The Na\textsuperscript{+}-K\textsuperscript{+}-ATPase as self-adhesion molecule and hormone receptor

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Cereijido M, Contreras RG, Shoshani L, Larre I. The Na\textsuperscript{+}-K\textsuperscript{+}-ATPase as self-adhesion molecule and hormone receptor. Am J Physiol Cell Physiol 302: C473–C481, 2012. First published November 2, 2011; doi:10.1152/ajpcell.00083.2011.—Thanks to the homeostasis of the internal milieu, metazoan cells can enormously simplify their housekeeping efforts and engage instead in differentiation and multiple forms of organization (tissues, organs, systems) that enable them to produce an astonishing diversity of mammals. The stability of the internal milieu despite drastic variations of the external environment (air, fresh or seawater, gastrointestinal fluids, glomerular filtrate, bile) is due to transporting epithelia that can adjust their specific permeability to H\textsubscript{2}O, H\textsuperscript{+}, Na\textsuperscript{+}, K\textsuperscript{+}, Ca\textsuperscript{2+}, and Cl\textsuperscript{−} over several orders of magnitude and exchange substances with the outer milieu with exquisite precision. This exchange is due to the polarized expression of membrane proteins, among them Na\textsuperscript{+}-K\textsuperscript{+}-ATPase, an oligomeric enzyme that uses chemical energy from ATP molecules to translocate ions across the plasma membrane of epithelial cells. Na\textsuperscript{+}-K\textsuperscript{+}-ATPase presents two types of asymmetries: the arrangement of its subunits, and its expression in one pole of the epithelial cell (“polarity”). In most epithelia, polarity consists of the expression of Na\textsuperscript{+}-K\textsuperscript{+}-ATPase towards the intercellular space and arises in part from the interaction of the extracellular segment of the β-subunit with another β-subunit present in a Na\textsuperscript{+}-K\textsuperscript{+}-ATPase molecule expressed by a neighboring cell. In addition to enabling the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase to transport ions and water vectorially, this position exposes its receptors to ouabain and analogous cardiotonic steroids, which are present in the internal milieu because these were secreted by endocrine cells.

Na\textsuperscript{+}-K\textsuperscript{+} pump; epithelial transport; ouabain; tight junction; gap junction; desmosomes; cilium; polarity

THE NECESSITY OF AN ION PUMP started to be recognized 65 years ago, when it was realized that certain asymmetric distribution of ions across the cell membrane could not be accounted for by fluxes driven by concentration gradients or by electrical potentials across the membrane. At that time, the plausibility of a pump began to be supported by increasing and compelling physiological as well as pharmacological evidence, but it received definitive support when Jens Christian Skou (95) demonstrated that the membrane enzyme Na\textsuperscript{+}-K\textsuperscript{+}-ATPase has most of the properties required to transport Na\textsuperscript{+} and K\textsuperscript{+} in opposite directions across the plasma membrane, using the energy released by the hydrolysis of ATP. Skou’s enzyme is specifically inhibited by ouabain and analogous cardiac glycosides. Today, we already know its crystal structure, the chemical composition as well as spatial arrangement of its three subunits (α, β, and γ), the relationship between ATP hydrolysis and ion movement, and several diseases related to its malfunction. The α- and β-subunits are required for ATP hydrolysis and ion pumping (10), and the γ-subunit is required for the modulation of pump activity (4). Amazingly, despite such detailed information, we keep finding new remarkable properties and physiological roles of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase. The present article is devoted to three newly found properties: its expression at the lateral membrane of epithelial cells due to the self-adhesive property of the β-subunit, its role as hormone receptor, and its ability to modulate several types of cell contacts.

