Contribution of monoaminergic modulation to the analgesic effect of tramadol

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Abstract

1. In humans, the central analgesic effect of tramadol 100 mg orally is only partially reversed by the opioid antagonist naloxone (0.8 mg intravenously). As suggested by in vitro and animal data tramadol analgesia may thus result from an action on opioid as well as monoaminergic pathways. We therefore investigated the effect of alpha 2-adrenoceptor antagonism with yohimbine on tramadol analgesia. 2. Healthy volunteers (n = 10) received tramadol (100 mg orally), followed (+3 h) by yohimbine (0.1 mg kg-1 intravenously), and yohimbine + naloxone (0.8 mg intravenously) and their respective placebo according to a randomized, double-blind crossover, placebo (P) controlled design. Analgesia was assessed over 8 h by subjective pain threshold (pain intensity numerical scale--PINS) and objective pain threshold (RIII nociceptive reflex--RIII) monitoring. 3. Tramadol induced a significant increase in both pain thresholds. Peak analgesic effect was observed at 3.7 h (RIII + 39.6 +/- 3.9% and PINS 50.1 +/- s.e.mean 5%) and the analgesia lasted about 6 h. 4. Yohimbine significantly reversed the analgesic effects of tramadol for 2.8 h [...]
Contribution of monoaminergic modulation to the analgesic effect of tramadol

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1 In humans, the central analgesic effect of tramadol 100 mg orally is only partially reversed by the opioid antagonist naloxone (0.8 mg intravenously). As suggested by in vitro and animal data tramadol analgesia may thus result from an action on opioid as well as monoaminergic pathways. We therefore investigated the effect of $\alpha_2$-adrenoceptor antagonism with yohimbine on tramadol analgesia.

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3 Tramadol induced a significant increase in both pain thresholds. Peak analgesic effect was observed at 3.7 h (RIII +39.6±3.9% and PINS 50.1±s.e.mean 5%) and the analgesia lasted about 6 h.

4 Yohimbine significantly reversed the analgesic effects of tramadol for 2.8 h with a maximum decrease of 97±4% (RIII) and 67±12% (PINS), whereas the addition of naloxone abolished tramadol effects throughout the study period with a decrease of 90±6% (RIII) and 79±9% (PINS), $P<0.05$.

5 Yohimbine alone did not significantly reduce pain thresholds.

6 $\alpha_2$-Adrenoceptor antagonism reverses tramadol effects, thus pointing to the significant role of monoaminergic modulation and the synergy with opioid agonism in tramadol antinociception after a single oral dose.

**Keywords** tramadol yohimbine opioids $\alpha_2$-adrenoceptor antagonist RIII reflex pain measurement pain threshold analgesics

**Introduction**

Tramadol hydrochloride, (1RS,2RS)-2-((dimethylamino)methyl)-1-(30-methoxyphenyl)cyclohexanol hydrochloride, is a central analgesic with low affinity for opioid receptors [1]. Tramadol analgesia following a single 100 mg oral dose, is only partially inhibited in humans by the selective $\mu$ opioid antagonist naloxone [2]. Besides the known opioid involvement in modulation of pain, the existence of a supraspinal monoaminergic neuronal system that modulates nociceptive processes in the spinal cord has been extensively documented [3]. Experimental data suggest that tramadol may exert part of its analgesic effect through activation of this central inhibitory monoaminergic pathway [4]. Tramadol has also been shown to exert some activity on the monoaminergic system since it is known to block noradrenaline and serotonin reuptake in rat synaptosomes, and its analgesic activity has been partially prevented or blocked by $\alpha_2$-adrenoceptor antagonists in behavioural experimental models [4-7]. In order to investigate the role of the monoaminergic system in tramadol antinociceptive effect in human at the recommended dose we used $\alpha_2$-adrenoceptor antagonism with yohimbine which is selective at low dosage and which easily crosses the blood–brain barrier [8, 9]. In addition naloxone was administered to measure the magnitude of opioid system participation in the antinociceptive effect of a single oral dose of tramadol.

This work was presented in part at the 95th Annual...

