PHYSIOLOGICAL ROLE OF HORMONE OUABAIN

M. Cereijido[1,]*, A. Ponce[1], M. I. Larre[1], A. Castillo[1] and L. Hinojosa[1]

[1]Center for Research & Advanced Studies (Cinvestav), México D.F.

***Correspondence to:**
Dr. Marcelino Cereijido (cereijido@fisio.cinvestav.mx)

ABSTRACT
Ouabain, a substance obtained from plant extracts, has been long known as cardiotonic, as well as by its high affinity to the Na-K- ATPase pump. The recent finding that ouabain is endogenously expressed in mammals has prompted research to determine its role as a hormone. We have shown that, in a physiological level (10 nM), it influences on three important features of epithelial physiology related to cell to cell contacts: (1) It modifies the Tight Junction integrity, as reflected by an increase in the Transepithelial Electrical Resistance (TER) of mature monolayers of MDCK cells. (2) It speeds up ciliogenesis, a feature closely related to epithelial (Apical/Basolateral) polarity and (3) It triggers Gap Junctional Intercellular Communication (GJIC). Since lack of GJIC has been associated to cancer, ouabain may be an interesting player on this issue.

Keywords: Ouabain, epithelia, tight junctions, gap junctions, connexins, cancer

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Introduction

Cells have an intense exchange of substances with the environment

When an organism consists of a single cell, and its environment is the whole ocean (**Figure 1A**), it cannot exhaust its nutrients nor pollute the immense amount of sea water (*external milieu*) with its wastes, regardless of how intense is the exchange. When the cell belongs instead to a multicellular organism, say a neuron in the brain (**Figure 1B, C**), the ocean is replaced by an extremely narrow extracellular space (*black*), less than a micron thick, that would be quickly exhausted and spoiled, were it not for a circulatory apparatus that shuttles the extracellular fluid to and from transporting epithelia, where the exchange with the outer milieu finally takes place. To give an idea of the amount and type of transporting epithelia involved in this exchange, the area of some of them is scaled with the silhouette of a man (**Figure 1D**). In turn, the exchange between blood and cells proceeds across endothelia, which have basically the same phenotype as epithelia, and occupy an even larger area (**Figure 1E, *pink***). **Fig 1F** depicts a transversal section of a nephron, i.e. a transporting epithelium, that is a continuous layer of cells with the shape of a hollow cylinder enclosing the lumen. Interestingly, in spite of this lumen running along the central axis of the tube, it is considered to be full of "external milieu" because it connects directly with the fluid bathing the environment of the animal.

Figure 1

The five cells in fig 1F illustrating a segment of the wall of the kidney tube, exhibit the two fundamental differentiated features of transporting epithelia: ***tight junctions*** (*red* belt of filaments) that encircle every epithelial cell at the outermost end of the intercellular space, thereby restricting the passage of substances between the cells through the so called "paracellular route" (*red arrow*) (Cereijido et al, 1988; 1989a), and ***apical/basolateral polarity***, i.e. a sharp difference in structure, chemical composition, and physiological properties between the domain of the cell membrane facing the lumen ("apical"), and the domain facing the blood side ("basolateral")

(Cereijido et al, 1989b). Most of the substances that are metabolically important (e.g. glucose, aminoacids, bicarbonate, vitamins) cross the cells by the "transcellular route", penetrating firstly into the cytoplasm (*gray*) through the apical domain, then the basolateral domain (*yellow arrows*).

Endothelia, epithelia and the fluids they separate

The body of metazoans contains aqueous compartments as big as the extracellular fluid (some 15 liters in humans), and as little as a kidney tube, and the acinus of a salivary gland (nano to microliters). The composition of the fluids contained in the lumen is in a steady state, kept through an intense but balanced influx and outflux of water, glucose, amino acids, ions, vitamins, etcetera. Transporting epithelia have to withstand the difference between the fluids bathing their two sides. The body side of a transporting epithelium always faces the interstitial fluid, which always has the same composition, but in the lumen it varies widely: urine in the urogenital tract, milk in the mammary glands of a breastfeeding woman, saliva in the salivary glands, bile in the gall bladder, intestinal content in a progressive degree of digestion, pancreatic juice, etc. Therefore, the steepness of the gradient is not the same when it contains a limpid glomerular filtrate, as in the proximal tube of the nephron, than when it is aggressive acid as in the stomach. Accordingly, the electrical resistance of the tight junction (TER) of the proximal tube of the nephron that has interstitial fluid (i.e. almost-plasma) on its outer side, and recently-filtrated-plasma in its lumen is merely 5-10 $\Omega.cm^2$; but the mucosa of the urinary bladder, that is interposed between interstitial fluid and urine in the lumen, reaches up to 100.000 $\Omega.cm^2$. A transporting epithelium has two permeation routes: one that proceeds firstly across the apical, then across the basolateral membrane (***trans***-cellular route), and another that bypasses the cell, but cannot avoid crossing the TJ (***para***-cellular route). The electrical resistance along the ***trans***-cellular route has almost the same resistance/permeability in all transporting epithelia, but the ***para***-cellular varies broadly. Just think of an endothelium so hermetic that does not allow the passage of a small sized molecule of toxins (in the order of picometers), but suddenly relaxes and lets through a huge macrophage (in the order of 10 micrometers, i.e. seven orders of magnitude bigger) en route towards a tissue infected with bacteria. This reveals that the organism has mechanisms to ***sense*** somehow the magnitude of the gradient, as well as mechanisms that regulate the permeability of the TJ accordingly. This insures that there must exist ***endogenous*** substances (i.e. not pharmacological ones) responsible for the degree of tightness. How could we learn about them?

To answer this question, we generated a series of working hypothesis and designed experiments to substantiate them. ***First hypothesis***: the plasma just-filtrated at the glomerulus contains a hypothetical substance (let's call it "S") with the ability to increase the hermeticity of the TJ in a concentration-dependent manner. ***Second hypothesis***: that as water is reabsorbed, the concentration of substance "S" in the lumen increases. ***Third hypothesis***: if this imaginary substance maintains its activity along the whole nephron, chances are that it will reach the urinary bladder and appear in the urine. Accordingly, we collected urine of a dog fractionated it on the basis of molecular weights. We called these extracts DLU (Dialyzed and Lyophilized Urine), and assayed them on the TER of a monolayer of MDCK cells (**Figure 2A, B**) mounted as shown in fig. 2B, in a transwell. We then took the extract of 30 kDa (the tallest column if **Figure 2A**) and subject the monolayers to a higher and higher concentration of this extract. In a series of monolayers DLU was added to the apical chamber (upper curve), and in another series to the basolateral side (lower one). Curiously enough, although the

hypothetical substance reaches the apical domain of the epithelial cells, the extract is more effective when added to the basolateral one (Gallardo et al.,1992). Epithelial cells have the ability to take molecules in the fluid bathing one side and deliver it on the opposite side ("transcytosis"). A familiar example is given by antibodies contained in maternal milk, that mucosal cells of the intestine of the baby transcytose towards his blood, thereby protecting his health until his own immune system matures and gets ready to take over.

