Themed Section: Opioids: New Pathways to Functional Selectivity

RESEARCH PAPER

µ-Opioid receptor activation and noradrenaline transport inhibition by tapentadol in rat single locus coeruleus neurons

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BACKGROUND AND PURPOSE

Tapentadol is a novel analgesic that combines moderate µ-opioid receptor agonism and noradrenaline reuptake inhibition in a single molecule. Both mechanisms of action are involved in producing analgesia; however, the potency and efficacy of tapentadol in individual neurons has not been characterized.

EXPERIMENTAL APPROACH

Whole-cell patch-clamp recordings of G-protein-coupled inwardly rectifying K$^+$ (K$_{IR}$3.x) currents were made from rat locus coeruleus neurons in brain slices to investigate the potency and relative efficacy of tapentadol and compare its intrinsic activity with other clinically used opioids.

KEY RESULTS

Tapentadol showed agonist activity at µ receptors and was approximately six times less potent than morphine with respect to K$_{IR}$3.x current modulation. The intrinsic activity of tapentadol was lower than [Met]enkephalin, morphine and oxycodone, but higher than buprenorphine and pentazocine. Tapentadol inhibited the noradrenaline transporter (NAT) with potency similar to that at µ receptors. The interaction between these two mechanisms of action was additive in individual LC neurons.

CONCLUSIONS AND IMPLICATIONS

Tapentadol displays similar potency for both µ receptor activation and NAT inhibition in functioning neurons. The intrinsic activity of tapentadol at the µ receptor lies between that of buprenorphine and oxycodone, potentially explaining the favourable profile of side effects, related to µ receptors.

LINKED ARTICLES

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Abbreviations

ACSF, artificial cerebrospinal fluid; K$_{IR}$3.x, G-protein-coupled, inwardly rectifying K$^+$ channels; LC, locus coeruleus; NAT, noradrenaline reuptake transporter

Introduction

Opioid analgesics are the mainstay of drug therapy for management of acute and chronic severe pain but their utility is limited by on-target side effects, as well as development of tolerance and dependence (Christie, 2008; Williams et al., 2013). One approach to limit side effects, tolerance and dependence clinically is to combine a µ-opioid receptor

agonist (nomenclature follows Alexander *et al.*, 2013a) and another analgesic drug with a different mode of action, which can enhance overall analgesic actions and produce opioid-sparing effects (Tzschentke *et al.*, 2007; Schröder *et al.*, 2011).

Tapentadol is a centrally acting analgesic that combines μ receptor agonism with a similar potency for noradrenaline transporter (NAT) inhibition in a single molecule (Tzschentke *et al.*, 2007). The rationale behind this particular combination effect is based on findings that both opioid and noradrenergic pathways contribute to production of analgesia in different ways, and can synergistically interact at spinal and supraspinal levels (Ossipov *et al.*, 1982; Fairbanks and Wilcox, 1999). Although tapentadol exhibits approximately 50-fold lower binding affinity for μ receptors than morphine, its analgesic potency is comparable with morphine for acute and chronic pain in animal models (Tzschentke *et al.*, 2007; Schiene *et al.*, 2011), and it has been widely shown that tapentadol has a favourable tolerability in humans, particularly with respect to gastrointestinal side effects (Hartrick *et al.*, 2009; Tzschentke *et al.*, 2009; Afilalo *et al.*, 2010; Lange *et al.*, 2010; Pergolizzi *et al.*, 2012).

The mechanisms of action of tapentadol have been studied *in vitro* (Tzschentke *et al.*, 2007), as well as in neurochemical and behavioural studies in animals *in vivo* (Schröder *et al.*, 2010; 2011; Bee *et al.*, 2011; Schiene *et al.*, 2011; Tzschentke *et al.*, 2012), but little is known about the mechanism of action in individual neurons. The locus coeruleus (LC) is the major source of noradrenergic innervation of the brain and spinal cord (Foote *et al.*, 1983). LC neurons express both μ receptors and α_2-adrenoceptors that converge via G-protein βγ-subunits to activate a single population of inwardly rectifying K^+ ($K_{IR}3.x$) channels (North and Williams, 1985; nomenclature follows Alexander *et al.*, 2013b). The LC also expresses a high density of NAT (Schroeter *et al.*, 2000). As such, the LC is also a useful model to study inhibition of NAT by assaying enhancement of actions of α_2-adrenoceptors on $K_{IR}3.x$ channels (Surprenant and Williams, 1987).

