Abstract The objective of the study was to clarify the postnatal development of the following transmitter release-modulating receptors of noradrenergic neurons in mice: $\alpha_2$-adrenoceptors, muscarinic, opioid and cannabinoid receptors (inhibitory), $\beta$-adrenoceptors and receptors for angiotensin II and bradykinin (facilitatory). Wildtype (NMRI) and in some cases $\alpha_2A/D$-adrenoceptor-deficient mice aged 1 day (P1) or 8–16 weeks (adults) were used. Hippocampal and occipito-parietal cortex slices and sympathetic innervated tissues (atria and vas deferens) were preincubated with $[^3H]$-noradrenaline and then superfused and stimulated electrically. Stimulation led to distinct increases in tritium efflux which were abolished by tetrodotoxin or removal of calcium. Concentration-response curves of appropriate agonists and in the case of $\alpha_2$-autoreceptors antagonists were determined. For $\beta$-adrenoceptors and angiotensin receptors, the interaction of agonists with antagonists was also examined. Results demonstrate that $\alpha_2A/D$-autoreceptors operate already at P1 whereas non-$\alpha_2A/D$-autoreceptors, presumably $\alpha_2C$, develop later. Of the various heteroreceptors, those of brain noradrenergic neurons (OP3 and ORL1) modulate the release of $[^3H]$-noradrenaline at least as effectively at P1 as in adults. Those of peripheral sympathetic neurons (muscarinic, probably mainly M3, OP1, OP2, OP3, CB1, AT1 and B2), in contrast, operate less effectively or not at all at P1, with one exception: $\beta_2$-adrenoceptors increase the release of $[^3H]$-noradrenaline (atria) to the same extent, irrespective of age. Overall, results indicate that brain and peripheral noradrenergic neurons release their transmitter already shortly after birth. Presynaptic receptor mechanisms mature differentially in the brain and the periphery. Moreover, the various presynaptic receptors differ in their postnatal development and may play differential roles at different ages.

Keywords Presynaptic receptors · Noradrenaline release · $\alpha_2$-Adrenoceptors · $\alpha_2$-Autoreceptors · Muscarinic receptors · Opioid receptors · Cannabinoid receptors · Postnatal development

Introduction

Presynaptic receptors contribute to the function of central as well as peripheral neuron systems (see Starke 1981; Starke et al. 1989; Fuder and Muscholl 1995; Powis and Bunn 1995; Langer 1997; Bennett 1999). Considering the universal occurrence of presynaptic receptors, little is known about their ontogenesis. Presynaptic release-inhibiting dopamine D2-autoreceptors operate in the rat striatum at embryonic day 17 (De Vries et al. 1992). Presynaptic muscarinic autoreceptors, in contrast, develop in rat brain only after birth and later than the release of acetylcholine (Marchi et al. 1983; Goldbach et al. 1998; but see De Vries et al. 1992). Presynaptic GABA_A-receptors are present on hippocampal GABAergic terminals of rats at birth, but an autoinhibition by endogenous GABA begins later, apparently because the concentration of released endogenous GABA at the autoreceptors remains too low at the time of birth (Caillard et al. 1998).

In the present work we have studied the development of presynaptic, release-modulating receptors at cerebral and peripheral noradrenergic neurons of mice. Noradrenergic axons from the locus coeruleus innervate the brain cortex and the hippocampus of rats at embryonic day 18 (see Marshall et al. 1991). Although the high affinity uptake of $[^3H]$-noradrenaline in rat brain tissue increases greatly after birth, release of $[^3H]$-noradrenaline is well detectable within 24 h after birth (P0; Wemer and Mulder 1981). In the periphery, few postganglionic sympathetic noradrenergic axons have reached the heart and vas defer-
ens of rats and the vas deferens of mice at birth, and the noradrenaline content per g tissue increases greatly in postnatal life (Yamauchi and Burnstock 1969; Owman et al. 1971; see Burnstock and Costa 1975; Hill et al. 1999). The axons possess transmitter storage vesicles, however, and like the brain neurons release previously incorporated [3H]-noradrenaline within the first 24 hours (present study; see also Moura et al. 1993). As in the case of other neurons, little is known about the development of presynaptic receptors at noradrenergic neurons. The first study seems to have been that of Wemer and Mulder (1981) in rat brain cortex. Wemer and Mulder showed that in cortical slices from P0, P7 and P17 rats noradrenaline reduced, and in cortical slices from P7 rats phenolamine enhanced, the release of previously stored [3H]-noradrenaline essentially as in slices from adult animals, indicating an early operation of presynaptic α2-autoreceptors in the brain.

We chose the mouse because of its importance for genetic studies and examined presynaptic α2-autoreceptors as well as several heteroreceptors, namely muscarinic (mainly M2), opioid OP1 (δ), OP2 (κ) and OP3 (μ) receptors, the opioid receptor-like ORL1 receptor, cannabinoid CB1 receptors, β2-adrenoceptors, angiotensin AT1 receptors and bradykinin B2 receptors. All occur in at least some tissues of adult mice (Cox et al. 1999; Trendelenburg et al. 2000; Zhou et al. 2001). Presynaptic β2-adrenoceptors are reckoned among the heteroreceptors here because they do not fully satisfy the autoreceptor definition (p. 872 of Starke et al. 1989). Experiments concentrated on two age groups: P1 and adults.