As a coarse first characterization, we processed the extract to a series of treatments before assaying it on monolayers of MDCK cells (**Figure 2D**). White column indicates the spontaneous TER of the monolayers; the first gray column when treated with the 30 kDa DLU, the second column, gray, shows the TER-increasing power when DLU was attacked by a protease and boiled for 10 min. Since heat and protease cancel the TER-increasing effect, we suspected that the active substance is a peptide. To find it out, in the third, gray, experiment the extract was boiled but not treated with protease. The peptide resisted heating and increased TER from 200 to 400 Ωcm^2. In fact, since there TER-increasing substances are also found in other extracts we anticipated that the filtrated fluid in the nephron may have a variety of such substances. The essence of physiological regulations is that whenever the organism has a substance that enhances a given function, it also has another that decreases the same function. It is convenient to have these ideas in mind, because one would say that, for instance, the fraction signaled by the green arrow (**Figure 2A**) contains no TER-modifying substances. However we cannot exclude the possibility that it contains two very powerful ones, one increasing and the other decreasing TER in an equivalent amount. But in those days (before year 2000) methods to separate substances circulating in the blood did not have the power and delicacy to encourage us to keep working along that line. Nevertheless, we made some promising observations (**Figure 2E**): the active substances contained in the extract of 30 kDa must be universal, as DLU prepared with urine from dogs were active on epithelial monolayers derived dog, humans, rabbit, cat, pig, etc.

Figure 2

Convergence of the studies on urine with those investigating a newly recognized hormone

Figure 3A is an old drawing showing a person suffering dropsy with a marked edema of the legs, and a voluminous abdomen due to ascites (accumulation of fluid in the peritoneum). Physicians tried to drain the fluid accumulated through holes pocked in her belly, legs and feet. Dropsy is a symptom that may have different causes; one of the most common is heart failure: the tricuspid valve, on the right hand side of the heart, closes during systole, preventing the return of blood from the right ventricle to the auricle. When this valve fails the organism receives from the heart a smaller amount of blood that it needs, and sends signals that force this organ to overwork and compensate. When the failure worsens the heart "attempts" to make itself stronger by increasing the amount of myocardium (hypertrophy), a condition in which the heart is no longer able to pump out enough oxygen-rich blood and pathological consequences ensue. Although in 1771 William Withering (**Figure 3B**) ignored the existence of such hemodynamic situation, he prescribed a tea of foxglove[1], a local plant that, chemistry demonstrated in the following century, contains ouabain[2], that for this reason became a celebrate cardiotonic (**Figure 3C**) (Schatzmann, 1953). In the last two decades it was demonstrated that ouabain is not only produced by plants, but there is an endogenous one synthesized in the hypothalamus and the suprarenal glands, that fulfills all requirements to be classified as a "new" hormone (**Figure 3D,E,F**) (Schoner et al., 2003). Quotation marks indicate that the hormone must be millions of years old, what is ***new*** is the recognition of its hormonal status. Its concentration in humans is around 1-10 nM under physiological conditions, but it increases under physiological conditions (i.e. exercise, salty food, **Figure 3G, I**) (Bauer et al., 2005), as well as pathological, such as arterial hypertension, eclampsia and renal failure (**Figure 3H**).

Figure 3

[1] So called because children use to put a flower in the tip of each finger, and imagine that they wear the gloves of a fox. Even today the official Latin name, *Digitalis purpurea*, evokes the old, ingenuous origin.

[2] From Somali waabaayo, "arrow poison" through French ouabaïo, also known as g-strophanthin, is a plant derived toxic substance that was traditionally used as an arrow poison in eastern Africa for both hunting and warfare.

Given that ouabain is a recently recognized hormone: what is its physiological role?

Figure 4A shows a Petri dish with a confluent monolayer of MDCK cells, that maintain a high concentration of K^+, and low of Na^+ and Ca^{2+} (**Figure 4B**). Ouabain has an exquisite affinity for the Na^+,K^+-ATPase, that (in the micromolar range of concentrations) inhibits ATP hydrolysis, which in turn abolishes ion pumping; cytoplasmic K^+ decreases whereas Na^+ and Ca^{2+} increase, causing cell death (**Figure 4C**). Physicians prescribe smaller doses that, of course, do not completely stop pumping nor kill the cells of the patients, but in whose pharmacological details we do not need to digress. But alas! in our laboratory we were surprised to discover that toxic levels of ouabain do not ***directly*** nor ***immediately*** kill the cells, only provoke retrieval of molecules involved in cell contacts with neighboring cells and the substrate (represented in green in **Figure 4D,E**), and cells detach from the monolayer (**Figure 4F,G**) (Contreras et al, 1999). Yet, during the time that cells are in suspension, the lack of contact with neighbors activates mechanisms of apoptosis that end up killing them anyhow. Of course the simplest assumption was that the same site in the α-subunit that has affinity for toxic levels of ouabain, would be available to bind ouabain at hormonal levels 100-times lower, only that the strong interaction at toxic levels would cause retrieval of the site from the plasma membrane, while the subtle hormonal levels will be just strong enough to trigger the interaction of Na^+,K^+-ATPase with the first station of a signaling pathways including cSrc, later on ERK1/2 and, finally, to a given type of cell contact. To check whether this is a valid interpretation, we explored the effect of 10 nM ouabain on a series of cell contacts and contact-associated processes.

