British Journal of Pharmacology (2000) 130, 321–330

www.nature.com/bjp

Modulation of ^{3}H-noradrenaline release by presynaptic opioid, cannabinoid and bradykinin receptors and β-adrenoceptors in mouse tissues

*,[1]A.U. Trendelenburg, [1]S.L. Cox, [1]V. Schelb, [1]W. Klebroff, [1]L. Khairallah & [1]K. Starke

[1]Pharmakologisches Institut, Universität Freiburg, Hermann-Herder-Strasse 5, D-79104 Freiburg i. Br., Germany

1 Release-modulating opioid and cannabinoid (CB) receptors, β-adrenoceptors and bradykinin receptors at noradrenergic axons were studied in mouse tissues (occipito-parietal cortex, heart atria, vas deferens and spleen) preincubated with ^{3}H-noradrenaline.

2 Experiments using the OP_1 receptor-selective agonists DPDPE and DSLET, the OP_2-selective agonists U50488H and U69593, the OP_3-selective agonist DAMGO, the ORL_1 receptor-selective agonist nociceptin, and a number of selective antagonists showed that the noradrenergic axons innervating the occipito-parietal cortex possess release-inhibiting OP_3 and ORL_1 receptors, those innervating atria OP_1, ORL_1 and possibly OP_3 receptors, and those innervating the vas deferens all four opioid receptor types.

3 Experiments using the non-selective CB agonists WIN 55,212-2 and CP 55,940 and the CB_1-selective antagonist SR 141716A indicated that the noradrenergic axons of the vas deferens possess release-inhibiting CB_1 receptors. Presynaptic CB receptors were not found in the occipito-parietal cortex, in atria or in the spleen.

4 Experiments using the non-selective β-adrenoceptor agonist isoprenaline and the β_2-selective agonist salbutamol, as well as subtype-selective antagonists, demonstrated the occurrence of release-enhancing β_2-adrenoceptors at the sympathetic axons of atria and the spleen, but demonstrated their absence in the occipito-parietal cortex and the vas deferens.

5 Experiments with bradykinin and the B_2-selective antagonist Hoe 140 showed the operation of release-enhancing B_2 receptors at the sympathetic axons of atria, the vas deferens and the spleen, but showed their absence in the occipito-parietal cortex.

6 The experiments document a number of new presynaptic receptor locations. They confirm and extend the existence of marked tissue and species differences in presynaptic receptors at noradrenergic neurons.

British Journal of Pharmacology (2000) **130,** 321–330

Keywords: β-adrenoceptors; B_2 receptors; CB_1 receptors; mouse; noradrenaline release; opioid receptors; ORL_1 receptors; presynaptic receptors

Abbreviations: BNTX, 7-benzylidenenaltrexone maleate; CGP 20712A, (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]propyl]amino]ethoxy]-benzamide methanesulphonate; CP 55,940, (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl)cyclohexanol; DAMGO, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin; DPDPE, [D-Pen^{2,5}]-enkephalin; DSLET, [D-Ser^2]-Leu-enkephalin-Thr^6; Hoe 140, D-Arg[Hyp^3,Thi^5,D-Tic^7,Oic^8]bradykinin; ICI 118,551, (±)-1-[2,3-(dihydro-7-methyl)-1H-inden-4-yl)oxy]-3-[(1-methylethyl)amino]-2-butanol HCl; ORL_1 receptor, opioid receptor like$_1$ receptor; PheΨ, [Phe^{1}Ψ(CH_2-NH)Gly^2]nociceptin(1-13)NH_2; SR 141716A, N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-3-pyrazole-carboxamide; U50488H, *trans*-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide methanesulphonate; U69593, (5α,7α,8β)-(+)-N-methyl-N-(7-(1-pyrrolidinyl]-1-oxaspiro[4,5]dec-8-yl)-benzeneacteamide; WIN 55,212-2, (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl)pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone

Introduction

Neurotransmitter release is subject to modulation by presynaptic receptors. Central and peripheral noradrenergic neurons, for example, possess release-inhibiting α_2-autoreceptors and many other presynaptic receptors which either induce, enhance or inhibit transmitter release (for review see Starke, 1977; Langer, 1981; Fuder & Muscholl, 1995). Presynaptic receptors have been extensively studied in many human and animal tissues. Molecular genetic techniques have now moved the mouse species into the centre of interest: transgenic animals are almost exclusively mice. However, in contrast to other species, little information is available about presynaptic receptors in mice.

*Author for correspondence; E-mail: trendele@ruf.uni-freiburg.de

Presynaptic α_2-autoreceptors are relatively well studied in the mouse. Like the majority of presynaptic α_2-adrenoceptors across mammalian species, they belong predominantly to the $\alpha_{2A/D}$ subtype (Limberger *et al*., 1995; Wahl *et al*., 1996), but α_{2C}-autoreceptors exist as well (Altman *et al*., 1999; Hein *et al*., 1999; Trendelenburg *et al*., 1999). Other presynaptic receptors at noradrenergic neurons demonstrated in mice include the opioid receptors in the vas deferens, which were among the first release-inhibiting opioid receptors detected (Henderson *et al*., 1972). All three classical opioid receptors, OP_1 (δ), OP_2 (κ) and OP_3 (μ), as well as the recently identified opioid receptor-like ORL_1 receptor, inhibit sympathetic neurotransmission in the mouse vas deferens (Berzetei-Gurske *et al*., 1996; Calò *et al*., 1996; Illes, 1989). Moreover, release-inhibiting muscarinic

(possibly M_2; Costa & Majewski, 1991), histamine H_3 (Schlicker *et al*., 1992), P2 (von Kügelgen *et al*., 1989), adenosine A_1 (Blakeley *et al*., 1988), neuropeptide Y (Foucart & Majewski, 1989), prostanoid EP_3 (Exner & Schlicker, 1995) and cannabinoid CB_1 receptors (Pertwee *et al*., 1992) as well as release-facilitating β-adrenoceptors (Johnston & Majewski, 1986), muscarinic (possibly M_1; Costa & Majewski, 1991), angiotensin (Rajanayagam *et al*., 1989) and bradykinin receptors (Llona *et al*., 1991) have been found at mouse noradrenergic neurons. However, the tissue distribution and subtype of these presynaptic receptors have not been systematically examined.

In a first attempt to enlarge our knowledge about receptors on noradrenergic neurons in the mouse, we recently investigated presynaptic receptors for angiotensin in several tissues (Cox *et al*., 1999). The aim of the present study was to search for, and if detected subclassify, two release-inhibiting receptors, namely opioid and cannabinoid receptors, and two other release-enhancing receptors, namely β-adrenoceptors and bradykinin receptors, on the noradrenergic axons of the mouse occipito-parietal cortex, atria, vas deferens and spleen.

Methods

Tissues and superfusion

Male NMRI mice weighing 35–45 g were killed by cervical dislocation. Either six to seven slices of the occipito-parietal cortex (Limberger *et al*., 1995), six to eight pieces of the atria (Wahl *et al*., 1996), eight to 12 pieces of the vas deferens (Trendelenburg *et al*., 1999), or 12 to 16 pieces of the spleen (Cox *et al*., 1999) were obtained from one animal. The tissue pieces were preincubated in 2 ml medium containing 0.2 μM ^{3}H-noradrenaline for 30 min at 37°C. One tissue piece was then placed in each of 12 superfusion chambers between platinum electrodes, where it was superfused with ^{3}H-noradrenaline-free medium at a rate of 1.2 ml min^{-1}. Successive 2-min samples of the superfusate were collected from $t=50$ min onwards ($t=0$ min being the start of superfusion). At the end of the experiments, tissues were dissolved and tritium was determined in superfusate samples and tissues.

The superfusion medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3 (brain slices) or 2.5 (peripheral tissues), $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium EDTA 0.03 and desipramine 0.001. The medium for preincubation with ^{3}H-noradrenaline contained no desipramine and, for peripheral tissues, only 0.2 mM $CaCl_2$.

Experimental protocol

There were seven periods of electrical stimulation. Each stimulation consisted of rectangular pulses of 1 ms width and 35 V cm^{-1} (brain slices) or 47 V cm^{-1} (peripheral tissues) voltage drop between the electrodes of each chamber, yielding a current strength of 60 and 80 mA, respectively. The first stimulation period was delivered at $t=30$ min and was not used for determination of tritium overflow. The subsequent stimulation periods (S_1 to S_6) were applied at $t=54$, 72, 90, 108, 126 and 144 min and differed, depending on the tissue and type of experiment, as indicated in the Results section.

Agonist concentration-response curves were obtained by introducing the agonist at increasing concentrations after S_1, 12 min before S_2, S_3, S_4, S_5 and S_6. Antagonists were present throughout superfusion at a fixed concentration.

