Br. J. Pharmacol. (1993), **110**, 169–176

Effects of the central analgesic tramadol and its main metabolite, O-desmethyltramadol, on rat locus coeruleus neurones

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1 Tramadol is a centrally acting analgesic with low opioid receptor affinity and, therefore, presumably additional mechanisms of analgesic action. Tramadol and its main metabolite O-desmethyltramadol were tested on rat central noradrenergic neurones of the nucleus locus coeruleus (LC), which are involved in the modulation of nociceptive afferent stimuli.

2 In pontine slices of the rat brain the spontaneous discharge of action potentials of LC cells was recorded extracellularly. (−)-Tramadol (0.1–100 μM), (+)-tramadol (0.1–100 μM), (−)-O-desmethyltramadol (0.1–100 μM) and (+)-O-desmethyltramadol (0.01–1 μM) inhibited the firing rate in a concentration-dependent manner. (+)-O-desmethyltramadol had the highest potency, while all other agonists were active at a similar range of concentrations.

3 (−)-Tramadol (10, 100 μM) was less inhibitory in brain slices of rats pretreated with reserpine (5 mg kg^{-1}, 5 h before decapitation) than in controls.

4 The effect of (−)-tramadol (10 μM) was abolished in the presence of the α_2-adrenoceptor antagonist, rauwolscine (1 μM), whilst that of (+)-O-desmethyltramadol (0.3 μM) virtually disappeared in the presence of the opioid antagonist, naloxone (0.1 μM). (+)-Tramadol (30 μM) and (−)-O-desmethyltramadol (10 μM) became inactive only in the combined presence of naloxone (0.1 μM) and rauwolscine (1 μM).

5 In another series of experiments, the membrane potential of LC neurones was determined with intracellular microelectrodes. (−)-Tramadol (100 μM) inhibited the spontaneous firing and hyperpolarized the cells; this effect was abolished by rauwolscine (1 μM). (+)-O-desmethyltramadol (10 μM) had a similar but somewhat larger effect on the membrane potential than (−)-tramadol. The (+)-O-desmethyltramadol- (10 μM) induced hyperpolarization was abolished by naloxone (0.1 μM).

6 The hyperpolarizing effect of noradrenaline (30 μM) was potentiated in the presence of (−)-tramadol (100 μM), but not in the presence of (+)-O-desmethyltramadol (10 μM). There was no potentiation of the noradrenaline (30 μM) effect, when the cells were hyperpolarized by current injection to an extent similar to that produced by (−)-tramadol (100 μM).

7 Both noradrenaline (100 μM) and (−)-tramadol (100 μM) decreased the input resistance.

8 The results confirm that the analgesic action of tramadol involves both opioid and non-opioid components. It appears that (−)-tramadol inhibits the uptake of noradrenaline and via a subsequent increase in the concentration of endogenous noradrenaline indirectly stimulates α_2-adrenoceptors. (+)-O-desmethyltramadol seems to stimulate directly opioid μ-receptors. The effects of (+)-tramadol and (−)-O-desmethyltramadol consist of combined μ-opioid and α_2-adrenergic components.

Keywords: Tramadol; O-desmethyltramadol; locus coeruleus neurones; firing rate; membrane potential; noradrenaline uptake blockade

Introduction

Tramadol is a centrally acting analgesic (Friderichs *et al.*, 1978) with a limited range of side effects (Vogel *et al.*, 1978; Flohe *et al.*, 1978). It binds to opioid μ-receptors with an approximately 100-times lower affinity than morphine (Hennies *et al.*, 1988), while there is a much smaller difference between the analgesic potencies of these compounds (Friderichs *et al.*, 1978). Hence, it was concluded that non-opioid mechanisms are likely to be involved in tramadol analgesia (see e.g. Carlsson & Jurna, 1987). In fact this opioid has been shown to inhibit the uptake of noradrenaline and 5-hydroxytryptamine (Driessen & Reimann, 1992; Raffa *et al.*, 1992) thereby increasing the concentration of the two neurotransmitters in the central nervous system (CNS). Endogenous noradrenaline and 5-hydroxytryptamine participate in pain modulation (Besson & Chaouch, 1987; Jones, 1991) and may, in consequence, mediate the analgesic effect of tramadol.

The nucleus locus coeruleus (LC) is situated in the pons and consists of a compact group of noradrenergic cell bodies, which project into various areas of the central nervous system (Foote *et al.*, 1983). LC neurones possess somatic (and/or dendritic) α_2-adrenoceptors (Aghajanian & Vander-Maelen, 1982; Williams *et al.*, 1985) and opioid μ-receptors (Williams & North, 1984). Stimulation of either receptor-type increases the same potassium conductance (North & Williams, 1985) and, subsequently, leads to hyperpolarization and inhibition of spontaneous firing. α_2-Adrenoceptors may be tonically activated by endogenous noradrenaline after uptake blockade by desipramine (Egan *et al.*, 1983) or cocaine (Surprenant & Williams, 1987).

The LC is involved in the control of various cognitive and vegetative functions (Olpe *et al.*, 1985), including the modulation of pain perception (Jones, 1991). The aim of the present study was two fold. Firstly, the effects of tramadol and its main metabolite O-desmethyltramadol (Lintz *et al.*, 1981) were investigated on the firing rate and membrane potential of LC neurones. Secondly, the possible involvements of α_2-adrenoceptors and opioid μ-receptors in the effects of

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tramadol and O-desmethyltramadol were studied. Both (−)- and (+)-enantiomers of tramadol and O-desmethyltramadol were used, since opioid receptors (Höllt & Wüster, 1978) and the noradrenaline carrier (Graefe & Bönisch, 1988) bind ligands in a stereoselective manner.

