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Involvement of Intracellular Calcium in the Phosphate Efflux from Mammalian Nonmyelinated Nerve Fibers

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Summary. Phosphate efflux was measured as the fractional rate of loss of radioactivity from desheathed rabbit vagus nerves after loading with radiophosphate. The effects of strategies designed to increase intracellular calcium were investigated. At the same time, the exchangeable calcium content was measured using ^{45}Ca. Application of calcium ionophore A23187 increased phosphate efflux in the presence of external calcium in parallel with an increase in calcium content. In the absence of external calcium, there was only a late, small increase in phosphate efflux. For nerves already treated with the calcium ionophore, the phosphate efflux was sensitive to small changes in external calcium, in the range 0.2 to 2 mM calcium, whereas similar increases in calcium in absence of ionophore gave much smaller increases in phosphate efflux. Removal of external sodium (choline substitution) produced an initial increase in phosphate efflux followed by a fall. The initial increase in phosphate efflux was much larger in the presence of calcium, than in its absence. The difference was again paralleled by an increase in calcium content of the preparation, thought to be due to inhibition of Na/Ca exchange by removal of external sodium. Measurements of ATP content and ATP, ADP, phosphate and creatine phosphate ratios did not indicate significant metabolic changes when the calcium content was increased. Stimulation of phosphate efflux by an increase in intracellular calcium may be due to stimulation of phospholipid metabolism. Alternatively, it is suggested that stimulation of phosphate efflux is associated with the stimulation of calcium efflux, possibly by cotransport of calcium and phosphate.

Key Words nerve fibers · membrane · transport · phosphate · calcium · Ca ionophore · Na/Ca exchange

Introduction

The influx of inorganic phosphate in mammalian nonmyelinated nerve fibers, as in other tissues, is mediated by a Na-dependent and saturable mechanism [3]. On the other hand, the efflux of phosphate shows a much more complicated dependence on the ionic composition of the extracellular medium. As for the influx, there is a dependence on the extracellular sodium and phosphate concentrations; this effect is probably due to transstimulation of the transport mechanism, but it is possible that changes in the internal phosphate and sodium concentrations are also involved [3, 21]. The transstimulation by external phosphate is abolished during prolonged exposure to Na-free solutions [9].

In addition to the sodium and phosphate dependence of the phosphate efflux, we have found a transient release of phosphate when the potassium of the extracellular medium is reduced as well as a decrease of phosphate efflux in high potassium [15]. Phosphate efflux is also modified by changes in extracellular calcium concentration [16], and as briefly reported, there is a stimulation of phosphate efflux on application of the calcium ionophore A23187 [13]. The dependence of the phosphate efflux on the ionic composition of the incubation medium is thus much more complicated than in the case of the influx. The present experiments were carried out in order to study the Ca-dependence of the phosphate efflux in more detail.

Materials and Methods

Preparation of Nerves and Loading with ^{32}P

Rabbits were sacrificed and their cervical vagus nerves rapidly removed and desheathed. The nerves were then incubated in physiological solution with approximately 3 μCi ^{32}P ml^{-1} for 150 min at 37°C.

Efflux Measurements

After loading, the nerves were mounted in a small polyethylene tube, through which physiological or a modified solution was perfused at 37°C at a rate of about 1 ml min^{-1}. The effluent was collected in glass vials and counted by Cerenkov radiation. At the end of the experiment the nerves were removed, the water-soluble phosphates extracted as described previously [4], and their radioactivity counted.

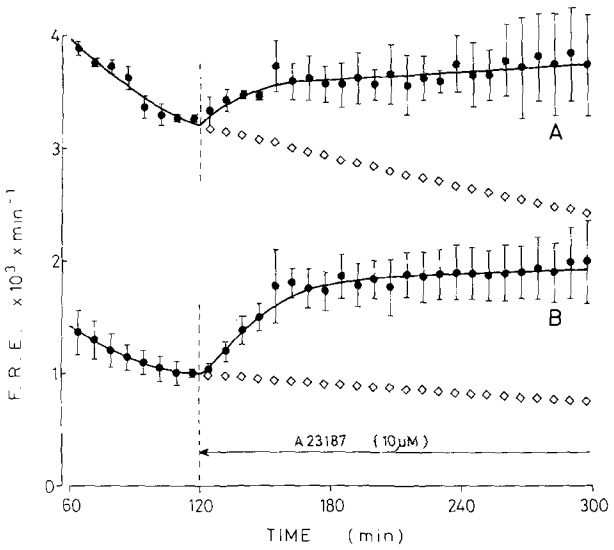

Fig. 1. Effect of calcium ionophore A23187 (10 μM) on phosphate efflux. Top curve was obtained in presence of 2 mM phosphate in washing solution, bottom curve in absence of phosphate. Points are means of three experiments, bars correspond to SEM. Diamonds indicate phosphate efflux in the absence of ionophore. Calcium concentration was 0.9 mM. Abscissa is time after beginning of washing; ordinate is fractional rate of efflux

Calculation of the Efflux

The efflux was expressed as the fraction of the radioactivity lost from the tissue during each collection period. The radioactivity of the water-soluble extract was used as the basis for this calculation, since most of the phosphate released during the washing comes from this phosphate pool [9]. During the first hour of washing the fractional rate of efflux (F.R.E.) fell rapidly; after this initial period of washing the average rate of decrease of the F.R.E. was nearly constant and much smaller (9% per hour, [16]). The decrease was not significantly different for solutions containing reduced or increased calcium. In order to compare the efflux in modified solutions with the efflux which would have occurred without a change, the theoretical "control" efflux was calculated in some experiments by extrapolation from the first hour of efflux in physiological solution using the known value (9% per hour) of the rate of decrease of the F.R.E.