Methods

Protocol

The experimental protocol was approved by the institutional ethics review board (Department of Medicine, University of Geneva) and conducted in accordance with the Good Clinical Practice Guidelines [10]. Ten healthy male volunteers (medical staff and students) with a mean age of 25.6 year (s.d. 4.5), a mean body weight of 70.9 kg (s.d. 11) and a mean height of 177 cm (s.d. 9.7), participated in the study. No subject had clinically abnormal findings on history or physical examination and none had taken any drugs within the past month. At least 1 week prior to the study they were phenotyped as extensive or poor metabolizers for the debrisoquine-type polymorphism by means of dextrometorphan/O-demethylated dextrometorphan metabolic ratio determination [11].

Medications

Each volunteer received according to a randomized double-blind, crossover placebo controlled design, on five occasions (at least 72 h apart), two 50 mg capsules of tramadol orally (Tramal®, Grünenthal Gmbh, Stolberg Germany) or placebo followed 3 h later either by placebo injections or by yohimbine 0.1 mg kg\(^{-1}\) plus placebo or yohimbine plus naloxone (Narcan®, DuPont de Nemours) 0.8 mg intravenously.

Pain threshold determination

The pain threshold was assessed by means of a validated technique measuring both subjective pain threshold (pain intensity numerical scale = PINS) and objective antinociception effect (RIII thresholds) for 8 h after tramadol intake [12–17]. Briefly, subjects lie comfortably in order to obtain muscular relaxation. A standardized meal and glucose perfusion (1000 ml glucose 5%/h) was administered [15]. The sural nerve was stimulated with cutaneous electrodes applied on the degreased skin in its retromalleolar pathway. The electrical stimulus consisted of single rectangular impulses (0.5 ms) delivered by a constant current stimulator at variable intensity (1–100 mA) (Neuroport, Biotron, Miehlen, Germany). Electromyographic responses were recorded using a pair of surface electrodes placed over the tendon of the ipsilateral biceps femoris. The RIII reflex (objective threshold) was identified as a multiphasic signal appearing between 90 and 250 ms after each stimulation and considered present when the corrected computed surface was \(>0.5 \text{mV}\cdot\text{ms}\). Cutaneous temperature was maintained constant by using thermal insulation. The subjective sensation was described by means of a 0–10 numerical scale. Subjective and objective pain thresholds are defined as the intensity of current inducing 50% of positive responses to a series of 40 stimulations and were obtained by fitting the percentage of positive responses to the Hill's equation. Subjective and objective pain thresholds were measured for 8 h after drug intake (\(t=0; 0.5; 1; 2; 3; 3.25; 3.5; 4; 5; 6; 7; 8\) h).

Data analysis

Differences between treatments were analysed by one factor repeated measures analysis of variance (ANOVA) followed by multiple comparisons of the means (Scheffe's test analysis). Values of peak effect (mA) and time to peak (h) were determined directly from the data and expressed as the maximal increment between the control threshold and the peak value for tramadol treatment and as the maximal diminution between \(t=3\) h values and the lowest values when antagonists were used (yohimbine and naloxone). The percent decrease of pain thresholds was determined from values obtained just before the antagonism's reversal.

Results

Ten healthy male volunteers completed the study. All were extensive metabolizers for the debrisoquine-type polymorphism. One subject withdrew during the fifth session because of side effects (nausea, drowsiness). His results were included with the exception of the interrupted session (tramadol with yohimbine and naloxone).

The pre-treatment pain thresholds were not significantly different (Student's paired t-test) on each separate occasion (Table 1). Mean measures during the placebo session \((n=13)\) on the same individual revealed a low intrasubject variability for both pain threshold measurements as reflected by the small s.d. (Table 2). There was a close individual correlation between objective and subjective pain threshold measurements. The mean intra-individual correlation is \(r = 0.66\) (s.d. \pm 0.18).

