

# **Assessment of inflow rate, fractional outflow rate and steady-state cellular pool of ions based on two measurements of radioactive tracer net uptake**

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*Abstract:* - A simple procedure, based on the measurement of the net uptake of a suitable radioactive tracer by cells incubated for two distinct periods, is proposed to assess the inflow rate, fractional turnover rate and steady-state value at isotopic equilibrium for the cellular pool of selected ions. Emphasis is also placed on the estimation of the parameter of dispersion for each of the variables under consideration. In addition to its great simplicity, the proposed design offer the advantages of indeed allowing the quantification of several parameters providing suitable results for comparison of data obtained under distinct experimental conditions. Potential limitations, e.g. extracellular radioactive contamination, and the suitable approach to correct for such a contamination are also considered.

*Key-Words:* Cellular ionic handling, Experimental design, Inflow-outflow, Fractional turnover rate, Cellular pool

## **INTRODUCTION**

The assessment of the several variables relative to the handling of a given ion (e.g.  $K^+$  or  $Ca^{2+}$ ) by cells is currently based on different types of measurements. First, the inflow rate may be judged from the net uptake of a suitable radioactive tracer over short periods of incubation [1]. Second, the steady-state value for the intracellular pool(s) is often based on the net uptake of the same tracer after prolonged incubation, i.e. when isotopic equilibrium is virtually reached [2]. Last, the estimation of fractional outflow rate may require the measurement of effluent radioactivity from prelabelled and perfused cells [3]. Even so, some limitations of these experimental procedures should not be ignored. To cite only one example, the fractional turnover rate of the intracellular pool(s) for the ion under consideration may be too high to allow a reliable estimation of fractional outflow rate in prelabelled and perfused cells.

The major aim of the present report is to draw attention to a simple experimental design that could be used to characterize the above mentioned variables, with emphasis on both the estimation of the parameter of dispersion for each variable under consideration and the unavoidable limitations of such a design.

## **MATERIALS AND METHODS**

The few experimental data used to illustrate the proposed experimental design were taken from a recent study of  $^{45}\text{Ca}^{2+}$  net uptake by rat pancreatic islets incubated for 10 and 60 min in a salt-balanced medium containing 8.3 mM D-glucose (unpublished data). The net uptake of  $^{45}\text{Ca}^{2+}$  was measured after separation of the islets from the surrounding incubation medium by centrifugation through a layer of dibutylphthalate [1].

## **RESULTS**

Let us assume that the net uptake ( $U$ ) of a radioactive tracer (e.g.  $^{45}\text{Ca}^{2+}$ ) by a given tissue sample (e.g. an isolated pancreatic islet), expressed by reference to its specific radioactivity in the incubation medium (e.g. dpm/pmol), is ruled, as a function of the length of incubation ( $t$ , expressed in min) by equation (i) :  $U = U_{\max} (1 - e^{-Kt})$ , in which  $U_{\max}$  represents the maximal uptake at isotopic equilibrium and  $K$  the fractional turnover rate of the cellular calcium pool (expressed as  $\text{min}^{-1}$ ). Since  $U$  can be measured, for instance after 10 and 60 min incubation ( $U_{10}$  and  $U_{60}$ ), the question is raised how to calculate  $U_{\max}$  and  $K$  from the experimental measured values. For instance, in a study of  $^{45}\text{Ca}$  net uptake by rat pancreatic islets incubated in the presence of 8.3 mM D-glucose, the values for  $U$  ( $\pm$  SEM) averaged, after 10 and 60 min incubation,  $3.47 \pm 0.20$  and  $6.54 \pm 0.47$  pmol/islet respectively ( $n = 27$  in both cases).