Measurements of ^{45}Ca Movements

The details of the method have been published previously [14]. Briefly, the desheathed nerve was inserted into a small plastic scintillator tubing, where it was superfused with a physiological or modified solution containing 4 to 6 μCi ^{45}Ca ml^{-1}. The scintillator tubing was placed between two photomultipliers connected in parallel to a coincidence circuit and then in series to a pulse height discriminator and a ratemeter. The time course of the radioactivity of the preparation was registered on a pen-recorder.

Determination of ATP Content

The water-soluble extract of the nerve was separated into orthophosphate + creatine phosphate, ATP, ADP, and AMP fractions, by column chromatography, according to the method of Garrahan and Glynn [10] as modified by Anner et al. [4], and the radioactivity of the different fractions then counted. In some experiments, the total content of ATP in the water-soluble extract was determined by bioluminescence using the firefly luciferase-luciferin reaction [20].

Solutions

The composition of the physiological solutions was (in mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 0.9; $MgCl_2$, 0.5; Tris, 1.0; glucose, 5.0. When phosphate was present, it was added as a mixture of Na_2HPO_4 and NaH_2PO_4. The phosphate concentration indicated in the text refers to the total concentration of orthophosphate. The Na-free solutions were prepared by replacing NaCl by choline. The calcium concentration was changed by adding, or omitting $CaCl_2$ (Ca-free solution) or omitting $CaCl_2$ and adding EGTA, 1 mM (Ca-free EGTA solution). The pH of all solutions was adjusted to pH 7.4 at 37°C. ^{32}P and ^{45}Ca were obtained from National Instituut voor Radio-elementen, Fleurus, Belgium. The calcium ionophore A23187 was from Calbiochem-Behring. The ATP monitoring kit was from LKB-Wallac.

Results

Ca Dependence of Phosphate Efflux

We have observed recently that changes in extracellular calcium concentration modify the phosphate efflux in rabbit vagus nerve [16]. An increase in extracellular calcium to 20 mM produced a rapid increase of efflux of phosphate to a value about 40% higher than the efflux in 0.9 mM external calcium. This effect was reversible; on returning to the normal calcium concentration, the efflux fell to control levels within a short time interval. These findings suggest an involvement of the calcium ion in the transmembrane phosphate transport. It is not clear, however, whether the effect is due to an extracellular or an intracellular action of calcium. To study this question, we have now used, in a first series of experiments, the Ca ionophore A23187, which allows modifications of the intracellular calcium without changes in the extracellular calcium concentration.

As shown in Fig. 1, application of the Ca ionophore produced an increase in phosphate efflux to a new steady value. In zero phosphate, this was about twice the level of controls. The increase in efflux was not dependent on the external phosphate concentration. If the same experiment was done in

presence of 2 mM phosphate (curve *A*), the modification of the phosphate efflux was not different from the effect in zero phosphate (curve *B*).

The increase in phosphate efflux produced by A23187 was paralleled by an increase in the Ca content of the preparation, as seen in the top record of Fig. 2.

The stimulation of the phosphate efflux by the ionophore could have been due to an increase in the intracellular calcium or to an effect of the ionophore on the phosphate efflux mechanism. This latter possibility was excluded by studying the effects of A23187 in the Ca-free-EGTA solution. As shown in Fig. 3, the application of the ionophore was then not immediately followed by an increase in phosphate efflux; a small release of phosphate occurred only after 1 to 2 hr. This delayed increase in phosphate efflux may be due to a liberation of calcium by the ionophore from intracellular calcium stores [7].

The other results in Fig. 3 show that the main effect of the calcium ionophore depends on the presence of calcium and are thus in agreement with the hypothesis that the drug exerts its stimulatory effect on the phosphate efflux by a rise in the intracellular calcium. From the results presented in Fig. 3 it can also be seen that the maximal stimulation of the phosphate efflux by the ionophore was obtained at 0.9 mM of external calcium; in higher calcium concentrations the stimulatory effect of A23187 was not further increased.

Figure 4 shows results of experiments in which external calcium was added to nerves treated with A23187 in Ca-free-EGTA solution. For comparison, similar experiments but without the ionophore are shown. In the absence of external calcium, the ionophore was almost without effect. The addition of calcium to the nerves pretreated with the ionophore produced a rapid rise in phosphate efflux, whereas in controls the same changes of extracellular calcium were either without effect or the increase in phosphate efflux was much smaller.

Na Dependence of Phosphate Efflux

In view of these results, it was interesting to reevaluate the effects of changes of extracellular sodium on the phosphate efflux, since this ion is known to play an important role in the regulation of the intracellular calcium levels.

