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# Supplementary Materials for

## $V_2$ Receptor–Mediated Autocrine Role of Somatodendritic Release of AVP in Rat Vasopressin Neurons Under Hypo-Osmotic Conditions

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**The PDF file includes:**

**SUPPLEMENTARY MATERIALS**

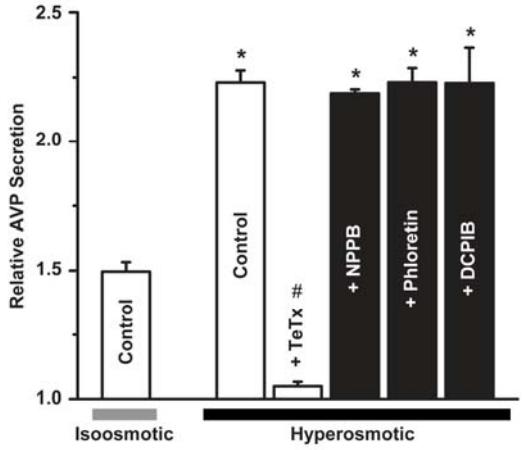


**Fig. S1.** Somatodendritic AVP release from dissociated AVP neurons in response to hyperosmotic stimulation.

Effects of pretreatment with tetanus toxin (TeTx: 15 nM for 70 min), and of treatment with an anion channel blocker (50 μM NPPB, 30 μM phloretin or 5 μM DCPIB), on the relative amount of AVP released 90 min after application of isoosmotic or hyperosmotic (136.5% osmolality) solution containing (in mM) 120 NaCl, 2.5 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 25 $NaHCO_3$, 10 glucose and 140 mannitol (adjusted to pH 7.4 with NaOH) at 37°C ($n$ = 5-10 samples). *$P < 0.05$ vs. the isoosmotic control data. $^{\#}P < 0.05$ vs. the hyperosmotic control.

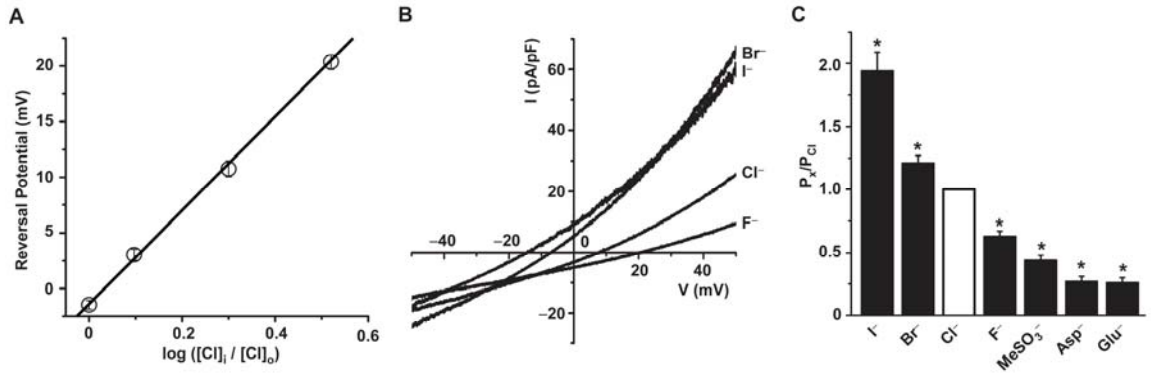


**Fig. S2.** Anion selectivity of swelling-activated currents.

(**A**) Relationship between the reversal potential ($E_{rev}$ : $n$ = 6 to 9 cells) and $\log([Cl]_i/[Cl]_o)$. The slope of a regression line drawn by linear fitting is 41.3 mV per one log unit. (**B**) Effects of extracellular anion substitution on the current-voltage relationships for swelling-activated currents observed under ramp voltage clamp. (**C**) Relative anion permeability of swelling-activated currents. Coefficients for the permeability of $I^-$, $Br^-$, $F^-$, methansulfonate$^-$ ($MeSO_3^-$), aspartate$^-$ ($Asp^-$), and gluconate$^-$ ($Glu^-$) relative to that of $Cl^-$ were calculated from reversal potentials using the Goldman-Hodgkin-Katz equation ($n$ = 5 to 14 cells). *$P < 0.05$ vs. the $Cl^-$ permeability.

