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Endogenous ouabain: Role in the pathogenesis of hypertension

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Endogenous ouabain: Role in the pathogenesis of hypertension. Substantial evidence implicates impaired renal excretion of sodium as the major culprit in the pathogenesis of hypertension. The key question is: How does the impairment of Na^+ excretion lead to increased peripheral vascular resistance and elevation of the blood pressure? Here we describe the evidence that elevated levels of a recently-discovered adrenal cortical hormone, endogenous ouabain, plays a central role in this process. This hormone inhibits the Na^+ pump and raises intracellular Na^+. Then, as a result of Na/Ca exchange, cytosolic Ca^{2+} and, more importantly, intracellular stores of Ca^{2+}, are increased in vascular smooth muscle (VSM), vasomotor neurons, and endothelial cells, as well as in many other types of cells. Consequently, these cells become hyper-responsive because the cytosolic Ca^{2+} transients induced by cell activation are enhanced. The synergy of augmented sympathetic neuron transmitter release and augmented VSM cell responsiveness may account for the increased arterial tone and peripheral vascular resistance that is the hallmark of hypertension.

In his classic monograph, *The Kidney*, Homer Smith [1] devoted a full chapter to the subject of "Essential Hypertension." He wrote that the enhanced arterial tone with increased total peripheral vascular resistance (TPR), the hallmark of essential hypertension, "*has a functional origin*." Nevertheless, due to the paucity of data then available, he was unable to draw firm conclusions about the pathogenesis of essential hypertension.

In the ensuing decade, a number of new studies, some of which involved the then recently-discovered mineralocorticoids, led Borst and Borst-de Geus [2] to re-examine the mechanisms responsible for the elevation of blood pressure. They suggested that "*hypertension is part of a homoeostatic reaction to deficient renal sodium output*." Subsequently, Guyton and his colleagues [3] re-emphasized the "*overriding dominance of the kidneys*" in the pathogenesis of hypertension. They concluded that "*All long-term arterial pressure regulation must involve the balance between intake and output of water and salt and almost invariably involves the kidneys*."

Coleman and Guyton [4] examined the hemodynamic changes that resulted from chronic salt and water retention and plasma volume expansion. They observed an initial rise in blood pressure due to increased cardiac output (CO) with a normal TPR. After several days of maintained volume expansion, however, CO declined and TPR increased to maintain the elevated blood pressure. This would be expected to induce a pressure natriuresis to help maintain salt balance and plasma volume. The shift from a high CO to a high TPR has been referred to as "autoregulation" [4, 5]. The view that the increased TPR of essential hypertension is a consequence of "autoregulation" does not, however, shed light on the precise mechanisms that underlie these hemodynamic changes.

The primary defect in the pathogenesis of essential hypertension thus appears to be an inability of the kidneys to handle the salt load with which they are faced in the presence of a normal blood pressure [2, 3, 6, 7]. Blood pressure therefore rises along the renal function curve, until the induced pressure natriuresis enables the kidneys to balance salt excretion with salt intake [2, 3, 5].

Endogenous ouabain and the pathogenesis of hypertension

In an effort to explain the origin of the increased TPR on the basis of functional changes, it was suggested [8] that the enhanced vascular tone might be the direct result of a slight excess of Ca^{2+} in the vascular smooth muscle (VSM) cells. The problem, then, was how to link this alteration in Ca^{2+} homeostasis to a primary defect in renal excretion of sodium. One proposed solution to this dilemma was the plasma membrane Na/Ca exchanger [8], which is a unique link between Na^+ and Ca^{2+} metabolism and, thus, between Na^+ and VSM contraction. This raised the possibility that the increased intracellular Ca^{2+} might be a secondary consequence of a slightly increased intracellular Na^+ concentration, $[Na^+]_{cyt}$. To explain the increased $[Na^+]_{cyt}$, the presence of elevated levels of a circulating endogenous digitalis-like substance was postulated (Fig. 1) [8, 9] . These concepts were later expanded to include the possibility that similar ionic alterations in vasomotor neurons and/or in endothelial cells might contribute to the enhanced vasoconstriction (Fig. 1, lower right quadrant, and see below) [10, 11].