As in the experiments described above, modified solutions were applied after an efflux period of 120 min in physiological solution. Experiments presented in Fig. 5 show, at two different external phosphate concentrations, the effects of sodium

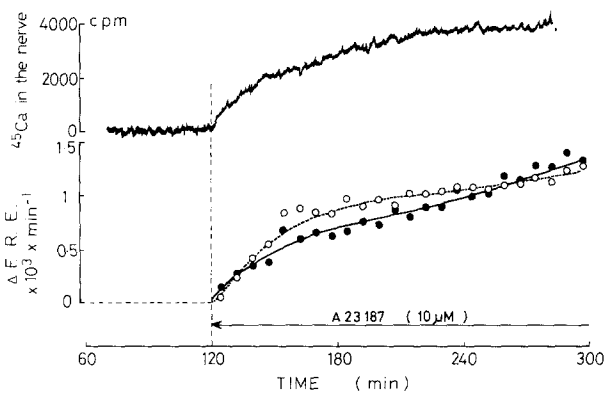

Fig. 2. Top curve is recorded of ^{45}Ca content after application of ionophore in preparation that was previously equilibrated in ^{45}Ca. Bottom curves are effluxes of phosphate at zero (○) and 2 mM (●) external phosphate calculated from Fig. 1 as the difference between control and means of ionophore-stimulated phosphate efflux. External Ca was 0.9 mM

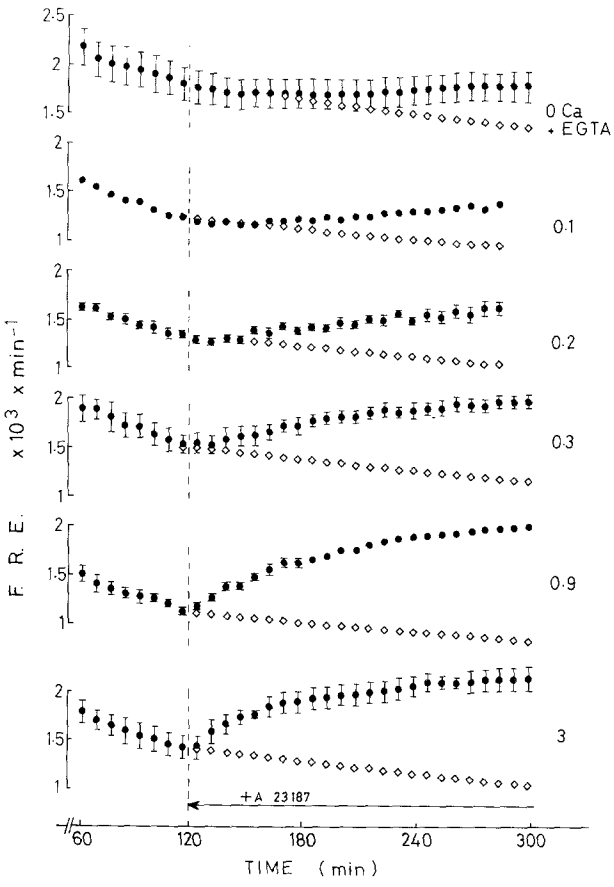

Fig. 3. Development of effect of Ca-ionophore at different extracellular calcium concentrations. The ordinate shows the fractional rate of efflux before and during the application of the drug; on the right, concentrations of calcium (in mM) in washing solution. Points, with bars indicating SEM, are means of three to seven experiments; diamonds represent effluxes without ionophore. Abscissa is time after the beginning of the washing

withdrawal (choline substitution) on the phosphate efflux. The omission of sodium produced a rapid initial rise of the phosphate efflux, which in high external phosphate was followed by an important decrease to values below the efflux in normal sodium concentrations.

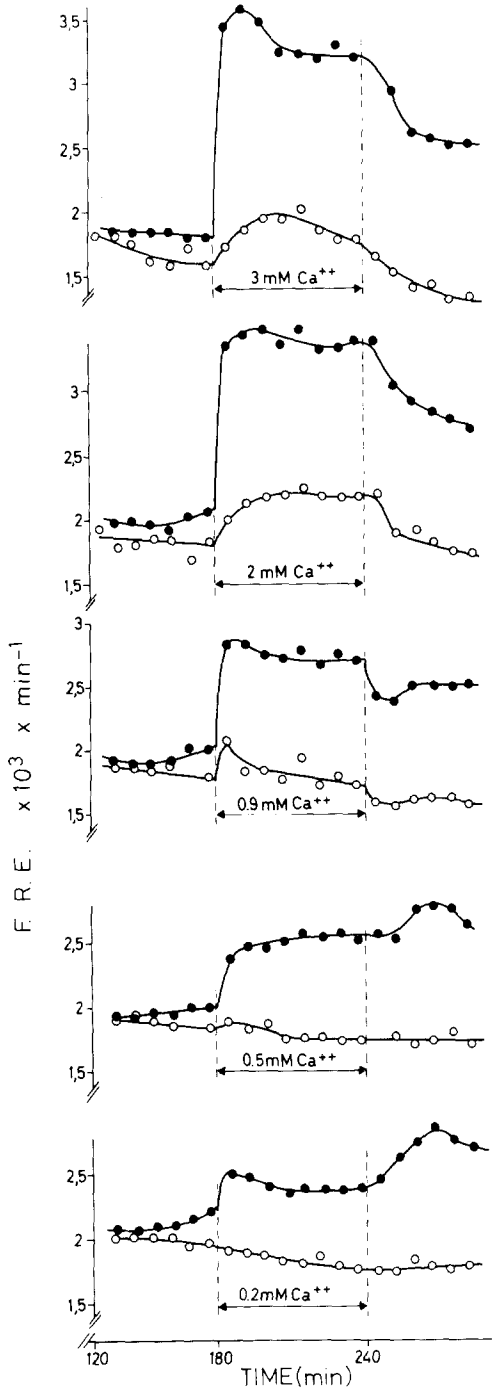

Fig. 4. Effects of increasing extracellular calcium in nerves pretreated with A23187 (●) in Ca-free EGTA solution. Ordinate shows the fractional rate of phosphate efflux before, during and after changes of the external calcium concentration to the value indicated on the Figure. Abscissa is time after beginning of the washing. Open circles show the results of similar experiments without the ionophore

