THE EFFECT OF BLOCKING ELECTRICAL ACTIVITY ON THE EFFLUX OF POTASSIUM FROM ELECTROPLAX

R. WHITTAM* AND M. GUINNEBAULT**

Departments of Neurology and Biochemistry, College of Physicians and Surgeons, Columbia University, New York, N.Y. (U.S.A.)

(Received March 28th, 1960)

SUMMARY

The effect of various factors which block electrical activity have been tested on the efflux of K from the electroplax of *Electrophorus electricus*.

1. Increase of K concentration from 5 m*M* to 110 m*M* in the Ringer's solution bathing the innervated site of the electroplax, with corresponding decrease in Na concentration, caused an immediate increase of the K efflux. Surprisingly, this increased rate was maintained for a few minutes only and then returned to about the original level. When the K concentration in the outside fluid was raised from 110 to 165 m*M*, no further change of the rate of K efflux was observed.

2. Addition of *d*-tubocurarine in high concentrations (1 mg/ml) did not affect the efflux, thus suggesting that the increased efflux with a high external K concentration must be attributed to an increased flow of K ions across the conducting membrane.

3. When the Ringer K concentration was increased to 110 or 165 m*M*, without corresponding removal of Na, the effects observed with this hypertonic solution were essentially the same.

4. Exposure of the electroplax to either carbamylcholine or to pyridine aldoxime dodeciodide, both known to block electrical activity and to depolarize the conducting membrane, as does high K concentration, affected the rate of efflux of K in essentially the same way as exposure to high K concentration.

5. In contrast, replacement of external Na by either sucrose or choline, in which case activity is blocked without depolarization, did not affect the rate of potassium efflux.

INTRODUCTION

It has previously been shown that during activity, an electroplax from the electric eel, *Electrophorus electricus*, loses 5–8 $\mu\mu$moles of K/cm^2/impulse[1]. This is the same order of magnitude as the K loss from cephalopod and crustacean giant axons[2,3] and suggests the basic similarity of the bioelectric currents propagating the action

Abbreviation: PAD, pyridine aldoxime dodeciodide.

* Present address: Department of Biochemistry, University of Oxford (England).

** Present address: Service de Biologie, Centre d'Etudes Nucleaires Saclay (S. – & –O.) (France).

potential in membranes of excitable cells from different species. It appeared pertinent to test this conclusion further by studying how various factors which block electrical activity affect the efflux of K. The electroplax preparation possesses certain features discussed by SCHOFFENIELS[4] which make it possible to obtain more information about the mode of action of certain compounds for which other preparations are unsuitable.

The response to direct and indirect stimulation in electroplax can be abolished by replacing the sodium in a saline medium with choline, sucrose or glucose, by increasing the external K concentration and by a number of drugs. By analogy with muscle and nerve, a small amount of Na probably enters the electroplax during stimulation, and its absence in the medium would prevent the inward Na current and the evanescent depolarization during the spike. The block of activity with sufficiently high external K concentrations may be explained by a depolarization of the membrane. The indirect response only is abolished by curare[5,6], which does not depolarize and does not affect the efflux of K from the innervated membranes of resting electroplax[1]. Carbamylcholine, a depolarizing agent, blocks the response to both indirect and direct stimulation although the latter effect is not produced by a direct action upon the conducting membrane but by an action upon the synaptic region[6,7]. A block of both responses is also obtained with lipid soluble quaternary nitrogen compounds such as dodecyl pyridinium chloride and PAD[8].

Several ways are therefore available to block conduction. Since electrical activity of the membrane is accompanied by changes in the efflux of K, it might be expected that a block of activity in various ways would produce changes in efflux characteristic of the mode of blocking which might be of help in the determining of the mechanism of action of drugs. This paper describes the effect of various methods of blocking conduction on the efflux of K from the innervated membranes of electroplax from the organ of Sachs of *Electrophorus electricus* L. The most interesting and unexpected finding is that block with depolarizing agents, like high external K or carbamylcholine or PAD, produces a transient increase in the efflux of K, suggesting that the membrane permeability does not reach a new constant level after depolarization.

METHODS

These were the same as those described in a previous paper[1]. Na free solutions were prepared by replacing Na_2HPO_4 and NaH_2PO_4 with K phosphates, and NaCl with the osmotically equivalent amounts of either sucrose, choline chloride, KCl or K_2SO_4. The pH was 7.2. When chemicals were added to the Ringer's solution, the pH was re-adjusted, if necessary, to 7.2 with either 0.1 *N* NaOH or 0.1 *N* HCl.

