

## **Inter- and intracellular communication in the cardiovascular system**

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The group consists of 5 senior scientists, 3 ph.d. students, 3 undergraduate students and 4 technicians. The group masters and has access to a wide variety of techniques, including quantitative fluorescence microscopy, confocal microscopy, one and two cell patch clamp, intra- and extracellular electrophysiology using sharp or blunt electrodes, microdissection of microvessels, intravital microscopy and other whole animal techniques, molecular biology including cell culture facilities.

**The role of gap junctions in heart arrhythmias:** It is becoming increasingly clear that disruption of the normal intercellular communication between cardiac myocytes plays a central role in the generation of several life threatening arrhythmias like for example ventricular tachycardia/fibrillation following ischemia. The substrate for these arrhythmias is a functional uncoupling of the myocytes leading to conduction block and re-entrance. The coupling between myocytes is mediated by gap junctions. The latter form pores between the cells that allow passage of both electrical current and signal molecules. Our research focuses on the mechanisms that act on gap junctions to change their permeability characteristics, and thus, the coupling between the cells. One central project is the study of the so called anti-arrhythmic peptides which is a group of endogenous peptides that modulate gap junction function.

**Vascular conducted responses:** When a microvessel is stimulated locally by either a vasodilatory or vasoconstrictory stimuli the response is not limited to the site of stimulation. Rather, it spreads for several millimetres in both the up- and downstream direction. The signal propagates between the cells of the vascular wall, i.e. the vascular smooth muscle cells and/or the endothelial cells. Like it is the case in the heart, it is most likely that gap junctions between the cells of the vascular wall play a central role in signal propagation. Our research focuses on 1) the signalling mechanisms, specifically the role of electronic conduction and its interplay with voltage operated calcium channels; and 2) the modulation of vascular conducted responses by either endocrine, pharmacologic and pathological factors.

**Regulation of intracellular calcium in mesenteric arterioles:** We have previously shown that mesenteric arterioles lack L-type voltage operated calcium channels, whereas T-type channels seem to be present. This is surprising since mesenteric arterioles are able to produce conducted vascular responses which are thought to depend on an electronic spread of a localized change in membrane potential which then alters vascular tone at the remote site by changing the influx of calcium through L-type channels. In order to understand how T-type channels can participate in a process that requires a sustained influx of calcium, we have undertaken a series of studies to characterize the mechanisms that regulate intracellular calcium in small mesenteric arterioles. Intracellular calcium is measured in microdissected rat mesenteric arterioles using fluorescence microscopy (Fura-2).

**Calcium metabolism in renal hemodynamics:** Hypertension is associated with increased peripheral resistance, and altered renal hemodynamics are also suggested to be a key factor in the development and maintenance of human essential hypertension. The microalbuminuria at the early stages of diabetes mellitus, preceding diabetic nephropathy, has been associated with a reduced afferent arteriolar resistance. The smooth muscle cell  $\text{Ca}^{2+}$  concentration  $[\text{Ca}^{2+}]_i$  is a second messenger mediating contraction. This concentration is in part controlled by other ions (e.g.  $\text{K}^+$  and  $\text{Cl}^-$ ) controlling the cell membrane potential. The general aim of this project is to evaluate the role of renal vascular smooth muscle cell calcium handling in the regulation of renal hemodynamics in health and disease. Renal blood flow is measured with an electromagnetic flow probe. A catheter is in the left renal artery is used for drug administration. This arrangement minimizes systemic effects of the drugs. Renal resistance vessels (afferent arterioles and interlobular arteries) are isolated from kidneys from normo- and hypertensive rats. The  $[\text{Ca}^{2+}]_i$  is measured using the  $\text{Ca}^{2+}$  sensitive dye Fura 2. Different pathways for  $\text{Ca}^{2+}$  entry and mobilization are going to be investigated. It is hypothesized that these pathways might differ in hypertension and diabetes mellitus. The role for different  $\text{Ca}^{2+}$  recruitment pathways will also be investigated in the conducted vasomotor response in renal resistance vessels. It will be examined whether  $\text{Cl}^-$  and  $\text{K}^+$  channels play a role in the vasodilation and vasoconstriction seen in early diabetes mellitus and hypertension, respectively. Also the role for these channels in autoregulation of renal blood flow and resetting thereof, will be evaluated.