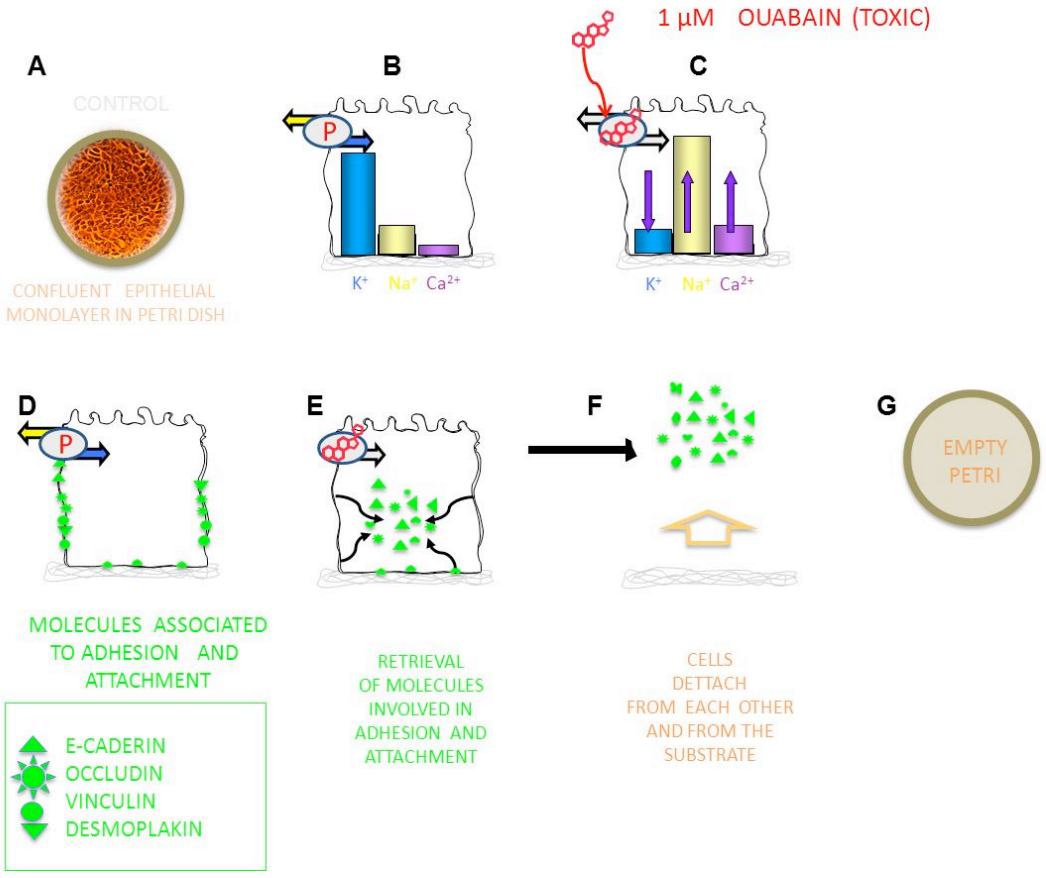

Figure 4

Before we describe the different types of cell attachments on which we tested hormone ouabain, let's mention some features that may be relevant (**Figure 5**). A basic profile of the molecules involved in adhesion/attachment is a transmembrane protein (*red*) with a long extracellular segment profusely glycosylated (*green circles resembling a bunch of grapes*) and a short intracellular one, connected through a segment that traverses the membrane matrix only once (*yellow*). The cytoplasmic segment interacts with highly specific proteins, some of which (*violet*) interact in turn with the cytoskeleton (*red fibers*) thereby anchoring the whole attaching complex to a given point of the membrane. These complexes also contain NACo proteins (from **N**uclear-**A**ttaching-**Co**mplex) so called because they travel from the nucleus to the attaching complex, depending on what the extracellular segment (*red*) is touching. When NACos detach from the complex, they move to the nucleus and penetrate through nuclear pores (Contreras et al., 2004), interact with protein related to the DNA, and turn on (or off) specific genes. Accordingly, the amount and species of messengers RNA that depart from the nucleus can be enhanced or depressed very specifically, because a given type of NACo acts on specific genes.

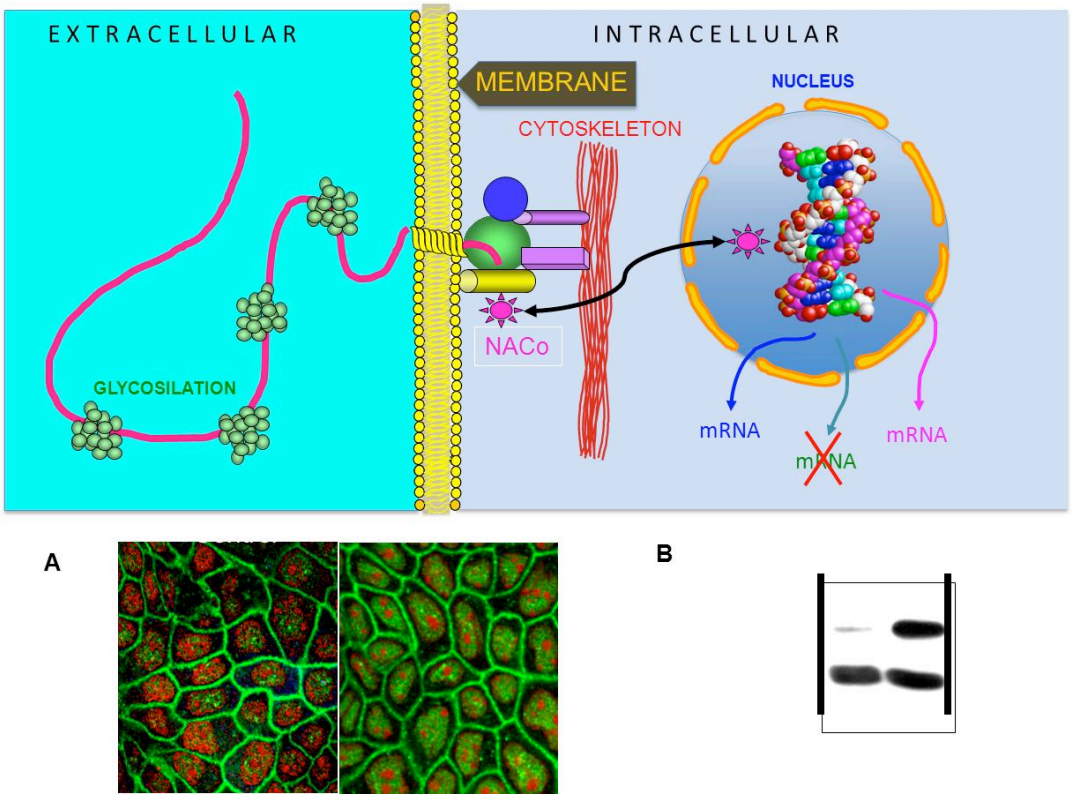

Figure 5

The bottom of **Figure 5A** shows a monolayer of MDCK cells under control (*left*) and 10 nM ouabain treated condition (*right*). Nuclei are treated with propidium iodide to stain them *red*, while β-catenin distribution is shown in *green* after treatment with combination of antibodies. This protein distributes at the periphery of the cells, looking like a chicken fence. Under control conditions, some of the nuclei have been stained with green specks, indicating that β-catenin is a NACo that has reached them. Treatment of the monolayer with 10 nM ouabain promotes changes in the distribution of β-catenin which is partially retrieved from the plasma membrane and sent to the nuclei, which becomes almost completely covered in green. Fig. 5B shows a Western blot analysis of the nuclear fraction of the cells, under control and ouabain challenge.

