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Noradrenaline release in rodent tissues is inhibited by interleukin-1β but is not affected by urotensin II, MCH, NPW and NPFF

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Abstract:
We studied whether noradrenaline release is affected by interleukin-1β and the neuropeptides urotensin II, melanin-concentrating hormone (MCH), neuropeptide W (NPW) and neuropeptide FF (NPFF). Rodent tissues preincubated with [^{3}H]noradrenaline were superfused, and the effect of peptides on the electrically-evoked tritium overflow ("noradrenaline release") was studied. In mouse brain cortex, interleukin-1β at 0.3 nM and the prostaglandin E_2 analogue sulprostone at 3 nM inhibited noradrenaline release by about 40%; the effect of interleukin-1β developed gradually, whereas the effect of sulprostone occurred promptly. Urotensin II at 0.001–1 μM did not affect noradrenaline release in rat kidney cortex, whereas 0.01 μM angiotensin II increased it (positive control). MCH at 0.01–1 μM did not alter noradrenaline release in the rat brain cortex, and NPW 1 μM did not affect noradrenaline release in the mouse hypothalamus or hippocampus. In each model, 0.1 μM sulprostone inhibited noradrenaline release (positive control). NPFF and the $NPFF_2$ receptor agonist dNPA (1 μM) did not affect noradrenaline release in the mouse atria; the inhibitory effect of the δ opioid receptor agonist 1 μM DPDPE on noradrenaline release in this tissue was not altered by NPFF or dNPA at 0.32 μM but was counteracted by the δ opioid antagonist naltrindole at 0.001 μM. In conclusion, interleukin-1β inhibits noradrenaline release in the mouse cortex; the effect develops gradually, suggesting that it affects protein biosynthesis. Noradrenergic neurons in various tissues from rodents are devoid of presynaptic receptors for urotensin II, MCH, NPW and NPFF. Finally, an interaction between a δ opioid agonist and NPFF could not be detected.

Key words:
interleukin-1β, melanin-concentrating hormone, neuropeptide FF, neuropeptide W, noradrenaline release, superfusion experiments, urotensin II

Abbreviations: dNPA – (*D*)-NP-(N-Me)AFLFQPQRFamide, DPDPE – ((*D*)-Pen^2, (*D*)-Pen^5)-enkephalin, IL-1β – interleukin-1β, MAP – mitogen-activated protein, MCH – melanin-concentrating hormone, NF-κB – nuclear factor "κ-light-chain-enhancer" of activated B-cells, NPFF – neuropeptide FF, NPW – neuropeptide W, NPY – neuropeptide Y, PSS – physiological salt solution, SEM – standard error of the mean

Introduction

Modulatory effects on noradrenaline release, although shown for many small molecules [21], have been studied less frequently for many peptides including

the neuropeptides urotensin II, melanin-concentrating hormone (MCH), neuropeptide W (NPW) and neuropeptide FF (NPFF). The present investigation aimed to study the effect of these neuropeptides and the protein interleukin-1β (IL-1β) on noradrenaline release in isolated rodent tissues. Because a modulatory effect could not be expected in each case, drugs known to facilitate or inhibit noradrenaline release were included as positive controls in the experimental series.

Urotensin II was first isolated from the urophysis of the goby fish [24], and its precursor mRNA was later identified in the human spinal cord [6]. In mammals, urotensin II is not only expressed in some regions of the central nervous system, but also in many peripheral tissues; it acts *via* the G_q protein-coupled UT receptor [11]. Activation of other G_q protein-coupled receptors, e.g., AT_1 receptors (activated by angiotensin II), leads to the facilitation of noradrenaline release, although this phenomenon is restricted to receptors in the periphery [36]. For this reason, we have examined the effect of urotensin II on noradrenaline release in the rat kidney, in which UT receptors occur in high density [10]. Angiotensin II, which is known to facilitate noradrenaline release in this tissue [37], served as a positive control.

The peptides MCH and NPW are implicated in the regulation of food intake, and their receptors occur in the hypothalamus [15, 32]. MCH was originally isolated from chum salmon pituitaries [20] and was later found in the rat hypothalamus [40]. A broad brain distribution, including the cerebral cortex, has been described for the MCH_1 receptors by which MCH acts [15]. For this reason, the rat cerebral cortex was chosen for our experiments because this area has frequently been used for the identification of presynaptic receptors on noradrenergic neurons in the past [29]. NPW was isolated first from porcine hypothalamus [31]. Because the distribution of this peptide and of its receptors is much more restricted, we have carried out our experiments with NPW in the mouse hypothalamus and hippocampus (in which mRNA for NPW is present [32]). $NPBW_1$ and $NPBW_2$ receptors (*via* which NPW acts [1]) and MCH_1 receptors are $G_{i/o}$ protein-coupled, and the possibility exists that they, like other $G_{i/o}$ protein-coupled receptors, lead to an inhibition of noradrenaline release. One example of a $G_{i/o}$ protein-coupled receptor that is involved in the inhibition of noradrenaline release on numerous central and peripheral sites of rodents is the prostanoid EP_3 receptor [14]. Sulprostone, an agonist at this receptor, served as a positive control in our experiments.

