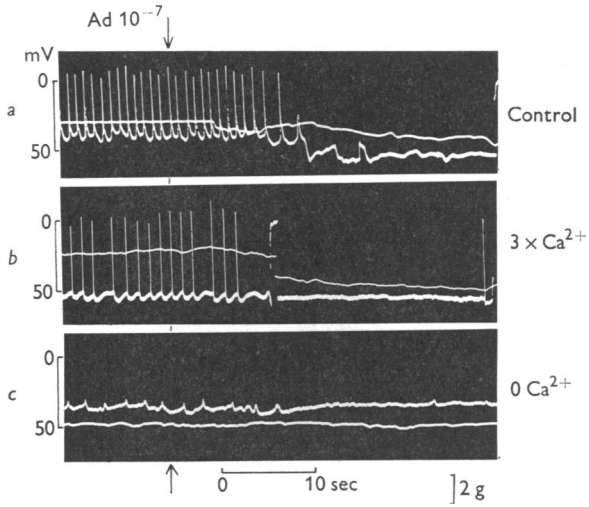

Fig. 11. The effect of adrenaline *a*, in normal solution; *b*, after 15 min in excess Ca^{+} (7·5 mM) (the micro-electrode was dislodged and was reinserted 11 sec later); *c*, after 25 min in Ca^{2+}-free solution.

DISCUSSION

The effect of acetylcholine

The view is generally accepted that acetylcholine at the motor end-plate increases the permeability to Na^{+}, K^{+} and possibly to other ions which are present on either side of the membrane (Fatt & Katz, 1951; del Castillo & Katz, 1954). That acetylcholine also increases the membrane permeability of taenia coli non-selectively is indicated by the observation that it still depolarizes the membrane of taenia coli in Na^{+}-free solution. This might be explained by an increased permeability of the membrane to the substitute for Na^{+}, e.g. Tris, as suggested by Katz (1962) (cf. Furukawa, Furukawa & Takagi, 1957) for various ammonium compounds. Durbin & Jenkinson (1961) observed the effect of carbachol on the depolarized taenia coli by tracer flux measurement in the absence of an electric field, using potassium-rich solution. Carbachol caused an increase in permeability to K^{+} (as well as Na^{+}, Ca^{2+}, Cl^{-} and Br^{-}) and the effect was reduced by lowering Ca^{2+}.

The evidence that the excitation by acetylcholine is mainly due to an increase in Na permeability is as follows: In the absence of Na^{+} acetylcholine fails to accelerate spike discharge though, as mentioned above, it still causes some depolarization. In contrast, the most striking potentiation of the acetylcholine effect is observed in excess sodium when both the depolarization and the spike acceleration are greater and the effect is

prolonged. Moreover, the effect of acetylcholine is potentiated by Ca^{2+} excess. This may be due to a greater availability of sodium carrier, a view which is supported by the observation that in the absence of Ca^{2+}, as in the absence of Na^{+}, acetylcholine is ineffective.

Both in Na^{+} excess and Ca^{2+} excess the post-excitatory inhibition (hyperpolarization and block of spike generation) is accelerated and greater. It is likely that this phase is related to the active sodium extrusion (Bülbring & Burnstock, 1960), which would be accelerated by an increased intracellular sodium accumulation during prolonged excitation. An electrogenic sodium pump may be involved (Keynes, 1960; Straub, 1961; Connelly, 1959*a*, *b*).

The effect of adrenaline

It is difficult to explain the action of adrenaline solely on the basis of a change in membrane permeability. Its inhibitory action is brought about by the stoppage of spontaneous spike discharge which, however, does not occur as the consequence of hyperpolarization but usually precedes it. Moreover, it was found in the present work that the degree of hyperpolarization depended on the membrane potential prevailing at the time of administration. When this was already 65–70 mV, adrenaline caused no further increase but still blocked spike discharge.

Evidence presented in a previous paper (Kuriyama, 1963) indicates that the low membrane potential of taenia coli is largely due to a high Na^{+} conductance, and that adrenaline, like excess Ca^{2+} in the medium, reduces this. Moreover, the slow rate of rise of the action potential may be the result of a poor fixation of calcium at the cell membrane (Bülbring & Kuriyama, 1962*b*). Adrenaline, like excess Ca^{2+} in the medium, accelerates the rate of rise.