The size and density of a band reflects the amount of a given peptide. Actin is a molecule which is not expected to change during this experiment, and is only measured to know exactly how much sample was loaded in each track, and used to normalize and compare the different bands. It is clear that the presence of ouabain has drastically increased the amount of β-catenin sent to the nucleus. At this point it is worth remembering that biological signals (in this case β-catenin) do not *instruct* the target how to do something, only tell the target that the moment to do it has arrived.

Hormone ouabain assayed on a first type of cell contact: the tight junction.

Figure 6A shows recordings of TER along 3 days. When ouabain is not present TER exhibits a subtle, non-significant decrease (*open circles*). When the concentration of ouabain in the culture medium was 1 μM, i.e. a toxic one, TER drops to zero (*red circles*); actually cells may be completely detached, suspended and gone, and the support left empty. But with a mere 10 nM ouabain (*light pink circles*) TER shows a significant increment with respect to control monolayers by the third day. At 50 and 100 nM (*pink* and *hot pink* circles respectively) a maximum effect is observed. Actually, in spite of calling it "maximum", curves show no tendency to saturate, indicating that a real maximum has not been reached yet. In a way, this is the only result we expected: 10 nM ouabain does modify the hermeticity of the TJ (Larre et al., 2010). But to gain a little information on the intrinsic mechanism we studied the amount and distribution of ***claudin 1, 2, and 4*** molecules. Claudins constitute a family of tetraspan proteins of TJs, responsible for the permeation of solutes, as well as ***occludin***, a typical TJ protein which is not involved in permeation, in monolayers exposed to 10 nM ouabain. Interestingly, ouabain modulates each claudin isoform independently, with its own kinetics (**Figure 6B**). Thus at the 2nd day claudin-2 (*green*) peaks, but claudin-4 is still at a low level (*blue*). Occludin (*magenta*), which is not a molecule involved in the hermeticity of the TJ, is not sensitive to the presence of 10 nM ouabain and remains constant.

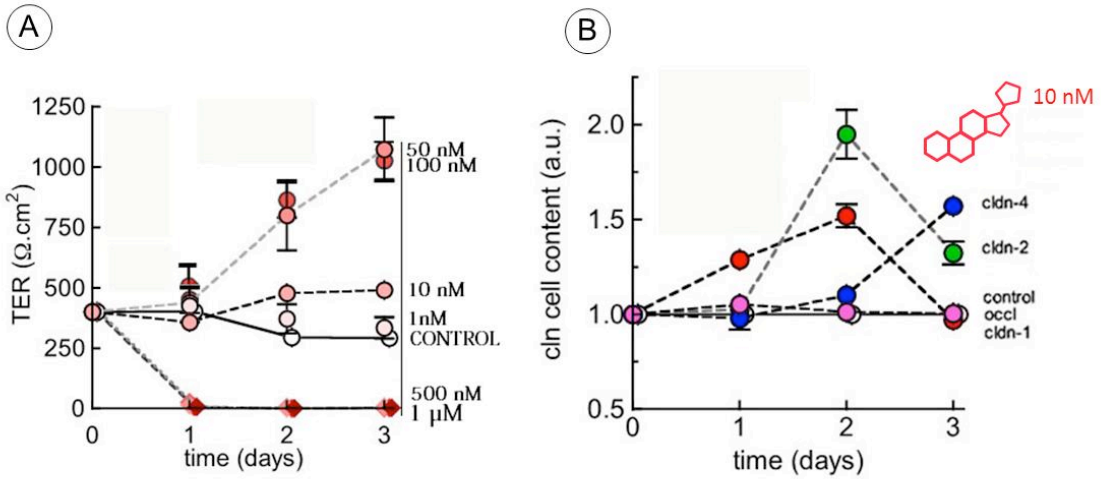

Figure 6

Hormone ouabain assayed on a second type of cell contact: the ones that intervene in the formation of the cilium.

The cilium is an eyelash-like filament that stems from the center of the apical domain of epithelial cells (**Figure 7A**). It is expressed once cells stop proliferating and achieve a considerable degree of packing between them. From then on, cells stop proliferating and start another –more advanced- degree of differentiation, that starts with pro-cilia (*yellow spots*, **Figure 7B**) and culminates with the development of one cilium per cell (**Figure 7C**) (Ishikawa and Marshall, 2011). We compared ciliogenesis in control (*open circles*) and in the presence of 10 nM ouabain (*red circles*), and observed that ouabain significantly accelerates ciliogenesis (Larre et al., 2011). Nevertheless notice that treatment does not alter the fact that each cell has a single cilium.

