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Effects of the central analgesic tramadol on the uptake and release of noradrenaline and dopamine *in vitro*

[1]B. Driessen, [2]W. Reimann & H. Giertz

Grünenthal GmbH, Abteilung Pharmakologie, Zieglerstr. 6, W-5100 Aachen, Germany

1 The centrally acting analgesic, tramadol, has low affinity for opioid receptors and therefore presumably other mechanisms of analgesic action. Neurotransmitter release and uptake experiments were used to characterize the effects of tramadol on the central noradrenergic and dopaminergic systems.

2 Tramadol inhibited the uptake of [^{3}H]-noradrenaline into purified rat hypothalamic synaptosomes with an IC_{50} of 2.8 μM; the (−)-enantiomer was about ten times more potent than the (+)-enantiomer. Results with the principal metabolite O-desmethyltramadol were very similar. Inhibition of dopamine uptake into purified rabbit caudate nucleus synaptosomes was very weak with 62% inhibition at 100 μM.

3 Rat occipital cortex slices were preincubated with [^{3}H]-noradrenaline and rabbit caudate nucleus slices with [^{3}H]-dopamine, then superfused and stimulated electrically. Tramadol, 1 and 10 μM, enhanced the stimulation-evoked [^{3}H]-noradrenaline overflow by 25 and 69%, respectively; the (−)-enantiomer was more potent than the racemate or the (+)-enantiomer. Tramadol, 10 μM, had no effect on dopamine release.

4 The effects of tramadol on the stimulation-evoked [^{3}H]-noradrenaline release were abolished when uptake sites were already blocked by a high concentration of cocaine.

5 The metabolite O-desmethyltramadol showed a slight facilitation of the stimulation-evoked noradrenaline release; the effect was more pronounced in the presence of a high concentration of naloxone. In the presence of cocaine, inhibition of the release was observed similar to the effect of morphine but less potent.

6 The results show that tramadol blocks noradrenaline uptake with selectivity as compared to dopamine uptake. The interaction with the noradrenaline transporter is stereoselective. The principal metabolite O-desmethyltramadol shows in addition to noradrenaline uptake inhibition, opioid inhibition of noradrenaline release.

Keywords: Tramadol; noradrenaline; dopamine; uptake; release

Introduction

Tramadol, (1**RS**; 2**RS**)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride, is a centrally acting analgesic with affinity for opioid receptors in the micromolar range (Hennies *et al.*, 1988; Raffa *et al.*, 1992), although its analgesic and antinociceptive potency is only five to ten fold lower than that of morphine. Actions other than opioid mechanisms may therefore contribute to its analgesic efficacy. This assumption is supported by the observation that in some experiments effects of tramadol are not fully antagonized by naloxone (Carlsson & Jurna, 1987; Kayser *et al.*, 1991; Raffa *et al.*, 1992). The noradrenergic system is involved in exogenously induced opioid and non-opioid antinociception (Sawynok, 1989), and a slight to moderate inhibition of noradrenaline uptake has been reported previously (Hennies *et al.*, 1982; Raffa *et al.*, 1992). Hence we investigated the interference of tramadol with the noradrenergic system more closely as regards specificity and stereoselectivity and examined in release studies, whether the effects were pure uptake inhibition since effects of tramadol in the 5-hydroxytryptaminergic system were more consistent with an action like an indirect mimetic (Driessen & Reimann, 1992).

Methods

General

Female Chinchilla cross rabbits weighing 2.5–4.2 kg and male Sprague Dawley rats weighing 180–380 g were killed by decapitation. The brain was quickly removed and chilled. Rabbit caudate nuclei were bluntly detached from the capsula interna, and rat hypothalami were dissected according to Glowinski & Iversen (1966). Slices from rabbit caudate nucleus (0.4 mm thick) and rat occipital cortex (0.4 mm thick, 5 mm diameter) were prepared as described and characterized previously (Starke *et al.*, 1978; Reimann *et al.*, 1981).