Since the phosphate efflux is stimulated by the intracellular calcium, the initial rise in phosphate efflux observed after the Na withdrawal could be due to an increase in the internal calcium pool, resulting from an inhibition of a Na/Ca exchange mechanism (for review, *see* [6]; and for vagus nerve, [17]). The next series of experiments was carried out in order to test this hypothesis. Nerves were superfused in absence of phosphate during 120 min with Ca-free solutions or with 2 mM added calcium; after this initial period, the Na from the washing solution was replaced by choline. The results of Fig. 6 show that in high Ca, Na-free solution produced a maintained increase in phosphate efflux, whereas in low external calcium only a smaller transient increase was observed after omission of Na. The calcium-sensitive component of the phosphate efflux after sodium withdrawal is shown in the bottom of Fig. 7, as the difference between the effluxes obtained in the presence of calcium and in the Ca-free solution. The top curve of this Figure shows a record of the ^{45}Ca content of the nerve, measured in the same conditions as the phosphate efflux experiments: there is an excellent correlation between the time courses of the Ca-stimulated phosphate efflux and the ^{45}Ca content.

Another experiment which showed that a part of the phosphate efflux in Na-free solution is Ca sensitive is illustrated in Fig. 8. In this experiment the sodium was first replaced by choline in the presence of 2 mM calcium, and later, after 1 hr of washing, the calcium from the Na-free solution was omitted. After the calcium withdrawal, the phosphate efflux fell to values close to control level. The Figure also shows the reversibility of the effect due to sodium readmission as well as the efflux in Na-free solution with 2 mM Ca.

Measurement of the Metabolic Effect of the Intracellular Calcium

It might be that an increase in the internal calcium concentration will stimulate the intracellular Ca-dependent ATPases, increasing breakdown of the cellular ATP pool. An increase of the phosphate efflux could in this case be explained by the increase of the intracellular inorganic phosphate concentration. This possibility was tested by measuring the ^{32}P-labeled intracellular ATP, ADP and phosphate and creatine phosphate, as well as the total ATP content in controls and in nerves treated with the calcium ionophore.

As in the efflux experiments described above, the nerves were first incubated during 150 min in ^{32}P-labeled solution. Controls were then washed during 240 min with nonradioactive physiological solution, whereas treated nerves were washed during 120 min with physiological solution and then 120 min with solution containing 10 μM of A23187. At the end of the experiment the labeled ATP, ADP and phosphate plus creatine phosphate as well as the total ATP content were determined as described in Materials and Methods. The results, presented in the Table, show that the ionophore-stimulated increase of intracellular calcium does not significantly modify the ATP turnover.

Discussion

Effects of A23187 on the Phosphate Efflux

As previously shown an increase in the calcium concentration in the external solution is followed by

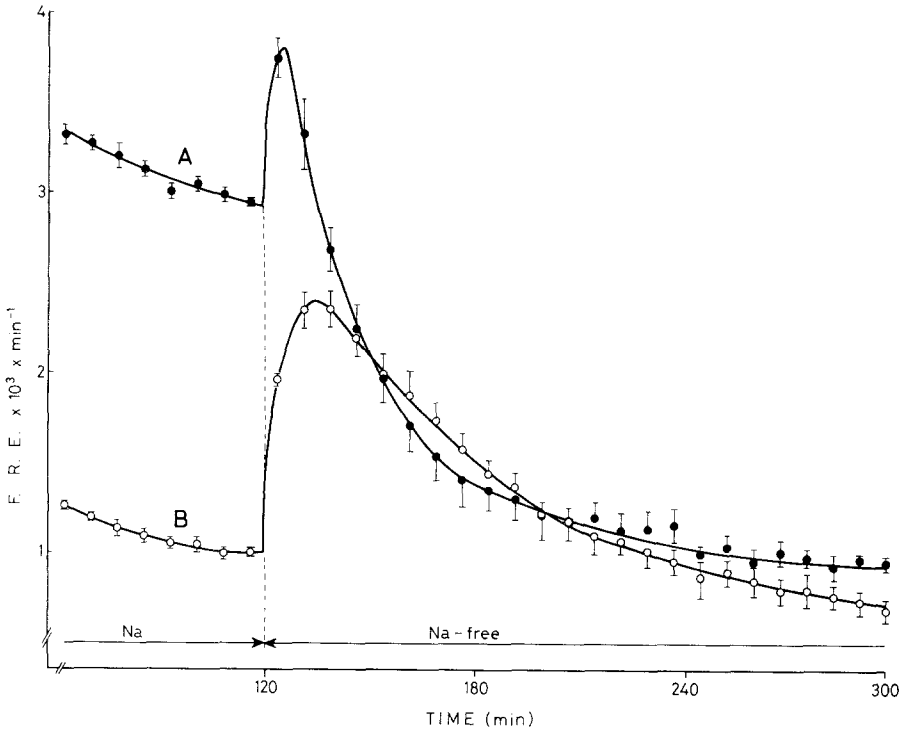

Fig. 5. Effect of replacing Na by choline in presence (●) and absence (○) of phosphate in washing solution. Ordinate shows the fractional rate of phosphate efflux before and after the removal of extracellular Na; abscissa is time after beginning of washing. Points are means of four (*A*) or three (*B*) experiments; bars indicate SEM

