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EFFECT OF CALCIUM, POTASSIUM AND OUABAIN ON THE OXYGEN CONSUMPTION OF EXTERNAL MEDULLA SLICES FROM DOG KIDNEY

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SUMMARY

Slices of external medulla from dog kidney were incubated in Krebs–Ringer solution with variable concentrations of Ca^{2+} ($[Na^+]$ and $[K^+]$ remaining constant) and K^+ ($[Na^+]$ and $[Ca^{2+}]$ remaining constant). It was found that K^+ and Ca^{2+} stimulated Q_{O_2} at low concentrations but inhibited it at high concentrations. The stimulation phase was studied for both cations by using three different Na^+ concentrations. Ouabain inhibited the Q_{O_2} of external medulla slices of kidney and this inhibition diminished with increasing concentrations of K^+ in the extracellular fluid. According to these results the interaction between K^+ and ouabain seemed to be competitive.

INTRODUCTION

The oxidative exergonic reactions occurring in the external medulla are essential for the formation of the renal interstitial osmotic gradient which is primarily made up of Na^+ accompanied by the chloride ion and urea. The present paper deals with the influence of Ca^{2+}, K^+ and ouabain on the O_2 consumption of external medulla slices from dog kidney. This O_2 consumption is basically associated with an active reabsorption of Na^+ (refs. 1–3); and, for this reason, the interaction of the previously mentioned agents with Na^+ has been investigated.

MATERIAL AND METHODS

Dogs were shot in the head, and the kidneys were removed immediately. After being placed on iced glass, the external medulla was cut with a razor blade. The slices were incubated in Krebs–Ringer solution buffered with Tris which also contained glucose and α-ketoglutarate[4].

The total osmolarity of the incubation medium was kept constant by adding choline chloride when needed. The Q_{O_2} was measured in a Warburg respirometer and was expressed in μl O_2/mg dry tissue per h and was transformed as fractions of basal consumption (consumption in a medium without sodium).

RESULTS

Calcium

Slices of external medulla incubated in Krebs–Ringer solution without Ca^{2+} showed a significant lowering in O_2 consumption[5]. In order to study the influence exerted by different extracellular Ca^{2+} concentrations, the Q_{O_2} was measured by incubating the slices in a Krebs–Ringer solution containing a constant Na^+ concentration (300 μequiv/ml) and variable Ca^{2+} concentrations (from 0 to 128.4 μequiv/ml).

When the Ca^{2+} concentration was raised from 0 to 25 μequiv/ml, a sharp increase in O_2 consumption was obtained, while from 25 to 128.4 μequiv/ml, the Q_{O_2} decreased with a shallow slope. The process of stimulation of oxygen consumption was also studied under a constant Na^+ concentration of 150 μequiv/ml and 94.5 μequiv/ml.

Hyperbolic curves were obtained when Q_{O_2} was plotted against $[Ca^{2+}]$ at the three Na^+ concentrations mentioned above. They could be transformed into straight lines by plotting $[Ca^{2+}]/Q_{O_2}$ against $[Ca^{2+}]$ (Fig. 1).

It seemed interesting to investigate whether the correlation between total O_2 consumption and Ca^{2+} concentration was also maintained between suprabasal* Q_{O_2} and $[Ca^{2+}]$. By plotting $[Ca^{2+}]$/suprabasal Q_{O_2} against $[Ca^{2+}]$ for the three Na^+ concentrations used, high correlations were obtained (Fig. 2). The slopes and intercepts of these regressions were linearly correlated with $1/[Na^+]$ (Figs. 8 and 9).

