Radioimmunoassays, Ouabain-Like Material, and Ouabain

To the Editor:

We read with interest the article by Bauer et al1 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 al1 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/kg2 and the 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.

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 beagle digitalislike factor from human plasma,2 there have been those who have questioned its existence of ouabain and ouabain analogs.4,5 We may not exclude that additional compounds of endogenous digitalislike factor from human plasma.5

Response

In their comment to the article of Bauer et al1 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 Reference5). There is a continuing debate on the existence of ouabain and ouabain isomers in mammals;3 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.4 PST 2238 apparently interferes with the natural circulating ouabain-like compounds at the sodium pump and lowers arterial hypertension in experimental animals.4,5 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.


P.J. Hilton
Renal Laboratory
St Thomas’ Hospital
London, UK

References


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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
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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