RESULTS

Effects of high external potassium concentration

Reversibility of the block of conduction: A single electroplax separating two pools of Ringer's solution in the apparatus developed by SCHOFFENIELS[4,6,9] was stimulated directly and the amplitude of the spike was about 60 mV (Fig. 1). To find the effect of an elevated K concentration on the amplitude of the spike, the fluid washing the innervated membrane was replaced with Ringer's solution in which sodium had been

replaced with potassium to give concentrations of 25, 45 and 80 m*M* K. Fig. 1 shows that the height of the spike was reduced by the raised K concentrations. It fell only about 5 mV with 25 m*M* K and on washing again with Ringer's solution, the spike immediately returned to its initial level. With 45 m*M* K the height of the spike was reduced by 50 % and on washing with Ringer's solution, it again recovered completely within about 3 min. 80 m*M* K caused a block of conduction and on washing with Ringer's solution afterwards, the height of the spike slowly increased until after 20 min it was 85 % of its initial level. Both the direct and indirect responses were also recovered after their abolition with 165 m*M* K, irrespective of whether NaCl was replaced by KCl (Fig. 2) or K_2SO_4 (Fig. 3). The recovery in the height of the spike was slower (40 min) and not so complete (75 %) in the presence of sulfate compared with the 85 % recovery in 20 min in the presence of chloride. Similar results were obtained when both sides of the electroplax were washed with high K Ringer's solution and show that the block of conduction was reversed when the electroplax was washed afterwards with Ringer's solution.

The efflux of K into isotonic solutions: To find the effect of the block of conduction by K on the efflux of K across the innervated membrane, electroplax previously soaked for 2–3 h in oxygenated labeled Ringer's solution were mounted in the apparatus with a pool of ^{42}K labeled Ringer's solution on the non-innervated side and a stream of inactive Ringer's fluid on the innervated side. The washing fluid was counted for ^{42}K until a constant rate was obtained; then it was replaced by a high K solution. The K concentrations chosen, of 110 and 165 m*M*, were obtained by a replacement of sodium of the saline with potassium. Fig. 4 shows that washing the innervated membrane with Ringer's solution containing 110 m*M* K caused an immediate increase in the loss of ^{42}K from the electroplax. Surprisingly, the high rate of loss of ^{42}K was not maintained for more than about 10 min, for after that time it fell slowly to the initial level. (The spike disappeared within about 30 sec after exposure to the high K.) To test whether a further transient increase in the loss of ^{42}K would occur on raising the external K concentration to 165 m*M*, the washing fluid was altered to contain 165 m*M* K and Fig. 4 shows that no further change in

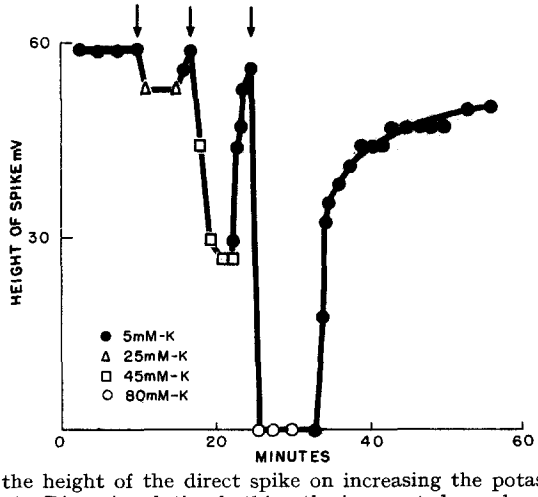

Fig. 1. Decrease in the height of the direct spike on increasing the potassium concentration in the Ringer's solution bathing the innervated membrane.

the efflux of K occurred. In 2 experiments the electroplax was bathed in high K Ringer's solution and subsequently washed with 5 m*M* K Ringer's solution until the electrical activity returned. The efflux of K was allowed to fall to the initial level, after the transient increase, before the start of washing with Ringer's solution. No further change occurred as the electrical activity was recovered after about 30 min washing.

