Sodium distribution in mesenteric arterial wall of rats with hypertension induced by drinking saline

JANE A. MADDEN, G. A. SMITH AND J. G. LLAUROADO
Nuclear Medicine Service, Wood Veterans Administration Medical Center, and Biomedical Engineering Group, The Medical College of Wisconsin and Marquette University, Milwaukee, WI, U.S.A.

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Summary

1. Hypertension was induced in rats by substituting 2% (w/v) sodium chloride solution for drinking water. Sodium distribution in isolated mesenteric arterial wall was studied with the aid of $^{22}$Na, which was continuously washed out. Data were analysed by digital computer simulation without recourse to ancillary chemical measurements of extracellular space.

2. A three-compartment model consisting of (i) extracellular, (ii) intracellular and (iii) subcellular space was found to represent adequately the kinetics of $^{22}$Na. Transport rate constants were chosen as primary parameters describing inter-compartmental sodium exchanges; the ratio of extra- to intra-cellular sodium compartments was calculated.

3. Results show the following significant changes in mesenteric arterial wall of salt-loaded hypertensive rats: (i) slowed sodium turnover; (ii) a decrease of the transport rate constant, which is presumed to reflect sodium movements from the intra- to the extra-cellular compartment; (iii) an increase of the transport rate constant, presumed to reflect sodium movements from the extra- to the intra-cellular compartment; (iv) a diminution of the extra- to intra-cellular compartment ratio for sodium.

4. The results suggest net movement of sodium into the cells; this change may be relevant to smooth muscle contraction and hence to the pathogenesis of blood pressure elevation in this model.

Key words: biomedical engineering, computer simulation, hypertension, kinetics, mesenteric artery, radionuclide tracers, sodium.

Abbreviations: ECF and ICF, extracellular and intracellular fluid respectively.

Introduction

Many studies (recently reviewed by Freis, 1976) have related particular forms of hypertension to changed sodium metabolism, but underlying mechanisms have proved elusive (Blaustein, 1977). As pointed out by Phelan & Wong (1968), there appears to be consensus in that 'only the vascular wall shows marked electrolyte changes in experimental hypertension'.

Although an increase in total sodium content of the arterial wall in hypertensive animals has been reported before (Tobian & Binion, 1954; Jones, Feigl & Peterson, 1964; Phelan & Wong, 1968; Nagaoka, Kikuchi & Aramaki, 1969; Friedman, 1974b), but not universally (Jones, 1973; Mas-singham & Shevde, 1973), definite information about the characteristics of the electrolyte alterations at the tissue level is lacking. The partition of electrolytes between extracellular fluid (ECF) and intracellular fluid (ICF) is of great importance in cell physiology (Swingle, Parkins, Taylor & Hays, 1937; Davson & Danielli, 1952; Adolph, 1968). Conventional approaches to study the partition of tissue electrolytes between ECF and ICF are based on determinations of bulk tissue electrolyte and on estimates of ECF volume derived from chemical indicators (chloride, inulin,
ties some inferences may be made about ICF space. Yet the variability of ECF space electrolytes. By suitably combining these two quantified by the ranges compiled by different authors (20–47% for smooth muscle (De La Riva, Blassier, Basso & Taquini, 1964); 8–35% for voluntary muscle (Ling & Kromash, 1967); 6–80% for liver (Williams & Woodbury, 1971) etc.).

In previous work (Llaurado, 1969), by employing solely a radionuclide of sodium as tracer, and resorting to digital computer simulation, a procedure was established for determining sodium transport rate constants in the arterial wall (Llaurado, 1970; Llaurado & Madden, 1975; Llaurado & Smith, 1978) and liver (Smith & Llaurado, 1974).

Most studies of arterial wall sodium have been carried out on relatively large arteries of animals with well-established hypertension (vide supra), whereas increased peripheral resistance (one of the determinants of hypertensive disease) is mainly related to a diminished calibre of small arteries and arterioles and much less to a contraction of aorta or carotid. Hence, it has been proposed that the early disturbances of electrolytes may be found in smaller, more distal arteries such as the mesenteric (Koletsky, Resnick & Behrin, 1959; Tobian, Janecek, Tomboulian & Ferreira, 1961). The present work was undertaken to study the tissue partition of sodium in the mesenteric arterial wall of rats with salt-induced hypertension.

