Phospholipase C Effect on Water, Sodium, Potassium Content and Amino Acid Transport of Kidney Cortex Slices[1] (35882)

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Transport of amino acids by kidney tubules is in part dependent upon the movement of Na and is ouabain inhibitable thus suggesting the importance of $(Na^+ + K^+)$-ATPase activity in the generation of the carrier system for amino acids (1). Recent work with a $(Na^+ + K^+)$-ATPase .preparation derived from rat kidney cortex indicated that phosphatidyl serine is essential for the activity of that enzyme (2). The inhibition of reabsorption of fluid droplets infused into rat proximal tubules (3) and the marked decrease of Rb^+ transport by kidney cortex tubules (4) after phospholipase C treatment implicate membrane associated phospholipids in the transport of monovalent cations.

In this study these relationships were further explored by examination of the effects of phospholipase C treatment of rat kidney cortex slices in terms of distribution of water, sodium, potassium and α-aminoisobutyric acid.

Materials and Methods. Male Sprague-Dawley rats (Simonson), weighing 200–225 g, were utilized in all experiments. Two 0.4-mm cortical slices obtained with a Stadie-Riggs microtome were utilized from each pole of the decapsulated kidneys after discarding the first slice. Incubations were performed in 25-ml Erlenmeyer flasks using 2 slices/2 ml of Krebs-Ringer bicarbonate buffer (KRB), pH 7.4 flushed with 95% O_2 and 5% CO_2 at 37° for 60 min after an initial preincubation at 22° for 10 min. Phospholipase C, *Clostridium welchii,* (Sigma, Type 1; 1 mg liberates 3.7 μmoles of water-soluble organic phosphorus from egg yolk lecithin/min at pH 7.3 at 37°) was added to KRB at concentrations of 7.5 or 75 μg/ml. In some instances, phospholipase C was dissolved in KRB and heated at 100° for 5 min prior to adding to KRB for the incubation with renal cortical slices.

Total tissue water was determined after drying 18 hr at 105° in a vacuum oven and expressed as percentage dry weight. Extracellular fluid volume was determined by incubation of the slices with 0.2 μCi/ml of [^{14}C] carboxy-inulin (New England Nuclear). Intracellular fluid (ICF) equals total tissue water minus extracellular fluid and is expressed as percentage wet weight. The distribution ratio of [^{14}C] 1-α-aminoisobutyric acid ([^{14}C]-AIB) (New England Nuclear) represents

$$\frac{\text{ICF (cpm/ml)}}{\text{incubation fluid (cpm/ml)}}$$

and is determined after incubation (37°, 60 min) using 0.25 μCi of [^{14}C] AIB/2 ml of KRB, quickly rinsing the slices in 0.9% NaCl, blotting gently, and extracting the isotope from the tissue by boiling it 10 min in 2 ml of distilled water. Efflux of [^{14}C] AIB from prelabeled slices (KRB, 37°, 60 min) was determined after rinsing twice in 0.9% NaCl and reincubation in KRB with, or without, added phospholipase C. One hundred microliters of incubation fluid were removed at each time interval for determination of radioactivity and the flasks were regassed prior to reincubation. The data were expressed as a fraction of the amount of [^{14}C] AIB present at 0 time at each inter-

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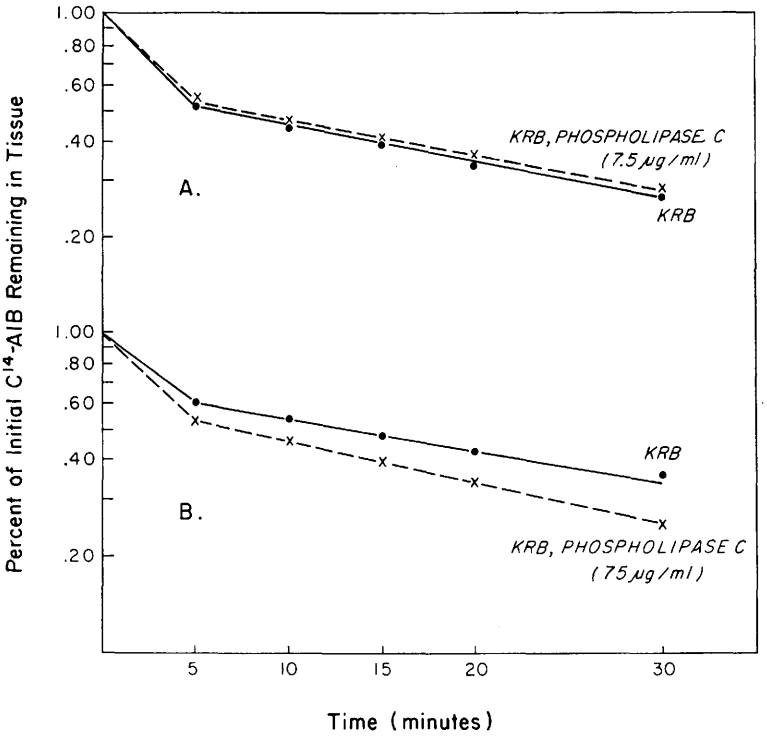

FIG. 1. Efflux of α-aminoisobutyric acid from prelabeled kidney cortex slices with and without varying concentrations of phospholipase C. Each point represents the mean of 3 incubations.

val. The slope was calculated by the method of least squares.

