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Reviews

Ouabain – a link in the genesis of high blood pressure?

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Abstract. Hypertension or high blood pressure is a risk factor that increases risk of myocardial infarction, renal failure or cerebral stroke. The pathogenesis of hypertension is due to a variety of causes, including inherited predisposition, dietary habits, especially salt intake, smoking, and also 'general lifestyle'. But for the scientist interested in the complex interplay of physiological and molecular factors, the actual causes of high blood pressure remain uninvestigated. The following article is concerned with new reports that ouabain, a plant derivative, occurs in human beings, in whom it appears to have a hormonal function; ouabain may even play a key role in the pathogenesis of hypertension. We are thus brought a step closer to the background of cardiovascular disease; we may also be afforded a lead to a new therapeutic principle.
Key words. Excretion; muscle cell; natriuretic hormone; glomerulus; tubule; Na^+,K^+-ATPase; ouabain; digitalis; blood pressure.

Cardiac glycosides are produced by plants

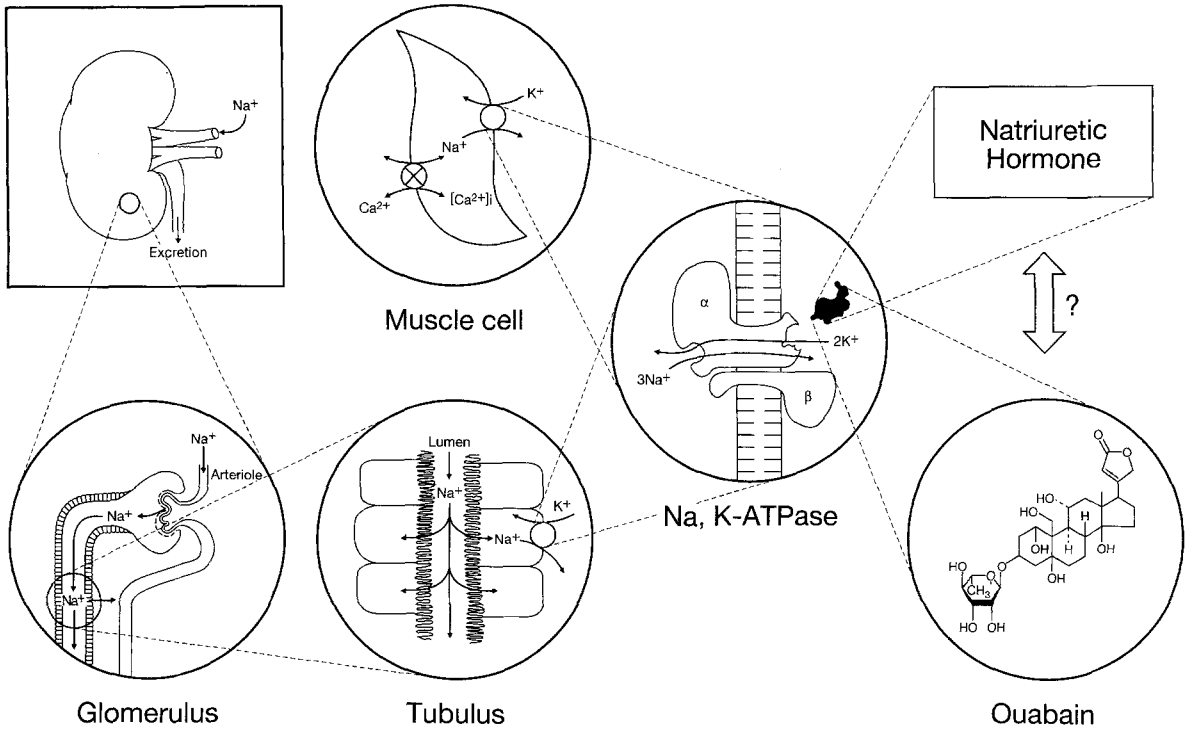
A large number of rare plants produce cardiac glycosides. The most familiar example is foxglove ('digitalis' in Latin). Preparations of digitalis have been used in medicine for more than two centuries to treat congestive heart failure, for one of the compound's predominant actions is to stimulate the force of myocardial contraction. However, the action of digitalis preparations is not confined to the heart alone but also affects other organs, a point to which we shall revert later.

