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Inhibition of the oxygen uptake of rat jejunum by Na^+ Deficiency in the medium

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The active transport of sugars by the intestine *in vivo* and *in vitro* is dependent on the Na^+ level in the medium (2, 3, 5, 6, 7, 13, 14, 15). Moreover it has been shown by CRANE (4) that Na^+ allows the penetration of actively transportable sugars into the cell in anaerobic conditions, by a process of facilitated diffusión probably mediated by a specific carrier. In this last sense also are the results of FAUST (9). These and other observations have suggested the hypothesis that active transport of sugars by the intestine has two components, one of penetration of the sugar acilitated by a carrier-transport mechanism and another of accumulation of the sugar into the epithelial cells. The both processes would be Na^+ dependents.

However, as it been previously pointed out (12), the influence of Na^+ on the process of sugar accumulation in aerobic conditions can be in part explained by some disturbance of the cellular metabolism due to the lack or insufficiency of this ion in the medium. In fact, it has been reported that oxygen uptake by kidney slices is depending on the extracellular Na^+ level (10, 17) and some previous experiments (12) indicated an inhibition of the oxygen uptake of jejunal mucosa by substituting Li^+ or mannitol for Na^+.

In this paper it is reported the inhibitory effect of variable substitution of Na^+ by Li^+ or mannitol, keeping constant the osmolarity of the medium, on the oxygen uptake of jejunum strips (whole wall) and mucosa, in absence of exterior substrate.

Material and methods

White rats, 120-180 g g weight, were used. A length of about 10 cm from the beginning of jejunum was removed and well rinsed with Krebs-Ringer-Tris solution. The intestinal segment was now lengthwise opened and several strips of 60-80 mg fresh weight were cut or the mucosa scraped and alloted in portions of a similar weight of tissue. The strips or mucosa preparates were

carried over Warburg flarks. The determination of oxygen uptake was done as described in a previous paper (1).

For a greater facility in the preparation of media with different concentrations of Na^+, a Krebs-Ringer-Tris solution was used, prepared as in (16) but changing the addition of 0.1 M buffer phosphate by the same volume of 0.2 M Tris (*p*H 7.4). Solutions with different levels of Na^+ from O up to 154 meq/1 were obtained on substituting the desired quantities of NaCl by LiCl or mannitol in the suitable proportion for isotony. It has been always operated without exterior substrate.

Mannitol (Merck) was tested for glucose by the glucose oxidase method and found it free of this sugar (less than 0,15 %).

The results are given as oxygen uptake during the first 60 minutes of incubation by 100 mg of fresh weight.

Comparative experiments with conventional Krebs-Ringer-Phosphate and Krebs-Ringer-Tris solutions gave very similar values of oxygen uptake, thus allowing to work usually with the last ones.

Results

1. Strips.

In Fig. 1 is show the influence of the Na^+ concentration in the medium on the oxygen uptake of jejunum strips. In all experiments was compared the respiration of strips taken from a same intestinal segment at 154 meq. Na^+/1 with that of the other concentrations. The results at the different Na^+ concentrations are given as per cent of that corresponding to 154 meq. Na^+/1. From each animal 3 strips have been taken for control (154 meq. Na^+/1 and one or two other groups of 3 strips for different conditions. Each point of the figure describes the average of experiments with at least six rats.

As it may be seen, the oxygen consumption diminishes at low concentrations of Na^+ either when the substituent is Li^+ or mannitol. In a total lacking of Na^+ the inhibition amounts to 32 % (Li^+) or 46 % (Mannitol). Substitution of Na^+ for only a 50 %, merely causes slight inhibitions (2-8 %) which are near the limits of accuracy of the methods.

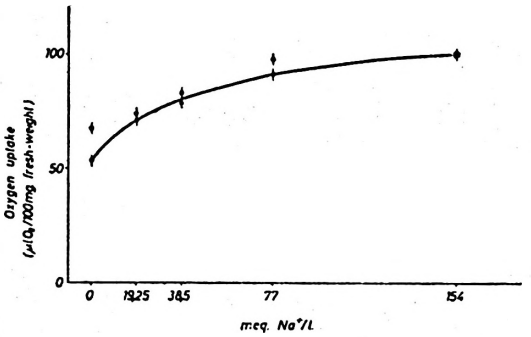

Fig. 1. Oxygen uptake by jejunum strips as a function of Na^+ concentration in the medium. Na^+ replaced by mannitol (■) or by Li^+ (o) for isotony. Control at 154 meq. Na^+/1 = 100.

If it is taken as «basal O_2 uptake» the O_2 consumption when the whole Na^+ is substituted by mannitol, values of «suprabasal O_2 uptake» may be obtained (total O_2 uptake-basal O_2 uptake). Now, plotting he logaritmus of suprabasal O_2 uptake against 1/[Na^+] a straight line is found, both either in the case of substitution of Na^+ by mannitol or by lithium (Fig. 2). A similar relation has been found in kidney slices (17).

2. Mucosa scraped.

The performance of these experiments was the same described for strips.

