Arginine Vasopressin in Extracts of Bovine Pituitary

Gitelman *et al.* (*1*) suggest the natural occurrence in the bovine posterior pituitary of two different *N*-terminal "hormonogen" forms of arginine vasopressin. Since both hormonogens were found to be natriuretic, there is an implication that they may be related to "natriuretic hormone." This stimulates not so much comment as questions, since were this concept shown to be valid much of what we thought we knew about these hormones would have to be modified.

1) The original isolation of vasopressin and oxytocin from the pituitary by du Vigneaud failed to find such hormonogen forms, but of course the original work was searching for maximum pressor and antidiuretic activities, not natriuretic. This same statement applies to many other laboratories about the world carrying out the same work. In 1974, we tried to isolate peptides using natriuresis as a criterion, also using freeze-dried posterior pituitaries as a starting material. The most active fractions contained two chromatographically clean spots on thin-layer chromatography; these spots were identified clearly as the nonapeptide vasopressin molecule and adrenocorticotropic hormone (1–13) with a nonacetylated *N*-terminus (*2*), again providing no evidence for the presence of vasopressin hormonogens. Could Gitelman *et al.* present evidence that what they were sequencing was in fact two pure substances?

2) Gitelman *et al.* comment that most oligopeptide hormones appear to be biosynthesized as larger molecules, but this is supposed to occur in the hypothalamus, not in the posterior pituitary. In fact, had the authors carried out their isolation from the hypothalamus, the results would be more convincing. As far as we know, once vasopressin is produced in the hypothalamus, it is only transported for storage to the posterior pituitary, not as a nonapeptide alone but in noncovalent binding to "carrier proteins"—neurophysins. It is well known, however, that vasopressin-neurophysin binding requires that the N^{α}-NH_2 group be immediately contiguous to 2-Tyr in order for binding to occur (*3*) and it has been shown experimentally that insertion of one or more residues between Tyr and the N^{α}-NH_2 group prevents binding, that is, the hormonogens of Gitelman *et al.* would not bind. Are they suggesting that vasopressin is biosynthesized in the posterior pituitary? Otherwise the hormonogens could not have reached that organ.

3) Genetic coding being what it is, there should be in a given species as rigid a sequence of a prohormone as there is for the "final" nonapeptide product. Why, then, are there two forms of the prohormone?

4) Such *N*-terminal "hormonogen" extensions of vasopressin (more than 15 in all) were synthesized as long ago as 1966 (*4*) with one to three added residues attached to 1-Cys. Provided the added residues are cleavable by aminopeptidases (that is, are alkyl, aromatic, or Gly, but not basic, acidic, or D-isomers) they are all natriuretic [for example, see (*5*)] just as the parent hormone is. How this relates to a "natriuretic hormone," however, is still not clear.

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References and Notes

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We disagree with several of Cort's comments. First, our speculations concerning the failure of other investigators to find more complex forms of arginine vasopressin in extracts of bovine pituitary glands include (i) the use of desiccated posterior pituitary glands as starting material in most of the early work rather than lyophilized, fresh-frozen glands that we employed, (ii) the use of more efficient chromatographic procedures than were available previously, and (iii) the elimination of exposure of the extracted material to bacterial peptidases throughout all phases of the purification procedure. We have not evaluated which of these differences may be critical to our results. The conclusion that we were dealing with "pure substances" was reached from amino acid analysis of the isolated materials. The amino acid composition matched precisely the amino acid sequence that was later obtained. There were no amino acids found by compositional analysis not found in the sequence and the molar ratios of the amino acids found by compositional analysis were identical (within 5 to 8 percent) to the molar ratios deduced from the sequence. In addition, we now find that synthetic Ala-Gly-[Arg^8]-vasopressin mimics the chromatographic behavior and physiological activity of the natural product with the same sequence.

Second, our observations do not contradict in any way evidence supporting a hypothalamic site or sites for vasopressin synthesis although our observations will necessarily alter our view of the chemical forms in which arginine vasopressin is transported. Third, the idea that prohormone sequences are invariant seems unwarranted, since there are clear precedents for variability in an individual or members of a species that could explain our observations. The question of "why" is a good question we cannot answer. We have simply reported an observation that we hope will help in finding an answer. Fourth, we agree with Cort that the physiological role of these peptides as natriuretic agents remains to be established.

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