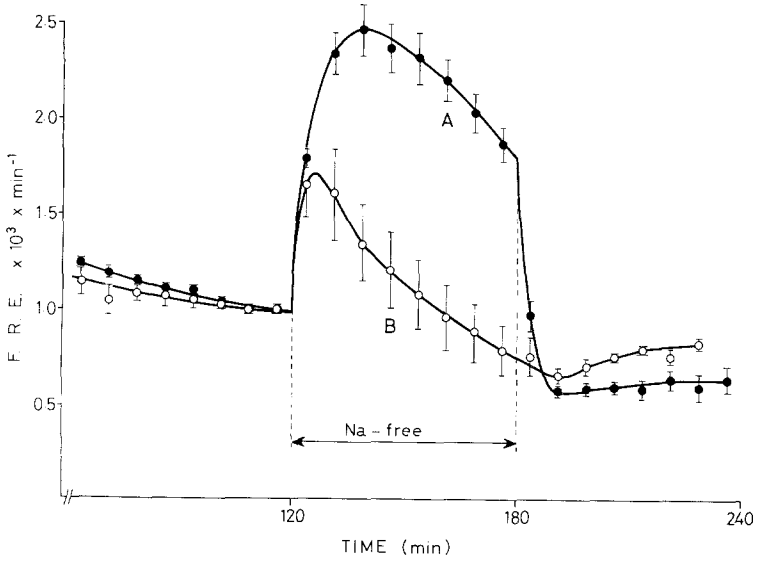

Fig. 6. Effect of substitution of Na by choline in presence of 2 mM Ca (●) and in Ca-free solution (○). Ordinate shows fractional rate of phosphate efflux before, during and after Na withdrawal; note reversibility of the effect. Abscissa is the time after beginning of the washing. Points are means of three or more experiments; bars indicate SEM

a rapid increase of phosphate efflux in rabbit vagus nerve [16]. This effect could be due to direct action of the extracellular calcium on the phosphate efflux mechanism or to an increase in intracellular calcium. In the present experiments, application of the calcium ionophore at constant external calcium was found to produce a large increase in phosphate efflux (Fig. 1). A possible direct effect of the ionophore on the phosphate transport system, or on the phosphate permeability of the membrane can be excluded, since in the absence of Ca the effect of the ionophore was strongly reduced. These results show that internal rather than external calcium affects the phosphate efflux. Moreover, experiments on Fig. 4, which show that the phosphate efflux becomes much more sensitive to changes in external calcium concentration when nerves are pretreated with A23187, are also in agreement with this idea. The idea is further supported by recordings of the ^{45}Ca content, which give an excellent agreement between the time course of the increase in calcium and the increase in phosphate efflux, during the treatment with the ionophore.

Figure 2 also shows that the external phosphate

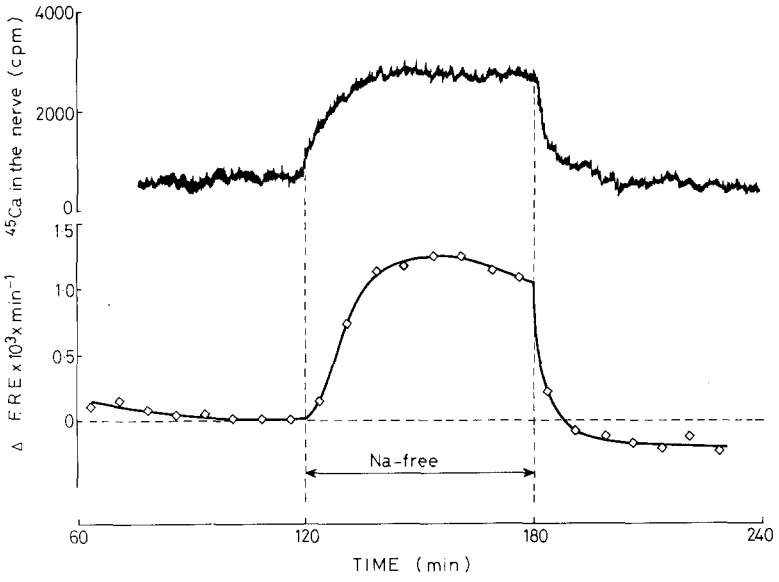

Fig. 7. Comparison of ^{45}Ca content and phosphate efflux during Na withdrawal. Top curve is record of ^{45}Ca content obtained after equilibration of the exchangeable Ca pool of the nerve with the labeled extracellular solution. Bottom curve represents difference between phosphate effluxes at 2 mM Ca and in Ca-free solution. Abscissa is time after application of radioactive solution (for top curve) or after beginning of the washing (bottom curve). Both were obtained in absence of phosphate

