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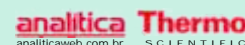
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Hypertensive effects of the *iv* administration of picomoles of ouabain

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Abstract

Ouabain, an endogenous digitalis compound, has been detected in nanomolar concentrations in the plasma of several mammals and is associated with the development of hypertension. In addition, plasma ouabain is increased in several hypertension models, and the acute or chronic administration of ouabain increases blood pressure in rodents. These results suggest a possible association between ouabain and the genesis or development and maintenance of arterial hypertension. One explanation for this association is that ouabain binds to the α -subunit of the Na^+ pump, inhibiting its activity. Inhibition of this pump increases intracellular Na^+ , which reduces the activity of the sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ exchanger and thereby reduces Ca^{2+} extrusion. Consequently, intracellular Ca^{2+} increases and is taken up by the sarcoplasmic reticulum, which, upon activation, releases more calcium and increases the vascular smooth muscle tone. In fact, acute treatment with ouabain enhances the vascular reactivity to vasopressor agents, increases the release of norepinephrine from the perivascular adrenergic nerve endings and promotes increases in the activity of endothelial angiotensin-converting enzyme and the local synthesis of angiotensin II in the tail vascular bed. Additionally, the hypertension induced by ouabain has been associated with central mechanisms that increase sympathetic tone, subsequent to the activation of the cerebral renin-angiotensin system. Thus, the association with peripheral mechanisms and central mechanisms, mainly involving the renin-angiotensin system, may contribute to the acute effects of ouabain-induced elevation of arterial blood pressure.

Key words: Ouabain; Hypertension; Vascular reactivity; Renin-angiotensin system

Introduction

Endogenous cardiac glycoside increases to nanomolar concentrations in the plasma of hypertensive humans and rats (1,2); in patients with diabetes (3), myocardial infarction (4), congestive heart failure (5) and other diseases, and after acute physical exercise (6). This substance was characterized as ouabain or as an isomer of ouabain (7) and is produced in the central nervous system (8,9), the adrenal cortex (10), and the heart (11). Acute administration of ouabain promotes a positive inotropic effect (12) and increases blood pressure and the contraction of vascular smooth muscle (13,14). These findings suggest that ouabain increases vascular resistance and induces hypertension (Figure 1).

In addition to its hypertensive effects, endogenous ouabain also modifies cardiac function and modulates cellular proliferation and differentiation in the heart (15) and vascular smooth muscle (16).

The primary site of ouabain action is generally assumed to be the α -subunit of Na^+,K^+ -ATPase, which is a heterotrimer composed of α -, β - and γ -subunits which exist as isoforms (17,18). Ouabain inhibits Na^+,K^+ -ATPase by binding mainly to the α_2 and α_3 isoforms of the α -subunit of the enzyme in rodents (13,19). This inhibition increases the Na^+ concentration in the cytoplasm, which reduces the activity of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger (NCX) and consequently increases the amount of Ca^{2+} available to activate contraction in tissues such as the heart, producing a positive inotropic effect (19,20). However, low concentrations of ouabain stimulate Ca^{2+} transients in arterial smooth muscle without raising cytosolic (Na^+) (21). This apparent contradiction can be explained by the co-localization of the α_2 and α_3 isoforms of Na^+,K^+ -ATPase and the NCX, which increases Na^+ concentration only at the plasmersome region in response to ouabain (19). The increase of Na^+ con-

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centration in the plasmersome region might reduce the activity of the NCX. As a result, intracellular calcium concentration increases, as do vascular tone and blood pressure (19,21). This suggests that ouabain acts as a pressor agent.

Xie and Cai (22) propose that low doses of ouabain, which are insufficient to inhibit Na^+, K^+ -ATPase activity, could bind the caveolar Na^+, K^+ -ATPase and activate some protein and lipid kinase cascades, thus generating several secondary messengers that, in turn, might modify cellular function. Thus, ouabain may modulate cellular proliferation and differentiation in the heart (15) and vascular smooth muscle (16), which could contribute to the maintenance of hypertension.

