ENDOGENOUS DIGITALIS-LIKE COMPOUND IN ESSENTIAL HYPERTENSION

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Introduction

Gradients in concentrations of sodium (Na⁺) and potassium (K⁺) between intracellular and extracellular fluids stem essentially from the activity of the sodium pump. The sodium pump may be altered in certain pathological conditions, including essential hypertension [1–3], and may originate from either intrinsic cell membrane alterations or the action of circulating factor(s) or both. Endogenous pump inhibitors have recently been described in biological fluids [4–7] and in tissues [8–11]. These factors appear to be increased by sodium loading and volume expansion [12–14]. Several clinical and experimental investigations suggest that the reduction in sodium pump activity in primary hypertension may be secondary to an increased activity of such an inhibitor [13, 15]. The resulting increase in intracellular Na⁺ may be of pathogenic significance.

The present study evaluates, by direct biochemical methods, the presence of Na⁺ pump inhibitor(s) in plasma from hypertensive patients and the influence of antihypertensive therapy on these inhibitors.

Material and methods

Preparation of plasma extracts

Blood sampling and preparation of heat stable plasma extracts were performed as previously described [16]. The clear supernatants of boiled plasma were either tested immediately, frozen, lyophilised or not, and kept at −80°C until analysis. Some plasma supernatants were subjected to the separation procedures previously reported [17]: briefly, a gel filtration where the inhibitory fraction was eluted just after the salt peak and anion exchange chromatography where the above fraction was resolved into three peaks. These three fractions, although not pure as shown by high pressure liquid chromatography on reverse phase, were characterised in terms of inhibition of ³H-ouabain binding, Na⁺,K⁺-ATPase activity and ³H-serotonin uptake by platelets.
3\textsuperscript{H}-ouabain binding to erythrocytes

Binding of 3\textsuperscript{H}-ouabain (17–32 Ci/mmol, Amersham) to erythrocytes was performed at equilibrium as described previously [16]. Number of pump units and affinity were calculated from Scatchard plots. The inhibition by plasma extracts was expressed as the decrease in apparent affinity given as per cent of the erythrocyte affinity in the absence of plasma.

\(\text{Na}^+\,\text{K}^+\text{-ATPase activity}\)

The activity of the enzyme (EC 3.6.1.3., prepared from dog kidney, Sigma) was determined by hydrolysis of \(^{32}\text{P}\)-ATP (Amersham, UK). At 80\(\mu\)l of the following medium: 100mM NaCl, 2.5mM EGTA, 4mM MgCl\(_2\), 2mM ATP Na (Vanadate-free, Sigma), 160nCi \(^{32}\text{P}\)-ATP and 80mM Tris-HCl buffered to 7.4, were added 10\(\mu\)l of plasma extracts corresponding to 190\(\mu\)l of boiled plasma supernate or ionic plasma-like solution and 10\(\mu\)l of enzyme suspension (3 \times 10\(^{-3}\) unit). After 30 minutes of incubation at 37\(^\circ\)C, the reaction was stopped by placing the tubes at 0\(^\circ\)C and adding 100\(\mu\)l of 23% HClO\(_4\). Liberated phosphates, separated from ATP adsorbed on acid-washed charcoal, were then counted.

\(\text{Controls and patients}\)

Seventy-one subjects on a free sodium diet were studied. Their blood pressure was recorded with a mercury manometer in the sitting position. They were divided into five groups as follows.

\textit{Group 1} comprises 21 normotensive healthy volunteers with no known family history of hypertension (13 males and 8 females, aged from 23 to 53 years, mean 32.1 \pm 2.0); their mean blood pressure was 90.8 \pm 1.9 mmHg.

\textit{Group 2} included 21 normotensive healthy volunteers who had at least one parent with high blood pressure (13 males and 8 females, aged from 23 to 50 years, mean 34.4 \pm 1.7); their mean blood pressure averaged 95.5 \pm 2.0 mmHg.

\textit{Group 3} comprises 21 hypertensive patients, untreated for at least two weeks (14 males and 7 females, aged from 22 to 71 years, mean 45.9 \pm 3.2). Essential hypertension was diagnosed after careful clinical investigation. In all patients plasma Na\(^+\) and K\(^+\) were in the normal range.

