Chronic Blood Pressure Effects of Bufalin, a Sodium-Potassium ATPase Inhibitor, in Rats

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Abstract

Endogenous Na+,K+-ATPase inhibitors may have a role in the mechanism of low-renin hypertension. Two such compounds have been characterized: ouabain from human plasma and resibufogenin from toad plasma. Previously, we examined the acute effects of ouabain and bufalin (which has the same structure as resibufogenin except for one H+) in normal rats. Bufalin raised blood pressure, but ouabain had little effect. In contrast, given chronically, ouabain substantially increased blood pressure in normal rats and 70% reduced renal mass rats on a salt-free diet. We have now examined the chronic effects of bufalin in rats. Normal rats received 14.8 μg/kg per day bufalin or an equimolar dose of ouabain intraperitoneally for 6 weeks; 70% reduced renal mass rats also received 14.8 μg/kg per day bufalin. Another group of normal rats received 29.6 μg/kg per day bufalin intraperitoneally for 6 weeks. Respective control animals received vehicle.

In contrast to ouabain, blood pressure did not increase in normal rats receiving the 14.8 μg dose of bufalin. However, normal rats receiving 29.6 μg bufalin and 70% reduced renal mass rats receiving 14.8 μg bufalin developed significant increases in blood pressure. Increases in blood pressure were associated with decreases in myocardial Na+,K+-ATPase activity and correlated with increased plasma Na+,K+-ATPase inhibitory activity. Thus, although bufalin is a more potent pressor agent than ouabain when both agents are given acutely, ouabain is at least as potent a vasopressor agent as bufalin when given chronically. Thus, both are pressor agents, more so in the presence of reduced renal mass, when given chronically in the rat. (Hypertension. 1994;23[suppl I]: I-106-I-109.)

Key Words • bufanolides • ouabain • Na+,K+-ATPase • sodium-potassium pump • blood pressure

Reports from several laboratories suggest the presence of a circulating Na+,K+-ATPase (NKA) inhibitor during acute volume expansion1-3 and in low-renin experimental4-6 and human essential7-9 hypertension. It has been hypothesized that this factor, through inhibition of NKA as with the cardiac glycosides or with decreased [K+], constricts blood vessels,10 enhances vasoconstrictor responses to agonists,11 increases cardiac contractility (dP/dt),12 and raises blood pressure (BP).13 Numerous investigators have attempted to isolate this endogenous NKA inhibitor. However, only two steroid NKA inhibitors have been purified and chemically characterized—resibufogenin, a bufodienolide derivative in toad skin and plasma,14 and a substance with the same basic structure as ouabain from human plasma.15 In an earlier study16 designed to determine whether such endogenous inhibitors in fact produce the above cardiovascular changes, we compared the acute effects of equimolar doses of commercially available bufalin (aglycone, identical to resibufogenin except for one H+) and ouabain (Fig 1) in normal rats. Bufalin infused intravenously into anesthetized rats increased BP, dP/dt, heart rate, urine volume, and urinary sodium excretion. When applied to tail arteries from normal rats in vitro, bufalin depolarized vascular smooth muscle cell membrane potentials. Although equimolar doses of ouabain caused the same directional changes in all parameters except heart rate and membrane potentials, the magnitudes of these changes were always less than those produced by bufalin (ouabain had no effect on heart rate or vascular smooth muscle cell membrane potentials). In fact, ouabain actually decreased BP at higher doses. In contrast, in a subsequent study17 we found that ouabain given chronically in the rat produced the above cardiovascular changes, and in theory should be more sensitive to pressor agents because of reduced pressure diuresis and renal clearance.

Methods

Male Wistar rats (150 to 200 g) were given free access to tap water and standard rat chow. When they reached the appropriate weight, they were entered into the study as 70% RRM rats or two-kidney (2K) normal rats.

Preparation of 70% Reduced Renal Mass Normotensive Rats

Under ether anesthesia, male rats (280 to 300 g) underwent subtotal nephrectomy (70±1% of the renal mass removed).4,5 The right kidney and both poles of the left kidney (20% of the total renal mass) were removed, for a total reduction of 70% of the renal mass. After recovery from surgery, the animals consumed a salt-free diet and drank distilled water. Indirect BP (tail plethysmography) was recorded weekly. As expected, the 70% RRM rats on salt-free food and water remained normotensive.

Normal Two-Kidney Rats

Normal male Wistar rats were entered into the study when they weighed 300 to 350 g and were maintained on normal rat

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Administration of High-Dose Bufalin

Because preliminary results showed that the low dose of bufalin had no effect on BP in normal rats, some normal 2K experimental and control group rats were treated with a higher dose of bufalin or vehicle, respectively, as follows: On day 1 experimental animals received a 36.0 µg/kg IP loading dose, followed by 29.6 µg/kg per day IP for 6 weeks. Control animals received an equivalent volume of vehicle only.

Indirect BP was recorded weekly during the treatment period. After 6 weeks of treatment, direct BP was measured with rats given intraperitoneally as a loading dose, followed by 29.6 µg/kg per day IP for 6 weeks. Control animals received vehicle intraperitoneally daily for 6 weeks as described below. Doses of ouabain and bufalin were the same as those used in our previous study.17

Administration of Low-Dose Bufalin or Ouabain

Experimental animals in each group (2K normal and 70% RRM rats) received a low dose of bufalin dissolved in 10% ethanol in saline or an equimolar dose of ouabain dissolved in normal saline as follows: On day 1, 18.0 µg/kg bufalin was given intraperitoneally as a loading dose, followed by 14.8 µg/kg per day IP bufalin for 6 weeks. Control animals received vehicle (10% ethanol in saline). Animals treated with ouabain received a loading dose of 34.0 µg/kg followed by 27.8 µg/kg per day ouabain for 6 weeks. Respective control animals received vehicle only.

