Effect of amiloride analogs on DOCA-salt-induced hypertension in rats

RICHARD F. KEEP, XIAOCHEM SI, PARVIN SHAKUI, STEVEN R. ENNIS, AND A. LORRIS BETZ

Departments of Surgery (Section of Neurosurgery), Pediatrics, and Neurology, University of Michigan, Ann Arbor, Michigan 48109-0532

Keep, Richard F., Xiaochen Si, Parvin Shakui, Steven R. Ennis, and A. Lorris Betz. Effect of amiloride analogs on DOCA-salt-induced hypertension in rats. Am. J. Physiol. Heart Circ. Physiol. 45: H2215–H2220, 1999.—Intracerebroventricular infusions of an amiloride analog, benzamil, reduce blood pressure in several rat models of hypertension. This effect has been attributed to an inhibition of amiloride-sensitive Na+ channels in the brain. This study examines whether intracerebroventricular benzamil would prevent the onset of deoxycorticosterone acetate (DOCA)-salt-induced hypertension in rats and whether this effect correlates with an inhibition of ion transport through the known amiloride-sensitive cation channels at the blood-brain barrier. We also examine whether the effects of benzamil on blood pressure are mediated by a Na+ channel by comparing the effects of different amiloride analogs. Benzamil (0.15 and 0.5 µg icv) did significantly attenuate the increase in blood pressure induced by DOCA treatment. This antihypertensive effect, however, was not associated with an alteration in a blood-brain barrier ion transport as assessed by measurements of blood-to-brain 22Na transport and cerebral spinal fluid Na+ and K+ concentrations. Indeed, intracerebroventricular infusion of dimethyl amiloride, an amiloride analog with low affinity for Na+ channels, also attenuated the increase in blood pressure induced by DOCA-salt treatment. Comparisons of the effects of benzamil, dimethyl amiloride, and 3,4-dichlorobenzamil, another amiloride analog, suggest that these antihypertensive effects are mediated by an inhibition of Na+/Ca2+ exchange in the brain.

Three recent studies have demonstrated that intracerebroventricular infusions of benzamil can prevent the onset of mineralocorticoid-induced and Na+-dependent hypertension (7, 8, 18). Benzamil, an amiloride analog, is a Na+-channel inhibitor (14), and this has been thought to be the mechanism by which it has its antihypertensive effects. However, the extent to which brain parenchymal cells express amiloride-sensitive Na+ channels is in question (5, 16, 27), although amiloride-sensitive nonselective cation channels are present at the cerebral endothelium (26), the site of the blood-brain barrier (BBB). Those endothelial channels are thought to be involved in the movement of Na+ and K+ between the blood and brain (6, 26), and changes in brain K+ regulation have been implicated in the genesis of deoxycorticosterone acetate (DOCA)-salt-induced hypertension (13).

This study examines the effects of intracerebroventricular infusion of benzamil on DOCA-salt-induced hypertension and whether intracerebroventricular benzamil alters BBB Na+ transport and brain K+ homeostasis. Finally, this study examines whether the effects of benzamil are mediated by a Na+ channel by comparing the effects of three different amiloride analogs, benzamil, dimethyl amiloride (DMA), and 3,4-dichlorobenzamil (DCB), which have markedly different affinities for amiloride-sensitive Na+ channels (14). Amiloride has effects on a number of transport systems, including Na+ channels, Na+/H+ exchange, and Na+/Ca2+ exchange (14). Benzamil is more selective than amiloride for the Na+ channel, but it also inhibits the other systems. By contrast, DMA preferentially inhibits Na+/H+ exchange, whereas DCB has a higher affinity for the Na+ and Ca2+ exchanger and a lower affinity for the Na+ channel than benzamil.

