CONTRIBUTION OF P.G. KOSTYUK TO PARADIGM OF CALCIUM SIGNALING

O.A. KRISHTAL

Bogomoletz Institute of Physiology, Ukraine
krishtal@biph.kiev.ua

The idea of the special role of calcium in biology dates back to the 19th century when almost immediately after the discovery of calcium as an inorganic element (Davy, 1808), its role was established in the formation of mammalian bones, as well as other mineralized tissues found in the biological diversity of the animal world. The next stage was the observation by the prominent English physiologist Sydney Ringer (S. Ringer), which he made closer to the end of the XIX century. Ringer found that isolated frog heart survives much better while maintaining contractile activity in tap water compared to distilled. It turned out that the reason for such a "life-giving" action of water from the London water supply system was its high calcium content (in a concentration, as it turned out, of the order of a millimole). The next breakthrough in understanding the role of calcium occurred already in the 40s of the last century when Heilbrunn (L. Heilbrunn) introduced calcium into muscle fibers through their trimmed ends and thus caused muscle contraction. In 1942, K. Bailey explained this phenomenon, showing that the ATPase activity of myosin is critically activated by calcium. Further research allowed the discoverer of the transmitter role of acetylcholine O. Levy (O. Loewy) to joke in 1959: "Calcium is everything!". However, it took another couple of decades to establish how this "everything" is everything.

The data on the key role of calcium in regulating the activity of many enzymes, including the mechanisms that control cell death, began to multiply. There is evidence of energy-dependent accumulation of calcium in the sarcoplasmic reticulum and mitochondria. The concept of calcium permeability of cell membranes arose almost simultaneously, that is, the participation of calcium in electrogenesis and neurosecretion (including secretion of neurotransmitters). The founders of this concept were, first of all, Bernard Katz and co-authors (V. Katz, R. Fatt, R. Miledi).

The first direct measurement of calcium current was made by Fatt (R. Fatt) and Ginsborg (B. Ginsborg) in 1958 on crustacean muscle cells. Giant muscle fibers of crustaceans possess a powerful system of calcium conductivity and are practically devoid of the ability to generate a sodium action potential. That allowed S. Hagiwara to produce the first description of the properties of calcium conductivity. At the same time, A. Hodgkin and co-authors showed that the calcium permeability of a giant nerve fiber is vanishingly small and cannot make any noticeable contribution to the electrogenesis associated with the transmission of a nerve impulse. World science faced the task of finding out whether nerve cells are equipped with calcium conductivity mechanisms. After decades of developing relevant scientific ideas, this question may seem academic, with each biology student knowing the positive answer. But it was necessary to obtain direct evidence of calcium electrogenesis in nerve cells by extrapolating to the general case of "ordinary" nerve cells information already obtained on exotic giant formations (giant axon, muscle fibers, synapse). Not to mention the fact that the concept of calcium channels was in its infancy.

Having shown genuine scientific vision, Platon Kostyuk became interested in this problem. In 1965, P.G. Kostyuk and co-authors (V.D. Gerasimov, V.A. Maisky) reported the ability of some giant neurons of freshwater mollusks to generate action potentials in the sodium-free environment. There was a need for direct measurement of the currents responsible for electrogenesis under these conditions, to identify the carrier ion. An attempt to solve this problem using two-microelectrode potential clamping on the membrane of giant mollusk neurons was encouraging. Still, it did not give an unambiguous answer to the question of the participation of calcium in the generation of a nerve impulse by the somatic membrane of a nerve cell. It could not be ruled out that the inward ion current observed when the near-pharyngeal ring of the ganglia was placed in a sodium-free solution was provided by sodium residues, which remained in the intercellular spaces enclosed by connective tissue membranes. The hope to solve the problem in connection with the development of the enzymatic isolation of individual neurons from the ganglion did not materialize either. It turned out that the inward (possibly calcium) current remaining in the sodium-free solution was masked by a powerful potassium outward current, which was only partially blocked by tetraethylammonium, the only blocker that existed at the beginning of the 70s. To solve the problem, a new way had to be found. The group engaged in the search for a solution included, in addition to Platon Grygorovych Kostyuk, Volodymyr Ivanovych Pidoplichko, and the author of these lines. Our efforts culminated in the development of the method of intracellular perfusion or, as it was "modestly" called in the first publication in the journal *Nature* (1975), "the method of intracellular dialysis". The cell was placed in a pore made in a plastic film; the "internal" part of the membrane was destroyed by a jump of hydrostatic pressure. Having removed potassium ions from the artificial intracellular environment, we saw how the outward potassium current gradually disappeared. Instead, a very slow inward current appeared, the amplitude of which was completely determined by the concentration of calcium in the extracellular solution. One can imagine the joy of the experimenters when seeing this for the first time.

The problem was solved: it turned out that the somatic membrane of nerve cells is equipped with a well-developed calcium conductivity system. Subsequent work carried out under the guidance of P.G. Kostyuk, allowed us to determine the most important characteristics of calcium channels in the membrane of a nerve cell. The articles published in the Journal of Physiology (London) in 1977 are included in the annals of the Citation Classics.

Subsequent publications were performed using an advanced intracellular perfusion method, which was the first version of the well-known patch-clamp technique, which appeared later. It was possible to make the world's first description of calcium currents in terms of the Hodgkin-Huxley model (m2h) and first record the gate currents of calcium channels (Nature, 1977).

P.G. Kostyuk and coworkers significantly contributed to the paradigm of calcium signaling in the nerve cell in subsequent years. In particular, factors were identified whose presence in the artificial intracellular medium provides the function of calcium channels (the famous "Kostyuk's cocktail" together with S.A. Fedulova and N.S. Veselovsky, 1981). With the same co-authors, as well as with Ya.M. Shuba and A.N. Savchenko, P.G. Kostyuk was among the first who managed to show the existence of several types of calcium conductivity, which laid the foundation for the modern classification of the types of calcium channels (1985, 1988).

As the author of numerous conceptual reviews based on his laboratory's successes at Bogomoletz Institute of Physiology, P.G. Kostyuk made an invaluable contribution to the formation of the paradigm of calcium signaling in the nervous system.

The brain is a unique device in which electrical signals interact with molecules, making changes in them, leading to changes in electrical signals. Calcium ions are the main driver of this interaction. Our teacher's talent made it possible to foresee the importance of research in this area and ensure their success. The contribution of P.G. Kostyuk to one of the most important problems of neurophysiology will forever remain in the history of science and the hearts of students.