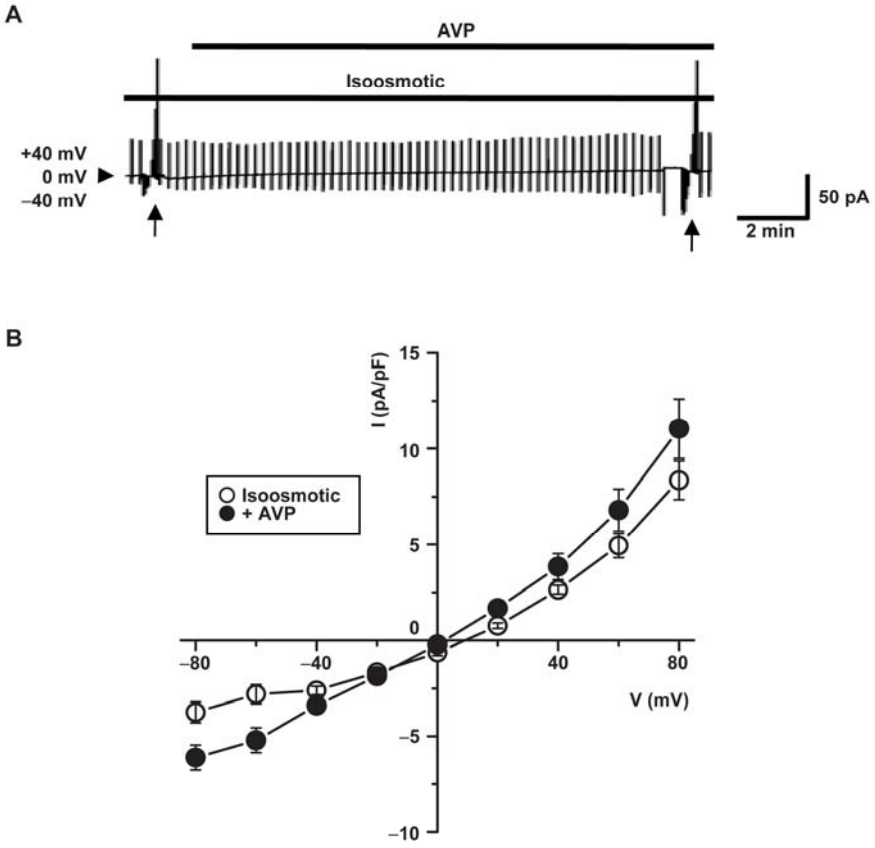


**Fig. S3.** Effects of AVP on basal anion currents in dissociated AVP neurons under isoosmotic conditions.

(**A**) Representative record of whole-cell anion currents during exposure to isoosmotic extracellular solution in the absence or presence of AVP (5 pg/ml). Alternating step pulses of ±40 mV or step pulses (at arrows) from –80 to +80 mV in 20 mV increments were applied from a holding potential of 0 mV. Arrowhead represents the zero-current level. (**B**) Current-voltage relationships of basal anion currents in the absence (open circles) or presence (filled circles) of AVP (5 pg/ml). There is no significant difference between the data with and without AVP stimulation in the voltage range studied (except at –80 and –60 mV).

