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THE EFFECT OF NORADRENALINE ON THE PERMEABILITY OF DEPOLARIZED INTESTINAL SMOOTH MUSCLE TO INORGANIC IONS

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SUMMARY

1. Radio-isotopes have been used to study the effect of noradrenaline on the permeability of the taenia of the guinea-pig caecum to inorganic ions. The preparations were bathed at either 10 or 20° C in solutions containing a high concentration of potassium, in order to depolarize the fibres and so avoid ionic movements secondary to changes in membrane potential.

2. Under these conditions noradrenaline increased both inward and outward fluxes of potassium whilst having little effect on the exchange of chloride.

3. No effect of noradrenaline on the uptake of sodium could be detected, whereas carbachol (carbamyl choline chloride), applied under identical conditions, caused a significant increase at a concentration chosen to match the effect of noradrenaline on potassium exchange.

4. These results are discussed in relation to the hypothesis that an increase in potassium permeability contributes to the inhibitory actions of noradrenaline on intestinal smooth muscle.

INTRODUCTION

Recent work has revealed several common features in the transmission of impulses at cholinergic and at certain adrenergic synapses. Thus stimulation of the adrenergic motor nerve supply to a variety of smooth muscles causes transient depolarizations of the muscle cells (Burnstock & Holman, 1961, 1966; Kuriyama, 1963*b*; Orlov, 1961, 1962; Speden, 1964). These depolarizations, if sufficiently large, initiate action potentials and resemble in their general features the synaptic potentials observed with other transmitters, e.g. acetylcholine. It therefore seems reasonable to assume as a working hypothesis that similar mechanisms are involved in both cases, and that noradrenaline causes excitation in such tissues by increasing the permeability of the membrane to Na, and probably also to K.

The electrical events underlying adrenergic inhibition in smooth muscle

have been studied in the distal colon of the rabbit (Gillespie, 1962) and in the taenia of the guinea-pig caecum (Bennett, Burnstock & Holman, 1966). In both instances, stimulation of the sympathetic nerve supply causes hyperpolarization of the cells, and reduces the frequency of action potentials. Similar changes follow application of adrenaline to the guinea-pig taenia (Bülbring, 1954, 1957; Bülbring & Kuriyama, 1963), and to both circular and longitudinal intestinal muscle of the cat (Sperelakis & Prosser, 1959; Bortoff, 1961). Although other factors may contribute, these effects can also be explained as a consequence of an increase in membrane permeability, in this instance to K alone. A mechanism of this kind has already been shown to underlie the inhibitory actions of acetylcholine on pace-maker and atrial tissue of the heart (Burgen & Terroux, 1953; Harris & Hutter, 1956; Hutter, 1957; Trautwein & Dudel, 1958).

The aim of the present work was to test the hypothesis that noradrenaline increases the K permeability of intestinal smooth muscle. The experiments were made with the guinea-pig taenia, which was chosen because it had been found suitable in an earlier study of the effects of carbachol on ionic permeability (Durbin & Jenkinson, 1961), and also because much is already known of its ionic composition and electrical properties. In particular, the effect of adrenaline on the exchange of K in the taenia has been examined by Born & Bülbring (1956), who observed an increase in uptake of ^{42}K, but little, if any, consistent change in efflux. Similar results were obtained by Hüter, Bauer & Goodford (1963). The failure of adrenaline to increase K efflux is not incompatible with the possibility that catecholamines may enhance K permeability, since such an action would be expected to increase the membrane potential and to reduce, or abolish, spike activity. Both effects would be expected to diminish the rate of loss of ^{42}K, and this reduction would tend to mask any increase in ^{42}K efflux resulting from the direct action of the drug.

In order to reduce the complexity of this situation we have made similar experiments, but using bathing fluids in which sodium chloride was largely replaced by potassium sulphate. Under these conditions the membrane potential is small (Evans, Schild & Thesleff, 1958; Burnstock & Straub, 1958; Falk & Landa, 1960; Kuriyama, 1963*a*), and also close to the equilibrium potential for the K ion, so that little potential change would be expected to result from alterations in K permeability. Changes in ion flux secondary to changes in membrane potential should then be negligible.