Evaluation

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; min^{-1}). The overflow elicited by electrical stimulation was calculated as the difference 'total tritium outflow during and after stimulation' minus 'basal outflow', and was then expressed as a percentage of the tritium content of the tissue at the time of stimulation (see Trendelenburg *et al*., 1997). For further evaluation, overflow ratios (S_n/S_1) were calculated. Overflow ratios obtained in the presence of agonist were also calculated as a percentage of the corresponding ratio in controls in which no agonist was added after S_1. Effects of agonists on basal tritium outflow were evaluated similarly (Trendelenburg *et al*., 1997).

Concentration-response data for agonists given alone were evaluated by sigmoid curve fitting (eq. 25 of Waud, 1976). This yielded the E_{max} (maximal effect) of the agonist and its EC_{50} (concentration causing a half-maximal effect) in the absence of antagonist. The EC_{50} values of agonists in the presence of antagonists were interpolated from the nearest points of the respective concentration-response curves, assuming that the E_{max} of the agonist had not changed. When only one or two concentrations of an antagonist were tested against an agonist, the negative logarithm of the apparent K_d value of the antagonist, i.e. the apparent pK_d, was calculated from the EC_{50} increase. When three concentrations of an antagonist were tested against an agonist (experiments on β-adrenoceptors in atria), results were evaluated as described by Arunlakshana & Schild (1959).

Unless stated otherwise, results are expressed as arithmetic means ± s.e. mean or, in the case of EC_{50} or E_{max} values, the standard errors as defined by Waud (1976). Groups were tested for significant differences by the Mann–Whitney test with Bonferroni correction. $P<0.05$ was taken as limit of statistical difference. *n* represents the number of tissue pieces.

Drugs

Drugs were (−)-[ring-2,5,6-^{3}H]-noradrenaline, specific activity 46.8–62.3 Ci mmol^{-1} (DuPont, Dreieich, Germany); bradykinin, bremazocine HCl, [D-Ala^2, N-Me-Phe^4, Gly^5-ol]-enkephalin (DAMGO), desipramine HCl, [D-Pen^{2,5}]-enkephalin (DPDPE), [D-Ser^2]-Leu-enkephalin-Thr^6 (DSLET), (±)-1-[2,3- (dihydro-7-methyl-1H-inden-4-yl) oxy]-3-[(1-methylethyl)amino]-2-butanol HCl (ICI 118,551), (−)-isoprenaline (+)-bitartrate, naltriben methanesulphonate, naltrindole HCl, salbutamol hemisulphate, *trans*-(±)-3,4-dichloro-N-methyl-N-(2-[1-pyrrolidinyl]cyclohexyl)-benzeneacetamide methanesulphonate (U50488H), (5α,7α,8β) - (+) -N-methyl-N-[7-[1-pyrrolidinyl] -1- oxaspiro [4,5] dec-8-yl) - benzeneacetamide (U69593; Sigma, Deisenhofen, Germany); 7-benzylidenenaltrexone maleate (BNTX), (−)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4- (3-hydroxypropyl) cyclohexanol (CP 55,940), nociceptin, [Phe$^1\Psi$(CH$_2$-NH)Gly^2]nociceptin(1-13)NH$_2$ (PheΨ), (R)-(+)-[2,3-dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo [1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone (WIN 55, 212-2; Tocris, U.K.). The following drugs were kindly provided by the producer: D-Arg[Hyp^3, Thi^5, D-Tic^7,Oic^8]bradykinin (Hoe 140; Hoechst, Frankfurt am Main, Germany); (±)-2-hydroxy-5-[2-[[2-hydroxy-3-[4-[1-methyl -4-(trifluoromethyl) - 1H-imidazol-2-yl] phenoxy]propyl] amino] ethoxy] -benzamide methanesulphonate (CGP 20712A), phentolamine methanesulphonate (Ciba-Geigy, Basel, Switzerland); naloxone HCl (Gödecke, Freiburg, Germany) and N-piperidino-5- (4-chlorophenyl)-1- (2,4-dichlorophenyl) -4-methyl-3-

pyrazole-carboxamide (SR 141716A; Sanofi, Montpellier, France). Drugs were dissolved in distilled water except naltriben (10 mM HCl) and CP 55,940, SR 141716A and WIN 55,212-2 (dimethylsulphoxide).

Results

General observations

Common features of the experiments will be summarized here. The operation of presynaptic opioid receptors, cannabinoid receptors and β-adrenoceptors is blunted whenever there is α_2-autoinhibition. Conversely, bradykinin requires ongoing α_2-autoinhibition for a major release-enhancing effect (see Schlicker & Göthert, 1998; Cox *et al*., 2000). For this reason, the former three receptors were studied with stimulation conditions leading to little autoinhibition, whereas bradykinin was studied under autoinhibition-rich conditions. Marked autoinhibition was created with 36 pulses (brain slices) or 120 pulses at 3 Hz (peripheral tissues) per stimulation period, conditions under which the α-adrenoceptor antagonist phentolamine (1 μM), when present throughout superfusion, greatly increased the overflow evoked by S_1 (Table 1). Minor autoinhibition was obtained either with single pulses (brain slices) or with brief trains of 20 pulses at 50 Hz (peripheral tissues) per stimulation period (see Singer, 1988; Limberger *et al*., 1995), conditions under which phentolamine (1 μM) increased S_1 only by maximally 70% (Table 1). Some tissue preparations were also superfused throughout the experiment with medium containing phentolamine (1 μM) which, of course, assured freedom from α_2-autoinhibition.

Table 1 Overflow of tritium (S_1) elicted by different pulse patterns in the absence and presence of phentolamine (1 μM)

Tissue (stimulation conditions)	*Evoked tritium overflow* (S_1,% of tissue tritium) *No phentolamine*	*Phentolamine* (1 μM)
Occipito-parietal cortex		
(Single pulse)	0.50±0.02 (76)	0.64±0.03* (9)
(36 pulses at 3 Hz)	2.50±0.28 (22)	N.T.†
Atria		
(20 pulses at 50 Hz)	0.38±0.01 (70)	0.52±0.03* (34)
(120 pulses at 3 Hz)	1.01±0.09 (19)	4.10±0.18* (47)
Vas deferens		
(20 pulses at 50 Hz)	0.29±0.01 (155)	0.49±0.03* (18)
(120 pulses at 3 Hz)	0.40±0.02 (13)	1.49±0.07 (11)
Spleen		
(120 pulses at 3 Hz)	0.50±0.06 (12)	1.35±0.10* (38)

Tissues were preincubated with ^{3}H-noradrenaline and then superfused with medium containing desipramine (1 μM). Phentolamine, when used, was present throughout superfusion. Values are means±s.e.mean from (*n*) tissue pieces. Significant differences from no phentolamine: $*P<0.001$. †N.T., not tested. Under the same conditions, phentolamine (1 μM) increased evoked tritium overflow from hypothalamus slices by 397% (Cox *et al.,* 1999).

Apart from phentolamine (Table 1), most of the other antagonists, when added throughout superfusion, did not *per se* change the evoked overflow of tritium (S_1). The greatest change was a 56% increase by 0.1 μM naltriben. In control experiments in which no drug was added after S_1, the overflow remained similar from S_1 to S_6 ($n=5-65$).

The basal outflow of tritium (for typical values see Cox *et al*., 1999) was not changed by the antagonists or agonists, with very few exceptions. The greatest change was a 50% increase by 1 μM of the cannabinoid (CB) agonist WIN 55,212-2.

Presynaptic opioid receptors

Presynaptic opioid receptors were investigated in the occipito-parietal cortex, in atria and in the vas deferens. Noradrenaline release was elicited either by single pulses (occipito-parietal cortex) or by 20 pulses at 50 Hz (peripheral tissues), conditions with little α_2-autoinhibition (Table 1). The following opioid receptor ligands were used: the OP_1 (δ) -selective agonists DPDPE and DSLET, the OP_2 (κ) -selective agonists U50488H and U69593, the OP_3 (μ) -selective agonist DAMGO, the ORL_1-selective agonist nociceptin, the OP_1-selective antagonists naltrindole, naltriben (δ_1) and BNTX (δ_2), the OP_2-selective antagonist bremazocine, the slightly OP_3-selective antagonist naloxone and the selective ORL_1 antagonist PheΨ.

