5. Summary

This thesis was aimed at studying the pharmacology of the contractile α_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 α_{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-methyl-urapidil ≥ 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 α_{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 monophasicly by 5-HT, 5-carboxamidotryptamine, almotriptan, frovatriptan, naratriptan, rizatriptan, and zolmitriptan. Eletriptan contracted the 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 side-effects of triptans. Contractions to 5-HT, 5-carboxamido-tryptamine, and triptans were antagonized by the 5-$HT_{1B/1D}$ 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 $PGF_{2\alpha}$ (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 antagonists 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 deferens (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 ergopeptine alkaloids ergotamine, ergocristine, and α-ergocryptine were found to be potent partial agonists at vascular α_{1B}- 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 $\Delta^{9,10}$ 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_{2A} receptors. The genuine clavine alkaloids lysergol and festuclavine possess a significantly lower affinity at subtypes of α_1-adrenoceptors *vs*. vascular 5-HT_2 receptors. Therefore, it has been concluded that the tricyclic peptide substituent is responsible for the increased affinity of ergopeptines at α_1-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_{2A} receptors. The affinity to α_1-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 α_1-adrenoceptors over 5-HT_{2A} receptors.

It is well known that *N*-1-methylergolines and *N*-1-isopropylergolines are potent antagonists with selectivity for rodent 5-HT_2 receptors over α_1-adrenoceptors. Therefore, the pharmacological interactions of *N*-1-allylergolines with 5-HT receptors and α_1-adrenoceptors were characterized. *N*-1 allyl-substitution of clavines, 8α-aminoergolines, and ergopeptines resulted in compounds with decreased or no agonist efficacy at α_1-adrenoceptors and 5-HT receptors. Furthermore, the 1-allylergolines possess a reduced affinity to guinea-pig 5-HT_{1B} receptors, porcine 5-HT_{2A} and porcine 5-HT_7 receptors. The affinity of these compounds at rat 5-HT_{2A} receptors was higher than that at porcine 5-HT_{2A} receptors. This discrepancy may be attributed to differences in the amino acid sequence of the 5-HT_{2A} receptor protein of rat and pig. The 1-allyl substituted ergopeptines 1-allyl-ergotamine and 1-allyldihydroergotamine, but not *N*-1 allyl-substituted 8α-aminoergolines and not *N*-1 allyl-substituded clavines possess an increased affinity at porcine 5-HT_{2B} receptors. Both, 1-allylergotamine and 1-allyldihydroergotamine, were identified as selective 5-HT_{2B} receptor-antagonists with 16 and 78-fold selectivity over other vascular 5-HT receptors and with 160 and 210-fold selectivity over α_1-adrenoceptors. The 5-HT_{2B} receptor-selectivity of the 1-allylergo-peptines 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.