Preliminary accounts of some of our results have already appeared (Jenkinson & Morton, 1965, 1966).

METHODS

The experiments were made with the taenia (often referred to as taenia coli) of the caecum of the guinea-pig. Usually males of the Porton strain, weighing between 300 and 500 g, were chosen. The procedures used were developed in an earlier study of the effect of carbachol on the exchange of ions in this tissue (Durbin & Jenkinson, 1961) and are described in detail only where changes have been made. It is to be noted that our preparations correspond to 'taenia strips' as described by Burnstock, Campbell & Rand (1966), although we removed, with the aid of fine scissors and a dissecting microscope, as much as practicable of the tissue underlying the longitudinal muscle.

Solutions. The muscles were dissected and mounted in a bathing fluid containing (mM): NaCl,133;KCl,5·6;$NaHCO_3$,16;$CaCl_2$,2·5;$MgCl_2$,1·15;Na phosphate buffer, 0·4; glucose, 6. They were then transferred for the remainder of the experiment to K-rich solutions of the following composition (mM): K_2SO_4,107;Na_2SO_4,2·5;$MgCl_2$,1·15;$KHCO_3$,16; $CaCl_2$,5; KCl,5; Na phosphate buffer, 0·4; glucose, 6. A modified form of this fluid, containing (mM):K_2SO_4, 84·5; Na_2SO_4, 25; $MgCl_2$, 1·15; $KHCO_3$, 16; $CaCl_2$, 5; KCl, 5; Na phosphate buffer, 0·4; glucose, 6, was used in some later experiments, as described in Results. The pH of these solutions was 7·2.

All solutions were bubbled with a 95 % O_2, 5 % CO_2 gas mixture.

Efflux experiments. Strips of taenia were stretched lightly under a tension of approximately 2 g before attachment by 38 s.w.g. stainless-steel wire hooks to frames made of 23 s.w.g. stainless-steel tubing through which the solutions were aerated. Several strips, to give a total weight of from 10 to 30 mg, were mounted on each frame.

The preparations were then transferred to K-rich solutions for an equilibration period of at least 2 hr. During part of this time ^{42}K or ^{36}Cl was included in the bathing fluid, care being taken to ensure that the active and inactive solutions were of the same chemical composition. After loading, the tissues were drained and then passed through a series of test-tubes containing a measured volume (usually 2 ml.) of inactive K-rich solution. The effect of noradrenaline on the rate of loss of radioactivity was tested by including a suitable concentration in three successive tubes. At the end of each experiment, the tissues were blotted carefully (using Whatman No. 1 paper) weighed and finally prepared for counting by exposure to a small volume of A.R. nitric acid. In later work it was found more convenient to destroy the muscles in distilled water, using an M.S.E. ultrasound disintegrator.

Knowing the quantity of radioactivity remaining in the tissue at the end of the experiment, and the amount lost into each tube, the tracer content of the muscle at any earlier time could be calculated. The rate of efflux could then be expressed as the proportion of tracer lost in unit time.

Uptake experiments. Six portions of taenia from the same guinea-pig were matched for size and shape to form either two or three groups. The strips in each group were then mounted on stainless-steel holders, as in efflux experiments, and equilibrated in K-rich solutions for at least 2 hr. Next, they were placed for 2–3 min in a loading solution of the same chemical composition, but which contained either ^{42}K, ^{24}Na, or ^{36}Cl. This was to allow tracer to enter the extracellular spaces, before application of drugs (Durbin & Jenkinson, 1961). The groups were then separated and exposed in different tubes to further samples of the same loading solution, to one of which noradrenaline had been added. In some experiments a second group was exposed to carbachol. The remaining preparation served as a control and was loaded for an identical time (9 min) in the absence of drug.

