Biochimica et Biophysica Acta, 598 (1980) 339–344

BBA 78705

A TRACER METHOD TO STUDY UNIDIRECTIONAL FLUXES OF LITHIUM

APPLICATION TO FROG SKIN

M. THELLIER [a], A. HARTMANN [a], J.P. LASSALLES [a] and J.P. GARREC [b]

[a] *Faculté des Sciences et Techniques, L.A. CNRS 203, C.R.B.P.C., Université de Rouen, 76130 Mont-Saint-Aignan and* [b] *Département de Recherches Fondamentales (Biologie Végétale), CEA, Centre d'Etudes Nucléaires de Grenoble, 85 X, 38041 Grenoble Cédex (France)*

(Received May 14th, 1979)
(Revised manuscript received November 11th, 1979)

Key words: Lithium transport; Unidirectional flux; Radiotracer; (Frog skin)

Summary

We describe a new tracer method to measure unidirectional fluxes of Li^+, despite the lack of any utilizable radioisotope of lithium. This method uses the purified stable isotopes, ^6Li and ^7Li, detected with an ion-probe microanalyser. The accuracy is comparable to that obtained for other ions (e.g., Na^+) with radiotracers.

The method has been applied to frog skin with both faces bathed in a 20% lithium/80% sodium medium. Sodium and lithium unidirectional fluxes have been measured simultaneously. The results are consistent with lithium being actively pumped, the outflux of lithium being, however, much larger than that of sodium.

Introduction

The discovery of the efficiency of lithium in the treatment of manic-depressive psychoses [1,2] focused attention on the physiological role of this element. It would be especially relevant to know about the mechanisms of lithium uptake by living cells. But, the study of cellular transport requires the measurement of unidirectional fluxes, whilst in the case of lithium, there is the severe methodological difficulty that no utilizable radioisotope exists. This prevents the determination of unidirectional lithium fluxes by conventional radiotracer methods. The difficulty can be overcome by using the stable isotopes of

lithium, ^6Li and ^7Li, as tracers. Two different means of detection have been attempted previously. We have used the nuclear reaction, $^6Li(n,\alpha)^3H$, which is specific for the isotope, ^6Li, of lithium [3]. Others have taken advantage of the isotopic shift between ^6Li and ^7Li in atomic absorption spectroscopy [4]. But, although some encouraging results had been obtained in both cases, the sensitivity was insufficient to give fully reliable information on the cellular unidirectional fluxes of lithium. In the present contribution we propose a third possibility of detection. It consists of measuring the isotopic composition of lithium in the studied samples with the aid of an ion-probe microanalyser, according to the theoretical possibility which we have previously discussed [5].

The method has been tested here in the experimental situation of the frog skin maintained under short-circuited conditions [6], because it is a case where the other ionic fluxes have already been most extensively studied [7]. Simultaneous use of our tracer method for lithium, and of a conventional double-labelling method for sodium (^{22}Na and ^{24}Na) has permitted determination of the four unidirectional fluxes (sodium and lithium influx and outflux).

Materials and Methods

Skin was excised from the abdomen of the frog (*Rana esculenta*), and an area of π cm^2 was maintained between two compartments, e and i, corresponding, respectively, to external and internal faces. The short-circuited conditions were maintained with an apparatus [8] slightly modified from that of Ussing [6]. During a 1 h pre-equilibration treatment, both compartments were filled with 8 ml of a conventional Ringer solution composed of 112 mM NaCl, 2.4 mM $NaHCO_3$, 2 mM KCl and 1 mM $CaCl_2$ (no radioisotope or lithium added). At time zero of the experiment, sodium radioisotopes and 2 ml of LiCl stock solutions were added (^{24}Na and ^6Li in compartment e, ^{22}Na and ^7Li in compartment i) so that the total salt concentration in both compartments remained unchanged, the volume became 10 ml in each compartment and the ionic composition 80% Na, 20% Li. Enriched isotopes of ^7Li at 99.9% and ^6Li at 99% purity, used in the present study, were obtained from the Commissariat à l'Energie Atomique, Saclay, France. The reinjection circuit kept the electric potential difference across the skin down to zero, and the overall electric intensity in the circuit was recorded. At given instants of time (1.5, 3, 4.6, 6 and 9 h), 1-ml samples were taken from both compartments and replaced by 1 ml of a solution identical to that present in the compartments at time zero.

