Effects of an Endogenous Ouabainlike Compound on Heart and Aorta

Sergio Bova, Mordecai P. Blaustein, James H. Ludens, Douglas W. Harris, Donald W. DuCharme, and John M. Hamlyn

An endogenous ouabainlike compound (OLC) has been purified from human plasma, and mass spectrometry has shown it to be indistinguishable from plant-derived ouabain. This human OLC was tested for its effects on evoked tension in guinea pig left atria and aortic rings. The tissues were incubated at 37°C in bicarbonate-buffered physiological salt solution gassed with 95% O₂-5% CO₂. In atria stimulated electrically at 1 Hz, 85 and 170 nM human OLC increased peak active force to 177±15% and 313±32% of control, respectively (n=3), with little effect on the duration of contraction. On washout of the OLC, peak systolic force returned to the control level with a half-time of 4.3±0.5 minutes. Similar results were obtained with 160 nM plant-derived ouabain: peak systolic force increased to 310±31% of control (n=4) and returned to the control level with a half-time of 3.8±0.2 minutes during washout. In aortic rings, neither 170 nM human OLC nor 160 nM plant ouabain (30-minute treatments) affected resting (unstimulated) tension, but they increased the contractions evoked by histamine (0.2-1.0 μM) to 156±13% (n=4) and 143±6% (n=4) of control responses, respectively. The mean half-time for washout of the OLC and plant ouabain-induced augmentation of histamine-evoked tension exceeded 35 minutes. These data show that human OLC has cardiotonic and vasotonic actions qualitatively and quantitatively similar to those observed with plant ouabain. Together with data on the mass spectrometry, chromatographic characteristics and interaction with Na⁺,K⁺-ATPase, and full cross-reactivity with polyclonal anti-ouabain antibodies, these physiological findings support the view that human OLC is ouabain. (Hypertension 1991;17:944–950)

Cardenolides of plant origin have a positive inotropic effect on cardiac muscle¹ and augment vasoconstriction in vascular smooth muscle.² These responses appear to be due to a secondary rise in cell calcium mediated by the sarcolemmal sodium/calcium exchanger,³ because the cardenolides directly inhibit the sarcolemmal sodium pump³ and thereby raise intracellular sodium in the cardiac and vascular myocytes.

An endogenous ouabainlike compound (OLC) now has been isolated from human plasma.⁴ It is isomeric with and similar to plant-derived ouabain in its molecular structure⁵,⁶ and biochemical action on the sodium pump⁴,⁷ Whether or not this human OLC also has cardiotonic and vasotonic effects similar to plant-derived ouabain has not been determined previously and is the subject of this report.

Methods

Tissues

Left atria and aortas were obtained from guinea pigs weighing 250–300 g. The animals were stunned by a blow to the head and killed by decapitation; the chest then was rapidly opened, and the heart and great vessels were removed. The left atrium or the aorta was carefully dissected free from surrounding tissue, and the atrium or a ring (2–3 mm long) of aorta was mounted between two platinum hooks in a 1.0-ml organ bath. One hook was anchored to the bottom of the organ bath, and the other was connected to a force transducer. In experiments on the left atrium, the anchoring hook also served as a stimulating electrode.

Reagents and Solutions

The tissues were incubated at 37°C in a physiological salt solution (PSS) containing (mM) NaCl 130,
Bova et al.
Actions of Human Ouabainlike Compound

A. Active Tension (F)

B. Active Tension (F)

C. dF/dt

Control 10 min 20 min 34 min 24 min 20 min 27 min

A = 10 sec
B, C = 100 msec

FIGURE 1. Representative recordings showing effect of human ouabainlike compound (OLC) on active contractions of guinea pig left atrium. A series of control contractions was obtained, and 85 nM human OLC (final concentration) then was added to the physiological salt solution in the tissue chamber; contractions were recorded at 10, 20, and 34 minutes. OLC concentration then was raised to 170 nM; tension recordings obtained 24 minutes later are shown. OLC then was washed out of the tissue chamber for 20 minutes, and 85 nM human OLC again was added to the tissue chamber. Representative tension records obtained at both slow (panel A) and fast (panel B) chart speeds are shown, as are rate of tension change (dF/dt) records at fast chart speed (panel C). Temperature, 37°C; preload tension, 350 mg.

KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.0, NaH₂PO₄ 0.4, NaHCO₃ 19, and glucose 5.4. The PSS was gassed continuously with 95% O₂-5% CO₂ to maintain pH at 7.3-7.4. The aortic rings were activated with 0.2-1.0 μM histamine (Sigma Chemical Co., St. Louis, Mo.), which was added from a fresh aqueous 0.1 mM stock solution directly to the PSS in the organ bath, as described in “Methods.” Both plant-derived ouabain (a gift from SIMES, Milan, Italy) and purified human OLC were dissolved in sufficient distilled water to provide stock solutions of 100 and 8.55 μM, respectively. Regrettably, only 1.0 μg of the human OLC was available for these experiments. Small aliquots of the stock solutions were added directly to the 1.0 ml of PSS in the tissue chamber; mixing was rapid because of the agitation produced by the small gas bubbles that entered from the bottom of the chamber through a fritted glass plate.

**Stimulation and Recording**

The guinea pig left atria were stimulated at 1 Hz with twice-threshold square pulses of 0.8-1.0-V intensity and 0.7-1.2-msec duration, using a model S88 stimulator (Grass Instrument Co., Quincy, Mass.). Resting (preload) tension was optimized for slightly submaximal isometric force by generating a Starling (length-tension) curve for each preparation. Isometric force was measured with a model TRB/200/2 force transducer (Battaglia-Rangoni, Bologna, Italy) and recorded on a Battaglia-Rangoni model KV 220 strip chart recorder. The preload tension for the atria ranged between 300 and 500 mg. Control records were obtained before the preload was stabilized (usually within 30-60 minutes after the tissue was mounted).

For the aortic rings, resting (baseline) tension was set at 1,000-1,500 mg so as to give an optimal force response with a submaximal dose of histamine (0.2-1.0 μM). After the baseline was established, the rings were stimulated with histamine (by adding aliquots of the stock solution directly to the PSS in the organ bath) for 7-minute periods separated by 35-minute washout periods in PSS alone.

**Statistics**

Student’s t test for paired or unpaired data was used when appropriate to determine the significance of differences between means.

**Results**

Comparison of the Effects of Human Ouabainlike Compound and Plant Ouabain on Guinea Pig Left Atria

Figure 1 shows data from an experiment in which the effect of human OLC was tested on the active tension in a guinea pig left atrium electrically driven at 1 Hz. Once the peak active tension reached a steady level (control), an aliquot of human OLC stock solution was added to the PSS in the tissue bath to give an OLC concentration of 85 nM. Peak active tension then rose toward a steady level over a 34-minute period. The concentration of human OLC in the bath then was increased to 170 nM, and peak active tension rose toward a new steady level over the next 24 minutes. The effect was reversible and repeatable; when the OLC was washed out, the peak active tension returned to the control level with a half-time (t_1/2) of approximately 3.5 minutes (Figure 2). Readdition of 85 nM human OLC again augmented the contraction (Figure 1). Data from this experiment and two similar experiments are summarized in Table 1 (atria 1-3). In addition, we tested the response of much higher concentrations of hu-
man ouabain in one other guinea pig atrial preparation and also observed a large positive inotropic response.

When the peak tension data for washout of OLC (Figure 2A) were plotted on semilogarithmic coordinates, the data fit a straight line with a t_{1/2} of 3.5 minutes and an off rate constant of 0.2 min⁻¹. These results imply that the cardiotonic action of OLC in the atria is due to binding of the OLC to a single class of sites, presumably on the sodium pump.

The positive inotropic effect of the human OLC was associated with an increase in the rates of rise and fall of tension. As illustrated in Figure 1C and Table 1, the maximal rates of force development during contraction and relaxation — (dF/dt)_max (contraction) and (dF/dt)_max (relaxation), respectively — both increased substantially. However, as seen in Figure 1B, human OLC had little effect on the time to peak tension or the duration of contraction. These data and (dF/dt)_max data from two other similar experiments also are summarized in Table 1.

For comparison, Figure 3 shows tension and dF/dt records from a guinea pig left atrium exposed to 160 nM plant ouabain. As seen here and in the data summarized in the lower part of Table 1 (atria 4–7), the positive inotropic effect obtained with 160 nM plant ouabain is very similar to that obtained with 170 nM human OLC (atria 1–3). Indeed, there is no significant difference between the two mean %C values, that is, the maximum peak active tension observed with 170 nM human OLC and with 160 nM plant ouabain expressed as percentages of the respective control values. The increase in mean normalized dF/dt values for both contraction and relaxation observed with plant ouabain also are very similar to the corresponding values obtained with human OLC; neither agent had a detectable effect on the time to peak tension. Moreover, both plant ouabain and human OLC washed out rapidly; in both cases, recovery t_{1/2} was shorter than 5 minutes (see Table 1).

The key observations on maximal active force development for both human OLC and plant ouabain are summarized in Table 1. These data indicate that the two agents have qualitatively and quantitatively similar effects on the guinea pig left atrium.

