Endogenous Marinobufagenin-like Immunoreactive Substance

A Possible Endogenous Na,K-ATPase Inhibitor With Vasoconstrictor Activity

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Vasoconstrictor and Na/K pump inhibitory properties of a bufodienolide Na/K-ATPase inhibitor, marinobufagenin, were studied in isolated rings of 2 to 3 order branches of human pulmonary arteries respectively. Marinobufagenin displayed concentration-dependent vasoconstrictor activity (0.01 to 10 mmol/L). In sarcolemma membranes prepared from pulmonary artery marinobufagenin inhibited Na/K-ATPase (IC_{50} = 50 nmol/L). In eight healthy male Caucasians, concentrations of marinobufagenin-like immunoreactive material in C-18 extracted plasma were 1.38 ± 0.60 nmol/L. Twenty-four-hour urinary release of marinobufagenin-like immunoreactive material in eight healthy males was 1.20 ± 0.95 nmol/day. Chloroform extract of human urine was fractionated using reverse-phase high-performance liquid chromatography (32% acetonitrile, Deltapak). The HPLC fraction co-eluting with marinobufagenin in 7 min, cross reacted with antimarinobufagenin and antidigoxin, but not antiouabain antibody. These results demonstrate that human plasma and urine contains a bufodienolide vasoconstrictor EDLF, marinobufagenin-like immunoreactive Na,K pump inhibitor. Am J Hypertens 1996;9:982–990

KEY WORDS: **Bufodienolides, Na/K-ATPase inhibition, human vasoconstriction, digitalis-like factors.**

Endogenous digitalis-like factors (EDLF), circulating inhibitors of Na/K-ATPase, have been implicated previously in the pathogenesis of various forms of human and animal hypertension.[1,2] It has been demonstrated that, in addition to recently purified ouabain,[3] several other endogenous Na/K-ATPase inhibitors exist in the plasma of mammals.[4,5] Other vertebrates, such as amphibia, have been shown to contain high plasma and tissue concentrations of Na/K-ATPase inhibitory steroids which, unlike digitalis glycosides, do not have cardenolide, but bufodienolide structure instead.[6–8]

In our previous experiments, a mixture of steroids from *Bufo marinus* toad venom and one of its ingredients, marinobufagenin, displayed vasoconstrictor and Na/K pump inhibitory activity in rat aorta.[9,10]

Recent evidence indicates that mammalian EDLF may also have a bufodienolide nature. Thus, antibod-

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ies against the bufodienolides bufalin[11,12] and proscillaridin[13] were shown to interact with the human-derived materials. Naomi et al[14] have analyzed cross-reactivity of various antidigoxin antibodies with various candidates for the role of EDLF, and have suggested that EDLF in human plasma shares immunoreactive properties with bufalin. Lichtstein et al[15] purified bufalin derivatives from human cataractous lenses. Antibodies against the mixture of bufodienolides[16] and marinobufagenin (3β5β-dihydroxy-14,15-epoxy bufadienolide) cross-reacted with EDLF from human and dog and rat plasma.[17,18] In our previous study, the concentration of marinobufagenin-like immunoreactivity in human plasma was in the nanomolar range.[19] High-performance liquid chromatography (HPLC) fractionation of human urinary extract demonstrated that endogenous-marinobufagenin-like immunoreactive material eluted from Deltapak column as a single peak having the same retention time as marinobufagenin purified from toad venom.[19]

The present study was undertaken in order to assess the vasoconstrictor and Na / K-ATPase inhibitory activity of marinobufagenin in human tissues, and to confirm the evidence that human EDLF may have a bufodienolide nature.

MATERIALS AND METHODS

Purification of Marinobufagenin Venom was collected from adult *Bufo marinus* toads of both sexes from Riga (Latvia) and St. Petersburg (Russia) Zoological Gardens, and marinobufagenin was purified by thin-layer chromatography as we reported recently in detail.[10] Briefly, chloroform-extracted venom was fractionated by thin-layer chromatography (Silica-gel 60, $F_{234+366}$, Merck) elution with ethyl-acetate. Marinobufagenin was separated from the other bufodienolides using ultraviolet light visualization and determination of its chromatographic mobility (R_f) and typical color reaction with $SbCl_3$.