\textit{Groups 4 and 5}: Ten hypertensive patients were under antihypertensive therapy for at least three months. Five (2 males, three females, aged 40–84 years) were on thiazide diuretics (group 4), and their mean blood pressure was 123.8 \pm 6.0 mmHg. Five (3 males and 2 females, aged 26–47 years) were on \(\beta\)-blocking agents (group 5); their mean blood pressure was 105.8 \pm 5.3 mmHg. None of the patients had significant change in plasma potassium.
Results

Inhibition of $^3$H-ouabain binding and Na$^+$,K$^+$-ATPase activity

Plasma extracts from nearly half of the normotensive subjects, offspring of hypertensive parents, and of the essential hypertensive patients exerted a marked inhibitory effect on the two biochemical parameters used as tests of interaction with Na$^+$,K$^+$ pumps. A significant correlation ($r = 0.74$, $n = 44$) was obtained between the inhibition of ouabain binding to erythrocytes and that of ATP hydrolysis obtained in individual plasma extracts. As shown on Table I, average affinities for ouabain binding and Na$^+$,K$^+$-ATPase activities were significantly decreased by plasma extracts of hypertensives and normotensives with family history of hypertension when compared to normotensive controls.

Plasma from patients under thiazide therapy did not significantly inhibit ouabain binding, whereas plasma from patients receiving β-blockers inhibited ouabain binding to a greater degree when compared to that of untreated hypertensive patients (Table I).

**TABLE I. Inhibition by plasma extracts of $^3$H-ouabain binding, Na$^+$,K$^+$-ATPase activity and $^3$H-serotonin uptake by platelets**

<table>
<thead>
<tr>
<th></th>
<th>Number</th>
<th>$^3$-H-ouabain binding</th>
<th>Na$^+$,K$^+$-ATPase activity (%)</th>
<th>Platelet serotonin uptake</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Boiled plasma supernatants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Normotensives</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Without family history of hypertension</td>
<td>21–19</td>
<td>0.21 ± 0.4</td>
<td>2.6 ± 1.9</td>
<td>–</td>
</tr>
<tr>
<td>With family history of hypertension</td>
<td>21–13</td>
<td>0.67 ± 14*</td>
<td>13.8 ± 3.3*</td>
<td>–</td>
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<tr>
<td>Hypertensives</td>
<td></td>
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<td></td>
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<tr>
<td>Untreated</td>
<td>21–19</td>
<td>0.61 ± 0.15*</td>
<td>12.8 ± 3.3*</td>
<td>–</td>
</tr>
<tr>
<td>Treated with diuretics</td>
<td>5</td>
<td>0.29 ± 0.34</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Treated with β-blockers</td>
<td>5</td>
<td>1.61 ± 0.42*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>Low molecular weight anionic fractions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>3</td>
<td>0.33</td>
<td>4.3</td>
<td>1.3</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>0.66</td>
<td>2.6</td>
<td>1.7</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>0.52</td>
<td>6.5</td>
<td>2.0</td>
</tr>
</tbody>
</table>

† Inhibition expressed as indicated in ‘Methods’
‡ Inhibition given as whole plasma equivalent concentration giving half maximal inhibition
* $p < 0.01$ by Student t-test when compared to normotensive controls

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Three fractions, extracted from plasma by gel filtration and anion exchange chromatography also inhibited significantly ouabain binding and Na\(^+\),K\(^+\)-dependent ATP hydrolysis (Table I). Their mean purification factors were estimated to reach 200, their inhibitory potency is given as the concentration of equivalent whole plasma giving half maximal inhibition, without taking into account possible losses during purification.

### Discussion

A defect in transmembrane sodium transport, secondary to a plasmatic factor, has been suggested as a possible mechanism of essential hypertension. The hypothesis that a circulating inhibitor of the sodium pump exists in essential hypertensive patients has been examined by analysis of the effects of plasma on two specific features of the pump: sodium and potassium-dependent ATP hydrolysis and specific ouabain binding.

Several investigators had previously measured the changes induced by plasma and/or urine in transmembrane sodium and potassium fluxes, in intracellular sodium content, in the activity of glucose-6-phosphate dehydrogenase whose stimulation has been suggested to reflect inhibition of Na\(^+\),K\(^+\)-ATPase [13], or recently in the activity of Na\(^+\),K\(^+\)-ATPase itself [18]. The present method offers the following advantages: 1) it directly measures the activity of plasma inhibitor on its target enzyme, the Na\(^+\),K\(^+\)-ATPase; 2) the simultaneous measurement of inhibition of ouabain binding on whole cells under conditions where an effect of plasma potassium is excluded establishes that this circulating compound either shares common structural determinants with the cardiotonic digitalis, or prevents ouabain binding by allosteric modification of the pump structure. It also confirms that the inhibition of the Na\(^+\),K\(^+\)-ATPase activity is not due to circulating inhibitors such as vanadate or calcium ions which did not inhibit ouabain binding under our experimental conditions. Plasma values of this inhibitor were high in some essential hypertensives and in some normotensives born of hypertensive parents. This suggests that presence of the inhibitor is not secondary to high blood pressure and must either act in conjunction with other prohypertensive systems or induce long-term modifications before it raises blood pressure.

