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MYO-INOSITOL TRANSPORT IN SLICES OF RAT KIDNEY CORTEX

II. EFFECT OF THE IONIC COMPOSITION OF THE MEDIUM

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SUMMARY

The effect of changes in the ionic composition of the incubation medium on the active uptake and retention of *myo*-inositol by cortex slices from rat kidneys has been assessed. The Na^+ requirement could not be satisfied by Li^+, K^+, Rb^+, Cs^+, choline$^+$ or Tris$^+$. K^+ (> 4 mM) was necessary for optimal uptake and retention of inositol, but could be replaced completely with Rb^+. Cs^+ was only partly effective in place of K^+, whereas Li^+ and NH_4^+ were ineffective. The effects on inositol uptake and leakage produced by the substitution of choline$^+$ for Na^+ were largely reversible, indicating that choline had not permanently damaged the slices.

Neither glucose nor ATP restored the values of the two parameters examined to normal in the absence of K^+ or at low levels of Na^+. Na^+ alone was not able to maintain the integrity of the system.

Both Ca^{2+} and Mg^{2+} at optimal concentrations of 1 mM and 1–2 mM, respectively, were required, but could be replaced by Sr^{2+} concentrations as low as 0.75 mM.

The inhibition of inositol uptake by ouabain in Krebs–Ringer bicarbonate media also occurred, when K^+ was either omitted or replaced by other monovalent cations. It was partly prevented by increased concentrations of K^+, Cs^+ or, especially, Rb^+, but not by Ca^{2+}.

The effects of cations are discussed in terms of the mechanism of active transport of non-electrolytes and cations and of the integrity of renal tubular membranes.

INTRODUCTION

Studies on the active transport of a variety of substances have revealed not only a requirement for Na^+ and coupling with sodium transport, but also an influence of the K^+ concentration in the incubation medium[1–7]. Although no effective substituent for Na^+ has been reported, both Rb^+ and Cs^+ are at least partly effective in replacing K^+. In addition to these monovalent inorganic cations, requirements for divalent cations have been demonstrated in most metabolic events, although studies directly related to transport systems are less prevalent for Ca^{2+} and Mg^{2+} than for Na^+ and K^+.

Previous experiments have shown that *myo*-inositol uptake into cortical slices

of rat kidney occurs and is dependent on metabolic energy and Na^+ (ref. 8). Parallel observations have also been made on the maintenance of high inositol concentrations in kidney slices, leakage being affected by essentially the same factors which influence the active transport system[9]. This report deals with the effect of changes in the ionic composition of the medium, notably the partial or complete omission of Na^+, K^+, Ca^{2+} and Mg^{2+} and their replacement by other mono- and divalent cations. All four of these physiological ions were required for optimal uptake and minimal leakage of inositol, although replacement of K^+ by Rb^+ and of Ca^{2+} and Mg^{2+} by Sr^{2+} maintained normal operation of the system.

A part of this study has been presented previously[10].

METHODS AND MATERIALS

Incubations. Cortical slices of kidneys from adult male rats were prepared and incubated as previously described[8,9]. The cation content of the Krebs–Ringer bicarbonate media was modified as indicated in the text, tables and figures by increasing, reducing or omitting the salt in question or by replacing it with suitable amounts of the desired substituent. Osmolarity was preserved by adjusting the NaCl concentration, which does not affect the distribution ratios as long as the Na^+ concentration is greater than about 110 mM [8]. When Na^+ was to be replaced, this was achieved by substituting isomolar amounts of the chloride and bicarbonate salts of the cation under study.

The preincubation in the absence of labeled inositol was carried out in all cases for 45 min in medium of the same composition as the one used subsequently for incubation with labeled inositol.

The treatment of slices and media at the end of the incubation period, the deproteinization of the slice extracts and media and the conditions for the determination of radioactivity were unchanged from earlier experiments[9].

Inositol determinations were made by bioassay using *Kloeckera apiculata* as described by CAMPLING AND NIXON[11]. [2-^{3}H]Inositol was obtained from New England Nuclear Corp., Boston, Mass.