In the occipito-parietal cortex, DAMGO caused concentration-dependent inhibition of the evoked overflow of tritium, whereas the OP_1-selective agonists DPDPE and DSLET and the OP_2-selective agonist U50488H were inactive (Figure 1A). The EC_{50} and E_{max} values are shown in Table 2. The antagonists at classical opioid receptors naltrindole, bremazo-

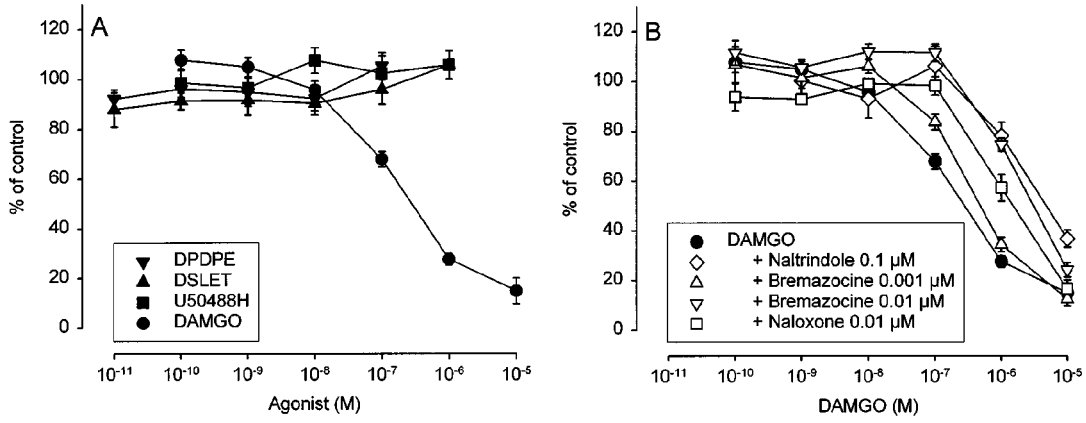

Figure 1 Effects of the opioid receptor agonists DPDPE, DSLET, U50488H and DAMGO on the evoked overflow of tritium from mouse occipito-parietal cortex slices (A), and interaction of DAMGO with antagonists (B). S_1 to S_6 each consisted of a single pulse. Agonists were added at increasing concentrations before S_2-S_6. Agonists were given either alone or (DAMGO) with the antagonists indicated, which were present throughout superfusion. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means±s.e.mean from 6–22 brain slices.

cine and naloxone all shifted the concentration-response curve of DAMGO to the right (Figure 1B). Apparent pK_d values are in Table 3. Bremazocine was tested at two concentrations, 0.001 and 0.01 μM; the identical apparent pK_d values are compatible with a competitive mode of interaction (Figure 1B, Table 3). Naltrindole, bremazocine and naloxone were approximately equipotent against DAMGO (Table 3).

The ORL_1-selective agonist nociceptin was studied in the presence of 1 μM naloxone, a concentration blocking all classical opioid receptors but devoid of a noticeable effect at ORL_1 receptors (Schlicker *et al*., 1998). Like DAMGO, nociceptin caused concentration-dependent and marked inhibition in brain cortex slices (Figure 2A, Table 2). Still in the presence of naloxone, the ORL_1-selective antagonist PheΨ shifted the concentration-response curve of nociceptin to the right (Figure 2A, Table 3).

A different pattern of effects was found in atria. U50488H again was inactive, but DAMGO was a weaker agonist than in the brain cortex, and DPDPE and DSLET potently reduced the evoked overflow of tritium (Figure 3A, Table 2). Naloxone (0.1 μM) abolished any effect of DAMGO (not shown); although the interaction did not permit an exact calculation, the pK_d value of naloxone against DAMGO clearly was >8 (Table 3). The concentration-inhibition curves of DPDPE and DSLET were shifted to the right by all antagonists at classical opioid receptors, naltriben, naltrindole, BNTX, bremazocine and naloxone (curves not shown; apparent pK_d values in Table 3). The two concentrations of bremazocine, 0.01 and 0.1 μM, yielded similar apparent pK_d values, in accord with a competitive antagonism. The potencies of each antagonist against DPDPE and DSLET were close to each other, and in contrast to the brain cortex, potencies now differed greatly between antagonists, with an order naltriben, naltrindole > BNTX, bremazocine, naloxone (Table 3).

Nociceptin, in the presence of naloxone, had a lower maximal effect than in the brain cortex (Figure 2B). PheΨ antagonized its effect (Figure 2B), but due to the small agonist E_{max} the apparent pK_d value is a little in doubt (Table 3).

Finally, the pattern differed again in the vas deferens. All agonists, including the selective OP_2 agonists U50488H and U69593 and (in the presence of naloxone) the ORL_1 agonist nociceptin, caused considerable inhibition (Figures 2C and 3B, Table 2). Wherever tested, antagonists shifted agonist concentration-inhibition curves to the right (for example

Table 2. EC_{50} and E_{max} values of agonists at presynaptic opioid receptors

	*Occipito-parietal cortex**		*Atria†*		*Vas deferens†*	
Agonist	EC_{50} (nM)	E_{max} (% inhibition)	EC_{50} (nM)	E_{max} (% inhibition)	EC_{50} (nM)	E_{max} (% inhibition)
DPDPE	>100	no inhibition	10.2±1.3	72±4	2.9±0.8	86±4
DSLET	>1000	no inhibition	1.3±0.3	83±3	0.56±0.26	85±4
U69593	—	—	—	—	92±20	61±4
U50488H	>1000	no inhibition	>1000	no inhibition	206±46‡	77±33‡
DAMGO	179±51	86±5	940±511‡	39±9‡	61±17‡	67±4‡
Nociceptin	3.5±1.2	86±3	1.4±1.3	35±5	8.0±3.5	51±5

Experiments with nociceptin were carried out in the presence of naloxone (1 μM). Values are mean±s.e. from 6–22 tissue pieces. *Stimulation with single pulses. †Stimulation with 20 pulses at 50 Hz. ‡The concentration-response curves did not approach an asymptotic maximum (Figure 3), but sigmoid curve fitting yielded the values indicated here.

Table 3. Apparent pK_d values of antagonists at presynaptic opioid receptors

				pK_d values against		
Antagonist (concentration used)		*DPDPE*	*DSLET*	*U50488H*	*DAMGO*	*Nociceptin*
				*Occipito-parietal cortex**		
Naltrindole	(0.1 μM)	–	–	–	8.3	–
Bremazocine	(0.001 μM)	–	–	–	9.0	–
	(0.01 μM)	–	–	–	9.0	–
Naloxone	(0.01 μM)	–	–	–	8.6	–
PheΨ	(0.3 μM)	–	–	–	–	7.8
				Atria†		
Naltriben	(0.0001 or 0.00001 μM)	13.0	12.3	–	–	–
Naltrindole	(0.0001 or 0.00001 μM)	12.9	11.6	–	–	–
BNTX	(0.01 μM)	9.0	9.1	–	–	–
Bremazocine	(0.01 μM)	8.1	8.8	–	–	–
	(0.1 μM)	8.4	9.0	–	–	–
Naloxone	(0.1 μM)	7.7	7.9	–	>8	–
PheΨ	(0.3 μM)	–	–	–	–	8.8
				Vas deferens†		
Naltriben	(0.1 μM)	11.3	10.9	–	–	–
Naltrindole	(0.01 or 0.001 μM)	11.2	10.7	8.5	8.7	–
	(0.1 μM)	–	–	8.6	9.0	–
BNTX	(0.1 μM)	9.3	9.1	–	–	–
Bremazocine	(0.001 μM)	9.2	9.0	10.3	9.2	–
	(0.01 μM)	–	8.8	–	9.5	–
Naloxone	(0.1 or 0.01 μM)	8.0	7.7	8.4	9.2	–
PheΨ	(0.3 μM)	–	–	–	–	7.4

Experiments with nociceptin and PheΨ were carried out in the presence of naloxone (1 μM). Each pK_d value is based on 5–21 tissue pieces, controls and preparations that received agonist only not included. *Stimulation with single pulses. †Stimulation with 20 pulses at 50 Hz.

