J. Physiol. (1961), **157**, *pp.* 74–89
With 10 *text-figures*
Printed in Great Britain

THE EFFECT OF CARBACHOL ON THE PERMEABILITY OF DEPOLARIZED SMOOTH MUSCLE TO INORGANIC IONS

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(*Received* 28 *November* 1960)

Recent evidence, principally from electrical measurements, indicates that transmitter substances (e.g. acetylcholine) act at synapses by altering the permeability of the post-synaptic membrane to one or more inorganic ions. Such a mechanism was first proposed by Fatt & Katz (1951) to account for the depolarizing action of acetylcholine (ACh) at the end-plate region of skeletal muscle. The aim of the present work was to obtain more information about the nature of the change in permeability underlying the action of a stable analogue of ACh, carbachol, on mammalian smooth muscle. For this purpose, the movement of labelled inorganic ions was examined in preparations bathed in solutions containing sufficient potassium to depolarize the muscle fibres; the effects of carbachol on permeability could then be studied without the secondary changes in ion flux associated with the depolarization, and consequent increase in spike activity, which otherwise result from the action of the drug. A preliminary account of part of these results has been given to the Physiological Society (Durbin & Jenkinson, 1959).

METHODS

The taenia coli of the guinea-pig was used in these experiments, which were made at room temperature (20–23° C). The animal was killed by a blow on the head and the required amount of the taeniae excised immediately. It was found difficult to separate the muscle from the large intestine without removing at the same time some of the underlying tissue. Most of this was subsequently detached with fine scissors under a dissecting microscope. During dissection, the strips of taenia were bathed in a modified Krebs's solution which had the following composition (mM): NaCl 133, KCl 5·6, $NaHCO_3$ 16, $CaCl_2$ 2·5, $MgCl_2$ 1·15, glucose 6, Na phosphates 0·5, and was bubbled with a gas mixture containing 95 % O_2 and 5 % CO_2. After preparation, the strips were stretched to approximately their *in situ* length on frames of stainless-steel wire. They were then placed in a solution in which all but 5 mM of the NaCl had been replaced by K_2SO_4. It was found in preliminary experiments that immersion in potassium-rich fluids containing the full osmotic equivalent of the replaced NaCl caused the muscles to lose weight rather rapidly. For this reason, less K_2SO_4 was included in the potassium-rich solution finally employed, which had the following composition (mM); K_2SO_4 76, $KHCO_3$ 16, $CaCl_2$ 7·5, Na_2SO_4 2·5, $MgCl_2$ 1·15, glucose 6, Na

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phosphates 0·5. This was also bubbled with 95 % O_2 + 5 % CO_2 gas mixture, and was used throughout the present work, except in experiments with ^{82}Br, when 5 mM chloride was replaced by bromide. Strips of the taenia bathed in this solution maintained their weight, and responded reproducibly to carbachol, for many hours.

Efflux experiments. It was important for the present work that any net movements of ions should have been largely complete by the time of application of carbachol. The preparations were therefore bathed in the potassium-rich solution for several hours before commencing the experiment proper. During part of this equilibration period tracer was included, care being taken to ensure that active and inactive solutions were of the same chemical composition. In experiments with ^{42}K or ^{36}Cl, the preparations were loaded for 1–4 hr. Shorter loads (17–40 min) were employed in measurements with ^{82}Br.

In a number of experiments efflux of ^{42}K in exchange for inactive potassium was followed simultaneously with tension developed by the taenia. For this purpose the strip of muscle was mounted in a bath similar to that described by Born & Bülbring (1956). It consisted of a block of Perspex through which a horizontal channel, 4 cm long and 0·7 mm in diameter, had been drilled. One end of the strip was fixed at the beginning of the channel, the other being attached by silk thread to a mechano-electric transducer valve (RCA 5734), the output of which was fed, after amplification, to a pen recorder. An appropriate bathing solution flowed past the muscle at approximately 1 ml./min and was collected in bottles which were changed at intervals of 2–8 min. The amount of solution in each was determined by weighing, and 1 ml. samples taken for counting. At the end of the experiment, the activity of the muscle itself was determined, so that its tracer content at any earlier time could be established. To prepare the muscle for counting, it was either dissolved in 0·25 ml. of hot concentrated nitric acid, and the solution evaporated on a platinum planchette, or it was heated with 0·5 ml. of 0·2 N sodium hydroxide on a water bath for several hours, and the fluid finally transferred to a nickel planchette. The latter procedure was used in experiments with ^{36}Cl and ^{82}Br.

With ^{45}Ca and ^{36}Cl the specific activity of the tracer used was so low that it became necessary to use most of the taeniae coli of one guinea-pig for a single measurement. In such experiments the strips (of total weight from 60 to 120 mg) were tied to stainless-steel frames as before, and the loss of the isotope was followed by moving the pieces as a group into successive test-tubes containing known volumes (1·1–3 ml.) of inactive solution.

Uptake experiments. To study the effect of carbachol on the uptake of labelled ions, strips of the taeniae from one guinea-pig were first separated into groups, each containing pieces chosen so as to be similar in size and shape. After dissection each group was carefully blotted and weighed as a unit. The strips were then attached to frames and equilibrated in the potassium-rich solution for at least 2 hr. Subsequent procedures varied. In some experiments with ^{42}K two groups of strips were exposed for a short time, usually 7 min, to separate radioactive potassium-rich solutions, one of which contained carbachol. The muscles were then transferred to inactive fluid, and the amount of radioactivity which each group had acquired during the load period was determined in separate efflux measurements, as described in Results.