Outflow of tritium from slices after preincubation with ^{3}H-labelled catacholamines

Slices from rat occipital cortex were preincubated with 0.1 μM (−)-[^{3}H]-noradrenaline, and slices from rabbit caudate nucleus with 0.1 μM [^{3}H]-dopamine at 37°C for 30 min. After incubation, the slices were rinsed 3 times with medium and transferred to glass superfusion chambers equipped with platinum electrodes. They were superfused at 37°C with ^{3}H-catecholamine-free medium at a rate of 1 ml min^{-1} for 115–165 min. Five minute samples of the superfusate were collected 50 min after starting the superfusion. The slices were stimulated electrically for two periods of 2 min each, after 60 and 95 min ((−)-[^{3}H]-noradrenaline) or 60 and 120 min ([^{3}H]-dopamine) of superfusion (S_1, S_2). Rectangular pulses of 2 ms duration, 24 mA current strength and a frequency of 3 Hz were delivered from a stimulator constructed by the Biomedical Technics Department of Grünenthal. The slices were solubilized in 0.5 ml Soluene 350 (Packard) at the end of the experiment. The radioactivity in superfusates and slices was measured after addition of Ready Gel (Beckman) or Ready Safe (Beckman), respectively.

The incubation and superfusion medium contained (mM): NaCl 118, KCl 4.8, $CaCl_2$ 1.3, $MgSO_4$ 1.2, $NaHCO_3$ 25,

[1] Present address: Pharmakologisches Institut, Hermann-Herder-Str. 5, W-7800 Freiburg i. Br., Germany

[2] Author for correspondence.

KH_2PO_4 1.2, glucose 11, ascorbic acid 0.57, disodium EDTA 0.03; the medium was saturated with 5% CO_2 in O_2. The pH was adjusted to 7.4 by the addition of NaOH. Drugs were added 15 min before S_2 or were present throughout superfusion as indicated.

Accumulation of ^{3}H-labelled catecholamines in synaptosomes

Rat hypothalami or rabbit caudate nuclei were homogenized in 0.32 M ice-cold sucrose (3 ml 100 mg^{-1} tissue) in a glass homogenizer with a loosely fitting teflon pestle using ten full up and down strokes at 800 revolutions per min. The homogenate was filtered through Thomapor gauze 100 μm mesh and centrifuged at 1000g for 15 min at 4°C. Further subfractionation was performed according to Dodd *et al.* (1981). The supernatant was layered on 4.5 ml of 1.2 M sucrose and centrifuged at 190 000 g for 25 min. Particles at the gradient interface were collected in a volume of about 2.5 ml and diluted with ice-cold 0.32 M sucrose to a final volume of 8.0 ml. This suspension was layered on 4.5 ml 0.8 M sucrose and centrifuged again for 25 min at 190,000 g. The resulting synaptosomal pellet was gently resuspended in 4 ml ice-cold incubation medium/100 mg original tissue weight by use of a teflon/glass homogenizer.

The composition of the incubation medium was as follows (mmol l^{-1}): NaCl 119, KCl 3.9, $CaCl_2$ 0.51, $MgSO_4$ 0.65, Na_2HPO_4 15.6, NaH_2PO_4 3.4, glucose 10, ascorbic acid 0.57. Nialamide, 0.0125 mmol l^{-1}, was added for inhibition of monoamine oxidase. The pH was adjusted to 7.4 by addition of NaOH.

The synaptosomes were preincubated for 5 min at 37°C in the absence or presence of the drugs to be tested. The uptake was started by addition of 10 μl (−)-[^{3}H]-noradrenaline or [^{3}H]-dopamine yielding a concentration of 0.1 μM. The final incubation volume was 1 ml, and the protein concentration, determined according to Lowry *et al.* (1951), was 99–135 μg ml^{-1} in noradrenaline experiments and 21–74 μg ml^{-1} in dopamine experiments. The incubation lasted for 2 min in noradrenaline and 30 s in dopamine experiments if not stated otherwise. It was stopped by addition of 6 ml ice-cold medium, immediately followed by vacuum filtration through nitrocellulose membranes (pore size 0.65 μm; Sartorius) presoaked in incubation medium. Filters were washed 3 times with 4 ml cold medium. For determination of radioactivity, the wet filters were solubilized in 2 ml ethylene glycol monoethyl ether. Radioactivity was counted for after addition of 10 ml Ready Safe (Beckman). Values were corrected for the respective accumulation of radioactivity in synaptosomes incubated at 0°C. All assays were performed in triplicate.