The action of an extract of the foxglove plant was first described in 1785 by William Withering in his book 'An Account of the Foxglove'. The property he noted first was the ability of digitalis to increase the flow of urine (diuretic action). In the same work he also called attention to the hazards of an overdose of digitalis.

The therapeutically most important compounds contained in the digitalis plant, digoxin in digitoxin, are chemically very similar. They consist of a sterol skeleton attached to three sugar residues. Steroid hormones, which occur in animals and man, also have a sterol skeleton as their basic structure. Ouabain is a vegetable derivative with a marked cardiac action and is obtained from the waba yo tree (*Strophanthus gratus*) which grows exclusively in East Africa. Ouabain contains only one sugar residue, but it is one that isn't commonly found in the animal world, namely rhamnose (see diagram).

The action of ouabain in human beings has been known for very much longer than its chemical structure. Both the structural analysis and chemical synthesis of ouabain were described in the 40s and 50s of this century by the team working with Professor T. Reichstein at the University of Basel.

The present article is concerned with this 'African' ouabain. Ouabain was recently detected in human blood and indeed in concentrations capable of eliciting a reaction. Therefore, it is legitimate to ask whether ouabain is a hormone.



The pathway from organ to hormone or compound
On the left is a diagram of the kidney and two sections taken from it. In the first window there is an extremely fine blood vessel which, in the glomerulus, excretes part of its content by ultrafiltration into the beginning of the proximal tubule. Its solutes include sodium, which may be reabsorbed back into the blood from the tubule. In the detail drawing this Na^+ is shown to be transported into the epithelial cells lining the tubule. In the side facing towards the blood the Na^+,K^+-ATPase is located in the cell membrane. It pumps Na^+ out of the cell in exchange for K^+. If the Na^+,K^+-ATPase is inhibited by the natriuretic hormone circulating in the blood, less Na^+ is transported from the primary urine into the cell interior and from there back into the blood. This is described as a natriuretic action.
Na^+,K^+-ATPase, a protein located within the cell membrane (hatched double line), consists of two subunits α and β. The 'sodium pump' transports per 'revolution' 3 sodium ions (Na^+) from the cell interior (left) to the outside (right) in exchange for 2 potassium ions (K^+). On the outside is located the binding site for digitalis-like compounds and hence also for ouabain. Binding normally leads to inhibition of the pump function. One cell has about 100000 individual sodium pumps in its membrane.
Na^+,K^+-ATPase is also present in the muscle cells of the heart or the blood vessels (top window). In these cells inhibition of the sodium pump causes an increase in the calcium concentration ($[Ca^{2+}]_i$), since elevated cytosolic Na^+ now seeks to leave via the exchanger (cross) in exchange for Ca^{2+} entering the cell. This causes muscle contraction with vascular constriction and hence an elevation of the blood pressure.
On the right is the chemical structure of ouabain showing the typical four interconnected rings of the sterol skeleton and the sugar residue, rhamnose. According to the latest findings ouabain is identical with natriuretic hormone.
The scale in the windows is approximately 1 : 4 for the (human) kidney, 1 : 200 for the section below and 1 : 1000 for the section at the side. The Na^+,K^+-ATPase is enlarged by a factor of 1 : 2000000 and the chemical structure of ouabain by 1 : 30000000.

Mechanism of action of cardiac glycosides

Cardiac glycosides induce their action by inhibiting the sodium pump or Na^+,K^+-ATPase, an enzyme which is embedded in the cell membrane and extrudes sodium ions out of the cell interior in exchange for potassium, which the pump transports into the cell[1,4,9]. Na^+,K^+-ATPase is a protein consisting of two subunits: α and β (see diagram). Such sodium pumps are present in the membranes of almost all cells. A cell could not possibly live without them, since they are responsible for maintaining the sodium and potassium concentration gradient, which is vital to many cell functions.

