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COMPARISON OF RATES OF ELUTION OF $^{24}Na^+$ FROM THE MUCOSAL AND SEROSAL SIDES OF THE TOAD BLADDER

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SUMMARY

1. The efflux of radioactive Na^+ from the mucosal and serosal sides of the toad bladder in which only the Na^+ transport pool had been labeled was determined between 5 and 19 min after onset of the elution.

2. Kinetic theory predicts that if there were a single compartment from which Na^+ moved to either side of the bladder, then the elution rates from each side, when plotted against time on semi-logarithmic paper, should result in parallel lines.

3. Data from eight bladders were tested for parallelism and the hypothesis of parallelism is rejected for the entire set of lines ($P < 0.005$). It is concluded that the sodium being eluted must be coming from more than one compartment.

4. A statistical method for combining the probabilities of individual experiments of a like kind which test a single hypothesis is well established. It has been infrequently applied to biological data. It was used for statistical analysis of the results of these experiments.

INTRODUCTION

The generally accepted concept of active transport of sodium by the urinary bladder of *Bufo marinus* states that sodium enters a single compartment from the mucosal side and is actively transported from this compartment to the serosal side of the bladder[1]. Recently VANATTA AND BRYANT[2] reported the elution pattern of $^{24}Na^+$ from bladders in which the transport pool of Na^+ had been labeled. They described fast rates of elution occurring from each side and these are virtually complete in less than 5 min. In addition, they describe a slow rate of elution from each side of the bladder, and these rates are best studied after the initial 5 min of elution. The authors could not determine from their data whether the slow rate of elution from the mucosal side and the slow rate of elution from the serosal side were from the same or from different compartments.

This washout technique has been used by FRAZIER AND HAMMER[3] with slight differences in the shape of the chambers. It is noted that the data they published for the period of elution from 7 to 26 min give rates in agreement with the rates obtained in this laboratory[2]. This agreement is encouraging and it reinforces our faith in the validity of the analysis here reported.

This is a report on the determination of the slow rates of elution from each side of eight bladders in a more precise manner and an evaluation as to whether the rates could be from the same compartment. It is concluded that the elution during the time interval involved must be from two different compartments.

METHODS

The methods are as previously described[2] except that the bladder was exposed to $^{24}Na^+$ after it was mounted between the chambers. The specific activity of the Na^+ was 790000–1000000 counts/min per μequiv Na^+. Collections of elution fluid were from 5 to 19 min following the onset of the exposure.

Elution rates were determined in eight consecutive experiments in which all collected samples counted greater than 2 times background, and to a statistical accuracy of at least 3.4 %.

THEORETICAL CONSIDERATIONS

The kinetic theory of the elution of a radioisotope from two sides of a single compartment predicts that the rate of change of the elution of the isotope from the two sides will be identical, under the conditions of this experiment. The above statement can be proven by considering the following model (see Fig. 1).

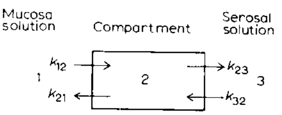

Fig. 1. A model used for kinetic analysis. Compartment 2 represents the portion of the toad bladder which might contribute $^{24}Na^+$ during the 5–19-min elution period.

Let 1 and 3 be the mucosal and serosal baths, respectively.

Let 2 be the compartment from which the isotope is being eluted.

Let k_{21} and k_{23} be rate constants for Na^+ movement out of Compartment 2 as indicated. k_{12} and k_{32} are rate constants for movement of Na^+ into the compartment in question.

P_i = total radioactivity in Compartment i, in counts/min corrected for decay. S_i = total Na^+ in Compartment i in μequiv. P_i^* = specific activity of Na^+ in Compartment i in counts/min per μequiv.

Now throughout the experiment the quantity of Na^+ in the compartment is not changing. Although the Na^+ concentration of the mucosal bath is changed from 60 to 117 mM, it has been established by FRAZIER *et al.*[1] that this does not change the quantity of Na^+ in the transport pool. Therefore, $dS_2/dt = 0$ throughout the experiment.

The amount of radioactivity in the compartment will be changing according to the following:

$$\frac{dP_2}{dt} = -(k_{21} + k_{23})S_2P_2^* + k_{12}S_1P_1^* + k_{32}S_3P_3^* \qquad (1)$$

Since during elution, $P_1^* = 0$, and $P_3^* = 0$, Eqn. 1 becomes

$$\frac{dP_2}{dt} = -(k_{21} + k_{23})S_2P_2^* = -\lambda P_2 \tag{2}$$

Now the rate of isotopic elution into Bath 1 is

$$\frac{dP_1}{dt} = k_{21}S_2P_2^* = k_{21}P_2 \tag{3}$$

and likewise, the elution rate into Bath 3 is

$$\frac{dP_3}{dt} = k_{23}S_2P_2^* = k_{23}P_2 \tag{4}$$

Here we define $\lambda = k_{21} + k_{23}$ and $P_2 = S_2P_2^*$.