Calculations and statistics

The fractional rate of tritium outflow in release experiments was calculated by dividing the tritium content in the superfusate by the tritium content in the slice at the start of the respective collection period. The stimulation-evoked overflow of tritium was calculated by subtraction of the estimated basal outflow and was expressed as a percentage of the tissue tritium content at the start of stimulation.

K_m and V_{max} were estimated by adaptation of the data to the Michaelis-Menten hyperbola and IC_{50}s by adaptation to the IB_1 or IB_2 model of the TOPFIT programme package (Thomae) on a Wang 2236-D type computer.

Means ± s.e.mean are given throughout. Differences between means were tested for significance by the Mann-Whitney test.

Compounds

(−)-[ring-2,5,6-^{3}H]-noradrenaline, specific activity 40.8 and 42.1 Ci $mmol^{-1}$, 3,4-[ring-2,5,6-^{3}H]-dopamine hydrochloride, specific activity 41 and 45.7 Ci $mmol^{-1}$ (New England Nuclear); cocaine HCl, morphine HCl (E. Merck); desipramine HCl (Sigma); naloxone HCl (DuPont); yohimbine HCl (Roth); tramadol HCl, (−)- and (+)-enantiomer (E 381, E382), O-desmethyltramadol HCl, (−)- and (+)-enantiomer (EM 724, EM 723; Grünenthal) were used. All drugs were dissolved in distilled water.

Results

Effects on tritium overflow from slices preincubated with [^{3}H]-noradrenaline

Slices from rat brain occipital cortex were preincubated with (−)-[^{3}H]-noradrenaline, then superfused with either drug-free medium or medium containing the drugs indicated and stimulated electrically for two periods (S_1, S_2). The test drugs were added to drug-free or drug-containing medium 15 min before S_2. The basal outflow of tritium was monitored in the collection periods preceding the stimulation. Drugs added before S_2 had only minor effects on the basal outflow of tritium not exceeding 12% deviation. Electrical stimulation at S_1 enhanced the overflow of tritium, about 5% of the tritium in the tissue appeared in the medium when no drug was present, and stimulation at S_2 induced on overflow of about the same amount as shown by the ratio S_2/S_1 near unity when no further drug was added. Tramadol, 1 and 10 μM, enhanced the stimulation-evoked overflow by 25 and 69%, respectively (Table 1; Figure 1). When the enantiomers were tested, the (−)-enantiomer was more active then the (+)-enantiomer, which showed significant effects only at 10 μM. Effects by tramadol were not changed, when α_2-adrenoceptors were blocked by a high concentration of yohimbine (Table 1). However, blockade of the noradrenaline uptake mechanism by cocaine abolished the facilitatory effects on the stimulation-evoked overflow almost completely (Table 1; Figure 2).

The O-demethylated metabolite of tramadol had only a weak facilitatory effect on the stimulation-evoked overflow, 17% at 10 μM, when no further drug was present (Table 1). The facilitatory effect became more prominent when naloxone was added to the medium as shown in Figure 3. An inhibition by O-desmethyltramadol of the stimulation-evoked overflow, 12% at 10 μM, became apparent in the presence of cocaine (Figure 3) and a similar effect was also observed, when desipramine, 1 μM, was added to the medium (not shown). In the presence of both drugs, cocaine and naloxone, no further effect by O-desmethyltramadol could be observed (Figure 3). In contrast, combination of the α_2-blocker yohimbine with naloxone did not attenuate the facilitation induced by O-desmethyltramadol (Table 1).

Morphine when added at S_2 depressed the stimulation-evoked overflow in a concentration-related manner; this effect was completely antagonized by the presence of naloxone (Table 1; Figure 3). The presence of cocaine had no distinct effect on the action of morphine. Naloxone, added at S_2, had no influence on the stimulation-evoked overflow, and cocaine itself effected a pronounced facilitation which was already observed at 0.1 μM and reached 58% at 1 μM (Table 1).

