Mechanism of Extracellular Zinc Induced Release in Neurohypophyseal Terminals

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Abstract

Zinc is a trace metal with many important roles in the human body. Recently it has been shown that it is important for processes in the nervous system including at synapses modulating neurotransmission and plasticity (Hershfinkel, et al., 2011). Studies demonstrate that, among other receptors, there is a G-coupled protein receptor known as GPR39 (see Fig. 1), that when it interacts with zinc triggers intracellular calcium release (Popovics & Stewart, 2011). This receptor has been evaluated in different cell models and based on calcium responses produced, it has been determined that GPR39 desensitizes during a long zinc exposure (Sharir & Hershfinkel, 2005) and that works at its best when exposed to a pH of 7.4 (Ganay, et al., 2015). Because zinc and this receptor have been found in terminals from the neurohypophysis (NH) or posterior lobe of the pituitary and its mechanism is not well understood (Pérez-Castejón, et al., 1994), we studied its role in oxytocin (OT) and vasopressin (AVP) release. Experiments evaluated possible desensitization, pH effects, and importance of extracellular calcium on OT and AVP release. Both OT and AVP are hormones that can be found stored and when necessary released at the NH (see Fig. 2). These hormones play important roles, such as water and sodium balance, in the human body. OT is also important for sexual behavior and female and male reproductive physiology. Furthermore, both hormones have important roles in social behavior, possibly mitigating diseases such as ASD. For this purpose, we isolated neurohypophysis terminals (NHT) and perfused them to evaluate OT and AVP release when induced by extracellular zinc. Hormone release was determined with an ELISA that is selective for OT vs. AVP. Results demonstrated that there is both a decrease and an increase of hormone release when pH is altered, showing that it is pH-dependent. Extracellular calcium is not necessary for zinc-induced AVP or OT release. Finally, that there is a decrease in both AVP and OT release when NHT were exposed multiple times to zinc, suggesting that GPR39 is involved in the mechanism of action.

Background

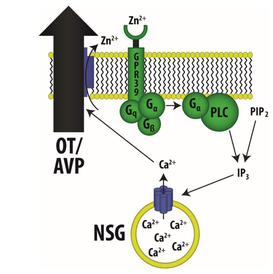


Figure 1: Mechanism of G-coupled protein receptor GPR 39. When zinc binds to GPR39 receptor it activates Gq and, then the Gα portion of the Gq activates PLC. Then, PIP2 is converted to form IP3 that causes calcium to be released from intracellular vesicles (NSG) and calcium subsequently causes hormones to be released (Popovics & Stewart, 2011) .

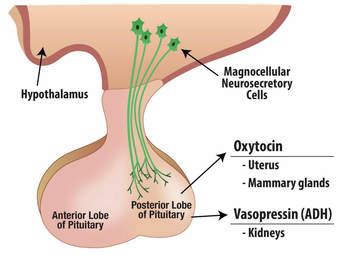
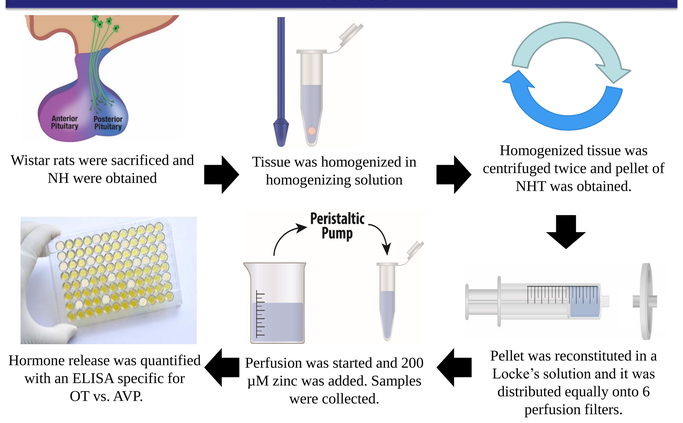


Figure 2. Pituitary gland. The pituitary is a pea sized gland located at the base of the brain and it is very important for hormone production. Vasopressin and oxytocin are both synthesized in the hypothalamus and stored and released from the posterior lobe of the pituitary. Oxytocin and vasopressin hormones are important for water and sodium balance, reproduction, social and sexual behavior.

Methods



Terminal isolation and perfusion (see Lemos et al. 2014) - NH from male Wistar rats were isolated and homogenized in a solution containing 270 mM sucrose, 10 mM HEPES, 10 µM EGTA with a pH of 7.0. Then it was centrifuged twice (100xg for 2 min; 2400xg for 6 min) and pellet of isolated NHT was obtained. Pellet was reconstituted into 1mL with Locke's buffer containing 140 mM NaCl, 5 mM KCl, 10 mM HEPES, 10 mM Glucose, 2 mM $CaCl_2$, and 10 mM $MgCl_2$ with a pH of 7.4. Once reconstituted it was distributed equally onto 6 perfusion filters and were perfused in the same Locke's solution with a pH of 7.4.