The Polarized Distribution of the Na\textsuperscript{+}-K\textsuperscript{+}-ATPase

The study of the intracellular routes and sorting compartments involved in apical-basolateral plasma membrane protein distribution in epithelial cells has shown that apical and basolateral plasma membrane proteins synthesized in the endoplasmic reticulum are sorted in the trans-Golgi network into different carrier vesicles for delivery to their corresponding plasma membrane domains (21–23, 102). For each of these routes, polarity depends critically on the existence of specific signals encoded within the membrane proteins themselves. Once at the target domain, the asymmetric distribution of membrane proteins is reinforced by selective retention either by anchoring to components of the cytoskeleton, clumping in the same plasma membrane, or, as it happens with most cell adhesion molecules, by molecular homotypic or heterotypic interactions between adjacent cells (11, 23, 47, 56, 101).

Na\textsuperscript{+}-K\textsuperscript{+}-ATPase has been shown to be polarized to the basolateral membrane of most epithelial cells (Fig. 1, A and B).
This polarization is so resilient that, in cells harvested with trypsin-EDTA, Na⁺-K⁺-ATPase randomizes and stays homogeneously distributed over the whole plasma membrane, even in cells reseeded at confluence and kept in the absence of Ca²⁺, but regains its typical lateral polarization upon addition of this ion (24, 33, 36, 53, 94). Addition of Ca²⁺ to cells that have randomized membrane proteins provokes a quick assembly of tight junctions (TJ) that poses a barrier to the lateral diffusion that precludes a horizontal displacement of the Na⁺-K⁺-ATPase over the plasma membrane (31).

The basolateral sorting signals are short peptide sequences most often found within the cytoplasmic domain of the membrane protein. Some basolateral sorting signals resemble endocytic signals, e.g., variations of the canonical endocytic dileucine, YXXΦ and NPXY motifs (51). Other basolateral signals are unrelated to endocytic signals, e.g., the tyrosine motifs in the low-density lipoprotein receptor (76) and the G protein of the vesicular stomatitis virus (103).

Early studies demonstrated that the Na⁺-K⁺-ATPase, composed of α- and β-subunits, is sorted in the trans-Golgi network and delivered directly to the basolateral membrane without significant appearance at the apical surface in certain strains of Madin-Darby canine kidney (MDCK) cells (17, 54, 114). Therefore, a basolateral signal was assumed to exist in the α-subunit of the Na⁺-K⁺-ATPase (81). Yet no sorting signal for the polarized distribution of Na⁺-K⁺-ATPase was identified, a situation that prompted us to propose (26) and demonstrate (86, 94) a plausible mechanism, which is depicted in the present article.

The Na⁺-K⁺-ATPase and H⁺-K⁺-ATPase are highly homologous ion pumps, yet in LLC-PK1 cells the former is polarized to the basolateral domain whereas the latter is localized to the apical plasma membrane. To localize the sorting signals of these ion pumps in LLC-PK1 cells, Caplan's group examined the polarized expression of chimeric constructs of the α-subunit of the H⁺-K⁺-ATPase and the Na⁺-K⁺-ATPase. They identified apical sorting information within the fourth transmembrane domain of the α-subunit of the H⁺-K⁺-ATPase and the Na⁺-K⁺-ATPase. This information resides in the fourth transmembrane domain of the α-subunit of the Na⁺-K⁺-ATPase (31). Yet no sorting signal for the polarized distribution of Na⁺-K⁺-ATPase was identified, a situation that prompted us to propose (26) and demonstrate (86, 94) a plausible mechanism, which is depicted in the present article.

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Clathrin plays a fundamental role in basolateral sorting (39). It does not interact directly with endocytic or basolateral proteins but rather through a variety of clathrin adaptor protein complexes (13). The epithelial specific adaptor AP-1B discovered by Bonifacino and coworkers (84) was shown to be required for efficient trafficking of several different proteins to the basolateral plasma membrane (45, 55). Interestingly, the basolateral localization of the Na⁺-K⁺-ATPase is independent of µIB expression because its localization was not signifi-
cantly affected by knocking down clathrin expression (39), and it remained localized to the basolateral surface in the μ1B-deficient cell line LLC-PK1 (40), the kidney proximal tubule, a native epithelium lacking AP-1B (92), and in MDCK cells in which μ1B expression had been suppressed via interfering RNA (55).