Calculations. The distribution ratios for radioactivity (or inositol) are as previously defined, namely, counts/min (or μmoles inositol)/ml intracellular fluid: counts/min (or μmoles inositol)/ml extracellular fluid and were calculated on the assumption that the slices contain 82 % total water and 26 % extracellular fluid[12,13]. Although under certain extreme conditions substantial changes in the size of the fluid compartments occur—for example, in an all K^+ medium the total water increases to 87 % and the extracellular fluid drops to 17 % (ref. 5)—in virtually all media used in this study the changes were minimal and no adjustments were made. Even in all K^+ media calculations based on the fluid volumes in the absence of water imbibition would be at most 20 % too high. However, in those instances where gross changes did take place, as indicated by the weights of slices before and after each of the two incubation periods, dry weights were obtained and extracellular spaces determined with [^{14}C]inulin[12]. The values of the distribution ratios used in the tables and figures represent the averages of two or more experiments. In most instances at least 4 experiments were carried out, giving standard deviations of $\pm$ 5–15 % of the mean value.

RESULTS

Replacement of sodium by other monovalent cations. Previous experiments demonstrated that the establishment of a radioactivity distribution ratio of 7–8 and the prevention of inositol leakage from the slices required the presence of Na^+ (ref. 8) and that both the inositol and the radioactivity ratios decreased progressively as Na^+ was reduced below physiological levels and the deficit made up with choline. These effects are reversible as shown by the data in Table I. Almost twice as much inositol is found in the medium after preincubation in choline media than after precubation in Na^+-media and the inositol appearance is increased when the slices are reincubated in a fresh portion of the same medium. At the same time virtually no radioactivity enters the slices, not even as much as would be expected from free diffusion. Thus, both the ability to take up radioactive inositol and to retain endogenous inositol are affected by the absence of Na^+. Na^+ is capable of partly preventing the leakage and of restoring the ability to establish a radioactivity gradient. The somewhat higher

TABLE I

REVERSIBILITY OF SODIUM REPLACEMENT

Incubations were under standard conditions except for the substitution of $choline^+$ for Na^+ where indicated. Average values are reported. The sum of the amounts of inositol in the two media and in the slices after incubation was set equal to 100%.

Major cation precent during		*% Inositol in medium*		*Distribution ratios*	
Preincubation	*Incubation*	*Preincubation*	*Incubation*	*Radioactivity*	*Inositol*
Na^+	Na^+	9.1	7.7	7.94	240
Na^+	$Choline^+$	9.2	21.1	0.58	76
$Choline^+$	$Choline^+$	15.9	21.0	0.14	65
$Choline^+$	Na^+	17.5	12.9	7.87	139

TABLE II

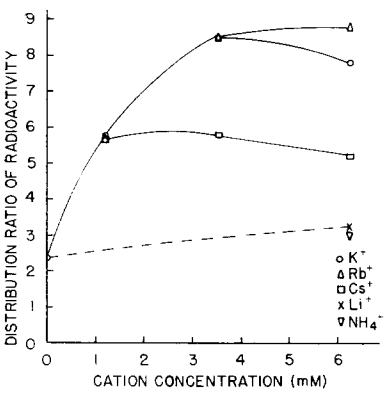
EFFECT OF SODIUM REPLACEMENT

The cation composition of the media was as follows: Na^+ *plus* its replacement, 144 mM; K^+, 6 mM; Ca^{2+}, 2.5 mM; Mg^{2+}, 1.2 mM. Otherwise incubations were carried out under standard conditions. Average values for two or more experiments are given.

