Actions of tramadol, its enantiomers and principal metabolite, O-desmethyltramadol, on serotonin (5-HT) efflux and uptake in the rat dorsal raphe nucleus

T. A. BAMIGBADE, C. DAVIDSON, R. M. LANGFORD AND J. A. STAMFORD

Summary

Tramadol is an atypical centrally acting analgesic agent with relatively weak opioid receptor affinity in comparison with its antinociceptive efficacy. Evidence suggests that block of monoamine uptake may contribute to its analgesic actions. Therefore, we have examined the actions of (±)-tramadol, (+)-tramadol, (−)-tramadol and O-desmethyltramadol (M1 metabolite) on electrically evoked 5-HT efflux and uptake in the dorsal raphe nucleus (DRN) brain slice, measured by fast cyclic voltammetry. Racemic tramadol and its (+)-enantiomer (both 5 μmol litre⁻¹) significantly blocked DRN 5-HT uptake (both \( P<0.05 \)) and increased stimulated 5-HT efflux (\( P<0.01 \)) (±)-tramadol; \( P<0.05 \) (+)-tramadol). The (−)-enantiomer and metabolite, O-desmethyltramadol, were inactive at the concentration tested (5 μmol litre⁻¹). For both (±)-tramadol and the (+)-enantiomer, the action on 5-HT efflux preceded an effect on 5-HT uptake, suggesting that uptake block was not the cause of the increased 5-HT efflux and that tramadol might therefore have a direct 5-HT releasing action. This activity, at clinically relevant concentrations, may help to explain the antinociceptive efficacy of tramadol despite weak μ-opioid receptor affinity and adds to evidence that tramadol exerts actions on central monoaminergic systems that may contribute to its analgesic effect. (Br. J. Anaesth. 1997; 79: 352–356).

Key words


Tramadol is a centrally acting analgesic agent, effective in the treatment of moderate to moderately severe pain with a relatively low addiction potential. A striking feature of the pharmacology of tramadol is its relatively weak opioid receptor affinity in comparison with its antinociceptive efficacy, indicative of the involvement of other mechanisms. Interestingly, its antinociceptive action in some animal models is only partially antagonized by naloxone, strongly suggesting a non-opioid component to its analgesic potency. Furthermore, tramadol has an active metabolite, O-desmethyltramadol.

Analgesia can be achieved both centrally and peripherally by interference with a variety of neurotransmitter systems. In particular, the control of pain is subject to descending modulation by brainstem cell groups such as the locus coeruleus/subcoeruleus and raphe complex. These nuclei contain mainly noradrenaline and serotonin (5-HT), respectively.

Drugs that block noradrenaline uptake (such as desipramine) or that stimulate \( \alpha_2 \) adrenoceptors (e.g. clonidine) are useful adjuncts to standard analgesic therapy in intractable pain and are also analgesic in their own right. Tramadol has been shown to block noradrenaline uptake in cortical synaptosomes and brain slices thereby increasing noradrenaline efflux. This and the fact that \( \alpha_2 \) adrenoceptor blockade reduces its analgesic efficacy indicates that enhancement of noradrenaline function contributes significantly to its analgesic profile.

The involvement of 5-HT in the descending control of pain is also widely recognized and tramadol has been shown to enhance basal efflux of [\( ^{3} \text{H} \)]-5-HT in frontocortical synaptosomes. Tramadol also blocks 5-HT uptake and it is thus possible that the effects of tramadol on cortical 5-HT efflux are mediated via uptake blockade. However, the synaptosome is such a highly reduced system, devoid of the transmitter interactions found in vivo, that it is not easy to draw conclusions on the relationship between 5-HT efflux and uptake inhibition.

Therefore, we chose to examine the actions of tramadol on 5-HT efflux and uptake in the dorsal raphe nucleus (DRN) brain slice, for four reasons. First, the dorsal raphe is part of the brainstem raphe complex and has been shown to have a role in the descending control of pain. Second, the brain slice retains much of the local synaptic integrity and interactions of the whole animal while being easier to manipulate. Third, drug concentrations may be controlled precisely in vitro, thus allowing the elimination of pharmacokinetic considerations that...
might confound data interpretation. Finally, the consequences of metabolism to active (or inactive) metabolites in the whole animal are excluded; this is particularly pertinent for tramadol.

