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Regulation of Water Channel Activity of Aquaporin 1 by Arginine Vasopressin and Atrial Natriuretic Peptide

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Aquaporin 1 (AQP1), a six-transmembrane domain protein that functions as a water channel, is present in many fluid secreting and absorbing tissues such as kidney, brain, heart, and eye. It is believed that among the five known mammalian aquaporins, kidney aquaporin (AQP2) is the only water channel that is regulated by arginine vasopressin (AVP). The present data suggest that AQP1 may also be regulated by AVP. The application of AVP to Xenopus oocytes injected with AQP1 cRNA increased the membrane permeability to water. In addition, our data reveal that atrial natriuretic peptide (ANP), a peptide hormone that plays an important role in the regulation of body fluid homeostasis, blocks the AQP1-mediated increase in water permeability. Incubation with 8-bromo-cAMP or direct 8-bromo-cAMP injection into oocytes expressing AQP1 cRNA significantly increased membrane permeability to water, suggesting that stimulation of AQP1 activity by AVP may involve a cAMP-dependent mechanism. Regulation of water permeability by AVP and ANP has potential relevance to active water transport in a variety of tissues that express AQP1 including kidney, brain, and eye.

The plasma membranes of all animal cells are at least moderately permeable to water and can maintain osmotic equilibrium in static external environments. However, the water permeabilities of many cell types have been found to exceed values that can be explained by lipid phase water permeation alone. The concept of a water channel was originated by Koefoed-Johnsen and Ussing in 1953 to explain the higher osmotic water permeability in frog skins (1). Subsequently, numerous other biophysical investigations supported the water pore concept (2). Water channels can raise plasma membrane water permeability required for efficient coupling between NaCl and water transport in epithelia that carry out isosmotic fluid transport and thus may provide a target for regulation of water transport in selective tissues.

Aquaporins (AQPs) are a rapid growing family of water channel proteins found in animals, plant and microorganisms (3, 4). AQP1 was the first identified and characterized homologue from mammalian red cells, and is found in other epithelia including renal proximal tubules and the ciliary processes of the eye (5-7). AQP2 was identified from renal collecting duct (8), and AQP3 was isolated from rat kidney (9). Other aquaporins have been cloned from brain (AQP4) (10, 11), and salivary gland, (AQP5) (12). AQP1, AQP2 and AQP3 have been shown to transport the nonionic small solutes such as urea and glycerol in addition to water (9, 13, 14). AQP4 and AQP5 are highly selective to water permeation and exclude small solutes (10-12). AQP2 and AQP 5 have consensus sites for cAMP-dependent protein kinase (12). However, only AQP2 is shown to be regulated physiologically via a cAMP dependent mechanism by arginine vasopressin (AVP) (15). AQP1, 3, and 4 lack the typical consensus sequences for phosphorylation by cAMP-dependent protein kinase. These aquaporins were thought to be constitutively active until the recent report by Yool et al which suggests that AQP1 is regulated physiologically via a cAMP-dependent mechanism by forskolin (16). Since vasoactive peptides such as AVP and atrial natriuretic peptide (ANP) play an important role in regulation of body fluids, such as formation of urine in the renal medulla, production of cerebrospinal fluid in the brain, and secretion of aqueous humor in the eye (17-19), we reasoned that these peptides may have regulatory roles on aquaporin homologs expressed in these organs. In this report, we examine the effect of AVP and ANP on the water channel activity of bovine AQP1 and demonstrate, that the AQP1 is an AVP- and ANP-regulated water channel.

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EXPERIMENTAL PROCEDURES

In vitro cRNA synthesis of AQP1. The EcoR1-BamH1 fragment of bovine AQP1 (CHIP29) containing 32 bp of the 5′-untranslated sequence, 205 bp of the 3′-untranslated sequence, and the entire aqp1 open reading frame (20) was blunt-ligated into the Bgl II site of the Xenopus expression construct pXbG, which contains the HindIII-Pst1 insert of pSP64T (an upstream Xenopus β-globin enhancer sequence) (5) in pBS-KS. Conventional molecular biology techniques along with commercially available restriction endonucleases were used to identify the orientation of the cDNA into the recombinant plasmid. Confirmation of the recombinant plasmid was made by nucleotide sequencing. Sense and antisense capped RNA transcripts of AQP1 were synthesized in vitro with T3 RNA polymerase using two recombinant plasmids with the AQP1 cDNA cloned in sense and antisense direction.