The peptide NPFF, first isolated from bovine brain [41], is known to modulate opioid receptor-related effects, and several mechanisms involved in the interaction have been studied [22]. Such an interaction might also occur in the heart and at least partially explain the catecholamine-dependent stimulatory effect of NPFF and a NPFF analogue on blood pressure and heart rate in anesthetized rats [2, 16]. NPFF immunoreactivity, NPFF receptors and δ opioid receptors have been identified in the heart of the rat and/or mouse [2, 30, 38]. The mechanism might implicate a direct interaction at the δ opioid receptor, which is known to inhibit cardiac noradrenaline release [30, 38]. On the other hand, one of the receptors activated by NPFF, the $NPFF_2$ receptor, is $G_{i/o}$ protein-coupled like the δ opioid receptor [1], and NPFF might inhibit noradrenaline release by itself. For this reason, we studied the following: (i) the effect of NPFF and a $NPFF_2$ receptor agonist on noradrenaline release in the mouse atrium and (ii) the possible interaction of the latter two compounds with the inhibitory effect of a δ opioid receptor agonist on noradrenaline release.

Finally, IL-1β was examined in the present study, which although peptidic in nature, differs from the aforementioned peptides with respect to its higher molecular weight, its function (cytokine) and the transduction machinery of the involved receptor, IL-1RI [9]. IL-1β and prostanoids of the E series (to which sulprostone belongs) not only share a proinflammatory effect, but also have marked effects on noradrenaline release [12, 14, 35]. Thus, we directly compared the effect of IL-1β and sulprostone on noradrenaline release. We used slices from the cerebral cortex from mice because in this species, the extent of the effect of sulprostone is stronger than in rats [12].

Materials and Methods

Chemicals

The following chemicals were used: (*R*)-(–)-[ring-2,5,6-^{3}H]noradrenaline (specific activity 53 Ci/mmol) (PerkinElmer, Zaventem, Belgium); angiotensin II (human) (Bachem, Weil am Rhein, Germany); recombinant human interleukin-1β (Innogenetics, Ghent, Belgium); naltrindole hydrochloride, tetraethylammonium chloride (Sigma, Munich Germany); DPDPE

(((*D*)-Pen^2, (*D*)-Pen^5)-enkephalin), melanin-concentrating hormone (human, mouse, rat), neuropeptide W-23 (rat) trifluoroacetate salt, urotensin II (human) (NeoMPS, Strasbourg, France); desipramine hydrochloride (Novartis, Wehr, Germany); dNPA ((*D*)-NP-(N-Me)AFLFQPQRFamide) and neuropeptide FF (FLFQPQRFamide) were synthesized in the laboratory of J.-M. Z. by manual solid-phase synthesis using Fmoc-amino acid chemistry; rauwolscine hydrochloride (Roth, Karlsruhe, Germany); sulprostone (Bayer Schering Pharma, Berlin, Germany). Stock solutions of the drugs were prepared with dimethyl sulfoxide (DMSO) (naltrindole), ethanol (sulprostone), methanol (dNPA, NPFF) or distilled water and diluted with physiological salt solution (PSS; see below) to the concentration required. The organic solvents did not affect basal and evoked tritium outflow by themselves.

Animals

All procedures complied with German animal welfare regulations (Tierschutzgesetz). Male Wistar rats weighing 200–300 g and male NMRI mice (Charles-River, Sulzfeld, Germany) and C57BL/6J mice of either sex (Jackson, Bar Harbor, ME, USA) weighing 20–30 g were housed in the animal facilities of the Institute of Pharmacology and Toxicology with free access to water and food pellets.

