

Proteomics: Inspiring New Hypotheses in the Vasopressin System

The vasopressin neurons of the rat supraoptic nucleus (SON) are among the most intensively studied neurons in the brain, and, although it does not necessarily follow, they are probably also among the best understood. The advantages of this system for the experimental neuroscientist are many and manifest; about 70% of the neurons in the SON make vasopressin, and the others make the closely related peptide oxytocin. All of these project to the neurointermediate lobe (NIL) of the pituitary gland, where they release their products from their nerve endings into the circulation in amounts that are readily measurable by RIA. As a consequence, it is possible to study the cellular and molecular properties of vasopressin cells cleanly and efficiently and relate these readily to physiological function. But it is in the seductive nature of science that the more we know, the more we need to know. My car is something that I understood; one pedal makes it go and another makes it stop; the wheel makes it turn and the radio tells me the cricket scores. When I open the hood it gets more complicated; I just don't want to know what is happening there, but, like Pandora, I sometimes can't resist looking anyway.

Until quite recently, our understanding of vasopressin cells was driven by hypothesis-led studies. From these, we gained a very clear account of how electrical activity is generated in these cells, how they transduce osmotic stimuli into patterns of discharge that efficiently control secretion, and of the afferent pathways that are important for this (1). But as our understanding grew, the things we did not know glowered at us ever more oppressively. For example, when a vasopressin cell is driven to secrete, it must replace that which is secreted, but what is the signal that increases synthesis? How, exactly, does osmotic stimulation result in an increase in vasopressin mRNA expression?

We had hoped that the new approach of genomic analysis might lead us to the answer to this and many similar questions, and perhaps it will, though perhaps not as swiftly and directly as we would wish. Gene microarray studies have seemed to tell us that, however simple we might have thought that the SON is, almost every gene in the genome is expressed there. Many of these genes are differentially expressed compared with other regions of the hypothalamus, and a frighteningly large percentage of them change in expression during chronic osmotic stimulation. Instead of looking for a few genes whose responsiveness to osmotic stimulation marks them as special, we were forced to look for

those genes that change most and hope that these were the most important ones.

This approach gave us new candidates to inspire a new generation of hypotheses and, indeed, suggested answers to many questions that we hadn't even thought to ask. But this approach, although unbiased in some sense, embodies many assumptions that we know to be unsound. First, the correlation between mRNA expression and protein expression is complex and gene specific, and it is the proteins, not the mRNA, that are the "business end" of things, as Gouraud *et al.* (2) put it in their paper in the current issue. Second, the relative change in abundance of anything is a weak indicator of its importance; after all, even a small percentage increase in salary may mean a large increase in disposable income and, possibly, a considerable change in quality of life. Particular proteins that are abundant in a vasopressin cell are probably important to it, and even a small proportionate change in their abundance in response to osmotic stimulation might be a lead worth pursuing.

Gouraud *et al.* (2) begin with a proteomic approach, using two-dimensional fluorescence difference gel electrophoresis to identify proteins in the SON and NIL changes after 3 d of dehydration. This allowed them to look at about 2000 of the most abundant proteins, and it excluded proteins of relatively low or high molecular weight because of technical limitations. In the SON, they found 25 protein "spots" that were appreciably different in intensity and 45 spots for the NIL. They identified nine of the proteins in the SON and six in the NIL, selected five of these for further study, and confirmed the changes in abundance by Western blotting. These five proteins are all interesting, and each reminds us of important things that we have still to learn about the vasopressin system.

NAP22, in the brain, is generally localized to structures associated with synapses, including the synaptic vesicles, and is reported to be associated with synaptogenesis. Dehydration is associated with considerable changes in the innervation of supraoptic neurons (3), but the functional implication of these changes is not clear. It is possible that they confer a change in neuronal sensitivity to particular afferent inputs, or they might be reactive changes, to maintain the efficacy of afferent pathways in the face of changing passive electrophysiological changes that are a direct consequence of the hypertrophy of vasopressin cells. It is also not clear whether these changes in innervation are the result of altered activity in the afferent neurons or of a retrograde signal from the magnocellular neurons. The abundance of NAP22 falls in the SON after dehydration: what might this mean? Does it reflect an up-regulation masked by an increase in turnover? Possibly, as Gouraud *et al.* also report that NAP22 mRNA expression is increased. However, the protein in the SON may be more in afferent nerve endings than in the

Abbreviations: NIL, Neurointermediate lobe; SON, supraoptic nucleus.

