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Effects of two endogenous Na^+,K^+-ATPase inhibitors, marinobufagenin and ouabain, on isolated rat aorta

Alexei Y. Bagrov [a,b,*], Natalia I. Roukoyatkina [b], Alexander G. Pinaev [b], Renata I. Dmitrieva [b], Olga V. Fedorova [a,b]

[a] *Laboratory of Behavioral Sciences, National Institute on Aging, National Institutes of Health, 4940 Eastern Avenue, Baltimore, MD 21224, USA*
[b] *Laboratory of Pharmacology, I.M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, 194223, Russian Federation*

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Abstract

Previously, we reported that the venom of *Bufo marinus* toad contains a Na^+,K^+-ATPase inhibitor with potent vasoconstrictor activity. In the present study, using thin-layer chromatography in Silicagel 60 $F_{254+366}$, we separated a vasoactive substance from a mixture of steroids from *Bufo marinus* venom. Based on chromatographic mobility of this substance and typical color reaction after its vizualization with $SbCl_3$, we identified it as a previously described steroid, marinobufagenin. Vasoconstrictor and Na^+,K^+ pump inhibitory properties of marinobufagenin were studied in isolated rat aortic rings and compared with those of ouabain. Ouabain (10–100 $\mu mol \cdot l^{-1}$) produced weak vasoconstriction, which was blocked by 2 $\mu mol \cdot l^{-1}$ phentolamine. 10 $\mu mol \cdot l^{-1}$ ouabain stimulated, and at higher concentrations inhibited, the Na^+,K^+ pump. 2 $\mu mol \cdot l^{-1}$ phentolamine abolished the activating effect of 10 $\mu mol \cdot l^{-1}$ ouabain on the Na^+,K^+ pump, but did not alter the inhibitory action of higher concentrations of ouabain. By contrast, marunibufagenin elicited rapid and strong vasoconstriction and inhibited ouabain-sensitive ^{86}Rb uptake. Antidigoxin antibody antagonized the vasoconstrictor responses to marinobufagenin, but not to ouabain. 2 $\mu mol \cdot l^{-1}$ phentolamine did not alter the constrictor effect of marinobufagenin. In solid-phase digoxin immunoassay, marinobufagenin demonstrated higher digoxin-like immunoreactivity than ouabain.

Keywords: Na^+,K^+-ATPase; Bufodienolide; Marinobufagenin; Ouabain; Smooth muscle, vascular; Vasoconstriction

1. Introduction

The relevance of Na^+,K^+ pump inhibitors to the regulation of blood pressure and to the genesis of hypertension is suggested by data showing that under the conditions of plasma volume expansion during salt loading and in some forms of human and animal hypertension, plasma concentrations of one or more endogenous digitalis-like factors (EDLF) (Fig. 1) increase and may contribute to vasoconstriction (Pamnani et al., 1981; Goto et al., 1992; Hamlyn and Manunta, 1992). In human and animal hypertension, plasma concentrations of digitalis-like immunoreactive material and the ability of plasma to inhibit Na^+,K^+ ATPase have been shown to correlate positively with blood pressure (Hamlyn et al., 1982; Gruber et al., 1985; Masugi et al., 1987).

Recent findings demonstrated that several EDLFs may exist in mammalian plasma (Kelly et al., 1985; Shaikh et al., 1991; Doris, 1994). The candidates for vasoactive EDLF include endogenous ouabain (Ludens et al., 1991), bufalin (Kieval et al., 1988; Cress et al., 1991), resibufagenin (Lichtstein et al., 1986) and a digoxin-like immunoreactive factor (Shaikh et al., 1991).

Many of the effects of EDLF have been demonstrated in the rat, even though Na^+,K^+-ATPase in rat cardiovascular tissues is relatively insensitive to inhibition by digitalis glycosides. Concurrent inhibition of the Na^+,K^+ pump in rat tissues and increased plasma concentrations of EDLF have been shown in volume-dependent hypertension (Pamnani et al., 1990; Clough et al., 1983), in spontaneously hypertensive rats (Rosati et al., 1988; Wauquier et al., 1988), during acute saline

* Corresponding author.

