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.

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 study we found that ouabain given chronically raised BP in both normal and 70% reduced renal mass (RRM) rats consuming salt-free food and distilled water. This suggested that the chronic and acute responses to the administration of these endogenous NKA inhibitors may not be the same. In the present study we therefore examined the chronic effects of bufalin in normal and 70% RRM rats. The latter rats were used because they have elevated plasma levels of an Na⁺-K⁺ pump inhibitor 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). 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
Chronic Treatment with Bufalin or Vehicle

Using the same protocol as in our previous chronic ouabain treatment study, we divided 2K normal and normotensive 70% RRM rats into experimental and control groups. Experimental groups received ouabain or bufalin (equimolar doses), and control groups 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.

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 Kg/kg per day and control group rats were treated with a higher dose of bufalin or vehicle, respectively, as follows: On day 1, 18.0 μg/kg bufalin was given intraperitoneally as a loading dose, followed by 29.6 μg/kg per day IP bufalin 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 under thiobutabarbital anesthesia. Blood samples then were taken and hearts removed for the measurement of plasma NKA inhibitory activity and myocardial NKA activity, respectively.

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.

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, 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. This 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 also 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 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 plasma NKA inhibitory activity, suppressed cardiovas-
cular NKA activity, and hypertension. In contrast, 70% RRM rats consuming a salt-free diet and water do not develop hypertension.45 However, the serum creatinine levels of these rats is increased,5 indicating impaired renal function. Thus, these animals are a model of maximally compensated normotension and are probably more sensitive to raised plasma levels of NKA inhibitor after exogenous administration, perhaps because of reduced renal clearance and pressure diuresis. These findings, along with those of our previous studies,16,17 suggest that in rats bufalin is a more potent vasopressor agent than ouabain on acute administration, but when effect of low-dose treatment In 70% reduced renal mass (RRM) rats. Pi Indicates inorganic phosphate.

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

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