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RATE OF ANTAGONISM OF TUBOCURARINE BY POTASSIUM IONS

BY

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The rate at which potassium ions antagonized the neuromuscular block produced by tubocurarine has been examined in isolated rat diaphragm muscle preparations. The half-time was dependent on the thickness of the muscle. In thick muscles (550 to 650 μ) the rate of action could be largely accounted for by the time which the potassium took to diffuse through the interstitial fluid to produce an increase in concentration in the immediate environment of the muscle fibre.

It is well known that addition of potassium antagonizes the blocking action of tubocurarine in skeletal muscle (Wilson & Wright, 1936; Quilliam & Taylor, 1947). When this is studied in isolated muscle, the process takes a measurable time for completion, and it was of interest to determine how far diffusion of potassium through the interstitial spaces to its site of action could account for the time relations.

METHODS

The solution bathing the diaphragm was similar to that used by Creese (1954) except that the potassium content was reduced to 1.5 mM. The fluid was bubbled with a mixture of 5% carbon dioxide and 95% oxygen, and the temperature of the bath was maintained at 38° C.

The left hemi-diaphragm from an albino rat weighing 100 to 150 g was removed, attached to a holder and immersed in the solution in such a manner that single maximal shocks could be delivered alternately to the nerve and muscle, using the procedure described by Holmes, Jenden & Taylor (1951) except that condenser shocks of 0.02 μF were used for the nerve and 2 μF for the muscle. The responses were recorded on smoked paper using a spring-loaded lever. Stimulation was continued throughout the experiments, and the magnitude of the responses obtained by direct and indirect stimulation was very similar. As direct stimulation was almost unaffected by tubocurarine, an estimate of the degree of block could be made at any time during the experiment.

The muscle was later removed from the bath, outlined on squared paper and weighed. The thickness was calculated, taking the specific gravity as 1.055 (Creese, 1954) and expressing the result to the nearest 5 μ.

RESULTS

The height of contraction of the muscle after immersion in the solution containing 1.5 mM potassium showed an initial decline. The solution was changed repeatedly until the height of contraction became steady, usually after 2 hr.

Sufficient tubocurarine was then added to produce a steady partial neuromuscular block. In the experiment illustrated in Fig. 1, 20 μg of tubocurarine had been added to the bath containing 40 ml. solution. The contractions which followed stimulation of the nerve became steady 2 hr after addition of the tubocurarine at a contraction height of 5% of the control obtained by direct stimulation of the muscle. At zero time, 1 ml. of a solution containing potassium chloride was added beneath the meniscus, so that the potassium content of the bath was suddenly raised from 1.5 to 7.4 mM. The solution which was added was obtained by dissolving potassium