Major cation	*Concentration (mM)*	*Distribution ratio*	
		Radioactivity	*Inositol*
Na^+	144	7.05	237
K^+	94	0.78	57
	144	0.10	11
Rb^+	144	0.13	54
Cs^+	144	0.27	78
Li^+	118	1.74	129
	144	0.63	99
NH_4^+	118	0.46	39
$Choline^+$	144	0.21	65
$Tris^+$	144	0.39	134

value for the distribution of radioactivity observed in the Na^+-preincubation, choline$^+$-incubation experiments, although still below 1, may be due to the introduction of a small amount of Na^+ into the incubation medium with the blotted slices. Replacement of Na^+ by other monovalent cations produced similar effects on the two ratios as did choline, although there were small differences between the cations employed (Table II). Potassium exerted the most deleterious effect, preventing inositol uptake and causing the inositol ratio to fall to the lowest level observed under any conditions. A contrasting situation obtained with $Tris^+$ which, although similarly abolishing inositol uptake, was relatively better able to preserve intracellular inositol. Li^+ was relatively less damaging than K^+ and permitted a radioactivity ratio greater than 1 even when it replaced 82 % of Na^+.

Replacement of potassium by other monovalent cations. Although omission of K^+ from the incubation medium substantially reduced the distribution ratios, they did not fall to the low levels seen in the absence of Na^+. Optimal levels of K^+ were about 4 mM, somewhat lower than those in Krebs–Ringer bicarbonate media (Figs. 1 and 2).



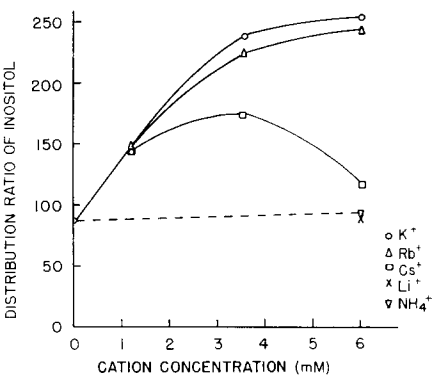


Fig. 1. Effect of variation of the concentration of K^+ and of its replacement with other monovalent cations on the distribution ratio of radioactivity. Incubations were carried out under standard conditions except for the concentration of K^+ or its substituent. Each point is the average of four or more experiments.

Fig. 2. Effect of variation of the concentration of K^+ and of its replacement with other monovalent cations on the distribution ratio of inositol. Conditions as in Fig. 1.

Replacement of K^+ with Cs^+ was effective up to 1.2 mM, but maximal ratios with optimal Cs^+ concentrations of about 3 mM were only 70 % of normal. In contrast, Rb^+ could substitute completely for K^+ and gave identical concentration response curves. At 20 mM, K^+, Cs^+ and Rb^+ gave values virtually the same as those obtained at 6 mM (Fig. 5). Li^+ or NH_4^+ had a minimal ability to maintain the radioactivity or inositol ratio, when used at concentrations isomolar with normal K^+ (Figs. 1 and 2). Intermediate concentrations were not tried, but would seem unlikely to be able to prevent marked changes.

Effect of exogenous energy sources. The phenomena under study require energy production by active metabolism, as shown by their susceptibility to disruption by a variety of agents, but are not materially affected by energy sources added to the medium[8,9]. Nonetheless, it seemed conceivable, that under conditions which prevent

the normal uptake and retention of inositol, an exogenous supply of metabolizable substrate or ATP might, at least in part, restore the functioning of the mechanisms involved. However, neither 10 mM glucose nor 5 mM ATP were able to do so, when K^+ was either completely absent or constituted two-thirds of the Na^+ *plus* K^+ concentration. The latter composition was chosen, since it promotes optimal respiration (high Q_{O_2}) of kidney cortex homogenates, although Ca^{2+} present in the modified Krebs–Ringer bicarbonate buffer used, but absent from the medium employed with the homogenates, is inhibitory[14].

TABLE III

EFFECT OF ALTERED CONCENTRATIONS OF Ca^{2+} *plus* Mg^{2+} AND REPLACEMENT BY Sr^{2+}

Incubations were carried out under standard conditions (see METHODS AND MATERIALS) except for the indicated adjustment of the divalent cation levels. Average values for at least 4 experiments are given.