Materials and methods

Fast cyclic voltammetry (FCV) at carbon fibre microelectrodes was used to determine the effects of tramadol, its enantiomers and principal metabolite on electrically stimulated 5-HT efflux and uptake in rat DRN brain slices. No Home Office-regulated procedures on live animals were performed.

PREPARATION OF SLICES OF THE RAT DORSAL RAPHE NUCLEUS

Male Wistar rats (150±25 g) were stunned and killed by rapid cervical dislocation. No prior anaesthesia was administered. The brain was excised rapidly and irrigated with ice-cold (−1 to +1°C) artificial cerebrospinal fluid (aCSF). A Campden 752M vibratome was used to prepare 350-μm thick brainstem slices (+1.0 to +1.3 mm vs the interaural line12) containing the DRN (seen as a dove-shaped translucent area, ventral to the cerebral aqueduct). The brain slice was secured in a 1-ml volume superfusion-type brain slice tissue bath13 by a nylon mesh drawn over a stainless steel grid. The internal temperature of the chamber was maintained at 32°C and the slice was superfused with oxygenated (95% oxygen-5% carbon dioxide) aCSF at 1 ml min⁻¹ throughout the experiment. Artificial CSF consisted of NaCl 124 mmol litre⁻¹, NaHCO₃ 25 mmol litre⁻¹, KCl 2 mmol litre⁻¹, CaCl₂ 2 mmol litre⁻¹, MgSO₄ 2 mmol litre⁻¹ and KH₂PO₄ 1.25 mmol litre⁻¹.

MEASUREMENT OF 5-HT EFFLUX AND REUPTAKE BY FAST CYCLIC VOLTAMMETRY

A glass-coated carbon fibre (8 μm in diameter, 50 μm in length) recording electrode14 was inserted 80 μm below the surface of the slice, approximately 200 μm from a bipolar tungsten stimulating electrode (A-M Systems, Seattle, USA). Figure 1A shows the basic set-up. The auxiliary (a 0.5-mm diameter stainless steel wire) and reference (Ag/AgCl) electrodes were positioned at a convenient location in the chamber.

Quantitative real time efflux of 5-HT was measured using FCV, as previously described.15 An input voltage (1.5 cycles of a triangular waveform, −1.0 to +1.4 V vs Ag/AgCl, scan rate of 480 V s⁻¹) was applied to the potentiostat (Millar Voltammetric Analyser, PD Systems, West Molesey, UK) every 500 ms. The working electrode was disconnected between scans. The current output of the carbon fibre microelectrode was displayed on a digital storage oscilloscope (Nicolet 310DD). Background (charging) current signals before a stimulation were saved and subtracted from those obtained after a stimulus. Subtraction of one current signal from the other yielded the current caused by 5-HT oxidation and its subsequent reduction. A sample and hold circuit monitored the current at the oxidation potential for 5-HT (+570 mV vs Ag/AgCl). Its output was displayed on a chart recorder and stored on a microcomputer using CED (Cambridge Electronic Design) “Chart” software.

ELECTRICAL STIMULATION OF 5-HT RELEASE

All electrical stimulations were generated with standard Neurolog modules and applied to the stimulating electrode via an NL 800 constant current isolator. The slice was allowed to equilibrate in the chamber for at least 1 h before stimulation was conducted. 5-HT efflux was evoked by trains consisting of 20 pulses (0.1 ms duration, 10 mA constant current at 100 Hz) applied every 10 min.

On each stimulation, two variables were recorded: 5-HT efflux and uptake (see fig. 1B). 5-HT efflux was taken as the peak extracellular 5-HT concentration attained after stimulation. On cessation of stimulation, 5-HT was removed from the extracellular space by uptake. The “half-time” (T½) of 5-HT uptake (the time taken for the extracellular 5-HT concentration to decrease to half of the peak concentration attained) was used as an (reciprocal) estimate of the rate of 5-HT uptake.