Methods

Brain slice preparation

Midpontine slices of the rat brain were prepared and maintained as described earlier (Henderson *et al.*, 1982; Regenold & Illes, 1990). In brief, male Wistar rats (150–220 g) were anaesthetized with ether and decapitated. Slices of 400 μm thickness, containing the caudal part of the LC were prepared in oxygenated medium at 1–4°C with a Lancer vibratome. A single slice was transferred to the recording chamber and was superfused at a rate of 2 ml min^{-1} with medium saturated with 95% O_2 plus 5% CO_2 and maintained at 35–36°C. The medium was of the following composition (in mM): NaCl 126, KCl 2.5, NaH_2PO_4 1.2, $MgCl_2$ 1.3, $CaCl_2$ 2.4, $NaHCO_3$ 25, glucose 11, EDTA 0.03 and ascorbic acid 0.3.

Recording techniques

Extracellular recording Glass microelectrodes filled with 4 M NaCl and having a tip resistance of 2–4 MΩ were used to record the firing rate. The electrode signals were passed through a Grass P16 high impedance amplifier, filtered and displayed on a Tektronix 5113 oscilloscope. The spikes were gated and counted by means of a WPI 121 window discriminator coupled to an electronic ratemeter and a Watanabe WTR 311 pen-recorder. Firing rate was recorded as consecutive 30 s samples.

Intracellular recording Recordings were carried out with glass microelectrodes filled with KCl 2 M (tip resistance 60–100 MΩ) using a high impedance pre-amplifier and a bridge-circuit (Axoclamp 2 A). In some experiments LC cells were constantly hyperpolarized (about 15 mV) by injecting current through the microelectrode. In addition, hyperpolarizing current pulses of constant amplitude and 250 ms duration were delivered at a frequency of 0.5 Hz. The input resistance was calculated from the peak potential change produced. The membrane potential was determined on withdrawal of the microelectrode from the cell at the end of each experiment. Changes in the membrane potential were displayed on a Gould RS 3200 pen-recorder and in addition stored on tape (Racal Store 4).

Identification of LC neurones

The LC could be easily identified under a binocular microscope as a translucent oval area at the ventrolateral border of the fourth ventricle. LC cells spontaneously fire with a constant rate of 0.2–5 Hz. The neurones were identified on the basis of their electrophysiological properties and their sensitivity to noradrenaline (Illes & Nörenberg, 1990; Regenold & Illes, 1990).

Application of drugs and evaluation of data

Drugs were applied by changing the superfusion medium by means of three-way taps. At the constant flow rate of 2 ml min^{-1} about 30 s were required for the drug to reach the bath.

Extracellular recording A first series of experiments was designed in order to find out whether the inhibitory effect of (−)-tramadol (10 μM) reaches a steady-state within 10 min. The depression of firing rate was measured 10 and 20 min after addition of tramadol (average of two counting periods each). These effects were expressed as percentages of the average firing during the 2 min immediately before addition of (−)-tramadol (average of 4 counting periods). (−)-Tramadol (10 μM) decreased the discharge of action potentials 10 min after its application to 44.0 ± 5.4% of the pre-drug value; the effect of (−)-tramadol did not increase further during the next 10 min of incubation (41.2 ± 5.2%; $n = 7$; $P > 0.05$). A few preliminary experiments indicated that the effects of (+)-tramadol (30 μM), as well as (−)- and (+)-O-desmethyltramadol (10 μM and 0.3 μM, respectively) also reached a maximum within 10 min of superfusion. Therefore, cumulative concentration-response curves of (−)- and (+)-tramadol as well as (−)- and (+)-O-desmethyltramadol were determined by applying increasing concentrations of each drug for 10 min. The depression of firing rate was measured again at its maximum. In every experiment, the IC_{50} value, i.e. the concentration that produced 50% inhibition of the spike discharge, was graphically estimated. Only one concentration-response curve for one drug was determined on a single cell of a brain slice. Concentration-response curves for (−)-tramadol were determined also by using brain slices of rats pretreated with reserpine (5 mg kg^{-1}, i.p., 5 h before decapitation).

The interaction of the agonists [(−)- and (+)-tramadol; (−)- and (+)-O-desmethyltramadol] with naloxone or rauwolscine was tested as follows. A concentration of the respective agonist was used that inhibited the discharge of action potentials by about 80%, as estimated from the concentration-response curves. At first the agonist was applied for 10 min. Then either naloxone (0.1 μM) or rauwolscine (1 μM) were co-applied in the continuous presence of the agonist for another 10 min. Finally, both antagonists together with the agonist were present in the medium for an additional 10 min; this period was followed by washout.

Intracellular recording (−)-Tramadol (100 μM) was applied to spontaneously firing LC neurones at the resting membrane potential. When the (−)-tramadol-induced hyperpolarization did not increase further, rauwolscine (1 μM) was added in the continued presence of the agonist until a complete recovery was achieved. In analogous experiments (+)-O-desmethyltramadol (10 μM) was used as an agonist and naloxone (0.1 μM) as an antagonist. Agonists were present for about 10 min alone and then for another 10 min in the presence of the respective antagonist.

The effect of noradrenaline (30 μM) was tested before, during and after the application of (−)-tramadol (100 μM) or (+)-O-desmethyltramadol (10 μM). At first noradrenaline (30 μM) was applied for 1.5 min. Then (−)-tramadol (100 μM) or (+)-O-desmethyltramadol (10 μM) was added for 10 min without, and subsequently for 1.5 min with noradrenaline (30 μM). After a washout period, during which the firing rate and membrane potential of LC neurones recovered to their pre-drug level, noradrenaline (30 μM) was applied again for 1.5 min. In some additional experiments, LC neurones were hyperpolarized (about 15 mV) by passing constant current through the microelectrode. The effect of noradrenaline (30 μM) was tested before, during and after hyperpolarization.

The effects of (−)-tramadol (100 μM; 10 min superfusion) and noradrenaline (100 μM; 1.5 min superfusion) on the apparent input resistance of LC neurones were determined on constantly hyperpolarized non-firing neurones (for experimental details see 'Recording techniques'). The two compounds were added in random order to the same cell; between applications sufficient time was allowed for full recovery of the membrane potential and input resistance. When the response to either drug reached a steady-state, the membrane potential was temporarily restored to its pre-drug value by depolarizing current injection. The input resistance measured during this procedure (manual clamp) was compared with the input resistance determined before the

application of noradrenaline (100 μM) or (−)-tramadol (100 μM).