Methods

Biological procedure

Male white Sprague–Dawley rats (200 g body wt.) were acclimatized to our animal quarters for at least 10 days before the beginning of the experiment. The animals were divided into two groups: the experimental group was given 2% (0-34 mol/l) NaCl solution as the only drinking fluid ad libitum, whereas the control group was given tap water (Sapirstein, Brandt & Drury, 1950). Both groups were fed on a standard Purina rat chow diet ad libitum. Weights were recorded once a week. Systolic blood pressure measurements were made periodically by the tail plethysmographic method, the Small Animal Study Unit (Narco Bio Systems, Houston, Texas), connected to a strip chart recorder, being used.

After 8 weeks on the above regimen, rats were guillotined, exsanguinated and their abdominal cavity was quickly opened. A specimen of anterior mesenteric artery of approximately 10 mg was dissected out and carefully stripped of extraneous connective tissue. The subsequent procedure (for further details see Llaurado, 1969; Llaurado & Madden, 1975) consisted of incubating the tissue segment for $^{22}Na$ uptake in electrolyte-containing medium (Llaurado & Madden, 1975) at 37.5°C under $O_2 + CO_2$ (95:5) atmosphere for 0.5 h, at which time the tissue had reached radioactive equilibrium. Then this tissue segment was subjected to a continuous outflow of $^{22}Na$ with isotopically inactive electrolyte solution bubbled with $O_2 + CO_2$ (95:5) in an apparatus specially built in the laboratory (Llaurado, 1969), which assures constant temperature (37.5°C) and flow (15 ml/s). The concentration (mmol/l) of electrolyte solution was as follows: NaCl, 118; NaHCO$_3$. 22; Na$_2$SO$_4$. 2-4; KCl, 3.3; KH$_2$PO$_4$. 1-2; CaCl$_2$. 1-9; MgSO$_4$. 1-2; glucose, 6; this gave Na$^+$ concentration 145 mmol/l and K$^+$ concentration 4-5 mmol/l.

Radioactivity counts in the tissue at time 0, i.e., before starting the washout, and every 5 s thereafter were recorded during the subsequent 15 min outflow or until background radioactivity level was reached with a model 530 Baird Atomic spectrometer attached to a model 620–2 printer. Previous work (Llaurado & Madden, 1975) indicated that this period was sufficient for adequate completion of the washout. Although data points were obtained every 5 s, the following utilization of data for computer input was selected: between 0 and 1 min every 5 s; between 1 and 3 min every 10 s, between 3 and 6 min every 20 s; between 6 and 12 min every 30 s; from 12 min on, every 60 s. This point selection provides a greater statistical weight on the initial part of the outflow curve. This is desirable for two reasons: (i) the higher radioactivity counts are more accurate; (ii) since the curve declines very fast at the beginning, important information would otherwise be missed.

Computational procedure

The print-out digital values of radioactivity counts in the course of time constituted the data input for the SAAM (Simulation, Analysis And Modeling) computer program developed by Bernan (1965). In the present study we found that the curve for $^{22}Na$ outflow from rat mesenteric arterial wall could be easily resolved into three exponentials. The number of exponentials determines
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(Berman & Schoenfeld, 1956; Solomon, 1960; Atkins, 1969) the minimum number of compartments needed to describe a kinetic model. It should be noted that an exponential fitting is simply a mathematical model which does not directly yield much information as to how compartments are biologically related. Compartmental analysis, on the other hand, serves to describe these interrelationships. A compartmental system is specified by a number of compartments and by intercompartmental relationships. The latter are used here as primary parameters and named transport rate constants ($k_{ij}$ values). Each $k_{ij}$ is defined as the fraction of compartment $j$ which enters compartment $i$ in unit time (Brownell, Berman & Robertson, 1968).

A simulation procedure for the ‘inflow’ is necessary to find the initial conditions for each compartment at the beginning of the outflow. The mathematical basis for the validity of this iterative simulation procedure is presented elsewhere (Llaurado, 1969). Later Gunn & Patlak (1970) added a physical argument for its justification. The questions of uniqueness (Llaurado, 1969; 1971) of the set of numerical values of the $k_{ij}$ and of sensitivity (Llaurado, 1970) of the model have also been discussed. The computer output for the outflow is therefore a double curve: (i) a theoretically calculated curve which is compared point by point with (ii) the experimentally obtained curve. Agreement between these two curves based on the ‘goodness’ of the least-squares fit is the criterion for acceptance of the set of $k_{ij}$ values. For further details of the computer program application see Berman & Schoenfeld (1956), Berman (1965) and Llaurado (1969, 1970, 1971).