Cation concentrations (mM)			*Distribution ratio*	
Ca^{2+}	Mg^{2+}	Sr^{2+}	*Radioactivity*	*Inositol*
0	0	0	2.60	48
0.25	0.12	0	7.76	144
0.50	0.24	0	9.58	163
1.0	0.50	0	9.48	319
1.0	1.2	0	10.81	323
2.5*	1.2*	0	7.78	254
2.5	2.4	0	9.00	239
5.0	1.2	0	7.37	184
5.0	2.4	0	7.71	192
0	1.2	2.5	10.62	266
2.5	0	1.2	5.81	262

* Concentrations in Krebs–Ringer bicarbonate media.

Requirement for divalent cations. In view of the apparent role of monovalent cations, it was of interest to test whether a requirement for divalent cations existed as well and, if so, what the specificities, and optimal concentrations were. That it does indeed exist is evidenced by the drop of the distribution ratios for radioactivity and inositol to 2.60 and 48, respectively, in the absence of both Ca^{2+} and Mg^{2+} from the medium (Table III). However, concentrations only one-tenth as great as those in standard Krebs–Ringer bicabonate media permitted normal radioactivity ratios. Doubling the concentration of either or both ions affected the ratios only to a relatively small extent. When K^+ was also left out of the medium, together with Ca^{2+} and Mg^{2+} so that Na^+ was the sole cation, a further deleterious effect was noted with the two ratios decreasing to 0.99 and 30, respectively.

If either Mg^{2+} or Ca^{2+} was omitted and the concentration of the other varied, the curves shown in Figs. 3 and 4 were obtained. Mg^{2+} alone could not take the place of both divalent cations and even at optimal concentrations, between 1 and 2 mM, caused marked reductions of both ratios. Ca^{2+}, on the other hand, could, in the absence of Mg^{2+}, preserve the integrity of the slices in terms of the parameters measured. Lower concentrations were required for the maintenance of the distribution ratio of radioactivity than for that of inositol. Sr^{2+} could be successfully substituted for

both Ca^{2+} and Mg^{2+} and was able to preserve, and perhaps even to raise slightly, both ratios (Figs. 3 and 4) at a concentration as low as 0.74 mM, 20% that of both physiological ions together. The highest ratios were obtained when optimal concentrations of Ca^{2+} and Mg^{2+} were combined (Table III, line 5). A curious, unexplained anomaly was observed, when isomolar Sr^{2+} was used instead of either Ca^{2+} or Mg^{2+} in the presence of the other. The radioactivity ratio was higher than usual, when Ca^{2+} was exchanged for Sr^{2+}, but markedly lower when Mg^{2+} was replaced. In neither case was the inositol ratio altered (Table III).

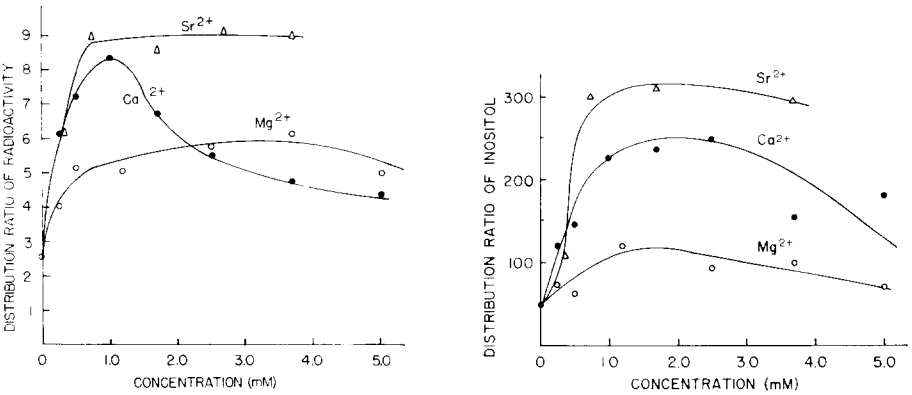


Fig. 3. Effect of the presence of Ca^{2+}, Mg^{2+} or Sr^{2+} as the sole divalent cation on the distribution ratio of radioactivity. Incubations were carried out under standard conditions except for the modification of the divalent cation levels. Each point is the average of four or more experiments.