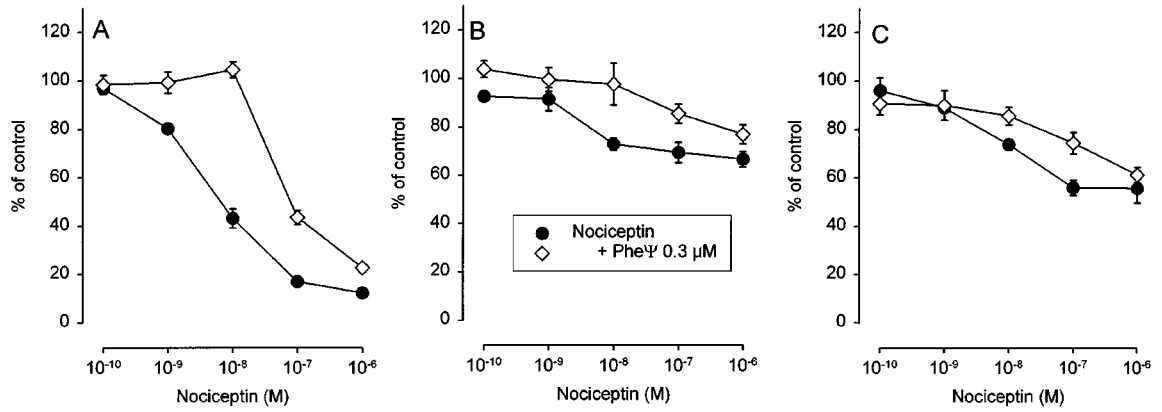

Figure 2 Effect of the ORL_1 receptor agonist nociceptin on the evoked overflow of tritium from mouse occipito-parietal cortex slices (A) and pieces of mouse atria (B) and vas deferens (C), and interaction with the antagonist PheΨ. S_1 to S_6 each consisted of either a single pulse (occipito-parietal cortex) or 20 pulses at 50 Hz (atria and vas deferens). Nociceptin was added at increasing concentrations before S_2-S_6. Nociceptin was given either alone or with PheΨ, which was present throughout superfusion. Naloxone (1 μM) was present throughout superfusion in all experiments. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means$\pm$s.e.mean from 5–8 tissue pieces.

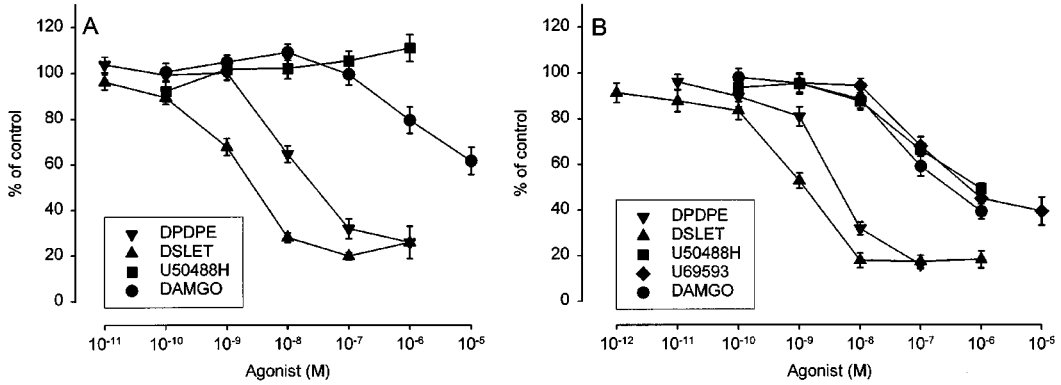

Figure 3 Effects of the opioid receptor agonists DPDPE, DSLET, U50488H, U69593 and DAMGO on the evoked overflow of tritium from pieces of mouse atria (A) and vas deferens (B). S_1 to S_6 each consisted of 20 pulses at 50 Hz. Agonists were added at increasing concentrations before S_2-S_6. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means$\pm$s.e.mean from 5–22 tissue pieces.

Figure 2C). Where two antagonist concentrations were examined (naltrindole against U50488H and DAMGO; bremazocine against DSLET and DAMGO), the shifts were compatible with a competitive interaction (Table 3). As in atria, the potencies of each antagonist against DPDPE and DSLET were close to one another. Apart from this, however, the potency orders of the antagonists depended greatly on the agonist; the antagonist potency order was naltriben, naltrindole > BNTX, bremazocine, naloxone against DPDPE and DSLET, as in atria; the order was bremazocine > naltrindole, naloxone against U50488H; and all antagonists were approximately equipotent against DAMGO, as in the occipito-parietal cortex (Table 3).

Presynaptic cannabinoid receptors

Presynaptic CB receptors were investigated in the occipito-parietal cortex, atria, vas deferens and spleen. Noradrenaline release was elicited by either single pulses (brain cortex), or 20 pulses at 50 Hz (atria and vas deferens), or 120 pulses at 3 Hz in the presence of phentolamine (1 μM; spleen), again conditions with no or little α_2-autoinhibition (Table 1). In the spleen, the brief high-frequency pulse trains used in atria and the vas deferens to avoid autoinhibition produced too small tritium overflow peaks – hence the 120 pulses at 3 Hz in the presence of phentolamine. The CB receptor ligands tested were the agonists WIN 55,212-2 and CP 55,940 and the CB_1-selective antagonist SR 141716A.

In occipito-parietal cortex, atria and spleen WIN 55,212-2 (0.1 nM – 1 μM) did not change the evoked overflow of tritium (data not shown; $n=6-10$).

In the vas deferens, WIN 55,212-2 (Figure 4) and CP 55,940 (not shown) produced concentration-dependent inhibition. The EC_{50} of WIN 55,212-2 was 2.7 ± 3.0 nM and its E_{max} 79 ± 11% inhibition; the EC_{50} of CP 55,940 was 0.27 ± 0.13 nM and its E_{max} 64 ± 5% inhibition ($n=10-12$). SR 141716A was examined against WIN 55,212-2 at concentrations of 0.001 and 0.01 μM and against CP 55,940 at concentrations of 0.01 and 0.1 μM. It shifted the concentration-inhibition curves of both agonists to the right (effect against WIN 55,212-2 in Figure 4). The four apparent pK_d values obtained were in the range of 9.7 and 10.5, i.e. similar, in accord with a competitive antagonism ($n=6-13$).

Presynaptic β-adrenoceptors

β-Adrenoceptors were investigated in the occipito-parietal cortex, atria, vas deferens and spleen. As in the CB receptor study, noradrenaline release was elicited by single pulses (occipito-parietal cortex), 20 pulses at 50 Hz (atria, vas

deferens) or 120 pulses at 3 Hz (spleen). In contrast to the CB receptor part, *all* experiments were carried out in the presence of phentolamine (1 μM); this was done in order to prevent any activation by β-adrenoceptor agonists of presynaptic α_2-adrenoceptors, a known effect at least of isoprenaline (Endo *et al*., 1977). The presence of phentolamine of course assured autoinhibition-free release. For comparison, 120 pulses at 3 Hz were also applied to a series of atria (in the presence of phentolamine). The drugs tested were isoprenaline, the β_2-selective agonist salbutamol, the selective β_1 antagonist CGP 20712A and the β_2-selective antagonist ICI 118,551.

In the occipito-parietal cortex and vas deferens neither isoprenaline (0.1 nM – 1 μM; $n=5-7$) nor salbutamol (0.1 nM – 10 μM; $n=2-3$) had any effect on the evoked overflow of tritium (data not shown).

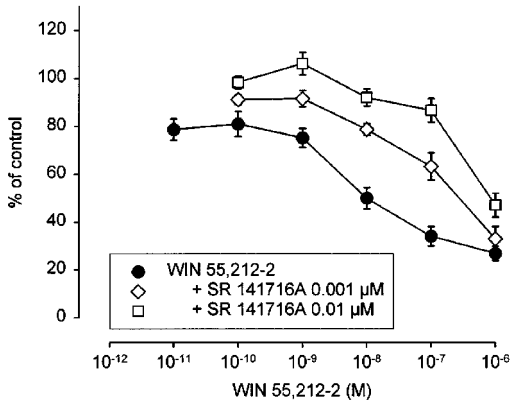

Figure 4 Effect of the CB receptor agonist WIN 55,212-2 on the evoked overflow of tritium from pieces of mouse vas deferens, and interaction with the antagonist SR 141716A. S_1 to S_6 each consisted of 20 pulses at 50 Hz. WIN 55,212-2 was added at increasing concentrations before S_2-S_6. WIN 55,212-2 was given either alone or with SR 141716A, which was present throughout superfusion. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means $\pm$s.e.mean from 6 – 10 tissue pieces.

In atria both isoprenaline (not shown) and salbutamol (Figure 5A) increased the overflow of tritium elicited by 20 pulses at 50 Hz. EC_{50} and E_{max} values are given in Table 4. The β_1-selective antagonist CGP 20712A (0.1 μM) did not alter the concentration-response curves of either agonist whereas ICI 118,551 (0.01 μM) produced a shift to the right (effects against salbutamol in Figure 5A). The apparent pK_d values against isoprenaline and salbutamol agreed well (Table 5). Similar concentration-response curves for isoprenaline and salbutamol were obtained when atria were stimulated by 120 pulses at 3 Hz; both EC_{50} and E_{max} values were somewhat lower than those found with 20 pulses at 50 Hz (Table 4). In experiments with 120 pulses at 3 Hz, ICI 118,551 was tested against salbutamol at three concentrations (0.001, 0.01 and 0.1 μM), and results were subjected to a Schild analysis: the slope of the Schild plot was 0.95 (95% confidence limits, 0.88 – 1.02), indicating a competitive antagonism, and the pA_2 value was 10.5 (10.3 – 10.7), identical with the pK_d value against salbutamol found with 20 pulses at 50 Hz (Table 5).

