Synergistic interaction between the two mechanisms of action of tapentadol in analgesia

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Abbreviations: ANOVA, analysis of variance; CI, confidence interval; CL, confidence limits; ED_{50}, median effective dose; IP, intraperitoneal; IV, intravenous; K_i, dissociation constant for inhibitor binding; MPE, maximal possible effect; MOR, μ opioid receptor; NA, noradrenaline; NAT, noradrenaline transporter; NRI, noradrenaline reuptake inhibition; SNL, spinal nerve ligation; tapentadol HCl, (-)-(1'R,2'R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride

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Abstract

The novel centrally acting analgesic tapentadol combines two mechanisms of action, μ-opioid receptor (MOR) agonism and noradrenaline reuptake inhibition (NRI), in a single molecule. Pharmacological antagonism studies have demonstrated that both mechanisms of action contribute to the analgesic effects of tapentadol. This study was designed to investigate the nature of the interaction of the two mechanisms. Dose-response curves were generated in rats for tapentadol alone or in combination with the opioid antagonist naloxone or the α2 adrenoceptor antagonist yohimbine. Two different pain models were used: (1) low-intensity tail flick and (2) spinal nerve ligation (SNL). In each model, we obtained dose-effect relations to reveal the effect of tapentadol based on MOR agonism, based on NRI, and based on unblocked tapentadol. Receptor fractional occupation was determined from tapentadol’s brain concentration and its dissociation constant for each binding site. Tapentadol produced dose-dependent analgesic effects in both pain models, and its dose-effect curves were shifted to the right by both antagonists, thereby providing data to distinguish between MOR agonism and NRI. Both, isobolographic analysis of occupation-effect data and a theoretically equivalent methodology determining interactions from the effect scale demonstrated very pronounced synergistic interaction between the two mechanisms of action of tapentadol. This may explain why tapentadol is only 2-3 –fold less potent than morphine across a variety of preclinical pain models despite its 50-fold lower affinity for the MOR. This is very
likely the first demonstration of a synergistic interaction between the occupied receptors for a single compound with two mechanisms of action.
Introduction

Monoamine reuptake inhibitors (tricyclics, non-tricyclic serotonin-noradrenaline reuptake inhibitors) are among the first-line treatment options for chronic pain. These drugs are generally tolerated relatively well. However, analgesic efficacy of such drugs is often not satisfactory (Fishbain, 2000). Opioids also play an important role in the treatment of chronic pain and can produce potent analgesia (Kalso, et al., 2004). However, opioids are often faced with tolerability problems. In particular, gastrointestinal side-effects such as nausea, vomiting and constipation can be troublesome with opioid treatment (Moore and McQuay, 2005).

Tapentadol ((-)-(1R,2R)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride) is a novel centrally acting analgesic combining two mechanisms of action, µ-opioid receptor (MOR) agonism and noradrenaline reuptake inhibition (NRI), in a single molecule (Tzschentke, et al., 2006; Tzschentke, et al., 2007; Tzschentke, et al., 2009). The rationale behind this combination is that beyond the fact that both mechanisms of action can produce analgesia in their own right, they also interact synergistically at the spinal and supraspinal level. The NRI component can produce an ‘opioid-sparing effect’, such that a moderate MOR-agonistic activity is sufficient (in concert with the moderate NRI activity) to produce potent analgesia, thus reducing opioid-induced side effects. Indeed, despite a 50-fold lower affinity for the MOR, tapentadol is only 2-3 –fold less potent than morphine across a variety of preclinical pain
models (Tzschentke, et al., 2006), clearly implicating that a) the NRI component of tapentadol contributes to its analgesic effect, and b) does so in a supra-additive/synergistic manner. Consistent with the rationale above, tapentadol has demonstrated potent analgesia in acute and chronic pain along with a substantially improved gastrointestinal side-effect profile in clinical studies (Hale, et al., 2009; Hartrick, et al., 2009; Hartrick, 2009).