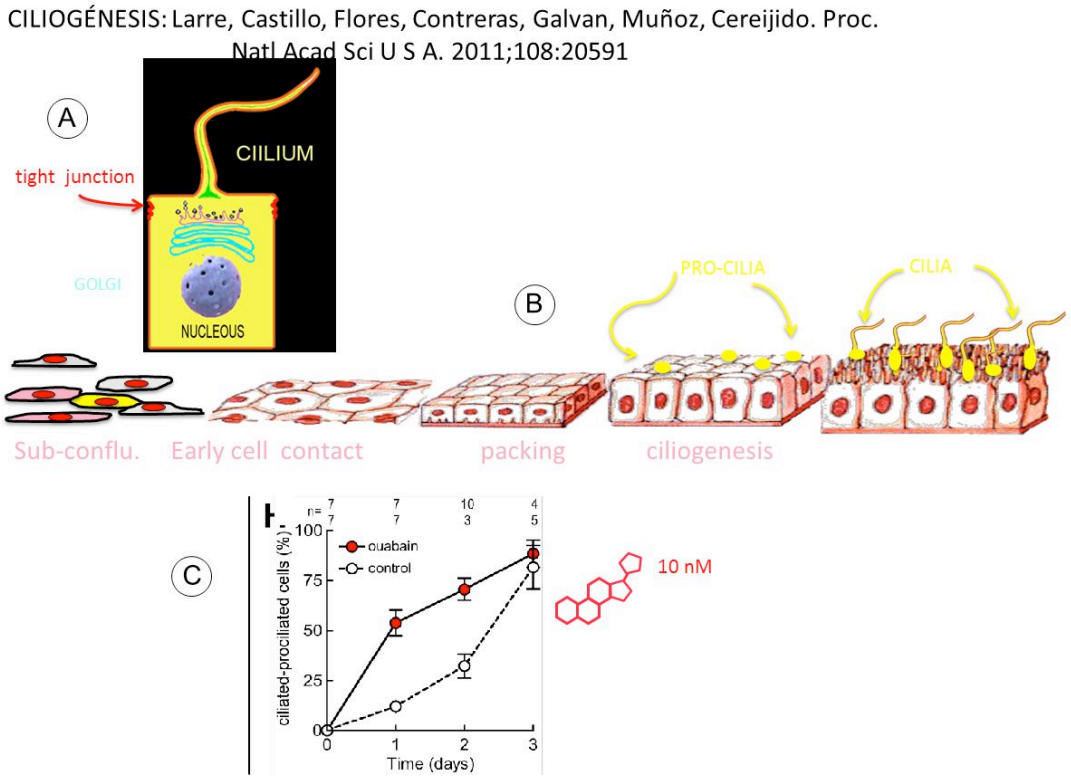

Figure 7

Figure 8 shows another peculiarity of the relationship between claudin-2 and hormone ouabain: at a time when cells express claudin-2, an antibody that stains this claudin-2 in *red*, insures that it is found where expected: at the TJ, exactly above the intercellular space, forming the well-known image of a "chicken fence". A second type of antibody, this time against α-acetylated tubulin, a specific marker of procilia and cilia (*green*), indicates that this structure is not present in the monolayer at this time. Accordingly, the merge of both images shows that claudin-2 seems to be by itself in the chicken fence. The second row of pictures in figure 8 corresponds also to a confluent monolayer of MDCK cells, but incubated with 10 nM ouabain. The main difference with the control condition (*the picture exactly above*), is the presence of a button of claudin-2 (*red*) mixed with α-acetylated tubulin (*green*), so the merge of the two images (*second row, right*) shows the chicken fence of claudin (*red*), as well as a *yellow* button exactly at the center of the apical domain containing both: claudin (*red*) and α-acetylated tubulin

(*green*) i.e. is a pro-cilium that contains claudin. The row of pictures at the bottom of fig. 8 shows a monolayer one day later, i.e. more mature. The *green* arrow corresponding to α-acetylated tubulin indicates that the tenuous filament is a cilium; the *red* arrow on the picture at the left tells us that this cilium has claudin-2, and the *yellow* arrow on the picture on the extreme right, insures that it is in fact a full-fledged cilium containing claudin-2. This is a very surprising finding, because the cilium does not separate two aqueous compartments, so in this position claudin-2 cannot possibly participate in the passage of Na^+ ion through a biological membrane. What other function may claudin-2 fulfill? When it is located at the TJ, claudin-2 intervenes in the permeation of Na^+ because it has a fine affinity for this ion. But, since the cilium does not separates two fluid compartments, the presence of claudin-2 at the cilium suggests that it may be a sensor of the concentration of Na^+ of the fluid bathing the apical domain of the cells. In summary, when ciliated cells in a nephron wave the cilium in the lumen, they may be sensing the concentration of Na^+ in the fluid, a parameter frequently associated to high arterial pressure.

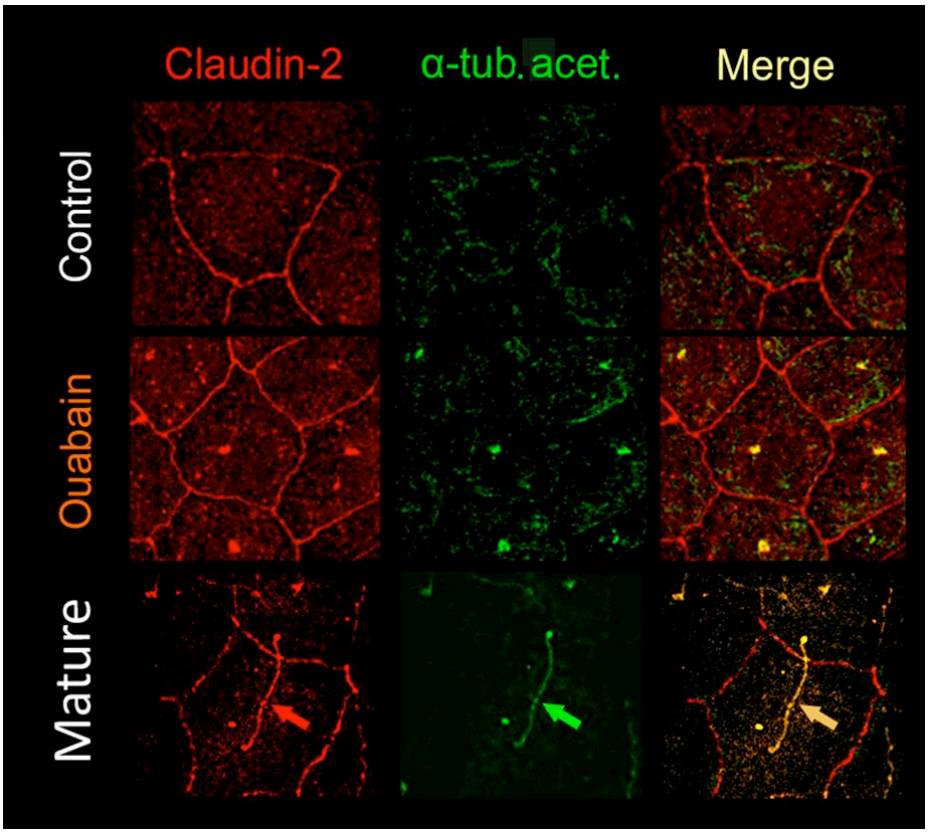

Figure 8

Hormone ouabain assayed on a third type of cell contact: gap junctions

To further test our hypothesis that hormone ouabain modulates cell contacts, we choose a third type of contact: gap junctions that enable the cell to communicate with its neighbors (**Figure 9A**, *red arrows*), through a process called ***gap junctional communication*** (GJC). The plasma membrane of the two neighboring cells approach each other and appear in freeze fracture replicas as two roughly circular areas (**Figure 9B,C**) formed by the tip of hemi-connexons integrated by six proteins (connexins) (**Figure 9D**) that contact a similar hemiconnexon in register belonging to the neighboring cell (**Figure 9E**). When hemi-connexons are simultaneously open, they

