The following is the abstract of the article discussed in the subsequent letter:

Fitts DA, SN Thornton, AA Ruhf, DK Zierath, AK Johnson, and RL Thunhorst. Effects of central oxytocin receptor blockade on water and saline intake, mean arterial pressure, and c-Fos expression in rats. Am J Physiol Regul Integr Comp Physiol 285: R1331–R1339, 2003. First published August 7, 2003; 10.1152/ajpregu.00254.2003—Central injection of ANG II has been proposed to have dual effects on salt appetite including a direct stimulatory effect and an indirect inhibitory effect through an activation of central oxytocinergic neurons. The inhibition was demonstrated by pretreating rats with central ornithine vasotocin (OVT; oxytocin antagonist) 30 min before a central ANG II injection. The OVT pretreatment produced a large increase in ANG II-induced saline intake. The present paper reports a failure to replicate that influential experiment. However, we also report for the first time that OVT by itself: 1) provokes drinking of both water and saline solution with a latency almost as short as that produced by ANG II; 2) produces a mild pressor response; and 3) increases c-Fos expression in the organum vasculosum laminae terminalis (OVLT) and the median preoptic nucleus (MnPO). Oxytocin activity may provide an inhibitory control of drinking responses as has been suggested, but the inhibition is tonic and includes both water and saline drinking. Inhibition of this tonic activity may stimulate drinking by increasing neural activity in the OVLT and MnPO.

Inhibition of salt appetite in rats by central oxytocin

To the Editor: It has long been known that intracranial injection of ANG II stimulates marked increases in water ingestion but relatively small increases in the intake of concentrated NaCl solution (5, 6). On the basis of an extensive series of investigations (see Ref. 7 for review), we had proposed that in addition to its accepted role in stimulating thirst, central ANG II provides a mixed signal for salt appetite with both excitatory and inhibitory components and that central oxytocin (OT) participates in mediating this inhibition. In one relevant experiment (1), we administered a dose of 5 ng ANG II into the cerebral ventricles of rats that stimulated intakes of ~15 ml water and ~3 ml saline in 1 h and found meaningful the significant increase of ~10 ml of 0.3 M NaCl that resulted when central OT receptors were blocked by intracerebroventricular treatment with ornithine vasotocin (OVT).

Recently, Fitts and colleagues (4) reported that intracerebroventricular OVT did not increase intake of NaCl solution by rats given intracerebroventricular ANG II. They emphasized that this was an important finding because our work had never been replicated. We disagree. The findings in our paper were confirmed twice in the initial report (1), including the use of a structurally different OT receptor antagonist, and we confirmed them again in a later study (2) using a ricin conjugated compound to destroy brain neurons that contained OT receptors. We also reported the results of numerous experiments in which ANG II-induced NaCl intake was inhibited in adult rats by treatments that stimulated central OT secretion and was enhanced by treatments that inhibited central OT secretion (7). Others have extended these findings to preweanling rats (3).

Fitts et al. (4) sought to replicate the disinhibitory effects of intracerebroventricular OVT on NaCl intake in their experiments 1A and 1B, but unfortunately they did not reproduce the typical effects of intracerebroventricular ANG II on water and saline intake. Specifically, 5 ng ANG II stimulated only small intakes of both water and saline in their experiment 1A, whereas 5 or 500 ng ANG II each stimulated sizeable intakes of both fluids in their experiment 1B. Their experiment 2A cannot be considered as an attempt to replicate our work because intracerebroventricular OVT was administered using a testing protocol and strain of rats that were different from ours.

Our hypothesis is that ANG II provides a dose-related stimulus of thirst and NaCl appetite, but the increased salt intake may not be expressed because of the inhibitory effects of an associated stimulation of central OT neurons. This hypothesis cannot be tested either when there is little stimulation of salt appetite (presumably the case when intracerebroventricular ANG II provides little stimulation of thirst, as in experiment 1A in the study by Fitts et al.) or when the salt appetite already is highly expressed (presumably indicating the relative absence of inhibition, as in experiment 1B in the study by Fitts et al.). In short, we believe that Fitts et al. (4) did not rigorously evaluate our hypothesis or comprehensively consider the broader scientific literature regarding the role of central OT in mediating inhibition of salt appetite and therefore had insufficient basis for their conclusions.