Vasoconstrictor Activity Experiments were carried out on the rings of 2 to 3 order branches of human pulmonary artery. The protocol of the study was approved by the Research Council of Dzhanelidze Research Institute of Emergency Medicine (St. Petersburg, Russia). Tissues were obtained from 12 male patients (50 ± 5 years) undergoing surgery due to pulmonary adenocarcinoma. None of the patients was receiving radiation therapy or chemotherapy before surgery. Vessels were dissected from the tissue that was not affected by malignant growth. Vascular rings (2.5 to 4.0 mm diameter) were suspended under a resting tension 1.0 *g* in a 10.0 mL organ bath perfused by a 37°C medium (mmol / L) NaCl 130, KCl 4.0, $CaCl_2$ 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 (Izhevsk, Russia). After 60 min equilibration, arterial rings were contracted twice with 80 mmol / L potassium, and after 60 min, concentration–response curves of vasoconstrictor effects of marinobufagenin were plotted.

Na/K ATPase from Human Pulmonary Artery Tissues were collected from 4 patients to detemine vasoconstrictor activity. Two to three order branches of pulmonary artery were removed from the surrounding tissues, and sarcolemmal membrane fraction was purified as described by Allen et al[20] with minor modification: 1 to 2 cm segments of 2 to 3 order branches of pulmonary were excised from the surrounding tissues at t 4°C in physiological salt solution (in mmol / L: NaCl, 130; KCl, 5.4; $CaCl_2$, 1.8; $MgCl_2$, 1; glucose, 5.4; KH_2PO_4, 1.1; $NaHCO_3$, 24; pH = 7.4; t = 4°C), and repeatedly washed by the solution of the same composition and cut into 1 to 2 mm rings.

A substantial portion of Na / K-ATPase in cardiovascular tissues is associated with adrenergic neural endings.[12] In order to denervate the vascular segments, rings of pulmonary artery were treated with 6-hydroxydopamine (6'OHDA) exactly as reported previously by Apregliano and Hermsmeyer.[22] 6'OHDA (300 mg / μL) was applied to the vascular rings twice for 10 min with a 30 min interval.

Next, pulmonary artery rings were placed into the flasks containing 250 mmol / L sucrose and 5 mmol / L histidine (t = 4°C; pH = 7.4) and were processed with Polytron 20S homogenizer (Kinematica, Basel, Switzerland). The tissue was further homogenized in a glass homogenizer (Glas-Col, Terre Haute, IN) with a tight-fitting Teflon pestle. Then the homogenized tissue was centrifuged (6,000 *g*, 15 min, t = 4°C) in a Sorvall RC-5B centrifuge (Du Pont Instruments). The supernatant was centrifuged again (20,000 *g*, 30 min, 4°C), using the same centrifuge. The supernatant was centrifuged in a Beckman L8-N centrifuge (48,000 *g*, 90 min, 4°C) and the resultant pellet was suspended in a homogenizing medium. The mixture was placed on 0.32, 0.8, 1.0, 1.2, and 1.4 mol discontinuous sucrose gradient prepared in 5 mmol / L histidine and was centrifuged at 48,000 *g* for 90 min (Beckman L8-N SW28, t = 4°C). A clear band appeared at the 0.8 mol interface and was aspirated with a Pasteur pipette. The membrane fraction was centrifuged at 48,000 *g* for 90 min again, and the pellet was resuspended in 1 mL of histidine–sucrose buffer and stored in liquid nitrogen for 5 to 10 days.

Na / K-ATPase activity in sarcolemma fraction was measured as described below.

High-Performance Liquid Chromatography Five liters of urine was extracted with 7.5 L of chloroform.

The chloroform was then removed under vacuum, and the dry residue was dissolved in acetonitrile. The fraction having R_f similar to marinobufagenin was isolated by thin layer chromatography as reported previously,[23] with minor modifications.[20] The partially purified material was further fractionated by HPLC on Deltapak C18 columns (3.9 × 150 cm, 300 A) using Gilson HPLC pump (model 303, detector model 116). The columns were equilibrated with 0.1% trifluoroacetic acid and developed with a linear gradient of acetonitrile over 0% to 80% acetonitrile containing 0.1% trifluoroacetic acid for 1 h at a flow rate 1 mL/min.