The tissues were finally returned to inactive solution, and the loss of tracer was followed as in efflux experiments. The amounts of tracer taken up by the test and control preparations could then be compared.

Measurement of radioactivity. Samples of fluid were placed on aluminium planchettes and dried under an infra-red lamp. Radioactivity was determined using either a G-M tube, or a

gas-flow proportional counter mounted in a Frieseke & Hoepfner automatic sample changer.

Drugs. Carbamyl choline chloride (carbachol) was supplied by B.D.H., and (−)-noradrenaline bitartrate by Koch-Light Ltd. A further sample of (−)-noradrenaline bitartrate was kindly donated by Winthrop Laboratories. Standard solutions containing 1 mg/ml. were made up freshly each day. In order to increase the stability, ascorbic acid (B.D.H.) was included at a similar concentration in the noradrenaline stock solution, and final dilutions were always made within a few minutes of exposure of the tissues to drug.

All drug concentrations are expressed in terms of the corresponding salts.

RESULTS

Effect of noradrenaline on the efflux of ^{42}K

It has previously been shown (Durbin & Jenkinson, 1961) that there are two phases in the loss of labelled K, Br and Cl ions from strips of taenia equilibrated at room temperature in solutions containing sufficient K to depolarize the muscle fibres. The first phase is rapid, being complete within a few minutes, and represents loss of radioactivity from the extracellular spaces. Thereafter, the tracer content of the tissue falls much more slowly,

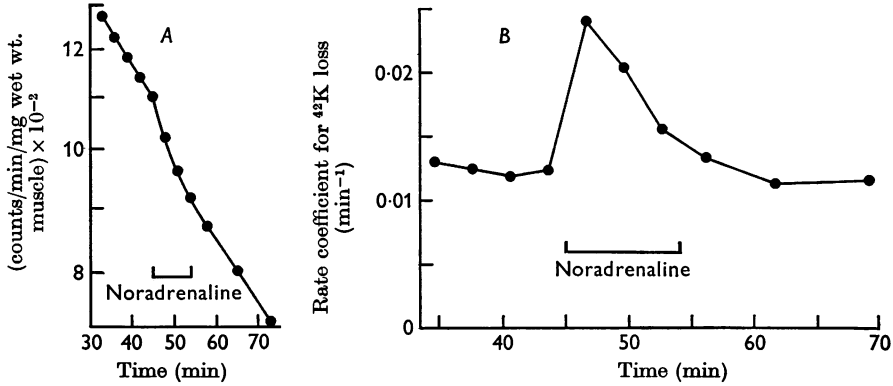

Fig. 1. The effect of (−)-noradrenaline bitartrate (3×10^{-7} g/ml.) on the rate of loss of ^{42}K from a strip of taenia bathed at 20° C in a solution containing sufficient K (235 mM) to depolarize the muscle fibres.

A. Portion of washout curve from which *B* was constructed. Ordinate, the amount of tracer remaining in the muscle; abscissa, time in min after transfer of the tissue from active to inactive solution. $3{\cdot}1 \times 10^4$ counts/min correspond to 1 μ mole K. Semilog scale.

B. Ordinate, rate coefficient for ^{42}K efflux, defined as $\Delta C/\Delta t . C_m$, where ΔC is the amount of radioactivity lost during the period Δt, and C_m is the mean of the ^{42}K contents of the muscle at times t and $t+\Delta t$. Abscissa, as in *A*. The points are drawn to correspond to midway in each collection period.

following an approximately exponential time course. This phase can be assumed to correspond mainly to exchange of ions across the cell membrane.

An experiment to test the effect of noradrenaline on the second phase

of ^{42}K loss is illustrated in Fig. 1. It is seen that the rate of efflux becomes considerably greater, as would be expected were noradrenaline to have increased the permeability of the membrane to K ions. This effect was consistently observed.