A previous study of electrical activity of LC neurons *in vivo* (Torres-Sanchez *et al.*, 2013) reported that systemic administration of tapentadol inhibits spontaneous action potential activity. The authors suggested that the actions of tapentadol on these neurons are mediated predominantly by activation of α_2-adrenoceptors but the direct membrane actions and relative potencies for μ receptors and NAT could not be determined using this experimental approach. The aim of the present study was to investigate the relative potency and efficacy of tapentadol in functioning LC neurons using patch-clamp recording in brain slices. The results establish that tapentadol is an agonist at μ receptors with lower intrinsic activity than morphine and oxycodone, but greater than that of buprenorphine. Tapentadol has similar potency in single LC neurons as an activator of μ receptors and inhibitor of NAT. At the cellular level, the interaction between these mechanisms on activation of $K_{IR}3.x$ channels is additive.

Methods

Preparation of brain slices

The Animals Ethics Committee of the University of Sydney, Sydney, NSW, Australia, which complies with the National Health and Medical Research Council 'Australian code of practice for the care and use of animals for scientific purposes', approved all experiments. All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). A total of N = 114 animals were used in the experiments described here.

Male Sprague Dawley rats (3 to 5 weeks old, *n* = 114) were used in this study. Animals were housed in groups of two to four under a 12 h/12 h light–dark cycle at 22 ± 2°C with environmental enrichment and free access to food and water. LC slices were prepared as described previously (Dang *et al.*, 2011; 2012). Briefly, rats were anaesthetized with isoflurane (4% in air), decapitated and the brains rapidly removed and blocked. Horizontal brain slices (260–300 μm thick) containing LC were obtained from the pons, using a vibratome (Leica biosystem, VT100, Wetzlar, Germany) in cooled artificial cerebrospinal fluid (ACSF) containing the following (in mM): 126 NaCl, 2.5 KCl, 2.4 CaCl$_2$, 1.2 MgCl$_2$, 1.2 NaH$_2$PO4, 21.4 NaHCO$_3$, 11.1 glucose, 95%O$_2$-5%CO$_2$. Slices were incubated in warm (37°C) oxygenated ACSF for at least 30 min before recording.

Electrophysiological recordings

Whole-cell voltage-clamp recordings of visualized LC neurons (infrared Dopt optics, Multiclamp 700B amplifier from Molecular Devices, CA, USA) were acquired at holding potential of –60 mV using Axograph X (Axograph Scientific, Sydney, Australia) as previously described (Dang *et al.*, 2012). Data were sampled at 10 kHz and filtered at 20–50 Hz. Series resistance (≤15 MΩ) was compensated by 75% and monitored throughout experiments. Patch pipettes with resistance of 2–4 MΩ were filled with a solution containing the following (in mM): 115 K MES (2-[morpholino]- ethane-sulfonic acid), 20 NaCl, 1.5 MgCl$_2$, 10 BAPTA, 5 HEPES, 4 Mg- ATP and 0.4 Na-GTP and pH 7.3–7.4 was adjusted using KOH. All drugs were applied by superfusion of the recording chamber (~2 mL min^{-1}, 35°C). Bestatin (10 μM) and thiorphan (1 μM) were included in all experiments using [Met]enkephalin to limit degradation of the peptide. In all experiments involving application of noradrenaline, prazosin (1 μM) was included for at least 5 min before superfusing noradrenaline to prevent potential effects of α_1-adrenoceptor activation on $K_{IR}3.x$ channels in these neurons (Osborne *et al.*, 2002). Solutions of noradrenaline were freshly prepared when reapplication time exceeded 5 min to avoid oxidative decomposition. A single concentration of tapentadol was applied per slice.

Data analyses

Data were analysed using Graph-Pad Prism (Graph-Pad V5, San Diego, CA, USA), except for isobolographic analysis. All data are presented as mean ± SEM. Significant differences were analysed by Student's *t*-test (paired or unpaired two-tailed Student's *t*-tests) or, where appropriate, one-way ANOVA followed by Dunnett's test, or two-way ANOVA followed by Bonferroni post-test. Concentration-response curves were calculated for each group and fitted to a logistic equation using Graph-Pad Prism, with minimal constrained to zero. Isobolographic analysis was performed using a modification of the method of Tallarida (2007). By this method, additivity is based on the concept of concentration equivalence for drug A

and B (or dual mechanisms) follows from individual concentration-effect curves (Grabovsky and Tallarida, 2004), that is a concentration α of drug A is equal to a concentration of drug B denoted b_{eq} (α). So combinations of these two concentrations (a,b) would be b_{eq} (α) + b. The theoretical additive EC_{50} value was compared with the observed EC_{50} for the combination by *t*-test using FlashCalc 4.5.3 (Dr Michael Ossipov, University of Arizona, Tucson, AZ, USA).