Effects on tritium overflow from slices preincubated with [^{3}H]-dopamine

Slices from rabbit caudate nucleus were preincubated with [^{3}H]-dopamine, then superfused with drug-free medium and stimulated electrically for two periods (S_1, S_2). Stimulation at S_1 evoked an overflow of 7.8 ± 0.3% ($n = 9$) of the tritium in the tissue. When no drug was added at S_2, the ratio S_2/S_1 was 0.98 ± 0.02 ($n = 3$). After addition of 10 μM of either tramadol or O-desmethyltramadol 15 min before S_2 the S_2/S_1 ratios were 1.04 ± 0.03 ($n = 3$) and 0.98 ± 0.00 ($n = 3$), respectively. Thus no changes of the stimulation-evoked overflow were

Table 1 Effects of tramadol, O-desmethyltramadol and reference compounds on the stimulation-evoked overflow of tritium from rat cerebral cortex slices preincubated with (−)-[^{3}H]-noradrenaline

Drug added at S_2	*Drug added throughout superfusion*	*S_2/S_1 at a drug concentration of* 0	0.1 μM	1 μM	10 μM
Tramadol	–	0.96 ± 0.01 (7)	0.96 ± 0.01 (7)	1.20 ± 0.06 (8)*	1.62 ± 0.03 (8)*
Tramadol (−)-enantiomer	–	0.94 ± 0.03 (6)	0.99 ± 0.04 (6)	1.18 ± 0.05 (6)*	1.82 ± 0.02 (6)*
Tramadol (+)-enantiomer	–	0.99 ± 0.02 (6)	0.96 ± 0.04 (6)	0.99 ± 0.03 (6)	1.17 ± 0.03 (6)*
Tramadol	Yohimbine 1 μM	0.96 ± 0.01 (5)		1.23 ± 0.02 (5)*	1.94 ± 0.06 (5)*
Tramadol	Cocaine 30 μM	1.03 ± 0.01 (6)	1.01 ± 0.02 (6)	1.03 ± 0.01 (6)	1.07 ± 0.01 (6)*
O-Desmethyltramadol	–	0.94 ± 0.01 (12)	0.91 ± 0.02 (5)	0.96 ± 0.03 (10)	1.10 ± 0.04 (11)*
O-Desmethyltramadol	Cocaine 30 μM	1.01 ± 0.01 (6)		0.96 ± 0.02 (6)*	0.89 ± 0.01 (6)*
O-Desmethyltramadol	Naloxone 1 μM	0.94 ± 0.02 (6)	0.95 ± 0.02 (5)	1.07 ± 0.02 (5)*	1.36 ± 0.06 (5)*
O-Desmethyltramadol	Cocaine 30 μM + Naloxone 1 μM	1.04 ± 0.03 (6)		0.99 ± 0.02 (6)	1.03 ± 0.03 (6)
O-Desmethyltramadol	Yohimbine 1 μM + Naloxone 1 μM	0.91 ± 0.01 (4)		1.06 ± 0.01 (5)*	1.51 ± 0.03 (6)*
Morphine	–	0.91 ± 0.02 (6)	0.83 ± 0.03 (6)	0.72 ± 0.03 (6)*	0.67 ± 0.03 (6)*
Morphine	Naloxone 1 μM	0.98 ± 0.02 (8)	0.95 ± 0.03 (6)	0.95 ± 0.02 (7)	0.94 ± 0.01 (8)
Morphine	Cocaine 30 μM	1.00 ± 0.04 (6)	0.97 ± 0.01 (6)	0.86 ± 0.01 (6)	0.83 ± 0.04 (6)*
Naloxone	–	1.01 ± 0.03 (4)	0.97 ± 0.01 (4)	1.06 ± 0.07 (4)	0.99 ± 0.04 (4)
Cocaine	–	0.96 ± 0.02 (6)	1.09 ± 0.01 (6)*	1.52 ± 0.07 (6)*	

After preincubation, the slices were superfused with medium containing no drug or the drugs indicated, and were stimulated electrically for two periods (S_1, S_2). Drugs given at S_2 were added to the medium 15 min prior to the onset of stimulation. Stimulation at S_1 evoked an overflow of 4.8 ± 0.1% (no drug added, $n = 174$), 9.3 ± 0.3% (yohimbine, $n = 15$), 9.2 ± 0.1% (cocaine, $n = 66$), 5.2 ± 0.1% (naloxone, $n = 50$), 8.2 ± 0.2% (cocaine and naloxone, $n = 18$) and 9.1 ± 0.4% (yohimbine and naloxone, $n = 18$) of the tritium in the tissue at the start of stimulation. Significant differences from corresponding controls (concentration of drug added at $S_2 = 0$): $^*P < 0.05$. Means ± s.e.mean of numbers of experiments indicated in parentheses.