The elution of the standards (marinobufagenin, ouabain, digoxin, and bufalin, Sigma Chemicals, St. Louis, MO) was monitored at wavelength 300 nm as previously reported.[24] Partially purified chloroform urinary extract was fractionated in 32% acetonitrile. One-minute fractions were tested for their ability to inhibit purified dog kidney Na/K ATPase, and to react with antimarinobufagenin, antiouabain, and antidigoxin antibodies.

Immunoassays Concentrations of marinobufagenin-like immunoreactivity were measured in C-18 extracted plasma and urine (Waters, 80% acetonitrile). The HPLC fraction from chloroform-extracted urine was tested for its ability to interact with antimarinobufagenin, antiouabain, and antidigoxin antibodies. The cross-immunoreactivity of the assays was expressed as the ratio of the amount of cross-reactant required to displace 50% of the antimarinobufagenin, antiouabain, or antidigoxin antibody from immobilized conjugate, to the amount of the cross-reactant to give the same 50% displacement.

Marinobufagenin-like immunoreactivity was analyzed using a solid-phase fluoroimmunoassay as reported previously in detail.[19] The method is based on a competition between the immobilized conjugate (marinobufagenin-3-glycoside-RNAase) and a sample of EDLF for rabbit polyclonal antimarinobufagenin antibody. Marinobufagenin was separated from the venom in parotid glands of *Bufo marinus* toads using thin layer chromatography, as previously reported.[10] Marinobufagenin-3-glycoside was synthesized, as described by Koenigs and Knorr, with some modifications.[25,26] Marinobufagenin-3-glycoside-BSA and marinobufagenin-3-glycoside-RNAase conjugates were prepared, and rabbits were immunized with marinobufagenin-3-glycoside-BSA, as previously reported by Curd et al for digoxin.[27] The mixture of immunoglobulins was separated from the serum using caprylic acid.[28] Marinobufagenin-3-glycoside-RNAase conjugate was immobilized on the bottom of NUNC microtitration strip wells as reported in detail previously.[19] One microgram of conjugate in 100 μL of phosphate buffered saline per well was used in the measurements in HPLC fractions. For the measurements in plasma and urinary samples, we were coating the solid phase with 0.2 μg/well conjugate in 100 μL of TSA buffer: 50 mmol/L Tris HCl buffer, 145 mmol/L NaCl, 0.5 g/L NaN_3, 5 g/L BSA and 0.1 mL/L Tween-20. We added 20 μL of marinobufagenin standards and unknown samples to the coated wells, followed by 100 μL of marinobufagenin antibody. After 1 h incubation, the strips were washed twice (Delfia wash solution, Wallac OY, Turku, Finland), following which 100 μL of secondary antibody (europium-labeled goat antirabbit antibody, Wallac OY, Turku, Finland) was added. After 1 h incubation, the wells were washed six times with the wash solution. Next, 200 μL of enhancement solution, which releases the europium conjugated with the secondary antibody (Wallac OY, Turku, Finland) was added into each well, the strips were shaken for 5 min, and after 10 min more the fluorescence of free europium was measured (Delfia 1234 Arcus Fluorometer, Wallac OY, Turku, Finland). The sensitivity of the immunoassay was 0.001 nmol. The cross-reactivity of antimarinobufagenin antibody was (%): marinobufagenin 100, ouabain 0.1, digoxin 1.0, digitoxin 3.0, bufalin 1.0, cinobufagin 1.0, prednisone <0.1, spironolactone <0.1, proscillaridin <1.0, progesterone <0.1, mixture of bufodienolides from *Bufo marinus* venom except marinobufagenin <5%.

Ouabain-like immunoreactivity in HPLC fraction from extracted urine was measured using a modified New England Nuclear ouabain ELISA kit, based on the competition between immobilized ouabain conjugate and sample ouabain for rabbit antiouabain antibody. The bound rabbit antibody was then detected, using labelled secondary (europium-labelled goat antirabbit) antibody (Wallac Oy, Turku, Finland). The sensitivity of the ouabain assay was 0.01 nmol/L. Cross-reactivity with marinobufagenin was less than 1.0%.

Digoxin-like immunoreactivity was measured using Delfia fluoroimmunoassay developed by Helsingius et al[29] with minor modifications.[19] Antidigoxin antibody (Sigma Chemicals, St. Louis, MO) was used 1:8,000. Cross-reactivity of digoxin antibody was (%): digoxin 100, marinobufagenin 0.2, ouabain <0.01, digitoxin 10.0, bufalin 0.01, cinobufagin <0.01. Sensitivity of immunoassay was 0.01 nmol/L.