Materials

The following drugs were used: reserpine (Serpasil, 0.1%; Ciba-Geigy, Wehr, Germany); naloxone hydrochloride (Du Pont, Wilmington, DE, U.S.A.); (−)- and (+)-tramadol hydrochloride [(1**RS**;2**RS**)-2-[(dimethylamino)methyl]-1-(3-methoxyphenyl)-cyclohexanol hydrochloride], (−)- and (+)-O-desmethyltramadol hydrochloride (Dr W. Reimann; Grünenthal, Aachen, Germany); (−)-noradrenaline hydrochloride (Hoechst, Frankfurt am Main, Germany); rauwolscine hydrochloride (Roth, Karlsruhe, Germany).

Stock solutions (1–10 mM) of all drugs were prepared with distilled water. Further dilutions were made with medium. Equivalent quantities of the solvent had no effect.

Statistics

Arithmetic means ± s.e.mean are given throughout except in the case of IC_{50} values, when geometric means and 95% confidence limits are presented. The paired or unpaired Student's *t* test (as appropriate) was used for comparison of the means, and the paired Student's *t* test was used for comparison of the means with zero. A probability level of 0.05 or less was considered to be statistically significant.

Results

Extracellular recording

Effects of (−)-tramadol, (+)-tramadol, (−)-O-desmethyltramadol and (+)-O-desmethyltramadol on the discharge of action potentials All LC neurones included in this extracellular study fired spontaneously with an average rate of 1.00 ± 0.04 Hz ($n = 88$). (−)-Tramadol (0.1–100 μM) and (+)-tramadol (0.1–100 μM) inhibited the firing rate in a concentration-dependent manner and with similar potencies (Figure 1a). When the experiments were performed on brain slices of rats pretreated with reserpine (5 mg kg^{-1}), both 10 μM and 100 μM (−)-tramadol produced less inhibition than on brain slices of untreated rats (Figure 1a). (−)-O-desmethyltramadol (0.1–100 μM) and (+)-O-desmethyltramadol (0.01–1 μM) also depressed the discharge of action potentials in a concentration-dependent manner. The (+)-isomer had a considerably higher potency than the (−)-isomer (Figure 1b). The highest concentrations of all agonists abolished the firing. The IC_{50} values determined from the concentration-response curves were 6.0 (2.6–13.4) μM for (−)-tramadol ($n = 5$), 11.6 (5.7–23.7) μM for (+)-tramadol ($n = 8$), 2.1 (0.3–14.2) μM for (−)-O-desmethyltramadol ($n = 5$) and 0.15 (0.11–0.22) μM for (+)-O-desmethyltramadol ($n = 6$). Thus, (+)-O-desmethyltramadol had the highest potency, while all other agonists were active at a similar range of concentrations.

Interaction of tramadol with rauwolscine and naloxone Based on the concentration-response curves (Figure 1a), concentrations of (−)-tramadol (10 μM) and (+)-tramadol (30 μM) were chosen that inhibited the firing rate by about 80%. The effect of (−)-tramadol (10 μM) was not changed in the presence of naloxone (0.1 μM), but was abolished in the combined presence of naloxone (0.1 μM) and rauwolscine (1 μM) (Figure 2a, left panel) as well as in the presence of rauwolscine (1 μM) alone (Figure 2a, right panel). On the other hand, the effect of (+)-tramadol (30 μM) was greatly reduced in the presence of naloxone (0.1 μM) (Figure 2b, right panel) and abolished in the combined presence of naloxone (0.1 μM) and rauwolscine (1 μM) (Figure 2b, left and right panels). However, rauwolscine (1 μM) failed to attenuate the effect of (+)-tramadol (30 μM), when there was no naloxone (0.1 μM) in the perfusion medium (Figure 2b, left panel).

Interaction of O-desmethyltramadol with rauwolscine and naloxone The experimental schedule was the same as described above. The effect of (+)-O-desmethyltramadol (0.3 μM) was not influenced by rauwolscine (1 μM), but was abolished in the combined presence of rauwolscine (1 μM) and naloxone (0.1 μM) (Figure 3a, left panel). When naloxone (0.1 μM) was applied first, the effect of (+)-O-desmethyltramadol (0.3 μM) was nearly fully antagonized, and there was only a very slight further antagonism by rauwolscine (1 μM) (Figure 3a, right panel).

The effect of (−)-O-desmethyltramadol (10 μM) was only moderately attenuated in the presence of rauwolscine (1 μM), but disappeared in the combined presence of rauwolscine (1 μM) and naloxone (0.1 μM) (Figure 3b, left panel). On the other hand, naloxone on its own was capable of markedly diminishing the effect of (−)-O-desmethyltramadol (10 μM) (Figure 3b, right panel).

In accordance with previous results (Illes & Nörenberg, 1990) neither rauwolscine (1 μM) nor naloxone (0.1 μM) altered the firing on its own (not shown).