**Comparison of the Effects of Human Ouabainlike Compound and Plant Ouabain on Guinea Pig Aortic Rings**

The action of human OLC on the vasoconstrictor response evoked by 1.0 μM histamine in a ring of guinea pig aorta is illustrated in Figure 4. The upper record shows one of the three control responses to histamine, that is, the response in the absence of OLC; these three 7-minute exposures to histamine were separated by 35-minute washout periods in PSS. After washout of histamine following the third control response, the tissue was reexposed to 170 nM human OLC for 30 minutes, and the tissue again was activated with 1.0 μM histamine. The middle record shows that human OLC augmented the histamine-evoked contraction by approximately 66%. The histamine and human OLC then were washed out with PSS for 35 minutes, and the tissue was reexposed to histamine. Note that in this experiment, there was little evidence of a return to the pre-OLC histamine response.

Data from this and three other, similar experiments are summarized in the upper part of Table 2 (aortic rings 1–4). On average, 170 nM human OLC increased the histamine-evoked tension by approximately 56% (i.e., to 156% of control). The washout of the effect of human OLC was variable. Nevertheless, it was apparent that the washout was much slower and less complete than the corresponding effect in the atria (Figure 2 and Table 1).

Very similar results were obtained when plant ouabain was used. This is illustrated by the data in
### Table 1. Effects of Human Ouabainlike Compound and Plant Ouabain on Guinea Pig Left Atrium

<table>
<thead>
<tr>
<th>Atrium</th>
<th>Control peak active tension</th>
<th>Human OLC (85 nM)</th>
<th>Human OLC (170 nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no.</td>
<td>(mg) (mg) (mg)</td>
<td>(mg) (mg) (mg)</td>
<td>(mg) (mg) (mg)</td>
</tr>
<tr>
<td>1</td>
<td>140 240 171</td>
<td>380 271 247</td>
<td>313 32 278</td>
</tr>
<tr>
<td>2</td>
<td>75 120 160</td>
<td>270 360 310</td>
<td>278 129</td>
</tr>
<tr>
<td>3</td>
<td>110 220 200</td>
<td>340 309 278</td>
<td>291 4.0</td>
</tr>
</tbody>
</table>

Mean±SEM 177±15 163±8 175±4 313±32 278±22 293±34 4.3±0.5

---

## Bova et al. Actions of Human Ouabainlike Compound

### Table 2. Effects of Human Ouabainlike Compound and Plant Ouabain on Guinea Pig Aorta

<table>
<thead>
<tr>
<th>Aortic ring no.</th>
<th>Histamine (µM)</th>
<th>Control (AH)* (mg)</th>
<th>Human OLC (170 nM) (mg)</th>
<th>Washout† (mg)</th>
<th>(AH)† (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.25</td>
<td>130</td>
<td>170 131</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.40</td>
<td>410</td>
<td>460 112</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>350</td>
<td>570 163</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>460</td>
<td>580 126</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mean±SEM 156±13% 133±12%

---

## Discussion

We have characterized the actions of an OLC purified from human plasma on guinea pig left atria and aortas. This OLC has a molecular weight identical to plant-derived ouabain, and its acetylated derivatives are indistinguishable from those of plant-derived ouabain on fast atom bombardment mass.
**Human Ouabain-like Compound Augments Active Tension in Guinea Pig Atria**

Human OLC had a positive inotropic effect on the left atrium that was qualitatively and quantitatively comparable to the effect of plant-derived ouabain. Both substances increased the rate of rise and rate of fall of electrically activated atrial tension without significantly altering the contraction duration.

The effects of OLC appeared to be dose dependent, but more complete dose–response curves could not be obtained because of the limited supply of OLC. The atria rapidly recovered (apparent t½ < 5 minutes) when both the human OLC and plant ouabain were washed out; indeed, the rate constant for washout of OLC from the atria (0.2 min⁻¹) is only slightly smaller than that for washout of plant ouabain from guinea pig cardiac sarcolemmal Na⁺,K⁺-ATPase (0.66 min⁻¹).³

**Human Ouabain-like Compound Augments Vasoconstrictor-Activated Responses in Guinea Pig Aorta**

Plant ouabain is known to augment vasoconstrictor responses²⁻⁰ and, at high concentrations, increase resting (unstimulated) tension in vascular smooth muscle.¹¹,¹² In fact, a digitalis-like substance has been postulated to play a key role in the pathogenesis of some forms of hypertension, including some essential hypertension.⁹,¹³⁻¹⁶ Therefore, it was important to determine whether human ouabain also had an effect on vascular smooth muscle.