### References

8. Haupert GT, Sancho JM. Proc Natl Acad Sci USA 1979; 76: 4658
9. Fishman M. Proc Natl Acad Sci USA 1979; 76: 4661
10. Lichstein D, Samuelov S. Proc Natl Acad Sci USA 1982; 79: 1453
Open Discussion

JONES (Chairman) I imagine that most of the audience will be very familiar with your published work on the sodium potassium co-transport system and its defect in hypertension, and in the relatives of hypertensives. How do you see at present the relationship between the work you have described to us this morning and that particular transport system?

MEYER There are two basic properties in hypertension which may occur in human beings and we have to define in what proportion of patients we see one phenomenon or the other. One phenomenon is the presence of a membrane alteration as shown in the rat which may affect the membrane in a different way according to genetic determination. The other possibility is that there are some circulating factors or factors which are capable of affecting the pump or another system. One has to define exactly what is the proportion of patients belonging to the first category and the patients belonging to the second category.

WOODS (Oman) I have been following Dr Meyer’s work about the effects of the various sodium transport problems in hypertension. If the normotensives with a family history have the same markers on the membrane, whether it be a membrane defect or whether it be a circulating inhibitor as our hypertensives have, what is the suggested mechanism by which over a 10–15 year period this defect contributes to hypertension?

MEYER I don’t know. The most likely explanation is that the progressive sodium retention in the cells leads to some changes which might increase the contractility of the arterial cells. It is possibly related to the action of sodium per se or change in the conformation and the sensitivity of contractile protein. Is it in relation to calcium influx, because in excitable cells there might be sodium calcium exchange? It will require some time to answer your question, possibly because it is quite difficult to study vascular and contractile cells. A great hope, I think, stems from study of platelets as they have contractile properties and that they are avid for calcium which might be estimated in those platelets. According to the work of Fritz Buhler there is a very good correlation between the amount of calcium which is within the platelets and the elevation of blood pressure.

PAPADIMITRIOU (Thessaloniki, Greece) Are there any studies on sodium/potassium transport via the red cell membrane concerning captopril administration? I am asking this because there are some recent reports concerning the
direct effect of captopril on this transport system. This has been studied with propranolol a long time ago and it has been proven that there is massive efflux of potassium out of the cell. Are there any studies with captopril, concerning this effect?

MEYER No, I fully agree with the action of propranolol in vitro although this was tested at high doses. In vivo we have not made any systematic analysis of the variation of fluxes on isolated cells. With treatment, the only concern we have at the moment is to study the variation of the circulating inhibitor. Concerning your question on captopril I have no data, but in vivo I don't think it is of much importance. Again this has to be verified.

BERGSTRÖM (Stockholm) If you think that inhibition of the sodium pump is involved in the development of hypertension why are patients on chronic dialysis therapy not developing hypertension? We know that dialysis inhibits the pump and we know that you get an increase in intracellular sodium, so I think it's very puzzling why these patients do not develop hypertension.

MEYER Firstly I think we should remember a well established fact which is that in dogs or in humans having no renal function, the administration of ouabain or digitalin results in a marked, although transient, increase in blood pressure. In addition the administration of ouabain centrally administered in rats induces a rise in blood pressure both in the conscious and awake animal. Your question is somewhat similar to the one which was raised a few minutes ago. You are dealing with a phenomenon which is observed during a matter of months, or maybe years, but the time is something less important than the time which is necessary for high blood pressure to develop. This is one part of my answer, the second answer is that I do not think we should simplify something which is obviously very complicated. Hypertension is a polymorphic disease and it is possible that it represents several varieties of underlying diseases. The pump inhibitor may be involved but you have seen that it is increased in only half of the patients. There are other possibilities and your patients who do not develop hypertension may be lacking one of these other factors which have to be identified in the future.

BIANCHI (Chairman) It is important to recall here that the first idea that a natriuretic factor might have a role in essential hypertension stems from some data suggesting that there is a primary kidney defect in people with essential hypertension in that the kidney is unable to excrete sodium. This sodium retention is the first stimulus for the increase in natriuretic factors. Hypertension, according to this theory might be explained by both mechanisms, a defect in the kidney handling of sodium and a natriuretic factor that acts on the smooth muscle or at the sympathetic nerve terminal increasing blood pressure. Certainly it is really difficult to explain hypertension by the natriuretic factor alone, and I would be agreed with all of you that are rather doubtful about this as the only mechanism.
RITZ (Heidelberg) Dr Meyer, both you and other investigators of course use blood cells because of the ready availability and accessibility of this material. To what extent can this be extrapolated to resistant vessels? Recently work from Denmark* casts some doubt as to whether inhibition of the transport Na⁺,K⁺-ATPase in mesenteric arteries actually increases the contractile state of these resistance vessels.

MEYER Yes, a recent review by John Parker described extensively all similarities existing between the membranes of red blood cells and the membranes of other tissue investigated so far.†

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† Parker JC, Berkowitz LR. Physiol Rev 1983; 63: 261