Materials

Stock solutions of tapentadol HCl supplied by Grünenthal GmbH (Aachen, Germany), [Met[5]]-enkephalin (Sigma-Aldrich, Sydney, NSW, Australia), bestatin (Sigma-Aldrich), morphine HCl (GSK, Sydney, NSW, Australia), idazoxan HCl (Tocris, Bristol, UK), naloxone HCl (Sigma-Aldrich), NA (Sigma-Aldrich), cocaine (GSK) and oxycodone HCl (National Institute on Drug Abuse Drug Supply Program, Chapel Hill, NC, USA), were dissolved in distilled water. Other drugs included UK14304 (Tocris), prazosin HCl (Sigma-Aldrich), dissolved in 20% and 10% dimethyl sulfoxide respectively. Thiorphan (Sigma-Aldrich) was dissolved in 50% ethanol. (+)-Pentazocine from Research Biochemical (Natick, MA, USA) was dissolved in distilled water with one to two drops (almost 50 µL) of glacial acetic acid. Buprenorphine (National Institute on Drug Abuse Drug Supply Program) was dissolved in distilled water using HCl (1 M, one to two drops). The maximum concentration of dimethyl sulfoxide used in the superfusion solution was <0.01% and for ethanol was <0.0001%.

Results

Relative potency and efficacy of tapentadol at µ-opioid receptors

To determine the relative potencies and efficacies of tapentadol and several other opioids at µ receptors, whole-cell patch-clamp of $K_{IR}3.x$ currents were recorded at a V_H of –60 mV in the presence of idazoxan (1 µM) and prazosin (300 nM) to block any action of increased noradrenergic tone that might be caused by the inhibition of NAT by tapentadol. Concentration-response curves for [Met]enkephalin were constructed using two to three concentrations per cell. For morphine and tapentadol concentration-response curves, a single concentration of tapentadol or morphine was applied per slice to minimize any possibility that desensitization would confound potency or maximal responses. As shown in Figure 1A, a supramaximal concentration of tapentadol (30 µM) produced an outward current that was consistently smaller than the current produced by a supramaximal concentration of the full $α_2$-adrenoceptor agonist UK14304 (3 µM), in the same cells (Table 1). Naloxone (1 µM) fully reversed the effects of tapentadol (*n* = 12).

As expected from previous studies (Osborne *et al.*, 2000; Dang and Williams, 2005; Virk and Williams, 2008; Bailey *et al.*, 2009), the effect of morphine was lower than the maximal response produced by [Met]enkephalin in LC neurons (Figure 1B, Table 1), that is morphine acted as a partial agonist for activation of $K_{IR}3.x$ currents in rat LC neurons using whole-cell recording in brain slices. The maximum response to tapentadol was smaller than that
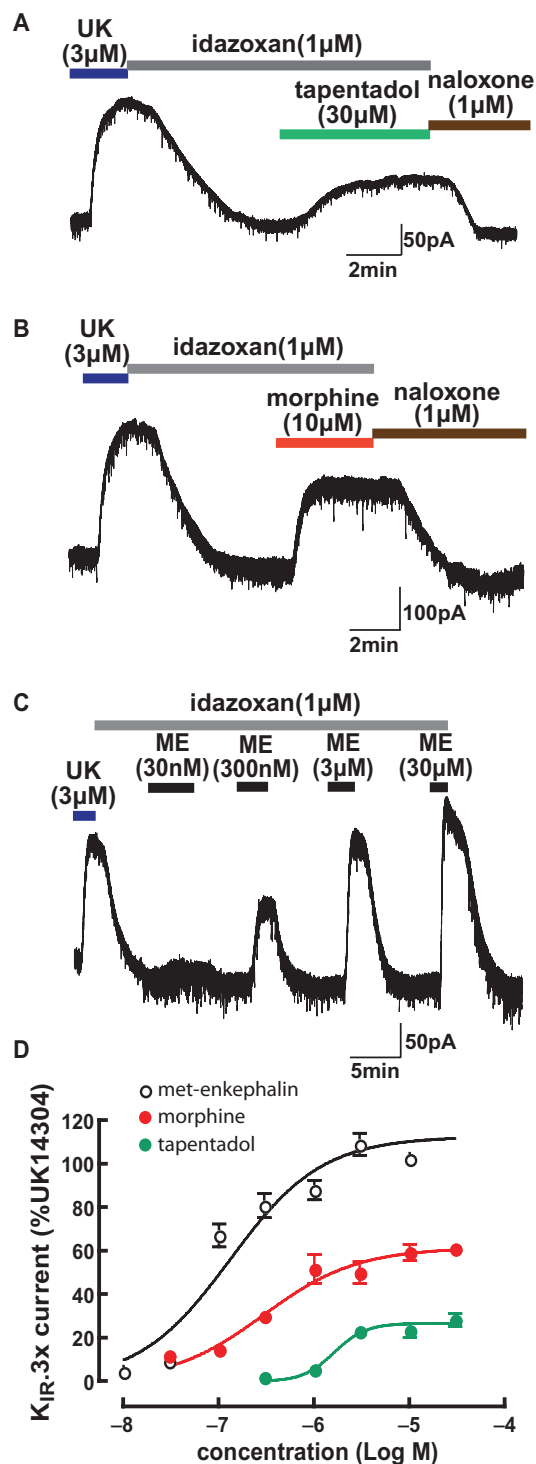
Figure 1