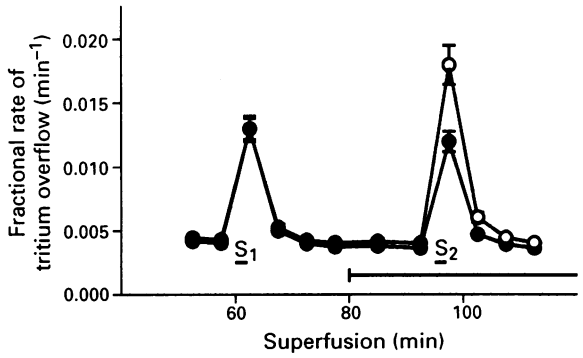

Figure 1 Effect of tramadol on the outflow of tritium from brain cortical slices preincubated with (−)-[^{3}H]-noradrenaline. After preincubation, slices were superfused with drug-free medium and were stimulated electrically for two periods (S_1, S_2). (●) Controls ($n = 7$); (○) tramadol, 10 μM, was added 15 min before S_2 as indicated by the bar ($n = 8$). s.e.mean shown by vertical bars.

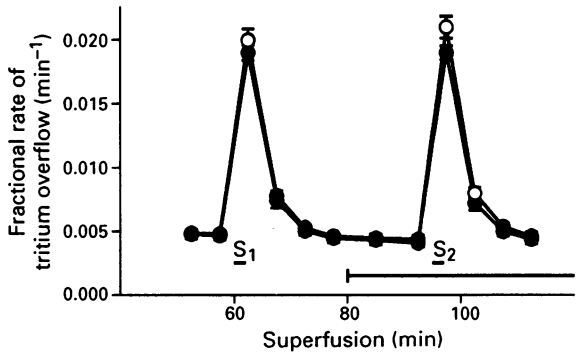

Figure 2 Effect of tramadol on the outflow of tritium from brain cortical slices preincubated with (−)-[^{3}H]-noradrenaline. After preincubation, slices were superfused with medium containing 30 μM cocaine and were stimulated electrically for two periods (S_1, S_2). (●) Controls ($n = 6$); (○) tramadol, 10 μM, was added 15 min before S_2 as indicated by the bar ($n = 6$). s.e.mean shown by vertical bars.

obvious, and nor were significant changes of the basal outflow observed.

Effects on the accumulation of [^{3}H]-noradrenaline in synaptosomes

Experiments evaluating the time course of (−)-[^{3}H]-noradrenaline accumulation in the purified hypothalamic synaptosomes showed linear uptake of tritium for 2 min (not shown). So this time period seemed adequate to approach the initial rate of uptake. Kinetic analysis showed an apparent K_m of 0.11 ± 0.02 μM and V_{max} was 16.2 ± 3.1 pmol 2 min^{-1} mg^{-1} protein ($n = 3$ with 7 concentrations per experiment).

Synaptosomes were incubated with (−)-[^{3}H]-noradrenaline either in the presence or absence of drugs. Accumulation of tritium under control conditions (no drug present) was 4.5 ± 0.2 pmol [^{3}H]-noradrenaline equivalent mg^{-1} protein ($n = 58$). The accumulation of radioactivity was potently inhibited by desipramine, the concentration-response relationship showed an obviously biphasic course (Figure 4). When the inhibition curve was analyzed with the IB2 model, the high potenty part of the inhibition with an IC_{50} value in the nanomolar range (Table 1) comprised 72 ± 2% of the total uptake; the other part of the uptake was inhibited with an IC_{50} in the micromolar range and constituted 28 ± 2% of the total uptake ($n = 6$). Cocaine, tramadol, O-desmethyltramadol (Figure 4) and both enantiomers of either tramadol or O-desmethyltramadol (not shown) displayed a steady inhibition of uptake in the concentration-range tested. IC_{50}s are summarized in Table 2. The uptake inhibition by the (−)-enantiomer of tramadol is about twice that of the racemate, and is about one order of magnitude more potent than that of the (+)-enantiomer. Results with O-desmethyltramadol and its enantiomers were very similar to those of