Intracellular recording

Effects of (−)-tramadol and (+)-O-desmethyltramadol on the membrane potential; interaction with rauwolscine and naloxone, respectively. The resting membrane potential (RMP) of the 30 LC neurones impaled by intracellular microelectrodes was −52.9 ± 1.5 mV. In 5 cells (RMP,

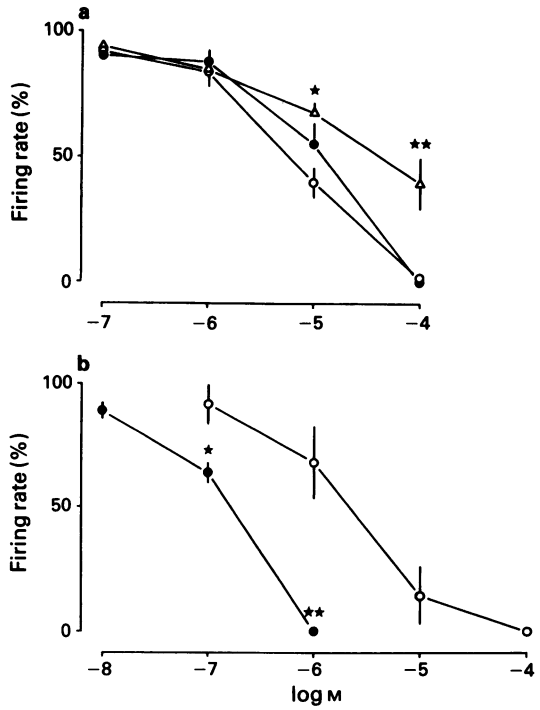

Figure 1 Inhibitory effects of (−)- and (+)-tramadol, and (−)- and (+)-O-desmethyltramadol on the firing rate of rat LC neurones. Extracellular recording. (a) Concentration-response curves of (−)-tramadol (○, $n = 5$) and (+)-tramadol (●, $n = 8$) on brain slices of untreated rats. Concentration-response curve of (−)-tramadol on brain slices of rats pretreated with reserpine (5 mg kg^{-1}) 5 h before decapitation (△, $n = 5$). Asterisks indicate significant differences from the effects of (−)-tramadol on brain slices of untreated rats (*$P < 0.05$; **$P < 0.01$). (b) Concentration-response curves of (−)-O-desmethyltramadol (○, $n = 5$) and (+)-O-desmethyltramadol (●, $n = 6$). Asterisks indicate significant differences from the effect of (−)-O-desmethyltramadol (*$P < 0.05$; **$P < 0.01$). Means ± s.e.mean from *n* slices each.

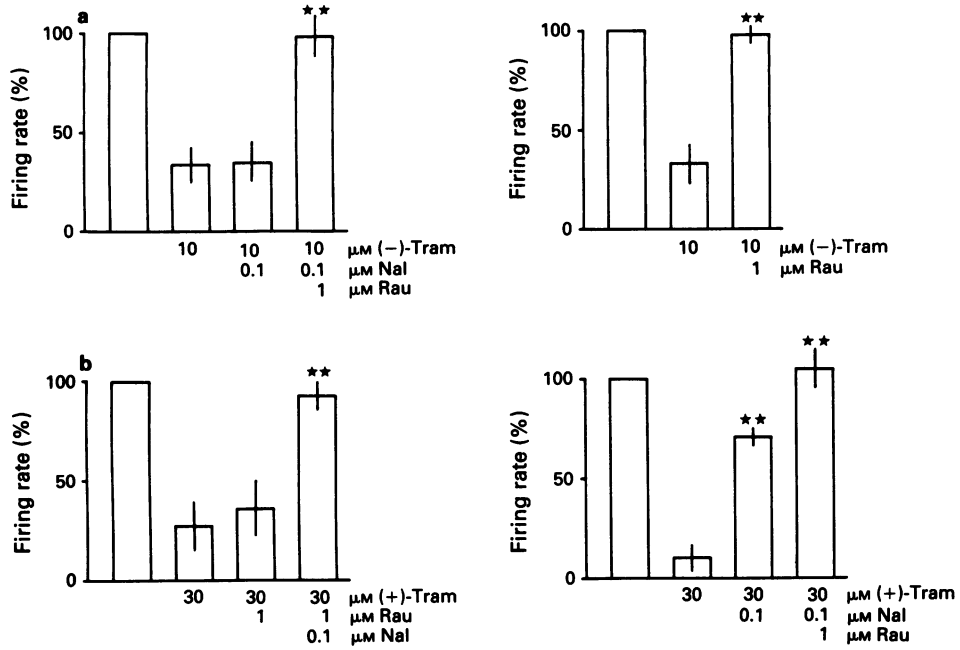

Figure 2 Inhibitory effects of (−)- and (+)-tramadol on the firing rate of LC neurones and antagonism by naloxone or rauwolscine. Extracellular recording. In this and the subsequent figure, the average firing rate during the 2 min immediately before addition of agonists was taken as 100% in each experiment (1st column). (a) Interaction between (−)-tramadol (Tram) and naloxone (Nal) or rauwolscine (Rau) (or both antagonists). Effect of naloxone (0.1 μM), and naloxone (0.1 μM) plus rauwolscine (1 μM) on the (−)-tramadol- (10 μM) induced inhibition (left panel). Asterisks indicate significant differences between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 5 slices. Effect of rauwolscine (1 μM) on the (−)-tramadol- (10 μM) induced inhibition (right panel). Asterisks indicate significant differences between the 2nd and 3rd columns (**$P<0.01$). Means ± s.e.mean from 5 slices. (b) Interaction between (+)-tramadol, and rauwolscine or naloxone (or both antagonists). Effect of rauwolscine (1 μM), and rauwolscine (1 μM) plus naloxone (0.1 μM) on the (+)-tramadol- (30 μM) induced inhibition (left panel). Asterisks indicate significant differences between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 6 slices. Effect of naloxone (0.1 μM), and naloxone (0.1 μM) plus rauwolscine (1 μM) on the (+)-tramadol- (30 μM) induced inhibition (right panel). Asterisks indicate significant differences between the 2nd and 3rd (**$P<0.01$) as well as between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 6 slices.

− 57.8 ± 1.1 mV), (−)-tramadol (100 μM) abolished the firing and caused a hyperpolarization of 12.3 ± 2.4 mV ($P<0.01$), which was abolished in the presence of rauwolscine (1 μM) (Figure 4) the membrane potential returning to its original value (RMP, − 53.5 ± 3.9 mV). Similarly, (+)-O-desmethyltramadol (10 μM) hyperpolarized another 4 cells (RMP, − 53.0 ± 3.8 mV) by 19.5 ± 1.7 mV ($P<0.01$); this effect was abolished by naloxone (0.1 μM) (RMP, − 53.2 ± 4.8 mV).