Two procedures were used to express the magnitude of the change in efflux. In the first, the rate of loss during the first period of drug application was divided by the corresponding value for the immediately preceding, pre-drug, period. In ten experiments of the type illustrated in Fig. 1 the mean value of this ratio came to $1{\cdot}66 \pm 0{\cdot}10$ (S.E. of mean). A second measure, which had the advantage that it allowed the effects of noradrenaline on inward and outward movement of ^{42}K to be compared, was obtained by dividing the amount of tracer lost from the tissue during the period of exposure to noradrenaline by the amount, as indicated by extrapolation, which would have been lost in the same period had the drug not been applied (cf. Durbin & Jenkinson, 1961). The average value of this ratio, termed R_e, was found to be $1{\cdot}31 \pm 0{\cdot}08$ (S.E. of mean) in the ten experiments.

Since there is mounting evidence, as discussed in the following paper (Jenkinson & Morton, 1967), that ganglion cells associated with smooth muscle also possess adrenergic receptors, it was necessary to consider whether the increase in ^{42}K exchange could have been a consequence of an action of noradrenaline on nerve rather than on muscle. This seemed unlikely, in view of the magnitude of the observed change in efflux, and the possibility was excluded by the finding that the effect of noradrenaline on the rate of loss of ^{42}K was unimpaired in preparations which had been dissected free of the myenteric plexus (which contains the bulk of the ganglion cells) in the manner described by Burnstock *et al.* (1966).

Effect of noradrenaline on the uptake of ^{42}K

A second series of experiments was made to test whether noradrenaline increased the inward movement of ^{42}K, as would also be expected if the permeability to K ions became larger. This was examined by exposing two preparations to separate loading solutions, one of which contained noradrenaline (see Methods). The subsequent procedure is illustrated in Fig. 2.

It may be seen that the noradrenaline-treated muscle had taken up a greater amount of ^{42}K. The magnitude of the increase was expressed by dividing the tracer content of the drug-treated muscles at the end of the load period by the corresponding figure for the controls. The average value for this ratio, R_u, was $1{\cdot}31 \pm 0{\cdot}11$ (S.E.: 8 expts.). This is significantly greater than unity ($0{\cdot}05 > P > 0{\cdot}02$), showing that the inward movement of K was also increased by noradrenaline.

Since the tissues were depolarized throughout each experiment, an increase in membrane permeability should affect the inward and outward movement of K ions to an equal extent; the values obtained for R_e and R_u should then be similar (Durbin & Jenkinson, 1961). It is therefore satisfactory that the observed figures were in such close agreement, although further experiments would be needed to establish the point with greater precision, in view of the relatively large scatter of our results.