Table 1 Mean pre-treatment RIII and PINS thresholds values for each treatment session expressed in absolute mA (s.d.). Thresholds were not significantly different (Student's paired t-test)

<table>
<thead>
<tr>
<th>Pre-treatment values (s.d.)</th>
<th>RIII (mA)</th>
<th>PINS (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (P)</td>
<td>25.6 (6.3)</td>
<td>22.6 (4.4)</td>
</tr>
<tr>
<td>Tramadol (T)</td>
<td>26.8 (8.7)</td>
<td>23.6 (6.4)</td>
</tr>
<tr>
<td>T + Yohimbine (Y)</td>
<td>26.2 (6.4)</td>
<td>24.1 (7.4)</td>
</tr>
<tr>
<td>T + Y + Naloxone</td>
<td>22.6 (6.8)</td>
<td>23.4 (3.4)</td>
</tr>
<tr>
<td>P + Y</td>
<td>26.0 (8.5)</td>
<td>24.0 (5.1)</td>
</tr>
</tbody>
</table>

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Table 2  Individual RIII and PINS thresholds measures (n=13) during the placebo session expressed in absolute mA (+s.d.)

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIII</td>
<td>24.5 (2.3)</td>
<td>27.4 (5.1)</td>
<td>22.6 (2.3)</td>
<td>19.2 (2.8)</td>
<td>27.9 (1.9)</td>
<td>33.2 (5.7)</td>
<td>20.3 (2.0)</td>
<td>22.3 (2.8)</td>
<td>20.5 (1.5)</td>
<td>29.6 (2.3)</td>
</tr>
<tr>
<td>PINS</td>
<td>36.0 (4.0)</td>
<td>20.0 (4.0)</td>
<td>22.5 (2.3)</td>
<td>24.1 (4.2)</td>
<td>16.1 (3.7)</td>
<td>24.5 (3.9)</td>
<td>26.8 (2.8)</td>
<td>29.0 (5.0)</td>
<td>18.5 (2.1)</td>
<td>22.1 (1.9)</td>
</tr>
</tbody>
</table>

The time course of objective and subjective pain threshold measurements in the 10 extensive metabolizers after tramadol intake and after subtraction of the values at time 0 is presented in Figures 1–4. Selective antagonism by yohimbine alone (Figures 1 and 2) and yohimbine plus naloxone (Figures 3 and 4) is also depicted.

After oral administration the three treatments including tramadol were significantly different from placebo, or placebo + yohimbine for RIII and PINS (P<0.001). Tramadol induces a significant and rapid antinociceptive effect with a 50% and 40% increase of PINS and RIII pain thresholds respectively (Table 3). Time of peak antinociceptive effect was 3.8 and 3.7 h for PINS and RIII after tramadol intake (Table 3). The antinociceptive effect of tramadol was almost completely antagonized (PINS = -68% and RIII = -97%) by yohimbine with a mean duration of 2.75 h for PINS and RIII respectively (P<0.05) (Table 3; Figures 1 and 2). Although not statistically different from yohimbine alone, the addition of naloxone significantly and almost completely (PINS = -79% and RIII = -90%) abolished tramadol antinociceptive effect on PINS and RIII (P<0.05) (Table 2; Figures 3 and 4).

The placebo session with yohimbine alone induced a non-significant decrease of nociceptive thresholds in

Figure 1  Time course of mean PINS thresholds (n=10) expressed in mA changes from pretreatment values (±s.e.mean) after tramadol intake (○), tramadol + yohimbine (●), placebo + yohimbine (□). Arrow represents the time of antagonism administration.

Figure 2  Time course of mean RIII thresholds (n=10) expressed in mA changes from pretreatment values (±s.e.mean) after tramadol intake (○), tramadol + yohimbine (●), placebo + yohimbine (□).
Discussion

