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Publications

This PhD has resulted in a number of manuscripts, of which three are presented in this thesis. Among the three manuscripts, two are published, and one is currently being reviewed.

Manuscripts included in this thesis:

Manuscript I:
van der Horst, J., Rognant, S., Abbott, G. W., Ozhathil, L. C., Hägglund, P., Barrese, V., Chuang, C. Y., Jespersen, T., Davies, M. J., Greenwood, I. A., Gourdon, P., Aalkjaer, C., & Jepps, T. A. (2021).
Dynein regulates Kv7.4 channel trafficking from the cell membrane
Journal of General Physiology, 153(3):e202012760

Manuscript II:
van der Horst, J., Rognant, S., Hellsten, Y., Aalkjaer, C., Jepps, T.A.
Dynein coordinates β2-adrenoceptor-mediated relaxation in normotensive and hypertensive rat mesenteric arteries
Hypertension. (2022), Accepted – Online ahead of print: DOI: 10.1161/HYPERTENSIONAHA.122.19351

Manuscript III
Ehlers, T. S.*, van der Horst, J.*, Møller, S., Gliemann, L., Aalkjaer, C., Jepps, T. A., Hellsten, Y.
Colchicine enhances β-adrenoceptor-mediated vasodilation in men with essential hypertension
Under review at Cardiovascular Research. (2022)
*Joint first authorship

Manuscripts not included in this thesis:

Manville, R.W.*, Redford, K.E.*, van der Horst, J.*, Hogenkamp, D.J., Jepps, T.A., Abbot, G.W. (2022).
KCNQ5 activation by tannins mediates vasorelaxant effects of barks used in Native American botanical medicine
Federation of American Societies for Experimental Biology
*Joint first authorship

van der Horst, J., Møller, S., Kjeldsen, S. A. S., Wojtaszewski, J., Hellsten, Y., Jepps, T. A. (2021).
Functional sympatholysis in mouse skeletal muscle involves sarcoplasmic reticulum swelling in arterial smooth muscle cells
Physiological Reports, 9(23):e15133
van der Horst, J., Manville, R. W., Hayes, K., Thomsen, M. B., Abbott, G. W., & Jepps, T. A. (2020). Acetaminophen (Paracetamol) Metabolites Induce Vasodilation and Hypotension by Activating Kv7 Potassium Channels Directly and Indirectly. *Arteriosclerosis, Thrombosis, and Vascular Biology, 40*(5), 1207–1219.

van der Horst, J., Greenwood, I. A., & Jepps, T. A. (2020). Cyclic AMP-Dependent Regulation of Kv7 Voltage-Gated Potassium Channels. *Frontiers in Physiology, 11*:727

Manville, R. W., van der Horst, J., Redford, K. E., Katz, B. B., Jepps, T. A., & Abbott, G. W. (2019). KCNQ5 activation is a unifying molecular mechanism shared by genetically and culturally diverse botanical hypotensive folk medicines. *Proceedings of the National Academy of Sciences of the United States of America, 116*(42), 21236–21245.
English summary

Hypertension is a chronic medical condition in which the systemic arterial pressure is persistently elevated and affects more than 1.4 billion people worldwide. Importantly, hypertension is a major risk factor for cardiovascular diseases, which remains one of the leading causes of global morbidity and mortality. Despite the existing therapies, hypertension remains poorly controlled. Therefore, it is crucial to improve our understanding of vascular physiology and the mechanisms involved in the pathophysiology of hypertension, which may provide new therapeutic targets to treat hypertension.