Results in the spleen were similar. Both isoprenaline (not shown) and salbutamol (Figure 5B) increased the overflow of tritium elicited by 120 pulses at 3 Hz. The EC_{50} and E_{max} values were lower than in atria stimulated by 20 pulses at 50 Hz or by 120 pulses at 3 Hz (Table 4). As in atria, CGP 20712A (0.1 μM) did not change the agonist concentration-response curves whereas ICI 118,551 (0.01 μM) caused a shift to the right (effect against salbutamol in Figure 5B); pK_d values were virtually identical with those found in atria (Table 5).

Presynaptic bradykinin receptors

These were once again studied in the occipito-parietal cortex, in atria, the vas deferens and the spleen. Noradrenaline release was triggered by either 36 (brain slices) or 120 pulses at 3 Hz (peripheral tissues) in the absence of phentolamine, i.e. under autoinhibition-rich conditions (Table 1). The drugs used were bradykinin and the B_2 receptor antagonist Hoe 140.

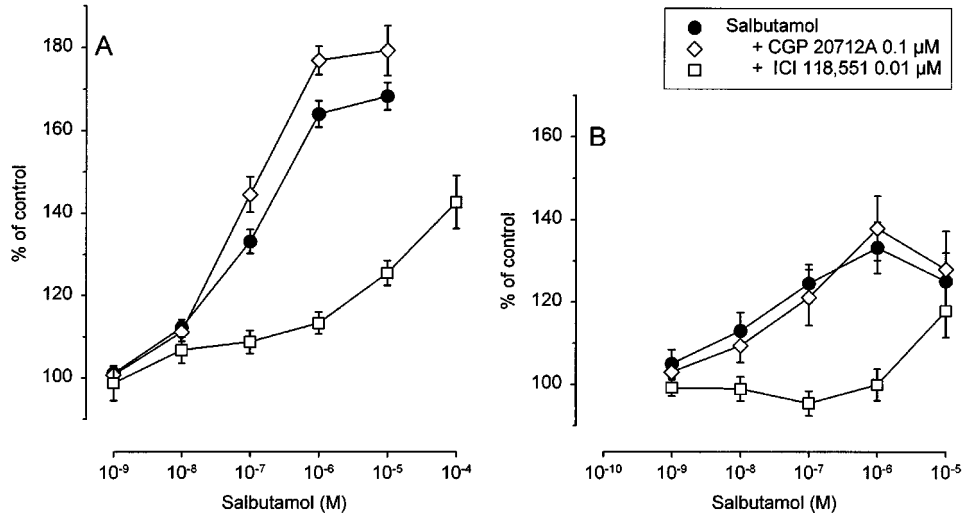

Figure 5 Effect of the β-adrenoceptor agonist salbutamol on the evoked. overflow of tritium from pieces of mouse atria (A) and spleen (B), and interaction with the antagonists CGP 20712A or ICI 118,551. S_1 to S_6 each consisted of either 20 pulses at 50 Hz (atria) or 120 pulses at 3 Hz (spleen). Salbutamol was added at increasing concentrations before S_2-S_6. Salbutamol was given either alone or with CGP 20712A or ICI 118,551, which were present throughout superfusion. Phentolamine (1 μM) was present throughout superfusion in all experiments. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means $\pm$s.e.mean from 4 – 14 tissue pieces.

Table 4. EC_{50} and E_{max} values of agonists at presynaptic β-adrenoceptors

	Atria		*Spleen*	
Agonist	EC_{50} (nM)	E_{max} (% increase)	EC_{50} (nM)	E_{max} (% increase)
Isoprenaline	4.2±0.5*	72±7*	1.2±0.3†	37±2†
	1.5±0.7†	41±4†		
Salbutamol	116±45*	71±5*	12.4±9.1†	30±4†
	31±12†	50±3†		

All experiments were carried out in presence of phentolamine (1 μM). Values are means±s.e.mean from 6–29 tissue pieces. *Stimulation with 20 pulses at 50 Hz. †Stimulation with 120 pulses at 3 Hz.

Table 5. Apparent pK_d values of antagonists at presynaptic β-adrenoceptors

Antagonist (concentration used)		pK_d *values against* *Isoprenaline*	*Salbutamol*
	*Atria**		
CGP 20712A	(0.1 μM)	<7	<7
ICI 118,551	(0.01 μM)	10.3	10.5
	Spleen†		
CGP 20712A	(0.1 μM)	<7	<7
ICI 118,551	(0.01 μM)	10.3	10.7

All experiments were carried out in the presence of phentolamine (1 μM). Each pK_d value is based on 6–14 tissue pieces, controls and preparations that received agonist only not included. *Stimulation with 20 pulses at 50 Hz. †Stimulation with 120 pulses at 3 Hz.

Bradykinin (0.01–100 nM) did not significantly alter the stimulation-evoked overflow of tritium in the occipito-parietal cortex (data not shown; $n=4-8$). In atria, vas deferens and spleen bradykinin caused increases (Figure 6A–C). It was distinctly more potent and had distinctly greater maximal effects in the atria and the vas deferens than in the spleen: the EC_{50} values in atria, vas deferens and spleen were 0.05±0.01, 0.05±0.01 and 0.3±0.2 nM, respectively; E_{max} values were 101±2, 87±3 and 55±8% increases, respectively ($n=5-33$). The concentration-response curves of bradykinin were shifted to the right by Hoe 140, but to a greater extent in atria and the vas deferens than in the spleen (Figure 6A–C). The apparent pK_d values were 11.4, 12.2 and 10.3 in atria, vas deferens and spleen, respectively.

Discussion

We made two attempts to ensure that our experimental conditions were adequate for the detection of presynaptic receptors and that the pK_d values were valid estimates of antagonist affinity. First, α_2-autoinhibition was minimized whenever opioid receptors, CB receptors and β-adrenoceptors were examined, which loose much of their release-modulating power when autoinhibition operates (see Schlicker & Göthert 1998); conversely, strong α_2-autoinhibition was created when bradykinin receptors were examined, which require autoinhibition for a major effect (Cox *et al*., 2000). Disregard of these conditions may lead to false negative results (Ramme *et al*., 1986; Cox *et al*., 2000). Second, although most pK_d values were obtained with a single antagonist concentration and hence were *apparent* pK_d values, we used two or three antagonist concentrations in several cases: almost identical apparent pK_d values (two antagonist concentrations), or a slope close to unity of the Schild plot (three antagonist concentrations), always confirmed a competitive kinetic and, hence, the validity of the pK_d as an affinity measure.

Presynaptic opioid receptors

Our results show that the noradrenergic axons of the occipito-parietal cortex of the mouse possess OP_3 (μ) and ORL_1 receptors, the sympathetic axons of atria OP_1 (δ) and ORL_1 receptors (OP_3 receptors are questionable), and those of the vas deferens OP_1, OP_2 (κ), OP_3 and ORL_1 receptors. The evidence comes from findings with both agonists and antagonists.

As to agonists, only the OP_3-selective DAMGO and the ORL_1 agonist nociceptin inhibited the release of noradrenaline in the occipito-parietal cortex (Figures 1A and 2A), whereas in atria the OP_1 agonists DPDPE and DSLET (Figures 2B and 3A), and in the vas deferens DPDPE, DSLET, and the OP_2 agonists U50488H and U69593 (Figures 2C and 3B) also caused presynaptic inhibition. In atria, the EC_{50} of DAMGO was five to 15 times higher than in the two other tissues (Table 2); since DAMGO also binds to OP_1 receptors at high concentrations (Emmerson *et al*., 1994), an action at the atrial presynaptic OP_1 receptors cannot be excluded.

