Largely from studies on isolated amphibian tissues—skin and urinary bladder—we know that vasopressin (AVP) exerts its antidiuretic action by making the responsive epithelium more permeable to water and urea. The nature of the membrane changes and the means by which the membrane changes are induced by the hormone remain to be elucidated. Of special interest is the finding of Orloff and Handler (1) that 3′5′ cyclic adenosine monophosphate (3′5′ cAMP), which has been implicated as an intracellular mediator of the action of several peptide hormones, mimics the action of AVP. Furthermore, increased tissue content of cAMP has been noted after exposures of responsive tissues to vasopressin (2)." The above text was published more than 30 yr ago (3) and this editorial, commenting on the manuscript of Li et al. in this issue of JASN (4), describes new developments in the cAMP–protein kinase A (PKA) signaling pathways.

**cAMP**

More than 20% of the human genome encodes proteins involved in transmembrane and in intracellular signaling pathways. The ubiquity of cAMP signaling in nature is remarkable because organisms as diverse as Paramecium, Dictyostelium, and man have developed the ability to convert ATP into cAMP. Invasive micro-organisms, such as Vibrio cholerae, Bordetella pertussis, and Bacillus anthracis are also able to subvert their host’s physiology by either synthesizing their own secreted form of the cyclases or various classes of activators of the enzyme (5,6).

**Catalytic Mechanisms of Mammalian Adenylyl Cyclase**

ATP, a nucleotide triphosphate composed of adenine, ribose, and three phosphate groups, is the principal carrier of chemical energy in cells. The terminal phosphate groups are highly reactive in the sense that their hydrolysis, or transfer to another molecule, takes place with large amounts of free energy. If the terminal phosphoanhydride bond of ATP were to rupture by hydrolysis to produce adenosine diphosphate (ADP) and inorganic orthophosphate (Pi), energy would be released in the form of heat. However, cells contain various enzymes that can couple ATP hydrolysis to other reactions, so that much of the released energy is converted to more useful forms. For example, the enzyme adenylyl cyclase can couple ATP hydrolysis to transfer phosphate groups to other reactants forming phosphorylated intermediates including PKA and the transcription factor cAMP responsive element (CRE)–binding protein (CREB) (7).

Adenylyl cyclase is the effector enzyme responsible for converting ATP to cAMP. Class III adenylyl cyclases, which are phylogenetically closely related to guanylyl cyclases, are found in prokaryotes and eukaryotes (8). $G_{max}$ (Figure 1) acts primarily as an allosteric activator (change in conformation) of adenylyl cyclase and helps the catalytic domain (C1 and C2) collapse around the substrate, whereas $G_{max}$ maintains the catalytic domain in an open conformation (9,10). The plant diterpene forskolin binds into the remnant pocket formed between C1 and C2 and allosterically activates most membrane-bound mammalian adenylyl cyclases.

The Most Common Downstream Effector of cAMP is PKA

The cAMP-PKA pathway is one of the most common and versatile signal pathways in eukaryotic cells and is involved in the regulation of cellular functions in almost all tissues in mammals, including regulation of cell cycle, proliferation, differentiation and regulation of microtubule dynamics, chromatin condensation and decondensation, nuclear envelope disassembly and reassembly, as well as regulation of intracellular transport mechanisms and ion fluxes (5). Because this single second messenger (the cAMP-PKA pathway) is involved in the regulation of so many diverse cellular processes, it must be highly regulated at several levels to maintain specificity. AVP-induced changes in cAMP concentration may vary in duration, amplitude, and extension in the principal cells. In addition, cAMP microdomains (a microdomain is represented as a red domain in the right part of Figure 1) are shaped by adenylyl cyclases that form cAMP, as well as phosphodiesterases (PDE) that degrade cAMP. Four PDE-4 genes (4A/B/C/D) give rise to 18 different isoforms in mammalian cells and one of these may be specifically associated with aquaporin-2 (AQP2)–bearing vesicles, a further specific compartmentalization of the specific expression of AQP2 (11).
**Figure 1.** Schematic representation of the effect of vasopressin (AVP) to increase water permeability in the principal cells of the collecting duct. AVP is bound to the V2 receptor (a G-protein–linked receptor) on the basolateral membrane. The basic process of G-protein–coupled receptor signaling consists of three steps: a hepta-helical receptor that detects a ligand (in this case, AVP) in the extracellular milieu, a G-protein (Gα) that dissociates into α subunits bound to GTP and βγ subunits after interaction with the ligand-bound receptor, and an effector (in this case, adenyl cyclase) that interacts with dissociated G-protein subunits to generate small-molecule second messengers. AVP activates adenyl cyclase, increasing the intracellular concentration of cAMP. The topology of adenyl cyclase is characterized by two tandem repeats of six hydrophobic transmembrane domains separated by a large cytoplasmic loop and terminates in a large intracellular tail. The dimeric structure (C1 and C2) of the catalytic domains is represented (see text). Conversion of ATP to cAMP takes place at the dimer interface. Two aspartate residues (in Cγ) coordinate two metal co-factors (Mg2+ or Mn2+, represented here as two small black circles), which enable the catalytic function of the enzyme (9). Adenosine is the large open circle and the three phosphate groups (ATP) are the three small open circles. Protein kinase-A (PKA) is the target of the generated cAMP. The binding of cAMP to the regulatory subunits of PKA induces a conformational change, causing these subunits to dissociate from the catalytic subunits. These activated subunits (C) as shown here are anchored to an aquaporin-2 (AQP2)–containing endocytic vesicle via an A-kinase anchoring protein (AKAP). The local concentration and distribution of the cAMP gradient is limited by phosphodiesterases (PDE). Cytoplasmic vesicles carrying the water channel proteins (represented as homotetrameric complexes) are fused to the luminal membrane in response to AVP, thereby increasing the water permeability of this membrane. The dissociation of AKAP from the endocytic vesicle is not represented. Microtubules and actin filaments are necessary for vesicle movement toward the membrane. When AVP is not available, AQP2 water channels are retrieved by an endocytic process, and water permeability returns to its original low rate. AQP3 and AQP4 water channels are expressed constitutively at the basolateral membrane.