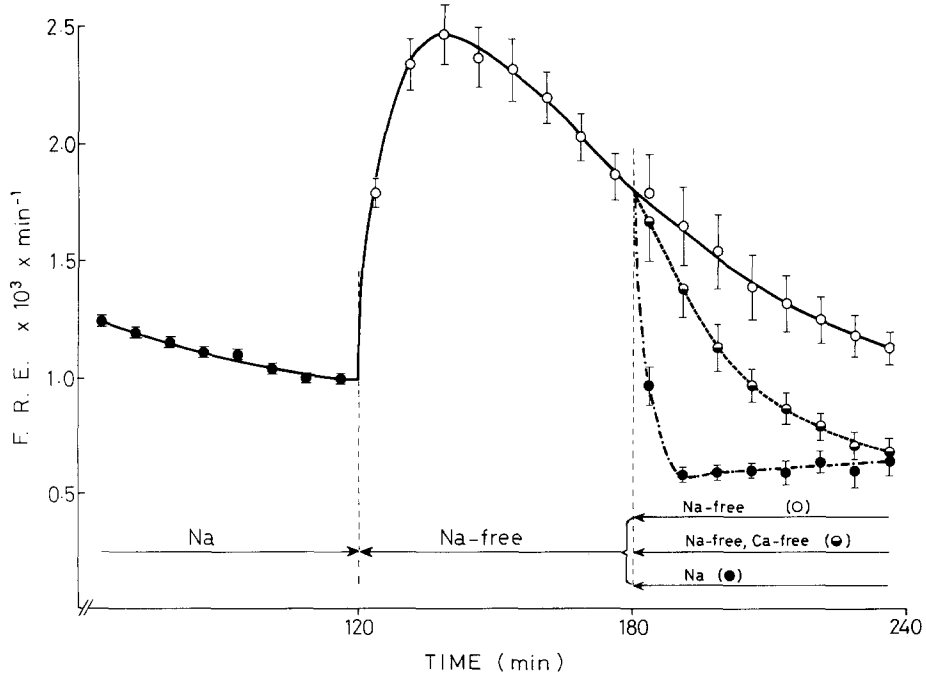

Fig. 8. Reversibility of the phosphate efflux in Na-free solution. Ordinate shows the phosphate efflux before and during the Na omission, as well as reversal of the effect by Ca withdrawal (◒) and Na readmission (●). The "control" efflux in Na-free solution is also shown (○). Abscissa is time after beginning of washing. Points are means of three to 12 experiments; bars indicate SEM. Ca concentration was 0.9 mM, except where otherwise indicated

concentration does not affect the ionophore-stimulated phosphate efflux. This suggests that the Ca-dependent part of the phosphate efflux is mediated by a mechanism distinct from the Na-dependent part of the phosphate transport system. This conclusion is confirmed by results obtained in Na-free solutions, where the Ca-dependence of the phosphate efflux is still present (*see below*, and Fig. 6).

Effects of Na-Free Solution of the Phosphate Efflux

Removal of extracellular sodium increases the membrane potential of rabbit vagus nerve by about 10 mV [15]. Qualitatively, this hyperpolarization could be at the origin of the observed increase in the efflux of the negatively charged phosphate ion in Na-free solution. Quantitatively, however, as we have shown previously [15], a hyperpolarization of 10 mV would produce an increase in phosphate efflux of only about 4 or 5%, a small fraction of the observed effect. Another mechanism must therefore be involved in the increase in phosphate efflux in Na-free solutions.

The results presented in this paper suggest that part of the effect of Na-withdrawal is related to alterations of the internal calcium. If we assume that in vagus nerve calcium fluxes are, at least partially, mediated by a Na/Ca countertransport, then an initial effect of removal of the extracellular sodium would be an inhibition of the calcium efflux, resulting in an increase of the intracellular calcium. At the same time, the intracellular sodium is progressively lost. The phosphate efflux after the Na withdrawal could thus present a biphasic pattern: in a first period there could be an increase of the Ca-dependent component of the efflux, followed by a decrease in the Na-dependent one.

The results presented in Figs. 5 and 6 show a good correlation with these predictions. Curve *A* of Fig. 5 was obtained in solution with 2 mM phosphate, curve *B* in the absence of external phosphate. In both cases, after removal of external sodium, the efflux first increased, which is in agreement with the hypothetical rise in intracellular calcium. In the presence of phosphate, the initial increase of efflux was followed by a decrease to values below the efflux in normal sodium concentrations. This decrease reflects the inhibition of the Na-dependent component of the phosphate efflux [9].

In low external phosphate (Fig. 5, curve *B*), the initial rise is usually larger than in high phosphate and the decrease that follows tends to values that remain close to the rates of efflux observed before the Na omission. This suggests that the lack of sodium in low phosphate does not inhibit the efflux of phosphate, indicating that only the efflux stimulated by the extracellular phosphate is Na dependent. In other words it signifies that both external phosphate and sodium stimulate the same component of the phosphate efflux mechanism. This is also confirmed by previous results [9], which showed that transstimulation of the phosphate efflux by external phosphate disappears in the absence of external sodium.

If then in the absence of phosphate the Na-dependent part of the phosphate efflux is abolished, an effect of Na withdrawal must be due to a component other than the Na-dependent part of the phosphate efflux. Figure 6 clearly shows that in the absence of phosphate there is an increase of efflux when sodium is omitted. Moreover, this increase depends on the external calcium concentration, increasing with increasing calcium in the perfusion solution. In the absence of Ca, application of choline causes only a short transient liberation of phosphate (Fig. 6*B*) which is probably due to the direct effect of choline rather than to the absence of Na, as described by Straub et al. [25].