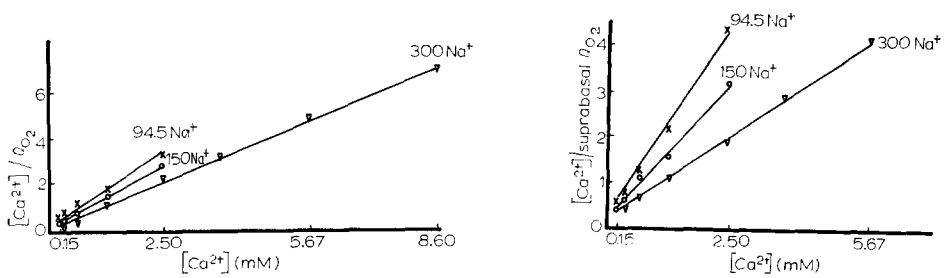

Fig. 1. Regressions of $[Ca^{2+}]$/total Q_{O_2} on $[Ca^{2+}]$ in the stimulation phase. With 300 μequiv Na^+/ml, $r = 0.96$ ($P<0.001$); with 150 μequiv Na^+/ml, $r = 0.99$ ($P<0.001$); with 94.5 μequiv Na^+/ml, $r = 0.99$ ($P<0.001$).

Fig. 2. Regression of $[Ca^{2+}]$/suprabasal Q_{O_2} on $[Ca^{2+}]$ in the stimulation phase. With 300 μequiv Na^+/ml, $r = 0.95$ ($P<0.001$); with 150 μequiv Na^+/ml, $r = 0.94$ ($P<0.001$); with 94.5 μequiv Na^+/ml, $r = 0.91$ ($P<0.001$).

When the correlation between $[Ca^{2+}]$ and Q_{O_2} was studied in the inhibition phase of the curve (25 μequiv/ml$<[Ca^{2+}]<$128.4 μequiv/ml; $[Na^+] = 300$ μequiv/ml), a hyperbolic function was obtained.

By plotting $[Ca^{2+}]\cdot Q_{O_2}$ against $[Ca^{2+}]$, a straight line was obtained (Fig. 3, A). The correlation was also maintained when plotting $[Ca^{2+}]\cdot$suprabasal Q_{O_2} against $[Ca^{2+}]$ (Fig. 3, B).

Potassium

It has been reported that a lack of K^+ in the extracellular fluid causes a significant lowering in Q_{O_2} in rat kidney cortex slices[6]. In order to discover whether the

* Suprabasal Q_{O_2} = total Q_{O_2} *minus* Q_{O_2} obtained in a medium without sodium.

same phenomenon is observable in the external medulla, suitable slices were incubated in Krebs–Ringer both with 5 μequiv K^+/ml and without K^+ during 10 min at 37° (10 ml of Ringer per 100 mg wet tissue) and were washed with the corresponding solution. (These steps were designed to remove the bulk of that ion which might have diffused into the medium during the K^+-free incubation). It was found that depletion of external K^+ causes a definite lowering in Q_{O_2} ($F = 19$; $P<0.001$).

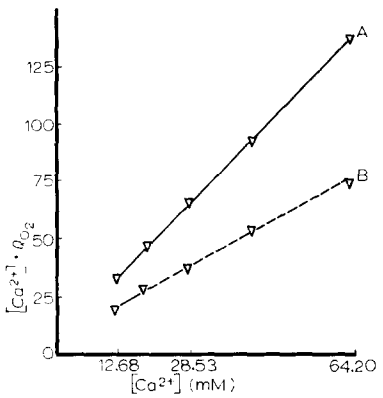

Fig. 3. Ca^{2+}: inhibition phase. (A) Regression of $[Ca^{2+}]$·total Q_{O_2} on $[Ca^{2+}]$; with 300 μequiv Na^+/ml, $r = 0.98$ ($P<0.001$). (B) Regression of $[Ca^{2+}]$·suprabasal Q_{O_2} on $[Ca^{2+}]$; with 300 equiv Na^+/ml, $r = 0.96$ ($P<0.001$).

The effect of variable K^+ concentrations on Q_{O_2} was studied by working with a constant Na^+ concentration of 300 μequiv/ml. O_2 consumption increased as the K^+ concentration was raised from 0 to 5 μequiv/ml, while larger concentrations induced a decrease in Q_{O_2}. In the stimulation phase, a hyperbolic curve was obtained when correlating Q_{O_2} with $[K^+]$. This was transformed into a straight line by plotting $[K^+]/Q_{O_2}$ against $[K^+]$. Similarly, straight lines were obtained when the medium had 94.5 μequiv/ml and 150 μequiv/ml Na^+ (Fig. 4). The effect of K^+ on Q_{O_2} in the inhibition phase was studied under 300 μequiv/ml Na^+; a hyperbolic function was obtained (Fig. 5).