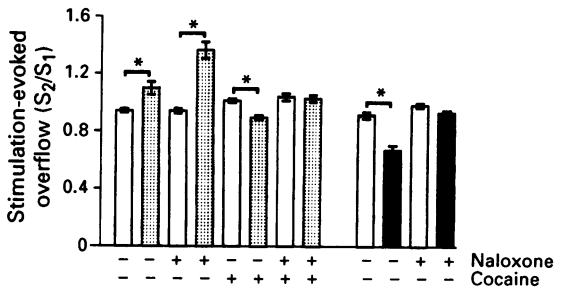

Figure 3 Effects of O-desmethyltramadol and morphine on the stimulation-evoked overflow of tritium from brain cortical slices preincubated with (−)-[^{3}H]-noradrenaline. After preincubation, slices were superfused with medium containing no drug or the drugs indicated below the abscissa scale. They were then stimulated electrically for two periods (S_1, S_2). Open columns represent control experiments with the indicated drugs contained in the medium. O-desmethyltramadol, 10 μM (stippled column) or morphine, 10 μM (solid column) were added 15 min before S_2. Columns represent mean ratios S_2/S_1 of 5–8 experiments; s.e.mean shown by vertical bars.

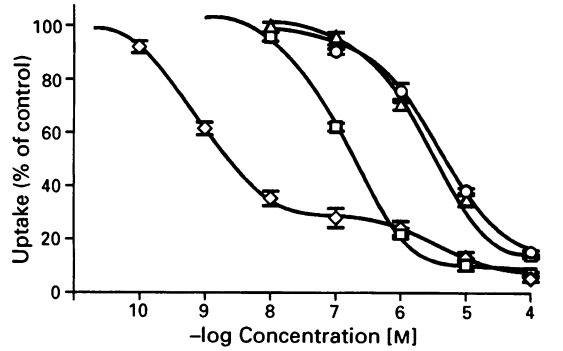

Figure 4 Effect of various drugs on the accumulation of tritium in hypothalamic synaptosomes incubated with 0.1 μM (−)-[^{3}H]-noradrenaline. The accumulation in the presence of desipramine (◇), cocaine (□), tramadol (△) and O-desmethyltramadol (○) is expressed as a percentage of control. Each data point represents the mean ± s.e.mean (vertical bars) of 4–10 experiments.

tramadol and its enantiomers (Table 2). Maximum inhibition at 100 μM amounted to 92.7 ± 0.7% with cocaine, 86.5 ± 0.5% with tramadol, 87.9 ± 0.8% with the (−)-enantiomer of tramadol, 75.1 ± 0.9% with the (+)-enantiomer, 82.7 ± 1.0% with O-desmethyltramadol, 86.5 ± 1.0% with its (−)-enantiomer and 60.5 ± 1.8% with its (+)-enantiomer. No inhibition of tritium accumulation by morphine could be observed (4.7 ± 4.6% inhibition at 100 μM; $n = 3$).

Effects on the accumulation of [^{3}H]-dopamine in synaptosomes

Preliminary experiments confirmed that the rate of accumulation of tritium in rabbit caudate nucleus synaptosomes was declining already at 30 s. This time period was therefore chosen for subsequent experiments. Kinetic analysis showed an apparent K_m of 0.11 ± 0.01 μM and V_{max} was 53.2 ± 15.9 pmol 30 s^{-1} mg^{-1} protein ($n = 4$ with 7 concentrations per experiment).

Synaptosomes were incubated with [^{3}H]-dopamine either in the presence or absence of drugs. Accumulation of tritium under control conditions was 21.7 ± 7.2 pmol [^{3}H]-dopamine equivalent mg^{-1} protein ($n = 9$). The accumulation was inhibited by nomifensine with an IC_{50} of 0.15 ± 0.02 μM ($n = 6$). Tramadol inhibited the uptake very weakly; only

Table 2 Effect of drugs on the accumulation of tritium in hypothalamic synaptosomes incubated with 0.1 μM (−)-[^{3}H]-noradrenaline

Drug	IC_{50} (μM)	n
Desipramine		
high potency range	0.00079 ± 0.00007	6
low potency range	7.2 ± 1.7	
Cocaine	0.16 ± 0.01	6
Tramadol	2.8 ± 0.3	6
Tramadol (−)-enantiomer	1.6 ± 0.3	6
Tramadol (+)-enantiomer	14.7 ± 0.7	6
O-Desmethyltramadol	4.8 ± 0.6	10
O-Desmethyltramadol (−)-enantiomer	1.4 ± 0.2	6
O-Desmethyltramadol (+)-enantiomer	41.4 ± 4.4	9
Morphine	>100	3

In each experiment, at least four drug concentrations were used. Means ± s.e.mean of numbers of individual experiments indicated.

small effects were observed at 1 μM and inhibition at 100 μM was 61.6 ± 5.9% ($n = 3$).