Some basolateral proteins travel from the Golgi to recycling endosomes (RE) before their surface delivery (51, 107). Using a novel system for pulse-chase microscopy, Caplan’s group have visualized the post synthetic route followed by a newly synthesized cohort of Na⁺-K⁺-ATPase. They found that the basolateral delivery of newly synthesized Na⁺-K⁺-ATPase occurs via a pathway distinct from that used by the vesicular stomatitis virus G protein (VSV-G). Na⁺-K⁺-ATPase delivery to the surface occurs at a faster rate than that observed for VSV-G. The Na⁺-K⁺-ATPase does not pass through the RE compartment en route to the plasma membrane, and Na⁺-K⁺-ATPase trafficking is not regulated by the same small GTPases as other basolateral proteins. Finally, Na⁺-K⁺-ATPase and VSV-G travel in separate post-Golgi transport intermediates, demonstrating directly that multiple routes exist for transport from the Golgi to the basolateral membrane in polarized epithelial cells (43). Plasma membrane proteins composed of two or more subunits, such as Na⁺-K⁺-ATPase and monocarboxylate transporters, are challenging to study in terms of sorting mechanisms as sorting signals may be present in one or more subunits which act hierarchically and can be recognized by different components of the cellular sorting machinery (18, 89).

While the answer to how the Na⁺-K⁺-ATPase achieves polarized distribution remains elusive, several clues have emerged on how this is accomplished. The first clue comes from the observation that the enzyme is not expressed in the apical or the basal domains but at the lateral membrane only (34, 59, 94) (Fig. 1, A and B, green). The second clue is that when epithelial monolayers of MDCK cells are treated with calcium chelating agents, cells detach from one another, each when epithelial monolayers of MDCK cells are treated with other agents, such as aminophylline or the μ1B subunit of Na⁺-K⁺-ATPase (49). Taken together, these clues indicated that this subunit is an adhesion molecule. In keeping with this attribute of the β-subunit, we have demonstrated that Chinese hamster ovary (CHO) cells transfected with the canine β1-subunit of Na⁺-K⁺-ATPase increase their tendency to attach to each other and form aggregates (Fig. 1F) (94). We also showed that β1-subunit immobilized on Ni beads could specifically bind to the soluble extracellular domain of β1-subunits of the same animal species (dog), and that β1-subunits of neighboring epithelial cells interact directly with each other, as assayed by fluorescence resonance energy transfer (FRET) and coimmunoprecipitation (Co-IP). Using cocultures of MDCK and CHO cells transfected with the canine β-subunit (CHO-β), we showed that the Na⁺-K⁺-ATPase of MDCK cells is now polarized to the lateral border even when the neighboring cell is of another species (94) (Fig. 1G). In crystal structures of the Na⁺-K⁺-ATPase, the β-subunit is mostly exposed towards the intercellular space, while most of the α-subunit is contained in the cytoplasm (80, 93). This position of the β-subunit favors the β-β association between Na⁺-K⁺-ATPases of adjacent cells at the intercellular space. On the basis of this structure, Karlsh’s group has confirmed that the β-subunit ectodomain contains an immunoglobulin-like structure that would be responsible for its adhesive properties (5). In this respect, several studies in mammals have shown that polarized targeting of the Na⁺-K⁺-ATPase in transporting epithelial cells is related to the expression of the β-isofrom. Hence, basolateral targeting is related to the expression of β1 and β2 while apical targeting is related to expression of the β2-isoform (15). Thus, it has been shown that in autosomal dominant polycystic kidney disease (ADPKD) the Na⁺-K⁺-ATPase is mislocalized to the apical surface (110). It was suggested that this is due to the inappropriate expression of the β2-isoform, and this idea was supported by the apical localization of heterologous expression of chicken β2- or human β2-subunits in MDCK cells (109). Nevertheless, similar expression of human β2 in MDCK cells by other groups resulted in basolateral delivery of the pump. This inconsistency has been clarified by Laughery and coworkers (67), who showed that the apical localization of β2-isoform in MDCK cells is due to treatment of the cells with butyrate. On the other hand, human gastric adenocarcinoma cell line (HGT-1) the pump is localized at the apical membrane domain and is constituted of the β2-isoform. When the β1-isoform is expressed in this cell line, the pump is delivered to the basolateral domain (106).