Preparation of oocytes and measurement of P_f. Defolliculated stage V and VI oocytes from female *Xenopus laevis* (21) were injected with 20 nl of water or cRNAs (1 mg/ml). After incubation in 200 mosM modified Barth's buffer at 18 °C for 72 h, oocytes were transferred to 7 0 mosM Barth's buffer diluted with distilled water, and the time course of osmotic volume increase was monitored at 20 °C (5). Oocytes were viewed by transmitted light on a Nikon inverted microscope using a X4 objective. Oocytes were illuminated with low-intensity (500 nm) monochromatic light. The oocyte was imaged on a CCD camera and the images were recorded by a PixelBuffer frame grabber and auxiliary processing board (Signal Analytics Corp. USA) in a computer. IPLab Spectrum P computer software (Signal Analytics) was used to determine the cross sectional oocyte area from the image. Relative volume (V/V_0) was calculated from the area at *time 0* (A_0) and at *time t* (A) from the relation

$$V/V_0 = (A/A_0)^{3/2}. \quad (1)$$

Osmotic water permeability (P_f) was calculated from initial oocyte volume ($V_0 = 9 \times 10^{-4}$ cm^3), initial oocyte surface area (S = 0.045 cm^2), molar volume of water (V_w = 18 cm^3/mol) (22), and osmolarity inside (osm_{in}) and outside (osm_{out}) the cell as shown:

$$P_f = \{V_0 X d(V/V_0)dt\}/\{S \text{ X } V_w \text{ X } (osm_{in} - osm_{out})\}. \quad (2)$$

Because the time course of cell swelling was principally linear during the initial 30 s, P_f of oocytes was calculated from this 30-s response. The effects of AVP, ANP, 8-bromo-cAMP, 8-bromo-cGMP and $HgCl_2$ were examined by incubating oocytes in Barth's buffer containing appropriate concentrations of the reagent for 15 min prior to P_f measurements. In some experiments, oocytes were directly injected with 20 nl of 1 mM 8-bromo-cAMP or 8-bromo-cGMP before the assay.

RESULTS AND DISCUSSION

The cDNA for bovine analog of the AQP1 (CHIP29) has been isolated and the deduced amino acid sequence predicts an integral membrane protein with six bilayer spanning domains (20). We prepared an expression construct by inserting the AQP1 coding sequences between 5′ and 3′ untranslated sequences of the Xenopus β-globin cDNA as described in the Experimental Procedures. Defolliculated oocytes were microinjected with 10 ng of in vitro transcribed AQP1 RNA. Osmotic water permeability after transfer of oocytes from a 200 to a 70 mosM solution was determined by monitoring changes in cell volume as described in the Experimental Procedures. Figure 1 shows the effects of AVP and ANP on the osmotic water permeability of oocytes expressing AQP1. Oocytes incubated for 15 min in 200 mosM Barth's buffer containing 10 μM AVP showed the greatest subsequent rate of swelling in 70 mosM buffer and the oocytes ruptured within 1 min. Oocytes incubated for 15 min in 200 mosM Barth's buffer containing 10 μM ANP showed significantly lower subsequent rate of swelling in 70 mosM buffer. These oocytes failed to rupture, even after 10 min. Ethanol (0.1%) was used to make a 1 mM stock solution of AVP and ANP, and had no effect alone on swelling. Oocytes expressing AQP1, but not treated with AVP or ANP, showed an intermediate rate of swelling and ruptured within 3 min. Swelling of oocytes expressing AQP1 was blocked by $HgCl_2$ (100 μM), the only known blocker of water channels (5). Oocytes injected with water or antisense cRNA showed a very low swelling rate that