Bülbring (1960) and Axelsson *et al.* (1961) postulated that the inhibitory action of adrenaline was brought about by an increase in the rate of metabolic energy supply required for stabilizing the membrane. This increased energy supply may affect membrane permeability, for example, by fixing calcium at the membrane, as has been suggested by Shanes (1958). The observation that adrenaline has no effect on the membrane potential after prolonged exposure to Ca^{2+}-free solution would be consistent with the view that it caused a fixation of calcium. Moreover, the blocking action of adrenaline is potentiated by excess Ca^{2+} without further hyperpolarization, as the membrane potential is already high. Metabolic energy is also required for active ion transport, and Burnstock (1958*b*) concluded from his results that adrenaline may stimulate an active transport mechanism involving an electrogenic extrusion of sodium, which would raise the membrane potential and hence accelerate the potassium influx. Evidence in favour of this hypothesis is the finding by Born & Bülbring (1956) that

adrenaline increases the rate of inward movement of potassium, but does not decrease the rate of loss of K. Furthermore, Bülbring & Goodford (unpublished) observed recently that the rate of loss of sodium is accelerated by adrenaline, to the same extent in the presence as well as in the absence of potassium.

Adrenaline still produces hyperpolarization in K^+-free solution, but its effect is completely abolished in Na^+-free solution (cf. Axelsson *et al.* 1961). An increase in K permeability is a possible mechanism for the hyperpolarization. However, the observation that, in the absence of Na^+, adrenaline causes neither a block of spike discharge nor a hyperpolarization, is consistent with the view that it acts chiefly by a modification of Na^+ movement across the cell membrane.

Chloride seems to be of minor importance for the action of adrenaline, as well as of acetylcholine.

SUMMARY

1. The effects of acetylcholine and adrenaline on membrane potential, electrical activity and tension have been observed in the isolated taenia coli of the guinea-pig.

2. The degree of depolarization caused by acetylcholine depended on the initial membrane potential prevailing at the time of application. During the acceleration of spike discharge the amplitude and the rates of rise and fall of the action potential were decreased.

3. In K^+-free solution the depolarization caused by acetylcholine was abolished. When the membrane was previously depolarized by excessive K^+ acetylcholine still caused depolarization, but it became ineffective when the potential was reduced to 15 mV. At this level acetylcholine sometimes increased the membrane potential.

4. In Na^+-free solution acetylcholine did not accelerate the spike discharge but still depolarized the membrane. In Na^+ excess (204 mM) its effect was potentiated and the acceleration of spike frequency was greatly increased and prolonged.

5. In Ca^{2+}-free solution acetylcholine had no effect; in excess Ca^{2+} (7·5 mM) its effect was potentiated.

6. The degree of membrane hyperpolarization caused by adrenaline depended on the membrane potential at the moment of administration, and no hyperpolarization was observed when the membrane potential was 65–70 mV. The block of spike discharge preceded the hyperpolarization or occurred without it. The rates of rise and fall of the action potential were greatly increased just before block occurred and immediately after.

7. In K^+-free solution after the membrane was hyperpolarized and all activity had ceased, adrenaline hyperpolarized the membrane still further.

In excess K^+ (> 30 mM), when the membrane was depolarized to about 30 mV, adrenaline abolished spike discharge but failed to cause hyperpolarization.

8. In Na^+-free solution (Tris) adrenaline had no effect; in Na^+ excess its effect was less than in normal solution.

9. In Ca^{2+}-free solution adrenaline was also ineffective. In the presence of excess Ca^{2+} adrenaline blocked the spike discharges and the duration of inhibition was longer than in the Krebs's solution. The membrane potential remained unchanged.

10. Cl^--deficient solution ($C_2H_5SO_3$) had little effect on the action of acetylcholine or adrenaline.

11. The observations are consistent with the view that acetylcholine exerts its effect by a non-selective increase of membrane permeability, while adrenaline affects chiefly the Na conductance during the active and resting state of the membrane, and modifies the movement of sodium across the membrane.

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Note added in proof. We have recently found that the effect of adrenaline is associated with an increase in the adenosine triphosphate and creatine phosphate content of the taenia (E. Bueding, E. Bülbring, G. Gercken & H. Kuriyama (1963), *J. Physiol.* **166**, 8*P*. This is, however, not the result of an activation of phosphorylase, which does not occur during the physiological effect of adrenaline (E. Bueding, E. Bülbring, H. Kuriyama & G. Gercken (1962), *Nature, Lond.* **196**, 944–946).