In the nearly two decades since these ideas were first put forth, several key features of the hypothesis have been directly tested. Most importantly, the critical missing link, the endogenous digitalis-like substance was purified from human plasma. Astonishingly, this substance was identified as either identical to, or a closely-related isomer of, the plant-derived cardiac glycoside, ouabain [12]. The endogenous ouabain co-chromatographs with plant ouabain, and has an identical molecular weight and very similar mass spectroscopic properties [12, 13]. The presence of an endogenous ouabain-like compound in mammals has been confirmed by several investigators [14–18].

Endogenous ouabain and plant ouabain (hereafter, "ouabain") have virtually identical vasotonic and cardiotonic properties [19], and comparable ability to inhibit the Na^+ pump [12]. Even in the rat (which is widely recognized as "insensitive" to cardiotonic steroids) both neural tissue [20] and VSM [21] are sensitive to nanomolar concentrations of ouabain. This can be explained by

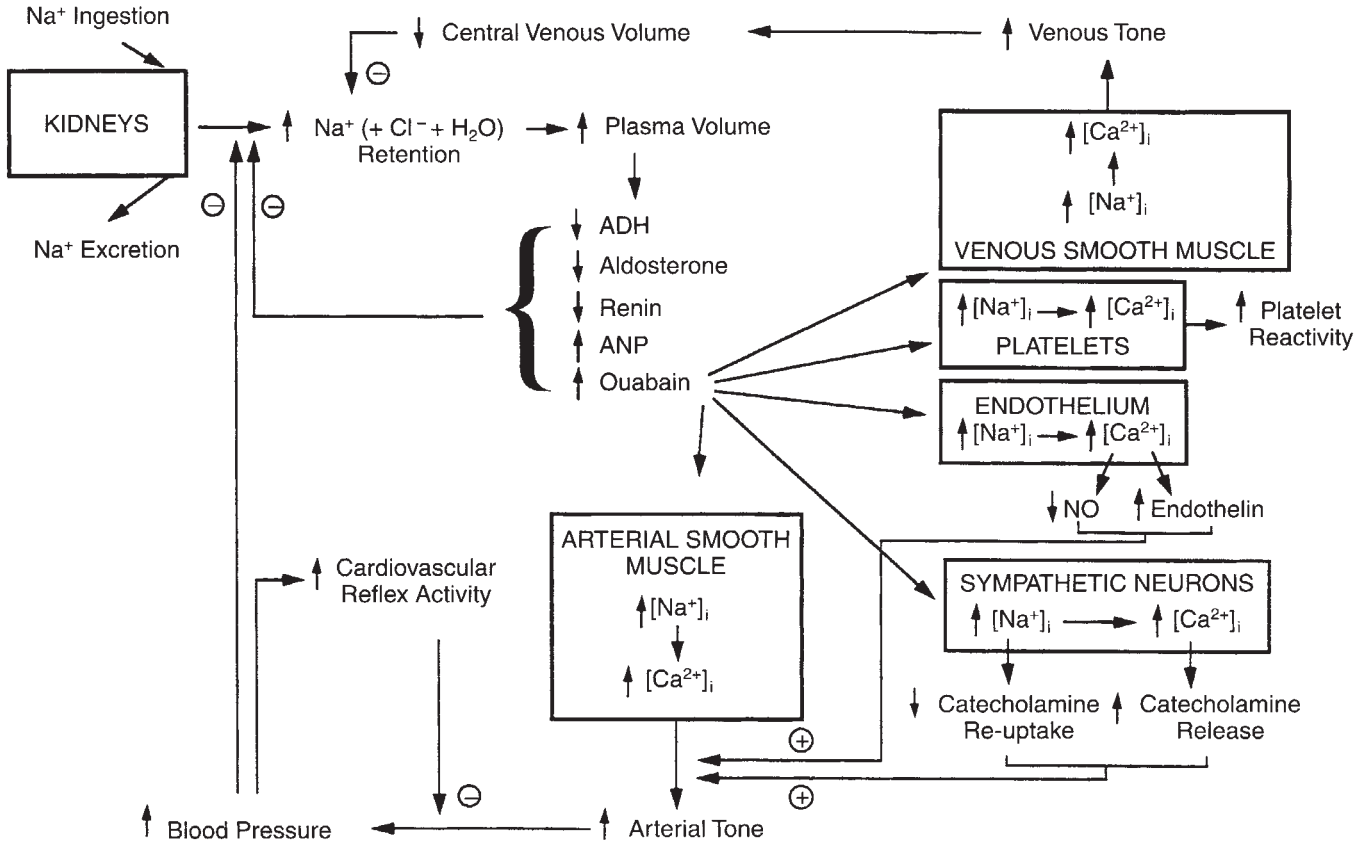

Fig. 1. *Diagram showing some of the major feed-back loops that help prevent plasma volume expansion when excessive Na^+ is ingested, relative to the kidney's innate ability to excrete the Na^+ load.* Signs (+) and (−) are positive and negative feedback loops, respectively. Abbreviations are: ADH, antidiuretic hormone; ANP, atrial natriuretic peptides; $[Na^+]_I$ and $[Ca^{2+}]_i$, intracellular (cytosolic) Na^+ and Ca^{2+} concentrations, respectively. The diagram is self-explanatory, but further descriptive information is provided in the text and in [11]. From [11] with permission of the American Physiological Society.

the presence of some Na^+ pump molecules with α subunits that are highly ouabain-sensitive (that is, the α_3 isoform) [22, 23].

Endogenous ouabain is found in high concentration in the adrenal glands of mammals [12, 14, 15]. It is synthesized and secreted by adrenal cortical cells in culture [24]. Indeed, a new syndrome has been recognized in a patient with elevated plasma endogenous ouabain and hypertension associated with an adrenocortical tumor that contained an exceptionally large amount of ouabain (that is, a "ouabainoma") [25].