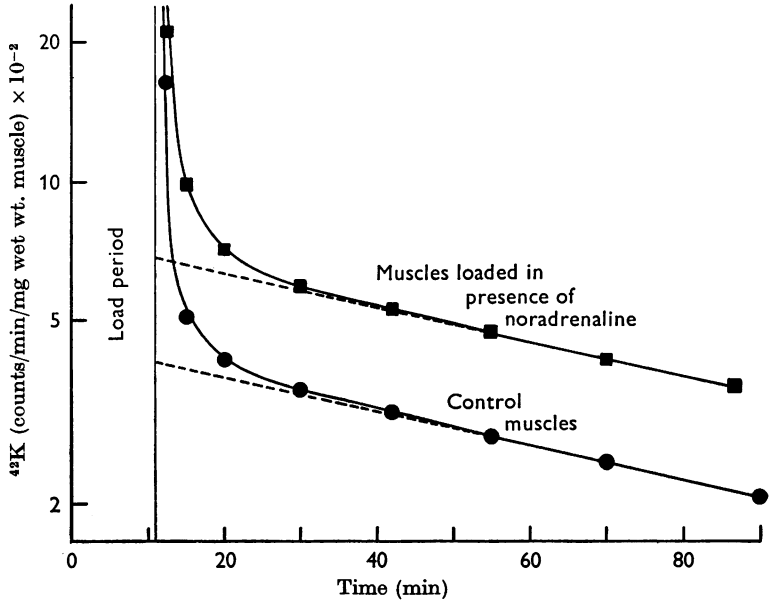

Fig. 2. The effect of noradrenaline on ^{42}K uptake. Two preparations from the same guinea-pig were loaded in separate solutions, identical except that one contained noradrenaline (3×10^{-7} g/ml.). They were then returned to inactive solutions, free of noradrenaline, and the loss of radioactivity was followed. Extrapolation of the linear parts of the curves, corresponding to loss of tracer from the cells, to the time of removal of the preparations from the load solutions allows the amounts of ^{42}K taken up by the tissues to be compared. $3{\cdot}8 \times 10^4$ counts/min correspond to 1 μmole K. Semilog scale.

Effect of noradrenaline on exchange of ^{36}Cl

Figure 3 illustrates the combined results of five experiments to determine the effect of noradrenaline on the efflux of ^{36}Cl from taenia bathed in K-rich solution. The results of ten similar experiments with ^{42}K have been included for comparison.

The effect of noradrenaline on ^{36}Cl uptake under these conditions was examined in a further four experiments which provided a mean value for R_u of $1{\cdot}11 \pm 0{\cdot}17$ (S.E.).

Taken together, these results suggest that any effect of noradrenaline on Cl exchange is small in comparison to that on the movement of K.

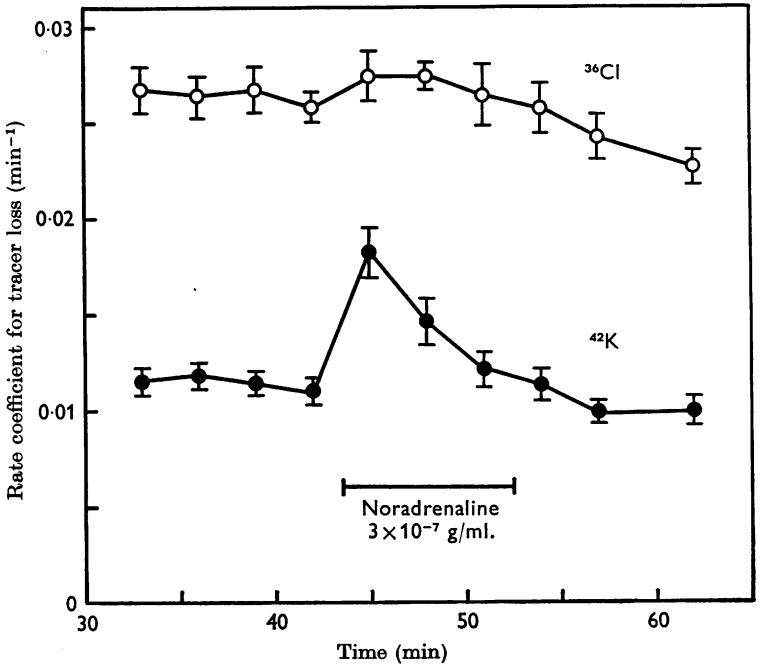

Fig. 3. The effects of noradrenaline on the rate of loss of ^{42}K (●) and ^{36}Cl (○). Each point represents the mean value (±S.E.) of the rate coefficients observed in the corresponding periods in a number of experiments (five with ^{36}Cl, ten with ^{42}K) of the type illustrated in Fig. 1.

Effect of noradrenaline and carbachol on the uptake of Na ions

Further experiments were made to determine whether the effect of noradrenaline on membrane permeability extended to Na ions. As noted by earlier workers, the study of Na flux in the taenia is complicated by the rapidity of exchange not only in Krebs solution at 35° C (Goodford & Hermansen, 1961; Nagasawa, 1963) but also in K-rich solution at room temperature (Durbin & Jenkinson, 1961). Under the latter conditions, the tracer content of muscles loaded with ^{24}Na falls to less than 5% of the initial value after only 1 hr in inactive solution. Also, in contrast to the time courses observed with ^{42}K and ^{36}Cl, it is difficult to distinguish a phase in the loss of ^{24}Na which might reasonably be assumed to correspond to exchange of the Na content of the cells.