In humans, Collart et al. [2] have shown that naloxone produced only a partial decrease of tramadol's antinociceptive effect on pain thresholds (circa 30%). The study's main objective was to evaluate the impact of monoaminergic system modulation on tramadol analgesia since experimental animal data suggested that part of tramadol's analgesic effect is mediated through monoaminergic activation [1, 4–6]. Yohimbine, at 0.1 mg kg\(^{-1}\) i.v. maintains its selectivity as an p1-adrenoceptor antagonist [8]. At this dosage it almost totally reversed the subjective (67%) and the objective (96%) antinociceptive effect of 100 mg tramadol for more than 2 h. This is strong but indirect evidence for the role of p2-adrenoceptors in tramadol's analgesic effect at therapeutic dose. This observation is also in agreement with in vitro and animal data suggesting that tramadol's analgesic effect may be mediated, at least in part as activation of the central monoaminergic inhibitory system [1, 2, 4–6]. Numerous studies have shown that p2-adrenoceptor agonists mediate a pronounced and selective antinociception on their own at the spinal, diencephalic periventricular region, dorsal raphe nuclei, and periaqueductal grey matter [15, 18–22]. However, tramadol has never been shown to bind to p2-adrenoceptors [1]. Thus it probably acts through indirect activation of post-synaptic p2-adrenoceptors. In fact, tramadol is known to increase monoaminergic content in rat synaptosomes [5, 6]. Anatomical, electrophysiological and biochemical evidence demonstrates that noradrenaline as well as serotonin modulates nociception in the superficial layer of the dorsal horn of the spinal cord [23, 24]. Iontophoretic application of noradrenaline inhibits the spontaneous activity of dorsal horn neurons and the excitation of these neurons by noxious stimuli [25]. Furthermore evidence suggests that noradrenaline acts at the post-synaptic p2-adrenoceptor level, since clonidine inhibits these neurons, an effect which is selectively blocked by yohimbine but not by the p1-adrenoceptor antagonist phenotamine [24, 25]. Our results are in good agreement with these observations. They suggest that tramadol could increase the monoaminergic content in the synaptic cleft and that yohimbine blocks post-synaptic p2-adrenoceptors on dorsal horn neurons, thus explaining the reversal of the antinociceptive effect of tramadol in humans as well.

It has been argued that the monoaminergic system closely interacts with the opioid system [25–29]. Moreover the synergy between antinociception effects of monoamines and opioids is well known [26]. The addition of naloxone 0.8 mg intravenously, a dose which selectively reverses \(\mu\) opioid agonist activity, prolonged the antagonism induced by yohimbine but its effect did not reach significance. Tramadol has been shown to exhibit only low affinity for opioid receptors and like morphine, binds more selectively to \(\mu\) receptors than to \(\kappa\) or \(\delta\) opioid receptors [1]. Tramadol undergoes liver biotransformation to several metabolites and one of them, O-demethyl-tramadol (M1), is an active moiety

Table 3

<table>
<thead>
<tr>
<th>PINS threshold (s.e.mean)</th>
<th>Peak effect (mA)</th>
<th>Peak effect (%)</th>
<th>t peak (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tramadol (T)</td>
<td>12.3 (2)</td>
<td>50.12 (5.0)</td>
<td>3.7 (0.4)</td>
</tr>
<tr>
<td>Placebo + Y</td>
<td>-3.2 (1.1)</td>
<td>-66.70 (12.4)</td>
<td>0.9 (0.3)</td>
</tr>
<tr>
<td>T + Yohimbine (Y)</td>
<td>-8.1 (1.6)</td>
<td>-78.56 (9.4)</td>
<td>0.8 (0.2)</td>
</tr>
<tr>
<td>T + Yohimbine + Naloxone (Nx)</td>
<td>-6.5 (1.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIII threshold</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>14.0 (1.9)</td>
<td>39.57 (3.9)</td>
<td>3.8 (0.4)</td>
</tr>
<tr>
<td>P + Y</td>
<td>-4.4 (0.7)</td>
<td>-96.50 (3.5)</td>
<td>0.4 (0.1)</td>
</tr>
<tr>
<td>T + Y</td>
<td>-12.8 (2.8)</td>
<td>-90.22 (6.0)</td>
<td>0.6 (0.2)</td>
</tr>
<tr>
<td>T + Y + Nx</td>
<td>-9.6 (2.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
that has higher affinity for opioid μ receptors than tramadol itself [1, 7]. M1 production is controlled by the polymorphic cytochrome CYP2D6 [2], which is also responsible for the bioactivation of codeine to morphine [30]. However, in humans, after a single dose evaluation, it appears that poor metabolizers of debrisoquine type (CYP2D6) present an analgesia equivalent to that of extensive metabolizers [2]. All volunteers had extensive metabolizer phenotypes and consequently this genetic polymorphism could not account for the weak antagonism of naloxone observed in our study. Furthermore a single dose study showed that opioid system activation offers only a minor contribution to tramadol’s analgesia after a 100 mg dose, since naloxone 0.8 mg reverses the tramadol analgesic effect by less than 30% and only for less than 1 h [2]. However, the involvement of the opioid system in tramadol’s analgesic effect could increase at higher doses and after repeated administration.