Superfusion studies

Animals were sacrificed by decapitation, and slices from the cerebral cortex (0.3 mm thick, diameter 3 mm), hippocampus and hypothalamus (0.3 mm thick, diameter 2 mm) and tissue pieces from the atrium and renal cortex (dimensions approximately 1 × 1 × 1 mm) were prepared. Tissues were incubated (37°C) for 60 min with PSS (Ca^{2+} 1.3 mM) containing 0.025 μM [^{3}H]noradrenaline. Subsequently, the preparations were transferred to superfusion chambers and superfused with PSS (37°C) at a flow rate of 0.5–1.0 ml/min (for auxiliary drugs and Ca^{2+} concentration, see Tab. 1). Superfusate samples were collected every 5 min; experiments lasted for 110 min. Tritium overflow was evoked by two 2-min periods of electrical field stimulation (pulses of 2 ms were administered consistently; for stimulation frequency and current strength, see Tab. 1); the two stimulation periods (S_1 and S_2) started after 40 and 90 min of superfusion. The drugs under study were present in the medium either throughout superfusion or from 62 min of superfusion onward, as indicated in the figure legends. The PSS was composed as follows (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3 or 3.25 (as indicated in Tab. 1), KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25, ascorbic acid 0.06, disodium EDTA 0.03, glucose 10; the solution was aerated with 95% O_2 and 5% CO_2 (pH 7.4). The experimental protocol used for the fifth series of experiments differed in three respects. First, the superfusion experiments lasted for 210 min; second, two additional periods of stimulation were administered after 140 (S_3) and 190 min of superfusion (S_4); third, the drug under study was present in the medium from 77 to 110 min of superfusion.

Tritium efflux was calculated as the fraction of the tritium content in the tissues at the beginning of the respective collection period (fractional rate of tritium efflux). To quantify the effects on basal efflux, the ratio of the fractional rates in the 5-min period prior to S_2 (t_2) and in the 5-min period 15–20 min after the onset of S_1 (t_1) was determined for drugs added to the PSS from 62 min of superfusion onward. For drugs present in the PSS throughout superfusion, the t_1 values obtained in the absence or presence of the drug were directly compared to each other. Stimulation-evoked tritium overflow was calculated by subtracting basal from total efflux during the 2-min stimulation period and the subsequent 13 min and was expressed as the percent of the tritium present in the tissue at the onset of stimulation; basal efflux was assumed to decline linearly from the 5-min period before to that 15–20 min after the onset of stimulation. To quantify drug-induced effects on the stimulated tritium overflow, the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined (S_2/S_1) (for drugs added to the PSS from 62 min of superfusion), or the S_1 values obtained in the absence or presence of a given drug were directly compared to each other (for drugs present throughout superfusion). For the fifth series of experiments, S_3/S_1 and S_4/S_1 in addition to the S_2/S_1 ratios and t_3/t_1 and t_4/t_1 in addition to t_2/t_1 ratios were determined; t_3 and t_4 refer to the 5-min periods prior to S_3 and S_4, respectively.

Statistics

Results are given as the means ± standard error of the mean (SEM) of *n* experiments. For the comparison of mean values, a Student's *t*-test was used for unpaired data; the Bonferroni correction was used when two or

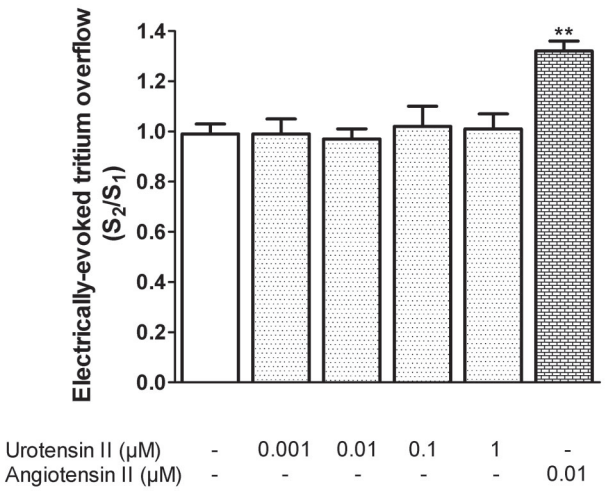

Fig. 1. Effect of urotensin II and angiotensin II on the electrically-evoked tritium overflow from superfused rat renal cortex pieces pre-incubated with [^{3}H]noradrenaline. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the tritium overflow evoked by S_2 over that evoked by S_1 is shown (S_2/S_1). The superfusion medium contained the drug under study from 62 min of superfusion onward and auxiliary drugs (see Tab. 1) throughout superfusion. The means ± SEM of 5 experiments each. ** $p < 0.01$ compared to control