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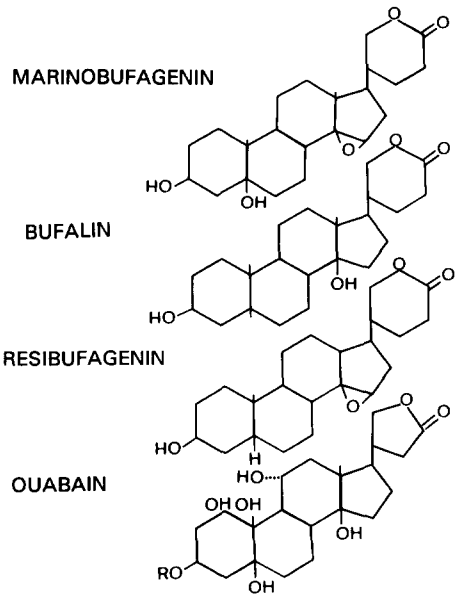

Fig. 1. Chemical structure of endogenous digitalis-like factors.

plasma volume expansion (Pamnani et al., 1981) and in acute myocardial ischemia (Bagrov et al., 1989, 1993a). Ouabain is the only EDLF which has been purified from mammalian plasma (Ludens et al., 1991). In isolated rat arteries, ouabain alone produces vasoconstriction only at very high concentrations, and its constrictor effects are relatively weak and slow (Karaki et al., 1978; Cutaia and Rudio, 1992). Unlike other species (e.g. guinea pig) norepinephrine release from perivascular adrenergic neural endings is likely to be important for the vasoconstrictor effect of ouabain in rat blood vessels (Karaki et al., 1978). Catecholamines have been shown to stimulate the Na^+,K^+-ATPase/Na^+,K^+ pump in various tissues (Desilets and Baumgarten, 1986) including rat vascular smooth muscle (Lynch et al., 1986), therefore, displaying the effect opposite to that of EDLF. Thus, reactivity of rat pulmonary artery to catecholamines was found to decrease following pretreatment with ouabain (Cutaia and Rudio, 1992). However, plasma volume expansion of normotensive rats (Pamnani et al., 1981) and rat models of hypertension with increased plasma EDLF (Pamnani et al., 1981; Songu-Mize et al., 1982; Huot et al., 1983) are associated with a rapidly developing inhibition of vascular Na^+,K^+-ATPase. In the rat ouabain administration causes hypertension, but only after several weeks (Yuan et al., 1993). These observations suggest that endogenous Na^+,K^+-ATPase inhibitor(s) other than ouabain may be responsible for the described rapid Na^+,K^+-ATPase inhibitory effects. Thus, Doris (1994) has recently shown that, although plasma levels of endogenous ouabain are increased in spontaneously hypertensive rats, other EDLFs are likely to be responsible for the ability of plasma to inhibit the sodium pump.

Previously, various tissues of *Bufonidae* toads have been shown to contain high concentration of bufodienolide Na^+,K^+-ATPase inhibitors which interact with digoxin antibody (Flier et al., 1980). Recently, we have shown that the digoxin-like immunoreactive material from *Bufo marinus* toad contains Na^+,K^+-ATPase inhibitor(s) which cause rapid, strong and sustained vasoconstriction in rat aorta (Bagrov et al., 1993b). This vasoconstriction developed independently from interactions of EDLF with the neural endings. Considering the recent evidence that antibodies against bufodienolides interact with the material from human (Lichtstein et al., 1993; Schoner et al., 1993), rat (Bagrov et al., 1993c) and dog (Bagrov et al., 1994) tissues, further study of the vasoconstrictor effect of EDLF from *Bufo marinus* toad was undertaken.

The present data (i) demonstrate that the potent vasoconstrictor and Na^+,K^+ pump inhibitory effects of the venom from *Bufo marinus* toad is likely to be due to the presence of a previously described steroid, marinobufagenin, and (ii) compares immunoreactivity, vasoconstrictor properties and interactions with the vascular Na^+,K^+ pump of marinobufagenin and ouabain.