The foregoing procedure may involve an error if carbachol acts on the muscle membrane before the full specific activity of tracer has been reached in the immediate vicinity; a rather similar situation has been examined by Bianchi & Shanes (1959) in experiments to determine the effect of increases in the potassium concentration on the uptake of ^{45}Ca by skeletal muscle. Accordingly, in most experiments both groups of muscle strips were 'pre-loaded' in radioactive potassium-rich solution for 2–3 min before exposure separately to either another 'control' or to the 'carbachol' load solution.

Measurement of radioactivity. Throughout this work radioactivity has been determined by evaporating portions of active solutions in nickel or platinum planchettes and counting under an end-window G–M tube. The total weight of material on the planchette, including a disk of lens paper to promote even spreading, was held constant to avoid differences in

self-absorption. Count rates were corrected for background, which included that due to the ^{40}K of the potassium-rich solution used. An allowance for differences in back-scattering was made if nickel and platinum planchettes were used in the same experiment.

The isotopes used fell into two classes, the first having a short half-life and energetic radiation (^{24}Na, ^{42}K, ^{82}Br), the second a longer half-life and consisting of weak beta-emitters (^{45}Ca, ^{36}Cl). Two isotopes, one from each class, were used in many experiments. In such 'double-tracer' measurements, the activity of the short-lived isotope was first determined by counting through an aluminium absorber thick enough to stop beta radiation from the long-lived tracer. Later the sample was counted without absorber, after allowing sufficient time for the decay of the short-lived isotope. The success of the method depends on the freedom of the latter from long-lived radioactivity; several consignments of ^{42}K, supplied as K_2CO_3 by A.E.R.E., Harwell, were found, in fact, to contain appreciable amounts of such a contaminant (^{35}S).

RESULTS

Effect of carbachol on the efflux of potassium, bromide and chloride

Preliminary experiments were made to determine the time course of loss of labelled potassium, bromide and chloride from isolated taeniae coli

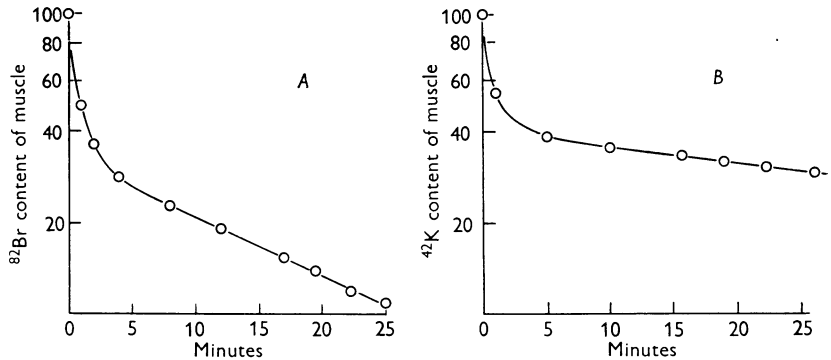

Fig. 1. Time course of loss of ^{82}Br (*A*) and ^{42}K (*B*) from separate portions of taenia coli bathed in inactive potassium-rich solution, at room temperature. Semilog. scales.

bathed in the potassium-rich solution used throughout the present work. Examples of the results obtained are illustrated in Fig. 1. Except for the first half-minute or so, the curves could be described as the sum of two exponential terms:

$$P = A\,e^{-\lambda_1 t} + B\,e^{-\lambda_2 t}, \qquad (1)$$

where P is the tracer content of the muscle at any time, t, and A, B, λ_1 and λ_2 are constants. Values obtained for λ_2 and, in a few cases, for λ_1 are listed in Table 1. After a few minutes the first term became unimportant, so that further loss of tracer followed a simple exponential law, as was found by Born & Bülbring (1956) for exchange of ^{42}K in taeniae coli bathed in Krebs's fluid at 37° C.

Carbachol consistently increased the rate of efflux, as is illustrated for ^{42}K in Figs. 2 and 3 (lower section). (In Fig. 3, and in some subsequent illustrations, the rate of loss has been plotted in terms of a coefficient, r, defined as the proportion of tracer lost in unit time, averaged over each collection period. r can be regarded as a convenient empirical measure of efflux, the use of which enabled results obtained with different isotopes to be expressed on a common basis. It may be noted that r approximates to λ_2 during the exponential phase of tracer loss.)

TABLE 1. Values of λ_1 and λ_2 ($\pm$S.E. of mean; no. of experiments in brackets) obtained in measurements of the rate of loss of ^{42}K, ^{36}Cl and ^{82}Br from the depolarized taenia coli, at room temperature

Ion	λ_1 (min^{-1})	λ_2 (min^{-1})
K	0·8, 0·7	0·012 $\pm$ 0·001 (9)
Cl		0·034 $\pm$ 0·002 (4)
Br	1·0, 1·0	0·040 $\pm$ 0·001 (5)

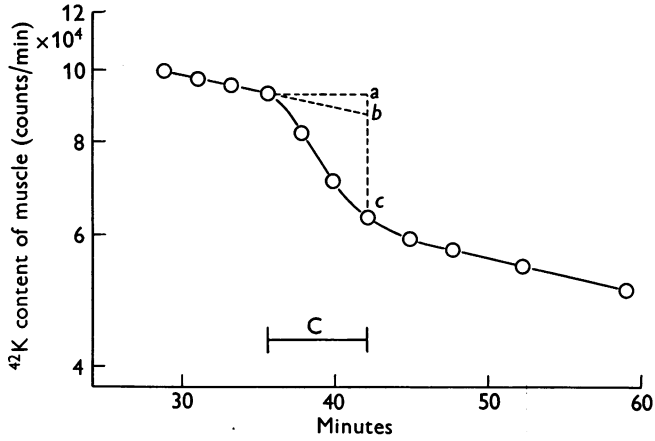

Fig. 2. Effect of 3×10^{-7} g/ml. carbachol (C) on loss of ^{42}K, plotted as in Fig. 1. A convenient measure of the action is given by the ratio, R_e, of the amounts of tracer represented by the distances *ac* and *ab* (see text). In this experiment R_e had the unusually large value of 4·9.