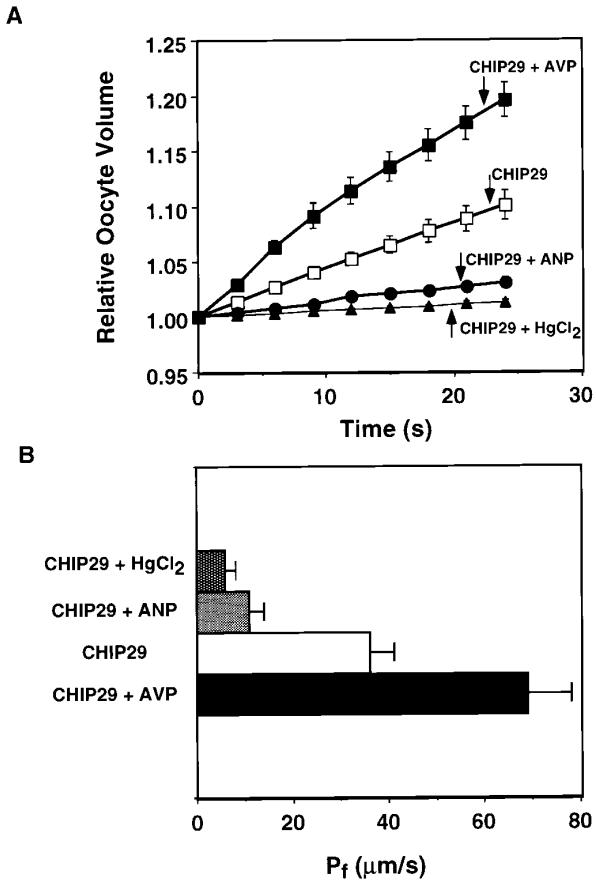

FIG. 1. Effect of AVP and ANP on the osmotic water permeability of oocytes expressing AQP1 RNA. Oocytes were injected with 20 nl of water containing 20 ng of AQP1 RNA. After 72 h, the oocytes were transferred from 200 to 70 mosM modified Barth's buffer, and changes in size were measured by videomicroscopy (A) and osmotic water permeabilities (B) were calculated—see "Experimental Procedures." The effects of AVP, ANP, and $HgCl_2$ were examined by incubating oocytes in Barth's buffer containing appropriate concentrations of the reagent for 15 min prior to P_f measurements.

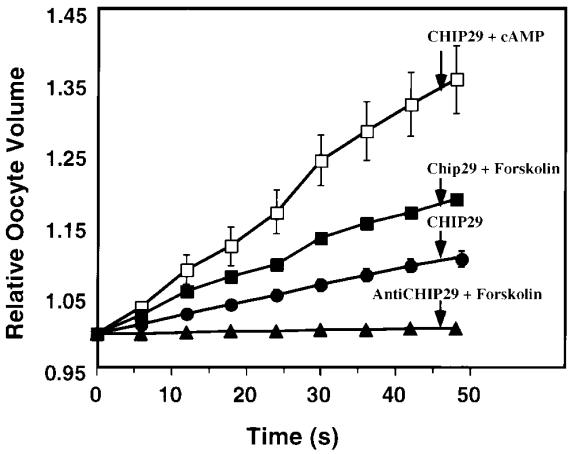

FIG. 2. Time course and c-AMP stimulation of osmotic swelling of oocytes expressing AQP1. Oocytes were injected with 20 nl of water containing 20 ng of AQP1 RNA. After 72 h, the oocytes were transferred from 200 to 70 mosM modified Barth's buffer, and changes in size were measured by videomicroscopy. Effect of cAMP was examined by directly injecting oocytes with 20 nl of 1 mM 8-bromo-cAMP before the assay (see "Experimental Procedures").

was unaffected by either AVP or ANP treatment. These oocytes failed to rupture even after 30 min (data not shown). The coefficients of osmotic water permeability (P_f) at 20 °C, calculated from the rates of swelling were 36 ± 5 μm/s (mean ± SE, n = 8) for untreated AQP1 injected oocytes, 69 ± 6 μm/s (mean ± SE, n = 10) for AVP stimulated oocytes (91% increase over control), and 11 ± 3 μm/s (mean ± SE, n = 12) for ANP treated oocytes (70% decrease over control), and 6 ± 2 μm/s (mean ± SE, n = 7) for $HgCl_2$ injected oocytes.

The effect of AVP on water permeability is believed to be mediated by cAMP (15). Therefore, we tested the effect of 8-bromo-cAMP (a membrane permeable analog of cAMP) on the water permeability of AQP1 expressing oocytes. Figure 2 shows the effects of 8-bromo-cAMP on the osmotic water permeability of oocytes expressing AQP1. The oocytes were injected with 8-bromo-cAMP or water 30 min before P_f measurements. The P_f values of oocytes expressing the AQP1 cRNA were increased from 38 ± 4 to 132 ± 6 (+247%) after the injection of oocytes with 8-bromo-cAMP. Whereas, the P_f values of oocytes injected with water were 42 ± 5 (data not shown) suggesting that microinjection itself had no effect on the oocyte swelling. The cAMP-dependent increase in water permeability suggests that AQP1 could participate in receptor-mediated regulation of water fluxes in a variety of tissues such as kidney, heart, brain, ear and eye in which it is widely distributed. Native oocyte follicles contain the AVP receptors (23). However, it is not clear whether these receptors are present on the oocyte membrane or follicular layer surrounding the oocyte. The data presented here suggest that AVP receptors may occupy the oocyte membranes. Alternatively, vasopressin may interact with AQP1 via vasotocin receptors found in amphibians. Amphibian vasotocin receptors are homologous to the vasopressin receptors of the mammals (23), and vasopressin is known to bind specifically to the amphibian kidney vasotocin receptors (24).