The physiological stimulus for secretion is uncertain. Increased dietary salt raises plasma endogenous ouabain levels in humans [26]. There is a question, however, about whether the secretion is mediated by increased plasma volume expansion [27]. In human congestive heart failure, plasma endogenous ouabain levels are inversely correlated with the cardiac index [28].

The hypothesis diagramed in Figure 1 leads to the prediction that ouabain ought to induce hypertension. This prediction has been directly tested, and the results are unequivocal: Chronic parenteral administration of ouabain to normal rats induces a sustained hypertension [29, 30]. Moreover, about 40% of human subjects with essential hypertension have plasma endogenous ouabain levels more than two SDs above the normal mean of ≈0.5 nM, and blood pressure is directly correlated with plasma endogenous ouabain [31, 32].

Taken together, these findings validate several key elements of the hypothesis illustrated in Figure 1. A critical unresolved issue, however, has been: What is the mechanism by which ouabain elevates blood pressure? We will focus on this issue in the ensuing discussion.

How does ouabain elevate blood pressure?

As described above, slight inhibition of the Na^+ pump by ouabain would be expected to increase $[Na^+]_{cyt}$ slightly. This should, via the Na/Ca exchange, concomitantly raise the cytosolic Ca^{2+} concentration, $[Ca^{2+}]_{cyt}$, in VSM cells. Most of the intracellular Ca^{2+} in these cells is sequestered in the sarcoplasmic reticulum (SR), where it is concentrated 1,000-fold or more. Thus, a sustained small increase in $[Ca^{2+}]_{cyt}$ should induce a large increase in SR Ca^{2+} [11]. In other words, the SR can be expected to amplify the (indirect) effect of the ouabain on cell Ca^{2+}. Then, because much of the Ca^{2+} that triggers contraction during VSM activation comes from the SR, these cells with increased SR Ca^{2+} ($[Ca^{2+}]_{SR}$) should contract much more vigorously in response to activation by neuroeffectors such as noradrenaline. This postulated sequence of events (ouabain → ↑ $[Na^+]_{cyt}$ → ↑ $[Ca^{2+}]_{cyt}$ → ↑ ↑ $[Ca^{2+}]_{SR}$ → ↑ ↑ VSM cell responsiveness) can be directly tested.