Interaction of (−)-tramadol and (+)-O-desmethyltramadol with noradrenaline Noradrenaline (30 μM) caused in 6 LC neurones a quickly developing hyperpolarization (14.2 ± 2.3 mV) (Figure 5). Subsequently applied (−)-tramadol (100 μM) evoked a hyperpolarization of comparable amplitude (12.3 ± 1.9 mV) but slower onset and offset. The effect of noradrenaline (30 μM) was more marked (19.1 ± 2.3 mV; $P<0.01$) and longer lasting in the presence of (−)-tramadol (100 μM) than in its absence. After the washout of (−)-tramadol (100 μM), the original sensitivity of LC cells to noradrenaline (30 μM) recovered (15.0 ± 2.8 mV hyperpolarization).

In another 5 neurones, (+)-O-desmethyltramadol (10 μM) produced a hyperpolarization of 14.2 ± 2.7 mV. Noradrenaline had the same effect before (9.7 ± 1.8 mV), during (7.3 ± 0.7 mV; $P>0.05$ from the preceding value) and after (8.3 ± 0.8 mV) the application of (+)-O-desmethyltramadol (10 μM). Finally, in 5 LC cells the membrane potential was hyperpolarized (about 15 mV) by passing current through the microelectrode. The effects of noradrenaline before (11.1 ± 1.4 mV), during (10.7 ± 1.3 mV; $P>0.05$ from the preceding value) and after (11.1 ± 1.0 mV) current injection did not change.

Effects of noradrenaline and (−)-tramadol on the input resistance Five LC cells were constantly hyperpolarized (about 15 mV) by injecting current through the microelectrode (Figure 6). In addition, hyperpolarizing current pulses of constant amplitude and 250 ms duration were delivered at a frequency of 0.5 Hz. The apparent input resistance of these neurones was 176.4 ± 16.9 MΩ. (−)-Tramadol (100 μM) caused a slowly developing hyperpolarization (10.8 ± 0.9 mV) and in addition decreased the input resistance by 18.4 ± 2.6% ($P<0.01$). Noradrenaline (100 μM) evoked a hyperpolarization of faster onset (13.3 ± 0.9 mV) than (−)-tramadol (100 μM), and decreased the input resistance by 34.6 ± 6.2% ($P<0.01$).

Discussion

The present experiments demonstrate that tramadol and its main metabolite, O-desmethyltramadol, depress the firing rate of rat LC neurones. This effect is stereospecific and consists of two components, one of them mediated by opioid μ-receptors and the other one by α_2-adrenoceptors. Two antagonists were used as experimental tools, namely naloxone and rauwolscine. Rauwolscine exhibits a high selectivity for α_2- over α_1-adrenoceptors (Weitzell *et al.*, 1979), while naloxone has only a slight preference for μ- over δ- and κ-opioid receptors (Illes, 1989). However, the limited selectivity of naloxone does not constitute a major problem, since LC neurones of rats are endowed with a homogeneous population of opioid μ-receptors (Williams & North, 1984). Furthermore, it is noteworthy that the only adrenoceptors present in the rat LC belong to the α_2-type (Williams *et al.*, 1985).

Noradrenaline released from dendrites or recurrent axon collaterals of LC neurones may regulate under *in vivo* conditions the spontaneous discharge of the action potentials (Aghajanian & VanderMaelen, 1982). However, under *in vitro* conditions there is probably no major α_2-adrenoceptor-mediated tonic control of neuronal activity, since rauwolscine did not increase the firing rate when given alone (Illes & Nörenberg, 1990). This may be due to the disruption of excitatory inputs to LC cells during the preparation of brain slices and the subsequent depression of the spontaneous dendritic release of noradrenaline. We suggest that (−)-tramadol induces an increase in the concentration of noradrenaline in