As to antagonists, the pK_d values (Table 3) show that the tissues contained all three classical opioid receptors, because the orders of potency of the antagonists against, first, DPDPE and DSLET, second U50488H, and third DAMGO, differed distinctly. A second revealing observation refers to the vas deferens. In that tissue, the OP_1-selective antagonist naltrindole acted with the highest potency against DPDPE and DSLET and with much lower potency against U50488H and DAMGO, confirming that the receptor for the former two peptides was OP_1 (the high potency of naltrindole against DPDPE and DSLET recurred in atria); the OP_2-selective antagonist bremazocine acted with the highest potency against U50488H, confirming that the receptor for U50488H was OP_2; and the OP_3-selective antagonist naloxone acted with the highest potency against DAMGO, confirming that the receptor for DAMGO was OP_3. Final support for the receptor diagnoses comes from a comparison with published affinity data: the present pK_d values agree excellently with those obtained, for example, by binding studies at monkey brain OP_1, OP_2 and OP_3 receptors (Emmerson *et al*., 1994). Similarly, the pK_d values of PheΨ against nociceptin in the occipito-parietal cortex (7.8) and vas deferens (7.4) agree reasonably with published values for ORL_1 receptors (6.8–7.2; Guerrini *et al*., 1998; Schlicker *et al*., 1998). Two uncertain antagonist results in atria remain to be mentioned. Due to the only weak inhibition by DAMGO, a pK_d value for naloxone against DAMGO could not be determined; the estimate of >8 (Table 3) is compatible with an OP_3 receptor but, like the effect of DAMGO itself (see above), does not rule out an action at the atrial presynaptic OP_1-receptors. The pK_d value of PheΨ against nociceptin (8.8) was higher in atria than in the two other tissues (Table 3), possibly an overestimation due to the only slight inhibition by nociceptin (Figure 2B).

It has been suggested that each of the three classical opioid receptors comprises several subtypes (see Dhawan *et al*., 1996). Our results permit speculation on the potential OP_1 subtypes (δ_1 and δ_2) and OP_2 subtypes (κ_1, κ_2 and κ_3) involved in presynaptic inhibition in atria and the vas

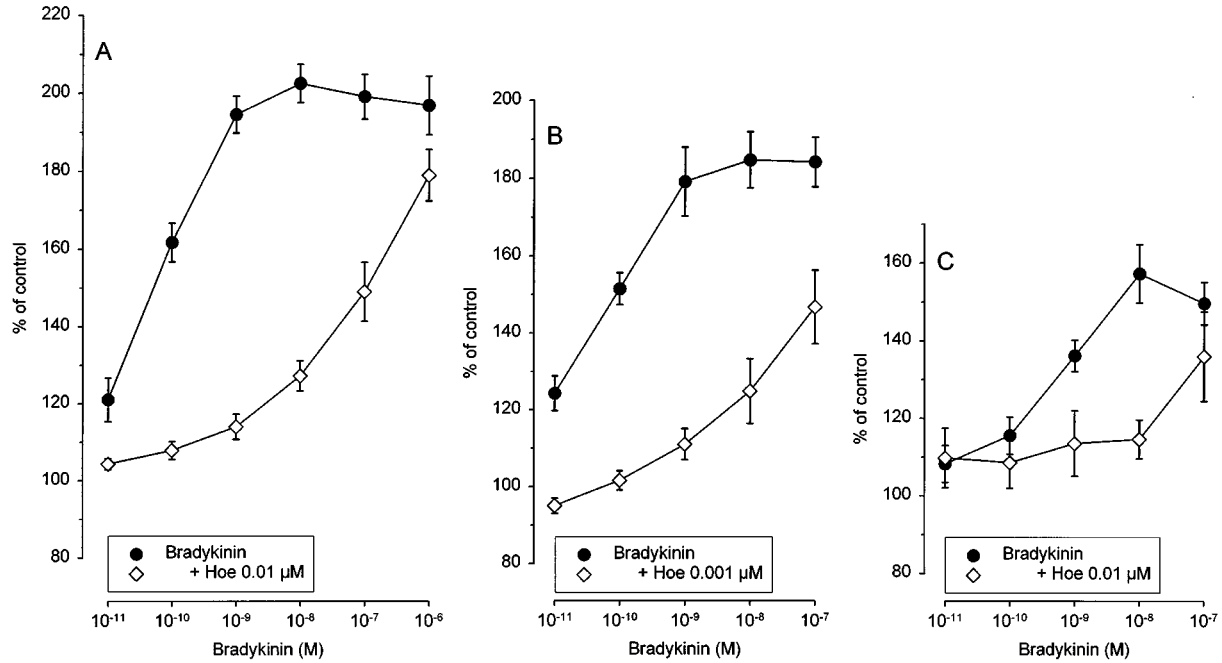

Figure 6 Effect of bradykinin on the evoked overflow of tritium from pieces of mouse atria (A), vas deferens (B) and spleen (C), and interaction with the antagonist Hoe 140. S_1 to S_6 each consisted of 120 pulses at 3 Hz. Bradykinin was added at increasing concentrations before S_2-S_6. Bradykinin was given either alone or with Hoe 140, which was present throughout superfusion. Ordinates, evoked overflow of tritium, calculated from S_n/S_1 ratios and expressed as a percentage of control. Values are means ± s.e.mean from 5–33 tissue pieces.

deferens. In either tissue, the δ_2-selective antagonist naltriben was much more potent than the δ_1-selective antagonist BNTX against DPDPE and DSLET (see Dhawan *et al.*, 1996), indicating that the OP_1 receptor was δ_2 (see also Wild *et al.*, 1993). In the vas deferens, not only U50488H but also U69593 inhibited the release of noradrenaline, indicating that the OP_2 receptor was κ_1, the only subtype sensitive to U69593 (see Dhawan *et al.*, 1996). The higher antagonist potency of both naltriben and naltrindole in atria than in the vas deferens (Table 3) remains unexplained.

Prior to our study there has been little work on presynaptic opioid receptors at noradrenergic neurons of mice. All four receptors, it is true, have been detected in the vas deferens, although mostly by measurement of contractions and rarely by determination of transmitter overflow (see Introduction and the review by Illes, 1989). However, presynaptic opioid receptors have never been shown in the heart, and only ORL_1 receptors (Schlicker *et al.*, 1998) but none of the classical receptors have been demonstrated at cerebral noradrenergic axons.

Presynaptic cannabinoid receptors

Presynaptic cannabinoid receptors were found only in the vas deferens, where the agonists WIN 55,212-2 and CP 55,940 decreased the release of noradrenaline (Figure 4). The effects were attenuated by the CB_1-selective antagonist SR 141716A, with apparent pK_d values (9.7–10.5) slightly higher than those reported in the literature both for the mouse vas deferens and for other tissues and species (7.7–9.2; Rinaldi-Carmona *et al.*, 1994; Pertwee *et al.*, 1995; 1996; Schlicker *et al.*, 1997). The noradrenergic axons innervating the occipito-parietal cortex, the atria and the spleen lacked CB receptors.

Presynaptic inhibition through CB_1 receptors, although a known mechanism in the mouse vas deferens, has not been shown in that tissue previously by measurement of noradrenaline overflow. Data concerning the occurrence of presynaptic CB receptors at noradrenergic axons in other mouse tissues have not been reported except for a study, with negative outcome, in the hippocampus (Schlicker *et al.*, 1997).

Presynaptic β-adrenoceptors

Presynaptic β-adrenoceptors were found in atria and the spleen but not in the occipito-parietal cortex or the vas deferens: isoprenaline and salbutamol increased the release of noradrenaline in the former but did not change it in the latter tissues (Figure 5, Table 4). Analogous experiments showed that the noradrenergic axons innervating the mouse hippocampus also lacked presynaptic β-adrenoceptors (Trendelenburg *et al.*, unpublished observation).

The receptors were β_2 as shown, first, by the effect of salbutamol, and second by results obtained with antagonists: only the β_2-selective ICI 118,551 but not the β_1-selective CGP 20712A antagonized the effects of the two agonists. In both tissues, and determined with two stimulation patterns (20 pulses at 50 Hz and 120 pulses at 3 Hz) and up to three antagonist concentrations, our pK_d values of ICI 118,551 (10.3–10.7) are consistently somewhat higher than literature values for other species (7.8–9.3; Bilski *et al.*, 1983; Molderings *et al.*, 1988).

The presynaptic β-adrenoceptors in mouse atria are well known (e.g. Johnston & Majewski, 1986) but their subtype has not been determined previously. Attempts to find presynaptic β-adrenoceptors in other mouse tissues have not been reported.

Presynaptic bradykinin receptors

Presynaptic bradykinin receptors were found in the three peripheral tissues but not in the occipito-parietal cortex:

bradykinin enhanced the release of noradrenaline only in atria, vas deferens and the spleen (Figure 6). Bradykinin also failed to change the release of noradrenaline in segments of the mouse hippocampus and hypothalamus (Cox *et al*., unpublished observation). Antagonism by the B_2-selective antagonist Hoe 140 indicated that the presynaptic bradykinin receptors were B_2. The pK_d values of Hoe 140 (10.3–12.2) are in the range of or slightly higher than literature values (8.4–10.9; Hock *et al*., 1991; Falcone *et al*., 1993). We do not know the reason for the large pK_d differences between atria (11.4), the vas deferens (12.2) and the spleen (10.3).

Previous studies on presynaptic bradykinin receptors in the mouse have been limited to atria (Chulak *et al*., 1998) and the vas deferens (Llona *et al*., 1991).