The presence of a Na/Ca exchanger in the plasma membrane of

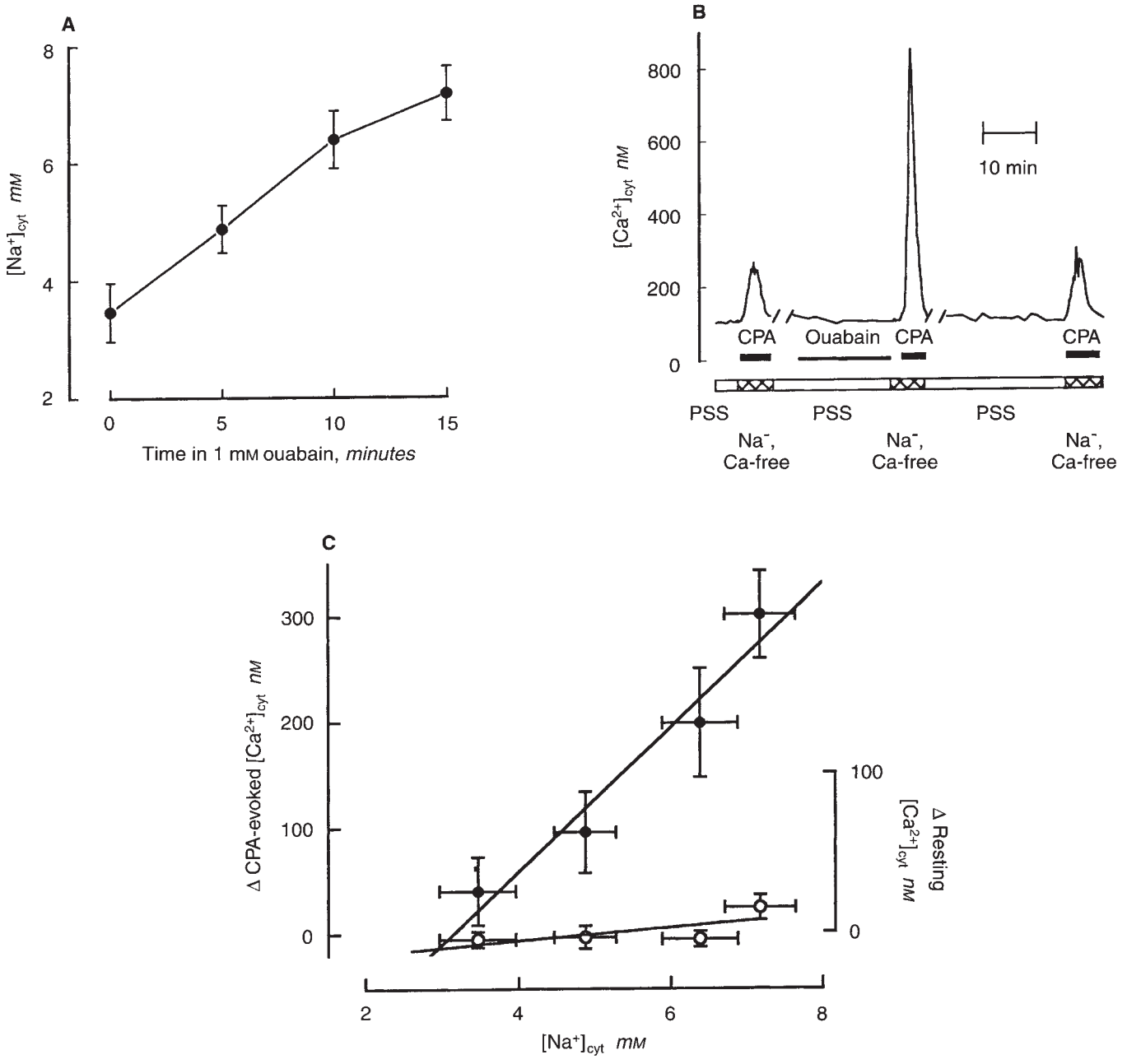

Fig. 2. *Effects of ouabain on $[Na^+]_{cyt}$ and $[Ca^{2+}]_{cyt}$ in rat aortic myocytes.* **A.** SBFI loaded cells were incubated in PSS with 1 mM ouabain to measure the time course of changes in $[Na^+]_{cyt}$. The data correspond to the mean values for 19 cells. **B.** Fura-2 loaded cells were incubated in PSS in the absence or presence of 1 mM ouabain, as indicated. Ca^{2+} release was evoked by 5 μM CPA (in Ca^{2+}-free PSS). Data correspond to mean values for 14 cells from a representative experiment. **C.** Graph of increases in resting $[Ca^{2+}]_{cyt}$ and CPA-evoked Ca^{2+} transients graphed as a function of $[Na^+]_{cyt}$. The abscissa values were obtained from A; ordinate values were obtained from experiments similar to the one shown in B, in which the duration of exposure to ouabain was varied. ΔResting $[Ca^{2+}]_{cyt}$ corresponds to the increase in $[Ca^{2+}]_{cyt}$ after a 0, 5, 10 or 15 minutes exposure to ouabain, relative to the $[Ca^{2+}]_{cyt}$ before the first (control) response to CPA. ΔCPA-evoked $[Ca^{2+}]_{cyt}$ corresponds to the increase in the amplitude of the second Ca^{2+} transient (after 0, 5, 10 or 15 min in ouabain) relative to the first (control) response to CPA. From [37] with permission.

