Activation of two inward chloride transport systems in rat femoral arterial smooth muscle in deoxycorticosterone acetate/salt hypertension

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INTRODUCTION

The Na⁺-K⁺-Cl⁻ co-transport system is a secondary active inward Cl⁻ transport system which exploits the Na⁺ electrochemical gradient to drive Cl⁻ into, for example, cardiac cells and smooth muscle [1, 2]. There are suggestions that there is an anomaly in co-transport in primary hypertension (see [1]), and our evidence is that, in the rat deoxycorticosterone acetate (DOCA)/salt model of hypertension, co-transport is more active in arterial smooth muscle, resulting in an elevated [Cl⁻]i. Consequently, the chloride equilibrium potential (Em) is less negative in hypertension, and this contributes to the depolarized membrane potential (Em) observed in this condition [3, 4]. The idea is, therefore, that the lower Em will make the muscle more prone to contract, and thus contribute to the generation and maintenance of hypertension.

There are, in fact, three processes by which Cl⁻ is transported into rat arterial smooth muscle. One is Na⁺-K⁺-Cl⁻ co-transport and another is Cl⁻/HCO₃⁻ exchange, but this is not functional in the HCO₃⁻-free media [5] in which the experiments just described were carried out. The third is an entity we call ‘pump III’ which is not dependent on Na⁺ or HCO₃⁻ and which is inhibited by acetazolamide [6, 7]. There was reason to suspect that a then unknown Cl⁻ transport system was activated in hypertension in addition to Na⁺-K⁺-Cl⁻ co-transport [4], and the purpose of this study is to find out whether this system is, in fact, ‘pump III’. A preliminary account has been published [8].

METHODS

Male Sprague–Dawley rats (150–400 g; Bantin & Kingman, Hull, U.K.) were used, and the means of induction, maintenance and monitoring of the development of DOCA/salt hypertension were as described previously [3, 4]. All procedures were carried out in accordance with the provisions of the Animals (Scientific Procedures) Act, 1986 (U.K.).

Recordings of Em and intracellular chloride ([Cl⁻]) in isolated femoral arterial smooth muscle were made using double-barrelled chloride-sensitive microelectrodes [4]. The muscle was superfused at 37°C by an oxygenated physiological salt solution...
containing (in mmol/l): Na\(^+\) 140, K\(^+\) 5, Ca\(^{2+}\) 2, Mg\(^{2+}\) 2, Cl\(^-\) 153, \(\alpha\)-glucose 10 and Hepes 5, pH 7.4. Chloride-free physiological salt solution (used for calibration of microelectrodes and correction for interference) contained (in mmol/l): Na\(^+\) 140, K\(^+\) 5, Ca\(^{2+}\) 12.5, Mg\(^{2+}\) 2, gluconate 17.5, glucuronate 140, sulphate 2, \(\alpha\)-glucose 10 and Hepes 5; calcium was increased to compensate for binding (see [4]). All values of [Cl\(^-\)]\(_i\) are given as concentration (mmol/l) and are corrected for intracellular interference, which was found to be 4 mmol/l in normotension [9] and in this study in hypertension. Inhibitors were added to the superfusion reservoir at the concentrations indicated.

All values are given as mean ± SD (n = number of observations), and statistical analysis was done using unpaired Student’s t-tests in Microsoft Excel. The target significance was P < 0.001.

RESULTS

Blood pressure, [Cl\(^-\)]\(_i\), co-transport and \(E_m\) in hypertension

The arterial systolic blood pressure rose linearly with time as DOCA/salt hypertension developed, and [Cl\(^-\)]\(_i\) in femoral arterial smooth muscle also increased linearly with time up to 6 weeks post-operative (Fig. 1). The increase in [Cl\(^-\)]\(_i\) was significant (P < 0.0001) and it was originally attributed to an increase in Na\(^+\)-K\(^+\)-Cl\(^-\) co-transport activity. This was because 10 μmol/l bumetanide, a specific inhibitor of co-transport at this concentration [3] and a greater fall in [Cl\(^-\)]\(_i\) in hypertension than in normotension [4]. The actions of bumetanide were confirmed in the present study by testing its effect on muscle isolated from unoperated controls and on 5–6 week post-operative hypertensive animals (Fig. 2 and Table 1).

In good agreement with earlier observations on the effect of bumetanide [4], [Cl\(^-\)]\(_i\) fell from 31 to 21 mmol/l in normotension and from 49 to 30 mmol/l in hypertension (Table 1). Moreover, \(E_m\) in hypertension (−56.1 ± 2.3 mV, n = 9) was less negative than in normotension (−64.3 ± 4.4 mV, n = 16, P < 0.0001), again in accord with previous work [3, 4].

Effect of acetazolamide in normotension and hypertension

In normotension, when 1 mmol/l acetazolamide (the 'pump III' inhibitor) and 10 μmol/l bumetanide were both present, [Cl\(^-\)]\(_i\) fell to 13 mmol/l (Fig. 2, Table 1). This was in exact agreement with the calculated equilibrium value at the observed \(E_m\) of −66.1 ± 2.0 mV (n = 7). In hypertension under the same conditions, [Cl\(^-\)]\(_i\) fell similarly to 15 mmol/l, which is not measurably different from the calculated equilibrium value of 14 mmol/l at the measured \(E_m\) of −63.1 ± 2.4 mV (n = 9). Thus acetazolamide caused a significantly greater fall in [Cl\(^-\)]\(_i\), in hypertension (16 mmol/l) than in normotension (7 mmol/l) and, in the presence of bumetanide and acetazolamide, there was no significant difference between either [Cl\(^-\)]\(_i\) in normotension and hypertension (P > 0.07) or \(E_m\) (P > 0.008).