$K_{IR}3.x$ currents induced by activation of µ receptors in LC neurons. Superfusion (shown by bars) of tapentadol (A), morphine (B) and [Met]enkephalin (ME; C) activate $K_{IR}3.x$ currents at holding potential (–60 mV) in the presence of the $α_2$-adrenoceptor antagonist, idazoxan. All actions are reversed by the µ receptor antagonist, naloxone. (D) Concentration-response curves for [Met]enkephalin, morphine and tapentadol. The amplitude of the hyperpolarization plotted as a percentage of the amplitude of a supramaximal concentration of UK14304 (UK).

Table 1

Membrane actions, MOPr agonism and potency of tapentadol and other opioids in LC neurons

	I_{max} (pA)	$I_{max}/I_{UK14304}$%	pEC_{50} (-log[μM])[a]	EC_{50} (μM)
Tapentadol	91 ± 15 ($n = 12$)	29 ± 4*†	5.8 ± 0.2	1.8
Morphine	151 ± 25 ($n = 8$)	59 ± 4*	6.5 ± 0.3	0.3
[Met]enkephalin	222 ± 13 ($n = 5$)	126 ± 7	6.9 ± 0.1	0.13

Data are expressed as mean ± SEM and the number of experiments is shown in parentheses.
[a]SEM of fitted curve.
*Significantly different from [Met]enkephalin (unpaired t-tests, all $P < 0.001$).
†Significantly different from morphine (unpaired t-test, all $P < 0.001$).

produced by morphine (Table 1) suggesting that tapentadol has lower intrinsic efficacy than morphine at the μ receptors.

[Met]enkephalin was a potent and efficacious agonist in LC (Figure 1D, Table 1), with a response amplitude similar to that produced by UK14304 (3 μM). The maximum responses relative to UK14304 (3 μM) for both morphine (30 μM) and tapentadol (30 μM) were considerably smaller than that produced by [Met]enkephalin (Table 1). As summarized in Table 1, tapentadol is approximately sixfold less potent than morphine. The EC_{50} for morphine was similar to that previously reported in LC neurons using patch-clamp recordings (Osborne et al., 2000).

Intrinsic activity of tapentadol relative to other μ receptor agonists using partial antagonism

If tapentadol does indeed have lower intrinsic agonist activity than [Met]enkephalin and morphine, it should partially antagonize both agonists. As shown in Figure 2, both morphine (Figure 2A) and tapentadol (Figure 2B) partially reduced the actions of a supramaximal concentration of [Met]enkephalin. Moreover, tapentadol partially reduced the effect of a supramaximal concentration of morphine (Figure 2C).

The relative intrinsic activity of tapentadol was further compared with several other clinically used μ receptor agonists including oxycodone, buprenorphine and pentazocine. Tapentadol partially reduced the effect of a supramaximal concentration of oxycodone (Figure 2D). Because buprenorphine and pentazocine are weak partial agonists at μ receptors in LC neurons (Virk et al., 2009), producing very small outward currents, they were both tested as partial antagonists, with tapentadol applied first. Buprenorphine (Figure 2E) and pentazocine (Figure 2F) both antagonized the actions of a supramaximal concentration of tapentadol, suggesting that tapentadol has greater intrinsic activity than these two partial agonists.

To test the possibility that tapentadol might inhibit $K_{IR}3.x$ channels in LC neurons, independently of its action on μ receptors [as does thadone; (Rodriguez-Martin et al., 2008)], slices were superfused with naloxone (1 μM), prior to and during application of tapentadol in the presence of a supramaximal concentration of UK14304 (Figure 2G) to fully activate $K_{IR}3.x$ currents via α_2-adrenoceptors. Prazosin (300 nM) was included in these experiments to prevent any

effects of the activation of α_1-adrenoceptors (Osborne et al., 2002). Application of a saturating concentration of UK14304 (3 μM) induced a slowly desensitizing outward current, as previously reported (Dang et al., 2012) but tapentadol had no apparent effect on the current (Figure 2G). To account for the decline in the UK14304-induced current because of desensitization, the slope of the declining current was fitted with a linear function prior to application of tapentadol and the increased slope produced by tapentadol was calculated. Superfusion of tapentadol in the presence of UK14304 did not have any effect on the steady decay of the outward current (t = 0.054, $P > 0.96$ paired t-test, d.f. = 6). These results establish that partial antagonism of [Met]enkephalin, morphine and oxycodone by tapentadol is due to its lower intrinsic activity rather than direct block of $K_{IR}3.x$ channels.