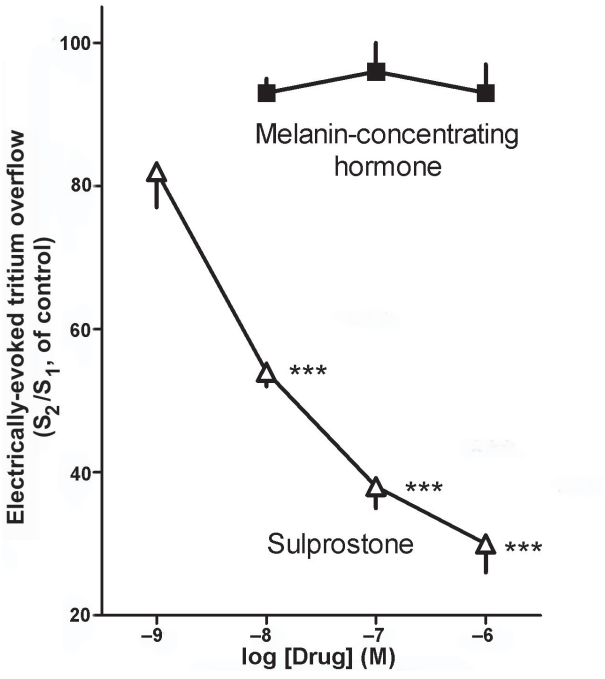

Fig. 2. Effect of melanin-concentrating hormone and sulprostone on the electrically-evoked tritium overflow from superfused rat brain cortex slices preincubated with [^{3}H]noradrenaline. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the tritium overflow evoked by S_2 over that evoked by S_1 is shown (S_2/S_1). Tritium overflow was expressed as the percent of the S_2/S_1 value in controls (not shown). The superfusion medium contained the drug under study from 62 min of superfusion onward and auxiliary drugs (see Tab. 1) throughout superfusion. The means ± SEM of 3–7 experiments. *** $p < 0.001$ compared to control

Tab. 1. Experimental details of superfusion studies and absolute values of basal and electrically-evoked tritium overflow

Experimental series	Results in Fig.	Species	Tissue	Auxiliary drugs (μM): Desipramine 1	$^1Ca^{2+}$ concentration (mM)	Frequency (Hz); current strength (mA)	^{1}Interacting drug (μM)	^{2}Basal tritium efflux (t_1) (min^{-1}) × 1000	^{3}Electrically-evoked tritium overflow (S_1) (% of tissue tritium)
1st	1	Wistar rat	Renal cortex	+ tetraethylammonium 320	3.25	3; 200	–	3.1 ± 0.4	4.11 ± 0.37
2nd	2	Wistar rat	Cerebral cortex	+ rauwolscine 1	1.3	0.3; 50	–	2.7 ± 0.3	5.70 ± 0.49
3rd	3	C57BL/6J mouse	Hippocampus	+ rauwolscine 1	1.3	0.3; 50	–	2.4 ± 0.3	8.13 ± 0.28
			Hypothalamus					1.9 ± 0.1	2.52 ± 0.17
4th	4, 5	C57BL/6J mouse	Atrium	+ rauwolscine 1	3.25	3; 200	–	2.1 ± 0.1	5.38 ± 0.50
							NPFF 0.32	2.3 ± 0.3	5.92 ± 0.94
							dNPA 0.32	2.0 ± 0.1	5.60 ± 1.03
							Naltrindole 0.001	2.2 ± 0.2	5.42 ± 0.93
5th	6	NMRI mouse	Cerebral cortex	+ rauwolscine 1	1.3	0.3; 50	–	2.3 ± 0.2	7.06 ± 0.46

[1]Present in the medium throughout superfusion. [2]Basal tritium efflux was determined in the 5-min collection period from 55 to 60 min of superfusion (t_1) and is given as a fraction of tissue tritium. [3]The first 2-min period of electrical stimulation (S_1) was administered 40 min after the beginning of superfusion. The means ± SEM of 3–11 experiments

more values were compared to the same control. To assess whether naltrindole significantly attenuated the effect of DPDPE (Fig. 5), the S_2/S_1 ratios obtained in the presence of DPDPE were divided by the S_2/S_1 ratios for the corresponding DPDPE-free controls, and the percentages obtained for the group with and without naltrindole were evaluated using a Student's *t*-test.

Results

The control values for basal and stimulation-evoked tritium overflow expressed as t_1 and S_1, respectively, are given in Table 1. Basal tritium efflux expressed as t_2/t_1, t_3/t_1 and t_4/t_1 was close to 0.8 in controls (not shown). The electrically-evoked tritium overflow expressed as S_2/S_1, S_3/S_1 and S_4/S_1 was close to one or somewhat higher in the control experiments (Figs. 1 and 3 and legend to Fig. 6; otherwise not shown). The drugs under study did not affect the basal tritium efflux (Tab. 1 or not shown). The effects of the drugs on the electrically-evoked tritium overflow are described below.

In the first series of experiments, the effect of urotensin II on the evoked overflow in rat renal cortex pieces was examined. Urotensin at 0.001–1 μM failed to affect the evoked overflow (S_2/S_1), whereas 0.01 μM angiotensin II, used as a positive control, increased it by about 35% (Fig. 1).