Purified Dog Kidney Na/K-ATPase HPLC fractions from urine were tested for their ability to inhibit purified Na/K-ATPase. Na/K-ATPase assay was performed using purified canine kidney Na/K-ATPase (Sigma Chemicals, St. Louis, MO) as reported previously,[2] with some modifications. ATP hydrolysis was assessed spectrophotometrically by measuring NADH oxidation at 340 nm using the linked enzyme system, pyruvate kinase (PK)-lactate dehydrogenase (LDH). The system contained 3 mmol/L $MgCl_2$, 0.2 mmol/L

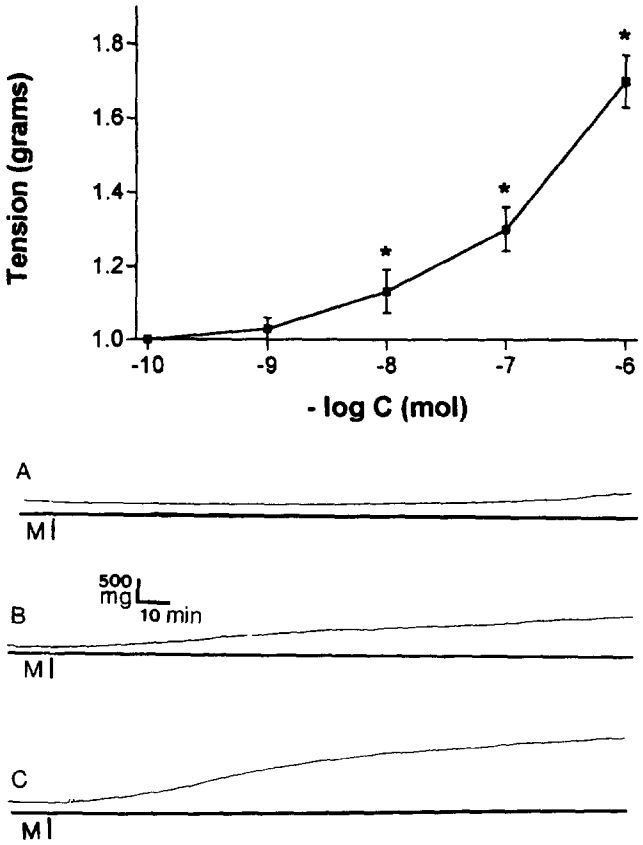


FIGURE 1. A. *Vasoconstrictor effect of marinobufagenin in isolated rings of 2 to 3 order branches of human pulmonary artery. Means ± SEM from six to nine experiments. (*P < .05 v baseline, Bonferroni test.* **B.** *Representative recordings from experiments in isolated rings of 2 to 3 order branches of human pulmonary artery. Vasoconstrictor effect of marinobufagenin* **(A)** *0.01 μmol/L,* **(B)** *0.1 μmol/L, and* **(C)** *1.0 μmol/L. M = administration of marinobufagenin.*

ATP, 100 mmol/L NaCl, 10 mmol/L KCl, 1 mmol/L phosphoenolpyruvate, and suspension of PK (2 units/mL) and LDH (95.5 units/mL). Na/K-ATPase was determined as the difference between oxidation of NADH with and without 0.1 mmol/L ouabain. Protein was maintained between 2 and 2.5 mg/cuvette in a volume of 1.0 mL.

Na/K-ATPase in Sarcolemmal Membrane Fraction Activity of Na/K-ATPase in plasmalemmal fraction from human pulmonary artery was measured as described above, except addition of alamethicin (0.5 mg/1 mg sarcolemmal protein) and NaN_3 (5 mmol/L, to block mitochondrial ATPase) to the incubation medium, as recommended by Dixon et al,[30] for cardiac sarcolemmal preparation. Protein was maintained between 7 and 10 μg/cuvette in a volume of 0.5 mL.

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

RESULTS

As presented in Figure 1A, marinobufagenin (0.01 to 10.0 μmol/L) constricted pulmonary artery rings in a concentration-dependent manner. As demonstrated in Figure 1B, the contractile response to marinobufagenin started developing 30 min after the addition of the substance to the bath, and reached a plateau after 1 h. The contracture was sustained for up to 3 h (Figure 1B) and was almost unaffected by washout. Phentolamine, 2 μmol/L, did not affect the contractions caused by marinobufagenin (data not shown).