**Methods**

**Preparations and protocols.** NMRI (Naval Medical Research Institute; genetically non-manipulated) or OP2 KO-adrenoreceptor-deficient (OP2−/−; KO) mice (Altman et al. 1999) aged 1 day (P1) or 8–16 weeks (adult) were killed by exsanguination. Unless stated otherwise, results refer to NMRI mice. It should be noted that the properties of presynaptic α2-autoreceptors are identical in NMRI mice and the wildtype strain from which the KO animals were generated (Scheibner et al. 2001; Trendelenburg et al. 2001b). The adult animals were males, whereas the young animals were of either sex except when the vas deferens was used. The brain, heart or vasa deferentia were removed and placed in ice-cold physiological salt solution. The preparations were then placed in 0.5 ml of [3H]-noradrenaline. Preparations were then placed in 0.16-ml superfusion chambers between platinum electrodes, 6–7 slices of the occipito-parietal cortex, the two hippocampi, the right and left parietal cortex, 6–7 slices of the occipito-parietal cortex, 6–7 slices of the atria and 12–14 pieces of the vasa deferentia of each chamber. The first superfusion period was delivered at t=0 min and was not used for determination of tritium overflow. The subsequent superfusion periods (S1 to S6) were applied at t=54, 72, 90, 108, 126 and 144 min. Agonist or antagonist concentration-response curves were obtained by introducing the drug at increasing concentrations after S1, 12 min before S2 to S6 (see Fig. 1). For interaction experiments, antagonists were present throughout the superfusion period. The reference value the average corresponding S/S1 ratio in control experiments in which no drug was given after S1. For release-enhancing drugs (phenolamine, rauwolscine, salbutamol, angiotensin II and bradykinin), logarithms of concentrations causing a p% increase in evoked tritium overflow (PECp%) were interpolated from the nearest points of the concentration-response curves. Apparent pKd values of antagonists were calculated from the shifts of agonist (salbutamol and angiotensin II) concentration-response curves to the right. The pKd values are apparent because only one antagonist concentration was tested and the competitive character of the interaction was not verified. Effects of drugs added after S1 on basal tritium outflow were calculated in the same manner, based on samples collected immediately before stimulation.

Data are expressed as mean±SEM; n denotes the number of superfusion chambers. The statistical significance of differences between groups was determined by Mann-Whitney test with Bonferroni correction. Error probabilities less than 5% (P<0.05) were taken to indicate significant differences.

**Materials.** The PSS for superfusion had the following composition (mM): NaCl 118, KCI 4.8, CaCl2 1.3 (brain slices) or 2.5 (peripheral tissues), MgSO4 1.2, NaHCO3 25, KH2PO4 1.2, glucose 11, ascorbic acid 0.57, ethylenediaminetetraacetic acid disodium salt 0.03 and desipramine 0.001. The PSS for preparation and for preincubation with [3H]-noradrenaline contained no desipramine and, for peripheral tissues, only 0.2 mM CaCl2.

(--)[2,5,6,6'3H]-Noradrenaline, specific activity 46.8–70.7 Ci/mmol, was from New England Nuclear, Köln, Germany. The following drugs were used: naloxone hydrochloride (gift from Gödecke, Freiburg, Germany); phenolamine hydrochloride, (±)-5-hydroxy-5-[2-[2-hydroxy-3-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy-benzenemethanesulfonate (CGB 20712A; gifts from Ciba-Geigy, Basel, Switzerland); 5-bromo-6-(2-imidazolin-2-ylidenamino)quinoxaline HCl (UK 14,304 or brimonidine; gift from Pfizer, Kent, U.K.); angiotensin II (human), bradykinin, [D-Ala2, N-Me-Phe4, Gly5-ol] enkephalin (DAMGO), desipramine hydrochloride, [D-Pen²,⁵]-enkephalin (DPDPE), (–)-2-(3,4-dihydro-7-methyl-1H-inden-4-yl)oxy-(3-[1-methyllethy]l)amino]-2-butanoic acid (ICI 118,551), oxotremorine methylhexylamino methanesulfate (OXM-150,000), oxotremorine methyl-3H]oxotremorine-M, S(+)-1-(4-4-chloro-2-butanoyl phosphoryl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1H-imidazo[4,5-c]pyridine-6-carboxylic acid di-trifluoroacetate (PD 123,319), rauwolscine HCl, salbutamol hemisulphate, trans-(–)-3,4-dichlo-3H]-noradrenaline contained no desipramine and, for peripheral tissues, only 0.2 mM CaCl2.

Analysis of data. The outflow of tritium was calculated as a fraction of the tritium content of the tissue at the onset of the respective collection period (fractional rate; per min). The overflow of tritium evoked by electrical field stimulation was calculated as the total tritium outflow during the collection period in which stimulation was applied and during the collection period thereafter, minus the estimated basal outflow. Basal outflow was assumed to decline linearly from the collection period before stimulation to the second collection period after stimulation. The evoked overflow was expressed as a percentage of the tritium content of the tissue at the time of stimulation. Overflow ratios (S/S1) were then determined. Percentage changes of S/S1 ratios caused by agonists or antagonists added after S1 were calculated for each preparation, taking as reference value the average corresponding S/S1 ratio in control experiments in which no drug was given after S1. For release-enhancing drugs (phenolamine, rauwolscine, salbutamol, angiotensin II and bradykinin), logarithms of concentrations causing a p% increase in evoked tritium overflow (PECp%) were interpolated from the nearest points of the concentration-response curves. Apparent pKd values of antagonists were calculated from the shifts of agonist (salbutamol and angiotensin II) concentration-response curves to the right. The pKd values are apparent because only one antagonist concentration was tested and the competitive character of the interaction was not verified. Effects of drugs added after S1 on basal tritium outflow were calculated in the same manner, based on samples collected immediately before stimulation.

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