MATERIALS AND METHODS

Experimental protocols. All procedures were approved by the University Committee on Use and Care of Animals at the University of Michigan. Two sets of experiments were performed on adult male Sprague-Dawley rats weighing between 275 and 300 g. The first set examined changes in blood pressure in conscious DOCA-salt- and sham-treated rats 2 wk postoperative. The rats received intracerebroventricularly either 300 mM mannitol or 300 mM mannitol with 1 mg/ml benzamil for the 2 wk. Six rats were used for each group. At the end of the infusion period, the rats were used to examine cerebral spinal fluid (CSF) K+ homeostasis and blood-to-brain 22Na transport. In the second set of experiments, the effect of intracerebroventricular infusions of different concentrations (0.1, 0.3, and 1.0 mg/ml) of either benzamil, DMA, or DCB on the blood pressure was examined in conscious DOCA-salt- and sham-treated rats. Five to six rats were used for each dose. Whether the effects of the highest dose (1.0 mg/ml) of DMA and DCB on blood pressure were mediated centrally was investigated by infusing the same dose subcutaneously. Four rats were used in each of these groups.

Animal preparation and blood pressure measurements. Rats were initially trained for tail-cuff systolic blood pressure measurement on at least three separate occasions to establish a baseline blood pressure. Systolic pressures were then measured every 3–4 days during the study.

After training for the blood pressure measurements, animals were anesthetized with ketamine (50 mg/kg) and xylazine (10 mg/kg) intramuscularly. All animals were uninephrectomized via a flank incision with care being taken to preserve the adrenal gland and its circulation. The animals designated as DOCA-salt also received Silastic implants impregnated with DOCA (200 mg/kg body wt), which were placed subcutaneously in the dorsal neck region. These animals were given...
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expressed as an influx rate constant ($K_i$) for brain uptake, calculated as

$$K_i = \frac{C_{br}}{\mathcal{A}C_{a}dt}$$

where $C_{br}$ is the concentration of extravascular tracer in the brain, and $\mathcal{A}C_{a}dt$ is the integral of the arterial tracer concentration. $C_{br}$ was calculated from total tracer counts ($C_{tot}$) in the brain samples, final tracer plasma concentration ($C_{pl}$) and plasma volume (PV) as

$$C_{br} = C_{tot} - (PV \times C_{pl})$$

PV was determined as the $[\text{H}]$inulin space of the brain samples from the $[\text{H}]$inulin concentration in the final plasma sample.

Materials. Rats were purchased from Charles River Laboratories (Portage, MI). Benzamil was obtained from Research Biochemicals International (Natick, MA), and dimethyl amiloride was from Sigma (St. Louis, MO). 3,4-Dichlorobenzamil was provided by Research Biochemicals International as part of a Chemical Synthesis Program of the National Institute of Mental Health (Contract N01MH30003). $^{22}$Na and $[\text{H}]$inulin came from New England Nuclear (Boston, MA).

Statistical analysis. The data are expressed as means ± SE. Statistical differences between groups were evaluated by ANOVA with a Newman-Keuls multiple comparison test. The effect of different amiloride analogs on blood pressure in sham-operated and DOCA-salt-treated rats was examined by ANOVA with a Dunnett’s post hoc test.

RESULTS

Effects of intracerebroventricular benzamil on DOCA-salt-induced hypertension. As expected, DOCA-salt treatment caused a marked increase in blood pressure over 2 wk (Fig. 1). At 2 wk mean systolic blood pressures were elevated by ~50 mmHg compared with controls ($P < 0.001$). Intracerebroventricular infusion of benzamil (1 mg/ml at 0.5 µl/h) had no effect on blood pressure in the sham-operated rats, but it significantly blunted the increase in blood pressure found in DOCA-salt-treated rats ($P < 0.001$; Fig. 1).

At the end of the 2-wk period, there was no significant difference in body weight between sham-operated and DOCA-salt-treated rats with or without benzamil (Table 1). Similarly, there were no significant differences among the four groups in blood $P_{CO_2}$ or hematocrit. DOCA-salt-treated rats, with or without benzamil, were alkalotic compared with sham-operated rats. There were also some minor variations in $P_{O_2}$ among the four groups, but these are likely to have no physiological significance.

The $K_i$ values for blood-to-brain and blood-to-CSF $^{22}$Na$^+$ transport were not significantly different in sham-operated and DOCA-salt-treated rats (Fig. 2). Intracerebroventricular benzamil (1 mg/ml) also did not significantly affect the $K_i$.