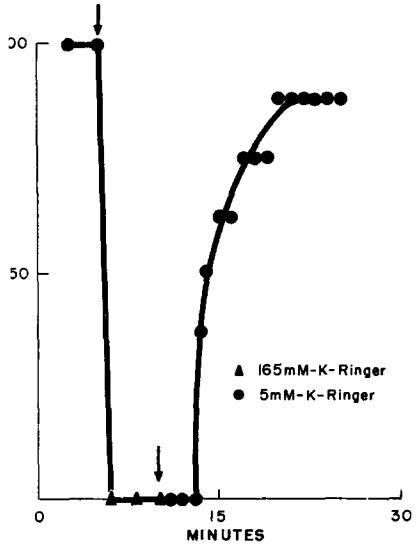

Fig. 2. Reversibility of the block of activity after bathing the innervated membrane with 165 m*M* K Ringer's solution.

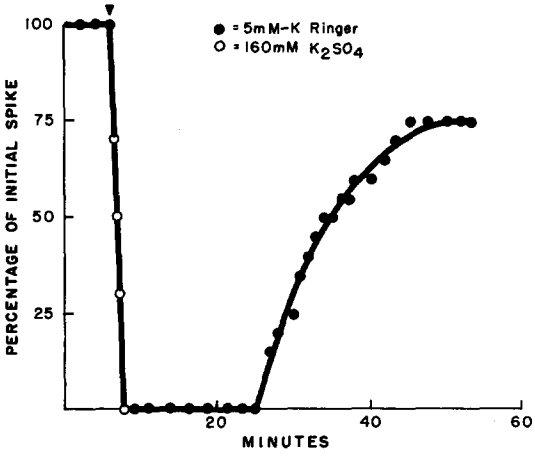

Fig. 3. The reversibility of the effect of 160 m*M* K_2SO_4 solution on the direct spike.

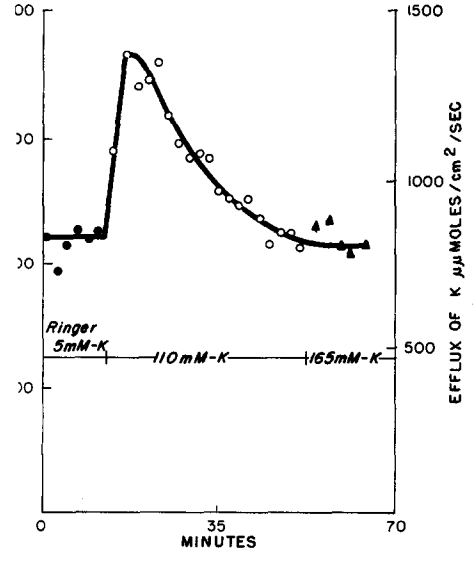

Fig. 4. The efflux of potassium from the innervated membrane of an electroplax on bathing with Ringer's solution in which sodium had been partially or completely replaced with potassium.

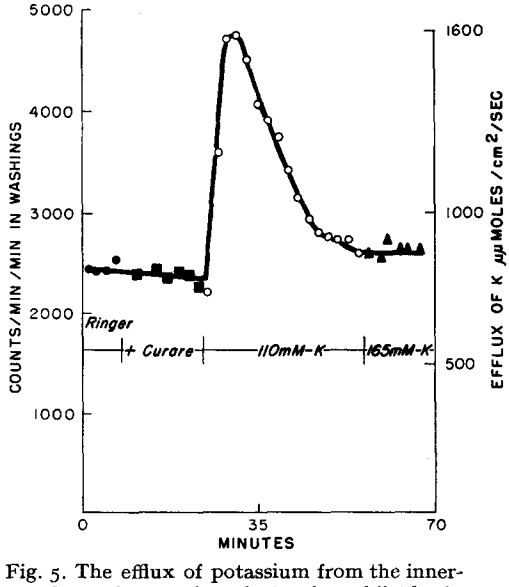

Fig. 5. The efflux of potassium from the innervated membrane of an electroplax whilst bathing it in the presence of curare and of high potassium concentrations.

Fig. 5 shows that the addition of *d*-tubocurarine (1 mg/ml) to the Ringer's solution bathing the innervated membrane had no significant effect on the efflux of K, although the indirect response was blocked, showing that nearly all the resting efflux is probably through the conducting membrane. This is not surprising since the area of the post-synaptic membrane at the synaptic junctions, in spite of their great number, forms only a small fraction, about 3–6 %, of the conducting membrane[10]. The previous abolition of the indirect spike with curare did not affect the transient increase in the loss of ^{42}K when the external K concentration was raised to 110 m*M*, and, as was found in the absence of curare, no further change in efflux occurred on increasing the K concentration from 110 to 165 m*M*.