The actions of tapentadol at the μ receptor are summarized in Table 2. The results suggest a relative order of intrinsic activity of [Met]enkephalin > morphine ≈ oxycodone > tapentadol > buprenorphine ≈ pentazocine.

Tapentadol enhances the action of exogenously applied noradrenaline in LC neurons

The potency of tapentadol to inhibit NAT and thereby potentiate the actions of noradrenaline on LC neurons was studied by blocking μ receptors, then superfusing a low concentration of noradrenaline with tapentadol as previously established for the actions of cocaine in LC neurons (Surprenant and Williams, 1987). In the presence of naloxone (1 μM) and prazosin (300 nM), without addition of noradrenaline, tapentadol (100 μM) produced little or no outward current (<5 pA, $n = 3$, data not shown), suggesting little basal noradrenergic tone in slices under the present recording conditions. However, as shown in Figure 3, tapentadol potentiated α_2-adrenoceptor responses when noradrenaline was exogenously applied. Responses to noradrenaline (3 or 10 μM) were variable from cell to cell and slice to slice, presumably because of variation in the diffusion path through slices to relevant α_2-adrenoceptors, which produced a large variation in subsequent NAT inhibition. Indeed, there appeared to be an inverse correlation between the amplitude of response to noradrenaline and extent of potentiation produced by tapentadol (not shown). To control for these differences, the extent of potentiation produced by different concentrations of tapentadol was determined only if the initial response to 3
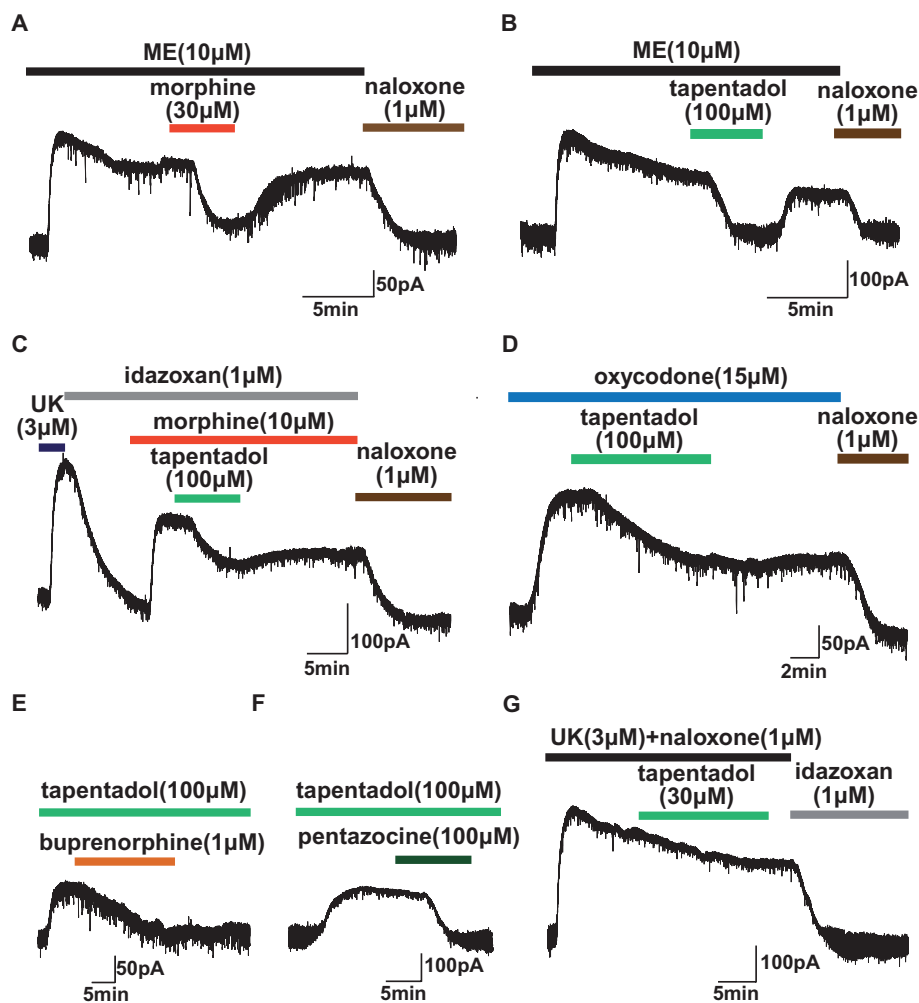
Figure 2