Development of model

The customary three-compartment model for arterial wall configuration proposed by many workers (reviewed in Llaurado & Madden, 1975) was adopted. The model describes sodium partitioned into an extracellular, an intracellular and a subcellular space (Fig. 1). Since by outflow studies only kinetic aspects of sodium exchange are detected, present results are not intended to add anything to the question of the osmotically inactive sodium found by others (Headings, Rondell & Bohr, 1960; De La Riva et al., 1964; Jonsson, Lundgren & Wennergren, 1975).

From numerical values for the transport rate constants it is possible to calculate the unlabelled sodium ratio of extracellular to intracellular compartments (Llaurado, 1970; 1971) according to the equation in Table 1. Throughout this report the terms ECF and ICF spaces are used by convention in the same way as results obtained from inulin or sucrose determinations are attributed to ECF. Strictly speaking, we should refer, for instance, to compartment 1 as ‘that compartment characterized by exchanging sodium with other compartments at such transport rate constants in and out’. The terms ECF and ICF are therefore used with this reservation. An objection sometimes raised against compartmental analysis is that it does not provide much insight into local mechanisms (such as permeability, diffusion etc.); instead, all delays that a particle undergoes in journeying from one compartment to another are reduced to a single transport rate constant value. On the other hand, transport rate constants as compartmental model parameters have the decisive advantage of concisely describing a set of data. Any change in state of corresponding descriptions between control and experimental groups will be discernible as a change in the numerical values of the descriptive parameters.

Results

Rats drinking 2% NaCl solution grew normally for 3 weeks but lost weight as compared with control animals for the remaining 5 weeks, so that the mean weight at 8 weeks was 219 ± SEM 1.7 g vs 364 ± 1.4 for the control group. Systolic blood pressure values at the week 8 are included in Table 1. Rats drinking salt water showed a 14% elevation in blood pressure as compared with control animals ($P < 0.02$).

Illustrative plots of kinetic studies of $^{22}$Na exchange in rat mesenteric artery wall are shown in Fig. 2 for a control specimen and in Fig. 3 for a specimen from a rat with hypertension induced by drinking 2% NaCl solution. The Figures illustrate the agreement for the outflow of $^{22}$Na between experimentally observed data and theoretical results calculated according to the numerical values...
Table 1. Comparative values of final blood pressure and transport rate constants \((10^3 k_{ij} \text{s}^{-1})\) for sodium distribution in mesenteric arterial wall of rats

Parameters are expressed as mean values ± SEM. Values of \(k_{ij}\) are multiplied by \(10^3\) to facilitate comparison. Significance levels \((P)\) are calculated according to t-test. The equation used (Llaurado, 1970; 1971) to calculate the ECF/ICF ratio for sodium was:

\[
\frac{Q_1}{Q_2} = \frac{k_{12}}{k_{21}} = \frac{Q_1}{Q_2 + Q_3} \frac{1 + Q_3/Q_2}{1 + 1/k_{21}}
\]

where each \(Q_j\) represents the quantity of unlabelled sodium in the respective compartment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control ((n = 10))</th>
<th>Hypertensive ((n = 6))</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mmHg)</td>
<td>139 ± 1.3</td>
<td>160 ± 6.2</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>(10^3 k_{01})</td>
<td>136.0 ± 11.0</td>
<td>92.6 ± 10.0</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(10^3 k_{11})</td>
<td>14.4 ± 1.5</td>
<td>10.7 ± 0.80</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(10^3 k_{12})</td>
<td>6.4 ± 0.58</td>
<td>8.92 ± 0.93</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>(10^3 k_{21})</td>
<td>2.74 ± 0.13</td>
<td>1.67 ± 0.62</td>
<td>N.S.</td>
</tr>
<tr>
<td>(10^3 k_{22})</td>
<td>0.45 ± 0.08</td>
<td>0.22 ± 0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Sodium ECF/ICF ratio</td>
<td>1.9 ± 0.21</td>
<td>1.1 ± 0.09</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Fig. 2. Kinetics of \(^{22}\)Na in mesenteric arterial wall of rat. This is an illustrative record for a control specimen. Left: computer simulated inflow only. Right: comparison of experimentally obtained outflow data (○) and computer simulated outflow (■); coincidental points are represented as ▲.