Discussion

Release of radiolabelled neurotransmitters from isolated tissues maintained *in vitro* is a frequently used model for the evaluation of presynaptic auto- and heteroreceptors (Langer, 1981; Starke, 1981; Chesselet, 1984; Starke *et al.*, 1989), and also of the noradrenaline uptake mechanisms as increased overflow can be observed with uptake inhibitors which reduce the fraction of the previously released transmitter which is normally subject to re-uptake (Starke *et al.*, 1971). The facilitating effect of the uptake inhibitor, cocaine, on the electrically-evoked noradrenaline overflow has been described (Starke & Montel, 1973) and was observed in the present experiments as well. An enhancement of the evoked overflow was also seen with tramadol. Differentiation between the possible mechanisms, α_2-autoreceptor antagonism or uptake blockade, was carried out either by means of receptor blockade by yohimbine or uptake site blockade by cocaine. Since effects were no longer observed when the function of the uptake sites was eliminated, in contrast to receptor blockade, evidence for an uptake blocking action of tramadol was provided. Efficacy seemed about one order of magnitude less than that of cocaine. The uptake inhibiting properties reside obviously in the (−)-enantiomer of tramadol. Direct α_2-adrenoceptor activation by tramadol which could have been masked by the effects of uptake inhibition was not observed in the presence of cocaine and can therefore be ruled out.

The naloxone-sensitive inhibition by morphine of noradrenaline release has long been known (Montel *et al.*, 1974; Henderson *et al.*, 1979) and was also observed in the present investigation. The lack of effect of naloxone alone (Montel *et al.*, 1974) indicates that noradrenaline release from cortical neurones is not tonically inhibited by endogenous opioids.

The principal metabolite O-desmethyltramadol has in [^{3}H]-naloxone binding experiments an approx. ten fold higher affinity for opioid receptors as compared to tramadol (IC_{50} 0.88 μM vs. 6.1 μM; Hennies *et al.*, 1988); it occurs in many species although in lower amounts in man as compared e.g. to rats and mice (Lintz *et al.*, 1981). In contrast to tramadol, its action on the stimulation-evoked noradrenaline overflow is apparently a combination of both noradrenaline uptake inhibition and opioid receptor activation. This becomes obvious when uptake sites are blocked by cocaine or opioid receptors are blocked by naloxone resulting in either inhibition or further enhancement of the evoked noradrenaline overflow. The assumuption of a dual mode of action is confirmed by the observation that in the presence of both

cocaine and naloxone, but not of yohimbine and naloxone, O-desmethyltramadol has no further effects on the release.

Actions by tramadol and its metabolite O-desmethyltramadol seem to be rather selective for uptake inhibition of the catecholamine noradrenaline since no effects were observed in dopamine release experiments. Previous experience, however, has shown that dopamine agonists and antagonists as well as uptake inhibitors have effects in these experiments (Starke et al., 1978; Reimann et al., 1979).

Although uptake inhibition can be specifically observed in functional studies such as neurotransmitter release experiments, quantification of the effects is usually performed in uptake experiments using synaptosomes. In order to approach true uptake, i.e. unidirectional transmitter flow, only the initial linear rate of uptake should be measured (Graefe, 1976). This precondition was met in the present investigation. Since transmitters may accumulate in other than their own terminals, e.g. noradrenaline is readily taken up in dopaminergic terminals (Iversen, 1974), regions for the preparation of synaptosomes were carefully chosen. The hypothalamus, which has a low amount of dopaminergic terminals, was used for noradrenaline uptake. The specific noradrenaline inhibitor desipramine inhibits the accumulation of [^{3}H]-noradrenaline in the nanomolar range and micromolar concentrations are necessary to inhibit the uptake in dopaminergic terminals. It has been shown that hypothalamic synaptosomes accumulate 74% of total [^{3}H]-noradrenaline in noradrenergic and 26% in presumably dopaminergic terminals (Michel et al., 1984). Our results concur with these findings and provide evidence that our synaptosomal preparation represented mainly noradrenergic terminals. Purification of the synaptosomes by gradient centrifugation was performed in order to minimize uptake in or attachment to other cell organelles.