Intrinsic activity of tapentadol at μ receptors (adrenoceptors were blocked from A to F with idazoxan, 1 μM and prazosin 300 nM). (A) Morphine (30 μM) reversibly antagonized the current induced by a supramaximal (and desensitized) concentration of [Met]enkephalin (ME) confirming the lower intrinsic activity of morphine. (B) Tapentadol had lower intrinsic activity than [Met]enkephalin and reversibly antagonized the current induced by a supramaximal (desensitized) concentration of [Met]enkephalin (C) Tapentadol partially and reversibly reduced the current induced by a supramaximal concentration of morphine. (D) Tapentadol partially reduced the current induced by a supramaximal concentration of oxycodone. (E) Buprenorphine and (F) pentazocine almost completely antagonized currents induced by a supramaximal concentration of tapentadol. (G) Tapentadol did not inhibit the current induced by supramaximal concentration of UK14304 (UK) in the presence of naloxone.

or 10 μM noradrenaline was between 5 (less than 5% was considered too small to calculate a reliable percentage of enhancement) and 20% of the maximum response produced by [Met]enkephalin. Tapentadol produced a concentration-dependent enhancement of noradrenaline-induced $K_{IR}3.x$ currents in the concentration range of 0.1–30 μM. In the presence of high concentrations of tapentadol (30–100 μM), no further potentiation of the response to noradrenaline could be achieved by further addition of a supramaximal concentration of cocaine [10 μM; (Surprenant and Williams, 1987) (101 ± 1% of tapentadol response, $n = 3$)], indicating that NAT inhibition was maximal at these concentrations. All subsequent data were normalized to the enhancement of outward current produced by a supramaximal concentration of cocaine (10 μM). The concentration-response curve for tapentadol is presented in Figure 4. The IC_{50} for tapentadol at NAT in LC slices was 2.3 μM ($pIC_{50} = 5.6 ± 0.1$).

Interaction between the two mechanisms of action of tapentadol in single LC neurons

To test how the combined actions of tapentadol on μ receptors and α_2-adrenoceptors, via NAT inhibition, interact with respect to $K_{IR}3.x$ currents, tapentadol was applied to LC neurons in the presence of a low concentration of noradrenaline (3 μM) without addition of antagonists of either receptor. To construct the concentration-response curve, a single concentration of tapentadol was applied per slice. Figure 4A shows the enhancement of the effect of noradrenaline (3 μM) under these conditions. Data were normalized to the maximum $K_{IR}3.x$ current induced by a saturating concentration of UK14303 (3 μM) after subtraction of the small current induced by noradrenaline (3 μM). As shown in Figure 4B, the potency of tapentadol acting on both μ receptors and NAT ($EC_{50} = 2.6$ μM, $pEC_{50} = 5.6 ± 0.1$, $n = 5$ for each concentration

Table 2

Relative intrinsic activity of tapentadol and other opioids in LC neurons

Opioid (n)	I_{opioid} (pA)	$I_{opioid+tapent}$ (pA)	$I_{tapent/opioid}$ (%)
[Met]enkephalin (4)	177 ± 6	26 ± 9	14 ± 5
Morphine (4)	153 ± 18	73 ± 9*	48 ± 3
Oxycodone (7)	209 ± 37	93 ± 27*	41 ± 5
Buprenorphine (5)	69 ± 6[a]	3.4 ± 1.7*	n.d.
Pentazocine (4)	117 ± 17[a]	1.3 ± 2.0*	n.d.

Data are expressed as mean ± SEM.
[a] $I_{tapentadol}$ applied first then combined with listed opioid.
*Significantly different from [Met]enkephalin (unpaired *t*-tests, all $P < 0.01$).
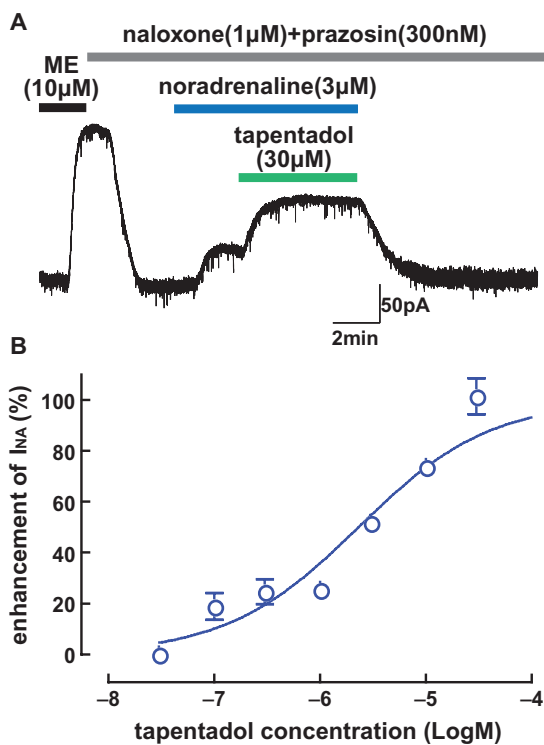n.d., not determined.