Baseline activity of sarcolemma Na/K-ATPase from human pulmonary artery was 7.8 ± 0.85 μmol ADP/1 mg protein/1 h. Mg-ATPase and Na/K-ATPase comprised 61% and 39% of total ATPase activity, respectively. As shown in Figure 2, marinobufagenin (1 nmol/L to 1 μmol/L) inhibited Na/K-ATPase activity in sarcolemma from human pulmonary arteries in a concentration-dependent manner.

Twenty-four-hour urinary output of marinobufagenin-like immunoreactive material by five healthy males comprised 1.2 ± 0.95 nmol/day. Plasma concentration of marinobufagenin-like immunoreactivity was 1.38 ± 0.60 nmol/L.

In linear gradient of acetonitrile, the marinobufagenin standard eluted from Deltapak column in 7 min and at 32.5% acetonoitrile. Ouabain and digoxin eluted at min 6.15 and 12.28, respectively. Figure 3 shows that authentic marinobufagenin purified from the venom of *Bufo marinus* toad eluted as a sharp single symmetrical peak (Figure 3A, isocratic elution with 32.5% acetonitrile). When the extracted urine was fractionated on

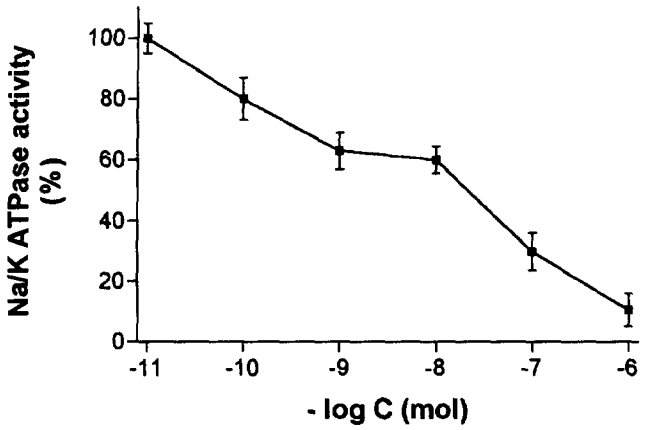
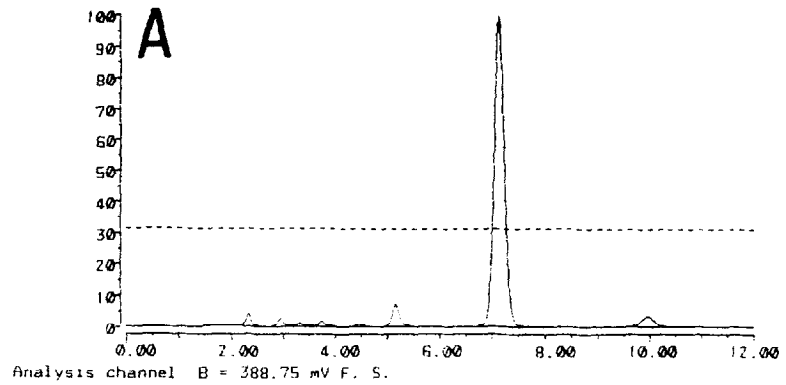
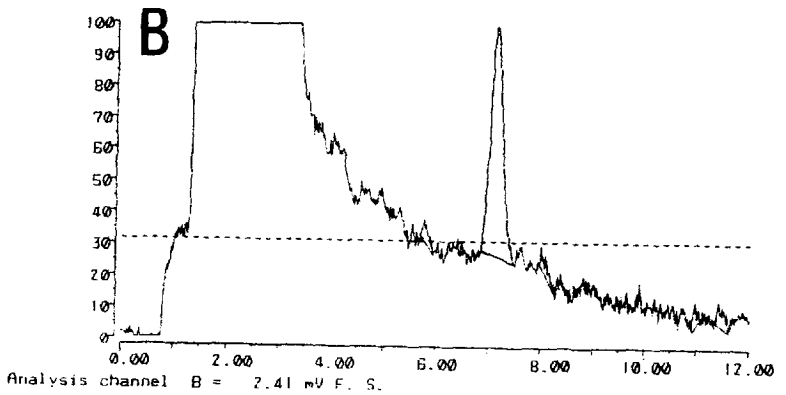