form a tube ("connexon") that allows the free passage of small molecules and ions from the cytoplasm of one to the cytoplasm of the neighboring cell. One way of studying this type of cell contact is to use a micropipette to inject Dextran of 220 Daltons tagged with a fluorescent probe (**Figure 10A**). 220 Daltons is far too big to pass through connexons, so it cannot escape from the cell where it was injected. Hence this probe just individualizes the cell that was injected, and at the end of the experiment it certifies that this cell did not burst during the experiment, thereby assuring that the spread of the dye to neighboring cells is due to its passage through connexons. The injected fluid also contains neurobiotin, a molecule small enough to cross freely through open connexons. The procedure is as follows: at the end of the experiment the cell is fixed and permeabilized, then treated with avidin, a substance that has a very specific affinity for the biotin that is part of neurobiotin. The monolayer is then observed with a fluorescence microscope. **Figure 10B** shows a control, and 10C a ouabain treated monolayer. Remember that the monolayer itself cannot be actually seen because this optics only sees fluorescent molecules. In the control monolayer both dyes (*green* fluorescent Dextran and *red* fluorescent avidin merge and appear *yellow*) remain in the injected cell, demonstrating that it has not established connections with its neighbors. In the ouabain-treated monolayer (**Figure 10C**) instead, Dextran has also remained in the injected cell, but neurobiotin by itself has also diffused to several neighboring cells, indicating that ouabain did stimulate connection through GJC (Ponce et al., 2014).

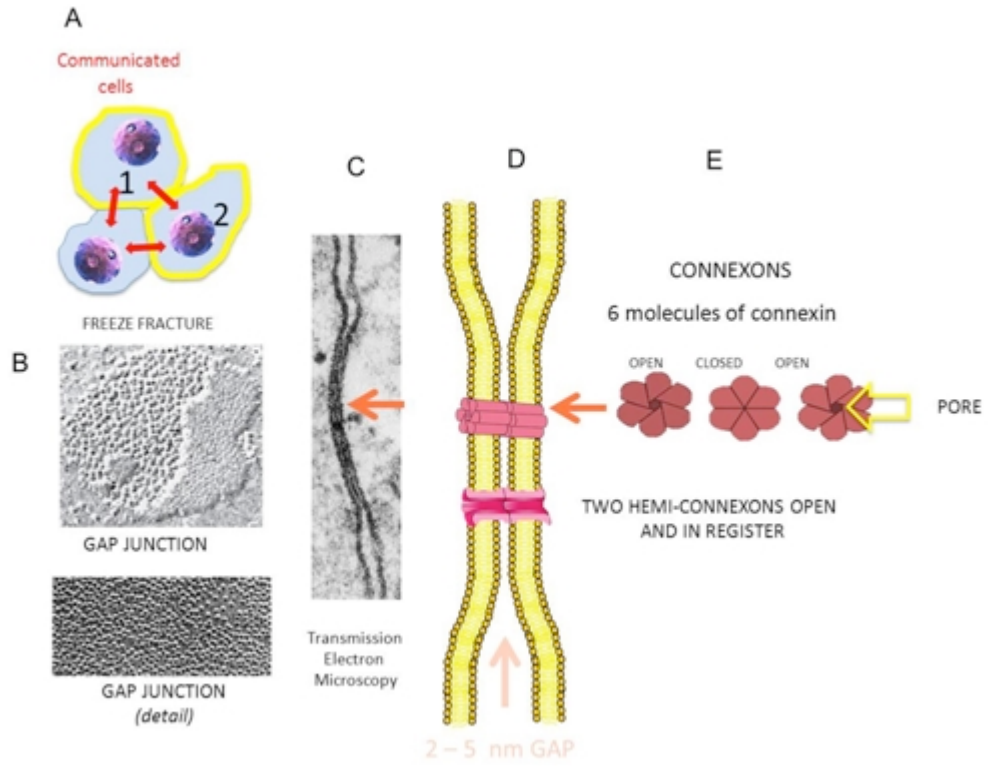

Figure 9

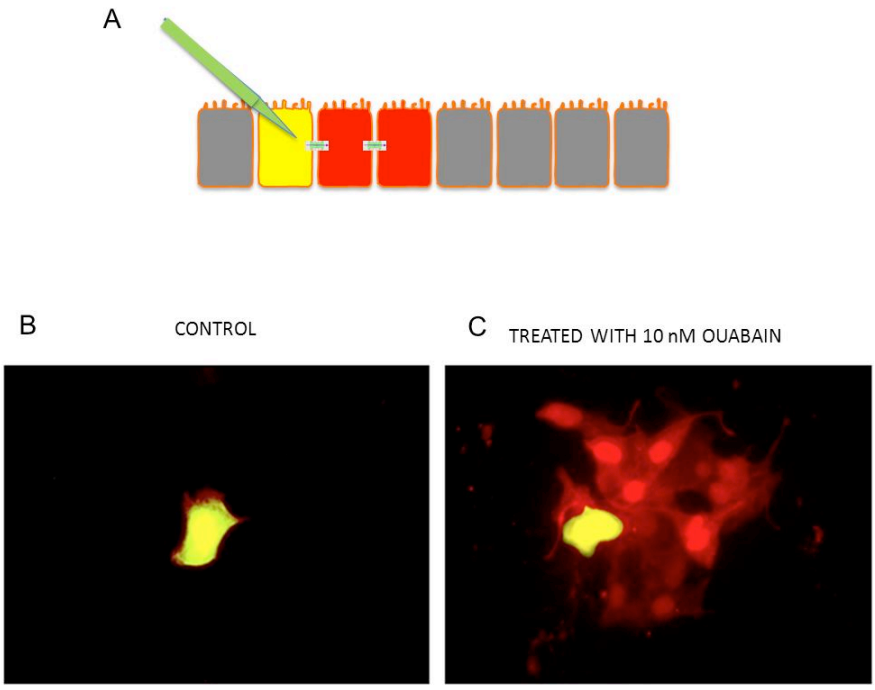

Figure 10

When tight junctions fail

It is a flagrant exaggeration to say that our species is 10% human and 90% microorganisms, but the expression may be forgiven because it is meant to call the attention to the extremely important fact that the flora of our digestive tract has 10 times more cells that all the rest of our body. If there were a democratic voting to decide who are "us" the somatic cells that constitute our body or the intestinal flora, they would hopelessly defeat us by an overwhelming majority. Microorganisms in the intestinal lumen produce a mind boggling number of peptides that cannot trespass the epithelium of our alimentary tract and gain access to our *milieu intérieure* because of the presence of TJs. (**Figure 11**). But –according to Ford Principle- TJs can and will fail. A peptide may intrude in between the cells of the intestinal mucosa, gain access to the *milieu intérieure*, and challenge the immunological system to generate specific antibodies against some of its epitopes. The problem arises when protein of our body has an analogous epitope, because in this case the immune system may end up attacking its own thyroid gland causing Hashimoto thyroiditis, the pancreas causing diabetes, triggering Crohn`s disease, multiple sclerosis of the nervous system, pemphigus, heart diseases, arthritis, etc. Not surprisingly, "permission" to cross a tight junction entails a heavily Kafkian molecular bureaucracy like the steps of diapedesis.