2. Materials and methods

2.1. Purification procedures

Venom was collected from adult *Bufo marinus* toads of both sexes from the Latvian Zoological Gardens (Riga). 600 mg of crystallized venom was subjected to a 2-week preextraction using 50% ethanol at $T = 30°C$. After this period, the mixture was filtered through Shott No. 4 filters, and the filter was washed with 50% ethanol. After removal of the filtrate, the residue was further extracted with a 1 : 1 solution of 50% methanol and chloroform followed by centrifugation ($1000 \times g$, 10 min). The chloroform phase was isolated and distilled under vacuum. The resulting 83 mg of the mixture of steroids was dissolved in ethyl acetate and further analyzed by thin-layer chromatography based on chromatographic mobility (R_f) of various steroidal fractions (Silica-gel 60 $F_{254+366}$, Merck, Darmstadt, Germany, elution with ethyl acetate) as reported previously by Meyer and Linde (1971). Detection of steroids was performed using ultraviolet light vizualization and spraying a saturated chloroform solution of $SbCl_3$ for color reactions (Ruckstuhl and Meyer, 1957).

2.2. Vasoconstrictor effects in isolated rat aorta

Experiments were carried out on 2–2.5 mm diameter rings of thoracic aorta from adult (180–300 g) male

Wistar rats killed by exsanguination under anesthesia with 50 mg · kg^{-1} pentabarbitone sodium. Aortic rings were suspended under a resting tension of about 1.25 N · m^{-1} in a 10.0 ml bath perfused by a 32°C medium (mmol · $^{-1}$) NaCl 130, KCl 5.4, $CaCl_2$ 1.8, $MgCl_2$ 1.0, NaH_2PO_4 0.4, $NaHCO_3$ 19, and glucose 5.4, and gassed with a mixture of 95% O_2 and 5% CO_2. Contractions were recorded isometrically using a force transducer based on KTD 2B tensoresisters and displayed on a pen oscillograph N-338. After a 60 min equilibration, concentration-response curves to the vasoconstrictor effects of ouabain and marinobufagenin were plotted.

2.3. Activity of the Na^+,K^+ pump in isolated rat aortic rings

Na^+,K^+ pump inhibitory effects of the compounds were studied in rat aortic rings using the ^{86}Rb technique as described previously by Songu-Mize et al. (1982) with minor modifications (Bagrov et al., 1993b). After the animals were killed, thoracic aortas were rapidly dissected and separated from the blood and adventitia, cut into rings 2–2.5 mm diameter and equilibrated for 15 min in the medium (mmol · l^{-1}) NaCl 120, KCl 4, $CaCl_2$ 2.5, $MgCl_2$ 2.0, NaH_2PO_4 1.1, $NaHCO_3$ 24, and glucose 5.6 gassed with 95% O_2 and 5% CO_2 at pH 7.4 and $T = 32$°C. The samples were then transferred to 2.0-ml flasks with the same medium, except that the KCl concentration was 2 mmol · l^{-1} (this procedure reduces the activity of the Na^+,K^+ pump and allows to enhance ^{86}Rb uptake during incubation). After 15 min preincubation, ^{86}Rb was added with unlabeled Rb to a final concentration of 0.1 mmol · l^{-1}. Aortic rings were then incubated for 30 min at 37°C in duplicate in the absence and in the presence of 1 mmol · l^{-1} ouabain. After the incubation the samples were rinsed 3 times in ice-cold physiological salt solution containing 4.0 mmol · l^{-1} KCl, blotted with filter paper, weighed, and counted in the gamma counter (Cherenkov radiation). Total ^{86}Rb uptake was determined on a wet weight basis, and the activity of the ouabain-sensitive Na^+,K^+ pump was measured as the difference between the total uptake of ^{86}Rb and this uptake in the presence of 1 mmol · l^{-1} ouabain and expressed in nmol of K^+ · 30 min^1 · 1 mg^{-1} of wet weight of tissue.

2.4. Digitalis-like immunoreactivity

Interactions of ouabain and marinobufagenin with digoxin antibody were studied using the DELFIA digoxin immunoassay 1244-09 (Wallac Oy, Turku, Finland), based on the competition between europium-labelled digoxin and sample digoxin for a limited amount of binding sites on digoxin-specific murine monoclonal antibody. A second (rabbit antimouse) antibody was coated to the solid phase, to bind the IgG-digoxin complex, providing separation of the antibody-bound and free antigen. Cross-immunoreactivity of digoxin antibody from the DELFIA immunoassy was expressed in percent, as the ratio of the amount of digoxin to displace 50% of europium-labelled digoxin from digoxin antibody to the amount of marinobufagenin and ouabain to produce the same displacement.