Plasma and CSF $[\text{Na}^+]$ were both unaffected by DOCA-salt treatment (2 wk), and intracerebroventricular benzamil did not alter either parameter in sham- or DOCA-salt-treated rats (Fig. 3A). Compared with sham-operated rats, DOCA-salt-treated rats were hypokalemic (Fig. 3B). The degree of hypokalemia was slightly less in the intracerebroventricular benzamil (1 mg/ml) group, but CSF $[\text{K}^+]$ was unaffected.
Hypertension. At a dose of 1 mg/ml (at 0.5 µl/h), the administration of amiloride analogs
Table 2. | Ion concentrations in sham and DOCA-salt-treated rats with and without intracerebroventricular administration of amiloride analogs |
| Blood pH | |
| Na⁺ | K⁺ | Ca²⁺ |
| sham | | | | | | |
| sham + DMA | | | | | | |
| sham + DCB | | | | | | |
| DOCA-salt | | | | | | |
| DOCA-salt + DMA | | | | | | |
| DOCA-salt + DCB | | | | | | |

Table 2 displays measurements of plasma and CSF ion concentrations after 2 wk of intracerebroventricular infusion of the highest dose of the amiloride analogs (1 mg/ml) compared with a vehicle infusion. Neither DMA nor DCB affected blood or CSF ion concentrations in sham-operated rats. Similarly, neither of these analogs affected ion concentrations in DOCA-salt-treated rats apart from a slight enhancement of the alkalosis generated by DOCA-salt treatment following intracerebroventricular infusion of DMA.

DISCUSSION

This study confirms the effect of intracerebroventricular benzamil on DOCA-salt-induced hypertension. However, this study found no evidence that intracerebroventricular benzamil affected BBB Na⁺ transport or brain K⁺ homeostasis, suggesting that it does not act by affecting the known amiloride-sensitive cation channel at the BBB. Although this result does not indicate whether intracerebroventricular benzamil might inhibit a parenchymal cell Na⁺ channel, a comparison of the effects of different amiloride analogs with those of benzamil suggests that the anti hypertensive effects are not mediated by inhibiting such a channel.

Effects of intracerebroventricular benzamil on DOCA-salt-induced hypertension. Gomez-Sanchez and Gomez-Sanchez (7, 8) first reported that intracerebroventricular infusions of benzamil could prevent mineralocorticoid-induced hypertension and salt-induced hypertension in Dahl salt-sensitive rats. Nishimura et al. (18) then expanded this work, demonstrating that intracerebroventricular benzamil could reduce blood pressure in a number of forms of Na⁺-induced hypertension, including DOCA-salt-induced hypertension. These blood pressure effects of benzamil were mediated centrally because systemic administration at the doses used had no effect on blood pressure (7, 8, 18).

In the current study, we have also shown that intracerebroventricular benzamil can prevent the effects of DOCA-salt treatment on blood pressure. These
results complement those of Nishimura et al. (18), who found that benzamil could reduce blood pressure in DOCA-salt-treated rats with established hypertension. The doses of benzamil that were effective in our study (0.3 and 1 mg/ml infused at 0.5 µl/h) were the same as those used by Gomez-Sanchez and Gomez-Sanchez in their studies (7, 8). The method used in this study to measure conscious systolic blood pressure (the tail cuff) can have limitations in studies where blood pressure changes are small. However, the effect of DOCA-salt treatment on blood pressure and the effect of amiloride analogs on that change are of a considerable magnitude (30–50 mmHg at 14 days), negating the need for measurements with indwelling catheters.

We next examined whether intracerebroventricular benzamil at these concentrations could affect the known amiloride-sensitive cation channel at the BBB (26). This channel is a nonselective cation channel (26), and although it appears to be involved in the movement of Na⁺ from blood to the brain (6), it may also be involved in K⁺ transport at the BBB (12). Infusing benzamil at 1 mg/ml at 0.5 µl/h failed to alter either blood-to-brain ²²Na transport or CSF K⁺ concentration, even though it did ameliorate the effects of DOCA-salt treatment on blood pressure, indicating that these antihypertensive effects are not mediated by the inhibition of the BBB ion channel.

In addition, although intracerebroventricular infusions of hypertonic NaCl (1, 25) induce increases in blood pressure, and changes in CSF Na⁺ concentration have been implicated in the genesis of Na⁺-dependent hypertension (17), we found no effect of benzamil (or any of the amiloride analogs) on CSF Na⁺. Indeed, in common with the findings of Takata et al. (23) and Nishimura et al. (18), we found no effect of DOCA-salt treatment on CSF Na⁺ concentration.