From equation (i), it can be derived (equation ii) that  $U_{10}/U_{60} = (1 - e^{-10K})/(1 - e^{-60K})$ . It is obvious, therefore, that the calculation of K is independent of the value for Umax. Yet, equation (ii) does not allow the calculation of K from  $U_{10}$  and  $U_{60}$  by an arithmetic approach. In other words, the value of K, and hence that of Umax, can only be found by successive approximations, using for instance an appropriate computer approach. Such an approach yielded, in the example mentioned above, for Umax and K mean values of 6.62 pmol/islet and  $7.43 \cdot 10^{-2} \cdot \text{min}^{-1}$ . The rate of  $\text{Ca}^{2+}$  inflow and outflow, at isotopic equilibrium, amounts in this example to  $6.62 \times 7.43 \cdot 10^{-2}$ , i.e.  $0.492 \text{ pmol} \cdot \text{min}^{-1}$  per islet.

The next question concerns the value of the dispersion parameter (e.g. SEM) for Umax and K

In the example mentioned above, the SEM on the  $U_{10}/U_{60}$  ratio can be calculated as being equal to  $\sqrt{(0.20/3.47)^2 + (0.47/6.54)^2}$ , i.e. 0.0921 times the  $U_{10}/U_{60}$  ratio (i.e. 0.5306) or 0.0489. Incidentally, if the numbers of individual determinations ( $n = 27$  in our example) were not identical in the two sets of measurements made after 10 and 60 min incubation, the following equation (iii) should be used to calculate the SEM on the  $U_{10}/U_{60}$  ratio (SEMr) from the experimental values (mean  $\pm$  SEM) of  $U_{10} + \text{SEM}_{10}$  ( $n = x$ ) and  $U_{60} + \text{SEM}_{60}$  ( $n = y$ ):

$$\text{SEMr} = \sqrt{\{[(\text{SEM}_{10} / U_{10})^2 \cdot x \cdot (x - 1)] + [(\text{SEM}_{60} / U_{60})^2 \cdot y \cdot (y - 1)]\} \cdot [(x + y) / (x + y - 2)] \cdot [1 / (x \cdot y)]}$$

( $U_{10}/U_{60}$ ).

Since the SEM on the  $U_{10}/U_{60}$  ratio is close to 9.21 % of such a ratio, it can be estimated from equation (ii) that the SEM on the mean K value ( $7.43 \cdot 10^{-2} \cdot \text{min}^{-1}$ ) is close to  $1.02 \cdot 10^{-2} \cdot \text{min}^{-1}$ . This estimation is reached as follows. Taking into account both the mean K value of  $7.43 \cdot 10^{-2} \cdot \text{min}^{-1}$  and the SEMr on the  $U_{10}/U_{60}$  ratio (i.e. 0.0489), the lower and upper limits (mean  $\pm$  SEM) for  $(1 - e^{-10K})$  are 0.4760 and 0.5726. The corresponding values for  $e^{-10K}$  are 0.5240 and 0.4274, yielding K values of 0.0646 and  $0.0850 \text{ min}^{-1}$ . The latter two

values differ by 0.0097 and 0.0107 from the mean K value of  $0.0743.\text{min}^{-1}$ . Hence, the SEM on such a K value is indeed close to  $(0.0097 + 0.0107)/2$ , i.e.  $0.0102.\text{min}^{-1}$ .

Taking into account the mean value for  $U_{10}$  (3.47 pmol/islet) and the lower and upper estimation of K, the corresponding limits for  $U_{\text{max}}$  are 7.29 and 6.06 pmol/islet, as distinct from a mean value of 6.62 pmol/islet. The SEM on  $U_{\text{max}}$  can then be taken as about  $(0.67 + 0.56)/2$ , i.e. 0.62 pmol/islet.

In these estimations of the SEM on both K and  $U_{\text{max}}$ , the d.f. should be taken as  $(x + y - 2)$ , i.e. in our example 52. With these informations available, it then becomes possible to assess the statistical significance of differences between the values for K and  $U_{\text{max}}$  found under different experimental conditions, e.g. at different D-glucose concentrations or in islets prepared from rats in distinct metabolic or hormonal situations.