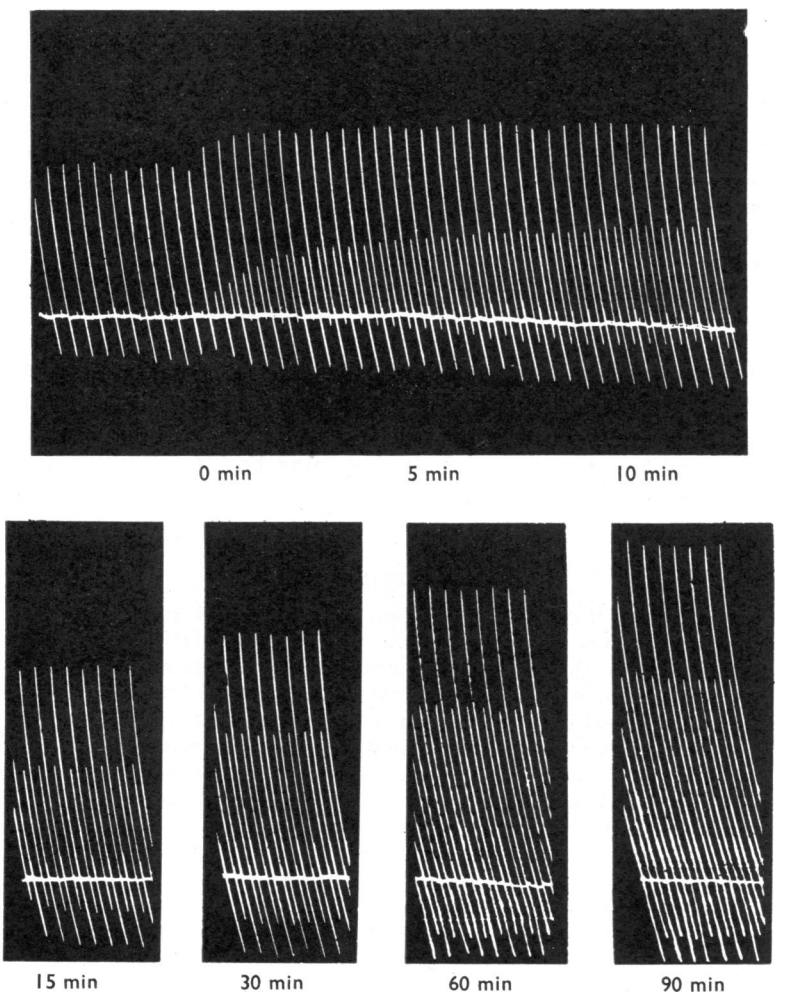

Fig. 1. Effect of potassium on the neuromuscular block produced by tubocurarine (0·5 μg/ml. administered 2 hr previously) in rat diaphragm. At zero time, the potassium concentration in the bath was increased from 1·5 to 7·4 mM. Bath volume, 40 ml. Temperature, 38° C. In this record stimulation was alternated to the muscle directly and to the phrenic nerve. The indirect responses, partially blocked by tubocurarine, are smaller than those in response to direct muscle stimulation.

chloride in some of the original solution which had been prepared ; it also contained tubocurarine (0.5 μg/ml.) and was warmed to 38° C before being added to the bath.

After addition of the potassium, the height of contraction produced by direct stimulation was slightly increased. There then followed a slow rise in the response to direct stimulation, taking about 1.5 hr to reach a maximum. The response to indirect stimulation also increased, rapidly during the first few min but slower later, reaching a maximum in 1.5 hr.

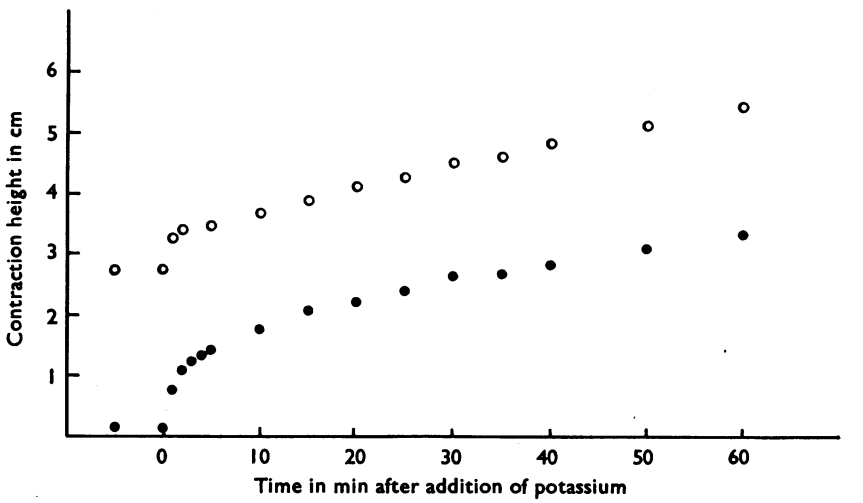

Fig. 2. Contraction heights plotted from tracing of Fig. 1. ○, Direct stimulation; ●, nerve stimulation. The abscissa gives the time after the increase of potassium concentration in the bath from 1·5 to 7·4 mM.

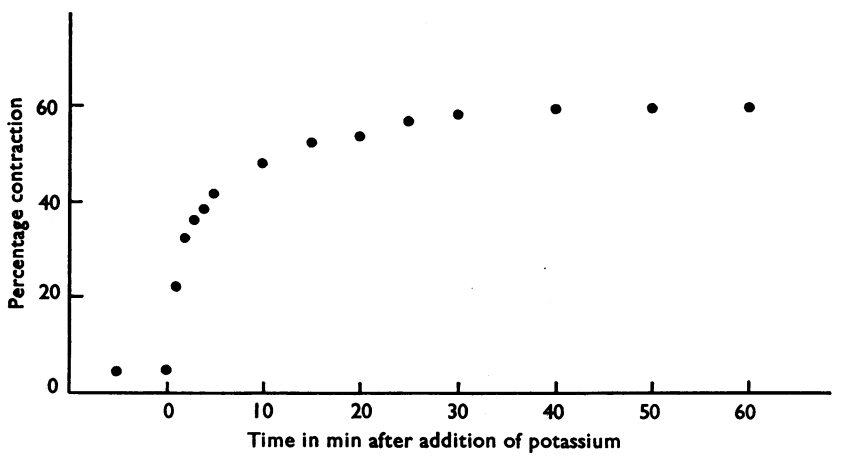

Fig. 3. Contraction height due to nerve stimulation expressed as a percentage of response obtained by direct stimulation of the muscle, from Figs. 1 and 2. For further explanation, see text. Abscissa gives time after the increase of potassium concentration in the bath from 1·5 to 7·4 mM.