In the second experimental series, the effect of MCH on the evoked overflow was studied in rat brain cortex slices. At concentrations from 0.01 to 1 μM, MCH did not affect the evoked overflow (S_2/S_1), whereas the prostanoid $EP_{1/3}$ receptor agonist sulprostone, used as a positive control, caused a concentration-dependent inhibition in the same concentration range (Fig. 2). The extent of inhibition obtained with 1 μM sulprostone was about 70%.

The third series, dedicated to the effect of NPW on the evoked overflow, was carried out in mouse hippocampal and hypothalamic slices. NPW at 1 μM did not affect the evoked overflow (S_2/S_1) in either tissue, whereas 0.1 μM sulprostone (again used as a positive control) caused an inhibition of 75 to 90% (Fig. 3).

In the fourth series of experiments, performed on mouse atrial tissues, the effect of NPFF on the evoked overflow and the interaction of NPFF with a δ opioid receptor agonist was examined. NPFF and the $NPFF_2$ receptor agonist dNPA failed to affect the evoked overflow (S_2/S_1), at 1 μM each whereas 0.1 μM sulprostone caused an inhibition of about 75%; the δ opioid receptor agonist DPDPE (1 μM) inhibited the evoked overflow by about 40%. In the interaction experiments, the effect of the latter compound on the evoked overflow *(*S_2/S_1) was not affected by NPFF or dNPA at 0.32 μM each but was markedly attenuated by the δ opioid receptor antagonist 1 nM naltrindole (Fig. 5). Administered alone, NPFF, dNPA and naltrindole did not affect the evoked overflow (S_1) (Tab. 1).

In the fifth experimental series, the effect of IL-1β on the evoked overflow was examined in mouse brain cortex slices. The evoked overflow was not only determined after 40 (S_1) and 90 min (S_2) (like in the above experiments), but also after 140 (S_3) and 190 min (S_4), i.e., after withdrawal of the cytokine from the me-

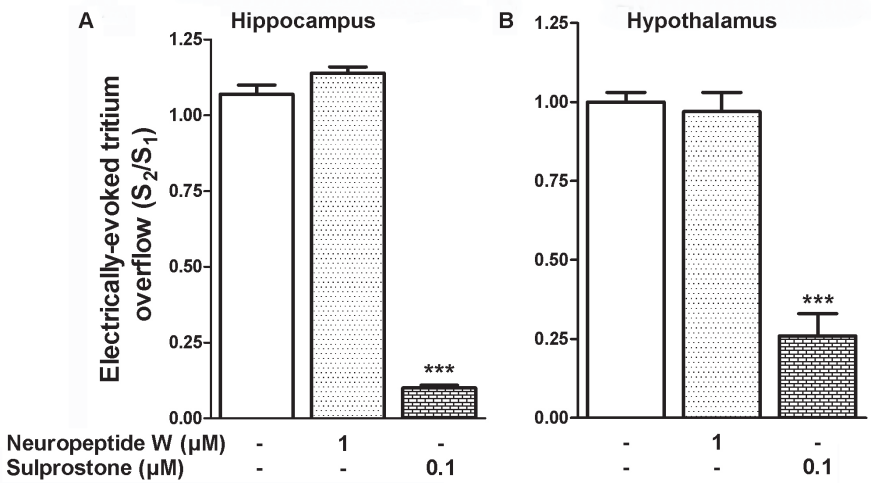

Fig. 3. Effect of neuropeptide W and sulprostone on the electrically-evoked tritium overflow from superfused mouse (**A**) hippocampal or (**B**) hypothalamic slices preincubated with [^{3}H]noradrenaline. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the tritium overflow evoked by S_2 over that evoked by S_1 is shown (S_2/S_1). The superfusion medium contained the drug under study from 62 min of superfusion onward and auxiliary drugs (see Tab. 1) throughout superfusion. The means ± SEM of 3–6 experiments. *** $p < 0.001$ compared to control

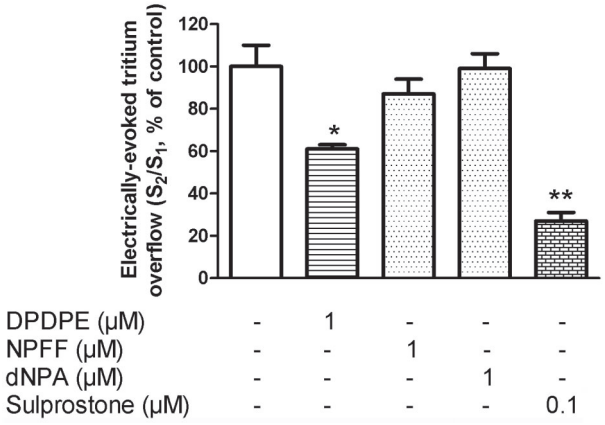

Fig. 4. Effect of DPDPE, NPFF, dNPA and sulprostone on the electrically-evoked tritium overflow from superfused mouse atrial tissue pieces preincubated with [^{3}H]noradrenaline. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Tritium overflow was expressed as the percent of the S_2/S_1 value in controls. The superfusion medium contained the drug under study from 62 min of superfusion onward and the auxiliary drugs (see Tab. 1) throughout superfusion. The means ± SEM of 4 experiments each. * $p < 0.05$, ** $p < 0.01$ compared to the corresponding control