Carbachol also caused the muscle to develop additional tension (Fig. 3, upper section). This response to depolarizing agents applied in potassium-rich solutions has been described for other varieties of smooth muscle by Evans, Schild & Thesleff (1958), who also showed that, as in the polarized muscle, the contractures elicited by ACh were reversibly eliminated by atropine. An experiment to test this action on the depolarized taenia is illustrated in Fig. 4. It can be seen that atropine (3×10^{-8} g/ml.) almost abolished the increase in ^{42}K efflux produced by carbachol (3×10^{-7} g/ml.). The effect was only slowly reversible.

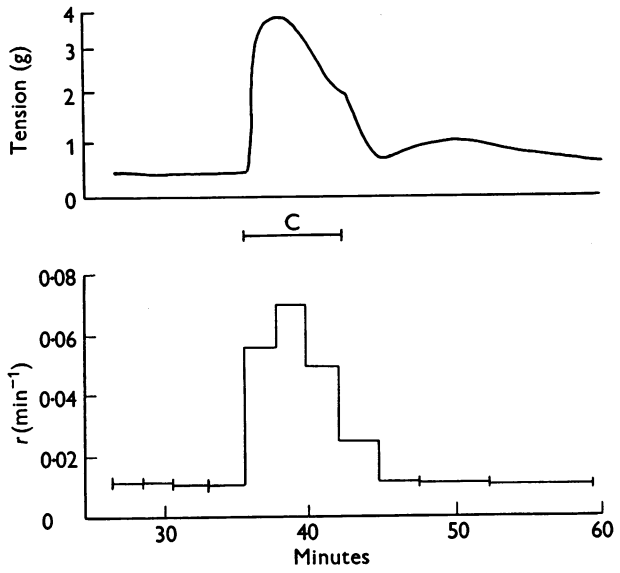

Fig. 3. Simultaneous determination of the effects of carbachol (3×10^{-7} g/ml.) on development of tension and on the rate of loss of ^{42}K. From same experiment as Fig. 2. Ordinates of upper and lower graphs respectively tension (grams) and rate coefficient, r (min^{-1}) defined as $\Delta c/\Delta t \,.\, c_m$, where Δc is the amount of tracer collected during a given period of duration Δt (indicated by the length of the horizontal bars), and c_m is the mean of the tracer contents of the muscle at the beginning and at the end of the period. Abscissa, time in min.

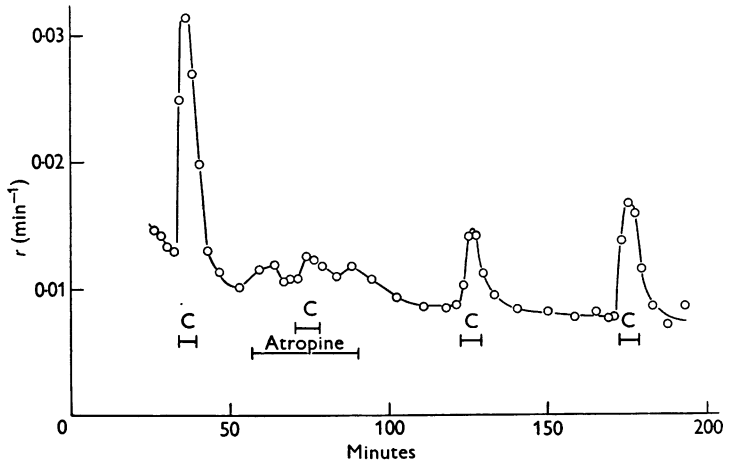

Fig. 4. Inhibition by atropine (3×10^{-8} g/ml.) of the increase in ^{42}K efflux produced by carbachol (3×10^{-7} g/ml.). Plotted as in lower part of Fig. 3, but with the rate of loss of tracer indicated for simplicity by points drawn at times corresponding to midway in each collection period. The additional tensions developed in response to the successive applications of the standard dose of carbachol were 2·9, 0·2, 0·3 and 0·8 g respectively.

To enable experiments of the kind illustrated in Figs. 2, 3 and 4 to be compared, carbachol was always applied in a concentration of 3×10^{-7} g/ml. for an arbitrary period of 7 min. A quantitative measure of the effect on ion flux was obtained by dividing the quantity of tracer lost during this standard application of the drug by the amount, estimated by extrapolation, which would presumably have exchanged had carbachol not been introduced (see Fig. 2). The average value of this ratio, R_e, obtained in nine measurements of the effect of the standard application of carbachol on ^{42}K efflux was $2{\cdot}32 \pm 0{\cdot}35$ (S.E. of the mean).