FIGURE 2. *Effect of marinobufagenin on the activity of sarcolemma Na/K-ATPase from 2 to 3 order branches of human pulmonary artery. Means ± SEM from four experiments.*





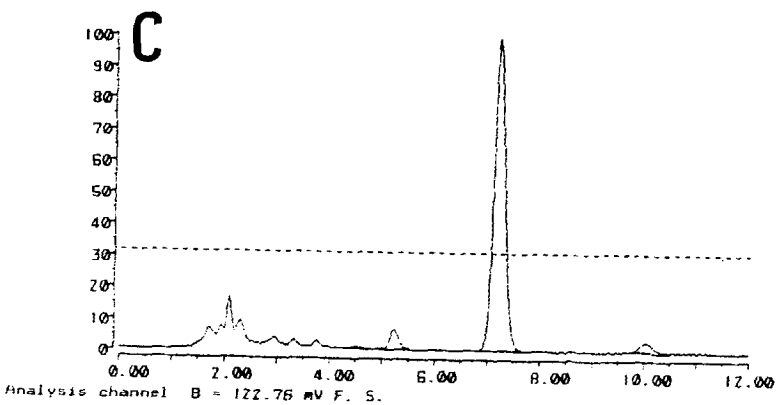


FIGURE 3. *HPLC fractionation of chloroform extracts from human urine. Isocratic elution, 32% acetonitrile.* **A.** *Marinobufagenin standard (100 μmol/L),* **B.** *Urinary chloroform extract,* **C.** *Urinary extract and marinobufagenin standard.*

Deltapak column (isocratic elution in 32.5% acetonitrile) more than 80% of marinobufagenin-like immunoreactivity eluted in 7 min, a significantly smaller portion of marinobufagenin-like immunoreactive material eluted in 8 min (Figure 4B).

Figures 3A and B demonstrate the elution profile of authentic marinobufagenin and chloroform extract in 32.5% acetonitrile. The addition of marinobufagenin standard to extracted urine resulted in a significant increase of the peak, which has the same retention time as the authentic marinobufagenin (Figure 3C). No separation of the peaks, corresponding to marinobufagenin standard and urinary extract, has been observed.

As presented in Figure 4B, when eluted with 32% acetonitrile, 71% of total marinobufagenin-like immunoreactivity eluted in fraction 7, and 18% in fraction 8. Digoxin-like immunoreactive material was present in all HPLC fractions (Figure 4D). The maximum of digoxin-like immunoreactivity eluted in