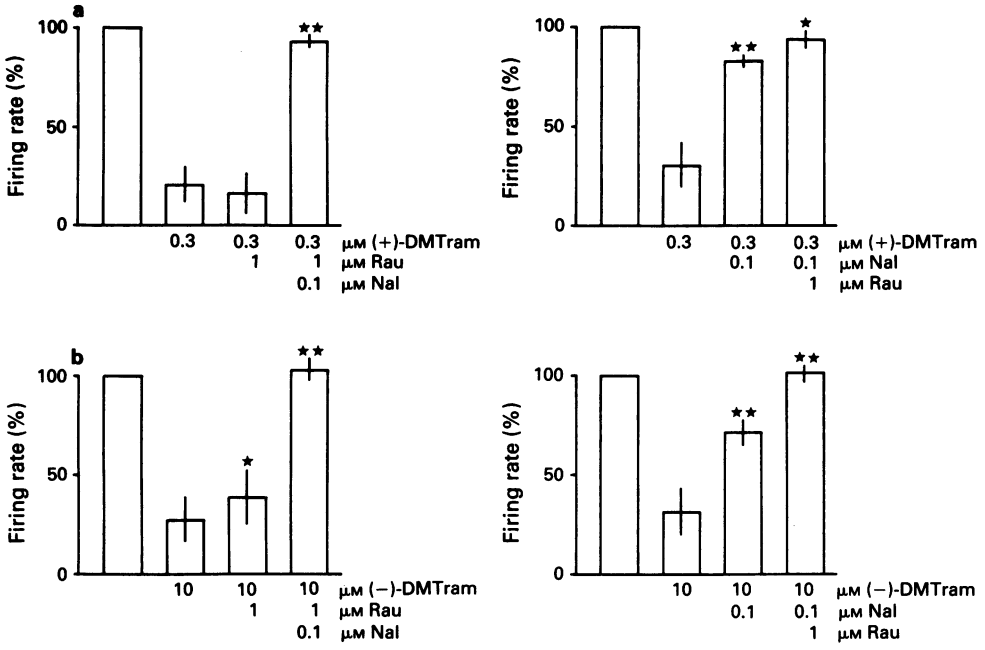

Figure 3 Inhibitory effects of (+)- and (−)-O-desmethyltramadol on the firing rate of LC neurones and antagonism by naloxone or rauwolscine. Extracellular recording. (a) Interaction between (+)-O-desmethyltramadol (DMTram) and rauwolscine (Rau) or naloxone (Nal) (or both antagonists). Effect of rauwolscine (1 μM), and rauwolscine (1 μM) plus naloxone (0.1 μM) on the (+)-O-desmethyltramadol- (0.3 μM) induced inhibition (left panel). Asterisks indicate significant differences between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 8 slices. Effect of naloxone (0.1 μM), and naloxone (0.1 μM) plus rauwolscine (1 μM) on the (+)-O-desmethyltramadol- (0.3 μM) induced inhibition (right panel). Asterisks indicate significant differences between the 2nd and 3rd columns (**$P<0.01$) as well as between the 3rd and 4th columns (*$P<0.05$). Means ± s.e.mean from 7 slices. (b) Interaction between (−)-O-desmethyltramadol, and rauwolscine or naloxone (or both antagonists). Effect of rauwolscine (1 μM), and rauwolscine (1 μM) plus naloxone (0.1 μM) on the (−)-O-desmethyltramadol- (10 μM) induced inhibition (left panel). Asterisks indicate significant differences between the 2nd and 3rd columns (*$P<0.05$) as well as between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 7 slices. Effect of naloxone (0.1 μM), and naloxone (0.1 μM) plus rauwolscine (1 μM) on the (−)-O-desmethyltramadol- (10 μM) induced inhibition (right panel). Asterisks indicate significant differences between the 2nd and 3rd (**$P<0.01$) as well as between the 3rd and 4th columns (**$P<0.01$). Means ± s.e.mean from 8 slices.

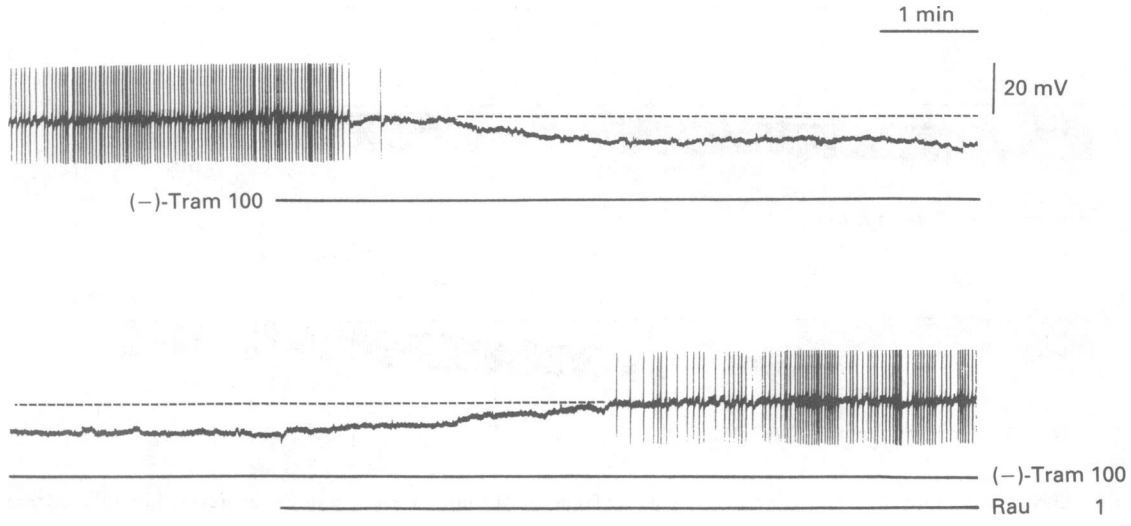

Figure 4 Effect of (−)-tramadol (Tram) on LC neurones. Intracellular recording. (−)-Tramadol (100 μM) abolished the spontaneous firing and hyperpolarized the neurone; its effect was antagonized by rauwolscine (Rau, 1 μM). The full height of action potentials was not reproduced by the pen-recorder. The resting membrane potential is indicated by the broken line. Representative experiment from 5 slices. Drugs were present for the periods indicated by the horizontal bars.