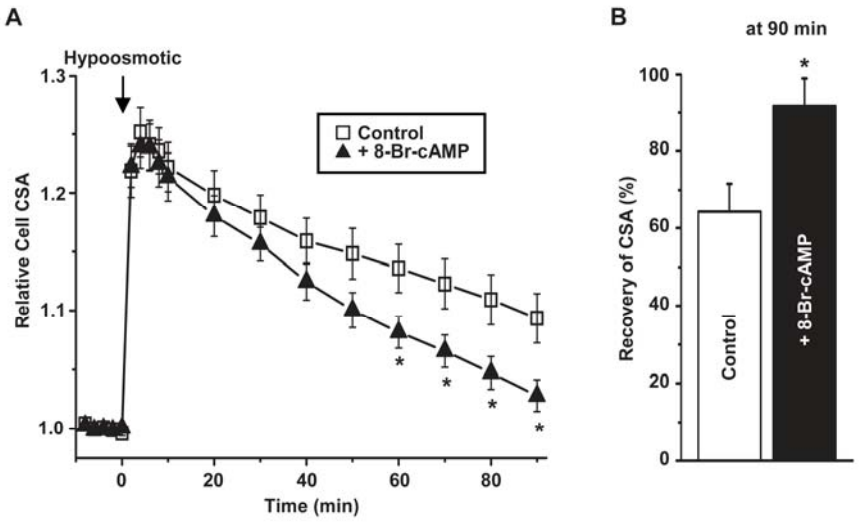


**Fig. S4.** Effects of 8-Br-cAMP on RVD after osmotic swelling in dissociated AVP neurons. (**A**) Time course of changes in the cross-sectional area (CSA) of the cell soma before and after application of hypoosmotic HBS (at arrow) in the absence (control; $n$ = 14 cells) or presence of 1 mM 8-Br-cAMP ($n$ = 13 cells). *$P < 0.05$ vs. the control data at a given time. (**B**) Percentage of CSA recovery (from peak osmotic swelling) at 90 min after application of hypoosmotic HBS in the absence (control; $n$ = 14 cells) or presence of 8-Br-cAMP ($n$ = 13 cells). *$P < 0.05$ vs. the control data.

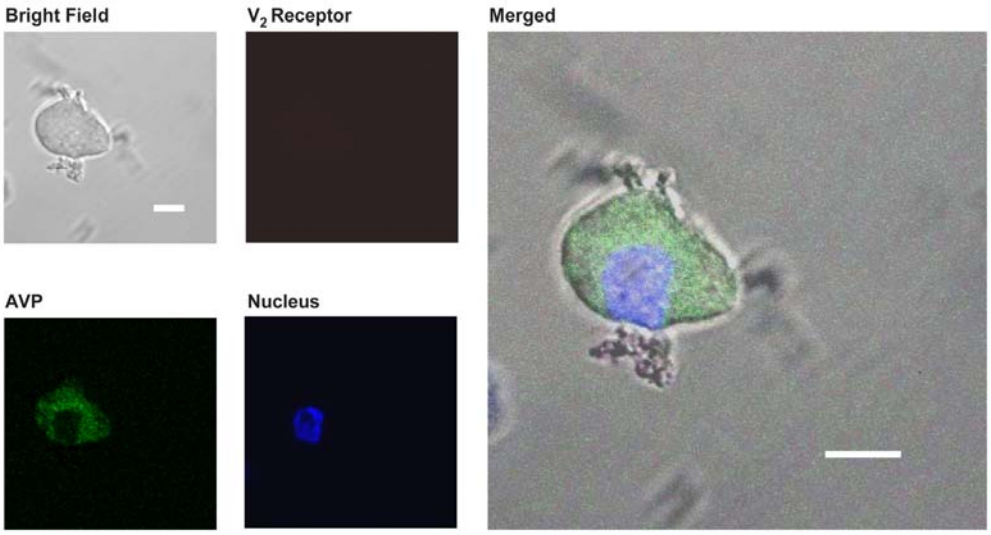


**Fig. S5.** Negative control experiments for immunostaining in dissociated rat AVP neurons. The primary rabbit anti-$V_2$ receptor antibody (2 μg/ml) was preabsorbed with the immunizing peptide (21 amino acid peptide from the 3rd cytoplasmic domain of rat $V_2$ receptor) (2 μg) for 120 min. The four photographs in the left panel are representative phase-contrast (Bright Field) and fluorescence microscopy images of dissociated AVP neurons that exhibit GFP fluorescence (green) indicative of AVP expression (AVP) and Hoechst 33342 fluorescence (blue) marking the nucleus (Nucleus) but not fluorescence (red) of Alexa Fluor 594 conjugated with anti-rabbit IgG antibody. The right panel is the merged image (Merged). The data is representative of similar experiments using 30 AVP neurons isolated from two AVP-eGFP transgenic rats. Scale bars, 10 μm.