There is preclinical and clinical evidence that opioid analgesia can indeed be augmented by noradrenergic compounds. For example, MOR agonists and noradrenaline (NA) reuptake inhibitors or \( \alpha_2 \)-adrenoceptor agonists additively or synergistically produced analgesia after systemic or intrathecal administration in models of acute and chronic pain. The NA reuptake inhibitor desipramine increased morphine analgesia after systemic and intrathecal administration (Ossipov, et al., 1982; Reimann, et al., 1999), and systemic as well as spinal combination of morphine with the \( \alpha_2 \)-adrenoceptor agonist clonidine resulted in synergistic antinociception (Ossipov, et al., 1990; Fairbanks and Wilcox, 1999). In clinical settings, morphine analgesia was potentiated by systemic tricyclic antidepressants (Levine, et al., 1986; Ventafridda, et al., 1990), and by spinal/epidural clonidine (Motsch, et al., 1990; Anzai and Nishikawa, 1995).

Isobolographic analysis is a method traditionally used to establish whether an interaction of two agonist drugs is sub-additive, additive or supra-additive/synergistic. In such applications the individual compounds are administered in graded doses by themselves and subsequently in dose combinations that are often fixed ratio combinations of the two compounds.
In the present case, we were concerned with a single compound whose effect is mediated through two distinct mechanisms. The analysis employed therefore consisted of a two-pronged approach: (1) a comparison of the observed and (calculated) additive effect magnitudes and (2) the use of isoboles based on the fractional occupancy of each binding site. Pharmacological antagonism studies have shown that both mechanisms of action of tapentadol (MOR, NRI) contribute to its analgesic effect (Tzschentke, et al., 2006; Tzschentke, et al., 2007; Schröder, et al., 2010). Accordingly, receptor specific antagonists were used to distinguish between the effects mediated by each component in relation to dose. Toward that end, dose-response curves were generated in the low-intensity tail flick model of acute nociception and in the spinal nerve ligation (SNL) model of chronic mononeuropathic pain in rats for tapentadol alone, tapentadol in combination with the MOR antagonist naloxone, and tapentadol in combination with the α₂ adrenoceptor antagonist yohimbine. The data from these experiments have been published in part previously in a different form and context (Schröder, et al., 2010). In additional experiments, for purposes of converting tapentadol doses to receptor occupation, brain concentrations of tapentadol associated with each intraperitoneal (IP) dose were determined in satellite groups of rats just at the same time point as the analgesic effect was quantified in the low-intensity tail flick test. This allowed a correlation of tapentadol brain concentrations with a given analgesic effect that was due to
both mechanisms, exclusively due to MOR agonism (under yohimbine antagonism), or exclusively due to NRI (under naloxone antagonism).
Methods

Behavioral testing

Animals

Male Sprague-Dawley rats (Janvier, Le Genest St Isle, France) were housed under a 12:12-hour light–dark cycle (lights on at 06:00 hours); room temperature 20°C to 24°C; relative air humidity 35% to 70%; 15 air changes per hour, air movement less than 0.2 m/s. The animals had free access to standard laboratory food and tap water. There were at least 5 days between the delivery of the animals and behavioral testing. Average weights were 160-240 g. All experiments were conducted according to the Declaration of Helsinki as well as the guidelines of the International Association for the Study of Pain (Zimmermann, 1983) and the German Animal Welfare Law.

Experimental procedures

Animals were assigned randomly to treatment groups. Different doses and vehicle were tested in a randomized fashion. Although the operators performing the behavioral tests were not formally “blinded” with respect to the treatment, they were not aware of the study hypothesis or the nature of the differences between drugs.
Low-intensity tail flick test

The tail flick test was carried out in rats using a modification of the method described by D’Amour and Smith (1941). The tail flick latency, defined by the time (in seconds) to withdraw the tail from a radiant heat source, was measured using a semi-automated device (Rhema Labortechnik, Germany). The rat was placed in a plexiglas restrainer, and a low-intensity radiant heat beam was focused onto the dorsal surface of the tail root. The stimulus intensity was adjusted to result in a mean pre-drug control latency of 7 s, thus also allowing a supraspinal modulation of the spinally mediated acute nociceptive reflex. A cutoff time of 30 s was applied to avoid tissue damage. The increase in tail flick latency was defined as anti-nociception and calculated as the percentage of maximum possible effect (MPE) according to the following formula: MPE [%] = (t_i – t_c)/(t_{cutoff} – t_c) \cdot 100\% (t_i, withdrawal latency; t_c, control latency; and t_{cutoff}, cutoff time).

Animals were tested before and 30 min after intravenous (IV) administration of tapentadol or vehicle. Naloxone (1 mg/kg) and yohimbine (2.15 mg/kg), or the respective vehicle was given IP 10 min prior to tapentadol.