On these bases and owing to the close β-β association (48), we proposed that the whole enzyme is anchored to the intercellular borders of this space as schematized in Fig. 1H. This arrangement was further demonstrated by protein-protein interaction assays by Co-IP and FRET experiments (86, 94).

This basic mechanism of lateral polarization seems to be reinforced through interactions with extracellular ligands or with intracellular scaffolds such as cytoskeletal elements or arrays of PDZ domain-containing proteins (30, 77). Na⁺-K⁺-ATPase has been shown to be retained at the basolateral membrane domain through binding to the ankyrin-fodrin cytoskeleton (59). Ectopic expression of E-cadherin in nonpolarized fibroblasts induces the assembly of a membrane cytoskeleton at cell-cell contact sites and leads to a restricted localiza-
tion of Na\(^+\)-K\(^+\)-ATPase to these sites (78); however, when a truncated E-cadherin form lacking the catenin-binding domain was expressed, neither fodrin nor Na\(^+\)-K\(^+\)-ATPase was localized to cell-cell contacts. Moreover, overexpression of ankyrin-binding and actin-binding domains of β-spectrin results in highly abnormal cells lacking polarized distribution of the Na\(^+\)-K\(^+\)-ATPase (28). Recent data indicate that ankyrin also has a role in vesicular traffic. The ankyrin-binding domain of the α\(_1\) subunit of Na\(^+\)-K\(^+\)-ATPase facilitates the entry of the α\(_1\)β\(_1\) dimer into the secretory pathway (100), indicating that the adapter protein ankyrin acts not only at the plasma membrane but also early in the secretory pathway to facilitate the intracellular trafficking of α\(_1\) and presumably other selected proteins. Conversely, intracellular Na\(^+\)-K\(^+\)-ATPase seems to be a key player in the regulation of endosomal pH and therefore in membrane traffic (44).

Studies in Drosophila have also shown that the β-subunit is a key determinant of the Na\(^+\)-K\(^+\)-ATPase subcellular localization as well as function. Of the three Na\(^+\)-K\(^+\)-ATPase β-subunits, Nrv1 and Nrv2 isoforms are localized in epithelia, while Nrv3 is expressed in the nervous system. Interestingly, Nrv1 is localized in the basolateral domain of almost all epithelial cells; by contrast, Nrv2 is expressed at the septate junctions (SJs) (the insects’ analog of the vertebrate TJ) and colocalizes with the SJ marker coracle. Moreover, it has been shown that Nrv2 controls, by its extracellular domain, the SJ functionality and the tracheal tube size in a pump-independent function (87).

In summary, the fact that the α- and β-subunits have a high affinity for each other and stay intimately associated after their synthesis, together with the fact that the extracellular moieties of β-subunits also have a high affinity for each other, indicates that the whole dimer Na\(^+\)-K\(^+\)-ATPase anchors to the lateral border of epithelial cells such as MDCK. As demonstrated with FRET analysis, the two external moieties belonging to neighboring cells achieve a close proximity (<10 nm) (62). This has profound functional consequences for the overall transport of Na\(^+\) in the inward direction, because Na\(^+\) pumped into the intercellular space can only diffuse towards the blood side, because of the fact that the outermost end of this space is closed by the TJ (Fig. 1H).

Hormone Ouabain Modulates Cell Contacts

The high affinity of ouabain for Na\(^+\)-K\(^+\)-ATPase led some investigators to suspect that there must be an endogenous analog. In 1991, Hamlyn et al. (57) and Mathews et al. (74) demonstrated the presence of a substance in plasma that they could not distinguish from ouabain of vegetal origin, a finding confirmed with more advanced methods such as 1H-NMR and mass ionization spectrometry (61, 63, 91, 105). Ouabain belongs to a group of several cardiac steroids circulating in the blood, along with isomers such as hypothalamic inhibitor factor (61, 105) and marinobufagenin, and appears to be the most prominent (7, 50). Marinobufagenin abounds in toad skin (8). Endogenous ouabain is a recently recognized hormone synthesized and secreted by the hypothalamus (105, 113) and the adrenocortical gland (68, 105). It increases during exercise (9), salty meals (73), and in pathological conditions such as arterial hypertension and myocardial infarction (6, 58, 79).

