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SHORT COMMUNICATION

ANOMALOUS BEHAVIOUR OF ^{86}Rb AS A TRACER FOR TRANSINTESTINAL POTASSIUM TRANSPORT IN THE FOWL, *GALLUS DOMESTICUS*

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Fluxes of potassium ions across biological membranes can be measured using ^{42}K as a tracer. This isotope, however, has a half-life of only 12·4 h and it is expensive. Rubidium belongs to the alkali metal series of elements and rubidium ions behave in many respects, physicochemically and biologically, like K^+. Furthermore, ^{86}Rb has a half-life of 18·7 days and is less expensive than ^{42}K. Thus ^{86}Rb has been used quite widely as a tracer for estimating K^+ transport. In mammals this usage has included erythrocytes (Beauge & Adragna, 1971), the crystalline lens (Becker, 1962), renal tubules (Ellison, Velazquez & Wright, 1986) and large intestine (Tannen, Marino & Dawson, 1986; Freel, 1987). In these instances, the ratios of the measured fluxes, J_K/J_{Rb}, were not found to differ significantly from unity. In non-mammals, ^{86}Rb has been shown to be a useful substitute for K^+ in transport studies using flounder intestine (Frizzell *et al.* 1984), salmon erythrocytes (Bourne & Cossins, 1984) and frog skin (Zerahn, 1983). In fish gills, the ratio J_K/J_{Rb} was consistently found to be about 1·3 (Sanders & Kirschner, 1983), thus allowing for correction of estimates of K^+ flux based on the use of ^{86}Rb as a tracer. In the American silkworm (*Hyalophora cecropia*) gut, this ratio was 1·1 in a flat-sheet preparation (Wood & Harvery, 1979), but in a spherical preparation it was rather unpredictable with a mean of 1·7 (Zerahn, 1980). None of these authors considered ^{86}Rb to be a reliable substitute for ^{42}K. Eddy (1985) has drawn attention to such possible disparities of K^+ metabolism estimated by the use of ^{86}Rb in fish. We have also found differences in estimates of K^+ transport using ^{42}K and ^{86}Rb as tracers in the avian intestine.

Unidirectional fluxes of K^+ and Rb^+ were measured, in both directions, across the jejunum, ileum and large intestine (colon) of White Rock domestic fowl. Intestinal segments were mounted (1 cm^2 surface area) in divided Ussing-type chambers and bathed on each side by 8 ml of a Krebs–bicarbonate solution of the following composition (mmol l^{-1}): Na^+, 141; K^+, 5·9; Ca^{2+}, 1·25; Mg^{2+}, 1·1; Cl^-, 127; HCO_3^-, 25; and glucose, 11. This solution was gassed with 95 % O_2–5 % CO_2. The pH was 7·4 and the temperature was maintained at 38 ± 0·1°C. Paired preparations

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from adjacent regions of the intestine were used, one for influx and the other for efflux measurements. Electrical short-circuit conditions were maintained with an automatic voltage clamp (DVC-1000 World Precision Instruments, see Grubb, Driscoll & Bentley, 1987). Simultaneous flux measurements were performed using ^{42}K and ^{86}Rb (New England Nuclear, Boston MA) as tracers. The isotopes ($0{\cdot}5\,\mu Ci\,ml^{-1}$) were simultaneously added to the same side of the preparation (the 'hot' side) and samples were collected from the opposite side for four successive 15-min periods. The relative contributions of the cellular and paracellular pathways to the movement of each ion appeared to be constant during the measurements, since the J_K/J_{Rb} ratios did not change over the four 15-min sample periods. These samples, as well as those from the opposite hot side, were initially counted in a scintillation counter (LKB, model 111), using a commercial scintillation cocktail, before being allowed to decay for 6 days. The radioactivity due to the ^{42}K had then declined to background levels and the samples were recounted by the same method for the residual ^{86}Rb. The latter was corrected for decay back to the time of the initial counting.

When using ^{86}Rb as a tracer, many investigators use the concentration of K^+ in the media to calculate the specific activity of Rb^+ and thus the apparent K^+ flux (see, for instance Sanders & Kirshner, 1983; Bourne & Cossins, 1984; Ellison *et al.* 1986; Tannen *et al.* 1986). This practice will give quantitatively correct results only if Rb^+ behaves identically to K^+ in the tissue. In an alternative procedure using ^{86}Rb, some investigators add nonisotopic Rb^+, in partial or complete substitution for K^+ (Beauge & Adragna, 1971; Wood & Harvey, 1979; Zerahn, 1980; Tannen *et al.* 1986). Then using the 'cold' Rb^+ (or a combination of Rb^+ and K^+), the specific activity of ^{86}Rb was calculated. Again, this will result in quantitatively correct K^+ flux values only if the tissue treats Rb^+ and K^+ identically. In our study, we used the concentration of 'cold' K^+ to calculate the specific activity for the Rb^+ fluxes. When comparing flux ratios it does not matter whether the ratios are calculated on the basis of the percentage of hot counts transported or absolute fluxes in terms of $\mu mol\,cm^{-2}\,h^{-1}$, as all units will cancel out.

