

Overall research theme:

- 1- Characterisation of i) CGRP receptors, ii) CGRP signal transduction and iii) the mechanism behind desensitisation of CGRP-induced responses in coronary small arteries.**
- 2- Change in vascular reactivity during ageing, diabetes and congestive heart failure.**
- 3- Investigation of cardiac pain during ischemia and diabetes.**
- 4- Alteration in coronary artery receptor expression during ischemia**

Latest update:

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Characteristics of the research group:

The group combines competences in in-vivo and in-vitro research. A unique feature is the combination of integrative research in whole animal models with isolated tissue preparations and methods in cellular and molecular pharmacology. The group has build up a fluorescence laboratory equipped with in-vitro and in-vivo imaging systems. In addition, the group has multi wire-myograph systems for studying isolated vessels and applying electric field stimulation. Animal models of interest include diabetes, congestive heart failure and neuropathy.

Running projects: Titles and abstracts:

Mechanism for desensitisation of CGRP-induced responses in rat coronary arteries

The project aims to elucidate the signal transduction behind the desensitisation of CGRP-induced responses (both tension and intracellular calcium) in isolated rat coronary arteries. We have recently demonstrated that the CGRP-induced desensitisations are homologous but the mechanism involved is more complicated. We are looking at the following: 1- Are CGRP receptors in fact being internalised in isolated arteries? 2- What kind of kinases are involved (G protein coupled receptor kinases or other kinases)? 3- Are potassium channels involved in CGRP signal transduction being phosphorylated /inactivated by kinases?

Effect of ischemia and diabetes on expression of c-fos in rat hearts

The project aims to elucidate the effect of ischemia (induced by coronary ligation) and diabetes neuropathy (type I and II diabetes) on the expression of c-fos in the heart. After retrograde labelling, the reactivity of isolated sensory cells from dorsal root ganglia (thoracic region) will be assessed. Nerve conduction studies will be performed along with the molecular pharmacological studies in order to assess the possible electrophysiological changes together with alterations in the activity of neurotropic factors and neuropeptides.

Signal transduction for Ca²⁺ in vascular tissue 1) during depolarisation with high extracellular potassium concentration and 2) during stimulation with receptor-dependent agonists (e.g. 5-HT, ATII, endothelin).

The project seeks to characterize alterations in excitation-contraction (EC) coupling for Ca²⁺ in resistance arteries. The rise and fall in intracellular free calcium ([Ca²⁺]_i) are therefore the principle mechanisms that initiate contraction and relaxation, respectively. There are also secondary mechanisms of regulation of the contraction that can modify, independently of intracellular Ca²⁺, the activities of the phosphorylating and dephosphorylating enzymes. It is generally accepted that phosphorylation of myosin light chain (MLC, 20kDa) by a Ca²⁺-calmodulin-dependent enzyme, MLCK, plays an important role in the regulation of smooth muscle contraction. However, a number of studies have been shown that the smooth muscle contraction and Ca²⁺-sensitivity is mainly regulated by the action of phosphatases, e.g. MLC phosphatase. These studies indicate that the balance between the activity of MLCK and MLC phosphatase is the major direct controller of contraction in the smooth muscle cells. Furthermore, the role of Rho-associated protein kinase (activated by RhoA) in the Ca²⁺-sensitization process has been examined by using the pyridine derivate Y-27632, and it was shown that Rho-associated protein kinase was able to

phosphorylate MLC-phosphatase causing its inactivation. RhoA is activated through a heterotrimeric GTP-binding protein, and during this process is translocated to the membrane, although the link between agonist activation and RhoA activation is not completely established. Finally, there are also other Ca²⁺-sensitizing mechanisms operating independently of MLC-phosphorylation, which include phosphorylation of caldesmon by mitogen-activated protein kinase (MAPK) or calponin by protein kinase C (PKC)

Receptor upregulation in coronary arteries during ischemic heart diseases

Our intentions are to initiate a series of experiments using isolated coronary arteries from rats with an acute MI or after reperfusion or ischemic preconditioning. We will then study the expression level of GPCRs (up-regulation of serotonin receptors and endothelin receptors), the signal-transduction (calcium sensitivity, PKCs, ERKs and MAPKs) and inflammatory cytokines. The techniques will include myograph studies, intracellular calcium measurements with FURA-2, ELISA, Western blot, ordinary RT-PCR and Real-time PCR.

Recent publications related to the projects described above:

- Sheykhzade, M.** and Nyborg, N.C. (1998). Caliber dependent calcitonin gene-related peptide-induced relaxation in rat coronary arteries: effect of K⁺ on the tachyphylaxis. *Eur. J. Pharmacol.*, 351: 53-55.
- Sheykhzade, M.** and Nyborg, N.C. (1998). Characterization of calcitonin gene-related peptide (CGRP) receptors in intramural coronary arteries from male and female Sprague Dawley rats. *Br. J. Pharmacol.*, 123:1464-1470.
- Sheykhzade, M.** and Nyborg, N.C. (2000). CGRP receptors in rat intramural left coronary arteries. In *The CGRP Family: Calcitonin gene-related peptide (CGRP), Amylin and Adrenomedullin* ed. Poyner, D., Marshall, I. & Brain, S.D. pp. 177-178. Texas: Landes Bioscience.
- Sheykhzade, M.** Dalsgaard, G.T., Johansen, T. and Nyborg, N.C.B. (2000). The effect of long-term streptozotocin-induced diabetes on contractile and relaxation responses of coronary arteries: selective attenuation of CGRP-induced relaxations. *Br. J. Pharmacol.*, 129: 1212-1218.
- Sheykhzade, M.** and Nyborg, N.C. (2000). Non-competitive antagonism of amylin on CGRP₁-receptors in rat coronary small arteries. *Br. J. Pharmacol.*, 130: 386-390.
- Sheykhzade, M.** and Nyborg, N.C. (2001). Mechanism of CGRP-induced relaxation in rat intramural coronary arteries. *Br. J. Pharmacol.*, 132: 1235 - 1246.
- Sheykhzade, M.** and Nyborg, N.C. (2004). Homologous desensitization of calcitonin gene-related peptide- induced relaxation in rat intramural coronary arteries. *Eur. J. Pharmacol.*, 484: 91 – 101
- Simonsen, A. H., **Sheykhzade, M.** and Nyborg, N.C. (2004). Age- and endothelium-dependent changes in coronary artery reactivity to 5-HT and calcium. *Vascular Pharmacology*, *in press*.
- Sheykhzade, M.**, Lind, H. and Edvinsson, L. (2004). Non-competitive antagonism of BIBN4096BS on CGRP-induced responses in human subcutaneous arteries. *Br.J. Pharmacol.*, *in press*.