Fig. 3. Kinetics of \(^{22}\)Na in mesenteric arterial wall of rat. This is an illustrative record for a specimen from a rat with hypertension induced by drinking 2% NaCl solution. Left: computer simulated inflow only. Right: comparison of experimentally obtained outflow data (○) and computer simulated outflow (■); coincidental points are represented as ▲.
of the transport rate constants \( k_{0j} \) values) arrived at by computer iteration.

The following statistically significant changes were observed in the hypertensive group (2% NaCl solution for drinking) as compared with the control group (Table 1): (1) transport rate constant \( k_{01} \), which is related to the overall outflow of sodium from the tissue, decreased by 32%, (2) transport rate constant \( k_{12} \), describing sodium movements from ICF to ECF, decreased by 26% and (3) its counterpart \( k_{21} \), representative of sodium movements from ECF to ICF, increased by 39%; (4) although \( k_{32} \) was also changed, the SEM of this value was high; (5) application of the equation in Table 1 yields the extra- to intra-cellular sodium ratio for the unlabelled sodium. This ratio was 1.9 for the control group and 1.1 for the salt-loaded group.

In Figs. 4 and 5 the outflow data (both experimental and theoretically calculated) from one control and one hypertensive specimen are plotted. The fraction of tracer in each compartment \( q_{j}, j = 1, 2, 3 \), as obtained from the computer output, is represented by the three curves obtained by computer and are interpreted as representing (1) extracellular, (2) intracellular and (3) subcellular compartments.

**Discussion**

**Justification of methodology**

Since the procedure employed in this work represents a relatively new approach, comparison with previous methods is necessary. First, in the current work values found for the compartmental distribution of sodium are based solely on kinetics of \(^{22}\text{Na} \) without requiring the use of auxiliary indicators of ECF such as chloride, inulin, sucrose, trivalent cations of ethylenediaminetetra-acetate (EDTA) or diethylenetriaminepenta-acetate (DTPA) etc., which are assumed only to penetrate the ECF. This is of importance, since different indicators give widely differing values for ECF (De La Riva et al., 1964; Ling & Kromash, 1967; Williams & Woodbury, 1971; Török, Nedergaard & Bevan, 1971). More specifically, Villamil, Rettori, Barajas & Kleeman (1968) reported a highly significant statistical difference between estimates of inulin and sucrose spaces in arterial wall; later, Harrison & Massaro (1976) found even qualitative differences between inulin and sucrose. It would appear, in principle, that a method based on the exclusive use of a radioactive sodium tracer to study sodium exchanges would not be subject to the fluctuations intrinsic to tracer dilution measurements of ECF.
The three-compartment configuration found by kinetic approaches has drawn strong support from morphological studies. Gabella (1971) demonstrated by electron microscopy four compartments in both smooth and striated muscle tissue: two compartments had the same electron density and both were penetrated by lanthanum nitrate, which is considered an ECF tracer. Thus there were only three compartments which were kinetically distinct. Obviously, models for arterial wall sodium distribution explicitly or implicitly consisting of only two compartments, as sometimes suggested, are inadequate. Without committing ourselves to the exact location of this additional sodium compartment within the cell (subcellular), it is noteworthy that its existence has been indicated by other workers (Gamble, 1957; Malbica & Hall, 1968; Villamil et al., 1968; Friedman, 1974a; Blaustein, 1977).

There was a very rapid uptake of $^{22}$Na, as indicated by the computer simulated inflow (Figs. 2 and 3). Obviously it is impossible to conduct a continuous inflow experiment, but by serial counting of radioactivity of samples Burnstock, Dewhurst & Simon (1963) and Garrahan, Villamil & Zadunaisky (1965) reported that 95% of the total sodium in smooth muscle had exchanged with the tracer in the first few minutes. This rapid uptake agrees with the information revealed by our theoretical inflow graph.

Our results in terms of extra- to intra-cellular electrolyte ratio may be compared with published data. First, the value of the ECF to ICF sodium ratio in arterial wall for normal animals can be used to show the consistency of our results. This ratio was calculated as being 1.3, 1.7 or 2.2 in rat aorta (Hagemeijer, Rorive & Schoffeniels, 1965), 2.0 in canine carotid (Garrahan et al., 1965; Harris & Palmer, 1972) but as large as 20.3 in sheep carotid (Keatinge, 1968). The ratio of 1.7 found earlier (Llaurado & Madden, 1975) for rat aorta and that of 2.3 found previously (Llaurado, 1970) for dog carotid falls clearly within the range of other workers' values.