A last point merits to be underlined. Under steady-state conditions, i.e. at isotopic equilibrium, the inflow and outflow rate of  $\text{Ca}^{2+}$  represents the product of  $U_{\text{max}}$  times K, i.e.  $0.492 \text{ pmol}.\text{min}^{-1}$  per islet (see above). Since, in relative terms, the SEM on either K ( $1.02/7.43 = 13.7 \%$ ) or  $U_{\text{max}}$  ( $0.62/6.62 = 9.4 \%$ ) are not vastly different from one another, an averaged percentage (in this case 11.6 %) could be used to estimate the SEM on the inflow and outflow rate. This would then yield a value of  $0.492 \pm 0.057 \text{ pmol}.\text{min}^{-1}$  per islet for such an inflow and outflow rate. As expected from the exponential time course for U, this value is higher ( $p < 0.02$ ) than the alternative estimation of inflow rate based on the  $U_{10}$  value, i.e.  $0.347 \pm 0.020 \text{ pmol}.\text{min}^{-1}$  per islet, the latter estimation being based on the assumption of a strict proportionality between U and t during the first 10 min of incubation.

## **DISCUSSION**

Both the advantages and limitations of the proposed experimental design should be underlined.

The positive aspects of such a design are that it allows from two sets of measurements made after suitable lengths of incubation by a simple radioisotopic procedure to assess the fractional cellular turnover rate and maximal value for the net uptake of the ion under consideration, as well as its monodirectional inflow rate. For instance, in the illustrative example considered in this report, the inflow rate ( $0.492 \text{ pmol}\cdot\text{min}^{-1}$  per islet) was, as expected from the exponential time course for the net uptake of  $^{45}\text{Ca}^{2+}$  somewhat higher than that calculated on the sole basis of the initial measurements made after 10 min incubation (i.e.  $0.347 \text{ pmol}\cdot\text{min}^{-1}$  per islet).

The three following limitations cannot be ignored, however. First, at variance with prelabelled and perfused cells, the present procedure does not allow to characterize the dynamics of changes in ionic handling resulting from a modification in environmental factors, e.g. an increase in D-glucose concentration. Second, the assumption is made of a single intracellular pool for the tracer under consideration. This obviously represents an oversimplification, especially in the case of the cellular handling of  $\text{Ca}^{2+}$ . Third, no correction was introduced for contamination of the tissue sample by extracellular fluid containing the tracer under study. Once again, this cause of error is not negligible with a tracer such as  $^{45}\text{Ca}^{2+}$ , since the apparent intracellular concentration at isotopic equilibrium (in our example about  $6.22 \text{ pmol}$  for an islet with an intracellular  $^3\text{HOH}$  space close to  $2.0 \text{ nl}$ , i.e. close to  $3 \text{ mM}$ ) is not vastly different from the extracellular  $\text{Ca}^{2+}$  concentration, i.e.  $1.0 \text{ mM}$ . Such a situation in turn accounts, but only in part, for the fact that the estimated fractional outflow rate ( $7.4 \cdot 10^{-2}\cdot\text{min}^{-1}$ ) is higher than that found in prelabelled islets placed in a perfusion chamber and examined from the 31<sup>st</sup> min of perfusion onwards [1, 3]. In such a case, a second factor accounting for the lower fractional outflow rate consists in the fact that the effluent radioactivity originates mainly from intracellular pools with a low fractional outflow rate. In considering the latter two objections, it should first be mentioned that a suitable

correction for extracellular contamination can easily be introduced, e.g. by measuring under the same experimental conditions the distribution space of an extracellular marker, e.g. L-[1-<sup>14</sup>C]glucose. Second, in addition to its great simplicity, the present approach offers the advantage of assessing the fluxes of Ca<sup>2+</sup> in cellular pools with a rapid turnover rate, whilst such an information is not readily accessible in prelabelled perfused cells. Last, whenever other ionic fluxes are examined, e.g. the inflow and outflow of K<sup>+</sup>, using <sup>86</sup>Rb<sup>+</sup> as the tracer, the results of the present approach are virtually identical to those obtained in prelabelled perfused islets [4].

In conclusion, therefore, the experimental design and analytical procedure proposed in this account represent useful tools, especially when the purpose of the study is to compare the behaviour of tissue samples (e.g. pancreatic islets) obtained from animals that were exposed *in vivo* to environmental factors susceptible to alter the handling of the ion under consideration.

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