In Fig. 2 the heights of contraction have been plotted during the first 60 min. If the height of the indirect response (Fig. 2, solid circles) is expressed as a percentage of the height of the direct muscle response (Fig. 2, open circles) at the time under consideration, a value for percentage contraction is obtained. Fig. 3 relates percentage contraction in response to nerve stimulation to time in the experiment illustrated in Fig. 1, and shows the rapid initial increase in contraction, followed by a slower

TABLE 1

OBSERVED HALF-TIMES SEEN WITH 7·4 mM POTASSIUM IN THE BLOCK BY TUBOCURARINE IN RAT DIAPHRAGM MUSCLE

Estimate of muscle thickness (μ)	Observed half-time (sec)
460	33
460	40
490	28
500	44
540	118
580	78
600	136
600	122
625	163
640	148
640	165
650	150

rise to maximum effect after the potassium concentration of the fluid bathing the preparation was suddenly raised to 7.4 mM at zero time.

In Fig. 3 the contraction following nerve stimulation increased from 5% at 0 min to a maximum of 59% of the control after 40 min. Half the maximum recovery of twitch height was 32% ([5+59]/2) and was achieved in 118 sec. Table 1 gives

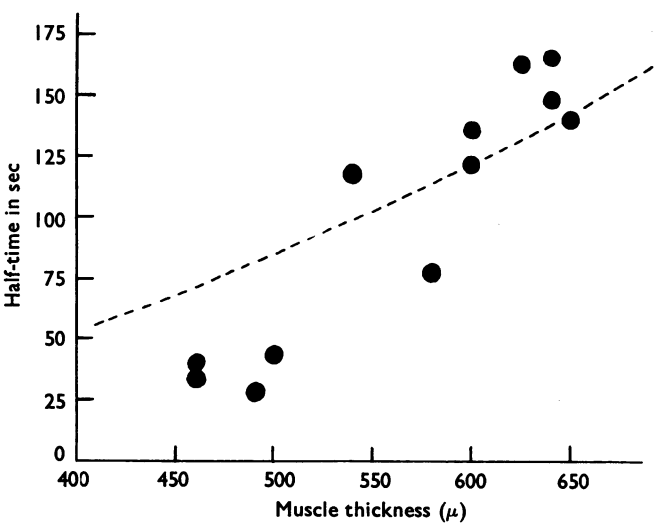

Fig. 4. The relation between calculated muscle thickness and the half-time required for the action of potassium on tubocurarine. The dotted line gives the time for the increase of potassium in the interspaces to reach half its final value, calculated on the basis described in the text.

the time of half-recovery in 12 experiments and the estimated thickness of the preparations. In Fig. 4 the results have been plotted to show the relation between the thickness of the muscle and the half-time for the action of potassium. It was found that the thickness of the muscle had a very marked effect on the time taken for potassium to antagonize tubocurarine.

DISCUSSION

The ratio of the height of contraction to indirect stimulation to that following direct stimulation was used as an indication of the fraction of fibres responding and hence the extent of the block. The increase of height of contraction to direct stimulation was assumed to be due to an increase in the force of contraction of each of the muscle fibres, rather than to an increase in the number of fibres responding because the directly applied shock was maximal. It was also assumed that the response plotted in Fig. 3 would be half-way to its final value when half the extra potassium had entered the tissue interspaces.

The time required for half the extra potassium to accumulate in the interstitial spaces may be calculated. It follows from Hill (1928) that for diffusion in the interspaces of a flat muscle Dt/d^2 is 0.196, where t is the half-time, d is the diffusion path and is half the thickness, and D is the relevant diffusion coefficient of potassium. Creese (1954) measured D', the apparent diffusion coefficient of potassium in diaphragm, and obtained the value of 5.2×10^{-6} cm^2 sec^{-1} by following the movements of ^{42}[K] into a solution containing high potassium (118 mM). In the experiment shown in Fig 1, some potassium would enter the fibres as well as the interspaces. It has been shown by Csapo & Wilkie (1956) that, under such conditions, the effective diffusion coefficient for potassium accumulation in the interspaces would be aD', where a is the fraction by volume of the interspaces. This fraction is 0.28 for diaphragm muscle (Creese, 1954). By applying these findings, theoretical half-times have been calculated for various thicknesses of the rat diaphragm muscle and are expressed by the dotted line in Fig. 4. In this procedure the muscle is treated as a slab of relatively large area. The experimental points fell near the calculated line with thick muscles (550 to 650 μ), but fell below with muscles thinner than 500 μ. Thus the unblocking action of potassium ions proceeds more rapidly in the case of thin muscles than can be accounted for by the simple theory.