EXPERIMENTAL PROCEDURE

After three stable consecutive 5-HT efflux events were obtained (a control period of 30 min), the agent to be studied (at a concentration of 5 μmol litre⁻¹) was added to the superfusate for 2 h. Five treatment groups were compared: (±)-tramadol, (+)-tramadol, (−)-tramadol, O-desmethyltramadol (M1 metabolite) and control. Controls received aCSF. Group sizes were: (±)-tramadol (n=5 slices), (+)-tramadol (n=6), (−)-tramadol (n=6), O-desmethytramadol (n=7) and control (n=5).

DRUGS

(±)-Tramadol, (+)-tramadol, (−)-tramadol and O-desmethytramadol were gifts from Grünenthal GmbH (Germany). Stock solutions of each drug were prepared in distilled water. Subsequent dilutions were made in aCSF.

STATISTICAL ANALYSIS

All drug effects on 5-HT efflux and reuptake were plotted against time. The area under the curve was calculated for each individual experiment and means (SEM) were determined for each series of experiments. The data for (±)-tramadol, (+)-tramadol, (−)-tramadol and O-desmethytramadol were tested for statistical significance compared with the control group using one-way analysis of variance (ANOVA) with post hoc application of Dunnett’s test.

Results

Electrical stimulation (20 pulses, 0.1 ms, 100 Hz) in the DRN evoked efflux of 5-HT that was detected at
an adjacent carbon fibre microelectrode. Figure 1 shows a typical rapid 5-HT efflux and reuptake profile after local stimulation in the DRN. Figure 2 shows the effects of tramadol, its enantiomers and main metabolite on stimulated 5-HT efflux. Data are expressed as mean (SEM) post-drug incremental area under the curve. (+)-Tramadol and its (+)-enantiomer significantly increased 5-HT efflux relative to aCSF controls (P<0.05 and P<0.01, respectively; Dunnett’s test). The (-)-enantiomer and O-desmethyltramadol had no significant effect compared with control.

Figure 3 shows the effects of tramadol, its enantiomers and metabolite on 5-HT uptake T₁/2. As before, data are expressed as mean (SEM) post-drug incremental area under the curve. Similarly, the racemic mixture and (+)-enantiomer significantly slowed 5-HT reuptake (both P<0.01 vs control; Dunnett’s test). O-desmethyltramadol and the (-)-enantiomer were inactive.

Figure 4 shows the time course of the effects of the active drugs, (+)-tramadol and (-)-tramadol, on stimulated 5-HT efflux and uptake T₁/2. For both (+)-tramadol and (+)-tramadol, there was a temporal dissociation between their effects on 5-HT efflux and uptake. In both cases, the drug effects on 5-HT efflux occurred before changes in 5-HT reuptake T₁/2.

Discussion

Release of neurotransmitter from isolated tissues maintained in vitro has long been a popular means of evaluating monoaminergic uptake mechanisms. However, problems with this technique have been highlighted regarding its applicability to neurotransmitter release within small anatomical regions in the brain. Furthermore, the neurotransmitter pool is frequently labelled with tritium which assumes, probably incorrectly, that release of the radiolabel accurately reflects true neurotransmitter release. We have therefore used FCV at carbon fibre microelectrodes to study the effects of racemic tramadol, its enantiomers and the major human metabolite, O-desmethyltramadol, on endogenous 5-HT neurotransmission in the DRN, a key nucleus in the regulation of afferent nociceptive transmission. An FCV scan takes only milliseconds and can be repeated many times per second, thus giving real time detection of neurotransmitter. This high
temporal resolution allows the relationship between 5-HT efflux and uptake to be determined precisely (see fig. 1).

In each case the drugs were applied at a concentration of 5 μmol litre\(^{-1}\). This approximates to the plasma concentration of tramadol after a therapeutically effective dose.\(^{18}\) Both (±)-tramadol and the (+)-enantiomer significantly enhanced stimulated 5-HT efflux in the DRN at this concentration. Neither (−)-tramadol nor the M1 metabolite, O-desmethyltramadol, had any effect (fig. 2). A qualitatively similar picture was obtained for the effects on 5-HT uptake (fig. 3). Again, both the (+)-enantiomer and the racemate were effective while the metabolite was devoid of action. Of interest, (−)-tramadol delayed 5-HT uptake in some slices although the group data were not statistically significant.