R_e may be expressed in terms of the rate constant, k, for exchange of tracer. To a first approximation, the loss of activity from the tissue is proportional to its tracer content, P:

$$-\frac{dP}{dt} = kP. \tag{2}$$

The application of carbachol results in an increase in the rate of loss (see Discussion), which may then be written

$$-\frac{dP}{dt} = (k+\Delta k)\,P, \tag{3}$$

where $k+\Delta k$ is a composite rate 'constant' holding in the presence of the drug. However, the effect of carbachol on a whole muscle develops slowly and is not well maintained (Fig. 3), possibly owing to desensitization of the receptors (Katz & Thesleff, 1957). For this reason, and to enable the effects of carbachol on both inward and outward fluxes to be compared, it is convenient to introduce a quantity $\overline{\Delta k}$, the average of Δk over the total time (from t to $t+\Delta t$) of application of the drug;

$$\overline{\Delta k} = \frac{1}{\Delta t}\int_t^{t+\Delta t} \Delta k\, dt. \tag{4}$$

It may be noted that $\overline{\Delta k}$, being a time-average value, will underestimate the maximum extent of the increase in permeability.

Equation (3) may then be integrated to give

$$\Delta P' = P'\{1-e^{-(k+\overline{\Delta k})\,\Delta t}\}, \tag{5}$$

where P' and $P'-\Delta P'$ are the tracer contents of the muscle at the beginning and end of the carbachol period. If the drug had not been applied, the loss of tracer would have been ΔP, where

$$\Delta P = P'(1-e^{-k\Delta t}). \tag{6}$$

Dividing (5) by (6), we obtain

$$\frac{\Delta P'}{\Delta P} = \frac{1-e^{-(k+\overline{\Delta k})\,\Delta t}}{1-e^{-k\Delta t}} = R_e, \text{ the efflux ratio as previously defined.} \tag{7}$$

Carbachol also increased the rate of loss of ^{36}Cl and ^{82}Br, as is illustrated in Fig. 5. R_e was found to be, for ^{36}Cl, $1{\cdot}33 \pm 0{\cdot}08$ (4 expts.) and for bromide, $1{\cdot}36 \pm 0{\cdot}05$ (3 expts.).

The effect of carbachol on uptake of potassium, bromide and chloride

If carbachol increases the permeability of the smooth muscle membrane, the rate of uptake of tracer from radioactive solutions should also be enhanced. This point was tested by briefly exposing two groups of strips

of taenia coli to separate load solutions which differed only in that one contained carbachol. The amount of tracer which had been taken up by each group was then determined, as illustrated in Fig. 6. The carbachol-treated muscles were invariably found to possess more radioactivity.

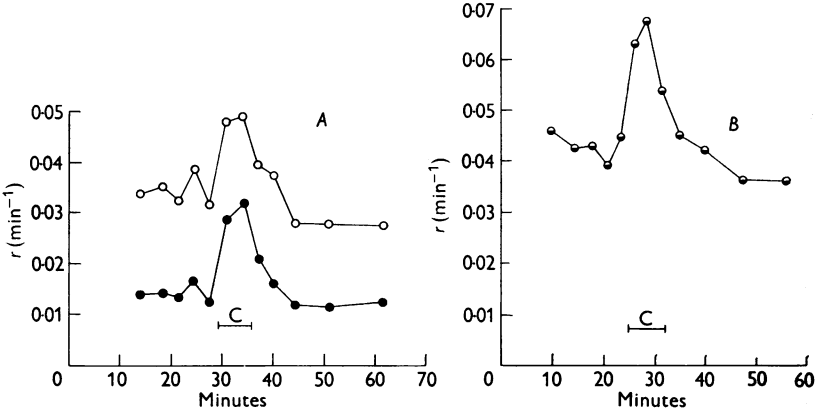

Fig. 5. The effect of carbachol (3×10^{-7} g/ml.) on the rate of loss of ^{36}Cl (○) and ^{42}K (●) (*A*, 'double tracer' technique employed), and ^{82}Br (◒) (*B*). Plotted as in Fig. 4.

The effect on uptake can be expressed in terms of the rate of exchange, k, just as in output experiments. For the control muscle, the rate of uptake of tracer is given by

$$\frac{dP}{dt} = k(P_f - P), \tag{8}$$

provided that the extracellular spaces have the same specific activity as the load solution. (P_f is the final steady state content of tracer, and other terms are as previously defined). Thus the amount of tracer, ΔP, taken up from time t to $t+\Delta t$ is

$$(P_f - P')\{1 - e^{-k\Delta t}\},$$

where P' is the activity of the muscle at time t.

The uptake, $\Delta P'$, by the carbachol-treated muscle during the same period is

$$(P_f - P')\{1 - e^{-(k+\overline{\Delta k})\Delta t}\},$$

where $\overline{\Delta k}$ is the time average of Δk, as before. Hence

$$\frac{\Delta P'}{\Delta P} = \frac{1 - e^{-(k+\overline{\Delta k})\Delta t}}{1 - e^{-k\Delta t}} = R_u, \tag{9}$$

the uptake ratio. Thus experimental determinations of R_u and R_e should provide similar values (cf. equation (7)).

In those experiments in which the muscles were 'pre-loaded' (see Methods) a correction was applied to allow for the fact that the carbachol-treated group were in contact with the drug only during 7 min of the total period (8–10 min) in load. It was assumed that, to a first approximation, the uptake of tracer was proportional to the load time; thus, for example, if the pre-loading period was 2 min, and the total time in load 9 min, 2/9 of the uptake by the control muscle was subtracted from the amounts of tracer taken up by the carbachol-treated group and by the control. The 'uptake ratio' was then expressed as the quotient of the differences.