It is readily seen that k_{21}, k_{23} and S_2 are all constant, so the change in the rate of elution of the isotope from both sides is due to the rate of change of P_2^*. Therefore, without ambiguity, we can use either P_2 or P_2^* in Eqns. 3 and 4.

A corollary of this statement would be that if the rates of change of the elution of radioactive sodium from the two sides are not the same, after evaluation for experimental errors, then the elution is not from a single compartment. This may be easily seen by solving Eqns. 2, 3 and 4 to obtain

$$P_2 = P_{20} \exp(-\lambda t) \tag{5}$$

where P_{20} is the value of P_2 at $t = 0$. Making substitutions and taking the logarithm of each of the Eqns. 5, 3 and 4 we obtain

$$\begin{aligned} \ln P_2 &= \ln P_{20} - \lambda t \\ \ln\left(\frac{dP_1}{dt}\right) &= \ln(k_{21}P_{20}) - \lambda t \\ \ln\left(\frac{dP_3}{dt}\right) &= \ln(k_{23}P_{20}) - \lambda t \end{aligned} \tag{6}$$

Thus we see a plot of P_2, dP_1/dt and dP_3/dt *versus* time on semi-log paper would result in three parallel lines. Any deviations from parallelism would indicate that the mathematical model is inadequate, perhaps that a two compartment model should be considered.

Although one expects the elution of $^{24}Na^+$ to give rise to equations in P_1 and P_3 that are the integrals of dP_1/dt and dP_3/dt found in Eqns. 3 and 4, an appeal to the relation

$$\int_{t_1}^{t_1+\Delta t} \left(\frac{dP}{dt}\right) dt = \frac{P(t_1 + \Delta t) - P(t_1)}{\Delta t}\,\Delta t \doteq \frac{dP(t_1 + \Delta t/2)}{dt}\,\Delta t$$

will allow the derivative to be used whenever the collection times Δt are small compared to the entire collection time. Therefore, the two equations in (6) including two derivatives are the equations of record for P_1 and P_3.

Eight pairs of equations in ln dP_1/dt and ln dP_3/dt from Eqn. 6 were fitted and with Student's t were tested pair-wise for parallelism. A mixture of "significant" and "nonsignificant" results lead to the following considerations[4].

Let p_j, $j = 1,2,\ldots,8$ be the probability that Student's t will exceed in absolute value the t_j actually calculated when testing for parallelism. That is

$$p_j = Pr\ (|t| > t_j),\ j = 1,\ 2,\ \ldots,\ 8 \tag{7}$$

which are the "tail" probabilities and are considered "significant" whenever p_j is small, say 0 05, 0.02, or 0.01. Now if it is true that the jth pair of lines are parallel, $-2 \ln p_j$ is exactly a X-square with two degrees of freedom. Thus, for the eight pairs of lines, which under the hypothesis are pair-wise parallel

$$X^2(16) = -2[\ln p_1 + \ln p_2 + \ldots + \ln p_8] \tag{8}$$

wherein X^2 (16) is a X-square with 16 degrees of freedom. The hypothesis of parallelism is rejected for the entire set of lines for a large $X^2(16)$. In this case with 16 degrees of freedom a value of 26.3 or 32.0 will reject the hypothesis at the 0.05 or 0.01 level, respectively.

As a last note the above statistical method of combining evidence collected from several independent experiments testing a common hypothesis is not unique to Student's t. It may be applied to any test from a continuous distribution which gives up exact tail probabilities.

Table I illustrates the above method. It is seen on inspection that the X-square is large which requires the rejection of parallelism and suggests that the slow rate of elution from each side must be from at least two different compartments.

TABLE I

RATES OF ELUTION OF $^{24}Na^+$ FROM MUCOSAL AND SEROSAL SIDES OF TOAD BLADDER WITH TEST FOR PARALLELISM

Bladders were exposed to $^{24}Na^+$-labeled Ringer solution in the mucosal chamber for 15 min. Following this exposure elution of $^{24}Na^+$ was determined by allowing a continuous flow of Ringer through chambers on both sides and then collecting samples. Rate constants reported are for the period of elution from 5 to 19 min, zero time being the onset of the elution.

Expt. No.	*Mucosal rate*	*Serosal rate*	*P value for parallelism*	*X-square* $-2\ (\ln P)$
1-21 A	0.0942	0.1208	0.73764	0.608
1-21 D	0.1025	0.0904	0.57938	1.091
2-04 D	0.0565	0.1765	0.01610	8.258
3-04 A	0.0527	0.2224	0.10280	4.550
3-04 D	0.1321	0.1014	0.59114	1.051
3-18 A	0.1665	0.2030	0.38414	1.913
4-01 A	0.2274	0.0744	0.00016	17.481
5-06 D	0.1322	0.1300	0.88344	0.248
Total X-square (df = 16)			$P < 0.005$	35.201

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