Fig. 6 shows the results with 3 electroplax of increasing the external K concentration and, for the sake of comparison of cells with different resting fluxes, the ordinate is given as the percentage of the initial efflux of K. The graph illustrates the variability of the increase in efflux with high external K, for the increase may be for a short

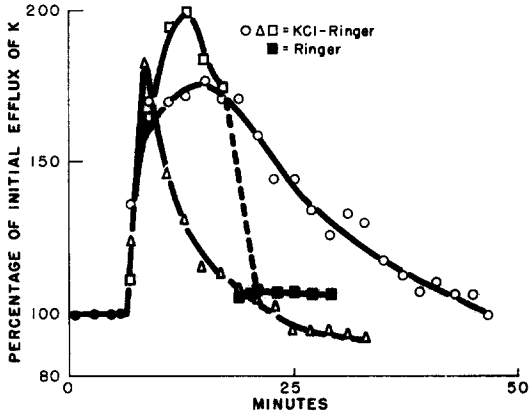

Fig. 6. The variability of the transient effect on the efflux of potassium of bathing the innervated membranes of electroplax with Ringer's solution containing a high potassium concentration.

period (2 min) in one cell, or more prolonged (12 min) in another. In each cell, however, the efflux returned to the initial level. In the curve of the cell with the biggest increase (□), the washing solution was changed to Ringer's solution before the initial level was reached again and the efflux seemed then to fall more quickly to the initial level than when the electroplax was washed with high K medium. No explanation is offered for the variability of the response.

Replacement of NaCl with K_2SO_4: Ringer's solutions in which NaCl is replaced with KCl probably allow K to enter the electroplax more freely, as well as causing an increase in efflux. It is unlikely that a net entry of K or of water occurred, however, because the highest external concentration (165 m*M*) was less than the internal concentration of about 175 mmoles/kg tissue water[11]. By replacing NaCl with K_2SO_4 instead of KCl it is to be expected that the same effects due to K itself would be found. Fig. 7 shows that this is so, for when the NaCl in Ringer's solution was replaced with 0.083 *M* K_2SO_4, the same transient increase in the efflux of K occurred as when NaCl was replaced with KCl.

Addition of KCl to Ringer's solution: To test the effect on the efflux of K of

maintaining the Na concentration in the Ringer's solution at 165 mM and of increasing the K concentration, hypertonic solutions were prepared by adding solid KCl to Ringer's solution to raise the K concentration to 110 or 165 mM K solutions. Fig. 8 shows a 110 % increase in the loss of ^{42}K from the electroplax when the direct spike was blocked with 110 mM K in a hypertonic medium and also the subsequent fall to the initial level. Washing with 165 mM K solution caused no further change in efflux from the initial level, after the pre-treatment with 110 mM K. This result is exactly the same as that found with isotonic high K solutions in which NaCl of the Ringer's solution was replaced with KCl.

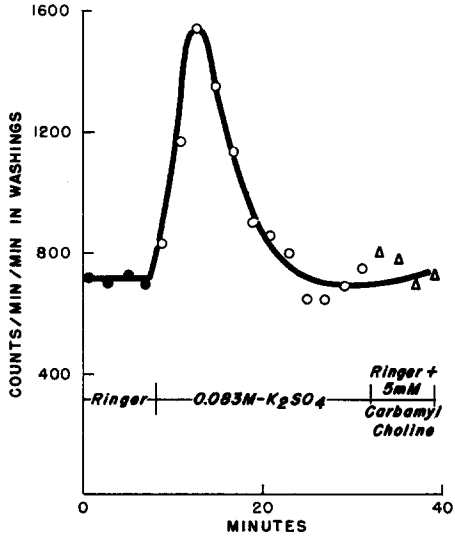

Fig. 7. The efflux of potassium from the innervated membrane of an electroplax bathed first with 0.083 M K_2SO_4 and then with Ringer's solution containing 5 mM carbamylcholine.