The curve presented in the bottom of Fig. 7 shows the "net" effect of calcium on the phosphate efflux during the Na withdrawal. Its time course corresponds closely to the increase in ^{45}Ca content of the nerve, measured in the same conditions and presented in the top of this figure.

The effect of Na-free solution on the phosphate efflux is perfectly reversible. After the reintroduction of sodium to the perfusion solution the phosphate efflux falls rapidly to control levels or even slightly below (Figs. 6, 7 and 8). A similar but somewhat slower reversal of the effect is obtained by withdrawal of calcium from the Na-free solution (Fig. 8). The difference between these two results suggests that, in the vagus nerve, the restorative movements of calcium from the cell by Na/Ca exchange mechanism is more effective than calcium extrusion via a Ca pump.

Table. Effect of A23187 (10 μM) on the labeling of nucleotides and on the total ATP content[a]

Incubating solution	Labeled		Total ATP (mmol/kg wet weight)
	ATP/ADP	ATP/ADP · (P_i + CrP)	
physiol. (3)	2.04 ± 0.35	27.22 ± 4.36	1.06 ± 0.11
A23187 (4)	2.37 ± 0.24	27.97 ± 3.55	1.02 ± 0.03

[a] The nerves were loaded during 150 min in ^{32}P-labeled physiological solution. Controls were then washed during 240 min in physiological solution, treated nerves 120 min in physiological solution and 120 min in solution containing 10 μM A23187. Number of experiments indicated in parentheses. Values are means ± SEM P_i = orthophosphate; CrP = creatine phosphate.

Mode of Action of Ca on the Phosphate Efflux

Data presented in the Table show that there is no significant modification of the ATP turnover when the intracellular calcium is increased. This observation, and the fact that a tenfold increase in extracellular calcium produces no increase (there is even a slight decrease) of the resting oxygen consumption in rabbit vagus nerve [23], show that the calcium-stimulated phosphate efflux is probably not due to an alteration of the ATP/phosphate balance, nor to a modification of the respiration-coupled transport of calcium by the mitochondria [19].

Calcium ions are known to interfere with a number of cellular functions, including the phosphorylation of membrane phospholipids (for review, *see* [12]). If, during their metabolic cycle the membrane phospholipids release some phosphate to the outside of the cell, it will contribute to the total phosphate efflux, and a modification of the phospholipid turnover could thus influence the measured efflux of phosphate. Rouiller [24] has shown an effect of tetracaine and other local anaesthetics on the labeling of different phospholipids, particularly the triphosphoinositol, which occurred in parallel with changes in the phosphate efflux. A similar stimulation of the phospholipid metabolism by the intracellular calcium [1, 2, 11] could be the cause of the calcium-stimulated phosphate efflux.

Another possible explanation of the results presented in this paper is to postulate that phosphate leaves the cell in cotransport with calcium. In efflux experiments with nerves loaded with ^{45}Ca the mean rate constant of calcium efflux (at 0.9 mM of extracellular calcium and in absence of phosphate) was about 1.25×10^{-4} sec^{-1}, and the steady value of the exchangeable calcium pool, calculated from our influx experiments was, for the same experimental conditions, 1.2 mM/kg wet weight (*unpublished results*). Assuming a membrane area of 60 cm^2/10 mg of nerve [18], we obtain for the efflux of calcium at the steady state a value of 25 fmol cm^{-2} sec^{-1}, which is similar to the value in squid axons reported by Mullins [22] and DiPolo and Beaugé [8]. The corresponding mean rate constant of phosphate efflux and the exchangeable phosphate pool are 1.7×10^{-5} sec^{-1} (this paper) and 6.9 mmol/kg wet weight [3], respectively. These values correspond to an efflux of phosphate of 20 fmol cm^{-2} sec^{-1}, which is surprisingly close to the efflux of calcium calculated above. Coupling between phosphate and calcium effluxes in rabbit vagus nerve is therefore not excluded on quantitative grounds. A further argument for this possibility is that low concentrations of lanthanum, which block the Na-independent part of the phosphate efflux [16], in squid giant axon inhibit the residual calcium efflux that persists in absence of external sodium and calcium almost completely [5]. In this residual calcium efflux system, calcium is extruded neither in exchange for an external calcium ion, nor for another external cation. The possibility that it is extruded with an anion, such as phosphate, could be a possible explanation.

Finally in internally dialyzed squid axons it has been found that *internal* calcium activates the exchange between external calcium and internal sodium (*see* [8]). The results presented in this paper show that whenever the intracellular calcium is increased, either by application of the calcium ionophore (Figs. 1–3) or by removal of external sodium (Figs. 5–8), an important increase in phosphate efflux is seen. It is thus possible that the Ca-dependent component of the phosphate efflux is linked to the operation of this Na_{in}/Ca_o exchange mechanism. This hypothesis seems to be the most attractive one, since besides the Ca dependence of the phosphate efflux, it also explains its Na dependence. Indeed, in the absence of extracellular Na, intracellular sodium will be progressively lost, so decreasing the Na_{in}/Ca_o exchange, and consequently the phosphate efflux.