Ouabain might increase blood pressure via different mechanisms on different tissues and systems. In this review, we shall focus on the acute effects of ouabain on vascular reactivity and the central nervous system, mechanisms which might contribute to the development of hypertension.

Acute effects of picomole quantities of ouabain on vascular reactivity

In normal human volunteers (23) and rats (14,24), short-term *iv* administration of ouabain in the range of 10-30 nmol/kg produces a pressor response that is peripheral in origin.

In addition, an *iv* subpressor dose of ouabain, 0.1 mg/m², enhances the ability of a single dose of norepinephrine to increase mean arterial pressure (25). Indeed, low doses of ouabain (6 and 18 µg/kg, which correspond to approximately 10 and 30 nmol/kg) increase the vasoconstrictor response to phenylephrine (24).

In the reflex-blocked anesthetized rat, ouabain increases diastolic pressure by phentolamine-sensitive and -insensitive mechanisms (26,27). At the doses used, ouabain induced increases in diastolic pressure but did not alter the dose-response curve for phenylephrine or the responsiveness to submaximal doses of norepinephrine. Because reflexes were blocked, the authors concluded that the short-term pressor effects of ouabain are not related to its direct central nervous system actions. Moreover, the authors suggested that the pressor actions of ouabain are mediated by norepinephrine released from sympathetic nerves as well as by a direct action on vascular smooth muscle cells. Presumably, both actions are due to the inhibition of Na^+, K^+ -ATPase. Thus, the role of endogenous ouabain in hypertension would also include the amplification of contractile responses.

In some rat models of hypertension, including the SHR model (28), renovascular hypertension and L-NAME-induced hypertension (29), acute ouabain administration