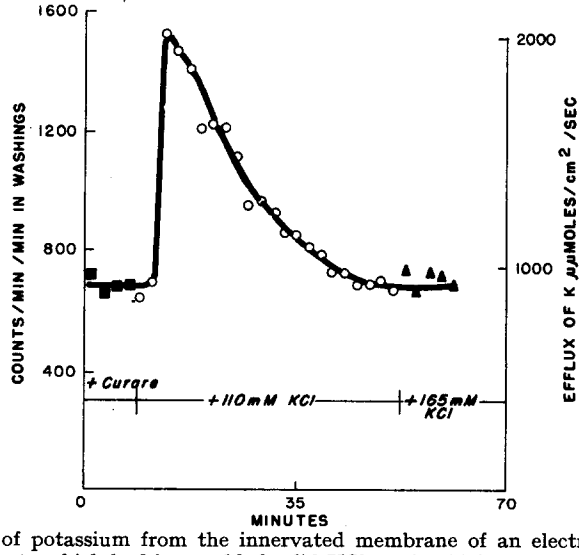

Fig. 8. The efflux of potassium from the innervated membrane of an electroplax bathed with Ringer's solution to which had been added solid KCl to give high potassium concentration.

Washing both membranes of the electroplax: The results already described in which high K Ringer's solution was added only to the innervated side could possibly be artifactual due to a net potential difference (p.d.) across the cell caused by a depolarization of only the innervated membrane which might cause a flow of current carried by K. (With high K on the innervated side only, a net p.d. would be expected across the cell since there would still be a p.d. across the non-innervated membrane

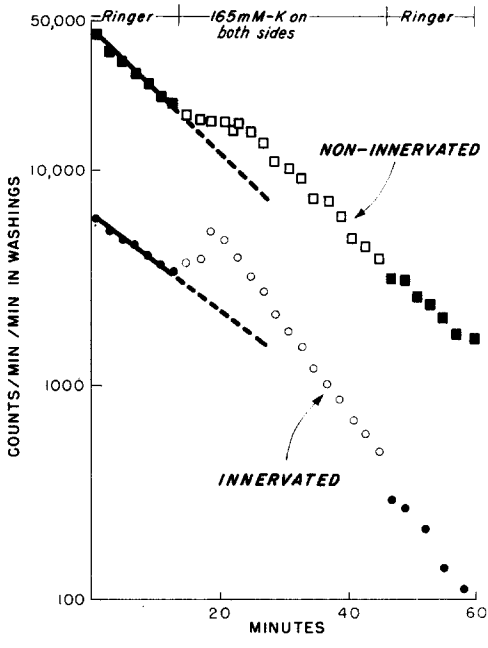

Fig. 9. The efflux of potassium from the two membranes of an electroplax on replacing Ringer's solution with 165 m*M* K-medium.

and the innervated membrane would be negative to the non-innervated.) To test whether the transient increase in the efflux of K across the innervated membrane might be due to some such cause, an electroplax was soaked for 3 h in labeled Ringer's solution and then washed on both sides, first with Ringer's solution, next with isotonic 165 m*M* K solution and finally again with Ringer's solution. The washings were counted for ^{42}K and the results are shown in Fig. 9. Initially, the rates of loss corresponded to losses of ^{42}K across the membranes by first order processes since straight lines are given in the semi-logarithmic plot. The loss from the non-innervated side was about tenfold that from the innervated. On replacing the Ringer's solution with 165 m*M* K solution, the loss of ^{42}K from the innervated membrane increased, and after about 6 min it was, for 4 min, about twice the rate it would have been with Ringer's solution. (The dotted line is a continuation of the linear slope with Ringer's solution.) However, the higher rate was not maintained and the rate fell to a level similar to that for Ringer's solution. Further washing with Ringer's solution again gave a linear semi-logarithmic rate of loss although the slope was a little steeper than during the first washing. In contrast to the marked rise and fall in the loss of ^{42}K found on the innervated side, no striking change was seen

in the washings from the non-innervated side. In 3 experiments made in this way the same effect was observed on the innervated side. In one experiment, the loss from the non-innervated membrane also showed a rise (middle section of upper curve in Fig. 9) although this was not so pronounced. The results therefore show that the same rise and fall in the loss of ^{42}K occurred from the innervated membrane when both sides were washed with 165 m*M* K as when only the innervated side was washed. The effect found when washing the innervated side alone is therefore unlikely to be an artifact and may be considered to be the typical way in which the efflux of potassium changes when the membrane is exposed to a high potassium concentration (110 or 165 m*M*) that blocks conduction.

