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Calcium efflux in giant axons

It is known that Ca^{2+} affects the permeability of cell membranes to ions and to water; it seems though, that the permeability of nerve membrane to Ca^{2+} itself is little understood, in spite of the fact that the problem has received some considerable attention[1,2]. It is claimed in the present paper that the permeability of the axon membrane to Ca^{2+} is many orders of magnitude higher than has been previously thought.

Giant axons from the squid *Dosidicus gigas* were used. Previous experiments[3] showed that their permeability to monovalent cations is the same as for Loligo. The axons were mounted in a chamber divided into three compartments separated by air gaps. The central part of the fiber lying in the middle compartment was internally loaded by means of a microinjection (effected as in ref. 4) with a tracer amount of $^{45}Ca^{2+}$ (about $4 \cdot 10^5$ counts/min per cm of fiber), dissolved in 0.6 M potassium glutamate at pH 7.2, to which a "pinch" of chlorphenol red was previously added. The amount of carrier in this solution was such that an increase in intracellular Ca^{2+} concentration of at most 0.01 mM was expected. The excitability was checked during the microinjection with external stimulation and recording, using a pulse frequency of 1 sec^{-1}. When the experiment was well advanced, a second test of activity was applied, stimulating for 30 min at 50 sec^{-1}. All axons employed here responded to

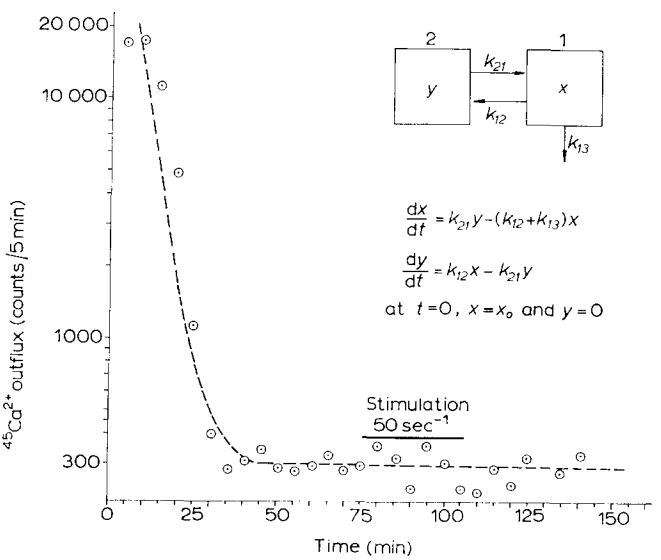

Fig. 1. Efflux of $^{45}Ca^{2+}$ as a function of time. Compartment 1 represents free, ionic calcium within the axon and compartment 2 represents the bound calcium. x represents the amount of free ionic $^{45}Ca^{2+}$ within 1 and y represents the amount of bound $^{45}Ca^{2+}$ within 2. A microinjection was effected in 1 at time $t = 0$, making $x = x_0$. Immediately after, $k_{13}x$, *i.e.* the efflux of $^{45}Ca^{2+}$, was measured in the external sea water which was collected every 5 min. The points represent the experimental results. The dashed line was obtained from an analog computer into which the differential equations and the k's calculated from this experiment were fed. No allowance was made in the model for diffusion within the axon.

this test and also to a final test of a 5-min stimulation period at 100 sec^{-1}. Immediately after the microinjection (no more than 15 sec elapsed), the external artificial sea water[5] from each of the three compartments of the chamber was removed every 5 min and replaced by an equivalent volume. Solutions from each compartment were collected separately but only the central part was used to determine the temporal course of $^{45}Ca^{2+}$ efflux. The lateral ones allowed us to exclude the possibility of contamination from the opening of the axon where the capillary tube with $^{45}Ca^{2+}$ solution was introduced. At the end of the experiment, the axon was dissolved in HNO_3 in order to determine the internal radioactivity.

The results of eleven experiments show consistently the same general picture: that is to say, two very different exponentials, a very fast one and a very slow one (see Fig. 1). The slow exponential seems to be in agreement with previous results[1]. Indeed, the latter gave a rate constant of the order of $7 \cdot 10^{-4}$ min^{-1} while in the present work the average rate constant for the slow exponential was about $2 \cdot 10^{-4}$ min^{-1}. The fast process was, however, missed by previous investigators because they allowed too much time to elapse between the microinjection and the beginning of re-collection of samples (15 min in the work of HODGKIN AND KEYNES), and also because the sampling interval was too long (30 min). It is clear that under those conditions the fast exponential would either not appear or would be only slightly manifested in a rise in the value of the first point, which is exactly what HODGKIN AND KEYNES obtained.

The interpretation of the present results was made in terms of a compartmental analysis which is schematized in the insert of Fig. 1 and which accounts for the known properties of Ca^{2+} within the axoplasm.

A preliminary inspection showed that k_{21} was much smaller than either k_{12} or k_{13}, which allowed us to simplify the solution of the differential equation system proposed in Fig. 1. The approximate function obtained is:

$$\text{Rate of } ^{45}Ca^{2+} \text{ efflux} = k_{13}x = k_{13}x_0 \cdot e^{-(k_{13} + k_{12})t} + k_{21}y$$

TABLE I

RATE CONSTANTS AND PERMEABILITY

Expt. No.	k_{13} *(min^{-1} × 10^2)*	k_{12} *(min^{-1} × 10^1)*	k_{21} *(min^{-1} × 10^4)*	*Axon diameter (mm)*	*P (cm/sec × 10^5)*
1	1.8	1.2	2.3	0.850	0.6
2	3.8	1.7	2.2	0.800	1.3
3	0.6	0.1	1.1	1.050	0.3
4	2.7	2.1	1.5	0.925	1.0
5	2.5	1.8	2.0	0.925	1.0
6	2.4	0.8	2.0	0.940	0.9
7	2.5	2.4	1.3	1.000	1.0
8	4.7	0.7	1.6	0.800	1.6
9	2.0	2.3	1.3	1.000	0.8
10	3.2	1.6	1.5	0.900	1.2
11	4.1	1.2	1.9	0.950	1.6
Average	2.8	1.4	1.7		1.0
S. D.	±1.0	±0.7	±0.4		±0.4

where the k's, x and y are explained in Fig. 1. In this system, y becomes constant after about 30 to 40 min and is much larger than x. Henceforth k_{21} was established from the constant flux which was obtained after a long time had elapsed and the internal radioactivity which was detected at the end of the experiment. While the slope of the fast exponential gave $k_{13} + k_{12}$, the extrapolation to zero time to obtain $k_{13}x_0$ was difficult because the position of the zero is uncertain. Analog computation based on the differential equations of Fig. 1 and tentative k's showed that about 70% of the tracer was already bound 10 min after the microinjection. Accordingly, a more reasonable k_{13} was obtained from the second point of the curve (which was usually the highest) and an x which was assumed to be $0.3x_0$. It seems clear that the permeability to Ca^{2+} is described by k_{13} and not by k_{21} which had been used by previous authors. This is why no changes in $^{45}Ca^{2+}$ efflux have been detected with stimulation or the presence of pump-inhibitors although such changes were expected. The P's tabulated in Table I correspond to the permeability coefficient defined by JACOBS and were calculated from the k_{13}'s, according to $P = k_{13}\ d/4$, where d is the diameter of the fiber[6].

From these results it appears that the permeability of the axonal membrane to Ca^{2+} as defined here is at least 20 to 30 times larger than the permeability to monovalent cations defined in the same way[3].

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