This prompted efforts to discover its physiological role. The last part of this review is devoted to our findings in this respect.

In addition to splitting ATP and pumping Na\(^+\) and K\(^+\), Na\(^+\)-K\(^+\)-ATPase integrates a membrane receptor whose α-subunit provides a binding site for ouabain and associates with molecules such as c-Src and phosphoinositide-phosphate receptor, constituting the signal transduction domain (104, 112). Because of this association, the enzyme also modulates different signaling routes involving ERK1/2 and oxygen-reactive species (3, 32, 111). Na\(^+\)-K\(^+\)-ATPase is able to interact with c-Src even in mutated pumps in which the pumping ability is suppressed (69).

Toxic levels of ouabain (≥200 nM) cause retrieval of molecules involved in cell-cell and cell-substrate contacts from the plasma membrane, resulting in cell detachment of MDCK cells (32, 33, 37). It also translocates β-catenin, an adherent junction-associated molecule, to the nucleus. It is possible to prevent this toxic effect of ouabain by coculturing wild-type MDCK (W-MDCK) cells with ouabain-resistant cells. In this case, it was observed that 1.0 μM ouabain promoted cell-cell communication through gap junctions (33). This suggested that the Na\(^+\)-K\(^+\)-ATPase is deeply involved in cell adhesion, a role further confirmed by other groups (90, 106). The possibility existed that, at concentrations below 200 nM, ouabain would influence several signaling routes triggered by this enzyme. On these bases we explored the effect of 10 nM ouabain on a series of cell contacts and contact-associated processes. This concentration is within the range found in humans under physiological and pathological conditions (9) and neither inhibits K\(^+\) pumping nor disturbs the K\(^+\) balance of the cell (65, 88). Hence, any effect of ouabain observed at 10 nM would be suspected of mimicking a hormonal effect.

Effect of ouabain on tight junctions. The TJ is a particular type of cell-cell contact that seals the intercellular space between epithelial cells, thereby transforming the layer of cells into an effective permeability barrier (19). Together with apical/basolateral polarity, TJs are a major component of the so-called “epithelial transporting phenotype” (21, 22, 25). We demonstrated that ≥200 nM ouabain opens the TJ and causes a drastic drop of TER, yet at 10–100 nM it has the opposite effect, as it increases the degree of sealing of the TJ (Fig. 2A) and decreases the paracellular flux of neutral 3 kDa dextran (J\(_{DEX}\)). This modulation is accompanied by changes in the levels and distribution patterns of specific claudins, each one describing a different kinetics [claudins (cldn) 1, 2, and 4; Fig. 2B]. While TER depends on c-Src and partially on ERK1/2, J\(_{DEX}\) depends on ERK1/2 but not on c-Src (65). The increase in cldn-1 depends on c-Src and ERK1/2, but that of cldn-4 depends on an as yet unidentified signal molecule and ERK1/2.

Effect of ouabain on ciliogenesis. The cilium is a slender protuberance polarizedly expressed at the center of the apical domain of most eukaryotic cells (96, 99). Therefore we gauged polarity through the expression of a cilium in MDCK cells (Larre I, Castillo A, Flores-Maldonado C, Contreras RG, Galvan I, Muñoz-Estrada J, and Cereijido M, unpublished observations). Ciliogenesis occurs in quiescent cells and is drastically accelerated by ouabain (Fig. 2, C–E). MDCK cells express cldn-2 at the TJ, where it accounts for specific cation permeation (46, 82). We observed that it can also localize at the cilium where it cannot possibly play a role in permeation, as cilia do not separate two liquid compartments from one an-
other. Nevertheless, claudin-2 may conceivably act as a sensor of Na\(^+\) concentration in the fluid bathing the apical domain. The effect of ouabain on the expression of cldn-2 at the TJ and the cilium involves ERK1/2 (Larre I, Castillo A, Flores-Maldonado C, Contreras RG, Galvan I, Muñoz-Estrada J, and Cereijido M, unpublished observations).