Effects of amiloride analogs. The absence of an effect of benzamil on BBB Na⁺ transport (and CSF Na⁺ and K⁺ concentration) suggests that benzamil does not have its antihypertensive effect by inhibiting the known BBB nonselective cation channel. A comparison of the effects of the different amiloride analogs on DOCA-salt-induced hypertension suggests that the antihypertensive effects are not mediated by the inhibition of a parenchymal cell Na⁺ channel but may rather be via an alteration in brain Ca²⁺ homeostasis. As with benzamil (7, 8, 18), the blood pressure effects of DMA and DCB appear to be mediated centrally because systemic administration did not affect blood pressure in DOCA-salt-treated rats.

Amiloride has effects on a wide range of ion transporters and ion channels, including Na⁺/H⁺ exchange and certain Na⁺ channels (reviewed in Ref. 14). Specific amiloride analogs have been developed that have different affinities for these transporters. Thus benzamil inhibits amiloride-sensitive Na⁺ channels at concentrations 10 times lower than with amiloride and 100 times lower than with DMA (14). In contrast, DMA inhibits Na⁺/H⁺ exchange at concentrations 10 times lower than with amiloride and 100 times lower than with benzamil (14). The fact that benzamil and DMA have potencies for the Na⁺ channel and the Na⁺/H⁺ exchanger that differ by two orders of magnitude but have almost the same potency in inhibiting the hypertensive effect of DOCA-salt treatment suggests that this antihypertensive effect is not mediated through a Na⁺ channel or Na⁺/H⁺ exchange.

Amiloride and its analogs are also inhibitors of Na⁺-K⁺-ATPase and Na⁺-coupled transport (e.g., Na⁺-PO₄³⁻ transport). However, these actions are unlikely to be involved in the antihypertensive actions described in this study. Inhibition of these types of transport requires concentrations close to the millimolar range (14), and the concentration in CSF in the present experiments may, at the highest dose, be closer to 10–15 µM [rat CSF production of 2 µl/min (11) would be expected to dilute the infusate 240-fold]. In addition, central administration of a Na⁺-K⁺-ATPase inhibitor ouabain actually causes increases in blood pressure (9).

Amiloride analogs also inhibit Na⁺/Ca²⁺ exchange (14). Unlike potencies for the Na⁺ channel and the Na⁺/H⁺ exchanger, the potencies of DMA and benzamil for the Na⁺/Ca²⁺ exchanger differ by less than an order of magnitude with benzamil having the higher affinity. DCB has a higher affinity for the Na⁺/Ca²⁺ exchanger than either of the other amiloride analogs, but its potency is still only about 10 times that of DMA. Thus, unlike with the Na⁺ channel and the Na⁺/H⁺ exchanger, the relative potency of the different amiloride analogs for inhibiting Na⁺/Ca²⁺ exchange fairly closely matches their antihypertensive effects. In vitro with astrocytes and brain membrane preparations, DCB inhibits Na⁺/Ca²⁺ exchange with an IC₅₀ of about 15–30 µM (10, 24). These values are similar to the CSF concentrations expected from the intracerebroventricular infusions used in the experiments in this study.

There have been numerous studies in humans and animals examining the role of Ca²⁺ in the regulation of blood pressure (reviewed in Ref. 21) and intracerebroventricular infusions of Ca²⁺ lower blood pressure (4, 15, 22). Amiloride analog-induced inhibition of Na⁺/Ca²⁺ exchange might be expected to have a similar effect on blood pressure by raising intracellular Ca²⁺ concentrations. Amiloride analogs can also inhibit Ca²⁺ channels (3, 14), but such inhibition would be expected to lower intracellular Ca²⁺ concentrations.

In summary, these results support previous findings on the antihypertensive effects of intracerebroventricular benzamil but suggest that these are not mediated by the inhibition of brain Na⁺ channels. Rather, the intracerebroventricular administration of amiloride analogs may ameliorate the effects of DOCA-salt treatment on blood pressure by inhibiting Na⁺/Ca²⁺ exchange.
REFERENCES