Administration of High-Dose Bufalin

Microsomal fractions from left ventricles were prepared by our standard technique.5,18 Membrane ATPase activity was assayed by measurement of the amount of inorganic phosphate liberated from ATP (Tris-ATP, Sigma Chemical Co, St Louis, Mo) during 1 hour of incubation at 37°C in a water bath shaker using our standard technique.4,18 Plasma NKA inhibitory activity was measured using canine kidney NKA by a modification of the method of Masugi et al.19 The modification included the use of freshly prepared NKA and 2 mmol/L rather than 10 mmol/L KCl in the final assay medium, as we found that NKA inhibition by ouabain in 2 mmol/L KCl was much greater than in the presence of 10 mmol/L KCl.

Data Analysis

The unpaired t test was used to compare means of experimental and control group parameters. Analysis of variance with repeated measures followed by Duncan's multiple range test was used for among-group comparisons. Correlation coefficients were calculated to determine the functional relation of one variable to another. A value of $P<.05$ was considered significant.

Results

All animals entered in the study remained healthy throughout and gained weight normally. Body weights were not significantly different between animals receiving ouabain, bufalin, or their respective vehicles.

Animals receiving the low dose of ouabain developed a greater increase in BP than animals receiving vehicle (Fig 2A). This was not the case for normal rats receiving an equimolar dose of bufalin (Fig 2B). However, normal rats receiving the high dose (Fig 2C) and RRM rats receiving the low dose (Fig 2D) of bufalin had much higher pressures than their vehicle control animals. These findings were confirmed by direct measurements of BP at the end of the experiments (data not shown).

Plasma NKA inhibitory activities in normal rats receiving the high dose and RRM rats receiving the low dose of bufalin were significantly increased (Fig 3). The increases in plasma inhibitory activity were significantly correlated with the increases in BP ($r=.704, P<.005$ and $r=.72, P<.002$, respectively). Myocardial NKA activity was significantly decreased in normal rats receiving the high dose and in RRM rats receiving the low dose of bufalin (Fig 4).

Discussion

The lower dose of ouabain raised BP in normal rats and the lower dose of bufalin did not, suggesting that ouabain is more efficacious than bufalin when administered on a chronic basis. The lower dose of bufalin produced a rise in BP in the 70% RRM rats but not in the 2K normal rats, again suggesting that these NKA inhibitors are more effective vasopressor agents in RRM animals. The significant correlation between the increase in plasma NKA inhibitory activity and the increase in BP with the higher and lower doses of bufalin in the 2K normal and 70% RRM rats, respectively, associated with the decrease in myocardial NKA suggests that the vasopressor effect of chronic administration of bufalin is at least in part due to its NKA inhibitory activity.

These chronic effects differ from the changes we observed in our acute study,16 in which bufalin produced larger changes than ouabain. This difference could not be attributed to the fact that ouabain is a glycoside whereas bufalin is an aglycone because ouabagenin, the aglycone of ouabain, was without any effect. These findings led us to conclude that bufalin is a more effective pressor agent than ouabain, at least in rats. This is consistent with data which indicate that the
binding of cardiac glycosides, such as ouabain, to NKA from rat kidney is less stable than in other species more sensitive to these glycosides. It is also consistent with our finding that bufalin is a more potent inhibitor of rat myocardial NKA activity than ouabain in vitro.

However, these conclusions had to be revised when we showed that chronic intraperitoneal administration of ouabain daily over a 6-week period produces an increase in BP in 2K normal and 70% RRM rats. The present study reinforces the conclusion that the acute and chronic effects of ouabain may not be the same. The ability of ouabain to raise BP when given chronically but not acutely may be due to the fact that the association rate constant for cardiac glycosides such as ouabain is often several-fold less than that of aglycones such as bufalin. Acutely administered ouabain may not act as a vasopressor simply because of slow binding to rat NKA. This may explain the failure of ouabain to produce depolarization when applied to vascular smooth muscle cells in rat tail arteries in vitro. Indeed, prolonged incubation of human mesenteric arteries in ouabain produces a time-dependent increase in tone.

The difference may also be related to the route of administration. In the chronic study, unlike in the acute study, the agents were administered intraperitoneally. It is also possible that the absorption rates are different, or the rate and/or mode of metabolism is different as the agents pass portal circulation. Thus, the association rate constant of ouabain may be enhanced as it passes through the liver so that it binds more readily to NKA. Using a radioimmunoassay for ouabain, Hamlyn et al showed that ouabainlike immunoreactivity levels are higher in deoxycorticosterone acetate–saline hypertensive rats relative to control rats. Elevated levels of ouabainlike immunoreactivity have been found in patients with primary aldosteronism and essential hypertension. These findings suggest that ouabain or a closely related compound probably has a role in hypertension.

Our findings again show that RRM animals are more sensitive to endogenous NKA inhibitors. A dose...
of bufalin that is ineffective in normal rats raises BP in 70% RRM rats. These animals, if fed a regular salt-containing rat chow, develop increased extracellular fluid volume, depressed plasma renin activity, increased cardiovascular NKA activity, and hypertension. In contrast, 70% of bufalin that is ineffective in normal rats raises BP in normal rats after exogenous administration, perhaps because of renal function. Thus, these animals are a model of reduced renal mass hypertension.

Acknowledgment

Fig 1 was reproduced from Hypertension (1989;13:690-695).

References


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