As in the case of Ca^{2+}, the existence of a correlation between $[K^+]$/suprabasal

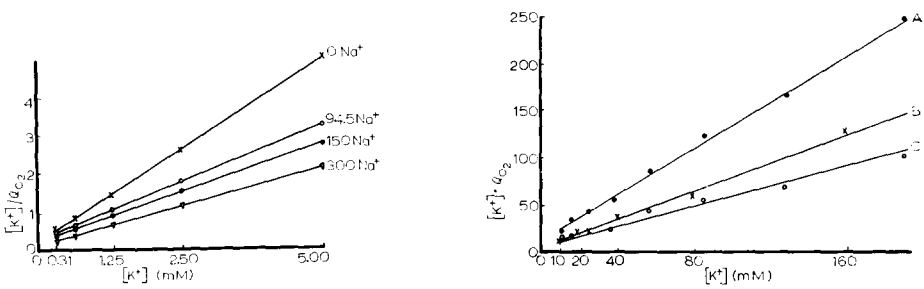

Fig. 4. Regressions of $[K^+]$/total Q_{O_2} on $[K^+]$. With 300 μequiv Na^+/ml, $r = 0.96$ ($P<0.001$); with 150 μequiv Na^+/ml, $r = 0.98$ ($P<0.001$); with 94.5 μequiv Na^+/ml, $r = 0.99$ ($P<0.001$); without Na^+, $r = 0.99$ ($P<0.001$).

Fig. 5. K^+: inhibition phase. (A) Regression of $[K^+]$·total Q_{O_2} on $[K^+]$; with 300 μequiv Na^+/ml, $r = 0.93$ ($P<0.001$). (B) Regression of $[K^+]$·basal Q_{O_2} on $[K^+]$; without Na^+, $r = 0.98$ ($P<0.001$). (C) Regression of $[K^+]$·suprabasal Q_{O_2} on $[K^+]$; with 300 μequiv Na^+/ml, $r = 0.79$ ($P<0.001$).

Q_{O_2} and $[K^+]$ was investigated. Using suprabasal Q_{O_2} instead of total Q_{O_2}, the correlation obtained was insignificant ($P>0.05$). The basal Q_{O_2} was measured by the O_2 consumption in a medium without Na^+ but rather with 5 μequiv/ml of K^+; therefore, K^+ probably could also influence the Q_{O_2} obtained in a medium without Na^+.

In order to investigate this point, the Q_{O_2} was measured while varying K^+ concentration in a medium without Na^+. It was found that when the K^+ concentration was raised from 0 to 10 μequiv/ml, an increase in O_2 consumption was obtained; from 10 to 160 μequiv/ml, there was a decrease in Q_{O_2}. Both curves represented hyperbolic functions and were rectified by plotting $[K^+]/Q_{O_2}$ against $[K^+]$ for the stimulation phase (Fig. 4) (0 μequiv/ml$<[K^+]<$10 μequiv/ml) and $[K^+]\cdot Q_{O_2}$ against $[K^+]$ (Fig. 5) (10 μequiv/ml$<[K^+]<$160 μequiv/ml) for the inhibition phase.

The value calculated from the regression line was then considered as basal oxygen consumption for every K^+ concentration used.

In the stimulation phase, by plotting $[K^+]$/suprabasal Q_{O_2} against $[K^+]$ a good correlation was obtained for the three Na^+ concentrations (Fig. 6). The slopes and intercepts of these regressions were linearly correlated with $1/[Na^+]$ Figs. 8 and 9).

In the inhibition phase, the suprabasal Q_{O_2} was calculated by deducing from the total O_2 consumption the Q_{O_2} obtained for every K^+ concentration in a medium without Na^+. The values obtained, when multiplied by $[K^+]$ plotted against $[K^+]$, gave straight lines (Fig. 5).

