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Invited review

Receptor for protons: First observations on Acid Sensing Ion Channels

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ABSTRACT

The history of ASICs began in 1980 with unexpected observation. The concept of highly selective Na^+ current gated by specific receptors for protons was not easily accepted. It took 16 years to get these receptor/channels cloned and start a new stage in their investigation. "The receptor for protons" became ASIC comprising under this name a family of receptor/channels ubiquitous for mammalian nervous system, both peripheral and central. The role of ASICs as putative nociceptors was suggested almost immediately after their discovery. This role subsequently was proven in many forms of pain-related phenomena. Many other functions of ASICs have been also found or primed for speculations both in physiology and in disease. Despite the width of field and strength of efforts, numerous basic questions are to be answered before we understand how the local changes in pH in the nervous tissue transform into electric and messenger signaling via ASICs as transducers.

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When starting this paper, I thought whether it is worth describing the role of a chance in the chain of events that led to the start of ASICs history. But then I recalled that the chance decides even more important things in science than a discovery of a new receptor. In 1980 I visited Erwin Neher in Gottingen several weeks after he found that sucking a cellular membrane with glass pipette isolated a patch of membrane with functional ion channels. Erwin told me that the finding was occasional. The same is mentioned in his Nobel lecture: "we noticed by chance …" Shortly after 1975, when we made the first intracellular perfusion of nerve cell body (Kryshtal and Pidoplichko, 1975; Kostyuk et al., 1975), our group, working at that time in the Department of Platon Kostyuk, started a search for a possibility of electric isolation of membrane patches with the same aim as Erwin Neher was pursuing already for some time, namely to monitor, if possible, currents through single ion channels. Erwin visited our lab for several occasions and we knew about his attempts to do this job using glass pipettes.

Intracellular perfusion was performed by making a hole in the plastic film (Fig. 1). Alternative method of intracellular perfusion suited specifically for large neurons of the mollusk. *Helix aspersa* was developed by using glass pipettes (Lee et al., 1978); it resulted in the leakage resistances similar to those attainable by using plastic materials. Knowing the difficulties experienced by Erwin at those times, we decided to continue the search for "patch clamp" making plastic pipettes. My colleague at that time, Vladimir Pidoplichko, who was extremely good in micromanipulations, elaborated such techniques. The system allowed adequate voltage clamp (Fig. 2), but we could not get input resistance of the membrane patch high enough to reveal elementary events associated with opening of individual ion channels.

When I heard from Erwin about "gigaseal" it immediately occurred to me that "the train has left" and we lost this game: borosilicate glass, not polyethylene could make molecular contact with membrane. Thus, our early success with plastic (intracellular perfusion) did not distract Erwin from going his way, but played Catch-22 with us: we concentrated on the "wrong" material which could not assure necessary tight molecular interaction with the cellular membrane. Accidental discovery of the "receptor for protons" remains in my memory as a sort of at least partial compensation for this lost game and a proof that one cannot be an eternal looser. Thus, in a couple of months after my return from Gottingen, Vladimir and myself were doing a routine experiment on neuroblastoma cells measuring dose–response relationship for serotonin. These responses were quite slow and it was possible to activate them using rapid superfusion of experimental chamber. Suddenly something strange occurred: in the sequence of solutions with progressively decreasing concentration of agonist the response to a lower concentration appeared dramatically larger than to a higher one. It immediately appeared that the responses we observed do not depend on the concentration of dissolved agonist. Short

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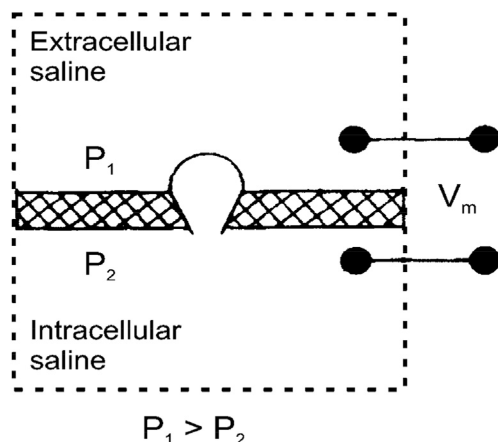

Fig. 1. Configuration of the planar perfusion system (according to Kryshtal and Pidoplichko, 1975). The cell was sucked by the saline flow into the hole in plastic film; the walls of the hole were covered with isolating grease. The method was modified first by using plastic pipettes (Fig. 2) and later abandoned for "patch-clamp". Amazing whirlpool of approaches: planar perfusion system returned with the development of high throughput patch clamp systems: all of them are based in this initial idea.

investigation revealed that the salines under scrutiny did not contain buffer due to a technical mistake and turned to be acidic. Mysterious response was transient and so its amplitude critically depended on the rate of solution exchange. We were witnessing something new and could not measure it properly. It was clear that the fastest perfusion of the experimental chamber we could master was way too slow to reveal more than a shadow of something mysterious occurring in the membrane of neuroblastoma subjected to external acidification. We needed a real pH drop, quasi-step change in this parameter. Enthusiasm prevailed and the problem of fast superfusion (called later "concentration clamp" or U-tube) was solved the same night: we made U-tube which is a microscopic variant of stop-flow approach. We needed some electrically driven relay to pinch a small silicone tube. To get this relay near midnight, there was no other way than to dismantle a local antique item: we sacrificed a trophy "Telefunken" telephone apparatus remaining in the lab from post-war times. Finally, we managed to observe a nice proton-activated inward current with activation kinetics much quicker than the kinetics of subsequent inactivation/desensitization indicating that the current peak was correctly measured.