Effect of carbamylcholine on potassium efflux

A high external K concentration depolarizes the membrane[12–14] and the same result is produced by carbamylcholine[6], although at a relatively slow rate with the concentrations usually used. In view of the transient character of the effect of high external K concentration on the efflux of K across the innervated membrane, it was clearly desirable to find whether the effect was due to K *per se*, or to its depolarizing action. An electroplax was therefore washed with Ringer's solution containing 5 m*M* carbamylcholine chloride, which was used in this high concentration in order to depolarize fairly quickly. The result (Fig. 10) shows that the drug blocked conduction and also caused a rapid rise in the loss of ^{42}K and, later, a slow fall approximately to the starting level. After the return to the resting level, the washing solution was changed to isotonic 165 m*M* K solution to see if the high external K concentration would still produce an effect on the efflux of K as it does before treatment with the drug. Fig. 10 shows that 165 m*M* K had no effect on the loss of ^{42}K across the innervated membrane once the direct spike had been abolished by carbamylcholine.

The converse experiment of adding carbamylcholine after high potassium showed

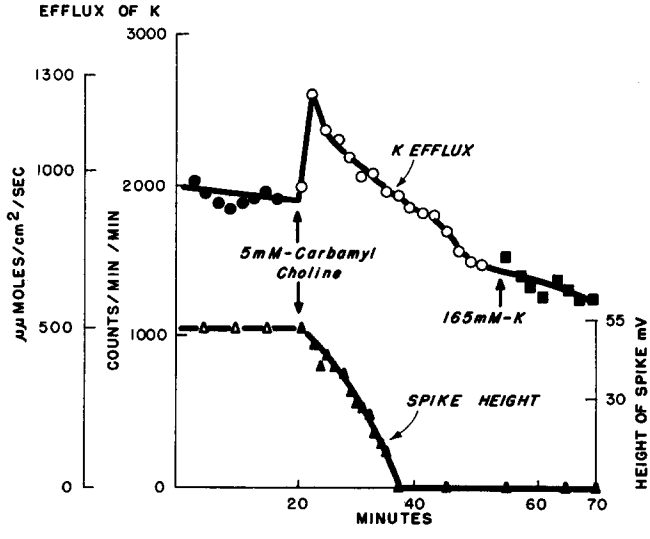

Fig. 10. The efflux of potassium and the height of the direct spike in the presence of 5 m*M* carbamylcholine and of 165 m*M* K in the Ringer's solution bathing the innervated membrane.

that no further change in efflux occurred with carbamylcholine once the spike had been abolished with 165 m*M* K solution. Since the peculiar effect on the efflux of K is the same with carbamylcholine as that with high external K concentrations, and since the response is abolished by pre-treatment with the other agent, the results suggest that the same reaction in the process of membrane depolarization is the cause of the phenomenon.

Effect of 2-PAD on potassium efflux

A drug that abolishes the direct spike even after the indirect spike has been abolished by curare in high concentrations, is 2-PAD, which therefore affects the conducting membrane directly[8]. Carbamylcholine affects the direct spike by way of the nerve synapses only[6] and was therefore used in the absence of curare. The innervated membrane of an electroplax was exposed to 1 mg/ml of curare, to abolish the indirect spike, and then to 400 μg/ml of 2-PAD. The effect of PAD on the efflux of K (Fig. 11) is the same as the effect of carbamylcholine and of a high external K concentration in producing a temporary rise in the loss of ^{42}K which then fell in about 10 min to the original level. The direct spike was abolished within 2 min after exposure of the innervated membrane to Ringer's solution containing PAD.

Block of conduction by removal of external sodium

The results have shown that, when activity was abolished by high external K, by carbamylcholine or by PAD, the efflux of K from the innervated membrane increased rapidly by about 70–100 % and then fell to the original level. The common feature of these blocking agents is membrane depolarization. To find the effect of block of activity without depolarization, the efflux of K has been measured from electroplax whose conduction had been blocked by replacing external Na ions with choline or sucrose. This procedure does not affect the resting membrane potential[12]. The results showed that the efflux of K from the resting cell was not affected by a removal of Na from the Ringer's solution, a procedure which blocked both the direct and indirect spikes. This shows that conduction can be blocked without changing the permeability of the membrane to K.