VSM cells, a central feature of this hypothesis, has been the subject of controversy. Recently, this questionable point was resolved by the direct identification of the exchanger gene, mRNA, and protein in VSM cells by immunocytochemical and molecular biological methods [33, 34].

$[Na^+]_{cyt}$ can be measured with the Na^+-sensitive fluorescent indicator, SBFI [35]. As illustrated in Figure 2A, treatment with ouabain causes $[Na^+]_{cyt}$ to rise in SBFI-loaded VSM cells. Ouabain also causes $[Ca^{2+}]_{cyt}$ to rise, as demonstrated in cells loaded with the Ca^{2+} indicator, fura-2; however, the rise in resting $[Ca^{2+}]_{cyt}$ is relatively small, even in presence of high doses of ouabain (Figs. 2 B, C and 3) [36, 37]. The rise in $[Ca^{2+}]_{cyt}$ is

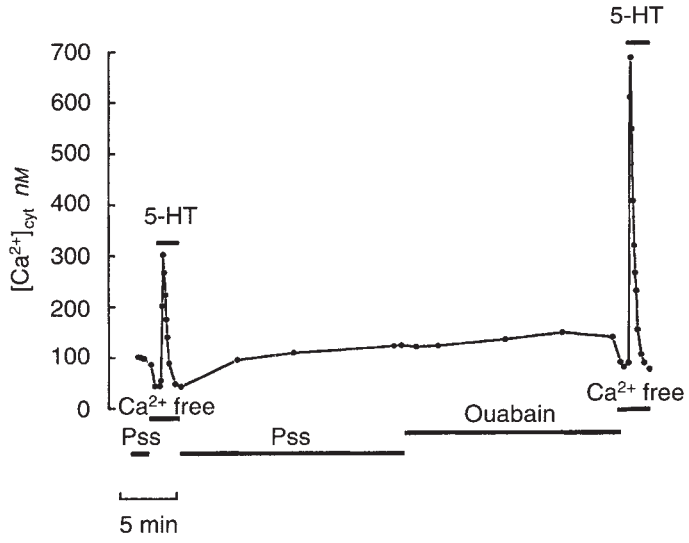

Fig. 3. *Effect of ouabain on serotonin (5-HT)-evoked Ca^{2+} transients in cultured A7r5 cells.* $[Ca^{2+}]_{cyt}$, was measured in fura-2 loaded A7r5 cells (derived from fetal rat aorta). The cells were superfused with standard physiological salt solution (PSS). As indicated by the appropriately-labeled bars below the graph, PSS was replaced by Ca-free PSS containing 0.5 mM EGTA one minute before introduction of 5-HT; note the rapid drop in $[Ca^{2+}]_{cyt}$ at this time. Subsequent addition of 1 μM 5-HT to the Ca-free PSS for 45 seconds (indicated by the short upper bars) induced a rapid, transient rise in $[Ca^{2+}]_{cyt}$. Ouabain (1 mM) was added to the PSS after recovery from the first Ca^{2+} transient. This caused $[Ca^{2+}]_{cyt}$ to rise slightly in the unstimulated cells; however, it greatly increased the amplitude of the subsequent 5-HT evoked Ca^{2+} transient. The data are the means from 12 cells. From [36] with permission.

attenuated by the rapid sequestration of Ca^{2+} in the SR as well as by Ca^{2+} extrusion via the plasma membrane ATP-driven Ca^{2+} pump.

The rise in Ca^{2+} within the intracellular stores can be visualized directly in cells loaded with the Ca^{2+}-sensitive fluorochrome, chlortetracycline (CTC) [36, 38]. CTC is lipophilic, and is concentrated in the intracellular (such as SR) membranes. It has a relatively low affinity for Ca^{2+} compared to fura-2 (K_d for the Ca^{2+}-CTC complex $\approx$ 50 μM vs. 0.25 μM for Ca^{2+}-fura-2), and is thus suitable for measurements of Ca^{2+} within the intracellular stores and not in the cytosol. CTC exhibits increased fluorescence when $[Ca^{2+}]_{SR}$ increases during ouabain treatment (Fig. 4). Evidence that this is SR Ca^{2+} is provided by the fact that the SR Ca^{2+} pump inhibitor, thapsigargin (TG), which depletes SR Ca^{2+} [39], reduces the fluorescence (Fig. 4 A, B).