These findings are broadly consistent with the literature. Tramadol inhibited 5-HT reuptake, the (+)-enantiomer being the active form.\(^9\) The hint of an effect on uptake with (−)-tramadol also supports the findings of other studies, using synaptosomes and radiolabelled neurotransmitter, indicating that it has approximately 25% of the activity of the (+)-enantiomer on the serotonergic system.\(^9\)

De-methylation of tramadol resulted in a marked reduction of activity on 5-HT reuptake inhibition. This is interesting for two reasons. First, the M1 metabolite is an effective analgesic and probably contributes significantly to the clinical profile of tramadol,\(^3\) presumably via μ opioid receptors.\(^3\)

Second, it is clear that the O-methyl function of the parent drug is therefore a structural determinant of 5-HT uptake block by tramadol.

As only those drugs that blocked 5-HT uptake— the (+)-enantiomer and the racemate—also increased 5-HT efflux, there is a prima facie case that uptake block is the cause of increased 5-HT efflux. However, such a conclusion is erroneous as the increase in 5-HT efflux after tramadol preceded reduced uptake (fig. 4). For both (±)-tramadol and its (+)-enantiomer, the effects on 5-HT efflux were essentially maximal on the first post-drug stimulation. Effects on 5-HT uptake \(T_{1/2}\) did not reach maximum until considerably later.

Furthermore, with paroxetine, the effect on 5-HT reuptake is much greater than that on 5-HT efflux because of activation of negative feedback mechanisms reducing efflux.\(^{15}\) Conversely, the effects of (+)-tramadol on 5-HT efflux and reuptake were of a similar magnitude.

The rapid effect of racemic tramadol and its (+)-enantiomer on 5-HT efflux cannot be caused by 5-HT\(_1\) autoreceptor block as the choice of short-duration stimulation variables precludes activation of the autoreceptor during the stimulation train.\(^{19}\) Furthermore, we have shown previously that the potent 5-HT\(_{1A}\) antagonist WAY 100135 did not increase 5-HT efflux in this experimental procedure.\(^{15}\)

Hence it appears that tramadol may have a direct releasing action. Other studies have shown that the tramadol-induced increase in 5-HT efflux does not occur if 5-HT reuptake sites are blocked by 6-nitroquipazine, before tramadol administration.\(^9\)
This suggests that tramadol can compete with 5-HT for the uptake mechanism and, when inside the presynaptic terminal, increase stimulation-evoked 5-HT release. A direct effect on 5-HT release, dissociated from reuptake inhibition, may be important to the multimodal analgesic actions of tramadol. It is unlikely that opioid receptor activation is responsible for the 5-HT releasing action observed with (+)-tramadol and the racemate as the M1 metabolite had no 5-HT releasing action despite being a more potent opioid than the parent drug.3

In conclusion, both racemic tramadol and its (+)-enantiomer blocked DRN 5-HT uptake and increased stimulated 5-HT efflux. The temporal dissociation between these actions suggests that uptake blockade was not the cause of the increased 5-HT efflux and that tramadol might therefore have a direct 5-HT releasing action. It should nevertheless be remembered that we have only examined a single, albeit clinically relevant, concentration of tramadol and it would be interesting to examine such actions over an extended concentration range. Nevertheless, the activity of such mechanisms at clinically pertinent concentrations may help explain the antinociceptive efficacy of tramadol despite weak µ opioid receptor affinity. This study adds to evidence that tramadol exerts actions on central monoaminergic systems and that this mechanism may contribute to its analgesic effect.

Acknowledgements

We thank Grüenthal GmbH (Germany) for gifts of (±)-tramadol, (+)-tramadol, (−)-tramadol and O-desmethyltramadol. This research was supported, in part, by Searle. We thank Paul Phillips for graphical assistance.

References