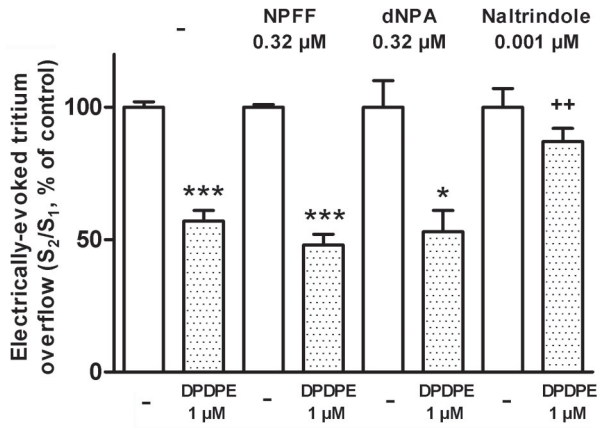

Fig. 5. Effect of DPDPE on the electrically-evoked tritium overflow from superfused mouse atrial tissue pieces preincubated with [^{3}H]noradrenaline and interaction with NPFF, dNPA or naltrindole. Tritium overflow was evoked twice, after 40 and 90 min of superfusion, and the ratio of the overflow evoked by S_2 over that evoked by S_1 was determined. Tritium overflow was expressed as percent of the S_2/S_1 value in controls. The superfusion medium contained DPDPE from 62 min of superfusion onward and NPFF, dNPA or naltrindole plus auxiliary drugs (see Tab. 1) throughout superfusion. The means ± SEM of 4–11 experiments. * $p < 0.05$, *** $p < 0.001$ compared to the corresponding control; $^{++}$ $p < 0.01$ compared to the effect of DPDPE obtained in the absence of naltrindole

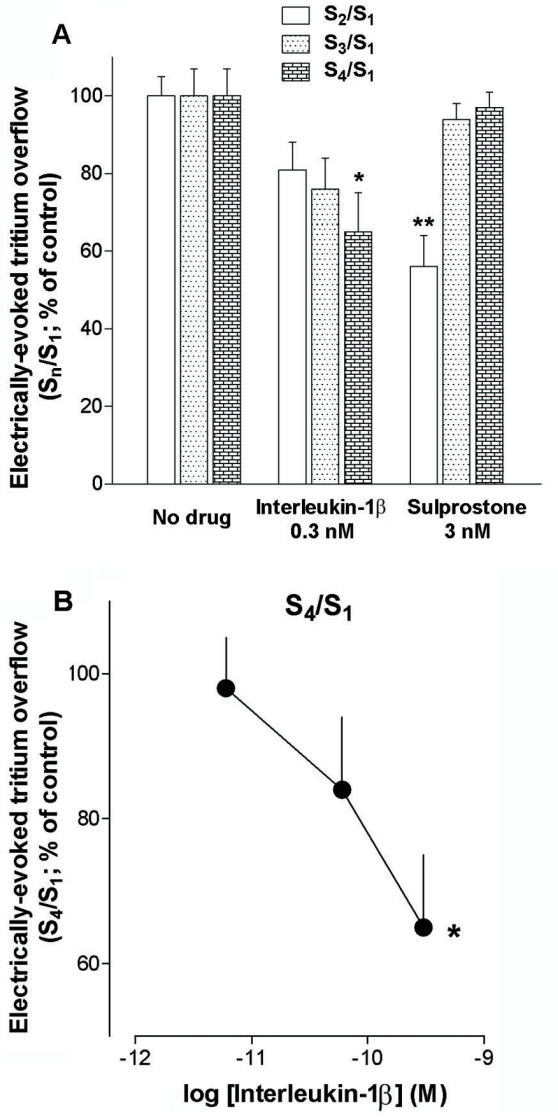

Fig. 6. Effect of interleukin-1β and sulprostone on the electrically-evoked tritium overflow from superfused mouse brain cortex slices preincubated with [^{3}H]noradrenaline. Tritium overflow was evoked four times, after 40, 90, 140 and 190 min of superfusion (S_1–S_4), and the ratio of the tritium overflow evoked by S_n over that evoked by S_1 was determined (S_n/S_1). Tritium overflow was expressed as the percent of the corresponding S_n/S_1 value in controls. The superfusion medium contained the drug under study from 77 to 110 min of superfusion and auxiliary drugs (see Tab. 1) throughout superfusion. Panel **A** shows the effect of 0.3 nM interleukin-1β and of 3 nM sulprostone on S_2/S_1, S_3/S_1 and S_4/S_1; in controls, the three ratios amounted to 1.24 ± 0.07, 1.29 ± 0.09 and 1.25 ± 0.08, respectively. In panel **B**, the concentration-response curve of interleukin-1β for its effect on S_4/S_1 is shown (control not shown). The means ± SEM of 7–8 experiments. * $p < 0.05$, ** $p < 0.01$ compared to control