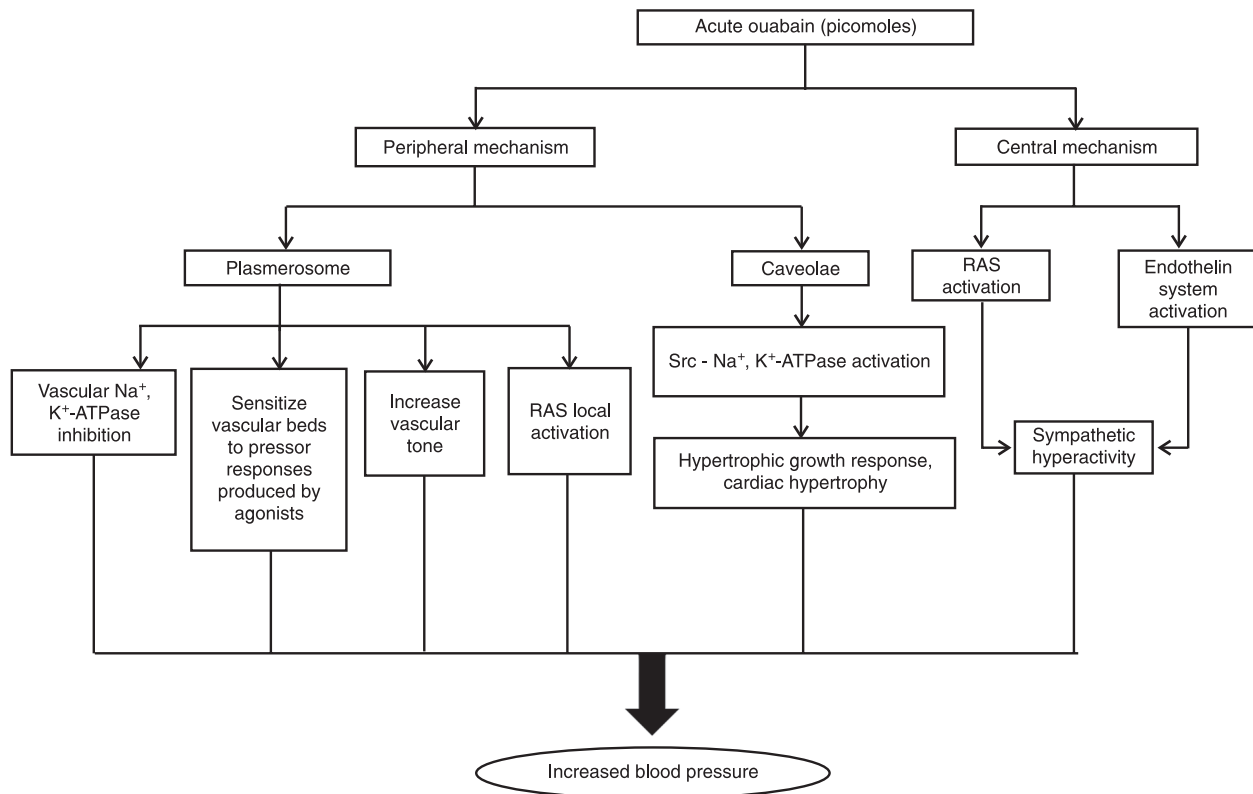


Figure 1. A generalized scheme showing the central and peripheral actions of acute ouabain. RAS = renin-angiotensin system; Src = sarcoma kinase.

increases blood pressure, whereas no change in blood pressure is observed in normotensive rats (14,30,31). These results suggest that the effects of ouabain are amplified in hypertensive conditions. We recently demonstrated in SHR that low picomole doses of ouabain (*iv*) increases systolic and diastolic blood pressure by a mechanism that seems to depend on activation of the renin-angiotensin system (31). However, because the effect of ouabain on diastolic blood pressure was not blocked by canrenone (1 mg/kg), we suggest that ouabain may have a mechanism of action that is independent of Na⁺ pump inhibition.

Ouabain and a series of structurally related analogs have hypertensinogenic activity, regardless of their potency as Na⁺ pump inhibitors (32). In addition, Ward et al. (33) suggested that adrenocortical cells express ouabain receptors that are distinct from the Na⁺ pumps. These findings could explain why the pressor effect of ouabain on diastolic blood pressure seems not to depend on its effect on the Na⁺ pump.

In the past few years, it has become apparent that binding of either endogenous or exogenous cardiotonic steroids, such as ouabain, to caveolar Na⁺,K⁺-ATPase evokes protein and lipid kinase cascades, thus generating several secondary messengers (22). This signaling pathway involves the association of protein tyrosine kinases (Src) with the Na⁺,K⁺-ATPase in a caveolar domain (34). Therefore, once the Na⁺,K⁺-ATPase/Src complex is activated by ouabain, Src is activated, initiating a cascade of signaling events. As a result, downstream effectors are activated, resulting in the activation of protein kinase cascades and the generation of second messengers. This leads to a cascade that involves the generation of reactive oxygen species (ROS), activation of extracellular signal-regulated protein kinase (ERK) through activation of its mitogen-activated protein kinase (MEK), activation of phosphatidylinositol 3-kinase (PI3K), stimulation of endocytosis and activation of Akt, as well as activation of protein kinase C (22,34,35). This binding of ouabain to Na⁺,K⁺-ATPase affects multiple cellular functions, thereby influencing cell growth, heart hypertrophy and apoptosis (22).

Ouabain can act directly on the vasculature to increase vascular resistance or to sensitize vascular beds to pressor responses produced by norepinephrine, angiotensin II, and phenylephrine (28,36-40). *In vitro*, picomole doses of ouabain enhance caffeine-induced contractures and increase the ability of phenylephrine to potentiate caffeine-induced contractions of rat isolated vascular preparations (41). These observations support the hypothesis that picomole quantities of ouabain should selectively interact with the high-affinity α_2 and α_3 isoforms of Na⁺,K⁺-ATPase to enhance the actions of vasoconstrictor agents.

Endothelium-derived factors might positively or negatively modulate the vascular actions of ouabain. Thus, the endothelium modulates the actions of picomole quantities of acutely administered ouabain in vascular preparations

from normotensive and hypertensive rats (28,29,40).