Fig. 4. Effect of the presence of Ca^{2+}, Mg^{2+} or Sr^{2+} as the sole divalent cation on the distribution ratio of inositol. Conditions as in Fig. 3.

Effect of cation concentration on ouabain inhibition. The inhibition of the uptake of inositol by ouabain has been noted before[8,9] and occurred at concentrations greater than 0.3 mM. However, even at 2.5 mM it was incomplete, permitting distribution ratios of radioactivity between 2 and 3. When K^+-free media were employed, in which the reduction was comparable to that achieved with 2.5 mM ouabain (*cf.* Fig. 1),

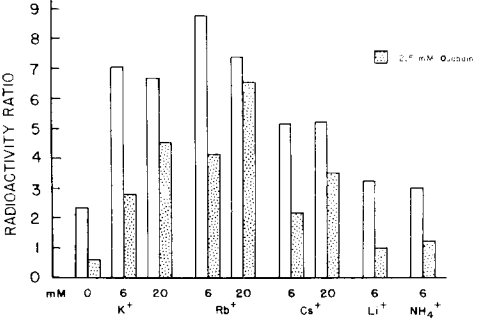


Fig. 5. Effect of K^+ and other monovalent cations on the inhibition of inositol uptake by ouabain. Incubations were carried out under standard conditions except for the concentration of K^+ or its substituent and the presence of ouabain where indicated. Each bar is the average of 2–6 experiments.

the addition of the cardiac glycoside lowered the radioactivity ratio still further, from 2.35 to 0.50 (Fig. 5). This was true also in media where Li^+ or NH_4^+ was used in place of K^+. If, on the other hand, K^+ was raised to 20 mM, the ouabain inhibition was only half as great as with 6 mM K^+ and similar protection was afforded by elevated levels of Rb^+ and Cs^+. However, increases in Ca^{2+} had parallel effects on both ratios under normal conditions and in the presence of ouabain (Table IV). The distribution ratio of radioactivity was lowered by 40–50%, that of inositol by about 60%.

TABLE IV

EFFECT OF ALTERED CALCIUM CONCENTRATIONS ON OUABAIN INHIBITION

Incubations in Krebs–Ringer bicarbonate media with Ca^{2+} concentrations as indicated.

Ca^{2+} (*mM*)	*Distribution ratio*			
	Radioactivity 2.5 *mM ouabain*		*Inositol* 2.5 *mM ouabain*	
	—	+	—	+
0	5.05	2.43	119	75
2.5	7.05	2.79	237	139
5.0	7.37	3.43	184	116

DISCUSSION

The coupling of non-electrolyte transport to that of sodium has been established in many instances and appears to be analogous in the case of inositol. It will not be dealt with further. The uniqueness of the sodium ion in providing the potential for the active uptake and retention of inositol has its parallel in numerous reported instances where monovalent substituents, especially inorganic cations, were tried and found to be ineffective in sustaining active transport (*e.g.*, refs. 5, 15–22). However, not in all instances is transport completely abolished, although a reduction has always been observed. Even when no accumulation against a concentration gradient occurs, sugar entry may be facilitated by Li^+, perhaps through its interaction with a hypothetical sugar carrier[19]. A similar effect may account for the somewhat higher distribution ratios obtained in the present experiments with Li^+ media (Table II) and may be related to the fact that it is the only monovalent cation with an ionic radius lower than that of Na^+ (ref. 23).

The size of the ion cannot provide the entire explanation of its ability to maintain physiological processes as illustrated by the replacement of K^+ with ions of similar radii. While Rb^+ is completely effective, NH_4^+, with the same radius of 1.48 Å as Rb^+, is as ineffectual as Li^+ with a radius of 0.60 Å (Figs. 1 and 2). Rb^+ and Cs^+ are usually thought of as being capable of replacing K^+ (refs. 24, 25), although in our experiments Cs^+ was only partly able to restore normal function.