Hormone release determination - OT and AVP release of samples obtained from the perfused terminals was determined with a specific enzyme-linked immuno-specific assays (ELISA) from Enzo Life Sciences, Inc. (Farmingdale, NY). Procedure was followed as established by the manufacturer and the hormone concentrations were determined by comparing samples to the appropriate standards. To determine differences the area under the curves were used and significance is indicated with *(P<.05).

Results

Effect of pH on Hormone Release

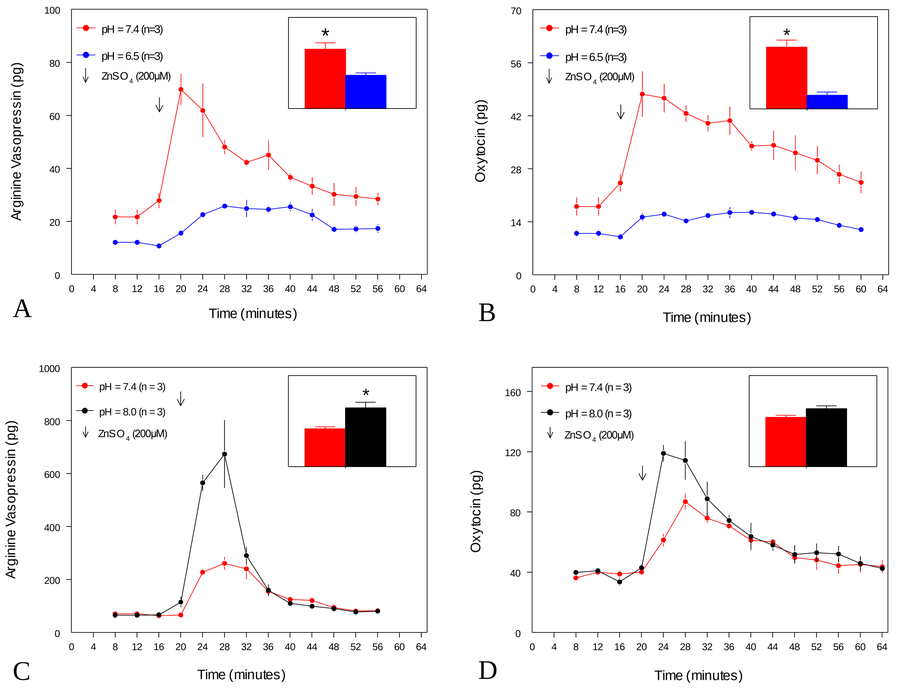


Figure 3: Effect of pH on OT and AVP release. Isolated NHT were perfused in Locke's solution with a pH of 7.4 and hormone release was induced with 200 µM $ZnSO_4$. A, B) Half of the samples (blue) were then changed to a Locke's solution with a pH of 6.5. C, D) Half of the samples (black) were then changed to a Locke's solution with pH of 8.0. In both cases control samples continued to be perfused in a 7.4 pH (red). The inset bar graphs demonstrate the differences in OT and AVP release between control and experimental pHs (*P < .05).

Effect of Extracellular Zinc Double Exposure on Hormone Release

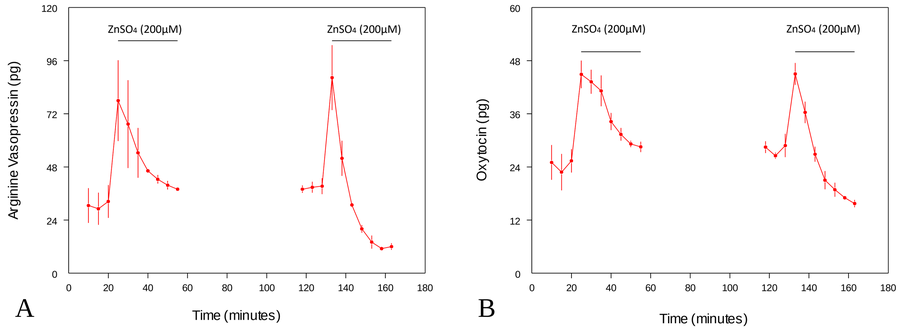


Figure 4: Effect of double zinc exposure on OT and AVP release. Isolated NHTs were perfused in Locke's solution and hormone release was induced with 200 µM $ZnSO_4$ for a period of 40 minutes. After a 60 minute rest period NHT were re-exposed to 200µM $ZnSO_4$. A) Arginine Vasopressin and B) Oxytocin were determined. Both have similar peaks, but the second exposure doesn't maintain OT or AVP release as in the first exposure.