Inhibition by desipramine and cocaine of noradrenaline uptake in our study was comparable to previously published data (Koe, 1976; Richelson & Pfenning, 1984). Inhibition by cocaine of total uptake was monophasic and almost complete; this is probably due to the potent and well-known interaction of cocaine with the dopamine transporter (Galloway, 1988). Tramadol inhibited more than 72% of the total uptake; the weak interference with the dopamine carrier observed in the dopamine uptake experiments is therefore presumed to contribute to the overall effect. If effects were only related to the noradrenergic proportion of the synaptosomes (= 72% of total uptake), a higher efficacy with an IC_{50} of about 1.5 μM became apparent. The efficacy in the uptake experiments is in good agreement with the effects observed in the release experiments. It can also be observed, that effects of the metabolite on uptake inhibition are very similar to those of the parent substance.

When the effects of tramadol on noradrenaline and dopamine uptake are compared, it is obvious that there is a selectivity of about two orders of magnitude towards the noradrenergic system. The noradrenergic carrier shows, in contrast to the dopaminergic carrier, stereoselectivity for noradrenaline uptake (Iversen, 1974); the stereoselective inhibition by tramadol and O-desmethyltramadol, the (−)-enantiomer being one order of magnitude more potent, provides further evidence for a specific interaction with the noradrenaline uptake system.

Apparent neurotransmitter uptake inhibition in experiments measuring accumulation of transmitter may be the result of the induction of neurotransmitter release (Baumann & Maitre, 1976; Maxwell et al., 1976). This misinterpretation of uptake inhibition can be avoided by the concomitant investigation of the release. These effects would impose as acceleration of the basal outflow. This effect of tramadol was observed in 5-hydroxytryptamine release experiments (Driessen & Reimann, 1992). However, concentrations of tramadol which inhibited the noradrenaline uptake in mainly noradrenergic synptosomes had no distinct effect on the basal outflow of noradrenaline in a tissue slice release model, providing evidence for a true interaction with the uptake mechanism. This assumption is further supported by the functional interaction of tramadol and O-desmethyltramadol with the uptake inhibitor cocaine.

Morphine exerts its antinociceptive effects in part by supraspinal activation of spinal descending noradrenergic pathways (Yaksh, 1988). Locally, at the nerve terminal, morphine interferes with the release of noradrenaline and may thus attenuate its own efficacy. Reinforcement of the noradrenergic neurotransmission might therefore add to opioid efficacy, and, in fact, it has been shown in rats, that the noradrenaline uptake inhibitor desipramine enhanced the antinociceptive actions of systemically or centrally administered morphine (Ossipov et al., 1982). Relevant tramadol concentrations in the CNS can be supposed, since experiments on antinociception in the mouse tail-flick test have shown, that plasma concentrations of tramadol range from 0.8 μM at the threshold to 10.8 μM at the maximum effective dose (Friderichs & Becker, 1991). Investigations of positron emission following [^{11}C]-tramadol injection showed, within minutes, at least four fold higher concentrations in the CNS as compared to plasma levels in the mouse and in man while O-desmethyltramadol levels only slowly approach those in plasma (G. Stoecklin, KFA Jülich, personal communication). Further support for the assumption of a participation of the noradrenaline uptake inhibition in the analgesic effect of tramadol is derived from the observation that spinal antinociceptive effects of tramadol are antagonized by the α_2-adrenoceptor blocker, yohimbine (Driessen et al., 1990; Raffa et al., 1992).

In conclusion, tramadol and its principal metabolite O-desmethyltramadol inhibited noradrenaline uptake in a synaptosomal preparation in a specific and stereoselective fashion. The functional consequences of this mode of action were confirmed in the release experiments. In these experiments the mode of action was further confirmed by the functional interaction of tramadol and O-desmethyltramadol with the uptake inhibitor, cocaine. Antinociception by opioids is in part mediated by supraspinal activation of spinal descending noradrenergic pathways (Yaksh, 1988; Sawynok, 1989). While morphine interferes locally with the release of noradrenaline and may thus attenuate its own effects, reinforcement of noradrenergic neurotransmission by tramadol may add to its analgesic efficacy.

Results are part of the doctoral thesis of B.D.

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