That K^+ itself is required is not a unique finding. A similar dependence of inositol accumulation has been reported for Ehrlich ascites cells[7] and exists also for the transport of other substances in different tissues[1–6], although K^+ removal may be without effect as well (*e.g.*, ref. 26).

The requirement for both Na^+ and K^+ which has been observed in this and other transport studies may be a reflection of the postulated model for the translocation of amino acids and sugars, notably those of its features which deal with the interaction of the hypothetical carrier, monovalent cations and the sodium pump[27,28]. Physiological extracellular levels of K^+ need to be maintained in order to preserve normal membrane function. The factors involved in this homeostasis and the state of K^+ within kidney slices are complex[29–32], but moderately high K^+ concentrations in the medium (20 mM, Fig. 5) in the presence of normal Na^+ seem to be less detrimental than low ones.

The obligatory presence of K^+ may perhaps be explainable in terms of active cation transport with consequent changes in energy-yielding and consuming processes[26,33,34]. In kidney slices ion transport acts as pace maker for respiration and decreased K^+ uptake is accompanied by a similar decrease in respiration[35–38]. Despite the loss in energy production in the absence of K^+, which is presumably a correlate of these observations, energy sources added to the incubation medium were of no avail in restoring normal distribution ratios. A parallel fall in K^+ concentrations and Q_{O_2} is also seen in the presence of ouabain[37]. This observation may be germane to the ability of increased medium K^+ to counteract the effect of ouabain (Fig. 5, refs. 39–44), perhaps through competition with the glycoside.

Considerably less speculation exists about the role of divalent cations in transport phenomena, but the limited experimental evidence indicates that not all substances are equally dependent on Ca^{2+} for translocation across membranes[1,5,45]. The physical, chemical and biological similarities of Ca^{2+} and Sr^{2+} have been extensively documented[46] with the present experiments adding another instance of comparable behavior.

In kidney slices Ca^{2+} is claimed to have little effect on Na^+ and K^+ transport *per se*[47] (and, in contrast to its action in homogenates[48], none on the respiratory rate[49,50]), but does prevent leakage of cations (and, apparently, of other substances as well), so that in its absence markedly depolarized cells with high Na^+ and low K^+ content are produced[51], similar to those obtained in the absence of K^+ (ref. 52). However, earlier evidence notwithstanding[47], activation of the cation pump by Ca^{2+} may also occur[51] and contribute to preserving the primary asymmetry required for the transport of inositol (and other substances). Both factors may be involved in the mechanism by which Ca^{2+}, Mg^{2+} and Sr^{2+} exert their influence on inositol uptake and leakage.

An additional point, possibly relevant in this context, is that Ca^{2+} is known to act on ATPases and is, in kidney, inhibitory for the Na^+–K^+-dependent component[53,54], while the Mg^{2+}-dependent component is activated[53]. It is not known what the relative importance of these two activities is and whether either is the locus through which divalent cations affect inositol penetration of the tubular membrane. Instead, membrane permeability might be modified, if these ions are available in abnormal concentrations and thus unable to maintain normal crosslinks within and between membrane fragments, as suggested by ROBINSON in the case of microsomal membranes of brain[55]. Further, the importance of calcium in maintaining normal intercellular communications of epithelial cells has been stressed by NAKAS, HIGASHINO AND LOWENSTEIN[56] (See also review by MANERY[57] on the effects of Ca^{2+} on membranes).

In the case of kidney slices, these considerations would apply primarily to the

luminal, rather than the peritubular cell membrane[58,21], since it is presumably this membrane which is involved in the studies reported here. The use of tubular fragments should reveal differences and similarities between the luminal and peritubular membranes in regard to inositol uptake and leakage, which might be phenomena primarily referable to the brush border of the luminal membrane and to the basal cell membrane, respectively.

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