U-tube immediately became in high demand: in the honeymoon of patch clamp Neher's group used it for pioneering measurements of the activity of single ligand-gated channels. Nontrivial moment in the construction of U-tube was to minimize a volume in which "previous" saline is intermixing with the next one to be rapidly applied. It is clear that rapid application can be achieved only if borderline between two salines is as narrow as possible. U-tube achieved this at the expense of constant flow of saline to be applied in front of a small hole used for application (Fig. 3).

Thus, we found that the cells of neuroblastoma clone N-18 demonstrate ionic conductance with the following properties:

This conductance is activated when external pH rapidly turns to be acidic. The activity becomes visible immediately in the acidic vicinity of neutral pH (at normal external Ca^{++}, as found later).

Activated conductance is transient and resembles in this quality (desensitization) the responses of the cells to transmitters.

This is a highly selective Na^+ conductance: as demonstrated later, selectivity between Na^+ and K^+ is as high as in the TTX-sensitive ion channel (Kovalchuk et al., 1990).

We got multiple questions to answer and some of them remain un-answered to-day. Reading textbooks in physiology we found that, although pH in big vessels is a sacred parameter, at the same time, virtually in any organ, in the course of its functioning, pH levels change and moreover, general tendency of these changes is such that pH fluctuates between normal weakly alkaline level and moderate acidification of extracellular space (working muscles etc.) which can activate "our" mechanism. But before hypothesizing that we have discovered a receptor for protons, it had to be demonstrated that this receptor is expressed not only in neuroblastoma tumor cells that originate from primitive neuroblasts not detectable in postnatal life (De Preter et al., 2006), but also in normal, hopefully sensory, neurons. With so many hopes expected to come true we developed a procedure of isolating sensory neurons from dorsal root ganglia (DRG) of rat and aimed a muzzle of U-tube at the first decent cell … This first one fulfilled all our hopes: we witnessed a similar characteristically transient response to pH drop, now in normal sensory neuron. We did not think long and published essentially similar story in two Journals (such trick would be next to a "gross misdemeanour" these days; but, from the other point of view, this would be impossible these days due to a Big Brother of Web). In both cases Chief Editors were benevolent to our courage and accepted a possibility of existence of a receptor for protons

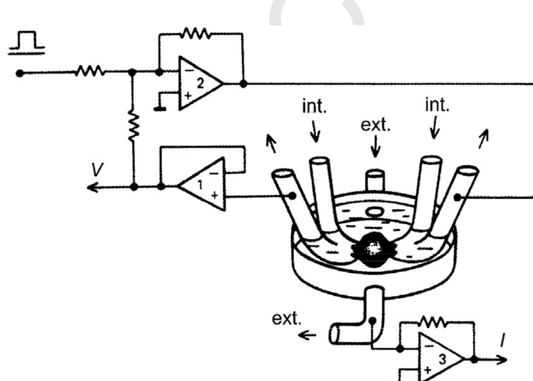

Fig. 2. The ultimate achievement in intracellular perfusion/voltage clamp: large nerve cell of mollusk is connected to voltage clamp via two plastic pipettes. This enables intensive internal perfusion and rapid voltage clamp with minimal series resistance (from Kostyuk and Krishtal, 1981).

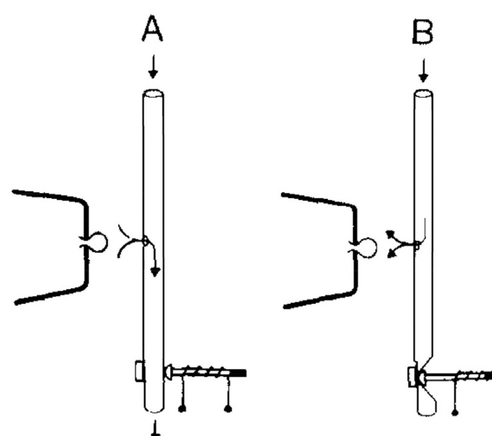

Fig. 3. The prototype of U-tube (it is not shown that thin polyethylene tube is U-bended to have the opening on the bottom of U making it easy to aim at the cell). A: the relay (taken initially from trophy telephone) is open and the opening in front of the cell sucks the external saline, while a saline to be rapidly applied is flowing in the tube, so that anytime it will be non-diluted when the relay is closed (B) and the saline comes from the hole. From Krishtal and Pidoplichko (1980).

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