The sample solutions were submitted to the usual $^{22}Na/^{24}Na$ double-labelling measurements with a γ-spectrometer (Gammatic S.A.I.P.). Taking into account the radioactive decay of both isotopes, the backgrounds of the radioactive measurements and the dilution effect of sampling for each considered interval of time, a straightforward calculation [3,5] gave an estimate of ^{22}Na radioactivity entering compartment e and ^{24}Na radioactivity entering compartment i, both values being normalized for time zero of the experiment. The previous estimations of the specific activities of ^{22}Na and ^{24}Na, at time zero of the experiment, respectively, in compartments i and e, allowed the exchanged radioactivities to be expressed in terms of moles of sodium unidirectionally exchanged. The corresponding mean unidirectional sodium fluxes were then given as $A \cdot cm^{-2}$.

After the sodium radioactivity measurements, 2% gelatin was added to the sample solutions. Droplets (0.5 μl) of these gelatinized solutions were taken up with aid of a Hamilton (The Hague, The Netherlands) microsyringe, deposited on an ultrasonically-cleaned tantalum plate and carefully dried [9]. The isotopic lithium composition of each droplet was estimated with the aid of the ion-probe microanalyser (Cameca ISM 300, The Nuclear Center of Grenoble, France). Briefly, the principle is as follows. A beam of accelerated Ar^+ (5.5 keV) is projected onto the sample, ejecting secondary ions which are selected by a mass-spectrometer. Each atomic mass is thus characterized in the apparatus by an ionic current, expressed in A, which is proportional to the percentage of that atomic mass in the sample. The sensitivity is remarkable: the measurable intensity ranges from $1 \cdot 10^{-13}$—$1 \cdot 10^{-18}$ A, which for lithium corresponds to approx. 10 ions per s, and hence, for a minimum value of 10 s of the measurement time and 1% counting efficiency, to a sensitivity as small as $1 \cdot 10^{-19}$ g (10 000 atoms!). The discrimination between the two lithium isotopes is good: isotopic ratios, $^6Li/^7Li$ or $^7Li/^6Li$, can be estimated down to values of $1 \cdot 10^{-5}$ before the signal of the minority isotope vanishes into the random noise of the recorder. The precision of a measurement is of the order of a few percent. In the particular use of the ion-probe microanalyser which we describe here, we have purposely defocused the beam of the bombarding particles so that it could cover entirely each droplet studied. Under these conditions, about 10 successive measurements could be made on each droplet before it was completely erased by the projected ions. The variation coefficient (100 × S.D./mean value) of the results thus obtained for each droplet was of the order of 5%. Taking into consideration the dilution effect of sampling, the ^6Li-contamination of the ^7Li-enriched isotope and the ^7Li-contamination of the ^6Li-enriched isotope, it was easy [3,5] to express the isotopic ratios, given by the ion probe, in terms of moles of lithium unidirectionally exchanged. The corresponding mean unidirectional lithium fluxes were then expressed in A · cm^{-2}.

Results and Discussion

Table I gives the values obtained for the four unidirectional fluxes under the short-circuited conditions. From these data, it is possible to calculate the overall ($Li^+ + Na^+$) current, and to compare it to the recorded electric intensity (Fig. 1). The results obtained for periods longer than 1.5 h should chiefly be considered, because during the first interval of time (0—1.5 h) it is likely that the steady-state conditions were not immediately satisfied. In fact, even after 1.5 h, the electric intensity, I, declined steadily during the experiment, instead of remaining stable. This was probably due to the combined effect of the short-circuiting conditions and the presence of lithium in the internal medium. Even so, the estimated overall current of sodium and lithium ($J^{ei}_{Na} + J^{ei}_{Li} - J^{ie}_{Na} - J^{ie}_{Li}$) appeared to be always similar in value to the electric current I. This is a direct verification that no ions other than Na^+ and Li^+ were actively pumped. Calculation of the average value for the flux ratio of sodium in the presence of lithium, between 1.5 and 9 h, gives 8545 J · mol^{-1}. This is of the same order as is usually found without lithium [6]. Hence, under the present experimental conditions