REFERENCES


Edward M. Stricker
Department of Neuroscience
University of Pittsburgh
Pittsburgh, PA 15260
E-mail: stricker@bns.pitt.edu

Joseph G. Verbalis
Department of Medicine
Georgetown University School of Medicine
Washington, DC 20005

REPLY

To the Editor: Blackburn et al. (1) demonstrated that a large salt appetite was produced by a central injection of ANG II in rats that had previously received a central injection of the oxytocin receptor antagonist ornithine vasotocin (OVT). The method was to inject 10 μg of OVT or vehicle, wait 30 min during which time the rats could not drink, then inject 5 ng of ANG II or vehicle and observe intakes of water and 0.3 M NaCl. Rats receiving no OVT drank ~15 ml of water and ~3 ml of saline in 60 min stimulated by ANG II alone. The rats in the critical condition that received OVT followed by ANG II
Drink ~18 ml of water and ~12 ml of saline—a robust increase in saline intake. Much intrigued by these results, our laboratories at the University of Iowa and the University of Washington set out independently to exploit and extend this phenomenon by conducting variations of the procedure. We were disappointed to find that this robust effect could not be repeated even with the same strain of rats and at the same dosages of OVT and ANG II that Blackburn et al. used. The phenomenon reported by Blackburn et al. had never been independently replicated and published, so we felt it was important to publish our findings (3).

It was neither the expressed nor implied intent of our paper (3) to deconstruct rigorously the entire hypothesis of oxytocin inhibition of salt appetite. In fact, our results could be interpreted favorably for that hypothesis in that OVT injections alone stimulated some water and saline intake even without an ANG II injection. This effect had been missed by Blackburn et al. because their experimental design did not allow the rats to drink for the 30 min between the OVT and ANG II injections. We do not know exactly how OVT causes this behavioral response. OVT may somehow directly stimulate drinking or else it may, acting through oxytocin receptors or some other mechanism, disinhibit drinking. Our discussion of these results was accordingly conservative, but one possible interpretation that we prominently mentioned was that OVT blocked a tonic inhibition of salt appetite by oxytocin.

In their criticism of our work, Stricker and Verbalis suggest that the dose of ANG II used in the experiment might be a critical factor affecting the results: too little stimulation would not be enough to trigger the response and too much stimulation might overdrive the system so that the inhibitory effect would be swamped by the stimulatory one. They also state that their observed drinking response to 5 ng of ANG II alone was “typical” and that the response to 5 ng observed by the laboratory at Iowa was too low to provoke the effect.

However, the Iowa researchers, based on their own extensive experience with intracranial injections of ANG II and dipsogenic responses, were not completely surprised to find that the 5-ng dose of ANG II alone, in their hands, did not produce the large intakes of water and saline observed by Blackburn et al. (1). Subsequently, they tried a larger dose, 500 ng, that they expected to produce intakes somewhere in the range of that observed by Blackburn et al., and this also did not produce the expected effect when combined with OVT. It is difficult to say whether the system was overdriven at this point, but the observed intakes were still less than those observed by Blackburn et al. after combined OVT and ANG II treatments. Independently, the Washington group decided not even to try the 5-ng dose based on their own experience and opted for a dose of 40 ng. In perusing the literature, it is obvious that the dose-response relation between ANG II and drinking differs widely among laboratories, so it is a fallacy to state that the response to 5 ng of ANG II observed by Blackburn et al. is typical of all laboratories. Selleck and Simpson (4) demonstrated within in a single experiment that the dipsogenic responses to individual doses of ANG II varied widely, but lawfully, when they were given in different volumes of injection. Furthermore, in a series of experiments to demonstrate a synergy of central ANG II and peripheral DOCA injections in provoking salt appetite, Epstein and coworkers (2) selected a “standard” dose of 6 ng of ANG II for a pulse injection precisely because it did not by itself, at least in their hands, induce any saline intake in rats.

In conclusion, we agree with Stricker and Verbalis (5) that our paper (3) did not rigorously deconstruct the oxytocin hypothesis or even consider other evidence for it. However, that was not our intent in publishing the data. Instead, the point of our paper was to demonstrate that a large and robust effect reported by them (1)—one that had excited the imaginations of a number of salt appetite researchers—was not easily replicable. If the effect exists, it is so restricted in its dependence on particular doses, laboratory methods, and strains of rats, that it is not as robust and as useful a model as it first seemed. It would be interesting to know if other investigators have made attempts and had similar experiences.

REFERENCES

Douglas A. Fitts
Alexandra A. Ruhf
Dannielle K. Zierath
Department of Psychology
University of Washington
Seattle, WA 98195-1525

Simon N. Thornton
Department of Psychology
University of Iowa
Iowa City, IA 52242-1407

Alan Kim Johnson
Departments of Psychology, Pharmacology, and the Cardiovascular Center
University of Iowa
Iowa City, IA 52242-1407

Robert L. Thunhorst
Department of Psychology and the Cardiovascular Center
University of Iowa
Iowa City, IA 52242-1407