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Presynaptic receptors in the vasculature of the freely moving rat

Remie, René

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7. SUMMARY

Until now modulation of neurotransmitter release by presynaptic receptors was almost exclusively studied in isolated tissues and organs using radiolabelled catecholamine as a marker for the release of endogenous noradrenaline (NA), other studies were done in anesthetized animals.

This thesis describes the development of an *in vivo* model allowing the study of presynaptic modulation of endogenous NA overflow in the vasculature of freely moving unanesthetized rats. In supplement 6.1 the technique is described in which a silicon cannula was used for the permanent cannulation of the portal vein, together with a bipolar platinum stimulation electrode around this vein. The method had a high success ratio of over 70% during a period of more than 2 months. The determination of the endogenous catecholamines was performed using 100 μ l plasma with high performance liquid chromatography with electrochemical detection. The final procedure showed a detection limit of 450 fg/ml NA and 1 pg/ml adrenaline (ADR). Enhancement of all electrical stimulation parameters proportionally increased the evoked NA release. The first experiments using 1 mg/kg yohimbine showed that blockade of the presynaptic α_2 -adrenoceptors resulted in a 4.4-fold rise of the basal portal NA level and an additional 4.7-fold facilitation of the electrically evoked release. Once developed, the technique was further refined by insertion of a second cannula distal from the stimulation electrodes, which allowed the injection and infusion of specific receptor agonists and antagonists for the pharmacological study of several types of vascular presynaptic receptors.

A facilitation of endogenous NA overflow was seen after stimulation of the presynaptic β_2 -adrenoceptors in the rat portal vein (suppl. 6.2). This facilitation, however, was much more pronounced than one would expect on the basis of previously reported *in vitro* studies (160%). A maximal enhancement of about 300% of the basal NA level was seen after a single dose of the β_2 -selective agonist fenoterol. In the

presence of the α_2 -adrenoceptor antagonist yohimbine, a low - in its absence uneffective - dose of fenoterol, was able to enhance the NA level to 1200% of the basal level, showing the pronounced counterregulatory role of the presynaptic α_2 -adrenoceptor. During electrical stimulation of the portal vein nervous plexus a comparable pronounced facilitation of evoked NA overflow was seen, both in the absence and in the presence of yohimbine.

In supplement 6.3 the nature of the vascular presynaptic β -adrenoceptors and the possible heterogeneity of the receptor population was investigated, using the differential blockade technique. The β_2 -selective antagonist ICI 118,551 was able to reverse the fenoterol-induced enhancement, both of basal and electrically evoked NA overflow, to control values. The β_1 -selective antagonist, CGP 20712 A, even at a high dose, was inactive. Neither antagonist influenced the effect of high synaptic concentrations of endogenous NA induced by administration of yohimbine. Since NA is a full β_1 -adrenoceptor agonist these results showed that no β_1 -adrenoceptor subpopulation was present in the portal vein of the unrestrained conscious rat. It could also be concluded that even at high concentrations, NA is unable to facilitate its own release via the homogenous presynaptic β_2 -adrenoceptor population.

In chapter 3, a number of experiments is described on the role of ADR as a possible cotransmitter in noradrenergic neurotransmission. It could be shown that infused ADR is taken up rapidly via cocaine-sensitive uptake carriers into portal vein sympathetic varicosities. Surprisingly, even low concentrations of ADR were capable of displacing endogenous NA from the vesicle. Following stimulation, both in absence and in presence of yohimbine, this ADR-pool was rapidly released without a concomitant facilitation of remaining endogenous NA overflow. In the presence of the β_2 -selective adrenoceptor antagonist ICI 118,551 a significant attenuation of the evoked ADR overflow was seen, indicating presynaptic β_2 -adrenoceptors also facilitating neuronal ADR release.

In supplement 6.4 we demonstrated that the spontaneously hypertensive rat

(SHR) respond to much lower dosages of fenoterol when compared to those of the Wistar rat (WR). This strongly pronounced facilitation could, however not be attributed to a dysfunction of the β_2 -adrenoceptor but rather to a dysfunctional α_2 -adrenoceptor, thus unmasking the β_2 -adrenoceptor-mediated facilitation of NA overflow.

Supplements 6.5 and 6.6 describe the investigations on the presence and the character of the presynaptic muscarinic receptors in the rat portal vein. Infusion of methacholine (MCh) had no effect on the basal NA level. During electrical stimulation, however, MCh strongly inhibited (95%) the evoked NA overflow, while atropine was able to reverse this inhibition. In the presence of the α_2 -adrenoceptor antagonist yohimbine, MCh again was able to reduce the yohimbine-induced enhanced NA overflow. Using selective antagonists like pirenzepine (M_1), AF-DX 116 (M_2) and 4-DAMP (M_3), the receptor subtype was determined. From the order of the pIC_{50} values it could be concluded that the presynaptic muscarinic receptor was of the cardiac M_2 -subclass.

Finally, some preliminary experiments using morphine showed the presence and functioning of inhibitory presynaptic opioid receptors in the vascular sympathetic varicosities of the unrestrained conscious rat. The precise nature of these presynaptic opioid receptor(s) remains to be elucidated.