TABLE I

ESTIMATION OF THE UNIDIRECTIONAL IONIC FLUXES

The resultant error for each unidirectional flux measurement was estimated with the aid of differential calculation: (a) from the errors of the volume determinations (pipettes, microsyringes) (b) from the standard deviations for the measurements of isotopic ratios ($^6Li/^7Li$) and of sodium radioactivity, these standard deviations being considered as the degree of error involved in the corresponding measurements. From the data in the table, it can be calculated that the concentrations of ^{24}Na and ^6Li in compartment e and those of ^{22}Na and ^7Li in compartment i changed less than 2% over the 9 h of the experiment. The errors on the four unidirectional fluxes, due to the backflow of each tracer, can thus be considered negligible.

Time interval	Sodium influx J^{ei}_{Na} $\mu A \cdot cm^{-2}$	Sodium efflux J^{ie}_{Na} $\mu A \cdot cm^{-2}$	Lithium influx J^{ei}_{Li} $\mu A \cdot cm^{-2}$	Lithium efflux J^{ie}_{Li} $\mu A \cdot cm^{-2}$
0 —1.5	36.4 ± 0.7	0.9 ± 2.8	4.5 ± 0.7	0.2 ± 0.8
1.5—3	24.3 ± 0.6	0.8 ± 0.6	3.7 ± 0.7	2.3 ± 0.9
3 —4.6	24.3 ± 0.8	0.8 ± 0.6	4.2 ± 0.7	1.3 ± 0.5
4.6—6	21.3 ± 0.6	0.9 ± 0.6	4.1 ± 1	2.7 ± 1.3
6 —9	12.9 ± 0.3	0.8 ± 0.3	3.1 ± 0.7	2.0 ± 0.8

(80% Na^+, 20% Li^+), lithium did not seem to inhibit significantly the active transport of sodium. As there was net influx of lithium in the short-circuited conditions this is consistent with lithium being actively transported.

A final interesting point is that lithium efflux (J^{ie}_{Li}) was very large. In the present experiment it was more than twice as large as sodium efflux (J^{ie}_{Na}), although lithium was only one quarter as concentrated as sodium. When the experiment was reproduced [5], the measurements being made with the same ion-probe method, Li^+ efflux was even larger than Li^+ influx. High values of

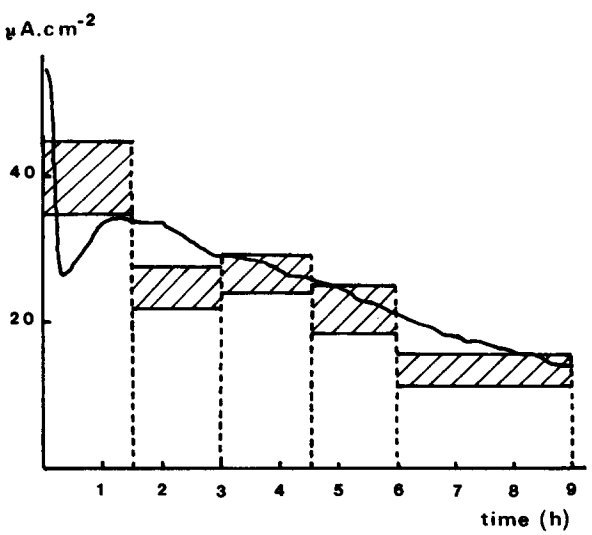

Fig. 1. Electric intensity and overall ionic current through the frog skin under short-circuited conditions. The recorded electrical intensity is represented by the solid line. For each interval of time, the mean value of the overall ionic current has been calculated from the data of Table I by the algebraic sum: $J^{ei}_{Na} + J^{ei}_{Li} - J^{ie}_{Na} - J^{ie}_{Li}$. The shaded area corresponds to the deviation of the estimate, as obtained by summing up the deviations (given again by Table I) for the different unidirectional fluxes.

