

Department of Cellular and Molecular Neuroendocrinology, Institute of Physiology (Academy of Sciences of the Czech Republic, Prague) – State of the Art

Laboratory is focused on the research of pituitary and hypothalamus. The main interest is on membrane receptors and ion channels that control intracellular calcium signaling, electrical activity and secretion of hormones from pituitary cells and hypothalamic neurons. Recent research is focused on purinergic P2X receptors that are highly expressed in endocrine cells, including pituitary. These receptors represent a new family of ligand-gated ion channels that conduct calcium and are activated by extracellular ATP, consequently they modulate synaptic transmission and biophysical properties and secretion in pituitary cell. The structure and function relationship of recombinant purinergic P2X receptors is also investigated. The work recently performed in the Laboratory resulted in publication in "Neuroscience" and "Journal of Neuroscience" in which the role of presynaptic P2X receptors in modulations of GABAergic and glutamatergic synaptic transmission in supraoptic and suprachiasmatic nuclei was described. These investigations contributed with highly precise and invaluable data to the P2X field, providing solid biophysical knowledge about function of native P2X receptors.

Cellular electrophysiology, molecular biology, cell cultures and transfection, and rat brain slices methods are routinely used in the Laboratory. Patch clamp techniques, microfluorimetric measurements of intracellular calcium, measurements of hormone secretion with radioimmunoassay and molecular biology methods such as RT-PCR and mutagenesis methods are available. The effect of activation of ligand-gated ion channels on GnRH-stimulated electrical activity and Ca²⁺ oscillations is studied in primary cultures of neonatal pituitary cells from rat, neurons of suprachiasmatic and supraoptic nuclei in rat brain slices are used for study of synaptic transmission, and molecular properties of P2X receptors are investigated using mutagenesis and expression system of transfected HEK293 cells.

The Laboratory is equipped for biochemical analysis, cell and tissue culture (CO₂ incubator and laminar flow box), and for molecular biology (centrifuges, electrophoresis etc); three electrophysiological setups, one with a calcium imaging system, are available, as well as inverted and up-right microscopes (Olympus), patch clamp amplifiers, Axopatch 200 B, Digidata and pCLAMP programs (all from Axon Instrument), MicroMAX digital CCD camera (Princeton Instruments), wavelength changer Polychrome II (T.I.L.L., Photonics), METAFUOR Imaging Software (Visitron Systems GmbH, Germany), micromanipulators (Burleigh and Sutter) and fast application system controlled by microcomputer. Shared are Sutter instrument P77 Micropipette puller and tissue slicer Vibratom Dosaka.

The Laboratory has long-term experience with in vitro studies on membrane receptors and established collaboration with the Section of Cellular Signaling, ERB/NICHD, National Institutes of Health (Bethesda, MD, USA) which is a distinguished laboratory focused on pituitary cells from adult rats and purinergic P2X receptors. Models of P2X structure are performed in cooperation with Dr. Tomas Obsil (Faculty of Sciences, Charles University, Prague) who is an expert in protein structure modeling.

The following personnel are involved in the research: RNDr. Hana Zemkova CSc., senior scientist; Ing. Irena Svobodova, PhD, scientist; Prof. Tomas Radil, DrSc, emeritus scientist; MUDr. Marie Jindrichova, PhD, postdoc ; Anirban Bhattacharyya, PhD, postdoc; Vojtech Vavra, PhD, postdoc; Vendula Tvrdonova, student research assistant (postgraduate), Marian Rupert, student research assistant (undergraduate); Katka Sprlakova, student research assistant (undergraduate), Ing. Hana Janousková, technician; Irena Kalová, technician.