Three caveats must be examined with regard to yohimbine antagonism on tramadol’s analgesic effect. Yohimbine is an indole alkaloid and therefore shares a functional group with serotonin, the indolamine. Experimental data have shown that yohimbine loses its selectivity acquiring antiserootoninergic, monoamine oxdase inhibitor or α2-adrenoceptor antagonist properties when dosages exceeded >2 mg kg−1; in our study 0.1 mg kg−1 was therefore employed in order to keep its selectivity [8]. The second caveat is that part of our argument depends on the absence of any change in morphine induced analgesia when yohimbine is administered. To the best of our knowledge there are no human data on the impact of yohimbine on morphine analgesia. However, a substantial number of investigations have addressed this topic with confounding results [6, 8, 31, 32]. Several studies have demonstrated either antagonism or enhancement of morphine analgesia whereas others have found no significant effect of adrenergic antagonists [8, 31]. Among the possible explanations for such discrepancies, species, behavioural test, site of administration and dosage seem critical. For instance, at 2 mg kg−1 i.p. (a dose at which yohimbine loses its selectivity) the adrenergic antagonism reduces the acute effect of high dose morphine (5 mg kg−1 i.v.) in the rat tail flick test [32]. In sharp contrast, recent observations in the same model showed that lower systemic yohimbine doses (1 mg kg−1) reversed intrathecal tramadol analgesia without affecting the effect of morphine [6]. Therefore it seems unlikely that 0.1 mg kg−1 of yohimbine, as used in our study, could influence the effect of an equipotent dose of morphine.

The third caveat emerged from the fact that whatever the treatment was, the maximum impact of yohimbine on subjective pain thresholds was uniformly less pronounced when compared with the decrease of RIII reflex. These differences apparently do not reflect the time to reach the effector site in the CNS since kinetic studies in healthy volunteers showed an extremely rapid and extensive tissue distribution (>90%), a high clearance and a very short half-life (0.4–18 min) after yohimbine intravenous administration [9]. Another explanation could be an effect of yohimbine on ventral horn motoneurons. In fact, many functional studies performed on the spinal effects of adrenergic agents have dealt with measures of reflex output, and since there are as many catecholaminergic binding sites in the ventral as in the dorsal horn, an effect at this level cannot be ruled out [24]. Some other indirect experimental evidence currently exist for such an occurrence [9, 23, 24, 33].

Taken together these observations suggest that yohimbine could act directly on presynaptic α2-adrenoceptors increasing noradrenaline content in the synaptic cleft of the ventral horn neurons. This could explain why a more rapid and intense maximum impact of yohimbine was observed on motoneurons of the nociceptive flexion reflex effector pathway than on subjective responses. Whatever the explanation, the magnitude of this effect is apparently not substantial since administration of yohimbine alone did not statistically differ from placebo baseline values. Our study was not designed to appraise a possible interaction between yohimbine and the nociceptive system. Thus our data attenuate, but certainly do not reject, the hypothesis of a putative tonic physiological monoaminergic control of pain nociceptive pathway which deserves further investigation. Finally the present study confirms the reproducibility and the close correlation of the objective RIII late polysynaptic reflex with subjective pain threshold measurement.

Conclusion

The central analgesic effect produced by a 100 mg oral dose of tramadol is significantly and transiently inhibited by yohimbine. This observation strongly supports that tramadol is not a classical weak opioid but that its analgesic effect might also be mediated through activation of the monoaminergic system probably through an indirect activation of post-synaptic α2-adrenoceptors in the central nervous system. The addition of naloxone at a dose which antagonizes tramadol action at the μ opioid receptor subtype, reinforces the inhibitory effect of yohimbine. These observations bring additional support to the close interactions between monoaminergic and opioidergic systems in pain modulation.

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References


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