Action on the kidney

Na^+,K^+-ATPase is therefore ubiquitous and thus occurs, for example, in the renal epithelial cells lining the tubule which serves as a conduit for primary urine (see diagram). Here part of the sodium is reabsorbed from the urine. It passes through the membrane facing towards the tubule and enters the cell interior, where it must be translocated to the other side of the cell via the sodium pump. In these cells the Na^+,K^+-ATPase is located exclusively on the side (called the basolateral side) facing towards the blood. Inhibition of Na^+,K^+-ATPase by cardiac glycoside therefore causes less sodium to return to the blood. The kidney then excretes more sodium, this process being known as the natriuretic action. Natriuresis and diuresis – water transport – are closely associated. This mechanism is also the one underlying the diuretic action of cardiac glycosides observed by Withering.

The natriuretic hormone

De Wardener in London postulated over 20 years ago that there must be a 'natriuretic hormone', i.e. a circulating messenger substance, which determines how much of

the dietary salt is excreted [5]. De Wardener did not know the precise point where this compound acted or, more importantly, what its chemical structure might be. A few years later the same author asked: 'Is a circulating sodium transport inhibitor involved in the pathogenesis of essential hypertension?' By the circulating sodium transport inhibitor De Wardener meant a hormone suspected of acting on Na^+,K^+-ATPase. The question which he posed – and answered in the affirmative – was whether this hormone plays a role in the pathogenesis of essential hypertension.

Hypertension is caused by an impairment of biological feedback mechanisms. Essential hypertension, as it is called, is a very common condition and is due to a number of factors which are poorly understood. An overworked kidney or a regulatory defect in its function appears to be involved: such patients are especially sensitive in their reactions to a high salt intake. They have a disturbed sodium metabolism; their blood pressure depends on the daily salt intake.

Inhibition of Na^+,K^+-ATPase produces increased contraction...

What can the link possibly be between contraction and Na^+,K^+-ATPase? The link connecting the two is the very ubiquity of Na^+,K^+-ATPase. If the kidney has to excrete more sodium, the body produces more natriuretic hormone. This acts not only on the Na^+,K^+-ATPase of the kidney but also on the same enzyme located in other cells in other organs.

...of the vascular muscle

Among the cells affected are the vascular myocytes. In muscle a rise in intracellular sodium concentration also raises the calcium level (see diagram). This increases contraction and augments vasoconstriction, thereby raising blood pressure.

It was established long ago that some patients with essential hypertension have elevated levels of intracellular sodium as well as calcium in circulating cells, i.e. in platelets or erythrocytes.These readily accessible cells can therefore be used in clinical tests to determine the degree to which hypertension is dependent on sodium.

...and of the cardiac musculature

The effect of the hormone is not confined to the vascular smooth muscle alone; it also acts on the heart. The increased force of contraction stimulated by sodium is called the positive inotropic effect. It is primarily for this reason that many physicians prescribe digitalis preparations for congestive heart failure: they boost the cardiac 'motor'.

The circle is thus closed. The control system may be said to start in the kidney, which is confronted with an increased quantity of salt and responds with increased production of natriuretic hormone. And there is in fact an increased output of sodium (and water) from the body. Almost everywhere the hormone has 'side effects'. Sodium concentration is raised in virtually all cells. In many of these there may be no perceptible effect, but in the myocardium the hormone produces intensified contraction. At each beat the heart contracts more forcefully and thus ejects more blood. On the same principle there is also a direct increase in blood pressure due to augmented vascular constriction.

What is the chemical structure of the natriuretic hormone?

For at least twenty years several teams have been at work elucidating the chemistry of the natriuretic hormone. There now seems to have been a breakthrough. In five publications, all appearing in mid-1991, one team at the University of Maryland in Baltimore and another with the pharmaceutical firm of Upjohn (both in the USA) announced their results, which are reproduced here with comments underlining their importance [3, 7, 8, 11, 16].