In this study, we compared the ratio of K^+ flux to Rb^+ flux to determine whether ^{86}Rb is a suitable tracer for K^+. Four 15-min flux periods were analysed, but the first 15-min period was not used in the data analyses. An analysis of variance (two-way) was used for the succeeding three periods. In none of the three intestinal segments studied did the ratios change with time, thus the values from the three 15-min sample periods were pooled and the means ± S.E.M. are shown (Table 1). In the jejunum there was no difference between the ratios of the K^+/Rb^+ flux (J_K/J_{Rb}) for the unidirectional fluxes in either direction. However, both ratios were significantly greater than 1·0, indicating that K^+ is transported at a significantly greater rate than is Rb^+. In the ileum and colon, the J_K/J_{Rb} ratios also exceeded 1·0 and it was interesting to observe that this ratio for the fluxes in the direction serosa-to-mucosa significantly exceeded those in the direction mucosa-to-serosa. The direction of the active K^+ secretion was from serosa to mucosa in both colon and ileum. In the jejunum, where the ratio in the direction $J_{m\rightarrow s}$ did not differ from the ratio $J_{s\rightarrow m}$, no

Table 1. *Comparison of ratios of* ^{42}K *to* ^{86}Rb *flux in the fowl* (Gallus domesticus) *intestines* in vitro

	Ratio J_K/J_{Rb}		
	Jejunum	Ileum	Colon
Mucosal → serosal ($J_{m\to s}$)	1·92 ± 0·33 ($N = 24$)	1·62 ± 0·26 ($N = 48$)	1·49 ± 0·25 ($N = 27$)
Serosal → mucosal ($J_{s\to m}$)	1·49 ± 0·18 ($N = 24$)	2·38 ± 0·27 ($N = 48$)	3·12 ± 0·53 ($N = 27$)
Statistical significance of ratios			
$J_{m\to s}$ *vs* $J_{s\to m}$	NS	$P < 0·001$	$P < 0·001$
Difference from 1·0			
$J_{m\to s}$	$P < 0·025$	$P < 0·025$	$P < 0·05$
$J_{s\to m}$	$P < 0·001$	$P < 0·001$	$P < 0·001$

Means are shown ± S.E.M. The numbers shown in parentheses are the number of observations made over three flux periods.

To determine actual number of preparations (from different birds) divide N by 3.

NS, not significant.

active K^+ transport appears to occur (data not shown). These differences in the ratio of the estimated unidirectional fluxes of K^+ and Rb^+ may reflect differences in the nature of the transport processes and the pathways, including the contribution of paracellular and intracellular routes in each direction across these tissues. As J_K/J_{Rb} differed significantly from 1·0 in all portions of the avian intestines studied, and in the colon and ileum the two unidirectional flux ratios differed significantly from each other, it does not appear that ^{86}Rb is a tracer suitable to give quantitatively accurate K^+ fluxes in these preparations.

If the variability in the ratio J_K/J_{Rb} is relatively small, a correction is sometimes applied in order to estimate the true flux of K^+ (for instance in fish gills, Sanders & Kirschner, 1983), but in other instances (for example in silkworm gut, Wood & Harvey, 1979; Zerahn, 1980) this adjustment is not considered to be practicable except for 'semi-quantitative work' (Wood & Harvey, 1979). In the fowl gut we also do not consider the application of such a correction factor appropriate, because of variability in the ratio J_K/J_{Rb} and because it depends on the direction of the flux measurements, $J_{s\to m}$ or $J_{m\to s}$. However, it may still provide a convenient procedure for initial screening experiments and it is notable that a process of net secretion of Rb^+ is still apparent in the colon and ileum, though it is somewhat less than that observed in measurements using ^{42}K. Eddy (1985) expressed reservations concerning the use of ^{86}Rb as a tracer for K^+ in some studies on fish. Interspecific and intertissue differences in fluxes of K^+ estimated by using ^{86}Rb as a tracer may be quite widespread and reflect differences in the intimate nature of the K^+ transport process in each instance.

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