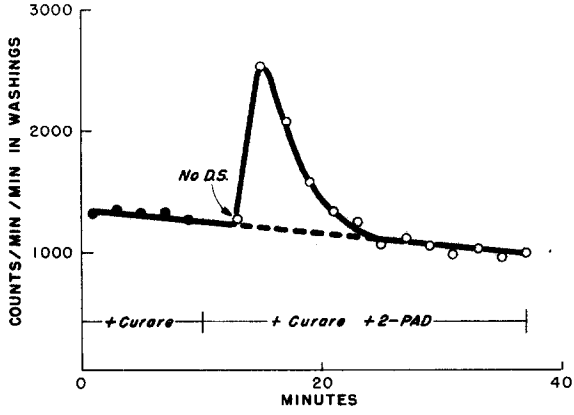

Fig. 11. The effect of 2-PAD (400 μg/ml) in the bathing Ringer's solution on the efflux potassium from the innervated membrane of an electroplax. (D.S. = direct spike).

DISCUSSION

The block and recovery of activity

The experiments described in this paper were designed to find out whether different methods of blocking electrical activity in electroplax from the organ of Sachs of *Electrophorus electricus* affected the efflux of K in characteristic ways. Block of activity has been obtained in two ways, viz. by removal of Na from the medium and by the action of depolarizing agents like K and drugs. Replacement of Na in Ringer's solution with choline or sucrose does not affect the resting potential[12]; presumably the driving force on K movements is essentially the same and it is not surprising that the efflux of K from the resting, yet unexcitable, electroplax was also unchanged. It was also shown[12] that the block obtained in sucrose medium was reversed on washing with Ringer's solution but not after immersion in choline medium; the reason for this difference remains to be explained.

Block of activity was obtained by exposing either the innervated membrane alone or both the innervated and non-innervated membranes to Ringer's solution in which NaCl had been partially (about 50 %) or completely replaced with KCl or K_2SO_4. The block was reversed on washing with the usual Ringer's solution containing 5 m*M* K. Loss of activity occurred within 1 min, in contrast to the longer time (about 30 min) needed to restore activity.

A similar long period of washing is needed to restore the resting potential of frog muscles after they have been immersed in high K solutions[15], and a slow repolarization of the membrane is the likely explanation for the slow recovery of excitability in electroplax.

The block of the direct response obtained with the lipid soluble quaternary nitrogen compound, 2-PAD, after the abolition of the indirect spike by the addition of *d*-tubocurarine, confirms SCHOFFENIELS *et al.*[8] and is probably due to the combination of 2-PAD with, and inactivation of, constituents of the membrane necessary for activity[10]. 2-PAD is a depolarizing agent[8] and so is carbamylcholine, but whilst 2-PAD acts after exposure of the electroplax to curare, carbamylcholine is then ineffective[6]. Carbamylcholine exerts its depolarizing action (and block of both the indirect and direct responses) upon the synaptic region of the membrane; the innervated membrane was therefore exposed to carbamylcholine in the absence of curare and the block of activity was observed.

Depolarizing agents and the efflux of potassium: The main results of this paper show that the depolarizing agents, carbamylcholine, 2-PAD and a high external K concentration are alike in blocking activity and in causing a rise in the efflux of potassium from the innervated membrane which later fell to the initial level. After the evanescent rise, the efflux from an inexcitable electroplax was thus identical with that from the same electroplax when it was excitable, either before block of activity or, after block with K, after washing and recovery of activity. The rise is to be expected because, as the membrane potential is reduced, so is the restraint of the membrane upon the movement of K in and out of the cell likely to be reduced. The subsequent return of the efflux to the initial level is unexpected, however, and is difficult to explain. Comparable effects have not been described in any other tissue and, indeed, the results with crab nerve showed that the efflux increased five-fold and remained constant at the high level when the external K concentration was

also increased five-fold[16]. In *Sepia* nerve axons poisoned with 2,4-dinitrophenol there was also a very rough proportionality between the external concentration and the efflux of potassium over the range from 10 to 207 m*M* K (see ref. 17). This work showed that depolarization increased the conductance of the membrane and that K ions carry the current; the experiments did not indicate transitory effects like those described here.