In six measurements of the effect of the standard application of carbachol on the uptake of ^{42}K, the mean value obtained for R_u was $1{\cdot}88 \pm 0{\cdot}12$. (In one of these experiments the muscles had been 'pre-loaded', and in another, three groups of muscles were used, to check the validity of the correction described in the preceding paragraph; the values obtained were respectively 2·29 and 2·00, and have been included with the others.) In the remaining uptake experiments to be described, the 'pre-loading' technique was employed.

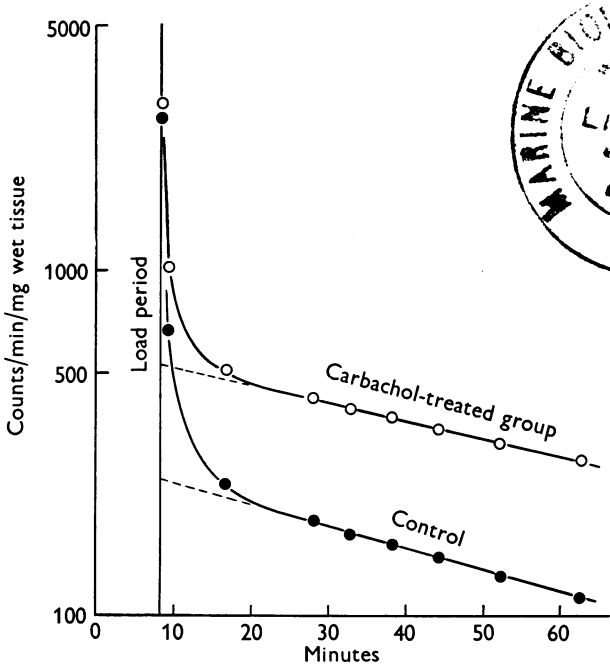

Fig. 6. The effect of carbachol on the uptake of ^{42}K. Two portions of the taenia have been exposed (without pre-loading, in this case) to separate load solutions, one of which contained carbachol (3×10^{-7} g/ml.). After 8 min both groups were transferred to inactive fluid and the loss of tracer was followed; the quantity remaining in each is shown as a function of time (cf. Fig. 1). The amounts which had been taken up can be compared by extrapolating the linear parts of the curves to time of removal from the load, which contained ^{42}K $2{\cdot}9 \times 10^4$ counts/min per μmole K. Semi-log. scale.

Carbachol also increased the influx of labelled chloride and bromide. In four experiments with ^{36}Cl, R_u came to $1{\cdot}38 \pm 0{\cdot}10$, and in three with ^{82}Br, $1{\cdot}28 \pm 0{\cdot}10$. These figures, together with the corresponding efflux ratios, are summarized in Table 2. It can be seen that for a given ion, R_e and R_u agree within the error of the measurements. Table 2 also lists values for $\overline{\Delta k}$, calculated, by equation (7), from the mean of R_u and R_e for

each isotope. The figures obtained are also in fair agreement. The possible significance of this is discussed later.

It has been assumed in applying equation (7) to the experimental data that k, the rate constant for exchange of tracer, is given by λ_2 (equation (1)). However, the two terms of equation (1) correspond to loss of tracer from interspaces and cells respectively only if these regions can be considered as being in independent equilibrium with the bathing fluid. In practice, it is likely that tracer from a proportion of the cells can gain access to the exterior only through the extracellular spaces, which thus have an appreciable specific activity. It can be shown that λ_2 may then differ substantially from k, and A and B from the original tracer contents of interspaces and cells, respectively, especially if the tissue is thick and if there is a large concentration gradient between the intra- and extracellular fluids (Harris, 1950). Under the conditions of the present experiments, however, the error introduced by equating k to λ_2 will be small (probably less than 10%) since $\lambda_2 \ll \lambda_1$ (Table 1), and since it is likely that the concentrations of potassium, chloride and bromide ions in the interspaces and cells are of the same order.

TABLE 2. The effect of a 7 min application of carbachol (3×10^{-7} g/ml.) on potassium, chloride and bromide fluxes, expressed in terms of R_c and R_u (pp. 79 and 80). The corresponding values of $\overline{\Delta k}$ are also given (see text)

Ion	R_c	R_u	$\overline{\Delta k}$ (min^{-1})
K	$2{\cdot}32 \pm 0{\cdot}35$	$1{\cdot}88 \pm 0{\cdot}12$	0·014
Cl	$1{\cdot}33 \pm 0{\cdot}08$	$1{\cdot}38 \pm 0{\cdot}10$	0·014
Br	$1{\cdot}36 \pm 0{\cdot}05$	$1{\cdot}28 \pm 0{\cdot}10$	0·015

Loss of labelled sodium and calcium from depolarized taeniae

Before examining the effect of carbachol on the rate of movement of sodium and calcium, preliminary experiments were made to determine the time course of exchange of these ions. Figure 7 illustrates the loss of ^{24}Na and ^{45}Ca from three portions of taenia coli which had been loaded for different periods. The shapes of the curves vary with the time of loading and differ markedly from those observed with potassium, chloride and bromide (cf. Fig. 1).