\(E_m\) in Cl\(^-\)-free media

If [Cl\(^-\)]\(_i\) is important in the depolarization of \(E_m\) in hypertension, then, in Cl\(^-\)-free conditions, the difference in \(E_m\) between normotension and hypertension will be diminished or abolished. In fact, \(E_m\) was routinely measured in Cl\(^-\)-free media in order to correct for interference by other anions (see Methods). In normotension, \(E_m\) was −59.3 ± 5.0 mV

![Fig. 1. Increases in arterial systolic blood pressure (A) and [Cl\(^-\)] (B) in rat femoral arterial smooth muscle during the development of DOCA/salt hypertension. Each point represents one animal. There was a linear correlation between blood pressure and [Cl\(^-\)] (r = 0.96).](image-url)
Hypertension and chloride in vascular smooth muscle

Fig. 2. Tracings of recordings of [Cl\textsuperscript{-}] in rat femoral arterial smooth muscle showing the effects of 10 μmol/l bumetanide and 1 mmol/l acetazolamide in normotension (NT, [5]) and hypertension (HT). Representative of seven and nine experiments respectively.

(n = 39) and, in hypertension \(-60.4 \pm 3.6 \text{ mV} (n = 26)\). Thus, there is no significant difference.

**DISCUSSION**

The main conclusion is that the increase in [Cl\textsuperscript{-}] in rat arterial smooth muscle in the DOCA/salt model of hypertension is due to the activation of two inwardly directed Cl\textsuperscript{-} transport systems. One, as shown previously [3, 4], is the well known Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} co-transport system and the other is an entity which we call ‘pump III’ [6, 7]. The amount by which the two transport systems are activated in hypertension cannot be exactly estimated from these experiments. However, the Cl\textsuperscript{-} permeability is not changed in hypertension (A.A. Harper, J.P.L. Davis and A.R. Chipperfield, unpublished work) and therefore it appears that the activity of both transport systems very roughly doubles. If there is a difference in activation of the two transport systems, then it is ‘pump III’ which shows greater activation.

Since the rationale of the investigation is that the depolarizing influence of [Cl\textsuperscript{-}] is more prominent in hypertension [3, 4], it follows that ‘pump III’, as well as co-transport, has a depolarizing influence on \(E_m\) in hypertension. Therefore, at least in the experimental model of hypertension used here, [Cl\textsuperscript{-}] may play a role in the development of hypertension, and there are three lines of evidence which bear out this contention. First, in the absence of Cl\textsuperscript{-}, the membrane potential was the same in normotension and hypertension. Thus, Cl\textsuperscript{-} is important in the depolarization of \(E_m\) in hypertension. Secondly, there was a clear parallel between the linear increases in blood pressure and [Cl\textsuperscript{-}] (Fig. 1) and, whilst the time resolution is not very precise, there must be a relationship if [Cl\textsuperscript{-}], \(E_m\) and blood pressure are linked in the way that is suggested. Thirdly, the regime for the induction of hypertension requires that the animals are given solutions of 1% NaCl/0.2% KCl to drink. If, however, the animals are given Cl\textsuperscript{-}-free (or Na\textsuperscript{+}-free) solutions to drink, then the evidence in the literature is that DOCA/salt hypertension either does not develop or is greatly attenuated [10, 11]. This suggests that in this experimental situation Cl\textsuperscript{-}, no less than Na\textsuperscript{+}, has an important role in the development of hypertension.

The reasons why the activities of co-transport and ‘pump III’ increase in hypertension is unknown, and there is also the possibility that the kidney might be involved. Thus, up-regulation of Na\textsuperscript{+}-K\textsuperscript{+}-Cl\textsuperscript{-} co-transport in the kidney would lead to volume expansion and hence hypertension [12] and, whilst there is no direct evidence that ‘pump III’ occurs in

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Normotension</th>
<th>Hypertension</th>
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<tbody>
<tr>
<td>Control</td>
<td>30.7 ± 1.3 (16)</td>
<td>48.7 ± 2.2 (9)</td>
</tr>
<tr>
<td>+10 μmol/l bumetanide</td>
<td>20.9 ± 2.4 (15)</td>
<td>30.4 ± 1.9 (9)</td>
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<tr>
<td>ΔBumetanide</td>
<td>9.8 ± 2.7</td>
<td>18.2 ± 2.9</td>
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<tr>
<td>+10 μmol/l bumetanide + 1 mmol/l acetazolamide</td>
<td>13.4 ± 1.6 (7)</td>
<td>14.7 ± 1.7 (9)</td>
</tr>
<tr>
<td>ΔAcetazolamide</td>
<td>7.4 ± 2.9</td>
<td>15.8 ± 2.5</td>
</tr>
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the kidney, a Cl\(^{-}\) transport system which is inhibited by ethacrynic acid with a very steep concentration dependence, which is characteristic of 'pump III' [7], does occur in the cortical thick ascending limb in rabbit kidney [13].

In conclusion, during the development of DOCA/salt hypertension in the rat, two inward Cl\(^{-}\) transport systems are activated in arterial smooth muscle. Whether these transport systems cause the blood pressure to rise, via the depolarizing influence of [Cl\(^{-}\)]\(_i\) on \(E_m\) in arterial smooth muscle, is not certain. On the other hand, there is compelling evidence that Cl\(^{-}\) plays an essential role in the development of DOCA/salt hypertension, although the idea that Cl\(^{-}\) is important in hypertension is not new (L. Ambard and E. Beaujard, 1904; cited in [10]).

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**REFERENCES**