Ouabain

It has been reported in different systems[7,8] that ouabain interferes with Na^+ transport and lowers the O_2 consumption.

The interaction of K^+ and ouabain in external medulla slices from dog kidney has also been investigated.

The Q_{O_2} of slices incubated in a medium with ouabain (10^{-3}–$4\cdot10^{-7}$ mmoles/ml) and $[Na^+]$ (300 μequiv/ml), was found to be lower than in the controls (Q_{O_2} without ouabain = 2.43; Q_{O_2} with $4\cdot10^{-7}$ mM ouabain = 1.23).

The interaction between K^+ and ouabain was studied by incubating slices in a medium with 300 μequiv/ml Na^+ and $4\cdot10^{-7}$ mmoles/ml ouabain. The K^+ concentration varied from 5 to 80 μequiv/ml. A definite rise in Q_{O_2} was observed up to 20 μequiv/ml K^+ followed by an inhibition of Q_{O_2} from 20 to 80 μequiv/ml (Fig. 7).

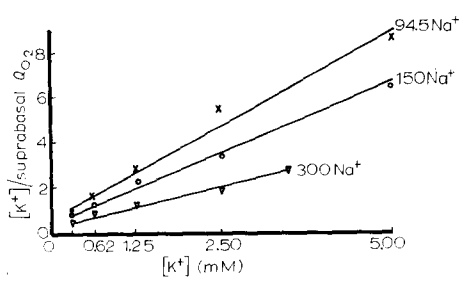

Fig. 6. Regressions of $[K^+]$/suprabasal Q_{O_2} on $[K^+]$. With 300 μequiv Na^+/ml, $r = 0.96$ ($P<0.001$); with 150 μequiv Na^+/ml, $r = 0.90$ ($P<0.001$); with 94.5 μequiv Na^+/ml, $r = 0.95$ ($P<0.001$).

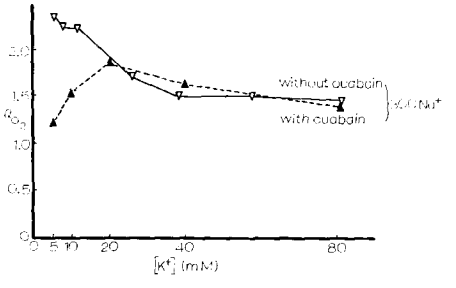

Fig. 7. Relationship between Q_{O_2} and $[K^+]$ in the absence and the presence of $4\cdot10^{-7}$ mmoles/ml ouabain. $[Na^+]$ = 300 μequiv/ml.

In the absence of ouabain, a lowering in Q_{O_2} is observed by varying the K^+ concentration from 5 to 20 μequiv/ml.

The stimulation phase caused by raising the K^+ concentrations with a constant concentration of ouabain may be compared with that obtained in the absence of ouabain. When $[K^+]/Q_{O_2}$ is plotted against $[K^+]$, both regressions have the same slope ($F = 0.03$; $P > 0.10$) but different intercepts ($t = 3.66$; $P < 0.001$).

Also the inhibitory phase in the presence of ouabain may be compared with that obtained in its absence; both lines have the same slope ($F = 0.33$; $P > 0.10$) and the same intercepts ($t = 0.19$; $P > 0.10$).

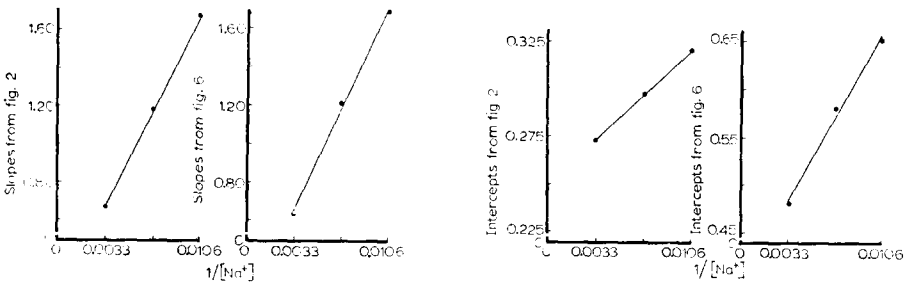

Fig. 8. Slopes from Figs. 2 and 6, plotted against $1/[Na^+]$.