In additional experiments focusing to correlate receptor occupancy with analgesic effects, animals were tested before and 10, 20 and 30 min after IP administration of tapentadol or vehicle. The antagonist naloxone (1 mg/kg), yohimbine (4.64 mg/kg) or the respective vehicle was given IP 10 min prior to tapentadol.

In the first set of experiments involving IV tapentadol administration, the minimal yohimbine dose (2.15 mg/kg IP) that produced complete antagonism of a maximally effective dose of the reference agonist reboxetine both in the low-
intensity tail flick test and the SNL model was used in order to compare the relative contribution of the NRI component to the effect of tapentadol in these two models (Schröder et al., 2010). In the second set of experiments involving IP tapentadol administration in the low-intensity tail flick test, the dose of yohimbine was further increased to a maximum of 4.64 mg/kg IP that did not yet produce confounding effects (i.e. due to behavioural side effects) in order to ensure complete antagonism of the NRI component of tapentadol.
Drugs

Tapentadol HCl (Grünenthal GmbH, Germany), naloxone and yohimbine (Sigma, Germany) were dissolved in saline (NaCl 0.9%). The volume of administration was 5 mL/kg. The antagonists naloxone and yohimbine or the respective vehicle were given IP 5 min (SNL) or 10 min (low-intensity tail flick) prior to IV or IP tapentadol treatment. Since mechanical hypersensitivity testing in the SNL model lasted about 5 min whereas testing in the tail flick assay was performed instantaneously, the administration time point of the antagonists relative to the agonists was adapted accordingly in the SNL model. Antagonist drug doses were carefully chosen to reach full antagonism of a maximally active dose of respective reference agonists (morphine, reboxetine) without confounding analgesic effect or behavioural side effects (Schröder et al., 2010). All doses refer to the respective salt form as indicated above.

Data Analysis

Data were analyzed by means of a 1- or 2-factor analysis of variance with or without repeated measures, depending on the experimental design, with a post hoc Bonferroni test. Significance of treatment, time, or treatment × time interaction effects was analyzed by means of Wilks Λ (lambda) statistics. In case of a significant treatment effect, pair-wise comparison was performed at the time of maximal effect by the Fisher least significant difference test. Results were considered statistically significant if $P < 0.05$. Median effective dose (ED$_{50}$) values and 95% confidence intervals (95% CIs) were calculated by linear regression.
using the percentage of MPE values obtained at 10 min after IP tapentadol administration. Median effective dose values with non-overlapping 95% CIs were considered to be significantly different. Each group included 10 rats.

**Determination of plasma and brain concentrations**

Six satellite groups of Sprague-Dawley rats (5 animals per dose group) were dosed with tapentadol in the same way as in the low-intensity tail flick experiment. Intraperitoneal doses of 1, 4.64, 10, 21.5, 46.4 and 68.1 mg/kg were administered; the highest two doses were given 10 min after prior administration of naloxone (1 mg/kg, IP) whereas the other doses of tapentadol were preceded by IP saline instead. Blood was collected from the orbital plexus under isoflurane anesthesia 10 min after IP tapentadol administration and samples were immediately transferred to ammonium heparin tubes. Immediately after blood sampling the rats were decapitated and the brain removed from the skull. After washing with 0.9% NaCl, the brains were swabbed dry with cellulose pulp, weighed and homogenized in 5 mL 100 mmol/L potassium phosphate, pH 7.4 using a Pro 200 hand-held homogenizer (Harvard Apparatus GmbH).

Ammonia (25 µL, 25% (w/v)), 25 µL internal standard (1 µM), and 500 µL tert-butyl-methyl ether were added to 50 µL aliquots of the tissue homogenate or plasma. Liquid-liquid extraction was performed by shaking robustly for 20 min at room temperature (Vibrax VXR basic, IKA). Then the samples were centrifuged (10 min, 4°C, 16000 rcf), the ether phase was transferred into an autosampler
vial, and dried under a stream of nitrogen. Samples were reconstituted in 125 µL 50% ACN + 0.1% formic acid, 50% H2O + 0.1% formic acid.