2.5. Digoxin antibody

Polyclonal antidigoxin antibody for experiments in isolated aorta was obtained by immunoaffinity chromatography from the serum of rabbits immunized with digoxin-bovine serum albumin conjugate as previously reported (Curd et al., 1971) with minor modifications (Bagrov et al., 1993a). F_{ab} fragments of sheep antidigoxin antibody (Digibind) were obtained from Burroughs Wellcome, Research Triangle Park, NC, USA.

2.6. Miscellaneous

The results were examined statistically using the paired and unpaired Student's t-tests and one-way analysis of variance with the Bonferroni test. Chemicals used were: bufalin, digoxin, ouabain, digitoxin, bufalin, cinobufagin, norepinephrine, phentolamine, rabbit digoxin antiserum (Sigma).

3. Results

3.1. Purification

35 mg of a steroidal substance with $R_f = 0.42$ showing a brown-green color after visualization by $SbCl_3$ and, typical for bufodienolides, maximal UV absorbance at 298 nm, was separated and further analyzed for its pharmacological effects. Chromatographic behavior of this substance ($R_f = 0.42$, Fig. 2, fraction No. 5) differed from that of commercially available bufalin ($R_f = 0.51$), and reduction of this compound with $NaBH_4$ resulted in the appearance of a steroid with $R_f = 0.37$. These characteristics allowed us to identify the separated substance as a previously described steroid, 3ß,5ß-dihydroxy-14,15-epoxy bufodienolide – marinobufagenin (Ruckstuhl and Meyer, 1957; Barbier et al., 1959; Zelnik and Ziti, 1962) (Fig. 2).

3.2. Vasoconstrictor effects of ouabain and marinobufagenin

As presented in Figs. 3 and 4A, the contractile response to ouabain (10–100 μmol · l^{-1}) was relatively weak, and reached its maximum at 15 min. In six

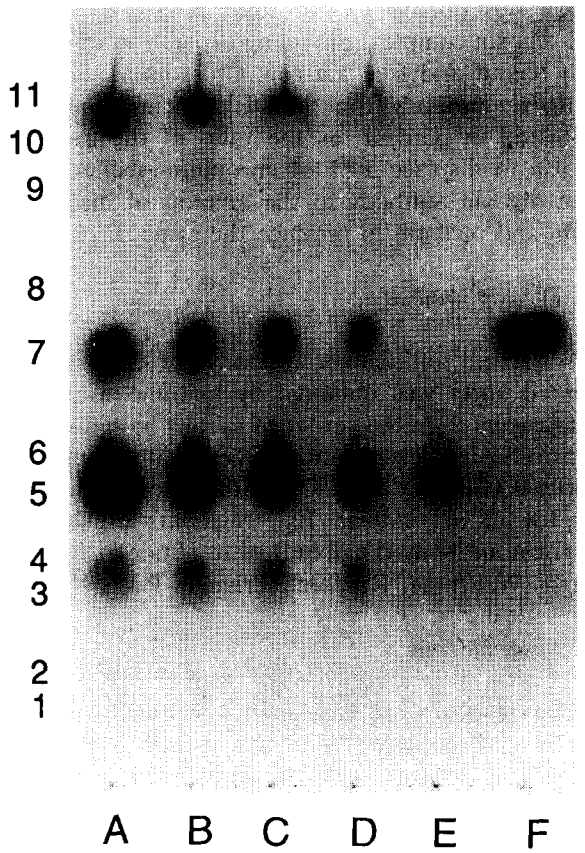

Fig. 2. Chromatographic separation of marinobufagenin from extracted venom of *Bufo marinus* toad in silica gel 60 $F_{254+366}$. Solvent: ethyl acetate, time of development: 60 min. 5: marinobufagenin, 7: bufalin. A–D: four different samples of extracted venom, E: separated marinobufagenin, F: bufalin (Sigma Chemicals).

experiments in the aortic rings pretreated with 2 μmol $\cdot l^{-1}$ phentolamine ouabain-induced contractions did not develop. The vasoconstrictor effect of ouabain was completely reversed by 2 μmol $\cdot l^{-1}$ phentolamine. In five experiments, polyclonal antidigoxin antibody had no effect on ouabain-induced aortic constriction (data not shown).