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magnocellular neurons, so we must be careful about this conclusion.

Heat shock protein 1 α (also known as Hsp90, Hsp86, and Hspca) is expressed in both vasopressin and oxytocin cells, and its abundance falls markedly in the SON after dehydration. This is one of a family of proteins commonly involved in promoting the assembly and folding of other proteins, so a fall in expression in conditions where vasopressin cells are hypertrophied is particularly surprising. Again, does this reflect increased turnover? Is it too localized in neurosecretory granules or part of the processes leading to aggregation of vasopressin for packaging into granules?

GRP58 (58-kDa glucose regulated protein), on the other hand, increases in abundance in the SON with dehydration. This protein is mainly localized in the endoplasmic reticulum of the magnocellular neurons, but has also been found in the cytoplasm and nucleus and can bind to DNA, suggesting that it might be a pathway whereby events in the endoplasmic reticulum affect gene expression. The endoplasmic reticulum contains intracellular calcium stores, and these regulate vasopressin release from the dendrites of SON neurons, release that is regulated semi-independently of secretion into the circulation (4). We still do not know how osmotic stimulation triggers changes in gene expression, but there is a close link between vasopressin secretion and vasopressin synthesis, suggesting a common link with either electrical activity or afferent signaling. However, we do not know whether the production of vasopressin granules that are destined for release from the dendrites is regulated independently of those destined for secretion from the pituitary.

ProSAAS (proprotein convertase subtilisin/kexin type 1 inhibitor) is widely involved in peptide processing in neuroendocrine tissues; its abundance increases in the SON during dehydration but falls in the NIL, consistent with a possible localization in neurosecretory vesicles. If so, and if it is involved in processing of provasopressin, the changes might simply parallel changes in vasopressin abundance. But if ProSAAS is secreted, might it also have an impact on peptide signaling? Vasopressin cells are regulated by other peptides both in the SON and in the NIL. Do vasopressin cells retrogradely regulate the influence of afferent signaling peptides by a secreted product that can cleave them? (See Ref. 5.)

Finally, in the NIL, there is an increased abundance of calretinin, confirming previous observations by Miyata *et al.* (6). Calretinin binds calcium with high affinity and so might have some impact on stimulus-secretion coupling. One of the major contributions that the vasopressin system brought to neuroscience was the recognition of the importance of stimulus patterning. In the dehydrated rat, vasopressin cells fire action potentials in a distinctive and characteristic phasic pattern that optimizes the efficiency of secretion (7). This

pattern is thought to be so efficient because the clustering of action potentials leads to a facilitation of calcium entry through voltage-gated channels. However, there is a converse that is less well explained: stimulus-secretion coupling “fatigues” when stimulation is maintained, and the nerve terminals need a period of quiescence to recover. Exactly what explains fatigue is not known, but one suggestion has been that calcium entry might itself be inactivated by raised intracellular calcium concentrations. If so, then an up-regulation of a calcium-binding protein might allow secretion to be maintained for longer before fatigue sets in. Interestingly, Miyata *et al.* reported that calretinin is expressed only in oxytocin nerve axons, and fatigue is known to be a much less prominent feature of oxytocin secretion than of vasopressin secretion.

What seems clear is that genomics and proteomics studies are not a substitute for hypothesis-driven research but have the power to invigorate such research by their ability to inspire new hypotheses and indeed to identify wholly new questions. One strength of the present paper by Gouraud *et al.* (2) is that their studies are based in such a well-defined model system, a system whose physiology is well enough understood that new hypotheses announce themselves with clarity.

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