Using a 10 billion-fold purification process, the groups were able to isolate from some 100 litres of blood obtained from hypertensives the infinitesimal amount of about 10 micrograms of a compound whose activity was followed throughout the various phases of purification with the aid of Na^+,K^+-ATPase inhibition. Purification was performed by several methods, especially high-pressure liquid chromatography and affinity chromatography [7, 11]. Analytical studies of the purified material showed that natriuretic hormone was not distinguishable from ouabain. The comparative mass spectra, which might be described as chemical fingerprints, are astonishing: they are virtually identical [16].

Biological tests, performed for example on cardiac tissue, showed that the effects produced are also largely identical. The increased contractive force of isolated heart muscle obtained with this factor was just as marked as with ouabain [3, 7, 8]. The slow 'wash-out' of the effect typical of ouabain was also clearly in evidence. I adduce no further proof of the structural identity with ouabain but rather summarize and report some new findings:

1. Ouabain or a very similar glycoside can be detected in human blood. The concentration ranges from below 1 nanomolar in most normal individuals to as high as 10 nanomolar or even higher in some patients [2, 6, 7, 14]. This is sufficient partly to inhibit sensitive forms of Na^+,K^+-ATPase.
2. Various considerations suggest that the adrenal gland is probably the site of ouabain production. Hamlyn and collaborators have recently cannulated arterial and venous blood of dog and human adrenal glands. Using their specific antiserum [8], they have found that the ouabain concentrations were 2–5 times higher in the venous effluent of the dog and higher in man [13]. The arterio-venous gradient persisted over several hours.

Using adrenal cortical cells in culture, they showed that ouabain was secreted by these cells over a period of several days and that depolarization by increasing the extracellular potassium concentration led to a reduced rate of ouabain production by these cells. The identity with ouabain was established by a specific radioimmunoassay and HPLC.

3. Ouabain blood concentrations are increased several-fold in certain forms of hypertension or heart failure[2,6]. Whether this increase is largely instrumental in precipitating the symptoms of disease or whether, on the contrary, it serves to remedy a deteriorating condition is still obscure.
4. In a patient with elevated blood pressure (170/102 mm Hg) that was under multiple – but unsuccessful – antihypertensive therapy, circulating ouabain (as assayed by RIA and HPLC) was 744 ± 104 nanomolar[15]. The patient was found to have an adrenal tumour. After removal of this tumour, the blood pressure decreased (135/80 mm Hg) and circulating ouabain was 255 ± 130 nanomolar. This suggests that the adrenal is secreting ouabain also in man and the parallel fall of blood pressure and of circulating ouabain indicated that elevated ouabain might cause hypertension in man.
5. Chronic administration of low doses of ouabain (3–10 μg/kg/day) to normal rats induces chronic hypertension in these animals[12]. The increased blood pressure could be reversed by giving canrenone, which has been postulated to be a ouabain antagonist.

To summarize, it would seem that endogenous ouabain has a hormonal function in the control of overall cardiovascular status. Is it not astonishing that a plant derivative which has previously been found only in an African tree should occur in human blood, where it apparently has a hormonal function?

Some critics, however, have objected that ouabain could enter the organism via the food chain (plant → cow → milk → human being) but there are potent arguments against this assumption, more especially that plants in our latitudes do not produce such a glycoside and the fact that the absorption of ouabain by the gastro-intestinal tract is poor. Moreover, plasma ouabain levels were not different in individuals maintained on semisynthetic diet for at least seven days[7].

Prospects

This is the state of our knowledge at present. I should now like to cast a glance at the future and look into the pharmacological possibilities. 'Shall we ever be able to achieve organ-specific effects on the basis of these new insights?' What is envisaged here is substances that are closely similar to ouabain in their effect but act only on the heart, or compounds which abolish in part the production or action of circulating ouabain, i.e. inhibitors. Compounds of this kind may be useful for a 'causal' therapy of high blood pressure.