Except for the first half-minute or so, the loss of tracer could be described as the sum of three exponential terms. In Fig. 7 the uppermost curves, which refer to the longest load times, have been drawn according to the expression

$$P = A\,e^{-\lambda_1 t} + B\,e^{-\lambda_2 t} + C\,e^{-\lambda_3 t}, \qquad (10)$$

where P is the tracer content of the muscle at any time, and A, B and C have the values (in counts/min/mg muscle) of 575, 360 and 11 (^{24}Na) and 150, 67 and 18 (^{45}Ca). The corresponding values of λ_1, λ_2 and λ_3 were 0·26, 0·086 and 0·0097 min^{-1}, for loss of ^{24}Na, and 0·21, 0·067 and 0·0061 min^{-1} for loss of ^{45}Ca. It was of some interest to compare values obtained for A in experiments with sodium and calcium on the one hand, and with potassium, chloride and bromide on the other. In the experiment of Fig. 7 the activity of the load solution was $1{\cdot}34 \times 10^6$ counts/min/ml. (^{24}Na) and

$4{\cdot}28 \times 10^5$ counts/min/ml. (^{45}Ca), so that the values of A corresponded to volumes which were respectively 43 and 35 ml./100 g wet tissue. These figures, and others obtained in measurements of this kind, are similar to the values observed in experiments with labelled potassium, chloride and bromide (Table 3). It thus seems reasonable to identify the first term in equation (10) with loss of tracer from the interspaces.

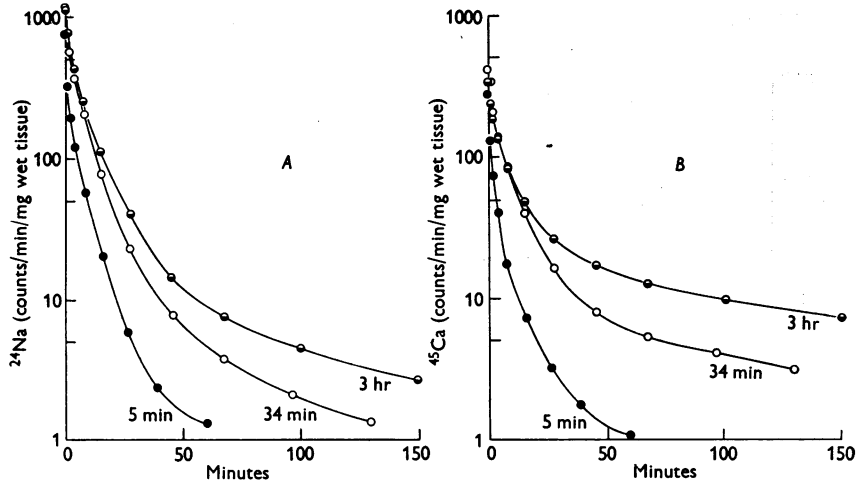

Fig. 7. 'Double tracer' experiment to demonstrate loss of ^{24}Na (A) and ^{45}Ca (B) from three portions of taenia from the same guinea-pig. The duration of the load period is given with each curve. $2{\cdot}35 \times 10^5$ counts/min ^{24}Na corresponded to 1μ-mole sodium, and $5{\cdot}7 \times 10^4$ counts/min ^{45}Ca to 1μ-mole calcium in the load solution. Semi-log. scales.

TABLE 3. Values for A, and in a few cases for B, obtained on fitting equation (10) to the efflux of different labelled ions. Both quantities have been expressed as the equivalent volumes of load solution (ml./100 g wet tissue). A value for B has been given only if the load period exceeded 2 hr (^{36}Cl) or 3 hr (^{42}K)

Isotope	A	B	Isotope	A
^{42}K	59, 39	32, 41, 53, 37	^{45}Ca	44, 32, 46, 44
^{36}Cl	43, 42	31, 47	^{24}Na	37, 44
^{82}Br	41, 39	—	—	—

It can be seen from Fig. 7 that the tracer content of muscles which had been loaded for as long as 3 hr fell to less than 5% of the original value after only 1 hr in inactive solution. Thereafter activity was lost much more slowly, indicating that part of the sodium and calcium in the muscle is relatively inexchangeable under the present conditions. Because this component itself may well be non-homogeneous, the second and third terms in equation (10) cannot be taken to correspond with any certainty to loss of tracer from distinct compartments or fractions.

Effect of carbachol on efflux of sodium and calcium

The results of experiments to test the action of carbachol on the rate of loss of ^{24}Na and ^{45}Ca are illustrated in Fig. 8. The effects observed were small and rather irreproducible. Because of the complexity of the efflux curves, no attempts were made to compute values for the efflux ratio, R_e, as previously defined. Instead, the actions were expressed as the maximum difference, Δr_m, between the rate coefficient in the presence of

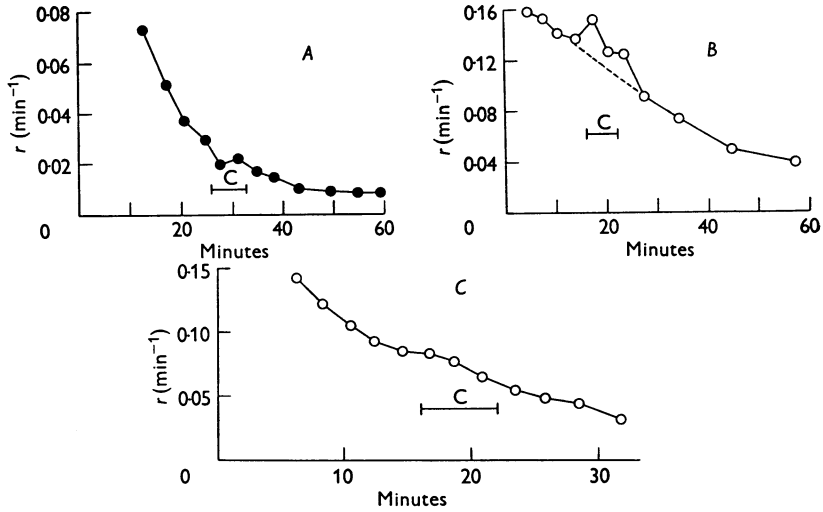

Fig. 8. Effect of carbachol (3×10^{-7} g/ml.) on the efflux of ^{45}Ca (●, *A*) and ^{24}Na (○, *B* and *C*) from depolarized taenia coli. Plotted as in Fig. 4.