Figure 3

Tapentadol potentiates responses of LC neurons to noradrenaline. (A). Typical recording showing that tapentadol in the continuous presence of noradrenaline enhances the outward current induced by noradrenaline (I_{NA}) via inhibiting the noradrenaline reuptake transporter (NAT). In these experiments, the μ receptors were blocked by naloxone (1 μM). (B). Concentration-response curve for enhancement of the noradrenaline current (I_{NA}) by tapentadol ($IC_{50} = 2.3$ μM).

of tapentadol) was not significantly different from the potency of tapentadol as a NAT inhibitor ($EC_{50} = 2.3$ μM, $P > 0.05$, two-way ANOVA, Bonferroni post-test). As confirmed by isobolographic analysis (Grabovsky and Tallarida, 2004;
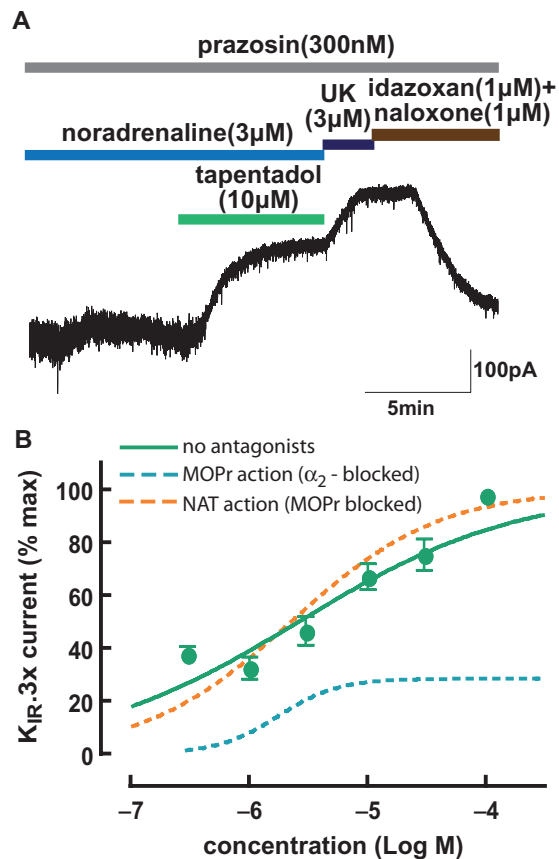

Figure 4

(A) Tapentadol with combined mode of action enhanced the effects of noradrenaline in single LC neurons. All data were normalized by the maximum effect induced by UK14304 (UK; 3 μM). (B) Comparison between the concentration-response curve of tapentadol as a μ receptor (MOPr) agonist alone (with adrenoceptors blocked; data from Figure 1), tapentadol as a NAT inhibitor alone (with μ receptors blocked; data from Figure 3) and tapentadol acting on both mechanisms in the absence of both μ receptor and α_2-adrenoceptor antagonists.

Tallarida, 2006), the interaction between these two mechanisms of action on a single neuron level was additive ($P > 0.15$), as the theoretical additive EC_{50} for independent actions on μ receptors and NAT (3.8 ± 1.2 μM) was not significantly different from EC_{50} for tapentadol with its combined mechanism (2.53 ± 1.4 μM).

Discussion

The present study demonstrates that tapentadol is a μ receptor agonist and NAT inhibitor in single LC neurons in brain slices. Tapentadol exhibited sixfold lower potency than morphine for activation of $K_{IR}3.x$ currents in LC neurons. A previous study (Tzschentke *et al.*, 2007) reported that the binding affinity of tapentadol for rat μ receptors was more than 40-fold lower than that of morphine ($K_i = 96$ nM for tapentadol vs. $K_i = 2.2$ nM for morphine). Similarly, in a

human recombinant μ receptor [^{35}S]GTPγS assay, tapentadol (EC_{50} = 670 nM) exhibited approximately 30-fold lower potency than morphine [EC_{50} = 22 nM; (Tzschentke *et al.*, 2007)]. From this, tapentadol would have been expected to exhibit substantially lower potency than morphine in LC neurons. A likely explanation for the smaller differences between the agonist potencies of tapentadol and morphine observed here compared with the previous study is that the μ receptor reserve is substantially lower in LC neurons than the heterologous expression system used previously (Tzschentke *et al.*, 2007). This interpretation is consistent with the overall potency difference for morphine (14-fold) between the two assay systems. The differences in relative intrinsic activities are also consistent with a large difference in receptor reserve. The maximal effect of tapentadol was 88% of that of morphine in the previous study (Tzschentke *et al.*, 2007) but less than 50% in the LC. Agonists with relatively high intrinsic efficacy (morphine) are predicted to exhibit greater reduction in EC_{50} than lower intrinsic efficacy agonists when receptor reserve increases (Strange, 2008). It is therefore likely that the relative potency and intrinsic activity differences between morphine and tapentadol reported here more closely reflect differences between μ receptor interactions in brain neurons and those in previous studies using heterologous expression systems, as discussed elsewhere (Law *et al.*, 2000; Morgan and Christie, 2011).