Our observations of regulation of water permeability of AQP1 due to vasopressin are contradictory to earlier reports (25, 26). It is believed that AQP2 is the only water channel that is physiologically regulated. Regulation of other aquaporin members was unknown until the recent report in which Yool et al (16) provide an evidence for AQP1 stimulation by forskolin or 8-bromo-cAMP in Xenopus oocytes microinjected with human AQP1 cRNA. As shown in Figure 2, we were able to reproduce these results in our laboratory. The α-TIP, a plant homolog of AQP1, is also stimulated by cAMP or forskolin (27). Other controversy with respect to AQP1 that remains unresolved is water selectivity. For example, AQP1 have been demonstrated by several investigators as water selective channels (5, 28). However, recent reports provide strong evidence that glycerol and certain small solutes permeate through AQP1 channels expressed in Xenopus oocytes or reconstituted in proteoliposomes (13, 14). Some of these discrepancies may be due to variation in oocyte batches undertaken for the studies. For example, studies with activation of ionic currents in Xenopus oocytes by AVP showed that not all donor frogs are responsive to this peptide and the response was variable between oocytes from a single donor (29). Furthermore, these studies indicated that there may be a seasonal variation in expression of the receptors for these neuropeptides.

AQP2-mediated water permeability of kidney collecting duct is regulated by AVP via cAMP dependent 'membrane shuttle' mechanism in which AVP increases the apical membrane water permeability by triggering exocytosis of intracellular vesicles containing water channels, delivering the vesicles with their water channels to the apical membrane (30, 31). The regulation of water transport in other tissues including heart, brain, eye and lung remains undiscovered. Our preliminary results obtained using the AQP1 antibody favors a cAMP-dependent membrane shuttle mechanism for AQP1 regulation. We detected a band of an expected molecular mass of 29-kDa in control oocytes as well as those treated with 8-bromo-cAMP or vasopressin for 30 min before membrane preparations. However, when equal amounts of membrane protein (50 μg) were separated on SDS-polyacrylamide gel electrophoresis, the 29 kDa band intensity corresponding to AQP1 in oocytes that were treated with 8-bromo-cAMP or vasopressin was 3 to 4 higher than the corresponding band in untreated oocytes, respectively (data not shown). It is difficult to conclude whether the increased AQP1 protein level in oocytes membranes is a direct response to AVP-induced intracellular signaling or to indirect

effects of AVP. AQP1 lack the typical cAMP dependent protein kinase consensus sequence Arg-Arg-X-Ser/Thr (where X is any amino acid) for phosphorylation. However, there are several proteins that are phosphorylated via cAMP dependent protein kinase that exhibit only Arg-X-Ser sequence (32, 33). Ser-238 of bovine AQP1 (20) exhibits the Arg-X-Ser sequence that could be the potential phosphorylation site in this aquaporin. This is further supported by a recent report which provides an evidence for the cAMP dependent phosphorylation of human AQP1 fusion protein (16) in which Ser 236 exhibits Arg-X-Ser sequence (34).

Another significant finding of this study is the demonstration of regulation of AQP1 by ANP. Most of the actions of ANP are accounted for by activation of membrane bound guanylate cyclase via ANP receptors, leading to generation of cGMP (35). Therefore, we tested the effect of 8-bromo-cGMP (a membrane permeable analog of cGMP) on the water permeability of AQP1 expressing oocytes. The oocytes were injected with 8-bromo-cGMP or water 30 min before P_f measurements. The P_f values of oocytes expressing the AQP1 cRNA were unchanged after the injection of oocytes with 8-bromo-cGMP or water suggesting that the decreased water permeability of AQP1 by ANP treatment is not cGMP-dependent. Several reports suggest that ANP inhibits adenylate cyclase in various tissue through an inhibitory G protein (36, 37), causing decreased cAMP level, which may explain in part the inhibitory effect of ANP on AQP1. The rat ANP used in current studies may interact via ANP receptors found on the Xenopus oocytes membranes (38) as the amino acid sequences of frog ANP and mammalian ANP are similar (39). Decrease in AQP1 water channel activity by ANP has potential relevance in many cells that express AQP1 since ANP is known to have important roles in the modulation of renal function and regulation of body fluid homeostasis (17-19). In the eye, ANP decreases intraocular pressure by binding to its receptors on ciliary epithelial cells and decreasing the production of aqueous humor (19, 40). Previous studies using in situ hybridization, cDNA cloning, and immunocytochemistry demonstrated abundant AQP1 in the ocular ciliary epithelium, a major site for the aqueous humor production (7, 20, 41). In brain, it has been reported that injection of ANP into the cerebral ventricles decrease the production of cerebrospinal fluid (42). AQP1 is present in the choroid plexus (7), a site for cerebrospinal fluid secretion. In kidney proximal tubules, where AQP1 is abundant (41), ANP has been shown to inhibit the sodium reabsorption (43). Thus, a more detailed understanding of ANP regulated water permeability of AQP1 would not only have physiological and pathophysiological significance, it would also add new therapeutic approaches to diseases that are accompanied by volume imbalance. In conclusion, the stimulation of AQP1 water permeability by AVP and it's inhibition by ANP suggest that neuropeptides have a regulatory role in bovine AQP1-mediated fluid transport.

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