carbachol, and that, r, which would have held had the drug not been applied; e.g. in the experiment of Fig. 8(*B*), Δr_m came to

$$0{\cdot}15\text{–}0{\cdot}12 = 0{\cdot}03 \text{ min}^{-1}.$$

This value is listed in Table 4 together with others obtained in a series of experiments in which carbachol was applied at different times after removal of the taenia from the load solution, in an attempt to determine whether a particular phase of tracer exchange was affected by the drug. The results suggest that this was not the case.

Effect of carbachol on the uptake of sodium and calcium

Figure 9 illustrates a typical experiment to test the action of carbachol on the uptake of ^{24}Na and ^{45}Ca by taenia coli. Because of the complex time course of exchange of these ions, it is difficult to make a direct comparison of the amounts of tracer taken up by the cellular material of the test and control muscles during the load period. Instead, the ratio of the total

tracer contents (per milligram wet tissue) of the two groups was computed for different intervals after the transfer of both from active to inactive solution. The mean values obtained in all experiments of this kind are shown plotted in Fig. 10. It can be seen that the carbachol-treated muscles contained more tracer than did the controls, from which it can be concluded that the drug affects the uptake of these ions.

TABLE 4. Effect of carbachol (3×10^{-7} g/ml.) on rate of loss of ^{24}Na and ^{45}Ca from the depolarized taenia coli. The time, t, from removal from load to the application of carbachol is listed in cols. 2 and 5 and the maximum increase, Δr_m, in rate coefficient in cols. 3 and 6. A — indicates a negligible effect

^{24}Na			^{45}Ca		
1	2	3	4	5	6
Expt.	t (min)	Δr_m (min^{-1})	Expt.	t (min)	Δr_m (min^{-1})
1	15	0·015	1	15	—
2*	16	0·030	2	16	—
3†	17	0·007	8‡	26	—
4	18	0·009	9	33	—
5	21	—			
6	46	—			
7	58	0·005			

* Fig. 8*B*; † Fig. 8*C*; ‡ Fig. 8*A*.

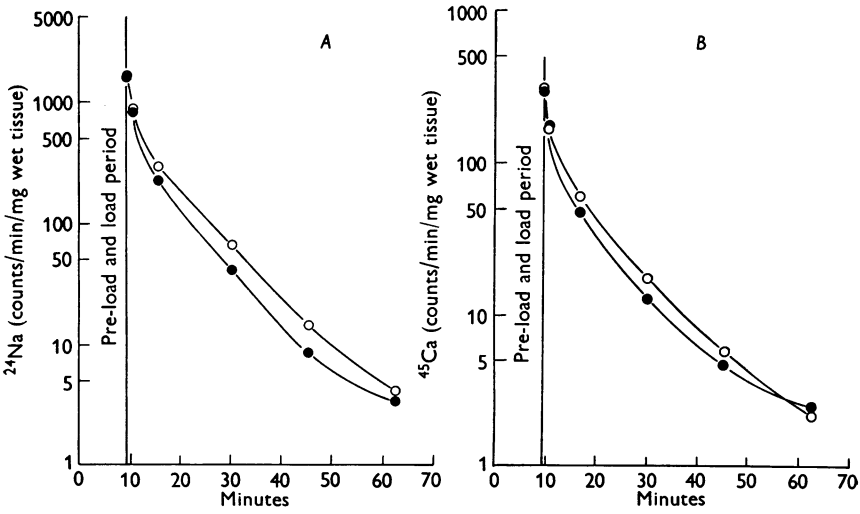

Fig. 9. 'Double tracer' experiment to test the effect of carbachol (3×10^{-7} g/ml.) on the uptake of ^{24}Na (*A*) and ^{45}Ca (*B*). Plotted as in Fig. 6. $4{\cdot}20 \times 10^5$ counts/min ^{24}Na correspond to 1μ-mole sodium, and $4{\cdot}10 \times 10^4$ counts/min ^{45}Ca to 1μ-mole calcium; it should be noted that in this early experiment the total calcium concentration was maintained at 10 mM. The carbachol-treated muscles (○) have taken up a slightly greater amount of both tracers than have the controls (●). Semi-log scales.

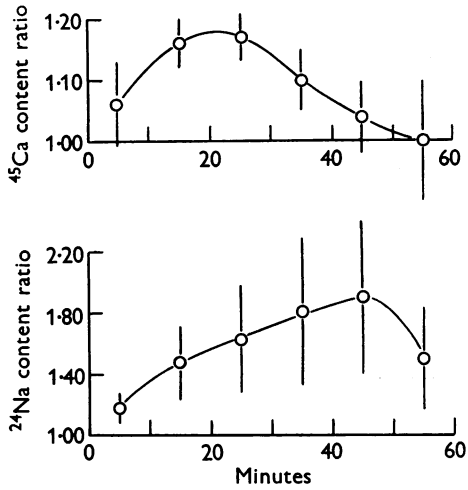

Fig. 10. Combined results of experiments (10 with ^{24}Na, 6 with ^{45}Ca) of the type illustrated in Fig. 9. The tracer contents of the carbachol-treated muscles have been divided by that of the controls, for various times after removal from load. Mean values, ± S.E. of means, plotted.

