ATP-DEPENDENT BINDING OF SODIUM BY MICROSOMES FROM BRAIN

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The active transport of sodium ions through cell membranes requires the presence in these membranes of sites which are capable of transient binding of sodium ions. Since active transport has been shown to be dependent on the availability of metabolic energy in the form of ATP (Hodgkin and Keynes 1955, Caldwell and Keynes 1957) the binding of sodium ions should also be dependent on ATP. Recently subcellular fractions of the microsome category have been described to contain an ATPase activity which is dependent on the presence of sodium ions (Skou 1957, 1960, Post et al. 1960, Järnefelt 1960, 1961a). This ATPase has been postulated to reflect a mechanism for active transport of sodium ions. Support for this view was obtained from inhibitor studies, which indicated the involvement of an intermediate in the ATPase reaction (Järnefelt 1961b).

It has now been possible to demonstrate that microsomes isolated from rat brain are capable of binding sodium ions in the presence of ATP. The properties of this binding indicate that sodium is bound to the intermediate formed in the ATPase reaction.

Experimental. Microsomes from rat brain were prepared as previously described (Järnefelt 1961b). An amount of the microsomal suspension in 0.25 M sucrose equivalent to one rat brain (4 - 5 mg of microsomal protein) was used in each experimental vessel, which consisted of a Spinco No. 40 tube. The incubation mixture contained: $MgCl_2$ 4 mM, Tris-HCl buffer 20 mM, pH 7.5, tris-ATP 5 mM (when present), 0.5 ml of the microsomal suspension and other

additions as indicated below in a total volume of 0.9 ml. At 0-time 0.1 ml of a 1 M sodium chloride solution containing ^{22}NaCl was added. After a suitable length of incubation at 30°C, usually 12 seconds, 10 ml of a chilled non-radioactive 1 M NaCl solution buffered with 20 mM tris-chloride, pH 7.5, was added rapidly. In the 0-time experiments the ^{22}NaCl was added together with this solution. The microsomes were centrifuged in the experimental tubes in the Spinco No. 40 rotor at 100000 g for 10 minutes, suspended in 11 ml of the washing solution and centrifuged again. The pellet was suspended in 2 ml of distilled water and the radioactivity was measured directly in the experimental tubes in a well type Scintillation counter.

Results. Experiments on the time course of sodium incorporation into the microsomes showed that in the absence of ATP the rate was slow and linear over the tested interval of one minute. In the presence of ATP, on the other hand, the incorporation rate was very rapid during the first few seconds after which time a saturation level was reached. It was therefore decided to study the reaction during a 12-second incubation, which permitted work in the rapid initial phase as well as reasonably good timing. Some of the more interesting results have been compiled in table 1. ATP produced a four to fivefold increase

Table 1. Incorporation of ^{22}Na into rat brain microsomes. The additions to and omissions from the standard incubation mixture given in the text are as indicated. Incubation time 12 seconds. The incorporation shown is that occurring in excess of the 0-time-value (1150 counts/10 min).

Additions (+) or omissions (-)	Counts/10 min
None	2863
- ATP	634
+ K^+, 5mM	2744
+ Ca^{++}, 1mM	2375
+ Ca^{++}, 1mM + K^+, 5mM	1725
+ Ca^{++}, 5mM	683
+ PAD, 10^{-4}M	3346
+ PAD, $2x10^{-4}$M	3787

in the binding of sodium. A slight decrease in the binding was caused by potassium ions in a 5 mM concentration. This decrease was small, but consistent and was larger in other experiments. A marked inhibition of the incorporation was caused by Ca^{++}. A small, but clear inhibition was obtained with 1 mM Ca^{++}, and this inhibition could be considerably increased if potassium was also present. Higher concentrations of calcium gave a complete inhibition of the incorporation. Pyridine aldoxime dodecyl iodide (PAD) which is an inhibitor of the microsomal ATPase caused a marked increase in the sodium binding if used in concentrations causing partial inhibition of the ATPase (Järnefelt 1961b).

Discussion. As a result of studies on the microsomal sodium-stimulated ATPase, the formation of an intermediate in the reaction was postulated and a general mechanism for the reaction suggested, as depicted in figure 1 (Järnefelt 1961b). Two separate reactions can lead to the formation of a phosphate

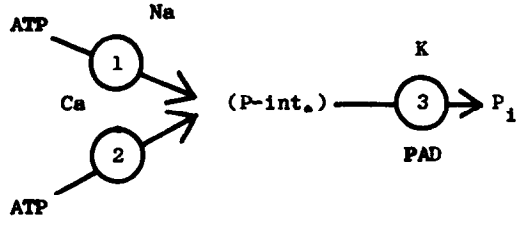

Figure 1. Proposed mechanism of microsomal ATPase with the binding of sodium to the intermediate.

intermediate from which phosphate is released through a single reaction. Reaction (1) is dependent on sodium ions and is inhibited by calcium, whereas reaction (2) does not have these properties. The release reaction (3) is stimulated by potassium and inhibited by PAD. The data on the incorporation of sodium can be easily explained if it is assumed that sodium is bound to the hypothetical intermediate. Thus potassium, which stimulates the rate of the overall ATPase by increasing the rate of reaction 3 would cause a more rapid release of the intermediate and therefore decrease its concentration. In an earlier communication (Järnefelt 1960b), the author has shown that potassium is able to cause a fall in the total amount of sodium bound to brain microsomes.

This observation is in good agreement with the data on sodium binding presented here. The effect of potassium on the release reaction (3) becomes even clearer in the presence of small concentrations of calcium as calcium is able to inhibit the formation of the intermediate at the same time as its destruction is stimulated by potassium. The stimulating effect of PAD on the sodium incorporation can be a result of the inhibition of reaction (3), thus leading to the piling up of the intermediate.

The data on the binding of sodium to brain microsomes are thus compatible with the properties of the ATPase. The reactions involving the ATPase and the sodium binding seem to stem from the same phenomenon. The hypothesis that this phenomenon represents the active transport of sodium ions across cell membranes becomes very plausible if all the above and following data are considered. 1, the morphological evidence indicates that the microsomal fraction studied here contains membrane fragments (Hanzon and Toschi, 1959). 2, the properties of the ATPase with respect to its sodium and potassium requirements indicate a close similarity to the known properties of active transport of sodium in more intact systems (Skou, 1957, 1960, Post et al. 1960, Järnefelt 1960a, 1961a). 3, the ATPase reaction involves an intermediate (Järnefelt 1961b), and 4 this intermediate binds sodium ions in the presence of ATP.

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