Abstract

The habilitation thesis entitled "***Original contributions in descriphering the cellular and molecular pathways with impact on the physiology and physiopathology of neuronal, interstitial uterine and endothelial cells***" describes *The scientific and profesional achievements and the planning for career development*, of the candidate for habilitation title Ms Beatrice Mihaela Radu, PhD, Assoc. Prof., Department of Anatomy, Animal Physiology and Biophysics, Faculty of Bioloy, University of Bucharest.

The core of the habilitation thesis *The scientific and profesional achievements and the planning for career development* is structured in three sections: **A) Teaching activity**, **B) Scientific activity** and **C) Plans for career development**.

Section A) Teaching activity presents the steps of the canditate's academic career, Ms Beatrice Mihaela Radu, indicating her academic positions: Junior reseach assistant (2003-2005), Research assistant (2006-2007), Lecturer (2008-2011), Research Associate (2012-2016), and the current position Associate Professor (2017-present). Additionally, this sections presents the list of bachelor and master degree thesis coordinated by Dr. Radu.

Section **B) Scientific activity** is structured in three subsections B1. Contributios in membrane biophysics, B2. Contributions in cellular biology and physiology and B3. Contributions in neurobiology, and each subsection describes the original contributions of the habilitation thesis author and presents her publications (24 ISI publications ISI, Hirsh index 10), scientific communications, and research grants on the described topics.

Subsection B1. contains chapter **B1.1.** *Model membranes that mimic the structure of the external membrane in procariotes. Mechanisms of transport and their role in resistance to antiobiotics.* Original contributions: (a) methodology for analyzing the transfer eficiency between tryptophan and TMA-DPH in proteoliposomes, (b) antibiotics (ciprofloxacin and ceftazidime) are quencing the fluorescence of tryptophan residues in porines in a dose-dependent manner, (c) for both antibiotics, the number of binding events is increased for negative membrane potentials in comparison with positive potentials, and the number andamplitudine of current fluctuations through porines is higher for ciprofloxacin than ceftazidime.

Subsection B2. Contains chapters **B2.1.** Molecular mechanisms in sensitive neuron of dorsal root ganglia from spinal cord and their alterations in experimental models of diabetes and **B2.2.** Calcium homeostasis in interstitial cells from human uterine myometrium.

Chapter B2.1 describes the topics:

B2.1.1. *The dual action of methylglyoxal (MG) on sensory neurons in dorsal root ganglia primary cultures*. Original contributions: (a) MG reduces the cell viability in neuronal primary cultures from dorsal root ganglia in a dose-dependent manner, (b) high MG doses reduce the number of viable neurons and glial cells, while low MG doses have a proliferative effect on glial

cells and a stimulative effect on adherent viable neurons, (c) the amplitude of calcium transients induced by depolarisation is increased by lower MG doses, while is reduced by higher MG doses, (d) medium neurons are more sensitive to higher MG doses than small neurons, (e) neurite outgrowth is stimulated at lower MG doses, while inhibited at higher MG doses.

B2.1.2. *Capsaicin modulates TRPV1 properties in sensory neurons from dorsal root ganglia primary cultures in experimental genetic diabetes mice, when administered intraperitoneal*. Original contributions: intraperitoneal capsaicin induces (a) reduction of the blood glucose levels in diabetic conditions, (b) TRPV1 upregulation in normoglycemic, but not in hyperglycemic conditions, (c) changes in the percentage of TRPV1-expressing small, medium and large neurons, (d) constant amplitude of capsaicin-induced TRPV1 currents, but an increase in their time recovery constant.

B2.1.3. *The functional expression of acid sensing ion channels (ASIC) is affected in sensory neurons from dorsal root ganglia primary cultures due to diabetic conditions.* Original contributions: (a) ASIC1, PcTx1-sensitive ASIC2, PcTx1-resistant ASIC2 and ASIC3 currents are expressed by sensory neurons in normoglycemic conditions, while PcTx1-resistant ASIC2 are absent in diabetic conditions, (b) PcTx1 has weaker inhibitory effects in diabetic conditions, while APETx2 has constant inhibitory effects, (c) ASIC expression in small sensory thoracic neurons is different in diabetic vs. normoglycemic conditions, (d) experimental diabetes upregulates ASIC1 and ASIC3 mRNA, and downregulates ASIC2 mRNA.