dium. IL-1β at 0.3 nM tended to decrease the evoked overflow when the cytokine was present in the medium (S_2/S_1) and 30 min after its withdrawal (S_3/S_1), and the effect became significant only 80 min after its withdrawal (S_4/S_1). The extent of inhibition amounted to 35% (Fig. 6A). Although 3 nM sulprostone, which was examined for the sake of comparison, inhibited the evoked overflow to about the same extent, the kinetics of the effect were entirely different. Thus, a statistically significant inhibition had already occurred

when the drug was present in the medium (S_2/S_1), but 30 min after its withdrawal (S_3/S_1), the effect was no longer detectable (Fig. 6A). The concentration-response curve for IL-1β is shown in Figure 6B and is based on the effect of IL-1β 80 min after its withdrawal (S_4/S_1). IL-1β (0.006 nM) was completely ineffective, whereas a ten-fold higher concentration tended to inhibit the evoked overflow; a five-fold further higher concentration (0.3 nM) significantly inhibited the evoked overflow (see also the sixth column from the left in Fig. 6A).

Discussion

The present study was dedicated to the search for release-modulating receptors for four neuropeptides and IL-1β, and was carried out on superfused rodent tissues preincubated with [^{3}H]noradrenaline. Electrical stimulation was used to induce quasi-physiological noradrenaline release [36]. Usually, the peptide was present in the medium shortly before and during the second period of stimulation (S_2). In the experiments with IL-1β, two additional stimuli (S_3 and S_4) were administered 30 and 80 min after the withdrawal of the cytokine from the medium because the effect of IL-1β or a partial effect has been shown to occur with some delay in some studies [4, 17].

In all experiments, desipramine was present in the superfusion medium in order to avoid the interference of the drugs under study with the neuronal noradrenaline transporter and to increase the amount of noradrenaline release. In the experiments with the neuropeptide urotensin II (the receptor of which is coupled to G_q proteins [1]), a facilitatory effect on noradrenaline release could be expected; for this reason, the K^+ channel blocker tetraethylammonium chloride was added to the medium in order to release a high quantity of endogenous noradrenaline and to induce a strong activation of the presynaptic α_2-autoreceptors by noradrenaline. Under this experimental condition, the effects of agonists at presynaptic facilitatory G_q protein-coupled receptors on noradrenergic neurons are particularly strong [7].

In those experiments in which inhibitory presynaptic receptors were expected (i.e., in the experiments with MCH, NPW and NPFF because the respective receptors are coupled to $G_{i/o}$ proteins [1]), rauwolscine was present in the medium to avoid the interaction of the drugs under study with the α_2-autoreceptor, to increase noradrenaline release and to increase the extent of inhibition of noradrenaline release. The latter phenomenon has been shown for many presynaptic inhibitory heteroreceptors on noradrenergic neurons of the peripheral or central nervous system [29]. Rauwolscine was also used as an auxiliary drug in the experiments dedicated to IL-1β.

Urotensin II has attracted much attention in recent years because it is a very potent constrictor of human vessels and is believed to play a role in pathophysiological conditions like hypertension, heart failure, renal dysfunction and diabetes mellitus [11]. It was tempting to assume that urotensin II, like angiotensin II (which acts *via* G_q protein-coupled AT_1 receptors [1]), might possess a dual vasopressor effect, namely *via* a postsynaptic site of action and additionally *via* a facilitatory effect on the release of noradrenaline that in turn contributes to the overall effect on the vessel *in vivo*. However, unlike angiotensin II (which served as a positive control [37]), urotensin II did not facilitate noradrenaline release in a concentration range of 1–1000 nM, including its K_i of 1.4 nM in a radioligand binding study in the rat kidney [10] and an EC_{50} of 1.9 nM in contraction experiments in the rat aorta [3]. In the concentration range used in the present study, urotensin II led to an increase in the release of noradrenaline and other amines in rat cerebrocortical slices [23]. In the latter study, noradrenaline release (determined by HPLC) was not evoked electrically or by another type of stimulation but occurred spontaneously as a result of exposure to urotensin II. Additional work is necessary to elucidate the mechanism of release and the location of the UT receptors found in that study.