Effect of Extracellular Calcium on Hormone Release

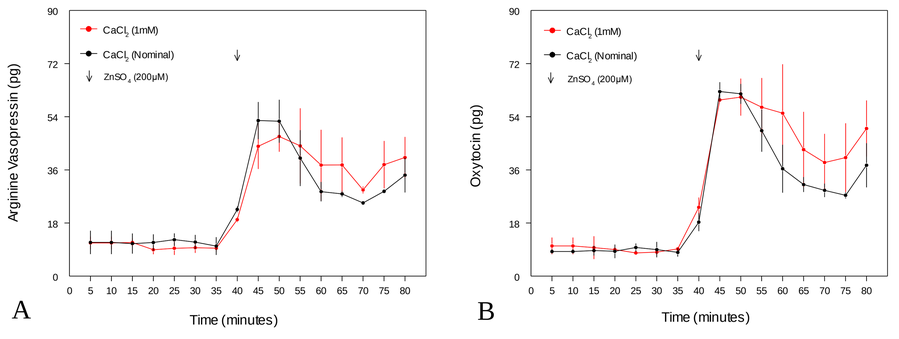


Figure 5: Effect of normal vs. nominal calcium on zinc induced OT and AVP release. Isolated NHTs were divided into two groups. One group was perfused in a Locke's solution with 1 mM calcium (red). A second group was perfused in Locke's solution without (nominal) calcium (black). OT and AVP release was induced with 200 µM $ZnSO_4$. A) Arginine Vasopressin and B) Oxytocin release was not affected by the absence of extracellular calcium (P > .05).

Conclusions

- Extracellular zinc induces release of both AVP and OT
- Both AVP and OT Zinc-induced release is pH dependent
- There is a decrease in hormone release (desensitization) with repeated zinc exposures
- Extracellular calcium is not necessary for AVP and OT Zinc-induced release
- Results indicate possible GPR39 involvement in zinc effects

Future Directions

Our next step is to use GPR 39 knockouts to evaluate and confirm that the responses we are getting are because of this specific receptor.

- We would start by repeating basic extracellular zinc induced release experiments to which we know what the response in rats is and evaluate what is the response in mice.
- We would repeat pH experiments in knockouts to evaluate if the change in release is only based on the GPR 39 receptor or not.
- We could alter the time in between of double exposure experiments to see the change and the recovery time (doesn't have to be on knockouts).

Also we would evaluate our model (see Fig. 6) by using normal mice vs. knockouts and applying P2X receptor blockers. This will tell us how much of the effect is based on GPR39 receptor and what effect the P2X2 and P2X7 are having. Also being able to compare these results between AVP and OT will help to see the effect of P2X7 since OT doesn't have P2X2.

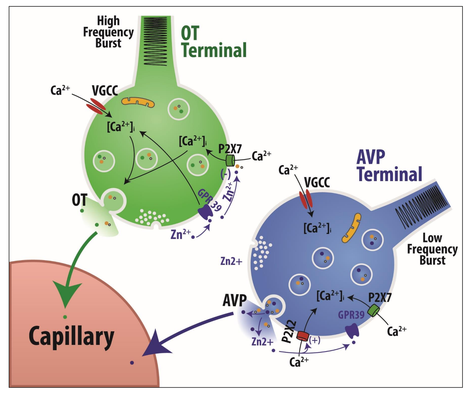


Figure 6: GPR39 and P2X receptor model. Different frequency bursts invade OT vs. AVP terminals and open the voltage gated channels (VGCC) permitting the entrance of calcium. Calcium induces hormone release. Also zinc and ATP would be co-released and by activating P2X and GPR39 receptors intraterminal calcium would increase thus facilitating even more hormone release.

Acknowledgements

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References

1. Ganay, T., Asraf, H., Aizenman, E., Bogdanovic, M., Sekler, I., & Hershfinkel, M. (2015). Regulation of neuronal pH by the metabotropic zinc-sensing Gq-coupled receptor, mZnR/ GPR39. *Journal of Neurochemistry*, 135, 897-907.

2. Hershfinkel, M., Aizenman, E., Andrews, G., & Sekler, I. (2011). Zinc bells rang in jerusalem. *Sci Signal*, 3(129), 1-13.

3. Lemos, J. R., Wang, G., Marrero, H., & Ortiz-Miranda, S. (2014). Neurophysiology of neurohypophysial terminals. *In Neurophysiology of neuroendocrine neurons* (pp. 165-188).

4. Pérez-Castejón, C., Vera-Gil, A., Barral, M.J., Pérez-Castejón, M.J. and Lahoz, M., (1994) Zinc in hypothalamus and hypophysis of the rat. *Histology and Histopathology*, (2):259-262.

5. Popovics, P. and Stewart, A.J., (2011) GPR39: a Zn2+-activated G protein-coupled receptor that regulates pancreatic, gastrointestinal and neuronal functions. *Cellular and Molecular Life Sciences*, 68:85-95

6. Sharir, H., & Hershfinkel, M. (2005). The extracellular zinc-sensing receptor mediates intercellular communication by inducing ATP release. *Elsevier*, 332, 845–852.

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