5. Summary

This thesis was aimed at studying the pharmacology of the contractile $\alpha_1$-adrenoceptors in rat tail artery, the contractile 5-HT$_1$-like receptors in guinea-pig iliac artery and the relaxant 5-HT receptors in pulmonary arteries of weaned pigs was studied in the present thesis. In addition, new functional in vitro assays were established and were used to investigate the pharmacology of genuine and partial synthetic ergolines.

The concentration-response ($E/[A]$) curves to noradrenaline were apparently monophasic but became biphasic in the presence of the selective $\alpha_{1A}$-adrenoceptor antagonist B8805-033. Whereas the first phase of the contraction to noradrenaline remained nearly unaffected in the presence of B8805-033 (0.03 – 3 µM), the second phase was shifted to the right in a concentration dependent manner. The receptors involved in the first phase of the contraction were further investigated in experiments in the presence of B8805-033 (3 µM). The second phase of contraction was investigated following pretreatment of arterial rings with B8805-033 (3 µM) and receptor inactivation with chloroethylclonidine (100 µM). The first phase of noradrenaline-induced contraction was antagonized in a competitive manner by prazosin, tamsulosin, WB 4101, spiperone, L-765,314, 5-methylurapidil, BMY 7378, and MDL 73005EF. RS-17053, however, antagonized the first phase of noradrenaline-induced contraction in a non-competitive manner. The rank order of antagonist potency was prazosin > tamsulosin > WB 4101 > spiperone > L-765314 > 5-methylurapidil ≥ RS-17053 ≥ BMY 7378 > MDL 73005EF. The $pK_B$ values obtained under these experimental conditions fitted best with antagonist affinities obtained at native and recombinant $\alpha_{1B/b}$-adrenoceptors. This argues for an involvement of $\alpha_{1B}$-adrenoceptors in the noradrenaline-induced contraction of the rat tail artery. The second phase of contraction was antagonized competitively by tamsulosin, 5-methylurapidil, RS-17053, B8805-033, BMY 7378, and L-765,314. Antagonist affinities for the second phase of contraction correlated highly with affinities at native and recombinant $\alpha_{1A/a}$-adrenoceptors.

The 5-HT$_1$-like receptor that mediates the contraction in precontracted guinea-pig iliac arteries was characterized using pharmacological and molecular-biological methods. RT-PCR studies in guinea-pig iliac arteries showed a strong signal for the 5-HT$_{1B}$ receptor while expression of 5-HT$_{1D}$ and 5-HT$_{1F}$ receptors was not detected in any sample. Following moderate precontraction with PGF$_{2\alpha}$ (0.1 – 1 µM), isolated rings of guinea-pig iliac artery were contracted monophasically by 5-HT, 5-carboxamidotryptamine, almotriptan, frovatriptan, naratriptan, rizatriptan, and zolmitriptan. Eletriptan contracted the guinea-pig iliac artery in a biphasic manner. The rank order of agonist potency was 5-carboxamidotryptamine ≥ 5-HT ≥ frovatriptan > zolmitriptan ≥ eletriptan (first phase of contraction) > rizatriptan ≥ naratriptan > sumatriptan > almotriptan. This rank order of agonist...
potency was similar to that observed in isolated human coronary arteries which are used by many
groups to predict cardiovascular sideeffects of triptans. Contractions to 5-HT, 5-carboxamido-
tryptamine, and triptans were antagonized by the 5-HT_{1B/D} receptor antagonist GR127935 (10 nM)
and by the 5-HT_{1B} receptor antagonist SB216641 (10 nM) but not by the 5-HT_{1D} receptor antagonist
BRL 15572 (100 nM). The present results demonstrate that triptan-induced contraction in guinea-
pig iliac arteries is mediated exclusively by the 5-HT_{1B} receptor. The guinea-pig iliac artery can thus
be used as a convenient in vitro assay to study the (cardio)vascular side-effect potential of anti-
migraine drugs of the triptan family.

In weaned pig pulmonary arteries with intact endothelium precontracted with PGF_{2α} (1 µM), the
relaxation to 5-HT consisted of an endothelium-dependent and an endothelium-independent
component. The endothelium-dependent relaxation was mediated via an activation of 5-HT_{2B}
receptors. In endothelium-denuded arterial rings, 5-HT, 5-carboxamidotryptamine, 5-methoxy-
tryptamine, and frovatriptan produced monophasic relaxations. Relaxant responses to the agonists
were antagonized by the selective 5-HT_{7} receptor antagonist SB 269970. The relaxant response to
the potent 5-HT_{7} receptor agonist 5-carboxamidotryptamine was also antagonized by methiothepin,
pimozide, mesulergine, methysergide, clozapine, and spiperone. The 5-carboxamidotryptamine-
induced relaxation was associated with an increase in cAMP. The estimated pK_{B} values for
agonists and the observed signal transduction argue in favor of an involvement of 5-HT_{7}
receptors in the direct vasorelaxant action of 5-HT in the pulmonary arteries of weaned pigs. The
present in vitro bioassay can be used to characterize new drugs with potential agonist or antagonist
properties at functional 5-HT_{7} receptors.