The peptides MCH and NPW are implicated in the regulation of food intake [15, 32]. Both neuropeptides did not affect noradrenaline release in our study. The concentrations were adequate because MCH 1 μM was the standard concentration for the electrophysiological identification of MCH_1 receptors in the rat lateral hypothalamus [13], and NPW 1 μM almost fully inhibited ^{125}I-NPW binding to rat amygdala membranes [33]. The possibility that noradrenaline release under the experimental conditions of our study is unresponsive to modulation *via* presynaptic inhibitory $G_{i/o}$ protein-coupled receptors can be discarded because the prostaglandin EP_3 receptor agonist sulprostone markedly inhibited noradrenaline release. Our results are also of interest for two reasons: (i) MCH in-

hibits the release of another two transmitters, GABA and glutamate, in the rat lateral hypothalamus [13]. (ii) Another neuropeptide – neuropeptide Y, which like MCH and NPW plays an important role in the regulation of food intake in the hypothalamus, inhibits noradrenaline release in numerous tissues *via* the $G_{i/o}$ protein-coupled Y_2 receptor [30].

NPFF has a modulatory and in many instances an inhibitory effect on the effect of opioids [22]. Like the $NPFF_2$ receptor, the three opioid receptor subtypes μ, δ and κ, are coupled to $G_{i/o}$ proteins [1]. Thus, it would be plausible that NPFF and the selective $NPFF_2$ receptor agonist dNPA [27] inhibit neurotransmitter release as well. In our model of the mouse atrium, the opioid δ receptor agonist DPDPE (as expected [30, 38]) inhibited noradrenaline release, whereas NPFF and dNPA had no effect. It is of interest that the inhibition of the release of another transmitter, GABA, *via* presynaptic receptors for NPFF has indeed been shown [18, 19]. In the second series of our experiments, we examined the possibility that the effect of DPDPE was modified by NPFF and dNPA. However, the latter two peptides were ineffective, although the opioid δ receptor antagonist naltrindole counteracted the inhibitory effect of DPDPE as expected [38]. In both experimental series, NPFF was used at concentrations high enough to activate $NPFF_1$ and $NPFF_2$ receptors [5] and dNPA was used at concentrations high enough to activate $NPFF_2$ receptors [27].

Interleukin-1β inhibited noradrenaline release in mouse brain cortex slices. The effect was concentration-dependent and occurred in a concentration range corresponding to the affinity of human IL-1β for the mouse IL-1RI receptor [8]. The effect developed gradually, and this is in harmony with the known fact that the kinetics of the transduction machinery associated with the interleukin-1 receptor (the transcription factor NF-κB, for example [9]) are much slower than those for G protein-coupled receptors. For the sake of comparison, the inhibitory effect of sulprostone, which acts *via* the prostanoid EP_3 receptor and causes an inhibition of noradrenaline release, has been studied in parallel. The effect of IL-1β on noradrenaline release has been studied under a variety of conditions and inhibitory (e.g., [4]), facilitatory (e.g., [26]) and biphasic effects (e.g., [17]) have been reported. Our study shows for the first time that IL-1β also affects noradrenaline release in the cerebral cortex, whereas in the studies by other authors, the brain stem and the hypothalamus (e.g., [39]) or peripheral tissues including the adrenal medulla (e.g., [26]), the myenteric plexus [17] and the spleen [4] have been considered. The fact that the effect of IL-1β in the present study was obtained on mouse tissue offers the possibility to do further experimentation on tissue from genetically manipulated animals. Furthermore, the present study might be the starting point for future work dedicated to the elucidation of the mechanisms involved in the effect of IL-1β on noradrenaline release and might be extended to other neurotransmitters.

With respect to the role played by IL-1β in the brain, one has to take into consideration that this cytokine can readily pass into the circumventricular organs but can also reach other areas of the brain by penetrating the blood-brain barrier *via* a transporter; moreover, IL-1β is secreted by the blood-brain barrier [25]. The inhibition of noradrenaline in the brain by IL-1β might account for affective symptoms such as anxiety that accompany the activation of the immune system, e.g., during an infection. In addition, IL-1β has been postulated as an etiological factor in the development of schizophrenia [34] and depression [35]. With respect to the latter disorder, the decrease in noradrenaline release we observed would fit well into the Schildkraut hypothesis of depression [28], which says that depression is associated with a depletion in noradrenaline.

In conclusion, our study shows that noradrenaline release in rodent tissues is not facilitated by urotensin II and is not inhibited by MCH, NPW and NPFF. Moreover, the frequently described interaction between opioids and NPFF does not occur with respect to noradrenergic neurons in the mouse atrium. On the other hand, IL-1β inhibits noradrenaline release in the mouse brain cortex; the inhibition, unlike that caused by sulprostone, develops slowly.

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