lithium efflux had also been found in three previous experiments, using the nuclear reaction method for detection of stable lithium isotopes [3]. This means that the overall mechanism of transepithelial transport is not identical for Na^+ and Li^+. The difference could be mainly in the passive transport, especially if lithium has an additional pathway to the outside via the cells, whereas sodium efflux goes chiefly through the cellular interspaces. If the outer membrane of the cell is not more permeable to lithium than it is to sodium [7], an alternative possibility could be a complex interaction between Li^+ and the active pump, with a competitive Na^+/Li^+ in the forward direction [10] and a competitive K^+/Li^+ in the backward direction [11]. This last possibility would allow for lithium efflux sometimes being larger than lithium influx.

Conclusion

Our data support the hypothesis of an active pumping of lithium and they show an unexpectedly high value for the efflux of lithium. The total number of skins studied is five if we consider both the cases when the isotopic measurements on lithium were performed with the ion-probe (the present paper) and with a nuclear reaction (our previous data [3]). This is still a small number, given the functional variability of the frog skin, but at least our data are consistent with each other. In any case, they are based upon unidirectional flux measurements performed, for the first time to our knowledge, with both media in contact with the skin (external and internal) containing a mixture of Li^+ and Na^+ salts.

The most important result is probably the methodological one. Until now, the main application of the ion-probe microanalyser had been to study the local elementary composition of specimens (most often metallurgic), both across their surface and in depth. We have shown here that this apparatus can also be used to obtain measurements of unidirectional fluxes using stable isotopic tracers, with a precision and a sensitivity comparable to that obtained with radioisotopes, when they exist. The method could easily be extended to measure lithium fluxes in other biological materials (erythrocytes, nerve cells, etc.). It could also be extended to measure unidirectional fluxes of elements other than lithium which are also devoid of utilizable radioisotopes (B, N, O, etc.).

Acknowledgements

This work was supported by grants of the CNRS (L.A. 203 and A.T.P. 'Temps') and of the D.G.R.S.T. (77 7 1272). We thank The Nuclear Center of Grenoble for the opportuinity to make the measurements with the ion-probe microanalyser, and especially B. Blanchard who monitored the apparatus. We also thank Dr. Durham for his help in the final preparation of the manuscript.

References

1 Cade, J.F. (1949) Med. J. Aust. 36, 349—352
2 Schou, M. (1968) J. Psychiatr. Res. 6, 67—95

3 Thellier, M., Stelz, T. and Wissocq, J.C. (1976) Biochim. Biophys. Acta 437, 604—627
4 Birch, N., Robinson, D., Inie, R. and Hullin, R. (1978) J. Pharm. Pharmacol. 30, 683—685
5 Garrec, J.P., Jourdan, J., Blanchard, B., Hartmann, A., Lassalles, J.P. and Thellier, M. (1977) C.R. Acad. Sci. Ser. D: 185, 579—582
6 Ussing, H.H. (1960) in Handbuch der Experimentellen Pharmakologie: The Alkali Metal Ions in Biology (Eichler, O. and Farah, A., eds.), pp. 1—195, Springer-Verlag, Berlin
7 Lindemann, B. and Voute, C. (1976) in Frog Neurobiology: Structure and Function of the Epidermis (Llinas, R. and Precht, W., eds.), pp. 170—210, Springer-Verlag, New York
8 Sarrau, J.M., Stelz, T., Ayadi, A. and Faict, A. (1974) C.R. Soc. Biol. 168, 102—107
9 Duval, Y., Meffroy-Biget, A.M., Stelz, T., Hartmann, A. and Thellier, M. (1977) Newsl. Appl. Nucl. Methods Biol. Agric. 8, 5—8
10 Beaugé, L. (1978) Biochim. Biophys. Acta 527, 472—484
11 Pandey, G., Sarkadi, B., Haas, M., Gunn, R., Davis, J. and Tosteson, D. (1977) Proc. Natl. Acad. Sci. U.S.A. 74, 3607—3611