**Effect of ouabain on cell-cell communication.** We have also tested the effect of ouabain on another type of cell-cell contact, i.e., gap junctions, which permit communication between neighboring cells (Fig. 2, E, F, and I). It is difficult to test 1.0 \(\mu\)M ouabain in W-MDCK cells because it detaches cells from one another as well as from the substrate in \(\sim 8\) h. Yet when these cells are cocultured with ouabain-resistant MDCK cells (R-MDCK), they are rescued from the effect of toxic levels of ouabain (12, 20). We observed that in these mixed monolayers, 1.0 \(\mu\)M ouabain promotes cell communication via gap junctions between W-MDCK cells as well as between W and R cells, and that this involves an increase of the specific synthesis of connexin 32 (66). Yet, as said above, 10 nM ouabain affects neither the K\(^+\) flux nor the K\(^+\) balance of the W-MDCK cells, and therefore W-MDCK cells do not need to be rescued. Nevertheless, we observe that even at nontoxic concentrations, ouabain promotes cell communication between W-MDCK cells (Fig. 2, E and F), suggesting that ouabain may have effects on normal tissues that are not necessarily related to rescuing (Romero A, Ponce A, Castillo A, Larre I, Contreras RG, and Cereijido M, unpublished observations). Of course, cell-cell communications have been observed in many cell functions other than the rescue of cells from toxic levels of ouabain. Thus Loewenstein and Kanno (72) found that liver cells communicate intercellularly, and that this communication is progressively lost in hepatic cancer cells in a direct proportion to their degree of dedifferentiation. While this tends to show an interesting correlation between communication, differentiation, and cancer, Ozawa et al. (85) have found that connexins have an antiproliferative role even when they are not expressed at gap junctions in the plasma membrane. However, it seems premature to elaborate on the uses of ouabain stimulation of the synthesis of connexins.

![Diagram of cell-cell communication](https://example.com/diagram.png)

**Fig. 2.** Ouabain modulates different types of cell contacts. A: at low concentrations (<100 nM), ouabain increases the hermeticity of the tight junction, whereas at higher concentrations (>200 nM), it completely relaxes it, as evidenced by the transepithelial electrical resistance (TER). B: ouabain (10 nM) modifies TER by modulating the amount as well as the pattern of distribution of individual claudins (not shown). AU, arbitrary units; cldn, claudin; occl, occludin. C and D: monolayers of MDCK cells stained with an antibody against \(\alpha\)-acetylated tubulin (red) 48 h after plating at confluence. Prociila can be barely seen in a control monolayer, whereas treatment with 10 nM ouabain accelerates ciliation and increases the length of cilia. E: a glass impaling microelectrode injected 10 kDa dextran (green) into a cell that, because of its molecular size, remained in the impaled cell. The same microelectrode was used to inject neurobiotin, which diffuses to neighboring cells provided that these are connected via gap junctions, and can be observed once the monolayer is treated with fluoresceinated avidin (red) 48 h after plating at confluence. Procilia can be barely seen in a control monolayer, whereas treatment with 10 nM ouabain accelerates ciliation and increases the length of cilia. F: a glass impaling microelectrode injected 10 kDa dextran (green) into a cell that, because of its molecular size, remained in the impaled cell. The same microelectrode was used to inject neurobiotin, which diffuses to neighboring cells provided that these are connected via gap junctions, and can be observed once the monolayer is treated with fluoresceinated avidin (red). G: a glass impaling microelectrode injected 10 kDa dextran (green) into a cell that, because of its molecular size, remained in the impaled cell. The same microelectrode was used to inject neurobiotin, which diffuses to neighboring cells provided that these are connected via gap junctions, and can be observed once the monolayer is treated with fluoresceinated avidin (red). H: a glass impaling microelectrode injected 10 kDa dextran (green) into a cell that, because of its molecular size, remained in the impaled cell. The same microelectrode was used to inject neurobiotin, which diffuses to neighboring cells provided that these are connected via gap junctions, and can be observed once the monolayer is treated with fluoresceinated avidin (red). I: a glass impaling microelectrode injected 10 kDa dextran (green) into a cell that, because of its molecular size, remained in the impaled cell. The same microelectrode was used to inject neurobiotin, which diffuses to neighboring cells provided that these are connected via gap junctions, and can be observed once the monolayer is treated with fluoresceinated avidin (red).
Effect of ouabain on adherens junctions. The tight junction is a very special form of cell-cell adhesion; therefore our demonstration that the hormone ouabain modulates this structure can be taken as a straightforward indication that it affects cell adhesion. Nevertheless, we have observed that ouabain also modulates adherens junctions. One of the scaffolding proteins of this junction is β-catenin, a key member of the Wnt signaling pathway. During the activation of this pathway (29), a condition that can be achieved by changes in cell adhesion (75), β-catenin is translocated to the nucleus, where it modifies gene expression. We have observed that 10 nM ouabain, as well as 1 μM (33), provokes the translocation of β-catenin to the nucleus (Fig. 2, G–I). In this regard, Na$^{+}$-K$^{+}$-ATPase not only sends β-catenin to the nucleus but is activated by the overexpression of β-catenin in oocytes (98). As previously shown by Nelson and coworkers (83), Liu et al. (71) have recently found evidence that Na$^{+}$-K$^{+}$-ATPase and E-cadherin are closely associated, indicating that the pump might be a component of the adherens junction.