Complementary evidence that ouabain increases SR Ca^{2+} is shown in Figure 2B. In these fura-2 loaded cells, we see that the rise in $[Ca^{2+}]_{cyt}$ (that is, the cytosolic Ca^{2+} transient), evoked by cyclopiazonic acid (CPA; which also blocks the SR Ca^{2+} pump [39]), is greatly augmented after a 15 minute exposure to 1 mM ouabain. The increase in the CPA-evoked Ca^{2+} transient as well as the increase in resting $[Ca^{2+}]_{cyt}$ are graphed as a function of $[Na^+]_{cyt}$ in Figure 2C. It is apparent that a large increase in the evoked Ca^{2+} transient (reflecting the increase in SR Ca^{2+}) is associated with only a small increase in resting $[Ca^{2+}]_{cyt}$, as anticipated (see above). Ouabain also greatly augments the Ca^{2+} transient evoked by the physiologic vasoconstrictor, serotonin (5-HT) (Fig. 3). These 5-HT evoked Ca^{2+} transients reflect only the relative SR Ca^{2+} content (and not Ca^{2+} entry) because extracellular Ca^{2+} was removed 30 seconds before the 5-HT was added.

The preceding experiments (Figs. 2–4) were all carried out with very high (non-physiological) concentrations of ouabain. To address this problem, some experiments were carried out with much lower doses of ouabain [21]. The experiment of Figure 5 shows that even 1 nM ouabain reversibly augments caffeine-evoked contractions in rat mesenteric artery. Comparable results have been obtained in rat tail artery and human subcutaneous artery (Bova and Blaustein, unpublished observations).

This functional relationship between the plasma membrane Na/Ca exchanger and SR has a structural correlate. The exchanger molecules are distributed in clusters arranged in reticular patterns on the VSM cell surface [33] that are co-localized with underlying (junctional) SR [37]. This raises the possibility that a major role of the Na/Ca exchanger is to modulate the Ca^{2+} content of the SR stores via a restricted portion of the cytosol that lies between the plasma membrane and the junctional SR.

Which cell types contribute to the augmented vascular tone?

It is essential to note that the ouabain-augmented responses illustrated by the data in Figures 2 to 4 are not limited to the VSM cells. Comparable augmentation of other Ca^{2+}-dependent physiological processes can be anticipated in most other types of cells in the body, which also have a plasma membrane Na^+ pump, a plasma membrane Na/Ca exchanger, and endoplasmic reticulum (ER) Ca^{2+} stores. Thus, vasomotor neurons and endothelial cells would also be expected to increase their ER Ca^{2+} stores, and to exhibit a heightened responsiveness, when they are treated with ouabain (Fig. 1). For example, ouabain antagonizes endothelium-dependent relaxation of VSM [40], and also augments synaptic transmission [41]. This leaves open the question of whether the elevated plasma endogenous ouabain in hypertensive subjects acts primarily on the VSM cells or on the vasomotor neurons and/or endothelial cells, to effect the elevation of blood pressure. A likely possibility, as in Figure 1 (lower right quadrant), is that the synergistic responses to ouabain in all of these cell types (and perhaps also in the central neurons that control blood pressure) culminate in the increased arterial tone and hypertension. Given the very steep relationship between blood vessel radius (r) and TPR (that is, resistance $\propto 1/r^4$), relatively slight changes in contractile activation of the myocytes in the small resistance vessels should have a substantial effect on the blood pressure. Thus, the hypothesis outlined in Figure 1 provides a specific mechanism to account for the "functional origin" of the increased TPR in essential hypertension mentioned by Homer Smith [1].