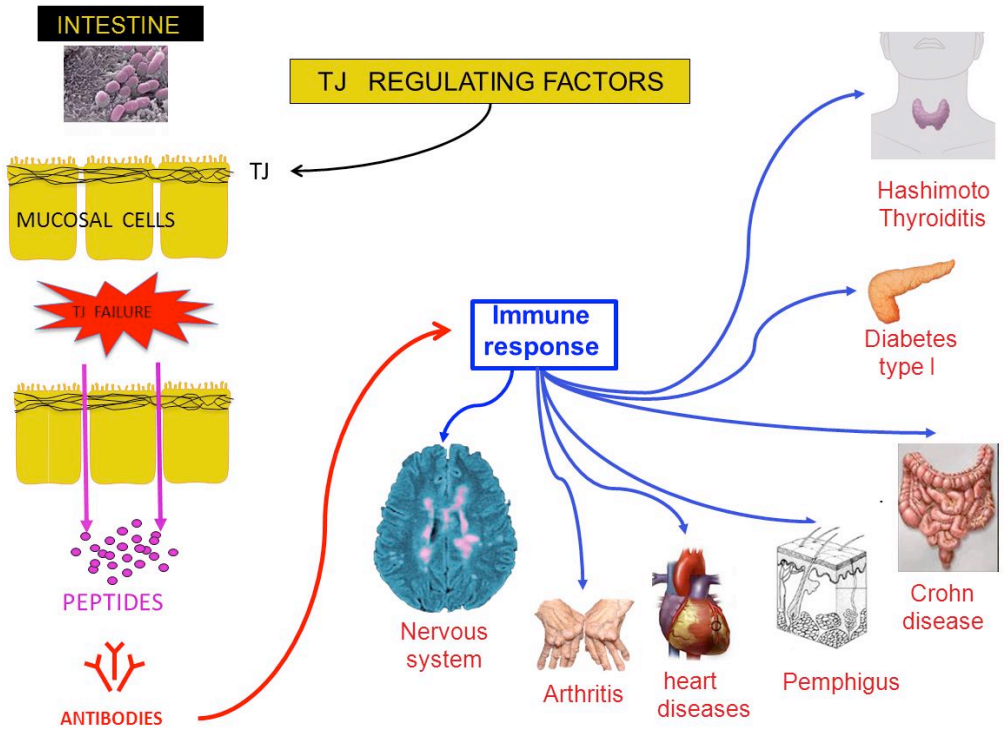

Figure 11

As discussed at the beginning of this review (**Figure 2**) a given epithelium has the degree of hermeticity it needs, i.e. according to the concentration gradients that they have to withstand between the two sides. So the mystery remains: what is the biological entity in charge of making a needed TJ?

Whatever the optimum of hermeticity and selectivity of a given TJ these parameters would certainly fluctuate, and of course the organism must have regulating systems to insure that the degree of departure would nevertheless remain within a physiological range. This prompted us to look for endogenous molecules that would participate in such regulations, and in figs. 1 and 2 we discussed our rational to look for them in urine. For technical reasons we could not pursue our search of such molecules in urine in spite of having solid arguments and evidences that urine does have them. Then, a coup of good luck enabled us to demonstrate that hormone ouabain is able to modulate highly specifically the degree of tightness of the TJ (**Figure 6**), as well as apical/basolateral polarity (**Figure 7**). So there are hosts of sick persons because of a faulty TJ, that would benefit if we were able to modulate it.

Cell signaling

The receptor of hormone ouabain is the α-subunit of the Na^+,K^+-ATPase (Larre and Cereijido, 2010) , but its effects can be observed in remote points of the cell, because the information travels through signaling pathways involving c-Src and ERK1/2 towards "groups" of cells expressing Cnx-43 that forms connexons at the cell border (*green*, indicated by *white arrows*). We know it, because when the monolayer is pretreated with their specific inhibitors (PP2, and PD respectively) the response is significantly smaller. **Figure 12** is a cartoon ordering this complex relationship between the plasma membrane, the different subunits of the Na^+,K^+-ATPase, c-Src, ERK1/2, effectors (in this case the connexons), and the inhibitors.