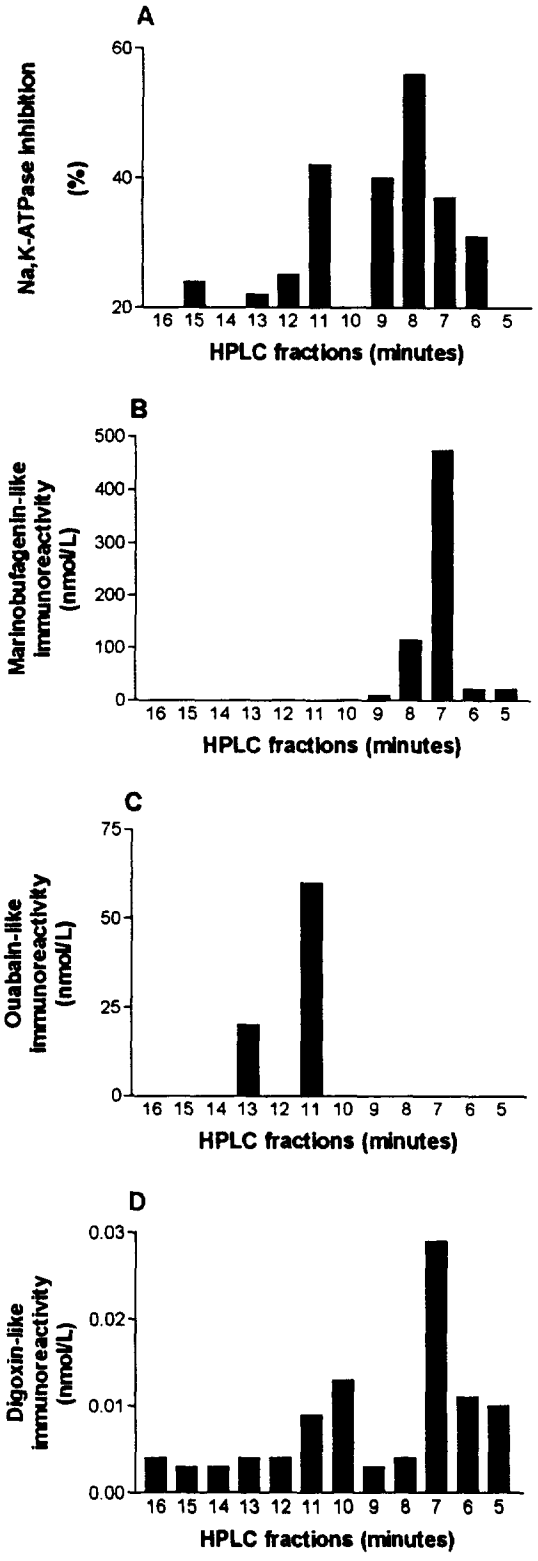


FIGURE 4. *Activity of HPLC fractions from chloroform-extracted human urine.* **A.** *Inhibition of dog kidney Na/K ATPase.* **B.** *Interactions with antimarinobufagenin antibody.* **C.** *Interactions with antiouabain antibody.* **D.** *Interactions with antidigoxin antibody.*

fraction 7, lesser amounts were detected in fractions 10, 6, 5, and 11. Ouabain-like immunoreactivity, 75%, was present in fraction 11 and 25 in fraction 13 (Figure 4C).

As shown in Figure 4A, the maximal inhibition of dog kidney Na / K-ATPase was produced by fractions 8, 11, 9, and 7 (56%, 42%, 40%, and 37%, respectively).

DISCUSSION

The results of the present study demonstrate that marinobufagenin acts as a vasoconstictor in isolated human pulmonary arteries, and provides further evidence that, like amphibia, mammals also have a bufodienolide EDLF.

In previous experiments with isolated rat aorta, marinobufagenin caused vasoconstriction at significantly higher concentrations, starting from 10 μmoles.[10] At the same time, in human pulmonary artery strips the contractile response to marinobufagenin developed more slowly, reaching a plateau in 2 h.

Previously, rat pulmonary arteries were found to be sensitive to the constrictor effect of hypothalamic Na / K pump inhibitor.[31] However unlike our experiment, these contractions were blocked by phentolamine.[31] Interestingly, the sensitivity of pulmonary arteries from spontaneously hypertensive rats was greater than that of normotensive animals and of the aortae obtained from both SHR and normotensive rats.[31] In rat aorta, the marinobufagenin-induced contraction reached its maximum within a period of minutes.[10] As in our previous observations in rats, marinobufagenin induced vasoconstriction was insensitive to adrenoceptor blockers. Therefore, the vasoconstrictor effect of marinobufagenin is likely to be due to the inhibition of Na / K pump in vascular smooth muscle cell membrane rather than due to interaction with adrenergic neural endings. Indeed, in sarcolemmal membrane fraction, prepared from the vascular rings treated with 6'OHDA, marinobufagenin inhibited Na / K-ATPase activity at the same concentration as it caused contractile response in isolated vascular rings (IC_{50} = 50 nmol / L).

Previously, effects of Na / K pump inhibitors were investigated in various human blood vessels. Mikkelsen et al[32] have studied effects of digoxin in isolated human crural arteries and veins. Digoxin constricted veins at concentration 0.1 μmol / L and was less active in the arteries. Similarly to our experiments, the effect of digoxin was sustained and was unaffected by the washout procedure.[32] In isolated human subcutaneous resistance arteries, ouabain produced vasoconstriction in micromolar concentrations.[33] The effect of ouabain reached its maximum 6 h after addition to the incubation bath. A bufodienolide Na / K-ATPase inhibitor, bufalin, inhibited endothe-

lium-dependent relaxation of human subcutaneous arteries precontracted with norepinephrine at concentration 1 nmol/L.[34] Cress et al[35] investigated the mechanism of vasoconstrictor effect of bufalin in canine saphenous vein. Bufalin inhibited norepinephrine uptake and increased norepinephrine overflow more than could be explained solely by the uptake inhibition. Clearly, vasoconstrictor activity of marinobufagenin merits further investigation in human resistance arteries.