Marinobufagenin elicited vasoconstriction in rat aorta in a concentration-dependent manner (Figs. 3 and 4A). The contractile response to marinobufagenin was sustained, developed rapidly, reaching a plateau 1–3 min after addition of this substance to the bath. 2 μmol $\cdot l^{-1}$ phentolamine did not alter vasoconstrictor responses to marinobufagenin (Fig. 4A). Polyclonal antidigoxin antibody (15 $\mu g \cdot ml^{-1}$) antagonized the contractile effect of marinobufagenin. Digibind at concentration as high as 40 $\mu g \cdot ml^{-1}$ did not alter the vasoconstrictor effect of 200 μmol $\cdot l^{-1}$ marinobufagenin ($n = 6$; 5.6 ± 1.0 and 5.1 ± 0.8 N $\cdot$ m^{-1}, respectively, $P > 0.5$, paired two-tailed t-test).

3.3. Effects of marinobufagenin and ouabain on the activity of the Na^+,K^+ pump in rat aortic rings

At a concentration of 10 μmol $\cdot l^{-1}$, ouabain stimulated, while at higher concentrations inhibited, the Na^+,K^+ pump in isolated aortic rings (Fig. 4B). Pretreatment of aortic rings with 2 μmol $\cdot l^{-1}$ phentolamine abolished the Na^+,K^+ pump-stimulating effect of 10 μmol $\cdot l^{-1}$ ouabain, but did not alter the inhibitory action of higher concentrations of ouabain. At

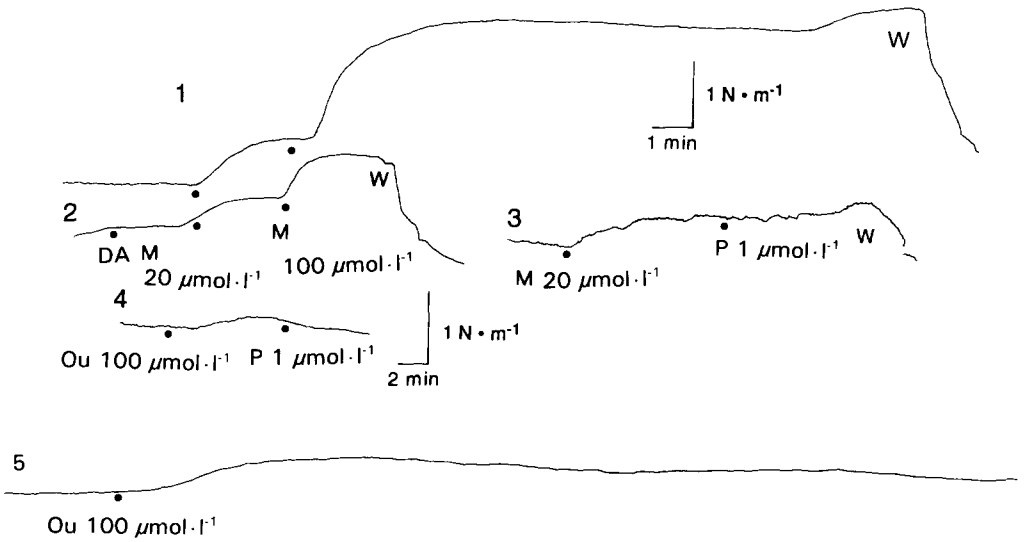

Fig. 3. Representative recordings from isolated rat aorta experiments. 1: Vasoconstrictor response to 20 and 100 μmol $\cdot l^{-1}$ marinobufagenin (M), 2: vasoconstrictor response to 20 and 100 μmol $\cdot l^{-1}$ marinobufagenin in the presence of 150 $\mu g \cdot ml^{-1}$ digoxin antibody (DA), 3: vasoconstrictor response to 20 μmol $\cdot l^{-1}$ marinobufagenin is unaffected by pretreatment of the tissue with 2 μmol $\cdot l^{-1}$ phentolamine (P), 4: vasoconstrictor effect of 100 μmol $\cdot l^{-1}$ ouabain (Ou) is blocked by 2 μmol $\cdot l^{-1}$ phentolamine, 5: vasoconstrictor response to 100 μmol ouabain (Ou). W = washout of the compounds.

the same time, phentolamine did not have any effect on the activity of total and ouabain-sensitive ^{86}Rb uptake in the control aortic rings.