The interaction of naturally occurring ergolines, dihydroergolines, 8α-aminoergolines,
6'-desoxoergolines, and 1-allylergolines with vascular and non-vascular α_{1}-adrenoceptors and with
subtypes of vascular 5-HT receptors was studied in the second part of this thesis. The ergolines were
characterized pharmacologically using approved and newly established methods in the rat vas
derefers (α_{1A}), guinea-pig spleen (α_{1B}), rat tail artery (α_{1A}, α_{1B} and 5-HT_{2A}), rat aorta (α_{1D}), guinea-
pig iliac artery (5-HT_{1B}), pig coronary artery (5-HT_{2A}), pig pulmonary arteries (5-HT_{2B}), and weaned
pig pulmonary arteries (5-HT_{7}).

The naturally occurring ergopeptide alkaloids ergotamine, ergocristine, and α-ergocryptine were
found to be potent partial agonists at vascular α_{1D}- and α_{1D}-adrenoceptors and at 5-HT_{2A}
receptors. At α_{1A}-adrenoceptors, ergotamine, ergocristine, and α-ergocryptine behaved as potent antagonists.
Their ability to discriminate between the subtypes of α_{1}-adrenoceptors and 5-HT receptors was
limited. A hydrogenation of the Δ^{9,10} double-bond of ergotamine (→ dihydroergotamine) resulted in
a moderate increase in affinity to vascular α_{1A}-adrenoceptors. The replacement of the C-2'-methyl
substituent by isopropyl (e.g. ergotamine → ergocristin) results in a 6-fold reduced affinity to 5-HT\textsubscript{2A} receptors. The genuine clavine alkaloids lysergol and festuclavine possess a significantly lower affinity at subtypes of \( \alpha \text{-adrenoceptors} \) vs. vascular 5-HT\textsubscript{2} receptors. Therefore, it has been concluded that the tricyclic peptide substituent is responsible for the increased affinity of ergopeptines at \( \alpha \text{-adrenoceptors} \).

The influence of the \( C-6' \)-amide was investigated following chemical reduction of the ergolines to \( 6' \)-desoxoergolines. In contrast to their parent compounds, the \( 6' \)-desoxoergolines lacked agonist efficacy and exhibited a 10 to 30-fold lower affinity to 5-HT\textsubscript{2A} receptors. The affinity to \( \alpha \text{-adrenoceptors} \) was similarly decreased. It is concluded that the chemical reduction of the \( C-6' \)-amide of ergolines does not result in an increase of selectivity for \( \alpha \text{-adrenoceptors} \) over 5-HT\textsubscript{2A} receptors.

It is well known that \( N \)-1-methylergolines and \( N \)-1-isopropylergolines are potent antagonists with selectivity for rodent 5-HT\textsubscript{2} receptors over \( \alpha \text{-adrenoceptors} \). Therefore, the pharmacological interactions of \( N \)-1-allylergolines with 5-HT receptors and \( \alpha \text{-adrenoceptors} \) were characterized. \( N \)-1 allyl-substitution of clavines, 8\( \alpha \)-aminoergolines, and ergopeptines resulted in compounds with decreased or no agonist efficacy at \( \alpha \text{-adrenoceptors} \) and 5-HT receptors. Furthermore, the 1-allylergolines possess a reduced affinity to guinea-pig 5-HT\textsubscript{1B} receptors, porcine 5-HT\textsubscript{2A} and porcine 5-HT\textsubscript{7} receptors. The affinity of these compounds at rat 5-HT\textsubscript{2A} receptors was higher than that at porcine 5-HT\textsubscript{2A} receptors. This discrepancy may be attributed to differences in the amino acid sequence of the 5-HT\textsubscript{2A} receptor protein of rat and pig. The 1-allyl substituted ergopeptines 1-allylergotamine and 1-allyldihydroergotamine, but not \( N \)-1 allyl-substituted 8\( \alpha \)-aminoergolines and not \( N \)-1 allyl-substituted clavines possess an increased affinity at porcine 5-HT\textsubscript{2B} receptors. Both, 1-allylergotamine and 1-allyldihydroergotamine, were identified as selective 5-HT\textsubscript{2B} receptor-antagonists with 16 and 78-fold selectivity over other vascular 5-HT receptors and with 160 and 210-fold selectivity over \( \alpha \text{-adrenoceptors} \). The 5-HT\textsubscript{2B} receptor-selectivity of the 1-allylergopeptines is comparable to that of lisuride, which was initially developed for the prophylaxis of migraine. Therefore, 1-allylergotamine and 1-allyldihydroergotamine may be effective drugs in the prophylaxis of migraine.