The initial increases in the 'content ratios' plotted in Fig. 10 are most probably due to progressive loss of tracer from the interspaces. Carbachol would be expected to enhance only that fraction of the total uptake which is associated with the cells, so that the presence of appreciable quantities of tracer in the extracellular spaces of both the drug-treated and the control muscles will mask the effect.

DISCUSSION

The present experiments show that carbachol increases the permeability of the taenia coli of the guinea-pig to potassium, chloride and bromide ions. Parallel studies of the action of the drug on the flux of sodium and calcium were complicated by the non-homogeneity, as indicated by the exchange kinetics, of the muscle content of these ions. Harris & Steinbach (1956) have shown that frog skeletal muscle possesses sites which can bind sodium rather firmly, and other evidence suggests that the same may hold for calcium. If such sites occur to an appreciable extent in the taenia coli, it is possible that after a short period in inactive fluid, the rate of loss of ^{24}Na and ^{45}Ca may be determined largely by desorption rather than by the permeability of the membrane. The failure to observe appreciable effects of carbachol on the efflux would not then be unexpected. However, this should not apply to the study of inward movement and in fact the uptakes of both sodium and calcium were enhanced by the drug, suggesting, although in a less conclusive manner, that the permeability to these ions is also increased by carbachol.

The analysis made by Fatt & Katz (1951) of the end-plate potential of frog skeletal muscle showed that ACh acts as though it created additional pathways for the movement of ions through the post-synaptic region. These would appear to differ from the normal channels in at least two respects; they permit the comparatively free passage of sodium, potassium and possibly some other ions (but not apparently of chloride (Takeuchi & Takeuchi, 1960); however, see Nastuk (1959)), and they allow externally-applied current to pass in both directions with much the same facility under circumstances in which the conducting channels normally present in the membrane show marked 'anomalous rectification' (del Castillo & Katz, 1955). This suggests that the ACh channels may be 'in parallel' with the others. It is of interest to express the present findings in terms of such a model. In all the experiments which have been described, the membrane was already depolarized by the application of potassium-rich fluids. It is then unlikely that the action of carbachol can give rise to an appreciable change in membrane potential, so that the additional ion flux produced by the drug might be expected to follow Fick's law, and so could be written as

$$\frac{\Delta A \,.\, D}{V \,.\, x} P,$$

where D is the effective diffusion coefficient in the carbachol-operated channels (of area ΔA, and thickness x), and P is the tracer content and V the volume, of the muscle fibres. Writing $\Delta A \,.\, D/V \,.\, x$ as Δk, the action of carbachol may be considered to increase the rate of exchange of tracer from $k \,.\, P$ to $(k + \Delta k)\, P$ (cf. equation (3)). If the value of D is similar, or proportional, to that in free solution, the quantity $\Delta A \,.\, D/V \,.\, x$ will be substantially the same for potassium, chloride and bromide ions. Experimentally determined values for Δk, and so most probably for $\overline{\Delta k}$, would then be expected to agree, as is in fact observed (Table 2). Although this finding is suggestive, its significance can only be decided by similar measurements with other varieties of smooth muscle. It may be noted that there is already much evidence to suggest that the effect of ACh on the ionic permeability of some other tissues may be more specific. For example, Harris & Hutter (1956) have demonstrated a striking increase in the rate of potassium exchange in the sinus venosus of the frog in response to ACh, but have not observed any comparable effect on the flux of sodium, chloride or bromide ions (personal communication). This difference in specificity most probably reflects the *inhibitory* action of ACh on the sinus (cf. Hutter, 1957).

Even when depolarized by immersion in potassium-rich solution, the taenia coli develops additional tension on the application of carbachol. The possibility arises that the observed changes in ion flux may have been

artifacts of the mechanical response, for example, due to 'squeezing out' of tracer from the extracellular spaces. However, the finding that inward and outward fluxes were increased to approximately the same extent suggests that this was not the case. Also, it was possible, by making a suitable reduction in the calcium concentration, to observe large effects of carbachol on ion movement in the almost complete absence of tension development. These experiments, which were made to obtain more information about the mechanism of the contracture, are described more fully in the following paper (Durbin & Jenkinson, 1961).

SUMMARY

1. Radio-isotopes have been used to study the effect of carbachol on the permeability of the taenia coli of the guinea-pig to inorganic ions. The preparations were bathed in potassium-rich solutions throughout in order to avoid secondary ionic movements associated with the changes in membrane potential which otherwise accompany the action of the drug.

2. Carbachol increased both inward and outward fluxes of potassium, chloride and bromide ions, and caused the muscle to develop additional tension. Both responses were reversibly abolished by atropine (3×10^{-8} g/ml.).

3. Similar experiments on ^{24}Na and ^{45}Ca flux were complicated by the relative inexchangeability of part of the muscle sodium and calcium. However, the results suggested that carbachol also affects the movement of these ions.

4. These findings support the hypothesis that depolarizing agents such as carbachol act by increasing the permeability of the muscle membrane to inorganic ions.

We are indebted to Professor B. Katz for constant help and encouragement, and to Mr J. L. Parkinson, Miss A. Paintin and Mr L. Ward for frequent assistance.

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