Comparison with other species

Tissue and species differences have been noticed ever since receptors modulating the release of noradrenaline became known. The present study adds or confirms some clear-cut examples, of which the examples for species differences will be discussed.

In rabbits, the major presynaptic opioid receptor at noradrenergic neurons is OP_2; presynaptic OP_1 receptors also occur, but presynaptic OP_3 receptors have not been found. In rats, in contrast, OP_3 receptors prevail at noradrenergic axons, with a minority of OP_1 but no OP_2 receptors (Illes, 1989). The mouse seems to resemble the rat in that presynaptic OP_3 receptors predominate (even though their presence in atria is questionable). Contrary to both rabbit and rat, however, all three classical opioid receptors appear to be used as presynaptic receptors at mouse noradrenergic neurons: OP_1 in atria and the vas deferens, OP_2 in the vas deferens, and OP_3 in the brain cortex, the vas deferens and possibly atria. The recently described ORL_1 receptors differs from the classical opioid receptors in that it inhibits noradrenaline release in each species and tissue examined (see Henderson & McKnight, 1997; Schlicker *et al*., 1998).

A known species difference in presynatic CB_1 receptors is their occurrence at the noradrenergic axons of human and guinea-pig but not rat and mouse hippocampus (Schlicker *et al*., 1997). Our experiments show an additional difference: CB_1 receptors modulate the release of noradrenaline in rat atria (Ishac *et al*., 1996) but not in mouse atria (this study).

Presynaptic, release-enhancing β_2-adrenoceptors have been detected in many but not all peripheral tissues; with few exceptions, they were found to be absent from brain tissues (see Starke, 1977; Langer, 1981; Majewski, 1983). A noticeable species difference exists between the mouse vas deferens, where we did not find the receptors, and the guinea-pig vas deferens, where they operate (see Driessen *et al*., 1996). It should be noted that the failure to detect the receptors can hardly be due to unsuitable experimental conditions: as mentioned above, the conditions of our experiments were optimal for presynaptic receptor operation.

A particularly remarkable species difference concerns the effect of bradykinin on cardiac sympathetic nerves. Bradykinin, acting on B_2 receptors, enhances the release of noradrenaline in human, rat, mouse and guinea-pig heart preparations (Chulak *et al*., 1998; Vaz-da-Silva *et al*., 1996; Rump *et al*., 1997; Seyedi *et al*., 1997; Cox *et al*., 2000; and this study). In contrast, bradykinin reduced the release of noradrenaline in rabbit hearts, perhaps because in rabbit hearts stimulation of prostaglandin synthesis by bradykinin and subsequent inhibition of noradrenaline release by prostaglandins outweighs the direct presynaptic facilitatory effect (Starke *et al*., 1977; Chulak *et al*., 1998).

Knowledge on presynaptic receptors in mice is a prerequisite to understand changes in neuronal functions in transgenic mice. For example, now that mice lacking α_{2A}-adrenoceptors (Altman *et al*., 1999) or both α_{2A}- and α_{2C}-adrenoceptors (Hein *et al*., 1999) are available, it will be interesting to see whether the loss of presynaptic α_2-autoreceptors leads to compensatory changes in other presynaptic receptors.

References

ALTMAN, J.D., TRENDELENBURG, A.U., MACMILLAN, L., BERNSTEIN, D., LIMBIRD, L., STARKE, K., KOBILKA, B.K. & HEIN, L. (1999). Abnormal regulation of the sympathetic nervous system in α_{2A}-adrenergic receptor knockout mice. *Mol. Pharmacol.,* **56,** 154–161.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol.,* **14,** 48–58.

BERZETEI-GURSKE, I.P., SCHWARTZ, R.W. & TOLL, L. (1996). Determination of activity for nociceptin in the mouse vas deferens. *Eur. J. Pharmacol.,* **302,** R1–R2.

BILSKI, A.J., HALLIDAY, S.E., FITZGERALD, J.D. & WALE, J.L. (1983). The pharmacology of a β_2-selective adrenoceptor antagonist (ICI 118,551). *J. Cardiovasc. Pharmacol.,* **5,** 430–437.

BLAKELEY, A.G.H., DUNN, P.M. & PETERSEN, S.A. (1988). A study of the actions of P_1-purinoceptor agonists and antagonists in the mouse vas deferens *in vitro*. *Br. J. Pharmacol.,* **94,** 37–46.

CALÒ, G., RIZZI, A., BOGONI, G., NEUGEBAUER, V., SALVADORI, S., GUERRINI, R., BIANCHI, C. & REGOLI, D. (1996). The mouse vas deferens: a pharmacological preparation sensitive to nociceptin. *Eur. J. Pharmacol.,* **311,** R3–R5.

CHULAK, C., COUTURE, R. & FOUCART, S. (1998). Modulatory effect of bradykinin on noradrenaline release in isolated atria from normal and B_2 knockout transgenic mice. *Eur. J. Pharmacol.,* **346,** 167–174.

COSTA, M. & MAJEWSKI, H. (1991). Evidence for facilitatory and inhibitory muscarinic receptors on postganglionic sympathetic nerves in mouse isolated atria. *Br. J. Pharmacol.,* **102,** 855–860.

COX, S.L., SCHELB, V., TRENDELENBURG, A.U. & STARKE, K. (2000). Enhancement of noradrenaline release by angiotensin II and bradykinin in mouse atria: evidence for cross-talk between $G_{q/11}$ protein- and $G_{i/o}$ protein-coupled receptors. *Br. J. Pharmacol.,* **129,** 1095–1102.

COX, S.L., TRENDELENBURG, A.U. & STARKE, K. (1999). Prejunctional angiotensin receptors involved in the facilitation of noradrenaline release in mouse tissues. *Br. J. Pharmacol.,* **127,** 1256–1262.

DHAWAN, B.N., CESSELIN, F., RAGHUBIR, R., REISINE, T., BRADLEY, P.B., PORTOGHESE, P.S. & HAMON, M. (1996). International Union of Pharmacology. XII. Classification of opioid receptors. *Pharmacol. Rev.,* **48,** 567–592.

DRIESSEN, B., BÜLTMANN, R., GONÇALVES, J. & STARKE, K. (1996). Opposite modulation of noradrenaline and ATP release in guinea-pig vas deferens through prejunctional β-adrenoceptors: evidence for the β_2 subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **353,** 564–571.

EMMERSON, P.J., LIU, M.R., WOODS, J.H. & MEDZIHRADSKY, F. (1994). Binding affinity and selectivity of opioids at *mu, delta* and *kappa* receptors in monkey brain membranes. *J. Pharmacol. Exp. Ther.,* **271,** 1630–1637.

ENDO, T., STARKE, K., BANGERTER, A. & TAUBE, H.D. (1977). Presynaptic receptor systems on the noradrenergic neurones of the rabbit pulmonary artery. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **296,** 229–247.

EXNER, H.J. & SCHLICKER, E. (1995). Prostanoid receptors of the EP_3 subtype mediate the inhibitory effect of prostaglandin E_2 on noradrenaline release in the mouse brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **351,** 46 – 52.

FALCONE, R.C., HUBBS, S.J., VANDERLOO, J.D., PROSSER, J.C., LITTLE, J., GOMES, B., AHARONY, D. & KRELL, R.D. (1993). Characterization of bradykinin receptors in guinea pig gall bladder. *J. Pharmacol. Exp. Ther.,* **266,** 1291 – 1299.

FOUCART, S. & MAJEWSKI, H. (1989). Inhibition of noradrenaline release by neuropeptide Y in mouse atria does not involve inhibition of adenylate cyclase or a pertussis toxin-susceptible G protein. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **340,** 658 – 665.

FUDER, H. & MUSCHOLL, E. (1995). Heteroreceptor-mediated modulation of noradrenaline and acetylcholine release from peripheral nerves. *Rev. Physiol. Biochem. Pharmacol.,* **126,** 265 – 412.

GUERRINI, R., CALO, G., RIZZI, A., BIGONI, R., BIANCHI, C., SALVADORI, S. & REGOLI, D. (1998). A new selective antagonist of the nociceptin receptor. *Br. J. Pharmacol.,* **123,** 163 – 165.

HEIN, L., ALTMAN, J.D. & KOBILKA, B.K. (1999). Two functionally distinct α_2-adrenergic receptors regulate sympathetic neurotransmission. *Nature,* **402,** 181 – 184.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972). A new example of a morphine-sensitive neuro-effector junction: adrenergic transmission in the mouse vas deferens. *Br. J. Pharmacol.,* **46,** 764 – 766.

HENDERSON, G. & MCKNIGHT, A.T. (1997). The orphan opioid receptor and its endogenous ligand - nociceptin/orphanin FQ. *Trends Pharmacol. Sci.,* **18,** 293 – 300.

