Letters to the Editor

Radioimmunoassays, Ouabain-Like Material, and Ouabain

To the Editor:

We read with interest the article by Bauer et al on the changes in the plasma concentration of a ouabain-like compound associated with vigorous exercise in both humans and dogs. The authors comment on the result that is most striking to us, namely the remarkably high concentrations of the immunoassayable substance achieved without apparent ill effect. Bauer et al suggest that this may be a result of either the transient nature of the high concentrations measured, the “slow on-rate in forming the ouabain-Na/K ATPase complex,” or by the ouabain-like substance being bound by proteins.

The most remarkable values of ouabain-like compound achieved were those in beagles with a mean “ouabain” concentration of 6882 ± 1436 nmol/L. The raw data are not given but it is reasonable to assume that the concentration in at least one animal reached approximately 8000 nmol/L (4.68 mg/L). At such a ouabain concentration, close to 100% inhibition of any accessible sodium pumps would occur almost instantaneously. Whereas the numbers in the non-trained humans are less dramatic (176 ± 68 nmol/L), it seems likely that the highest concentration seen in this population was in the region of 300 nmol/L (175 µg/L). This would inhibit approximately 50% of the sodium pumps in proximity to the plasma. If the immunoassayable “ouabain” was uniformly distributed throughout the plasma this would correspond to a ouabain “dose” of approximately 0.8 mg in humans and 4.5 mg in beagles. The rapid administration of such an intravenous dose of ouabain would not be expected to be uneventful for the human recipient and even less so in the case of the beagle because the LD50 is around 0.1 mg/kg and the animals weighed < 20 kg.

If we were to assume a distribution volume equal to the extracellular fluid and make no allowance for binding by sodium pumps, we can calculate the total amount of ouabain secreted by the beagle in 13 minutes from the formula: ECF volume/plasma volume × total dose in plasma; this is close to 13 mg. Given that significant amounts of endogenously secreted ouabain would have bound to the sodium pumps this is likely to be an underestimate.

Whatever the explanation for these results, they do provide an excellent opportunity to resolve a division that exists in relation to endogenous ouabain. Since the mass-spectrometric identification of ouabain (or closely related substance) in an extract of human plasma, there have been those who have questioned its endogenous origins, whereas others, relying principally on radioimmunoassay, have equated the immunoassayable substance with authentic ouabain and use the terms “ouabain-like” and “ouabain” interchangeably. The quantities of the immunoassayable substance seen in these humans and, to a far greater extent in dogs, fall comfortably within the range required for physical characterization by mass spectrometry and, in view of the milligram amounts reported in beagles, proton NMR. If the substance responsible is indeed authentic ouabain the skeptics probably have to revise their position. If not, then the significance of plasma immunoassayable “ouabain” would be called into question.

Response

In their comment to the article of Bauer et al on the changes of the plasma concentrations of a ouabain-like compound associated with vigorous exercise in humans and dogs, Hilton and McKinnon are wondering why dogs are apparently healthy although their plasma concentration under exercise exceeds that reported for the LD50 of ouabain. We do agree that ouabain circulating in blood plasma in such high concentrations of about 6 µmol/L should harm dogs and humans. We tested our antibodies for cross-reactivities, which are part of the article, and found them rather specific. We furthermore found a linear correlation between ouabain and the signal (Figure 3 in the Reference 1). Nevertheless, additional unknown compounds may circulate in blood competing with ouabain for ouabain antibodies and the cardiac glycoside receptor site of Na+/K+-ATPase. This is the reason why the compound was called ouabain-like.

The reader should be aware that in addition to the mechanism usually communicated in text books of pharmacology (ie, inhibition of the sodium pump), cardiac glycosides may also use Na+/K+-ATPase as a signal transducer of cardiac glycosides by a mechanism not inhibiting the pump to exert its inotropic effect (for a short review see Reference3). There is a continuing debate on the existence of ouabain and ouabain isomers in mammals; Ouabain has been identified by mass spectroscopy and proton NMR in bovine adrenals and hypothalamus; other studies analyzing the nature of the compound without application of proton NMR but mass spectroscopy and other techniques came to the conclusion that the isolated compound is either ouabain (human plasma, PC-12-cell media) or a closely related isomer (human plasma, bovine hypothalamus). No general agreement on the nature of the circulating ouabain-like compound has been reached so far. Interestingly, substances like PST 2238 have been synthesized that bind to the ouabain receptor of Na+/K+-ATPase without inhibiting the pump. PST 2238 apparently interferes with the natural circulating ouabain-like compounds at the sodium pump and lowers arterial hypertension in experimental animals. We may not exclude that additional compounds exist that interact with the cardiac glycoside receptor without inhibiting the pump but acting as a signal transducer. They may interact with antibodies against ouabain. Certainly, blood plasma from an exercise-stressed mammal would be a good source to isolate this ouabain analog, whose nature should be identified by mass spectroscopy and proton NMR.
WILHELM SCHONER  
Institute of Biochemistry and Endocrinology  
Justus-Liebig University  
Giessen, Germany


Radioimmunoassays, Ouabain-Like Material, and Ouabain

P.J. Hilton and W. McKinnon

Hypertension. 2005;46:e9-e10; originally published online August 15, 2005;
doi: 10.1161/01.HYP.0000180069.86224.57

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 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/46/3/e9

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/