Hypertension is usually the result of increased total peripheral resistance caused by an increase in arterial tone, i.e. arterial constriction. Vascular smooth muscle cells (VSMCs) within the wall of an artery facilitate constriction and dilation and thus play a key role in determining total peripheral resistance. The influx of extracellular Ca\(^{2+}\) is particularly important in initiating contraction of the VSMCs and is mainly controlled by membrane depolarization and activation of the voltage-dependent calcium channels (VDCCs). Ion channels in the plasma membrane contribute to regulating the membrane potential and therefore determine the Ca\(^{2+}\) influx and vascular tone. The resting membrane potential is predominantly dictated by K\(^{+}\) channels. Voltage-gated K\(^{+}\) channels from the Kv7 family, namely Kv7.4 and Kv7.5, are particularly important regulators of the resting membrane potential in VSMCs from different rodent and human arteries. Furthermore, Kv7 channels are downstream targets for cGMP and cAMP-dependent receptor-mediated vasodilation, including β-adrenergic receptors.

Ion channels and receptors found in the membrane of smooth muscle cells contribute to VSMC contractility. Therefore, the trafficking of ion channels and receptors into and away from the cell membrane must be carefully controlled to maintain an appropriate vascular tone. One mechanism of protein trafficking within the cell is along the microtubule network, which forms part of the cytoskeleton and consists of polymers of α- and β-tubulin subunits. The motor protein dynein can move along the microtubule network to transport cargoes away from the cell membrane towards the minus-end. Little is known about how microtubules control protein trafficking within VSMCs. Recently, it has been shown that the microtubules affect VSMC contractility by regulating the membrane abundance of Kv7.4 channels. However, the underlying mechanism responsible for this trafficking remains unknown.

The current PhD thesis aimed to investigate the role of the motor protein dynein on the microtubule-dependent trafficking of Kv7 channels in vascular smooth muscle and investigate whether pharmacologically interfering with dynein trafficking or disrupting the microtubule network could improve vascular tone in hypertensive arteries. In study I, we demonstrated that dynein binds to Kv7.4 channels through a recognition sequence located in the C-terminus of Kv7.4 channels. With isometric tension recordings, a physiological role for dynein was established in rat mesenteric arteries, where dynein inhibition increased the Kv7 channel...
Following dynein inhibition, the enhanced Kv7.4 channel function was associated with increased Kv7.4 channel membrane abundance in isolated VSMCs. These results highlight the important role of dynein in the trafficking of the Kv7.4 channels in rat mesenteric arteries. Furthermore, we identified this dynein-dependent trafficking of Kv7.4 to be dependent on cholesterol-rich caveolae, where Kv7.4 channels reside. In arteries from hypertensive rats, Kv7.4 protein expression and function are reduced, partially underlying the attenuated β-adrenoceptor-mediated relaxation. We found in study II, that microtubule depolymerization and dynein inhibition in arteries from hypertensive rats improved Kv7.4 channel function, thereby restoring the β-adrenoceptor-mediated relaxations. In addition, we identified that dynein inhibition increased the β2-adrenoceptors functional contribution to isoprenaline-mediated relaxations. This study highlighted the potential for targeting the microtubule network for the treatment of hypertension. We, therefore, conducted a human clinical trial in study III, providing translational evidence that acute colchicine treatment can potentially improve arterial responses to certain vasodilators in hypertensive humans. In this study, colchicine improved vascular conductance to certain vasodilators in a short-lasting acute effect. Hence, further investigations are required to determine the long-term effect of colchicine treatment on vascular function in hypertensive patients. Together, these studies suggest that colchicine may be a promising novel therapeutic agent for hypertension and thereby reduce the incidence of cardiovascular disease.
Danish summary

Kronisk forhøjet arterielt blodtryk, hypertension, påvirker flere end 1.4 milliarden mennesker i verden. Hypertension er en risikofaktor for kardiovaskulære sygdomme og en af de ledende globale årsager til morbidity og mortalitet. På trods af eksisterende medicinsk behandling er hypertension svær at kontrollere, især fordi viden om de cellulære årsager bag udviklingen af hypertension mangler og den medicinske behandling ikke er tilstrækkelig specifik. Det er derfor vigtigt at forbedre vores forståelse for de cellulære og molekylære mekanismer som ligger til grund for hypertension, så at mere effektive lægemidler kan udvikles.