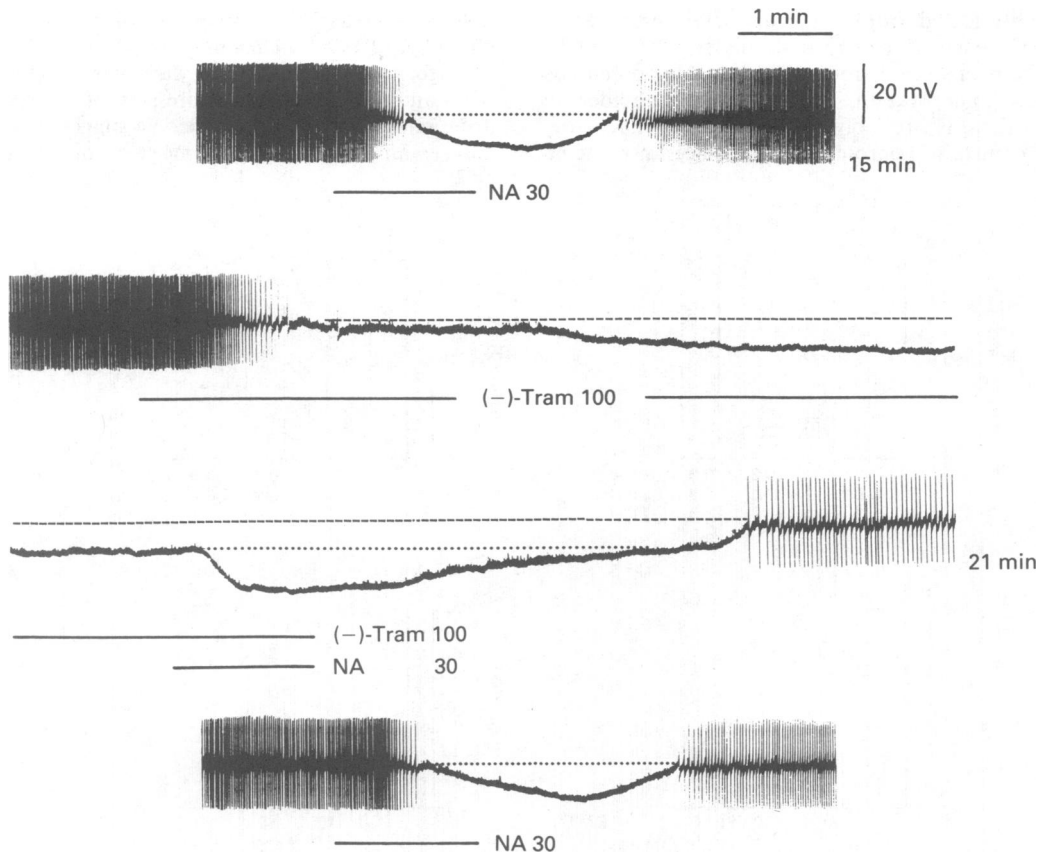

Figure 5 Potentiation of the effect of noradrenaline in LC neurones. Intracellular recording. Noradrenaline (NA, 30 μM) had a larger and longer-lasting effect during, than before or after the application of (−)-tramadol (Tram, 100 μM). The resting membrane potential is indicated by the broken line. The steady-state effect of (−)-tramadol is designated by the dotted line. Representative experiment from 6 slices. Drugs were present for the periods indicated by the horizontal bars. The intervals between the traces are shown.

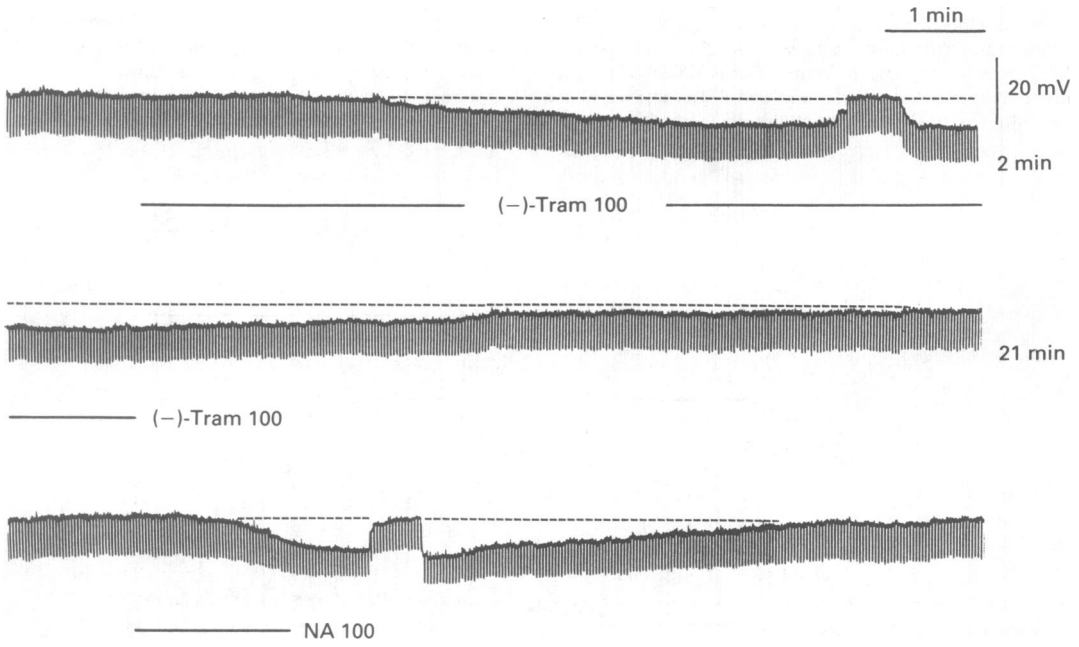

Figure 6 Effects of noradrenaline (NA) and (−)-tramadol (Tram) on the input resistance of LC neurones. Intracellular recording. The resting membrane potential of the neurone was slightly raised by continuous current injection in order to suppress spontaneous firing; this raised membrane potential is indicated by the broken line. Downward deflections represent electrotonic potentials caused by hyperpolarizing current pulses of constant amplitude. The electrotonic potentials are directly proportional to the input resistance. At the steady-states of the (−)-tramadol (100 μM) and noradrenaline (100 μM) responses the membrane potential was temporarily restored to its pre-drug value with depolarizing current. Representative experiment from 5 slices. Drugs were present for the periods indicated by the horizontal bars. The intervals between the traces are shown.

the vicinity of the α_2-adrenoceptors, and thereby inhibits the firing rate. In fact, the inhibitory potency of (−)-tramadol decreased in brain slices of rats pretreated with reserpine, a compound known to deplete noradrenaline pools. Moreover, (−)-tramadol caused a slowly developing depression of the firing, which was antagonized by rauwolscine, but not naloxone. The prototypic uptake inhibitor, desipramine, has been shown to cause a similar rauwolscine-sensitive inhibitory effect on LC neurones (Illes & Nörenberg, 1990).