The experimental conditions were changed in two respects in order to overcome these difficulties. First, the temperature was lowered to 10° C,

to slow the rate of Na exchange. Second, the Na content of the bathing fluid was raised from 6 to 51 mM, with the aim of increasing the proportion of Na ions within the cells. Details of this modified solution are given in Methods. It may be noted that the concentration of K, although less than that in the preceding experiments, was still sufficiently high (190 mM) to depolarize the muscle fibres almost completely (Burnstock & Straub, 1958; Kuriyama, 1963*a*).

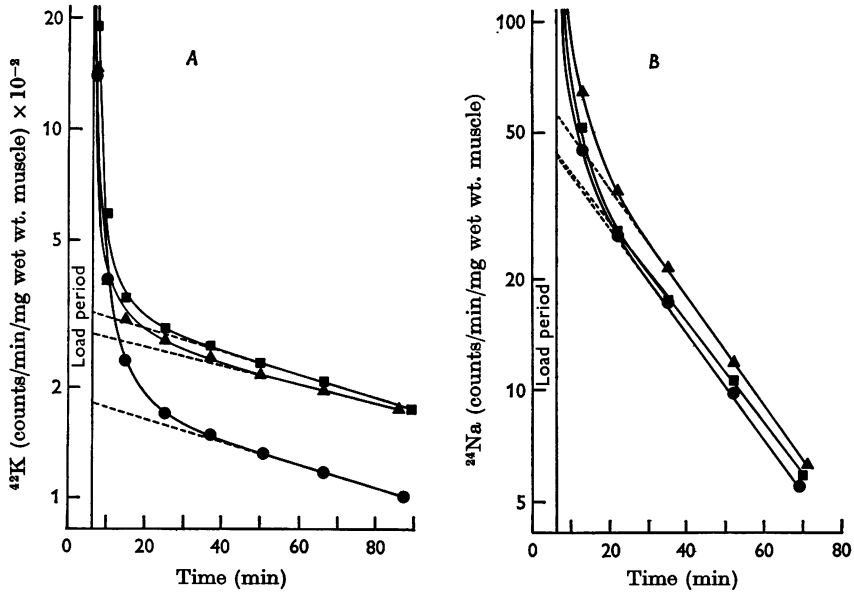

Fig. 4. Comparison of the effects of carbachol (5×10^{-7} g/ml.) and noradrenaline (3×10^{-7} g/ml.) on the uptake of ^{42}K (*A*), and ^{24}Na (*B*), by taeniae bathed in a solution containing 190 mM-K and 51 mM-Na, at 10° C. Procedure as in experiment of Fig. 2, except that the exposure to drug was reduced from 9 to 4·5 min, and the total period in radioactive solution from 11 to 6 min.

Symbols: carbachol (▲) noradrenaline (■) and control (●). $4{\cdot}6 \times 10^4$ counts/min correspond to 1 μmole K in (*A*), and $5{\cdot}1 \times 10^4$ counts/min correspond to 1 μmole Na in (*B*). Plotted as in Fig. 2.

It was found that under these conditions the efflux of ^{24}Na occurred in two distinct phases, as with ^{42}K and ^{36}Cl. The first was again complete within a few minutes. After this time, the loss of ^{24}Na became much slower, with a half-time of $23{\cdot}3 \pm 2{\cdot}6$ min (5 expts.). It thus became possible to test the effect of noradrenaline on ^{24}Na uptake by applying the procedure found suitable in earlier experiments with ^{42}K.

