Increased sodium content and altered sodium transport in thymocytes of spontaneously hypertensive rats

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Summary
1. The intracellular sodium content and the sodium efflux rate constant have been determined in vitro in thymocytes derived from the Okamato-Aoki strain of spontaneously hypertensive rats.
2. A strong positive correlation between the systolic blood pressure and the sodium content of thymocytes was observed ($r = 0.59$, $n = 39$, $P < 0.001$).
3. The rate constant for total sodium efflux was negatively correlated with systolic blood pressure ($r = -0.43$, $n = 45$, $P < 0.005$) and this was due to a fall in the ouabain-sensitive component of sodium efflux.
4. Sodium efflux, influx and the thymocyte potassium content were not related to the blood pressure.

Key words: intracellular sodium, sodium transport, spontaneously hypertensive rats, thymocytes.

Introduction
There have been numerous studies on the role of electrolyte metabolism in human hypertension. Defects in the sodium pump [1, 2], ouabain-insensitive sodium efflux [3] and in lithium–sodium cotransport [4] have all been reported. Attempts to investigate this phenomenon in more detail in man have been hampered by the scarcity of samples from untreated hypertensive subjects. For this reason we have developed a method for the study of cation transport in rat thymocytes [5] initially to investigate whether a defect in sodium transport, comparable with that seen in hypertensive man, could also be demonstrated in thymocytes derived from spontaneously hypertensive rats. Such a model would provide a system whereby the interactions of sodium transport and hypertension could be studied by observing the effects of manipulating either on the other.

Materials and methods
Studies were performed on the Okamato–Aoki strain of spontaneously hypertensive rats. In these animals blood pressure rises progressively with age until, by 15 weeks, a high proportion of the animals have a systolic pressure in excess of 180 mmHg. The animals were fed standard rat chow (Spillers LD1) and allowed water ad libitum. Blood pressures were measured in the conscious animal by means of a tail cuff and electronic pulse detector. Determinations were made at weekly intervals and the mean of three pressure measurements taken over approximately 10 min was considered to represent the systolic blood pressure.

All procedures and fluxes were carried out in Earle's balanced salt solution with a sodium and potassium concentration of 140 and 4.0 mol/l respectively. The animals were killed by cervical dislocation at various ages between 3 and 26 weeks. The thymus gland was removed intact, washed in Earle's buffer to remove any surface blood and finely chopped in buffer at 0°C. The thymocytes were separated from the gland...
stroma by filtration through a fine gauze mesh. The cells were incubated for 1 h at 37°C in fresh Earle’s buffer.

Intracellular sodium and potassium were measured as follows: an aliquot of the cell suspension was transferred to a weighed polythene lay-flat tube, centrifuged for 3 min at 0°C at 250 g, washed once in 2 ml of ice-cold iso-osmotic magnesium chloride solution (99 mmol/l) containing 51Cr-labelled ethylenediaminetetra-acetic acid (EDTA) as an extracellular fluid marker and centrifuged as before. The supernatant was poured off and the cell pellet was then weighed, its radioactivity measured, and then dried to constant weight at 100°C. The dried cell pellet was extracted in HNO3 (1·0 mol/l) and the sodium and potassium contents were determined by flame photometry.

The sodium efflux rate constant was determined by observing the loss of radioactivity of a second aliquot of cells which had been loaded with 22Na. The efflux was observed additionally in the presence of ouabain at a concentration of 2 × 10−3 mol/l, this high concentration being necessary because in the rat the thymocyte shares with the erythrocyte a relatively high degree of ouabain resistance.

Sodium influx was determined by observing the gain of radioactivity of cells exposed for 10 min to 22Na in the extracellular fluid. At the termination of the influx study, the cell specimen was placed into a polythene lay-flat tube, plunged into ice, centrifuged at 0°C for 3 min, washed in 2 ml of ice-cold magnesium chloride solution (99 mmol/l) and recentrifuged. From a knowledge of the counts in the cell pellet and in an aliquot of the extracellular fluid sodium influx can be determined. This value is corrected for the efflux which necessarily takes place during the study according to the equation: 

$$m = Kx/(1-e^{-Kt})$$

where m is the corrected influx, x the observed influx, K the efflux rate constant and t (h) is the time over which the influx was observed.

Results
The relationship between thymocyte sodium content and systolic blood pressure is shown in Fig. 1. A positive correlation between intracellular sodium and systolic pressure was observed which was highly significant ($r = 0.59, n = 39, P < 0.001$).

The relationship between the total sodium efflux rate constant and systolic blood pressure is shown in Fig. 2. The rate constant for total sodium efflux decreased significantly with increasing blood pressure ($r = -0.43, n = 45, P < 0.005$).