Intracellular recordings in LC cells also suggest that (−)-tramadol acts by the blockade of noradrenaline uptake. Such a mode of action is favoured by three fold evidence. Firstly, the effects of (−)-tramadol on the membrane potential and firing rate were abolished by rauwolscine. Hyperpolarizations sensitive to α_2-adrenoceptor antagonists developed in LC neurones also in the presence of the uptake inhibitors, desipramine (Egan *et al.*, 1983) and cocaine (Surprenant & Williams, 1987). Secondly, the hyperpolarizing effect of noradrenaline was potentiated in the presence of (−)-tramadol. In good accordance with this finding, the uptake inhibitor cocaine was reported to facilitate the noradrenaline-induced outward current (Surprenant & Williams, 1987). Since (−)-tramadol causes hyperpolarization when given alone, we shifted the membrane potential to a similar extent by passing constant current via the microelectrode. Under these conditions the effect of noradrenaline was the same as in the absence of (−)-tramadol at the original resting membrane potential. It is unclear, why the expected slight depression of the noradrenaline-induced response after hyperpolarizing current injection was not noticed (Williams *et al.*, 1985). Thirdly, (−)-tramadol may act in LC neurones by the same mechanism as noradrenaline. Both compounds caused hyperpolarization and a reduction of input resistance, which persisted when the membrane potential was manually clamped to its pre-drug value. Input resistance changes that are secondary to hyperpolarization are thereby eliminated (Williams *et al.*, 1984). The ions involved in the (−)-tramadol effect might be potassium and not chloride as the microelectrodes were filled with KCl. When Cl^- diffuses from the microelectrode into the cells, an increased permeability of the membrane to Cl^- depolarizes rather than hyperpolarizes LC neurones (Cherubini *et al.*, 1988).

(+)-O-desmethyltramadol depressed the firing rate of LC neurones in a naloxone-reversible manner; rauwolscine had only a slight antagonistic effect. The (+)-O-desmethyltramadol-induced hyperpolarization was also abolished by naloxone. In contrast to (−)-tramadol, (+)-O-desmethyltramadol did not potentiate the effect of noradrenaline. Hence, (+)-O-desmethyltramadol seems to stimulate directly opioid μ-receptors, while the activation of α_2-adrenoceptors by (−)-tramadol is indirect. This proposal is in good agreement with the previously published high binding affinity of (±)-O-desmethyltramadol to opioid μ-sites and its correspondingly strong analgesic potency (Hennies *et al.*, 1988). In contrast, (±)-tramadol blocked the neuronal uptake of noradrenaline with a dissociation constant of approximately 1 μM, although even much higher concentrations of the compound did not bind to α_2-adrenoceptors (Raffa *et al.*, 1992). Furthermore, (±)-tramadol facilitated the electrically-evoked release of [^{3}H]-noradrenaline from rat brain cortex slices in the absence, but not in the presence of the uptake inhibitor cocaine (Driessen *et al.*,1993).

The use of naloxone and rauwolscine demonstrated that (−)-tramadol acts exclusively via α_2-adrenoceptors, whereas (+)-O-desmethyltramadol activates mostly opioid μ-receptors (see above). The effects of (+)tramadol and (−)-O-desmethyltramadol consist of both opioid and noradrenergic components. The relative contribution of these components to the effect of agonists was determined by the application of rauwolscine or naloxone, and the subsequent application of both antagonists. With this experimental schedule the noradrenergic components of both (+)-tramadol and (−)-O-desmethyltramadol appear to be smaller, when rauwolscine is applied alone, than when rauwolscine and naloxone are present together in the medium (see Figures 2b, 3b). The underestimation of the noradrenergic component may be due to the unidirectional interaction between α_2-adrenoceptors and opioid μ-receptors (Illes & Nörenberg, 1990; Illes *et al.*, 1990): blockade of α_2-adrenoceptors enhanced μ-receptor-mediated effects, while blockade of μ-receptors did not alter α_2-adrenoceptor-mediated effects. Thus, rauwolscine may, on the one hand, potentiate the opioid component, and, on the other hand, counteract the noradrenergic component of both (+)-tramadol and (−)-O-desmethyltramadol.

Although O-desmethyltramadol is the main metabolite of tramadol in most species, in man there is only a slow biotransformation, and tramadol is excreted mainly unchanged (Lintz *et al.*, 1981). Hence, the production of metabolites with strong opioid activity (i.e. O-desmethyltramadol) occurs in man only to a minor extent.

Drugs that manipulate noradrenergic activity markedly influence opioid-induced antinociception (Bläsig & Herz, 1980). The LC is a main source of the ascending and descending noradrenergic systems in the CNS (Moore & Card, 1984). Noradrenaline administered into the spinal subarachnoidal space produces powerful analgesic effects (Reddy *et al.*, 1980). Electrical or chemical stimulation of the LC also causes analgesia of spinal origin (Segal & Sandberg, 1977; Jones & Gebhart, 1988). It is difficult to reconcile these data with the finding that opioids depress the firing rate of LC neurones both *in vivo* (Bird & Kuhar, 1977) and *in vitro* (Pepper & Henderson, 1980; North & Williams, 1985). The most likely explanation is that noradrenaline plays opposite roles in the brain and spinal cord; the descending noradrenergic system facilitates analgesia, while the ascending system inhibits it (Kuraishi *et al.*, 1987). Ascending nociceptive projections from the spinal cord transmit information via collaterals to the LC (Jones, 1991). Opioids, including tramadol may interrupt the subsequent noradrenergic activation of higher brain centres necessary for the perception of pain.

Potentiation of opioid analgesia by noradrenaline uptake blockers is a well documented clinical finding (Jaffe & Martin, 1990). It has been shown that the inhibitory effect of tramadol on the uptake of noradrenaline occurs at serum concentrations which are achieved with analgesic doses in the mouse tail-flick test (Friderichs & Becker, 1991). These observations support the proposal that inhibition of the activity of LC neurones by a combined stimulation of opioid μ-receptors and α_2-adrenoceptors may be one of the mechanisms by which tramadol exerts antinociception. Of course additional transmitter systems (e.g. 5-hydroxytryptamine; Driessen & Reimann, 1992) may also be involved.

J.S. is the recipient of a research fellowship from the Alexander von Humboldt-Stiftung. We are grateful to Drs W. Reimann and W. Nörenberg for many helpful discussions and to Dr U. Trendelenburg for critically reading the manuscript. The excellent technical assistance of Mr E. Schöffel is acknowledged with thanks. This work was supported by the Deutsche Forschungsgemeinschaft (SFB 325).

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(Received January 18, 1993
Revised March 30, 1993
Accepted April 29, 1993)