A-Kinase Anchoring Proteins, Further Compartmentalization

A-kinase anchoring proteins (AKAP) target PKA to specific substrates and distinct subcellular compartments, providing further spatial and temporal specificity to the cAMP-PKA pathway. The activation of AKAP-anchored PKA by cAMP in discrete microdomains has been visualized in neonatal cardiomyocytes (12) and AKAP 18δ (a splice variant of AKAP 18) has been found in principal cells of the inner medullary collecting duct in a distribution closely resembling the distribution of AQP2 (13), which could imply a role for AKAP 18δ in the AQP2 shuttle exocytic process.

In addition to the rapid signaling cascade described above, the long-term regulation of AQP2 expression involves the PKA-mediated phosphorylation of the transcription factor CREB (14,15).

Lithium Toxicity

In this issue of *JASN*, Deen’s group used mouse cortical collecting duct cells in culture to study and recapitulate some of the features of renal lithium toxicity in humans. This immortalized clonal collecting duct cell line, mpkCCDc14, was originally generated by Alain Vandewalle and colleagues (16). These cells exhibit electrogenic Na+ transport stimulated by aldosterone and AVP, maintain AVP-inducible AQP2 expression, and produce large amounts of both AQP2 mRNA and protein in response to physiologic concentrations of AVP (17). As demonstrated before in rat kidney medulla (18), lithium caused marked downregulation of AQP2 expression. Surprisingly, this diminution/suppression of AQP2 expression seemed to be independent of cellular cAMP levels, which were as or more elevated as compared with cells treated with 1-desamino [8-D-arginine] vasopressin (dDAVP) but not subjected to lithium treatment. Is the compartmentalization effect described earlier responsible for this apparent dissociation between cAMP concentration and AQP2 expression? Kramer and colleagues have pioneered the use of “patch cramming,” where a patch pipette containing a cAMP- or cyclic guanosine monophosphate (cGMP)-gated ion channel in a membrane patch is introduced into a recipient cell (19). Endogenous cyclic-nucleotide-gated channels have also been used to measure cAMP in neurons (20). Concentrations of cAMP in discrete cellular domains might be a better measurement of the cAMP-AKAP signaling pathway. Alternatively, the major effect of lithium could be at a step distal to the generation of cAMP. Forskolin stimulation and expression of AKAP18δ will be, in this context, experiments to pursue.

Could We Bypass the V2-Receptor Stimulation-Induced Cascade to Insert AQP2 in the Luminal Membrane?

Nitric oxide, atrial natriuretic factor, and the cGMP PDE inhibitor sildenafil citrate (Viagra) have been shown to stimulate AQP2 membrane insertion in renal epithelial cells in *vitro*. However, no change in urine osmolality was detectable in sildenafil-treated Brattleboro rats compared with dehydrated controls, although there was a trend toward an increased osmolality after drug treatment (21). More recently, methyl-β-
cyclohexadextrin, a cholesterol-depleting drug, was shown to induce AVP-independent apical accumulation of AQP2 in the isolated perfused rat kidney (22). These new developments could help bypass defective V2 receptors (X-linked nephrogenic diabetes insipidus [23]) or the complex signaling defect of lithium-induced nephrogenic diabetes insipidus.

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