An important assumption in this method is that the slow phase of efflux corresponds to exchange of the ^{24}Na content of the cells. This seemed

reasonable, by analogy with the interpretation of the closely similar curves observed with labelled K and Cl, and the procedure was validated by showing that a substance considered to increase Na permeability did in fact enhance the uptake of ^{24}Na, as estimated by the present method. Carbachol was chosen for this purpose since under physiological conditions it causes depolarization, presumably by increasing the Na permeability of the membrane. Also, earlier work had already provided evidence that the uptake of ^{24}Na (as well as of certain other ions) by the depolarized taenia becomes greater in the presence of carbachol applied at room temperature (Durbin & Jenkinson, 1961).

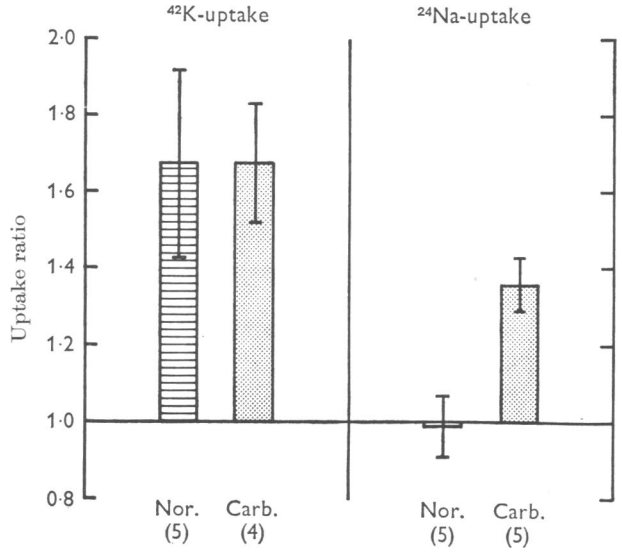

Fig. 5. Combined results of experiments of the type illustrated in Fig. 4. The heights of the rectangles represent the mean values of the ratio of the amount of tracer taken up by a drug-treated muscle to that by the control preparation in each experiment. The vertical bars indicate the S.E. about the mean, and the number of determinations is given below each rectangle.

We have therefore determined the effects of noradrenaline and carbachol on the uptake of ^{24}Na under the modified conditions described above. To increase the comparability of the results, tissues from the same animal were used in each experiment, and further, the drugs were applied at concentrations which were equally active in increasing the rate of exchange of ^{42}K. This was established in preliminary experiments which showed that noradrenaline (3×10^{-7} g/ml.) and carbachol (5×10^{-7} g/ml.) caused similar changes in ^{42}K efflux. The effects of these concentrations on the uptake of ^{42}K were then compared in experiments of the type illustrated in Fig. 4*A*. Having confirmed that the increases in K uptake were

of the same order, similar measurements were made with ^{24}Na. One of these is illustrated in Fig. 4*B*, and the results of five such experiments are combined in Fig. 5. It is seen that whereas carbachol significantly increases the uptake of ^{24}Na ($R_u = 1{\cdot}36 \pm 0{\cdot}07$), noradrenaline was without effect ($R_u = 0{\cdot}99 \pm 0{\cdot}08$).

The effects of similar concentrations of noradrenaline and carbachol on the efflux of ^{24}Na have not yet been determined.

DISCUSSION

The increase in the rate of exchange of ^{42}K caused by noradrenaline is most readily explained as a consequence of an increase in the K permeability of the muscle cell membrane. Before discussing the implications of such a permeability change, it is necessary to consider whether the observed effects might not be accounted for in other ways. Thus it could be suggested that the increase in K exchange results from an action of noradrenaline in accelerating active transport of K ions. This possibility is important since effects of catecholamines on the activity of enzymes controlling the production of energy-rich compounds have been described for many tissues, including cardiac, smooth and skeletal muscle (e.g. Ellis, 1956, 1959). However, although an action of this kind could perhaps explain the increase in the influx of ^{42}K, since the active transport of K ions is in the inward direction, the effect on efflux could then be accounted for only by assuming in addition either that the efflux of K ion is also dependent on energy supply, or that the increase in K influx had in some way caused an immediate change of similar magnitude in the rate of loss. Neither possibility seems likely, there being little evidence to suggest such intimate coupling between inward and outward movements of K.