It is worth examining the evidence against the view that the effect is artifactual since this possibility is the most obvious and least demanding. First the result could be spurious if depolarization allowed a net loss of K from the electroplax which might account for the extra radioactivity in the washings. However, since it may be calculated that the total radioactivity collected above the initial level represented only about 5 % of the intracellular K, the return of the efflux to the initial level cannot be explained by a loss of cell K. This result also rules out the possibility that the non-innervated membrane might become the limitation to the movement of tracer from the pool of Ringer's solution on the non-innervated side of the electroplax to the washing solution on the other side. A net loss of K from the electroplax is unlikely when bathing with 110 or 165 m*M* K solutions, but this calculation shows that it also was unlikely to occur as a result of the action of carbamylcholine or 2-PAD when the external K concentration was 5 m*M*.

Secondly, the fall in efflux might be due to a decrease in the specific activity of the intracellular K upon which the efflux is directly dependent. A fall in efflux due to a lowering of intracellular specific activity should be related to the external K concentration, which determines the amount of K that can enter the cell, since the internal specific activity would fall to a greater extent as more K entered. This possibility can be excluded because the efflux returned to the same initial levels during exposure of the electroplax to carbamylcholine, 2-PAD and high K concentrations, when different amounts of K would be expected to enter the cell because of the different external concentrations (5, 110 and 165 m*M*). The fact that the efflux did not fall to lower levels with 110 and 165 m*M* external K than with 5 m*M* shows that entry of external K could not have lowered the internal specific activity in a way that would explain the fall to the same initial levels. These two considerations argue that the effect of depolarizing agents on the efflux of K is not an artifact but the typical response of the innervated membrane.

Although at the moment there is no explanation for the phenomenon of the transient change in K efflux in the electroplax there are several hypotheses that can be tested and the isolated electroplax is interesting material for further study of the nature of the system controlling potassium permeability.

ACKNOWLEDGEMENTS

We are greatly indebted to Dr. D. NACHMANSOHN for his generous hospitality and much helpful discussion of the work. We also wish to thank Dr. C. W. COATES for his help in the procurement and maintenance of eels and Mr. J. ALEXANDER for maintenance of the electrical equipment. This work was supported by the Division of Research Grants and Fellowships of the National Institutes of Health, U.S. Public Health Service, Grant No. B-400, by the National Science Foundation, Grant No. G-4331, and by the Atomic Energy Commission, Contract No. AT(30-1)-1503.

REFERENCES

1 R. WHITTAM AND M. GUINNEBAULT, *J. Gen. Physiol.*, 43 (1960) 1171.
2 R. D. KEYNES AND P. R. LEWIS, *J. Physiol. (London)*, 114 (1951) 151.
3 R. D. KEYNES AND P. R. LEWIS, *J. Physiol. (London)*, 113 (1951) 99.
4 E. SCHOFFENIELS, *Ann. N.Y. Acad. Sci.*, 81 (1959) 285.
5 D. ALBE-FESSARD AND C. CHAGAS, *Compt. rend. soc. biol.*, 145 (1951) 248.
6 E. SCHOFFENIELS AND D. NACHMANSOHN, *Biochim. Biophys. Acta*, 26 (1957) 1.
7 M. ALTAMIRANO, W. L. SCHLEYER, C. W. COATES AND D. NACHMANSOHN, *Biochim. Biophys. Acta*, 16 (1955) 268.
8 E. SCHOFFENIELS, I. B. WILSON AND D. NACHMANSOHN, *Biochim. Biophys. Acta*, 27 (1958) 629.
9 E. SCHOFFENIELS, *Biochim. Biophys. Acta*, 26 (1957) 585.
10 D. NACHMANSOHN, *Chemical and Molecular Basis of Nerve Activity*, Academic Press, New York, 1959.
11 H. DAVSON AND H. V. LAGE, *Ann. acad. brasil. sci.*, 25 (1953) 303.
12 R. D. KEYNES AND H. MARTINS-FERREIRA, *J. Physiol. (London)*, 119 (1953) 315.
13 E. SCHOFFENIELS, *Biochim. Biophys. Acta*, 27 (1958) 660.
14 M. ALTAMIRANO AND C. W. COATES, *J. Cellular Comp. Physiol.*, 49 (1957) 69.
15 R. H. ADRIAN, *J. Physiol. (London)*, 143 (1958) 59P.
16 R. D. KEYNES AND P. R. LEWIS, *J. Physiol. (London)*, 113 (1951) 73.
17 A. L. HODGKIN AND R. D. KEYNES, *J. Physiol. (London)*, 128 (1955) 61.