In the tail vascular bed from normotensive Wistar rats, Rossoni et al. (40) demonstrated that the acute perfusion of ouabain (10 nM) induces the release of a potassium channel opener and that the effects of ouabain are partially modulated by the endothelium. On the other hand, in cultured rat aortic endothelial cells, ouabain (10 nM) contributes to the bradykinin-stimulated increase of nitric oxide (NO) production by inhibiting plasma membrane Na⁺,K⁺-ATPase activity and increasing intracellular Na⁺ concentration (42). Moreover, Eva et al. (43) demonstrated that small quantities of ouabain stimulate NO production in human umbilical artery endothelial cells via PI3K. The binding of ouabain to Na⁺,K⁺-ATPase initiates a signal via PI3K, which turns on the proliferation and survival pathways that involve Akt and that stimulate endogenous nitric oxide synthase (eNOS) and NO production (43). Because NO is an endothelial factor that activates Na⁺,K⁺-ATPase (44), we speculated that picomole quantities of ouabain could release NO, which, in turn, could stimulate Na⁺,K⁺-ATPase activity, reducing vascular reactivity in normotensive rats. In L-NAME-hypertensive rats, the acute administration of ouabain (10 nM) induces the release of endothelium-derived hyperpolarizing factor in the tail vascular bed (29). This and other results support the notion that the endothelium might modulate the action of acute and chronic ouabain administration (28,29,40,45).

In the aorta from SHR, ouabain at micromolar concentrations releases an unknown vasoconstrictor factor not related to products from the arachidonic acid pathway or free radical release (39). However, the perfusion with a lower concentration of ouabain (1 nM) increases the sensitivity to phenylephrine in the tail vascular bed of SHR by a mechanism involving increased activity of endothelial angiotensin-converting enzyme and the local synthesis of angiotensin II (28). Indeed, the role of low-concentration ouabain (10 nM) in the local renin-angiotensin system was demonstrated in bovine adrenal glomerulosa cells (46). In that report, the authors showed that ouabain interacts with angiotensin II to stimulate aldosterone production in bovine adrenal glomerulosa cells. On the other hand, the central mechanism described above suggests that ouabain stimulates the brain renin-angiotensin system, leading to the production of angiotensin II, which further increases sympathetic tone and ultimately increases arterial blood pressure (47). We also found that the increases in systolic and diastolic blood pressure induced by low-dose ouabain (~0.3 nmol/kg) were blocked completely by pretreatment (*iv*) with 10 mg/kg losartan, although these increases were not affected by ganglionic blockade with 5 mg/kg hexamethonium, suggesting that ouabain, at least at this concentration, does not act on preganglionic neurons (28,31). On the other hand, the increase of blood pressure induced by 0.18 μ g/kg ouabain in L-NAME-hypertensive rats is blocked by hexamethonium (29). Taken together, these

observations indicate that in SHR, ouabain activates the local renin-angiotensin system, which induces increases in vascular resistance and blood pressure. The classical effect of ouabain is the inhibition of Na^+, K^+ -ATPase activity by binding mainly to the α_2 and α_3 isoforms of the α -subunit of the enzyme in rodents (13,19). Indeed, we demonstrated that in SHR, 1 nM ouabain activates rather than inhibits Na^+, K^+ -ATPase activity in vascular smooth muscle (28), an observation similar to that described by Gao et al. (48) in cardiac myocytes. However, the angiotensin II released by the action of ouabain may also be able to stimulate Na^+, K^+ -ATPase activity (49).

Although the peripheral actions of ouabain could contribute to increased blood pressure, the central mechanisms, already well established, are also important mediators of increased pressure induced by this digitalis. Therefore, the central mechanism of ouabain is described below.

Acute effects of ouabain on the central nervous system

The effects of ouabain on the central nervous system have been well established. Neurons express the α_3 isoform of Na^+, K^+ -ATPase, the most ouabain-sensitive isoform of the α -subunits, and therefore neurons are very sensitive to the effects of this substance.