The concentration of marinobufagenin-like immunoreactivity in C-18 extracted plasma was 1.35 ± 0.60 nmol/L. In our previous study, the concentration of marinobufagenin-like immunoreactive material in protein-free plasma was lower, 0.4 nmol/L. Previously, plasma levels of EDLF (digoxin-like immunoreactivity) have been shown in various forms of human and animal hypertension to be increased by 50 to 250%.[3,36] In plasma volume expansion and saline loading, plasma EDLF may be increased by 10 times.[36,37] Such increases in plasma concentration of marinobufagenin could produce functionally significant inhibition of vascular Na/K-ATPase. In our previous experiments, we investigated a possible role of endogenous marinobufagenin-like immunoreactive substance during pressor response to voluntary hypoventilation.[19] A fourfold rise in plasma concentrations of marinobufagenin-like immunoreactivity was associated with a 40% inhibition of Na/K-ATPase activity in erythrocytes.[19]

Previously, it has been shown that the daily urinary output of a nonpolar digoxin-like immunoreactive factor comprises 12 ng digoxin equivalents per day.[38] Average urinary excretion of a polar ouabain-like substance was 1.8 pmol ouabain equivalents per day.[38] In our study, 24 h urinary output of marinobufagenin-like immunoreactivity in 5 healthy humans was greater (1.2 nmol) than reported above, but less than we have reported previously (8 nmol).[19] However, this number does not look unreasonably high, for example plasma concentrations of aldosterone (which has aproximately the same molecular weight as marinobufagenin, and therefore, similar renal clearence characteristics) is in picomolar range of concentrations.[39] At the same time, 24 h urinary release of aldosterone may be as high as 20 to 50 nmol per 24 hours.[39,40]

The observation of the presence of marinobufagenin-like immunoreactivity in human urine is supported by the results of analysis of HPLC fractions from chloroform-extracted urine. Marinobufagenin-like immunoreactivity eluted from Deltapak column in 7 min (fraction 7, Figure 4B); so did the marinobufagenin standard (Figure 3A). In our study, we were detecting EDLF at wavelength 300 nm (which is typical for bufodienolides). Previously, Lichtstein et al separated digitalis-like material from human cerebrospinal fluid showing absorption at 300 nm.[24] Fraction 7 also demonstrated the highest digoxin-like immunoreactivity (Figure 4D). At the same time, ouabain-like immunoreactivty eluted from chromatographic column 11 and 13 minute (Figure 4C, fractions 11 and 13). Therefore, endogenous digoxin-like immunoreactivity is likely to represent endogenous bufodienolide rather than endogenous ouabain. In our previous experiments, marinobufagenin also demonstrated digoxin-like immunoreactivity greater than ouabain.[10,19] When HPLC fractions were tested for their ability to inhibit purified Na/K-ATPase, three fractions (8, 11, 9 and 7, Figure 4A) demonstrated highest Na/K-ATPase inhibitory potency. Fractions 8 and 9, which inhibited Na/K-ATPase by 56% and 40%, accounted for 20% of marinobufagenin-like immunoreactivity. The nature of the compound(s), which was contained in fractions 8 and 9 and caused the strongest Na/K-ATPase inhibition, remains to be clarified. This substance could represent one of the metabolites of endogenous bufodienolide EDLF. Fraction 11 caused 42% Na/K inhibition and contained 75% of total ouabain-like immunoreactivity. Fraction 7, which contained maximum marinobufagenin-like immunoreactivity, caused 37% inhibition of canine kidney Na/K-ATPase. These observations show a dissociation between the ability of HPLC fractions to react with various antidigitalis antibodies and to cause inhibition of Na/K-ATPase, and support the view that human plasma contains several EDLFs.[4,5]

The present findings confirm previous reports that human EDLF may include a bufodienolide structure.[11–15,17,19] Although, chromatographically, marinobufagenin-like immunoreactive material was indistinguishable from the marinobufagenin standard, its chemical structure remains to be determined. Previously, it has been shown that in experimental animals bufodienolides may cause hypertensive responses after both acute and chronic administration. Thus, Eliades et al[41] have demonstrated that bufalin raises blood pressure after acute administration to anesthetized dogs. In rats, bufalin caused blood pressure elevation after a 6-week treatment.[42] Further studies will also show whether or not human bufodienolide EDLF plays a role in the pathogenesis of human hypertension.

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