Isotopes were counted by liquid scintillation techniques using 200-µl aliquots of incubation media and of tissue extract in Fluoralloy containing 20% Bio-Solv solubilizer (Beckman). Na^+ and K^+ content of incubated slices was determined by flame photometry with internal lithium standards after extraction of dried slices with 1.0 ml of 1.0 *N* nitric acid for 48 hr at room temperature.

The significance of all data was calculated using Student's *t* test.

Results and Discussion. The effects of varying concentrations of phospholipase C upon kidney cortex slices are described in Table I. A concentration of 75 µg/ml of the enzyme led to increases of both total tissue water and inulin space with marked increase of sodium content and reduction of potassium content of intracellular fluid. The reduction of the distribution ratio of AIB is also associated with a significant increase in the rate of efflux ($p<.05$) (Fig. 1) thus providing additional evidence of alteration of passive permeability by that concentration of phospholipase C.

On the other hand, incubation of kidney cortex slices in the presence of 7.5 µg/ml of phospholipase C (pretreated at 100° 5 min) was associated with no alterations of either total tissue water, inulin space, or Na^+ content. However, the K^+ content of the slices was significantly decreased. This observation is analogous to the observation that isolated renal tubules incubated with phospholipase C have decreased ability to concentrate Rb^+ (4) which is associated with instead of: despite the absence of a significant reduction of $(Na^+ + K^+)$ ATPase activity of either whole homogenates or crude membrane fractions from the treated tubules (5). A slight increase in total tissue water was noted in kidney cortex slices which were treated with 7.5 µg/ml of phospholipase C, which was not preheated. This may be due to other

TABLE I. Phospholipase C Effects on Kidney Cortex Slices.[a]

	KRB	KRB, PLC[b]	p	KRB	KRB, PLC[c]	p	KRB	KRB, PLC[d]	p
Total tissue water (%)	77.8 ± 1.0	83.8 ± 1.0	<.005	78.1 ± 0.5	80.0 ± 0.6	<.005	78.9 ± 0.5	78.1 ± 1.0	>.05
Inulin space (%)	29.4 ± 1.4	34.4 ± 3.1	<.05	25.3 ± 1.6	26.3 ± 1.6	>.05	24.1 ± 1.6	24.8 ± 1.6	>.05
[^{14}C] AIB distribution ratio	3.73 ± 0.57	0.91 ± 0.05	<.001	4.33 ± 0.88	2.48 ± 0.57	<.025	4.29 ± 0.31	2.88 ± 0.27	<.005
Sodium content (mEq/kg of ICF)	105 ± 16	137 ± 9	<.001				128 ± 8	130 ± 6	>.05
Potassium content (mEq/kg of ICF)	94 ± 5	28 ± 2	<.001				85 ± 4	73 ± 6	<.05>.01

[a] Total tissue water, inulin space, [^{14}C] AIB distribution ratio and Na^+ and K^+ content were determined after 60 min of 37° incubation of rat renal cortical slices in Krebs–Ringer bicarbonate buffer in the presence or absence of phospholipase C. All values represent the mean ± SD of 5 incubations.

[b] Phospholipase C, 75 μg/ml of KRB.

[c] Phospholipase C, 7.5 μg/ml of KRB.

[d] Phospholipase C, 7.5 μg/ml of KRB; PLC was dissolved in KRB and heated at 100° for 5 min prior to addition to incubation fluid.

enzymes which may contaminate the enzyme preparation (6).

Whereas there was a significant reduction of the ability of the treated slices to concentrate AIB after treatment with 7.5 μg/ml of preheated phospholipase C, there was no alteration in the rate of efflux of AIB from prelabeled slices (Fig. 1).

This study indicates an effect of phospholipase C on the transport of AIB by rat kidney cortical slices without alteration of permeability thus suggesting a phospholipid dependency of membrane function as related to the transport of that amino acid. This is in contrast with the reported lack of an effect of similar treatment of kidney tubules upon the transport of galactose (4). The probable relationship of reduction of potassium content of treated slices to reduction of Rb^+ uptake (4) and ATPase activity (5) provides further support for the potassium dependency of amino acid transport (7) and hence of the involvement of $(Na^+ + K^+)$ ATPase activity in the transport of amino acids by the kidney.

Summary. Treatment of rat kidney cortex slices with phospholipase C at high concentrations leads to increased total tissue water, inulin space, reduced α-aminoisobutyric acid (AIB) uptake as well as increased efflux. These observations are concluded to represent alterations in permeability characteristics of the slices. Treatment with doses of phospholipase C that cause no alteration in distribution of water or sodium is associated with reduction in potassium content of slices and reduction in AIB accumulation which is not associated with altered efflux. The relationships of the role of phospholipids to renal tubule transport systems for monovalent cations and amino acids are discussed.

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