In a separate series of four experiments, 2 μmol $\cdot l^{-1}$ norepinephrine within 30 min significantly stimulated ouabain-inhibitable ^{86}Rb uptake in rat aorta (11.7 $\pm$ 2.3 vs. 3.7 $\pm$ 0.4 nmol $K^+ \cdot 30$ min $\cdot$ mg^{-1}; $P < 0.001$).

Marinobufagenin inhibited ouabain-sensitive ^{86}Rb uptake in rat aorta in a concentration-dependent manner (Fig. 4B). Pretreatment of aortic rings with 2 μmol $\cdot l^{-1}$ phentolamine did not affect the Na^+,K^+ pump inhibitory action of marinobufagenin.

3.4. Digoxin-like immunoreactivity

As shown in Fig. 5, both marinobufagenin and ouabain cross-reacted with digoxin antibody in the solid phase fluoroimmunoassay. Cross-immunoreactivity of marinobufagenin and ouabain with digoxin antibody was 0.2 and 0.01%, respectively.

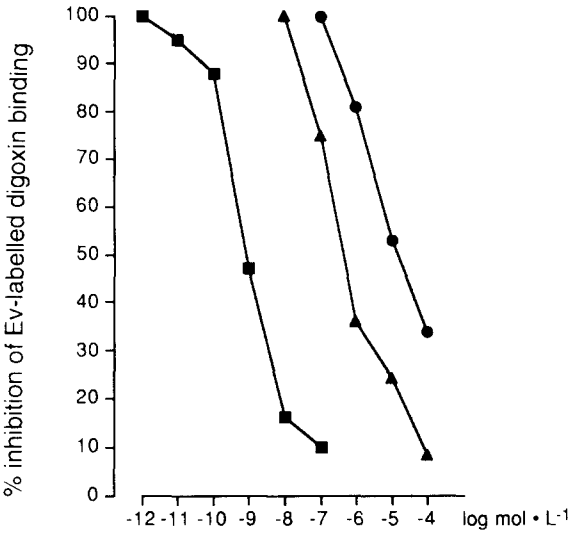

Fig. 5. Cross-reactivity of digoxin (■), ouabain (●) and marinobufagenin (▲) with mouse monoclonal antidigoxin antibody from the DELFIA immunoassay.