*Recent publications related to the projects described above:*

Gustafsson, F., and Holstein-Rathlou, N-H. Angiotensin II modulates conducted vasoconstriction to norepinephrine and local electrical stimulation in rat mesenteric arterioles. *Cardiovasc. Res.*, 44: 176-184, 1999.

Gustafsson, F., and Holstein-Rathlou, N-H. Conducted vasomotor responses in arterioles. *Acta Physiol. Scand.*, 167: 11-21, 1999

Salomonsson M, and Arendshorst WJ. Calcium recruitment in rat renal vasculature: Norepinephrine effects on blood flow & vascular calcium concentration. *Am. J. Physiol.* 276, F 700 - 710, 1999.

Salomonsson M, Brännström K, and Arendshorst WJ.  $\alpha_1$ -adrenoceptor subtypes in rat renal resistance vessels. An in vivo and in vitro studie. *Am. J. Physiol.* 278, F 138 -147, 2000.

Kornfeld, M., Salomonsson, M., Gutierrez, A., Persson A.E.G. The influence of  $\beta$ -adrenergic activation on noradrenergic  $\alpha_1$  activation of rabbit afferent arterioles. *Pflugers Arch.* 441: 25-31, 2000.

Salomonsson M, & Arendshorst WJ. Norepinephrine-induced calcium signaling pathways in afferent arterioles of genetically hypertensive rats. *Am. J. Physiol.*, 281: F264-F272, 2001.

Salomonsson, M., Oker, M., Faber, J., Zhang, H., and Arendshorst, W.J.  $\alpha_1$ -Adrenoceptor subtypes on rat afferent arterioles assessed by radioligand binding and RT-PCR. *Am. J. Physiol.*, 281: F172-F178. 2001.

Salomonsson M, Gustafsson F, Andreasen D, Jensen BL, and Holstein-Rathlou N-H. Local electric stimulation causes conducted calcium response in rat interlobular arteries. *Am. J. Physiol.* 283: F473-F480. 2002.

Ollerstam, A., Salomonsson, M., and Persson, A.E.G. Reduced rat renal vascular response to Ang II after chronic inhibition of nNOS. *Acta Phys. Scand.* 176, 245-252. 2002.

Gustafsson F, Andreassen D, Salomonsson M, Jensen BL, and Holstein-Rathlou N-H. Conducted vasoconstriction in rat mesenteric arterioles: a role for dihydropyridine insensitive Ca<sup>2+</sup> channels. *Am. J. Physiol.*, 280: H582-H590, 2001.

Gustafsson, F., Mikkelsen, H.B., Jensen, L.J., Arensbak, B., Thuneberg, L., Neve, S., and Holstein-Rathlou, N-H. Expression of connexin 37, 40 and 43 in rat mesenteric arterioles and resistance arteries. *Histochem. Cell Biol.*, 119: 139 - 148, 2003.

Xing, D., Kjølbye, A.L., Nielsen, M.S., Petersen, J.S., Harlow, K.W., Holstein-Rathlou, N-H., and Martins, J.B. ZP123 increases gap junctional coupling and prevents reentrant ventricular tachycardia during myocardial ischemia in open-chest dogs. *J. Cardiovasc. Electrophysiol.*, 14: 510 - 520, 2003.

Sørensen, C.M., Leyssac, P.P., Skøtt, O., and Holstein-Rathlou, N-H. NO mediates down regulation of RBF after a prolonged reduction of renal perfusion pressure in SHR. *Am. J. Physiol.*, 285: R329 - R338, 2003.

Steendahl, J., Holstein-Rathlou, N-H., Sørensen, C.M., and Salomonsson, M.: Effects of chloride channel blockers on rat renal vascular responses to angiotensin II and norepinephrine. *Amer. J. Physiol.*, 286, F323 – F330, 2003.

Sorensen, C.M., Leyssac, P.P., Salomonsson, M., Skott, O., and Holstein-Rathlou, N-H.: Angiotensin II induced down regulation of RBF after a prolonged reduction of renal perfusion pressure is due to pre- and postglomerular constriction. *Am. J. Physiol.*, in press, 2004.