Csapo & Wilkie (1956) studied the rate at which high concentrations of potassium ions produced reversible inexcitability in frog muscle, and concluded that interfibre diffusion was responsible. In their studies the concentration of potassium in the interspaces had to reach a critical value (9 mM) before a fibre was affected. In the experiment shown in Fig. 1, there was a critical concentration of tubocurarine present, so that any increase in the potassium would diminish the neuromuscular block, and hence the kinetics would be different. In other respects, however, the same assumptions have been made, and in the calculations it has been considered that the interaction between tubocurarine and potassium is rapid for any one fibre. If potassium acts by depolarization, then this assumption is an approximation, for Hodgkin & Horowicz (1959) have shown that an increase of potassium ion concentration with constant chloride (which resembles the experiment of Fig. 1) produces

immediate depolarization in the case of a single frog muscle fibre followed by a further slow and small depolarization so that the membrane potential eventually reaches the equilibrium value. This may explain the long time needed to produce a new steady neuromuscular block (Fig. 3). It would also be expected that the measured half-time would be somewhat greater than the calculated values, and the results of Fig. 4 for thick muscles (over 600 μ) are consistent with this.

In the case of thin muscle (below 500 μ) the potassium acted more rapidly than the simple theory predicted. Treatment in terms of a slab appeared to be no longer valid in the case of these small muscles with a relatively large surface-mass ratio. It was not possible to extend these studies over the range used by Conway & Fitzgerald (1942), who used diaphragm muscles up to 1 mm thick, because of the probability of anoxia occurring in the depths of the tissue (Creese, Scholes & Whalen, 1958) and consequent depolarization and potassium changes.

The potassium ions may exert their anti-curare action by depolarization, but other interactions or displacements of tubocurarine by potassium ions are not excluded by these experiments. Liley (1956) has found evidence that the liberation of transmitter may be increased by potassium. There is a resemblance between the factors governing the rate of action of tubocurarine and those governing the rate of action of potassium ions. It has already been pointed out (Holmes, Jenden & Taylor, 1951) that the site of action of tubocurarine is superficial; it probably does not penetrate any membranes which require an activation energy for the process, and the rate of action of tubocurarine can be accounted for largely by the time the drug takes to diffuse into the interfibre spaces (Creese, Taylor & Tilton, 1959).

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REFERENCES

CONWAY, E. J. & FITZGERALD, O. (1942). Diffusion relations of urea, inulin and chloride in some mammalian tissues. *J. Physiol.* (*Lond.*), **101**, 86–105.

CREESE, R. (1954). Measurement of cation fluxes in rat diaphragm. *Proc. roy. Soc. B.*, **142**, 497–513.

CREESE, R., SCHOLES, N. W. & WHALEN, W. J. (1958). Resting potentials of diaphragm muscle after prolonged anoxia. *J. Physiol.* (*Lond.*), **140**, 301–317.

CREESE, R., TAYLOR, D. B. & TILTON, B. (1959). The effect of curare on the uptake and release of a depolarizing agent labelled with ^{131}[I]. In *Curare and Curare-like Agents*, ed. BOVET, D., p. 386. Amsterdam: Elsevier.

CSAPO, A. & WILKIE, D. R. (1956). The dynamics of the effect of potassium on frog's muscle. *J. Physiol.* (*Lond.*), **134**, 497–514.

HILL, A. V. (1928). The diffusion of oxygen and lactic acid through tissues. *Proc. roy. Soc. B.*, **104**, 39–96.

HODGKIN, A. L. & HOROWICZ, P. (1959). The influence of potassium and chloride ions on the membrane potential of single muscle fibres. *J. Physiol.* (*Lond.*), **148**, 127–160.

HOLMES, P. E. B., JENDEN, D. J. & TAYLOR, D. B. (1951). The analysis of the mode of action of curare on neuromuscular transmission; the effect of temperature changes. *J. Pharmacol. exp. Ther.*, **103**, 382–402.

LILEY, A. W. (1956). The effects of presynaptic polarization on the spontaneous activity at the mammalian neuromuscular junction. *J. Physiol.* (*Lond.*), **134**, 427–443.

QUILLIAM, J. P. & TAYLOR, D. B. (1947). Antagonism between curare and the potassium ion. *Nature* (*Lond.*), **160**, 603.

WILSON, A. T. & WRIGHT, S. (1936). Anti-curare action of potassium and other substances. *Quart. J. exp. Physiol.*, **26**, 127–139.