Fig. 9. Intercepts from Figs. 2 and 6, plotted against $1/[Na^+]$.

DISCUSSION

The renal O_2 consumption can be divided into two parts: (1) the basal O_2 consumption which can be determined by the Q_{O_2} of kidney slices in a medium without Na^+ (ref. 1) and in the intact mammalian kidney by the O_2 consumption in the non-filtering and hence non-reabsorbing state[2] and (2) the suprabasal O_2 consumption correlated with Na^+ transportation in cortical slices[1] and also with Na^+ reabsorption in the filtering intact kidney[2].

Stimulation of suprabasal Q_{O_2}

From the experimental results obtained, it could be inferred that in the absence of Ca^+ and K^+ in the extracellular medium the suprabasal O_2 consumption would approach zero. WHITTEMBURY[9] and MACKNIGHT[10] found that slices of kidney cortex incubated in a K^+-free medium lost Na^+. KLEINZELLER *et al.*[11] incubated kidney cortex slices in the absence of Ca^{2+} and found that the slices gained Na^+ and lost K^+, but the Na^+ pump was not affected by the lack of Ca^{2+}. Apparently, the behavior of kidney cortex cells is different from that of external medulla slices.

In a previous work[5,6], a certain suprabasal O_2 consumption was obtained by using an extracellular solution without either K^+ or Ca^{2+}. It could be assumed that Ca^{2+} or K^+, diffusing from the cells to the extracellular fluid although in a small quantity, would be sufficient to justify this small suprabasal Q_{O_2}.

The stimulating effect of Ca^{2+} on Q_{O_2} is in agreement with the results obtained by DUMONT *et al.*[12] while measuring net Na^+ transport in the small intestine of rats and by BENTLEY[13] who reported that reduction of the Ca^{2+} concentration decreased the short-circuit current across the wall of toad bladder. ANDERSON AND TOMLISON[14]

also found that the short-circuit current was reduced when the Ca^{2+} concentration of the solution bathing both sides of the toad bladder was lowered. This effect of Ca^{2+} on epithelial membranes contrasts sharply, as pointed out by BLOND AND WHITTAM[15], with the inhibition of Q_{O_2} caused by the same ion on homogenates of kidney cortex and on ATPase activity of an enzyme preparation from rabbit kidney cortex[16]. These differences, in effect, could be explained[15–17] by supposing that only intracellular Ca^{2+} has an inhibitory effect. As pointed out by OPIT AND CHARNOCK[18], "the whole membrane structure is concerned in the transport process, although not directly involved in the chemical reaction". It seems likely, from our experimental results, that the properties of isolated enzymes could be different from the activity of the enzyme system when located in and precisely orientated at the membrane.

Inhibition of suprabasal Q_{O_2}

From the data obtained in the inhibition phase for Ca^{2+}, it can be assumed that when the "stimulation" sites are filled with Ca^{2+}, additional doses of this ion cause a general "stiffening" or impedance to Na^+ transportation such as postulated by DUMONT *et al.*[12] for rat small intestine, or that when the extracellular Ca^{2+} concentration is high, the intracellular Ca^{2+} concentration also increases and perhaps inactivates the Mg^{2+}-dependent enzyme system.

High K^+ concentrations inhibit ATPase activity of rabbit brain, kidney cortex[19], crab nerve[20] and brain[21] preparations. Apparently in all of these systems, the inhibition kinetics differ from the present experimental results. Probably the presence of intact cellular structures is likely to impose a difference on the total kinetics.

Ouabain

From the present experimental results obtained with external kidney medulla, it could be concluded that K^+ and ouabain compete for the sites in the membrane where K^+ activates the O_2 consumption or perhaps that the competitive inhibition obtained suggests that ouabain and K^+ cannot be attached to the membrane sites at the same time[21]. When all of the sites where K^+ stimulates the O_2 consumption are filled with K^+, the inhibition by an excess of K^+ proceeds as if ouabain were absent from the system.

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