Aliquots of 25 µL of the extract were analyzed by liquid chromatography−tandem mass spectrometry. Chromatography was performed on an AQUA 3 µ C18 125A (2 x 75 mm) column operated at 55°C. The mobile phase was a gradient using 0.5% acetic acid in water and 0.5% acetic acid in methanol at a flow rate of 0.5 mL/min. Detection was by tandem mass spectrometry (API-3000, Applied Biosystems) equipped with Turbolon Spray operated in the positive mode. Analytes and internal standards were monitored at mass transitions m/z 222.2 to 107.0, and m/z 228.2 to 109.0, for tapentadol and its deuterium labeled internal standard, respectively. Calibration and quality control samples were prepared in rat plasma.

**Theory**

**Isoboles**

Isobolographic analysis, introduced and used by Loewe (LOEWE, 1953;LOEWE, 1957), has a traditional application in describing the combination of two agonists drugs with overtly similar action (e.g., two analgesics). In this method the two agonist drugs (here denoted *drug A* and *drug B*) and their respective dose-effect relations allow a prediction of the combined effect *from their individual potencies*. From that relation one determines the combination dose pairs (a,b) that are calculated to give a specified level of effect (usually 50%
of Emax, although other effect levels can be used). This set of dose pairs constitutes the isobole for the selected effect level. This plot is almost always a line or curve having a negative slope in a rectangular coordinate plot of dose B against dose A. If each drug alone is capable of attaining the specified effect (e.g., 50% of Emax) then the intercepts of the graph denote the individual drug doses that give the half maximal effect (see Figure 1). If drug A alone does not reach the 50% effect level, then there is no intercept on the axis for any dose of drug A. The isobole may be interpreted as a visual that shows the diminution in the dose of drug B due to the presence of the dose of drug A and it is this diminution that accounts for its negative slope.

The exception to the negative slope is in that situation in which one of the drugs, say, drug A, lacks efficacy over some dose range. In this case there is no diminution in the needed dose of drug B; hence, in that case the isobole is a horizontal line (also shown in Figure 1). The isobole has an historical use in defining unusual interactions (Loewe, 1953, 1957) and, in recent years, has witnessed a much expanded usage and application (Tallarida, 2001; Tallarida, et al., 2003; Grabovsky and Tallarida, 2004; Tallarida, 2006; Tallarida, 2007; Braverman, et al., 2008; Tallarida and Raffa, 2010). When experiments with actual combinations show that a dose pair below the isobole gives the specified effect, this means that lesser quantities were needed because of a synergistic interaction. In contrast, an experimental point above the isobole means an antagonistic interaction between the constituent drugs. Experimental points that lie on the isobole are the expected dose pairs under conditions of zero interaction.
and we refer to this case as an “additive interaction.” Details are given in several reviews (Tallarida, 2006; Tallarida, 2007; Tallarida and Raffa, 2010).

The theoretical basis of the isobole is the concept of dose equivalence for drugs A and B. Dose equivalence is determined from the individual dose-effect curves, i.e., a dose $a$ of drug A will have a drug B-equivalent dose, $b_{eq}(a)$. Thus, an actual dose $b$ of drug B, when added to $b_{eq}(a)$, is effectively the same as the ED50 of drug B: $b + b_{eq}(a) = ED50$. This mathematical relation defines the isobole and, in its most common form (when the relative potency is constant) the $(a,b)$ dose pairs are given by the equation $a/ED50(A) + b/ED50(B) = 1$. (It is worthy of note that in these relations we have used the “dose”, but it is equally valid to analyze from the drug’s concentration when that is known.)