Tapentadol exhibited a relative intrinsic activity at μ receptors with respect to activation of $K_{IR}3.x$ currents that was less than [Met]enkephalin, morphine and oxycodone but greater than the weak partial agonists buprenorphine and pentazocine. This was confirmed, where possible, both by examining the relative maximal response to each agonist and partial antagonism. Furthermore, the present study confirmed that measurement of relative intrinsic activity of tapentadol was not confounded by direct block of $K_{IR}3.x$ channels, as was observed for other opioids such as methadone (Rodriguez-Martin *et al.*, 2008).

Many studies have sought to separate the analgesic effect of μ receptor activation from their side effects. It is well established that the μ receptor mediates both analgesic activity and adverse effects of μ receptor agonists (Matthes *et al.*, 1996). Agonists with a relatively low intrinsic activity at μ receptors could overcome some of the limitations of very high intrinsic efficacy μ receptor agonists in terms of therapeutic window and side effects. Indeed, buprenorphine is a very low intrinsic efficacy μ receptor agonist, but produces strong analgesia, and shows an improved tolerability profile compared with morphine (Cowan *et al.*, 1977; Jasinski *et al.*, 1978). In opioid tolerant individuals, buprenorphine also shows a greatly reduced overdose liability (Fatseas and Auriacombe, 2007). It is tempting to speculate that favourable tolerability of tapentadol in humans, particularly with respect to gastrointestinal side effects (Hartrick *et al.*, 2009; Tzschentke *et al.*, 2009; Afilalo *et al.*, 2010; Lange *et al.*, 2010; Pergolizzi *et al.*, 2012), could in part be due to its relative intrinsic activity at μ receptors which is intermediate between morphine and buprenorphine.

The present study has also established that tapentadol enhances the outward current induced by application of noradrenaline in LC neurons by effectively increasing the concentration of noradrenaline reaching postsynaptic

α_2-adrenoceptors, as do other NAT inhibitors (Surprenant and Williams, 1987). Tapentadol exhibited potency for NAT inhibition similar to that for activation of the μ receptor.

The finding that the interaction between the two mechanisms of action of tapentadol is additive with respect to activation of $K_{IR}3.x$ currents in LC neurons is not surprising because both mechanisms are known to converge via a common transduction system through G-protein βγ-subunits to activate a single population of $K_{IR}3.x$ channels (North and Williams, 1985; Stone and Wilcox, 2004). However, previous studies have demonstrated that the interaction between the two mechanisms of action of tapentadol *in vivo*, which is predominantly mediated at the spinal level, is synergistic rather than additive (Schröder *et al.*, 2011). Possible explanations for the synergistic interaction of tapentadol acting on both μ receptor and NAT *in vivo* could be due to receptor activation at multiple sites along the physiological pain-modulatory pathways, rather than being mediated at the single cell level (Stone and Wilcox, 2004). The synergistic interaction observed *in vivo* could include a network effect activation of the descending inhibitory pathways at supraspinal level via μ receptor activation together with potentiation of this effect via NAT inhibition at the spinal level, as well as inhibition of nociceptive transmission between primary afferent and spinal second order neurons via pre- and post-synaptic μ receptors and α_2-adrenoceptors.

Although the LC is useful to study the outcome of μ receptor pharmacology and NAT inhibition in neurons, the influence of neuronal activity in this nucleus in pain modulation is not fully understood. Electrical or chemical stimulation of the LC produces analgesia in acute and inflammatory pain states (Jones and Gebhart, 1986; Jones, 1991; West *et al.*, 1993) as would be expected from enhancement of spinal noradrenaline release. However, direct inhibition of LC activity by microinjection of μ receptor agonists *in vivo* is also antinociceptive (Jongeling *et al.*, 2009). The predicted overall effect of suppression of LC neuronal activity by tapentadol on pain modulation *in vivo* is therefore uncertain.

In conclusion, the present study has established that tapentadol has almost the same potency for NAT inhibition and μ receptor activation in single functioning neurons. Its reduced intrinsic activity at μ receptor compared with morphine, together with its inhibition of NAT may both contribute to improved tolerability profile.

Conflict of interest

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