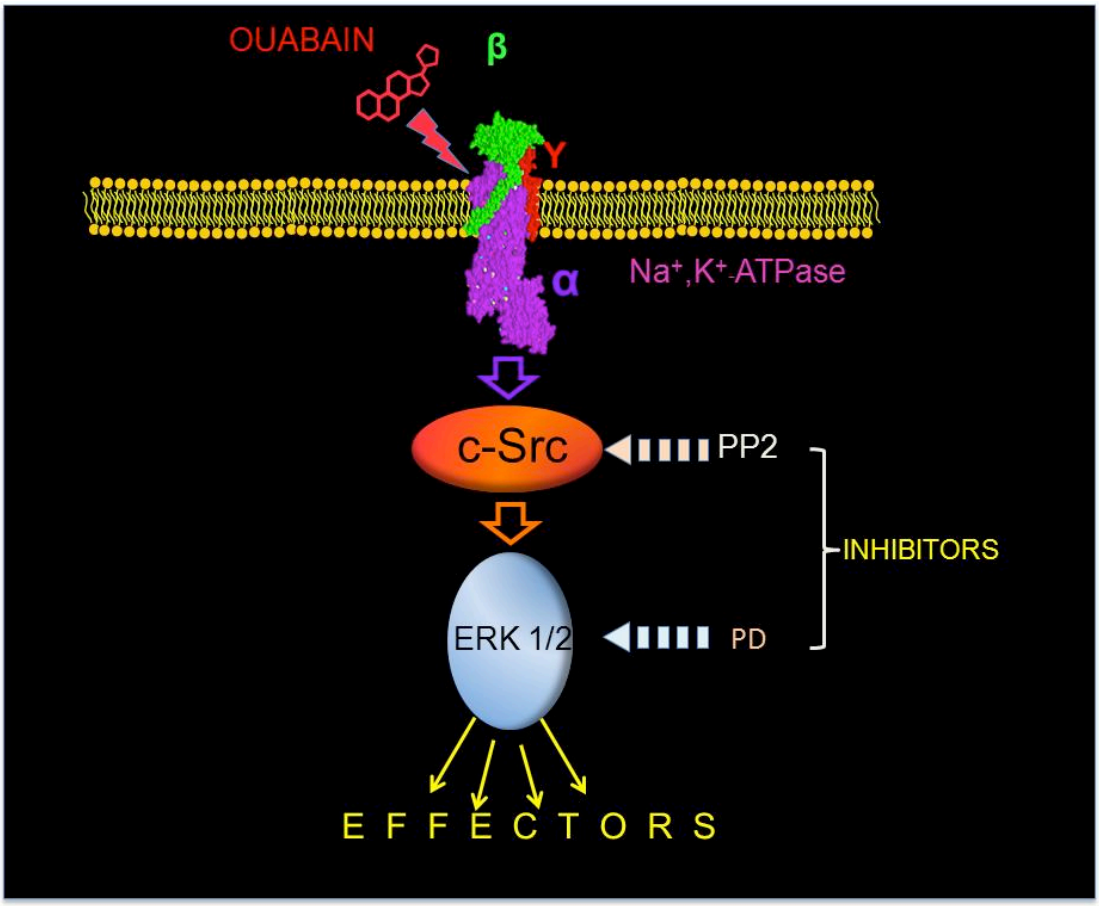

Figure 12

Hence, as initially postulated, ouabain hormone modulates cell contacts, a very important property indeed because, in the past, cell attachments were regarded as mere structural attributes to keep cells together, lest tissues and organs would disintegrate upon mechanical deformation. But today we know that besides of this coarse function of holding cells together, connections are involved in proliferation, cell cycling, differentiation, tissue architecture, cancer, metastases, activation/inactivation of genes in the same and neighboring cell, in metabolic cooperation, etcetera. In summary, ouabain hormone modulates fundamental phenomena of metazoan life. Sufficient to say that the most complex object in the Universe, the human brain, assembles itself, functions as a supreme control of our organism, feels and think depending of what cell contacts with a given neighbor, when and how.

Diseases associated to faulty gap junction communication?

Cancer cells exhibit a very poor level of GJC (**Figure 13**). Fig. 13A shows that when one passes an electrical pulse with microelectrode 1 in a layer of normal liver cells, the pulse, albeit attenuated, can be found in other cells thanks to GJC (**Figure 13B**). A similar experiment but using epithelial cells from liver cancer (**Figure 13C**) shows that that cells are poorly communicated (**Figure 13C**). This led to suggest that this lack of communication does not permit cells to organize and build up a supramolecular organization (Loewenstein and Kanno, 1966) (**Figure 13**, *right*). If these cell physiologists were right, cancer would be a consequence of a sort of cellular

schizophrenia. And we now add: this might be due to a lack of affinity for hormone ouabain, or to a combination of low affinity and low concentration level of this hormone in plasma. Of course we are now assaying if the property of hormone ouabain to provoke cell-cell communication might force cancer cells to communicate and has an effect on the evolution of a cancer.

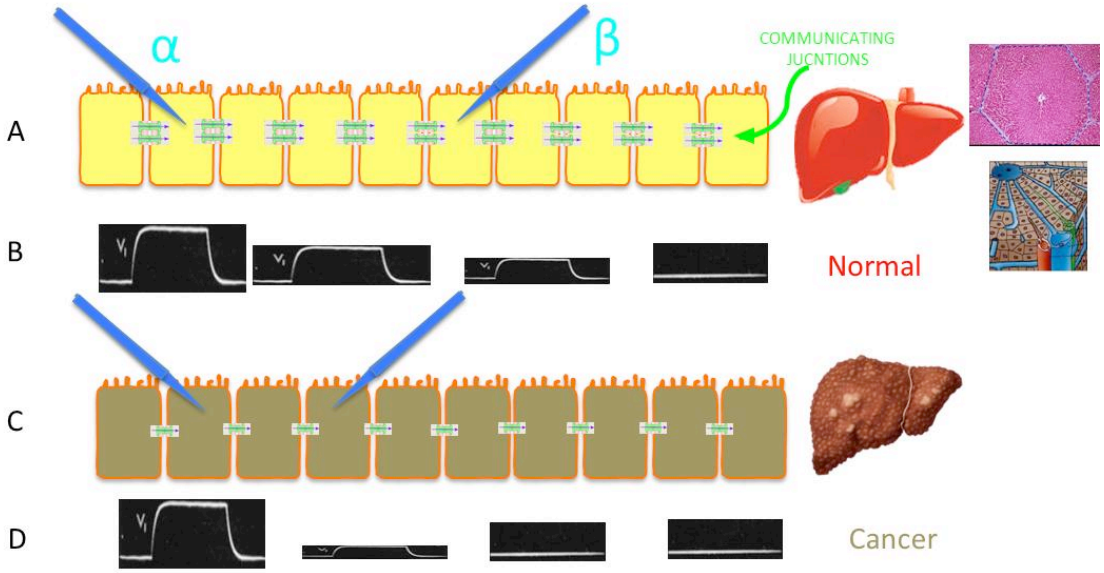

Figure 13

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About Authors

Dr. Marcelino Cereijido, is Emeritus Prof. of Cellular and Molecular Physiology of the Center for Research and Advanced Studies of Mexico, as well as Emeritus member of the National Research System (SNI). He developed the first model system of epithelial cells cultured on a permeable and transluscent support, and used it to elucidate the differentiation of the two fundamental features of transporting epithelia: apical/basolateral polarity & tight junctions. With his coworkers he demonstrated one of the main physiological roles of hormone ouabain: it modulates cell-cell contacts. He was awarded several fellowships and prizes in Argentina, Mexico, United States, like best doctoral thesis, National Medal, Guggenheim Foundation, NIH, etc.

Dr. Arturo Ponce is a full Professor at Department of Physiology, Biophysics and Neurosciences of Center for Research and Advanced Studies, National Polythecnic Institute, Mexico City, Mexico. As cell and molecular physiology specialist, Dr. Ponce is devoted to study biophysical aspects of membrane biology, such as ion channels and has been author or co-author of more than 50 peer-reviewed publications. He was awarded by the PEW latinamerican fellows program as well as the Human Frontiers Science Organization Program to perform postdoctoral research. Dr. Ponce is currently member of Mexico´s National Researchers System (SNI).

Note from the Editorial Committee: This article exceeds the number of Figures usually allowed for a Physiological Mini-Review. We decided to keep them, because we believe that reducing the number of Figures would also reduce the comprehensible and friendly way in which this important topic was presented.