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References

1. Akhtar, R.A., Abdel-Latif, A.A. 1978. Calcium ion requirement for acetylcholine-stimulated breakdown of triphosphoinositide in rabbit iris smooth muscle. *J. Pharmacol. Exp. Ther.* **204**:665–668
2. Allan, D., Michell, R.H. 1978. A calcium-activated polyphosphoinositide phosphodiesterase in the plasma membrane of human and rabbit erythrocytes. *Biochim. Biophys. Acta* **508**:277–286
3. Anner, B., Ferrero, J., Jirounek, P., Jones, G.J., Salamin, A., Straub, R.W. 1976. Sodium-dependent influx of orthophosphate in mammalian non-myelinated nerve. *J. Physiol. (London)* **260**:667–686
4. Anner, B., Ferrero, J., Jirounek, P., Straub, R.W. 1975. Uptake of orthophosphate by rabbit vagus nerve fibres. *J. Physiol. (London)* **247**:759–771
5. Bake, P.F., McNaughton, P.A. 1976. Kinetics and energetics of calcium efflux from intact squid giant axons. *J. Physiol. (London)* **259**:103–144
6. Blaustein, M.P., Nelson, M.T. 1982. Sodium-calcium exchange: its role in the regulation of cell calcium. *In:* Membrane Transport of Calcium. E. Carafoli, editor. pp. 217–236. Academic, London
7. Desmedt, J.E., Hainaut, K. 1976. The effect of A23187 ionophore on calcium movements and contraction processes in single barnacle muscle fibres. *J. Physiol. (London)* **257**:87–107

8. DiPolo, R., Beaugé, L. 1980. Mechanisms of calcium transport in the giant axon of the squid and their physiological role. *Cell Calcium* **1:**147–169
9. Ferrero, J., Jirounek, P., Rouiller, M., Straub, R.W. 1978. Efflux of inorganic phosphate from mammalian non-myelinated nerve fibres. *J. Physiol. (London)* **282:**507–519
10. Garrahan, P.J., Glynn, I.M. 1967. The incorporation of inorganic phosphate into adenosine triphosphate by reversal of the sodium pump. *J. Physiol. (London)* **192:**237–256
11. Griffin, H.D., Hawthorne, J.N. 1978. Calcium-activated hydrolysis of phosphatidyl-*myo*-inositol-4-phosphate and phosphatidyl-*myo*-inositol 4,5-bisphosphate in guinea-pig synaptosomes. *Biochem. J.* **176:**541–552
12. Hawthorne, J.N., Ansell, G.B. 1982. Phospholipids. J.N. Hawthorne and G.B. Ansell, editors. Elsevier Biomedical, Amsterdam
13. Jirounek, P., Jones, G.J., Pralong, W., Rouiller, M., Straub, R.W. 1982. Involvement of Ca ions with phosphate movements in rabbit vagus nerve fibres. *J. Physiol. (London)* **328:**52P–53P
14. Jirounek, P., Jones, G.J., Pralong, W.F., Straub, R.W. 1982. Continuous measurements of Ca movements in mammalian nerve fibres using ^{45}Ca. *J. Physiol. (London)* **334:**117P–118P
15. Jirounek, P., Rouiller, M., Ferrero, J.D., Straub, R.W. 1980. Role of potassium in the phosphate efflux from mammalian nerve fibers. *J. Membrane Biol.* **52:**75–82
16. Jirounek, P., Rouiller, M., Jones, G.J., Straub, R.W. 1982. Effects of calcium and lanthanum on phosphate efflux from nonmyelinated nerve fibers. *J. Membrane Biol.* **65:**125–130
17. Kalix, P. 1971. Uptake and release of calcium in rabbit vagus nerve. *Pflügers Arch.* **326:**1–14
18. Keynes, R.D., Ritchie, J.M. 1965. The movements of labelled ions in mammalian non-myelinated nerve fibres. *J. Physiol. (London)* **179:**333–367
19. Lehninger, A.L. 1974. Role of phosphate and other proton-donating anions in respiration-coupled transport of Ca^{2+} by mitochondria. *Proc. Natl. Acad. Sci. USA* **71:**1520–1524
20. Lundin, A., Rickardsson, A., Thore, A. 1976. Continuous monitoring of ATP-converting reactions by purified firefly luciferase. *Analyt. Biochem.* **75:**611–620
21. Maire, J.C., Straub, R.W. 1980. Release of inorganic phosphate during activity in mammalian non-myelinated nerve fibres. *J. Physiol. (London)* **304:**135–143
22. Mullins, L.J. 1981. Ca entry upon depolarization of nerve. *J. Physiol. (Paris)* **77:**1139–1144
23. Ritchie, J.M., Straub, R.W. 1980. Oxygen consumption and phosphate efflux in mammalian non-myelinated nerve fibres. *J. Physiol. (London)* **304:**109–121
24. Rouiller, M. 1982. Etude du transport du phosphate dans le nerf vague du lapin. Effets des anesthésiques locaux et modélisation. Thèse No. 2040, Université de Genève, Switzerland
25. Straub, R.W., Ferrero, J., Jirounek, P., Rouiller, M., Salamin, A. 1978. Action of acetylcholine and related compounds on efflux of phosphate from nerve fibers. *In:* Cell Membrane Receptors for Drugs and Hormones: A Multidisciplinary Approach. R.W. Straub and L. Bolis, editors. pp. 81–88. Raven, New York

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