Chapter B2.2. describes the topics:

B2.2.1. *Stimulation of interstitial cells from human uterine myometrium with low intensity NIR lasers.* Original contributions: (a) interstitial cells telopodes from non-pregnant and pregnant uterine myometrium have differences in reactivity when stimulated with low intensity NIR lasers, (b) the direction of telopodes' growth changes upon laser stimulation, (c) acute exposure to mibefradil, T-type calcium antagonist, significantly reduces the stimulation-induced telopode's rate of growth in pregnant uterine myometrium cultures, (d) chronic exposure to mibefradil stops the telopodes growth.

B2.2.2. *Description of T-type calcium channels in telocytes from interstitial cells in human uterine myometrium by immunofluorescence and patch-clamp techniques.* Original contributions: (a) human myometrial telocytes (TCs) from pregnant and non-pregnant uterus are immunopositive for CD34, PDGFRα, Cav3.1, and Cav3.2, (b) T-type and L-type calcium currents are present in human myometrial uterine TCs, (c) mibefradil significantly reduces T-type calcium currents in human myometial uterine TCs.

B2.2.3. *Beta-estradiol downregulates voltage-gated calcium channels and estrogen receptors in human myometrial uterine TCs.* Original contributions: (a) Cav3.1 channels are overexpressed, while Cav3.2 and Cav3.3 are downregulated in human myometrial TCs from human pregnant uterus samples, (b) estrogen receptors are overexpressed in TCs from pregnant versus non-pregnant human uterine samples, (c) beta-estradiol downregulates T-type calcium channels and estrogen receptors in human myometrial TCs from pregnant uterus, (d) beta-estradiol blocks calcium transients and L-type calcium currents, induced by BAY K8644, in human myometrial TCs from pregnant uterus.

Subsection B3. describes the topics:

B3.1. *Qtracker®800 are used for imaging cerebral vasculature although it interact with cerebral microvasculature endothelium*. Original contributions: (a) obtainn the values of the hydrodinamic diameter for Qtracker®800 in Ringer HEPES and saline solutions, (b) Qtracker®800 are uptaken in cerebral vascular endothelium after 3 hours from their intravenous

administration, (c) Qtracker®800 generate calcium transients in a percentage of endothelial cells from cerebral microvasculature or from human umbilical vein, (d) the percentage of sensitive endothelial cells from human umbilical vein is donor-dependent.

B3.2. *Functional expression of muscarinic receptors for acetylcholine in murine microvascular endothelial cells*: (a) evidencing all five muscarinic receptors gene expression in cerebral murine microvascular endothelial cells, (b) quantifying th expression sequence: *Chrm3* > *Chrm4* > *Chrm1* > *Chrm5* > *Chrm2*, (c) M_1, M_3, and M_4 receptors are highly abundent, (d) detecting the distinct cellular localisation of the muscarinic receptors, (e) selective antagonists for M_3 receptor, but not for M_1 are diminishing the intracellular calcium release induced by acethylcholine.

Section C) Plans for career development is structured in 2 subsections C1. Plans for teaching career development and C2. Plans for scientific career development. Subsection C1. contains chapters **C1.1.** Proposals for developing teaching disciplines, **C1.2.** Outreach of Master of Neurobiology, **C1.3.** Action contribution for developing the Department of Anatomy, Animal Physiology and Biophysics. In chapter **C1.1.** are presented the planning for developing three disciplines: Advanced methods in spectroscopy and microscopy with applications in neurobiology (Course), Medical Neurobiology (Course), and Biophysics (Hands-on sessions). Subsection C2. contains chapters **C2.1.** Consolidating the research topics of the department and **C2.2.** Introducing new research topics in the department.