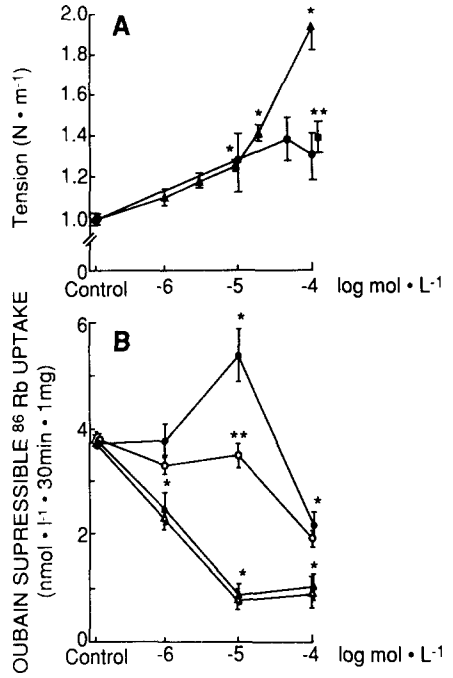

Fig. 4. A: Vasoconstrictor effects of ouabain and marinobufagin in rat aortic rings. (▲) Marinobufagenin, (■) marinobufagenin in the presence of antidigoxin antibody, (●) ouabain. Means $\pm$ S.E.M from 6–9 experiments. * $P < 0.05$ (one-way analysis of variance and Bonferroni t-test) as compared with the control values. ** $P < 0.01$ (unpaired two-tailed Student's t-test) as compared with the effect of marinobufagenin in the absence of antidigoxin antibody. B: Effects of ouabain and marinobufagenin on the activity of the Na^+,K^+ pump (ouabain-sensitive ^{86}Rb uptake) in rat aortic rings. (●) Ouabain, (○) ouabain in the presence of 2 μmol $\cdot l^{-1}$ phentolamine, (▲) marinobufagenin, (■) marinobufagenin in the presence of 2 μmol $\cdot l^{-1}$ phentolamine. Means $\pm$ S.E.M. from 6–9 experiments. * $P < 0.05$ (one-way analysis of variance and Bonferroni t-test) as compared with the control values. * $P < 0.05$ (unpaired two-tailed Student's t-test) as compared with the effect of marinobufagenin in the absence of 2 μmol phentolamine.

4. Discussion

Previously, we have shown that the venom from *Bufo marinus* toad evoked rapid vasoconstriction and inhibited the Na^+,K^+ pump in isolated rat aortic rings. The major observations of the present study are that (i) the digoxin-like immunoreactive vasoconstrictor substance from *Bufo marinus* toad venom is distinct from previously described vasoactive EDLFs (i.e. ouabain, bufalin and resibufagenin) and is likely to be marinobufagenin, and (ii) unlike the effects of ouabain, neither the vasoconstrictor nor the Na^+,K^+ pump inhibitory effects of marinobufagenin in rat aorta are sensitive to adrenoceptor blockade with phentolamine.

The present observations show that a rapidly acting vasoconstrictor and Na^+,K^+-ATPase inhibitor from the venom of *Bufo marinus* toad is identical to a previously described bufodienolide, marinobufogenin (3ß,5ß-dihydroxy-14,15-epoxy bufadienolide) (Barbier et al., 1959). Similarly to previous results of Barbier et al. (1959), marinobifagenin in our study represented 6% of the weight of the venom. The chromatographic mobility of marinobufagenin ($R_f = 0.42$) in our study coincided with the mobility of marinobufagenin when this steroid was separated by the same method from the venom of the Brazilian toads *Bufo ictericus Spix*, *Bufo crucifer* Wied and *Bufo parenchimus* Lutz (Zelnik and Ziti, 1962). Distinction of marinobufagenin from the previously described EDLFs with a bufodienolide structure, bufalin and resibufagenin, was demonstrated as fol-

lows. The chromatographic behavior of marunibufagenin was compared with bufalin (Sigma); as shown in Fig. 2, the mobility of marinobufagenin in Silica gel ($R_f = 0.42$) differed significantly from that of bufalin ($R_f = 0.51$). Like marinobufagenin (Fig. 1), resibufagenin contains a 14,15-epoxy group (Barbier et al., 1959). Reduction of 14,15-epoxy-containing bufodienolides by $NaBH_4$ is known to lead to the appearance of 14-hydroxybufodienolides (Ruckstuhl and Meyer, 1957). As can be seen from Fig. 1, and as has been demonstrated previously, the reduction of resibufagenin with $NaBH_4$ results in the appearance of bufalin (Ruckstuhl and Meyer, 1957; Barbier et al., 1959). In our experiments, however, reduction of marinobufagenin by $NaBH_4$ resulted in the appearance of a substance with $R_f = 0.37$ and, therefore, distinct from bufalin ($R_f = 0.51$). Identification of the separate substance as marinobufagenin was further confirmed by the fact that it demonstrated the typical brown-green color after the visualization with $SbCl_3$ (Ruckstuhl and Meyer, 1957; Siperstein et al., 1957).