Indeed, we found that the effects of human OLC on aortic rings also were qualitatively and quantitatively comparable to the effects of plant ouabain. Both agents increased the histamine-evoked contraction (on average, to approximately 156% and 143%
of control histamine-evoked responses, respectively) without affecting the resting (unstimulated) tension. We assume that inhibition of the sodium pump by OLC and plant ouabain did increase the intracellular sodium concentration and thereby promoted calcium entry via sodium-calcium exchange in the unstimulated rings. The lack of effect of these agents on resting tension then may be attributed to sequestration, in the sarcoplasmic reticulum, of most of the entering calcium (i.e., "calcium buffering"), so that the contraction threshold was not exceeded when these relatively low concentrations of OLC and plant ouabain were used.

The augmented histamine-evoked responses are qualitatively comparable to the augmented vasoconstrictor-evoked responses induced by sodium pump inhibition with plant ouabain or potassium-depleted media in rat and rabbit arteries, and to the augmented vasoconstrictor-evoked tonic tension responses induced by plant ouabain in human resistance arteries. Plant ouabain shifts the vasoconstrictor dose–response curve toward lower vasoconstrictor concentrations without significantly altering the maximal vasoconstrictor-evoked tension. Parallel studies with the calcium-sensitive fluorochrome fura-2 suggest that the augmented contractile responses are likely due to the increased availability of calcium to activate contraction, because inhibition of the sodium pump raises intracellular sodium, and this then leads to an increase in intracellular calcium as a result of sodium/calcium exchange.

Recovery of the control histamine response, after washout of both human OLC and plant ouabain, was much slower in the aortic rings than in the cardiac atria, and was, on average, less than 50% complete in 35 minutes. We previously have observed slow but complete recovery of control vasoconstrictor-activated responses in rings of rat aorta after exposure to plant ouabain (M.P. Blaustein, S. Bova, and X-J. Yuan, unpublished data and Reference 2). It remains to be elucidated whether this difference between the cardiac and vascular preparations is due to the greater thickness of the aorta or to differences in the ouabain receptors (sodium pumps) in these two tissues. However, there is a precedent for plant ouabain washout rate differences as high as 50-fold in different tissues from the same animal; this might be due to the presence of different Na+, K+-ATPase isozymes in the different tissues.

The slow washout of these substances from the aortic rings suggests that OLC may play an important, continuous role in the vasculature, even though plasma levels of OLC may fluctuate.

**Significance of These Findings**

Our observations on the augmentation of vasoconstrictor-evoked contractions (i.e., the vasotonic effect) by human OLC are particularly pertinent in light of the evidence that cardiotonic steroids enhance vascular contractility in normal humans, and that many subjects with essential hypertension have elevated levels of a circulating inhibitor of the sodium pump. Also, patients with essential hypertension and primary aldosteronism have elevated plasma levels of a substance with ouabainlike immunoreactivity. In all of these patients with established hypertension, the levels of the ouabainlike immunoreactivity and of the sodium pump inhibitor are likely to be directly correlated with blood pressure. Also, in the primary aldosterone patients, the level of the ouabainlike immunoreactivity and the sodium pump inhibitor were reduced after removal of the adrenal adenomas. Furthermore, in heminephrectomized pigs treated with deoxycorticosterone acetate, the plasma level of the sodium pump inhibitor rises markedly before the blood pressure goes up. These observations, coupled with the data in the present report, fit with the idea that human OLC may play a role in the pathogenesis of hypertension and may, in fact, lead to the elevation of blood pressure as a consequence of its vasotonic action.

The OLC used in these studies was isolated and purified from human plasma. Its chromatographic characteristics on high-performance liquid chromatog-
raphy are indistinguishable from those of plant ouabain, and OLC and ouabain bind to Na⁺,K⁺-ATPase in a very similar fashion.¹ The molecular ions, daughter fragments, and acetylated derivatives of OLC and plant ouabain are indistinguishable by fast atom bombardment mass spectrometry. Furthermore, polyclonal antibodies raised against plant ouabain exhibit full cross-reactivity with OLC.² Finally, our observations show that human OLC and plant ouabain induce qualitatively and quantitatively similar cardiotonic and vasoactive effects in guinea pig left atria and aortas, respectively. The sum of these observations suggest very strongly that human OLC is ouabain.

References


Key words: ouabain • ouabainlike compounds • cardiotonic agents • heart atrium • aorta
Effects of an endogenous ouabainlike compound on heart and aorta.
S Bova, M P Blaustein, J H Ludens, D W Harris, D W DuCharme and J M Hamlyn

Hypertension. 1991;17:944-950
doi: 10.1161/01.HYP.17.6.944

Hypertension is published by the American Heart Association. 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1991 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/17/6_Pt_2/944

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org/subscriptions/