Primær hypertension er til stor del et resultat af en forøget total perifer modstand årsaget af en forhøjet arteriel tonus. Arteriers vægge er opbygget af vaskulære glatte muskelceller som har kontraktile egenskaber og som, ved at øge eller mindske graden af konstriktion, ændrer på arteriernes diameter og dermed den totale perifer modstand. Intracellulære calciumniveauer er især vigtige for initiering af de glatte muskelcells kontraktion. De intracellulære calciumniveauer reguleres ved membranepolarisering og aktivering af spændingsafhængige calciumkanaler (VDCCs). Ionkanaler i plasmamembranen bidrager til at regulere membranpotentialet og er derfor med til at bestemme calciums influx og den vaskulære tonus. Der findes flere forskellige ionkanaler kanaler i de glatte muskelceller, hvor kalium (K⁺) kanalerne udgør den mest diverse gruppe. Den største familie af K⁺ kanaler er de spændingsafhængige Kv kalium kanaler, hvor Kv7 isoformerne Kv7.4 og Kv7.5 er de mest almindeligt forekommende i humane og murine arterier. Kv7 kanaler reguleres af cGMP og cAMP afhængige receptorer og bidrager blandt andet til β-adrenoreceptor medieret vasodilation.

Internalisering af ionkanaler og relaterede receptorer er en velreguleret proces. Transport af disse proteiner sker blandt andet via mikrotubuli som er en del af cyoskelettet og består af polymerer af α- and β-tubulin subenheder. Proteintransporten foregår ved at motor proteinet dynein bevæges langs med mikrotubuli netværket. Der mangler i dag viden om hvordan mikrotubuli kontrollerer protein transport i de glatte muskelceller, men der er indikationer i litteraturen på, at mikrotubuli kan internalisere Kv7.4 kanaler og derved påvirke cellernes kontraktilitet. Den bagvedliggende præcise mekanisme er dog uklar.

Denne PhD afhandling havde til formål, at undersøge motorproteinets dynein’s rolle for mikrotubuli-afhængig transport af Kv7 kanaler i glatte muskelceller. I studie I, viste vi at dynein binder til Kv7.4 kanaler ved en genkendelses sekvens i C-terminus. Ved brug af isometriske spændings målinger fik vi klarlagt at dynein inhibering øgede Kv7 kanalernes funktion. Dynein inhibering resulterede i en øget tilstedeværelse og funktion af Kv7.4 kanalerne i isolerede glatte muskelceller. Disse resultater understreger dyneins vigtige rolle i transport af Kv7.4 kanalen i murine arterier fra mesenterium. Studiet viste også at mekanismen var afhængig af kolesterolige caveolae, hvor Kv7.4 kanaler er lokaliseret. Ydermere blev det fundet at Kv7.4 proteinmængde og funktion var nedsat i arterier fra hypertensive rotter. I studie II fandt vi i arterier fra hypertensive rotter at depolymerisation af mikrotubuli og inhibering af dynein forbedrede Kv7.4 kanal funktionen samt β-adrenoceptor-mediated relaksation. Ydermere fandt vi, at inhibering af dynein øgede β2-adrenoceptorernes funktionelle bidrag til isoprenaline-medieret relaksation. Studiets fund pegede på, at mikrotubuli er et lovende mål
for farmakologisk behandling af hypertension. Derfor gennemførte vi et translationelt forsøg på mænd med hypertension for at belyse om colchicine kan forbedre β-adrenoceptor-medieret karudvidelse. Studiet viste at colchicine akut og transient forbedrede følsomheden for isoprenalin. Fremtidige studier bør undersøge langtidseffekten af colchicine behandling på vaskulær funktion hos individer med hypertension. Sammenlagt viser studierne i denne afhandling at colchine er en potentiel kandidat til behandling af hypertension.