The values of the activity of ouabain-sensitive ^{86}Rb uptake in rat aortic rings in our experiments were similar to previous observations (Lynch et al., 1986). Similarly to the previous experiments in isolated rat aorta, ouabain, even at very high concentrations, caused relatively weak vasoconstriction which was blocked by phentolamine (Karaki et al., 1978). In our study, ouabain stimulated the Na^+,K^+ pump in rat aorta at a concentration of 10 $\mu mol \cdot l^{-1}$ and inhibited its activity at higher concentrations. The fact that both the Na^+,K^+ pump stimulatory and vasoconstrictor effects of ouabain were blocked by phentolamine, suggests that (i) stimulation of the Na^+,K^+ pump was due to release of norepinephrine from perivascular neural endings, and (ii) that in rat aorta a neurogenic mechanism is important in the vasoconstrictor effect of ouabain. The observation that a norepinephrine concentration of 1 $\mu mol \cdot l^{-1}$ stimulated the Na^+,K^+ pump in rat aorta is consistent with this suggestion. Recently, as with ouabain, another bufodienolide Na^+,K^+-ATPase inhibitor, bufalin, was shown to stimulate the Na^+,K^+ pump in rat aorta at lower concentrations, while, at higher concentrations, it inhibited its activity. The vasoconstrictor effect of bufalin also was antagonized by phentolamine (Bagrov et al., 1993b). The stimulating effect of digitalis glycosides on the Na^+,K^+-ATPase/Na^+,K^+ pump was first described by Hougen et al. (1982) in guinea pig myocardium and was also attributed to the release of norepinephrine from adrenergic terminals. Later, stimulation of Na^+,K^+ pump activity by norepinephrine and phenylephrine has been reported in rat and rabbit aorta and was attributed to the stimulation of α_1-adrenoceptors (Lynch et al., 1986). The nature of the relationship between catecholamines and Na^+,K^+-ATPase depends on the species, types of blood vessels, and concentrations of Na^+,K^+-ATPase inhibitors. Na^+,K^+-ATPase inhibition has been shown to potentiate (Blaustein et al., 1987; Mikkelsen et al., 1979), as well as to decrease (Woolfson et al., 1991; Cutaia and Rudio, 1992) vasoconstrictor responses to catecholamines. Broekaert and Godfraind (1973) reported that in guinea pig and in rabbit aorta lower doses of ouabain enhanced the response to norepinephrine, while higher doses of ouabain inhibited norepinephrine reactivity. Our data are consistent with the results of Broekaert and Godfraind (1973).

Previously marinobufagenin was shown to inhibit myocardial Na^+,K^+-ATPase (Shimada et al., 1985) and to possess a positive inotropic effect in cardiac preparations (Chen and Kovarikova, 1967), but, to our knowledge, the mechanisms of the vasoconstrictor effects of marinobufagenin were not analyzed. Unlike ouabain, marinobufagenin showed no Na^+,K^+ pump stimulatory effect. Neither the contractile nor the Na^+,K^+ pump inhibitory effect of marinobufagenin was sensitive to phentolamine. Therefore, the vasoconstrictor effect of marinobufagenin may be attributed to the inhibition of the Na^+,K^+ pump in vascular smooth muscle. It remains unclear, however, why an increase in the concentration of marinobufagenin from 10 to 100 $\mu mol \cdot l^{-1}$ was associated with an increase in the developing tension of aortic rings, while no further inhibition of the Na^+,K^+ pump was observed (Fig. 4A and B). Previously, the absence of parallelism in Na^+,K^+ pump inhibition and vasoconstriction in placental arteries was attributed to the release of an unknown substance interacting with Na^+,K^+-ATPase from endothelium (Rodriguez-Manas et al., 1992). Since endothelin may stimulate Na^+,K^+-ATPase (Gupta et al., 1994) it could also antagonize the effect of Na^+,K^+-ATPase inhibitors.

Previous studies have shown that Na^+,K^+ pump inhibitor-induced vasoconstriction may be either 'myogenic' or 'neurogenic' in origin, depending on the animal species and the type of blood vessel studied (Adams et al., 1983; Marin et al., 1988). Here we present evidence that in rat aorta, different Na^+,K^+ pump inhibitors, marinobufagenin and ouabain, seem to evoke these differing effects. The differences in the mechanisms of vasoconstrictor effects of marinobufagenin and ouabain are supported by the differences in their immunoreactivity; marinobufagenin demonstrated 20 times higher cross-reactivity with digoxin antibody, as compared with ouabain.

The present observations show that a rapidly acting bufodienolide Na^+,K^+ pump inhibitor, marinobufagenin, may regulate vascular tone independently from neuroeffector interactions in the vascular wall. The possible importance of these results is illustrated in recent observations showing that, like Amphibia, mam-

mals may have a bufodienolide EDLF (Lichtstein et al., 1993; Schoner et al., 1993; Bagrov et al., 1993c, 1994).

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