HOCK, F.J., WIRTH, K., ALBUS, U., LINZ, W., GERHARDS, H.J., WIEMER, G., HENKE, S., BREIPOHL, G., KÖNIG, W., KNOLLE, J. & SCHÖLKENS, B.A. (1991). Hoe 140 a new potent and long acting bradykinin-antagonist: *in vitro* studies. *Br. J. Pharmacol.,* **102,** 769 – 773.

ILLES, P. (1989). Modulation of transmitter and hormone release by multiple neuronal opioid receptors. *Rev. Physiol. Biochem. Pharmacol.,* **112,** 139 – 233.

ISHAC, E.J.N., JIANG, L., LAKE, K.D., VARGA, K., ABOOD, M.E. & KUNOS, G. (1996). Inhibition of exocytotic noradrenaline release by presynaptic cannabinoid CB_1 receptors on peripheral sympathetic nerves. *Br. J. Pharmacol.,* **118,** 2023 – 2028.

JOHNSTON, H. & MAJEWSKI, H. (1986). Prejunctional β-adrenoceptors in rabbit pulmonary artery and mouse atria: effect of α-adrenoceptor blockade and phosphodiesterase inhibition. *Br. J. Pharmacol.,* **87,** 553 – 562.

LANGER, S.Z. (1981). Presynaptic regulation of the release of catecholamines. *Pharmacol. Rev.,* **32,** 337 – 362.

LIMBERGER, N., TRENDELENBURG, A.U. & STARKE, K. (1995). Subclassification of presynaptic α_2-autoreceptors: α_{2D}-autoreceptors in mouse brain. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **352,** 43 – 48.

LLONA, I., GALLEGUILLOS, X., BELMAR, J. & HUIDOBRO-TORO, J.P. (1991). Bradykinin modulates the release of noradrenaline from vas deferens nerve terminals. *Life Sci.,* **48,** 2585 – 2592.

MAJEWSKI, H. (1983). Modulation of noradrenaline release through activation of presynaptic β-adrenoreceptors. *J. Auton. Pharmacol.,* **3,** 47 – 60.

MOLDERINGS, G., LIKUNGU, J., ZERKOWSKI, H.R. & GÖTHERT, M. (1988). Presynaptic β_2-adrenoceptors on the sympathetic nerve fibres of the human saphenous vein: no evidence for involvement in adrenaline-mediated positive feedback loop regulating noradrenergic transmission. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **337,** 408 – 414.

PERTWEE, R.G., FERNANDO, S.R., NASH, J.E. & COUTTS, A.A. (1996). Further evidence for the presence of cannabinoid CB_1 receptors in guinea-pig small intestine. *Br. J. Pharmacol.,* **118,** 2199 – 2205.

PERTWEE, R.G., GRIFFIN, G., LAINTON, J.A.H. & HUFFMAN, J.W. (1995). Pharmacological characterization of three novel cannabinoid receptor agonists in the mouse isolated vas deferens. *Eur. J. Pharmacol.,* **284,** 241 – 247.

PERTWEE, R.G., STEVENSON, L.A., ELRICK, D.B., MECHOULAM, R. & CORBETT, A.D. (1992). Inhibitory effects of certain enantiomeric cannabinoids in the mouse vas deferens and the myenteric plexus preparation of guinea-pig small intestine. *Br. J. Pharmacol.,* **105,** 980 – 984.

RAJANAYAGAM, M.A.S., MUSGRAVE, I.F., RAND, M.J. & MAJEWSKI, H. (1989). Facilitation of noradrenaline release by isoprenaline is not mediated by angiotensin II in mouse atria and rat tail artery. *Arch. Int. Pharmacodyn.,* **299,** 185 – 199.

RAMME, D., ILLES, P., SPÄTH, L. & STARKE, K. (1986). Blockade of α_2-adrenoceptors permits the operation of otherwise silent opioid κ-receptors at the sympathetic axons of rabbit jejunal arteries. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **334,** 48 – 55.

RINALDI-CARMONA, M., BARTH, F., HÉAULME, M., SHIRE, D., CALANDRA, B., CONGY, C., MARTINEZ, S., MARUANI, J., NÉLIAT, G., CAPUT, D., FERRARA, P., SOUBRIÉ, P., BRELIÈRE, J.C. & LE FUR, G. (1994). SR 141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Letts.,* **350,** 240 – 244.

RUMP, L.C., BERLIT, T., SCHWERTFEGER, E., BEYERSDORF, F., SCHOLLMEYER, P. & BOHMANN, C. (1997). Angiotensin converting enzyme inhibition unmasks the sympathofacilitatory effect of bradykinin in human right atrium. *J. Hypertension,* **15,** 1263 – 1270.

SCHLICKER, E., BEHLING, A., LÜMMEN, G. & GÖTHERT, M. (1992). Histamine H_{3A} receptor-mediated inhibition of noradrenaline release in the mouse brain cortex. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **345,** 489 – 493.

SCHLICKER, E. & GÖTHERT, M. (1998). Interactions between the presynaptic α_2-autoreceptor and presynaptic inhibitory heteroreceptors on noradrenergic neurones. *Brain Res. Bull.,* **47,** 129 – 132.

SCHLICKER, E., TIMM, J., ZENTNER, J. & GÖTHERT, M. (1997). Cannabinoid CB_1 receptor-mediated inhibition of noradrenaline release in the human and guinea-pig hippocampus. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 583 – 589.

SCHLICKER, E., WERTHWEIN, S., KATHMANN, M. & BAUER, U. (1998). Nociceptin inhibits noradrenaline release in the mouse brain cortex via presynaptic ORL_1 receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **358,** 418 – 422.

SEYEDI, N., WIN, T., LANDER, H.M. & LEVI, R. (1997). Bradykinin B_2-receptor activation augments norepinephrine exocytosis from cardiac sympathetic nerve endings. *Circ. Res.,* **81,** 774 – 784.

SINGER, E.A. (1988). Transmitter release from brain slices elicited by single pulses: a powerful method to study presynaptic mechanisms. *Trends Pharmacol. Sci.,* **9,** 274 – 276.

STARKE, K. (1977). Regulation of noradrenaline release by presynaptic receptor systems. *Rev. Physiol. Biochem. Pharmacol.,* **77,** 1 – 124.

STARKE, K., PESKAR, B.A., SCHUMACHER, K.A. & TAUBE, H.D. (1977). Bradykinin and postganglionic sympathetic transmission. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **299,** 23 – 32.

TRENDELENBURG, A.U., HEIN, L., GAISER, E.G. & STARKE, K. (1999). Occurrence, pharmacology and function of presynaptic α_2-autoreceptors in $\alpha_{2A/D}$-adrenoceptor-deficient mice. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **360,** 540 – 551.

TRENDELENBURG, A.U., SUTEJ, I., WAHL, C.A., MOLDERINGS, G.J., RUMP, L.C. & STARKE, K. (1997). A re-investigation of questionable subclassifications of presynaptic α_2-autoreceptors: rat vena cava, rat atria, human kidney and guinea-pig urethra. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **356,** 721 – 737.

VAZ-DA-SILVA, M., MAGINA, S., DOMINGUES-COSTA, A., MOURA, D. & GUIMARÃES, S. (1996). The role of the endocardium in the facilitatory effect of bradykinin on electrically-induced release of noradrenaline in rat cardiac ventricle. *Br. J. Pharmacol.,* **118,** 364 – 368.

VON KÜGELGEN, I., SCHÖFFEL, E. & STARKE, K. (1989). Inhibition by nucleotides acting at presynaptic P_2-receptors of sympathetic neuro-effector transmission in the mouse isolated vas deferens. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **340,** 522 – 532.

WAHL, C.A., TRENDELENBURG, A.U. & STARKE, K. (1996). Presynaptic α_2-autoreceptors in mouse heart atria: evidence for the α_{2D} subtype. *Naunyn-Schmiedeberg's Arch. Pharmacol.,* **354,** 253 – 261.

WAUD D.R. (1976). Analysis of dose-response relationships. In: *Advances in general and cellular pharmacology*. Vol 1. ed. Narahshi, T. & Bianchi, C.P. pp 145 – 178. New York, London: Plenum.

WILD, K.D., CARLISI, V.J., MOSBERG, H.I., BOWEN, W.D., PORTOGHESE, P.S., SULTANA, M., TAKEMORI, A.E., HRUBY, V.J. & PORRECA, F. (1993). Evidence for a single functional opioid *delta* receptor subtype in the mouse isolated vas deferens. *J. Pharmacol. Exp. Ther.,* **264,** 831 – 838.

(Received January 5, 2000
Revised February 7, 2000
Accepted February 17, 2000)