Acknowledgments

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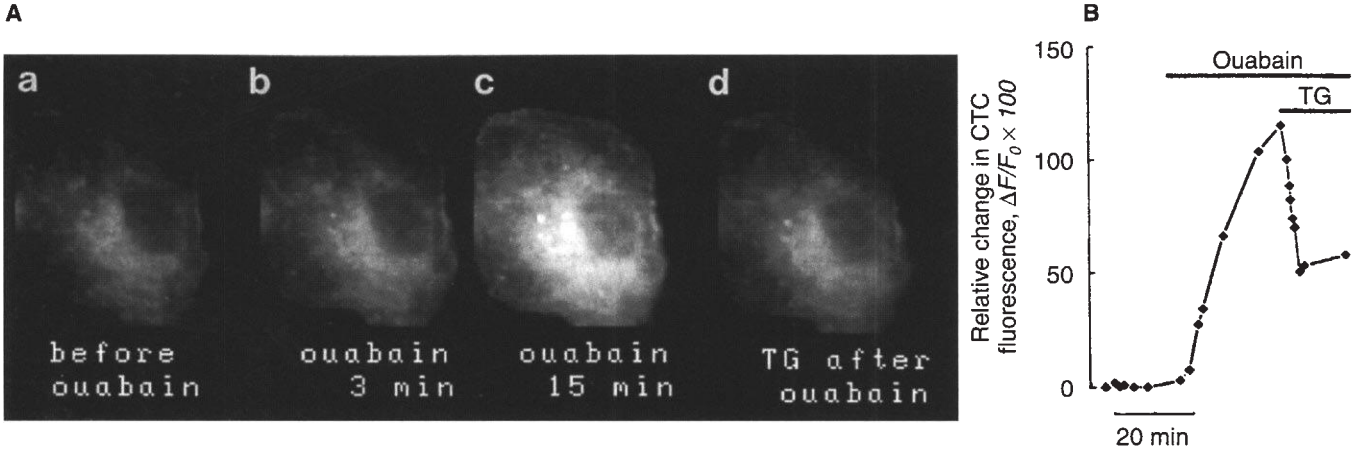

Fig. 4. *Effect of ouabain on Ca^{2+} accumulation in intracellular organelles measured with chlortetracycline (CTC).* Changes in CTC fluorescent emission reflect changes in the amount of Ca^{2+} stored in intracellular organelles. **A.** Representative images of a CTC-loaded A7r5 cell: Before ouabain (a), after three minutes (b), and after 15 minutes exposure to 1 mM ouabain (c); three minutes after addition of thapsigargin (TG) in the presence of ouabain (d). **B.** Time course of relative changes in CTC fluorescence (ΔF) evoked by ouabain and by subsequent addition of TG. Data are means from cytoplasmic areas of 12 cells from the experiment illustrated in A. Comparable results were obtained in two other, similar experiments. The data in B are normalized to initial fluorescence (F_0) as indicated on ordinate scales. From [36] with permission.

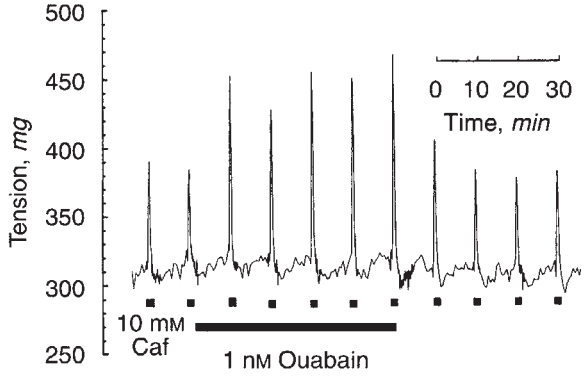

Fig. 5. *Effect of low-dose ouabain on caffeine-evoked contraction amplitude in a ring of rat small mesenteric artery.* Isometric tension was measured in a third order branch of the mesenteric artery. Initially (A), the tissue was bathed with standard PSS containing 3×10^{-8} M phenylephrine (PE) to increase the resting tone slightly; this PE concentration negligibly affected the resting tension. The standard PSS (with PE) was replaced by a similar solution containing caffeine (Caf, 10 mM) for 45 seconds periods as indicated by the short black bars. During the 50 minutes period indicated by the long black bar, the standard physiological salt solution was replaced by a similar PSS containing 10^{-9} M ouabain as well as the PE; at the end of this period, the original, ouabain-free PSS containing PE was restored. Note that the amplitude of the Caf-evoked contractions increased substantially in the presence of this low dose of ouabain, and gradually fell when the ouabain was washed out. From [11] with permission (and see [21]).

Note added in proof

Recently Zhao et al reported that the endogenous ouabain-like compound purified by Hamlyn et al [12] is an isomer of plant ouabain.

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