In summary, we have seen that ouabain, at nontoxic concentrations, does affect a number of different types of cell contacts: TJs, gap junctions, adherens junctions, and even ciliogenesis, a step in differentiation that depends on arrest of cell growth by contacting neighboring cells.

Na$^{+}$-K$^{+}$-ATPase As a Receptor for Ouabain

In addition to its role as transducer receptor, Na$^{+}$-K$^{+}$-ATPase itself is the target for a variety of signaling pathways that regulate its activity or expression in the plasma membrane as a result of stimuli as diverse as dopamine (27), hypoxia (108), ouabain (35, 66, 70), or salt (60). Ouabain only elicits the effects described above on the different types of cell contacts when added from the basal side, where it has easy access to the extracellular side of Na$^{+}$-K$^{+}$-ATPase (Fig. 2J) (64). The possibility that the receptor for the hormone ouabain is the same site of the enzyme where it acts at toxic levels is supported by the fact that it has no hormonal or toxic effects on cell growth by contacting neighboring cells.

The two basic features of the transporting epithelium phenotype (TEP) are TJs and apical/basolateral polarity. The fact that the hormone ouabain modulates TJs as well as cilia, which are a polarized feature of epithelial cells, suggests that ouabain specifically influences the TEP, which is essential to allow the existence of metazoan life.

Horizons and Perspectives

Na$^{+}$-K$^{+}$-ATPase is a multifunctional protein that, in addition to pumping ions asymmetrically, participates in cell contacts, acts as specific receptor for the hormone ouabain, and transduces extracellular signals. As illustrated in Fig. 2, Na$^{+}$-K$^{+}$-ATPase also participates in the modulation of cell contacts, the importance of which can hardly be overestimated, as the brain, the most complex object in the universe, assembles itself depending on which cells contact which other cells, when and how (i.e., the type of synapses established). Furthermore, cell contacts are crucially involved in gene expression, cell cycling, proliferation, differentiation, migration, tissue architecture, cancer, and metastases, suggesting that Na$^{+}$-K$^{+}$-ATPase and the hormone ouabain will be soon shown to play a major role in human health.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.C. conception and design of the research; M.C., R.G.C., L.S., and I.L. analyzed the data; M.C., R.G.C., L.S., and I.L. interpreted the results of the experiments; M.C. and R.G.C. prepared the figures; M.C. drafted the manuscript; M.C., R.G.C., L.S., and I.L. edited and revised the manuscript; M.C. approved the final version of the manuscript; L.S. and I.L. performed the experiments.

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