Secondly, values of the ECF to ICF sodium ratio in arterial wall of normal animals of different species can be compared with previously published data on inulin space (Harrison & Massaro, 1976). These data are listed in Table 2. The results for three species (rabbit, dog and rat) follow the same gradation in both procedures.

Thirdly, we showed previously (Llaurado & Madden, 1975) that the ECF to ICF sodium ratio was significantly increased in the arterial wall of spontaneously hypertensive rats, this finding pointing towards a diminution of intracellular sodium. Similar findings were reported later by independent groups (Friedman & Friedman, 1976; Altman, Garay, Papadimitriou & Worel, 1977).

Fourthly, when we studied the partition of $^{201}$Tl in rat myocardium we found (Llaurado, Madden, Meade & Smith, 1978) that more than 70% was intracellular, which is in agreement with the suggested (Lund, 1956) and inferred (Gehring & Hammond, 1967) tissue distribution of this ion.

Thus it appears that effects detected by the methodology employed in the present work truly reflect results generally found by other investigators using different methods. It has been pointed out (Brading, 1973) that the type of model used to characterize sodium outflow curves may possibly determine the calculated parameters to some degree. Even if absolute parameter values are difficult to achieve with certainty (Brading, 1973; Friedman, 1974a), it seems reasonable that values obtained from normal and hypertensive arteries by applying the same theoretical model after using the same experimental method lend themselves to a fairly reliable comparison.

**Interpretation of sodium partition changes**

The finding of a significantly decreased $k_{01}$, the transport rate constant from tissue to outside (Fig. 1), indicates a slowed sodium turnover in the mesenteric wall of salt-loaded rats. Furthermore, the finding of a decreased $k_{12}$, an increased $k_{21}$ and a diminished ECF to ICF sodium ratio indicates a relative accumulation of intracellular sodium. Other workers have studied mesenteric arterial wall sodium content in rats with hypertension induced by renal mechanisms. Thus Koletsky et al. (1959) and Tobian et al. (1961) found an increase in total sodium of mesenteric arteries. By using chloride space as an indicator of

| TABLE 2. Comparison of extracellular fluid/intracellular fluid sodium ratio and inulin space estimates reported for the arterial wall in different species |
|---------------------------------|---------|---------|---------|
| ECF/ICF sodium ratio            | Rabbit  | Dog     | Rat     |
| Inulin space $^\text{§}$ (ml/kg of wet tissue) | 3.6*    | 2.3†    | 1.7†    |

† Llaurado (1970).
‡ Llaurado & Madden (1975).
§ Grand mean of data collected in Harrison & Massaro (1976).
ECF volume they concluded that not all of the excess sodium could be located entirely within the ECF and some of it was intracellular. There was, however, some hesitancy in establishing a definite increase in ICF sodium owing to doubts as to whether chloride space is a reliable indicator of ECF space in smooth muscle.

To the best of our knowledge, the present work indicates for the first time a partition of sodium favouring the intracellular compartment in salt-induced hypertension. Although our findings may partially be a reflection of sodium loading and consequent pathophysiological effects upon the arterial wall, it should be noted that increased intracellular sodium has recently been invoked as one of the participating mechanisms in some forms of hypertension. Thus a plausible hypothesis at this time (Blaustein, 1977) is that increased peripheral tone reflects changes in intracellular calcium and that the sodium–calcium exchange plays an important role in regulating the calcium gradient across the sarcolemma. It has been further calculated (Blaustein, 1977) that an increase of only 5% in intracellular sodium may cause an increase of 15% in intracellular calcium, which in turn corresponds to an increase of 50% in the tension of vascular smooth fibres. In the present work we found an accumulation in intracellular sodium, which by the above hypothesis may be sufficient to account for the hypertension demonstrated.

In a previous study (Llaurado & Madden, 1975) in spontaneously hypertensive (190 mmHg) rats we found an augmentation of the ECF to ICF sodium ratio, which could be attributed to a relative diminution of intracellular sodium. Similar findings in spontaneously hypertensive rats have been subsequently reported (Friedman & Friedman, 1976; Altman et al., 1977). Results of the present study on development of hypertension in rats given excess sodium for drinking are the opposite, i.e., they imply a relative increase of intracellular sodium. The inescapable conclusion is that different models or types of hypertension may be associated with opposite changes in arterial wall electrolyte composition.

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