Also, it is shown in the following paper (Jenkinson & Morton, 1967) that the effect of noradrenaline on K exchange is mediated through the type of adrenergic receptor defined as α on Ahlquist's classification (1948), the β-receptors being inactive in this respect. However, it is known that the β-receptors alone are involved in the metabolic actions of catecholamines on cardiac (Mayer & Moran, 1960; Kennedy & Ellis, 1963) and skeletal (Ali, Antonio & Haugaard, 1964; Hornbrook & Brody, 1963) muscle. Further, it has recently been found that the action of adrenaline in activating phosphorylase in the rat uterus and in the taenia of the guinea-pig is also mediated by β-receptors (Brody & Diamond, 1966; Diamond & Brody, 1965, 1966). Our finding that α- rather than β-receptors are concerned in the effect of noradrenaline on K exchange thus supports the conclusion that this response is not a consequence of a change in cellular metabolism.

It was invariably found that the increase in K flux caused by noradrenaline declined with time despite the continued presence of the drug in the bathing fluid (e.g. Figs. 1, 3). A similar spontaneous fall in the effect of carbachol on the rate of loss of ^{42}K had been seen in earlier work (cf. Figs. 3 and 5 in Durbin & Jenkinson, 1961). No explanation for this phenomenon can yet be given, although it seems possible that it may be the result of receptor desensitization as described by Thesleff (1955) and analysed by Katz & Thesleff (1957).

Our results thus suggest that noradrenaline, and presumably therefore also adrenaline, increase the permeability of depolarized smooth muscle cells to K, but not, at least to any marked extent, to Na or to Cl. Under physiological conditions such a permeability change would be expected to hyperpolarize the membrane, inhibit the initiation and propagation of action potentials, and reduce the electrical excitability of the tissue. These effects have repeatedly been observed in intestinal muscle treated with catecholamines (e.g. Bozler, 1940; Bülbring, 1954; Burnstock, 1958).

Some other phenomena which can be explained by this effect on K permeability are as follows. (1) If the tissue is bathed in a solution without Na, but containing the normal concentration of K, the membrane potential will tend to approach the equilibrium potential for K ions, and so would be expected to change only slightly following an increase in K permeability. In fact, little change in potential is observed in response to adrenaline applied under these conditions (Bülbring & Kuriyama, 1963). (2) As a consequence of an increase in K permeability, the relation between resting potential and external K concentration should approach more closely to that predicted by the Nernst equation. An effect of this kind has also been observed in taeniae exposed to adrenaline (Kuriyama, 1963*a*).

However, it may be noted that these two findings can also be explained by postulating that adrenaline reduces the Na permeability of the membrane, as suggested by Bülbring & Kuriyama (1963), and by Kuriyama (1963*a*). These authors (see also Bueding & Bülbring, 1964) have proposed that catecholamines exert this action by increasing the concentration of Ca ions at a membrane site controlling Na permeability. The suggestion has also been made that the hyperpolarization caused by adrenaline is due to an increase in the activity of an electrogenic Na pump (Burnstock, 1958, Axelsson, Bueding & Bülbring, 1961; Bülbring, 1960, 1962). The relative contribution of these mechanisms remains to be determined, and it is clear that a complete account of the actions of catecholamines will not be possible until more is known of the properties of smooth muscle. Nevertheless, the hypothesis that noradrenaline and adrenaline cause an increase in K permeability would appear to provide a satisfactory explanation of many of the electrophysiological changes observed in response to these drugs.

It may be noted that this hypothesis, in common with others outlined above, clearly cannot account for the tension changes which have been found to occur when catecholamines are applied to smooth muscles which have been completely depolarized (Evans *et al.* 1958; Edman & Schild 1962, 1963). Further experiments (Jenkinson & Morton, 1967) showed that different receptors are involved in this action and in the effect on K permeability examined in the present work.

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