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## Prevention of ouabain-induced potassium loss by incubation of brain slices in low sodium media

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THE CARDIAC glycoside, ouabain, is a known inhibitor of the $Na^+$, $K^+$-stimulated adenosine triphosphatase [($Na^+$, $K^+$)-ATPase] system and also inhibits active $Na^+$ and $K^+$ transport in a variety of tissues, including brain.[1] When added to cerebral slices, this agent brings about a rapid fall in $K^+$ content and a rise in $Na^+$ which takes place somewhat more slowly.[2] This effect is in part reversible so that, when ouabain-treated slices were washed within minutes with ouabain-free medium, pretreatment levels of $K^+$ were restored.[3]

Recently, Albers *et al.*[4] examined the actions of ouabain on the ($Na^+$, $K^+$)-ATPase of *Electrophorus* electric organ and reported effects of $Na^+$ ions on ouabain inhibition of the enzyme and on binding of $^3$H-ouabain to microsomes. At 0°, inhibition of the enzyme in the presence of $Mg^{2+}$ and ATP was much more complete when $Na^+$ was added. Binding of $^3$H-ouabain was also increased by $Na^+$ ions.

The present experiments were carried out with isolated slices of guinea pig cerebral cortex prepared by standard methods.[5] The slices, held in quick transfer holders, were incubated at 37° in medium of the following composition: NaCl, 124 mM; KCl 5 mM; $KH_2PO_4$, 1·24 mM; $MgSO_4$, 1·3 mM; $NaHCO_3$, 26 mM; glucose, 10 mM; and $CaCl_2$, 0·75 mM. The medium was equilibrated with 5% $CO_2$–95% $O_2$. After preincubation for 30 min, the slices were rinsed twice in fresh medium buffered with Tris-HCl (30 mM, pH 7·4) instead of bicarbonate, and choline chloride was substituted for the deleted sodium. When indicated, ouabain ($10^{-4}$ M) was present in the Tris medium.

In the absence of ouabain and at low sodium concentrations, $K^+$ was lost from brain slices (Fig. 1).

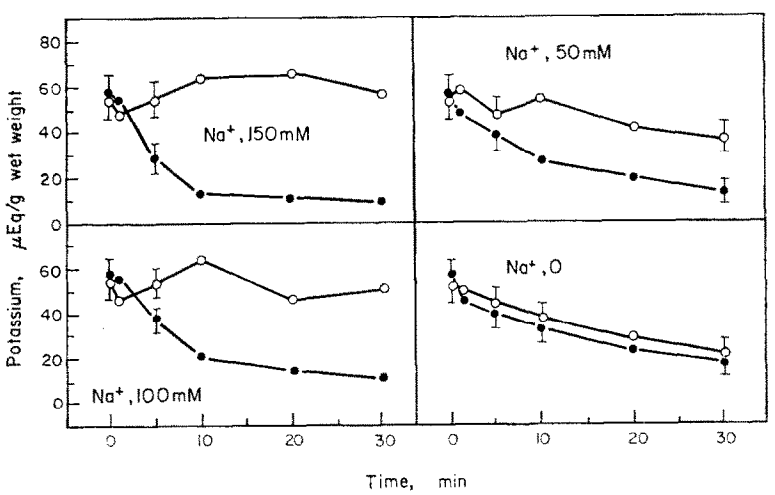


FIG. 1. Time course of ouabain effects on potassium content of guinea pig cerebral cortex slices. Slices were incubated in media containing varying concentrations of sodium ion. The slices were preincubated for 30 min in standard bicarbonate-buffered media which contained 0·75 mM $CaCl_2$. They were rinsed twice in fresh medium and transferred to beakers with oxygenated medium buffered with Tris-HCl, pH 7·4, containing indicated concentrations of NaCl with or without ouabain, $10^{-4}$ M. Choline-chloride replaced NaCl in the low sodium media. Each point represents mean values from two to eight slices, with S.D. bars. The diameter of the point approximates the S.E.M. ○——○, Ouabain absent; ●——●, ouabain ($10^{-4}$ M) present.

After 30 min, the loss of $K^+$ was about 20 per cent at 50 mM $Na^+$ and 60 per cent in sodium-free medium. This effect has been attributed to a less efficient $Na^+$, $K^+$ pump at low $Na^+$ concentrations.[6] However, rather than accelerate the loss of $K^+$, at low $Na^+$ concentrations ouabain ($10^{-4}$ M) became a much less effective inhibitor of cation transport, and in $Na^+$-free media failed to significantly accelerate the loss of $K^+$ from the slice. When the slice contents of $K^+$ in ouabain and control slices were compared after 5 min of incubation, the effect of ouabain on $K^+$ loss was almost negligible when choline-chloride replaced NaCl (Table 1). LiCl as a substitute for NaCl acted like choline-chloride.

TABLE 1. EFFECTS OF OUABAIN ON POTASSIUM CONTENT OF CEREBRAL CORTEX SLICES AT VARYING MEDIUM $Na^+$ CONCENTRATIONS*

| $Na^+$ concn. of medium ($\mu$equiv./ml) | Tissue $K^+$ content after 5-min incubation ($\mu$equiv./g wet wt.) | | |
|---|---|---|---|
| | a Without ouabain | b With ouabain ($10^{-4}$ M) | Ratio b/a |
| 150 | 56·2 ± 1·9 (6) | 29·5 ± 2·4 (6) | 0·52 |
| 100 | 55·5 ± 1·7 (5) | 39·7 ± 1·6 (5) | 0·72 |
| 50 | 51·7 ± 1·0 (8) | 40·3 ± 2·1 (8) | 0·78 |
| 0 | 44·3 ± 0·8 (7) | 43·9 ± 1·8 (7) | 0·99 |

* Slices were incubated as described in Fig 1. Values are means ± S.E.M. with the number of slices in parentheses. $K^+$ levels at zero time were: for column a, 55·9 ± 1·4 (12 slices); for column b, 58·6 ± 2·6 (8 slices).

These results suggest that the observations of Albers *et al.*[7] on subcellular fractions of electric organ are relevant to the effects of ouabain on cell-containing tissues. Ouabain is felt to inhibit ($Na^+$, $K^+$)-ATPase at a site which is in communication with the outside rather than the inside of the cell. Since $Na^+$ is likely to act internally,[7] the requirement of $Na^+$ for the action of ouabain may well indicate a $Na^+$-induced structural change in the ($Na^+$, $K^+$)-ATPase which allows accessibility of ouabain to its site of inhibition.

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