Indeed, the acute administration of ouabain in some regions of the central nervous system, such as the median preoptic nucleus (50), paraventricular nucleus (51) or rostral ventrolateral medulla (52), causes dose-dependent increases in sympathetic nerve activity, blood pressure and heart rate. Therefore, these findings suggest that endogenous ouabain might be a regulator of sympathetic tone and arterial blood pressure by influencing some areas of cardiovascular pressor control. This hypothesis seems to be confirmed by reports that the sympathoexcitatory and pressor effects induced by ouabain can be reversed and prevented by the intracerebroventricular administration of antibody Fab fragments, which bind ouabain, blocking its actions (9,53). The sympathetic hyperactivity induced by ouabain and the consequent increase in blood pressure appear to involve the central renin-angiotensin system. This observation is supported by reports that the sympathetic hyperactivity induced by ouabain is prevented by an AT1 receptor blocker (50-52), suggesting that the central renin-angiotensin system could be the sympathoexcitatory component involved in the hypertensinogenic effect of ouabain. In addition, the acute hypertensinogenic effect of ouabain is attenuated in transgenic rats that are deficient in brain angiotensinogen (54). Therefore, in some diseases that are accompanied by an increased level of endogenous ouabain, such as hypertension (1), heart failure (55) and diabetes (35), the increase in sympathoexcitatory activity could be due to the actions of ouabain on the central renin-angiotensin system. In addition to the sympathoexcitatory

activity induced by ouabain via the central renin-angiotensin-aldosterone pathway, D'Amico et al. (56) demonstrated that the central endothelin system (periaqueductal gray area) of rats is also involved in this sympathetic activation and in the ouabain-induced hemodynamic effects.

Ouabain release in the brain is very sensitive to sodium loading (9). This position is supported by the observation that the effects of sodium loading can be mimicked by intrahippocampal microinjection of ouabain (57). Indeed, acute intrahippocampal administration of an extremely low dose of ouabain (60 pg) in Dahl salt-sensitive rats (DSS) is associated with activation of the renin-angiotensin-aldosterone system in the hypothalamus and adrenal cortex, an increase in the adrenocortical level of marinobufagenin, inhibition of sodium pumps in the vasculature and kidney, and pressor and natriuretic responses (57). Moreover, the central administration of NaCl or NaCl loading in DSS or SHR rats induces increases in brain endogenous ouabain that, in turn, induce sympathoactivation and increase blood pressure (9,53). However, the evidence regarding the relationship between NaCl loading and increased ouabain level is contradictory. Manunta et al. (58) demonstrated in healthy humans that NaCl depletion produced an elevation in the plasma concentration of endogenous ouabain, but in the same study, 3 days of NaCl loading (171 mEq NaCl/day) caused a transient elevation in the plasma concentration of endogenous ouabain. In another report (59), acute NaCl loading in DSS and Dahl salt-resistant (DR) rats caused a transient increase in an ouabain-like compound in the pituitary gland, adrenal gland, and plasma, followed by a decrease to baseline. Therefore, it seems that the plasma level of endogenous ouabain may not be a good indicator of volume expansion because it displays transient response.

General considerations

Endogenous ouabain was discovered in humans 20 years ago, and its effects on sodium pumps have been widely explored, although much remains to be clarified. In general, endogenous ouabain participates in the control of blood pressure and sodium balance (1), but it also modifies cardiac function and modulates cellular proliferation and differentiation of the heart (15) and vascular smooth muscle (16).

Acutely, ouabain in picomole quantities increases blood pressure (24,28-31), pressor and vascular reactivity to vasoconstrictor agents (14,24,28-31,40,41), and sympathoexcitatory activity (9,53). These effects of ouabain are due at least in part to activation of the central and peripheral renin-angiotensin system (31,28,50-52).

Understanding the mechanisms of the effects of acute ouabain on the cardiovascular system is important because acute conditions of NaCl loading (13,57-59) or physical exercise (6) induce a sudden increase in the plasma level of endogenous ouabain. In some diseases, such as

hypertension (1,2), diabetes (3), myocardial infarction (4), and congestive heart failure (5), the basal plasma level of ouabain is increased. Therefore, acute conditions that elevate ouabain should be evaluated and studied to verify the systemic repercussions.

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