No conclusive answer can be given as yet. Previous functional investigations together with the results of molecular biology show that Na^+,K^+-ATPase occurs in several slightly different forms[4,9,10,17]. In the rat the various types of Na^+,K^+-ATPase show different sensitivities to ouabain inhibition. It should, in principle, be possible to achieve specific alterations in the structure of ouabain with the aid of molecular modelling for the purpose of obtaining therapeutic agents with selective actions.

This important breakthrough would not have been made without significant contributions from a variety of disciplines such as physiology, cell biology, chemistry and molecular genetics but it was the successful integration of key information in each area that led to the discovery of ouabain as a link in the pathogenesis of essential hypertension. More of such thinking will be required in the future in this and many other areas of research.

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Research Articles

Speed and consistency of human decisions to swallow or spit sweet and sour solutions [1]

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Abstract. Measurements of the frequency and speed of spitting or swallowing citric acid, sodium saccharin, or mixture solutions, using the taste of one of them as the definition of what was to be spit, revealed that 'correct' spits occurred on $\geq 70\%$ of trials with equal reliability and latency among the liquids, indicating that recognition-based rejection decisions in adult humans are as rapid and consistent for an arbitrary sweet taste as for a sour or mixed taste.
Key words. Taste; decisions; reaction time; sour; sweet; mixture spit; swallow.

The gustatory characteristic of regional or national human diets differ widely, but the children of individuals from one region, when brought up in another locale, will usually adopt the cuisine of their new habitat if they are regularly exposed to it, and may find the diet of their parents' homeland unacceptable [2–5]. These observations indicate that much human taste-dependent preference and rejection is experientially based. It is generally assumed that the primary means through which this experience acts is learning [6–9] (although learning-independent effects of prenatal experience on gustatory structure and function have also been demonstrated in a mammal [10]). Despite these experience-based differences in what are considered acceptable foods and beverages, a fundamental consistency in human taste preferences is often stated. The claim is that sweet things will be selected or accepted, while others, especially sour or bitter, will be rejected [11, 12]. It is argued that sour things, for example, will be much more likely to be immediately damaging than sweet things [13, 14]. When adults nonetheless accept or select sour items and reject sweet ones, this is taken to be a learned reversal of the 'natural' human pattern [15].

If 'natural', unlearned human behavior is to reject sour tasting substances and select sweet items, rejection of a sour tasting substance should be faster or more reliable than rejection of a sweet item, even in adults who have learned to accept particular regional cuisines. It is known that simple taste reaction time, i.e., detection that any taste is present, is generally more rapid for acids than for sweeteners [16, 17]. Faster or more reliable rejection would be predicted because taste-dependent behavior that rapidly and consistently removes from the oral cavity substances that are usually dangerous should be maximized through natural selection. We tested this hypothesis by providing 15 paid, screened [18], volunteer subjects (age 21 ± 6 years {mean ± SD}, 7 female) with a target taste sipped from a 100-ml drinking glass containing 80 ml of liquid (10 mM analytical reagent grade citric acid [citric] or 2 mM United States Pharmacopoeia sodium saccharin [NaSac] or a mixture containing both [mixture], prepared in distilled water (H_2O) (refractive index = 1.3330; conductivity < 1.5 μS), and instructing subjects to then take a sip from 100-ml drinking glasses containing 80 ml of test liquids. The sipped test liquids were to be spit out if they corresponded to the target taste, but were otherwise to be swallowed. Subjects used each of the three target tastes once in individual sessions separated by 10 min. Sessions began with a vigorous whole mouth rinse with H_2O, a practice series of sips and spits or swallows, and then another whole mouth rinse with H_2O. 10 min after the completion of the practice sips, the 8 data sips of each session were begun. No information whatsoever was given on the taste of the target or test tastes, or the accuracy or speed of spits or swallows. Two of the 8 test drinking glasses contained the sour target liquid citric, the equally intense [19, 20] sweet target liquid NaSac [21], the sweet/sour [19] mixture, or H_2O, in random order. Start of contact between