The oxygen uptake is also inhibited

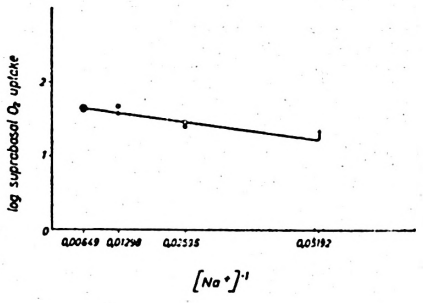

FIG. 2. Plotting of log suprabasal O_2 uptake by jejunum strips against the reciprocal of Na^+ concentration. Na^+ replaced by mannitol (■), or by Li^+ (o) for isotony. Suprabasal in per cent of the basal. As basal is taken the O_2 uptake when Na^+ has been completely replaced by mannitol.

by lowering the concentration of Na^+. Absolute lack of Na^+ produced somewhat stronger inhibitions than those observed wih strips. They were of 54 % (mannitol) or 39 % (Li^+). The substitutions of Na^+ in only a 50 % (77 meq. Na^+/1) gave inhibitions scarcely different from those with strips and it was not found significance in distinguishing between substitution by Li^+ or by mannitol at these levels of Na^+.

Discussion

The results show that on substituting Na^+ in variable proportions by Li^+ or mannitol in the medium, the oxygen consumption becomes inhibited. The inhibitions are specially apparent when the level of Na^+ is less than 77 meq/1.

The lacking of Na^+ produces higher inhibitions of the oxygen uptake when preparates of jejunum mucosa are used than with strips of the whole intestinal wall. This greater susceptibility of the mucosa may be related to its higher respiratory activity (8). In our experiments, the O_2 consumption of the strips per 100 mg of fresh weight was about a 70 % in respect to that of the mucosa. It can be calculated that about a 86-87 per cent of the total O_2 consumption of a strip is due to the mucosa respiration.

At Na^+ levels under 77 meq/l, the O_2 uptake is lower in the mannitol substitution for Na^+ than in substitution by Li^+. Neither Li^+ or mannitol are capable of completely substituting Na^+ in respect to the influence of this ion on the O_2 uptake, but it seems that in the case of Li^+ the lack of Na^+ is a little less inhibitory. This behaviour may be due to the fact that with substitution by Li^+ the medium is not only of the same osmolarity but also of the same ionic strength and of equal relation between monovalent and divalent cations.

Besides, as to the suprabasal O_2 uptake values is concerned, the differences between Na^+ replacement by Li^+ or by mannitol, are very small.

It is difficult to give at present some interpretation of this influence of the exterior Na^+ level on the O_2 consumption of the intestine, in absence of substrate in the medium. In frog skin, very careful studies have permitted to refer differences in O_2 consumption as due to changes in active transport of Na^+ (11, 18). Some results similar to those we have found, but in kidney slices and in the presence of substrate have been explained as differences in the active transport of Na^+ (10, 17). In fact, we have tested a linearity between log of suprabasal O_2 uptake and the reciprocal of Na^+ concentration. However, several other factors must be kept in mind, as the influence of the Na^+ level on the accumulation of many substrates into the cells, possible changes in the membrane permeability, disturbed metabolism, etc. It seems that if the required energy for the active transport of Na^+ would account for so important variations in O_2 uptake as have been found, this would mean that transport of Na^+ would be a major component of the

energy requirements of the cell. With intestinal mucosa we were not able to measure an increase of O_2 consumption related to the active transport of galactose or 3-methyl-glucose (1), suggesting that active transport of sugars «per se» represents an O_2 requirement of little importance in respect to the total consumption of oxygen of the cells. But in this case, Na^+ was always present in the medium at the same concentration.

On the other hand, since long the physiological disturbances produced in different organs and tissues by a change in the ionic composition of the medium are well known. This may be correlated to changes in the ionic distribution inside the cell and to different kinds of metabolic alterations, perhaps involving a diminution of the O_2 consumption, as we have tested in intestine. New experiments are in progress to better explain the effects of lacking of Na^+.

The inhibition of oxygen uptake at low levels of Na^+ must be kept in mind when the influence of Na^+ on the active transport of sugars and other substances is to be discussed. In fact, such an inhibition very probably involves a diminished energy production and therefore less energy available for active transport. If the hypothesis of two components for the active transport of sugars is accepted, the inhibition of the tissue respiration would not affect the facilitated diffusion mechanism for penetration, as it is independent of energy, but it would inhibit the accomulation component.

There is no doubt that active transport of sugars, as a whole, is more strictly dependent on the Na^+ concentration than respiration. As it has been shown, a 50 % substitution of Na^+ notably inhibits the active transport and produces only a small diminution of O_2 uptake (without substrate). The interpretation may be that at Na^+ levels from 77 upto 154 meq/l. the chief action of Na^+ deficiency would be upon the penetration mechanism (facilitated diffusion component) and from 77 down to A meq. Na^+/l. the accumulation component also becomes progressipely inhibited, both effects being added.

Summary

It has been studied the O_2 consumption by rat jejunum (strips and mucosa) without exterior substrate as a function of the Na^+ concentration in the medium (Krebs-Ringer-Tris). NaCl was variably substituted by LiCl or mannitol for isosmoticity.

The O_2 uptake diminishes as the Na^+ is progressively replaced. With total lacking of Na^+, the diminution was of 32 % (Li^+) and 46 % (mannitol) for strips, and of 39 % (Li^+) and 54 % (mannitol) for mucosa.

This effect of the Na^+ deficiency on the O_2 consumption of the intestine must be kept in mind when the Na^+ dependence of the active transport of sugars and other substances is discussed, as it implicates less availability of energy.

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