The mean value for the ouabain-insensitive sodium efflux rate constant was $1.52 \pm 0.06$ ($n = 32$) and was not correlated with systolic blood pressure ($r = -0.14, P > 0.55$). The decrease in the rate constant for total sodium efflux therefore appears to be confined to the ouabain-sensitive component of sodium efflux.

Sodium influx was not related to the blood pressure ($r = -0.104, P > 0.45$), the average value being $247 \pm 6.5$ mmol h−1 kg−1 dry weight ($n = 34$). Similarly, neither cell water ($r = 0.089, n = 39, P > 0.41$) nor intracellular potassium ($r = 0.039, n = 39, P > 0.80$) were related to the blood pressure, the mean values being $2.85 \pm 0.05$ litres/kg dry weight and $595 \pm 6.8$ mmol/kg dry weight respectively.

Systolic blood pressure is strongly correlated with age in this strain of spontaneously hypertensive rat ($r = 0.836, P < 0.001$) and both the sodium efflux rate constant and intracellular sodium were significantly correlated with age ($r = 0.505, P < 0.001; r = 0.470, P < 0.001$).
Bivariate analysis of the data revealed a weak negative correlation between the sodium efflux rate constant and systolic blood pressure after adjustment for the effect of age \((t = -1.56, \ 0.2 > P > 0.1)\). There was no suggestion of a residual effect of age on the sodium efflux rate constant after adjustment for the effect of blood pressure \((t = -0.67, P = 0.5)\).

The thymocytes of young normotensive spontaneously hypertensive rats have values for intracellular sodium content and sodium efflux rate constant indistinguishable from those of normotensive Wistar rats. Members of the latter strain of rat showed no tendency for intracellular sodium to rise nor for the sodium efflux rate constant to fall with increasing age up to 16 weeks.

**Discussion**

The present studies show that thymocytes from the Okamoto–Aoki strain of spontaneously hypertensive rats develop an abnormality of sodium metabolism with increasing age and blood pressure. This abnormality takes the form of an increase in intracellular sodium and a decrease in the rate constant for total sodium efflux. Sodium efflux (the product of the intracellular sodium and the rate constant) is unchanged.

Previous studies on cation transport in spontaneously hypertensive rats have reported a variety of abnormalities. Jones [6] observed an increased washout and decreased accumulation of potassium in aorta suggesting an increased permeability to potassium and reduced activity of the sodium pump. Ben-Ishay et al. [7] have reported an unchanged intracellular sodium and an increase in the rate constant for sodium efflux in erythrocytes in a Hebrew University strain of spontaneously hypertensive rats. The lithium permeability of both erythrocytes [8] and tail artery [9] of spontaneously hypertensive rats has also been shown to exceed that of normotensive controls. Postnov et al. [10] have observed a pattern of abnormalities of sodium and potassium metabolism in erythrocytes of spontaneously hypertensive rats which they interpret as a reduction in the activity of the sodium pump and an increased permeability to both ions. A reduced ratio of net sodium to potassium cotransport in erythrocytes from spontaneously hypertensive rats, similar to that which they have observed in human hypertension, has been reported by Garay et al. [11], though their results in the rat are not presented in detailed form. The results of the present study differ from these in that no increase in the permeability of the thymocyte to sodium, as measured by either the ouabain-insensitive efflux rate constant or sodium influx, was observed.

It is interesting to compare the changes seen in the thymocytes of spontaneously hypertensive rats with those reported in human essential hypertension. The original report of Wessels et al. [12], of an increased erythrocyte sodium content in physiological conditions, has not been confirmed by others. Other workers have shown a variety of abnormalities of sodium (and lithium) fluxes in erythrocytes exposed to unphysiological media. By contrast an increase in leucocyte sodium content as well as a defect in sodium transport has been shown by a number of groups in human hypertension [1, 13, 14]. The present study may be compared with that of Edmondson et al. [1] who demonstrated a decreased rate constant for ouabain-sensitive sodium efflux and an increased sodium content of circulating leucocytes. Araoye et al. [13] confirmed a raised leucocyte sodium in hypertension which decreased after effective treatment. Ambrosioni et al. [14] studying human lymphocytes also found an increase in cellular sodium which correlated with the severity of the hypertension. Neither Ambrosioni et al. [14] nor Araoye et al. [13] studied sodium transport and these studies, including that of Edmondson et al. [1], measured the sodium content of cells at room temperature.

Thymocytes of spontaneously hypertensive rats develop a pattern of abnormality of sodium transport that resembles closely that reported by ourselves and others in leucocytes of subjects with essential hypertension. These cells should prove to be a useful preparation with which to study the significance of sodium-transport defects in hypertension.

**References**