**Isoboles based on receptor occupation**

While the common use of the isobole is for two drugs, we showed that the same concepts apply when analyzing a single drug that acts through two (or more) receptors (Braverman, et al., 2008) and, therefore, this methodology is applicable to tapentadol. This approach begins with the conversion of concentrations to receptor occupations, thereby transforming concentration-effect data into occupation-effect data. The fraction of the receptors occupied is determined from mass action binding of the drug according to $[C] / ([C] + K)$, where $K$ is the drug-receptor dissociation constant for that receptor and $[C]$ is the drug concentration. When applied to the data for tapentadol we obtained the occupation-effect relations for both MOR and NAT (fractional) occupation as
described and illustrated in the Results. The fractional receptor occupation is ideally determined from the biophase concentration of the drug (as opposed to brain concentration). It is widely held that drug activity in the CNS is due to the unbound brain concentration, because it is that concentration that determines the drug’s occupation of the receptors. This widely accepted concept was confirmed by Liu, et al. (2009) who showed that this unbound (biophase) concentration is approximately 1/100 of the total brain concentration for a number of agents, e.g., serotonin and dopamine transporter inhibitors (substances with molecular weights similar to tapentadol). Based on this, by using the brain concentration data (ng/g) for tapentadol (Figure 5), when adjusted for the brain composition (22% tissue and 78% H2O), we calculate that the fraction of drug in solution (free drug) is 0.0335 which leads to a free concentration that is (0.0335/0.78) = 0.043 of that in the whole brain. Therefore, a factor 0.04 in determining biophase concentration was used and this same value was used for calculating both the observed and expected (additive) concentrations and the corresponding fractional occupancies. A different factor would lead to a different biophase concentration and fractional receptor occupancy, but the results of this analysis, which compares two receptors in the brain for interactions, are independent of the precise biophase values, i.e., this comparative analysis is sufficiently robust as to not require exact values of the occupancies.
Interactions viewed on the effect scale: an alternative to isobolographic analysis

An alternative to isobolographic analysis uses drug combination data and derives the expected (additive) effect of the dose combination \((a, b)\), i.e. an analysis on the effect scale. One might assume that the effect of the combination is a simple sum of the effects that each achieves alone, but that would be incorrect. For example, if the individual effects are, say, 70% and 55% of \(E_{\text{max}}\), the addition of these percentages has no meaning. Thus, we employed the concept of dose equivalence as follows: Using symbols previously defined, we denote the effective dose of the combination as \(b + b_{\text{eq}}(a)\), and this quantity is used in the dose-effect relation for drug B as its effective dose, thereby giving the additive effect of the combination. In the special case in which dose \(a\) alone lacks efficacy, then \(b_{\text{eq}}(a) = 0\), which means that this dose in the combination produces no change in the dose-effect relation of drug B.
Results

Interactions between the two mechanisms of action determined from the effects of tapentadol in two pain models

One view of the interactions between the two mechanisms of action of tapentadol action is afforded from a comparison of the observed and (calculated) additive effect magnitudes. To this end, tapentadol was administered IV in varying doses as the sole agent as well as under conditions of yohimbine block (2.15 mg/kg, IP) and naloxone block (1.0 mg/kg, IP). The former case reveals tapentadol agonism due to MOR stimulation, whereas the latter reveals agonism due to NRI. Agonism in these two conditions was assessed in both an acute pain model (low-intensity tail flick test) and in a model of chronic mononeuropathic pain (SNL).

Low-intensity tail flick test

A graded dose-effect relationship was found in this model, as shown in Figure 2A. It is evident from these relationships that tapentadol’s MOR-mediated action was more potent than that due to NRI in this test (Schröder, et al., 2010). It is also seen (Figure 2A) that the effect of NRI is not evident at tapentadol doses less than 4.64 mg/kg. Thus, in this low dose range the NRI component of action has no equivalent in terms of MOR agonism. It is therefore expected that, in this lower dose range, tapentadol’s antinociceptive dose effect will be the same as
that due to MOR agonism if the interaction is additive. It is seen, however, that the tapentadol effects are elevated above the MOR-mediated effects. This elevation is shown numerically in Table 1 which shows the effect (with 95% CL) for both conditions in the tapentadol dose range ≤ 4.64 mg/kg. From the concept of dose-equivalence the effects are expected to be the same for a simply additive interaction. However, they are seen to be different and the significant difference at each dose is a manifestation of synergism between these two mechanisms of action.

SNL

The dose-effect relationships from this model are shown in Figure 2B and reveal the interesting fact that tapentadol’s NRI-mediated action is more potent than the MOR component of action (Schröder, et al., 2010). This stands in contrast to the situation revealed in the acute pain model described above, where naloxone produced a greater rightward shift of the tapentadol dose-response relationship as yohimbine. It is also seen that in the lower tapentadol dose range (< 4.64 mg/kg) the MOR component of action is not evident and therefore that the NRI component is expected to be the same as that of unblocked tapentadol if there is no interaction. However, there is a prominent elevation in effect levels, e.g., 54.3% vs 6.6% at the 2.15 mg/kg tapentadol dose (Table 1). It is further seen that even at the 4.64 mg/kg dose, where MOR agonism is virtually undetectable in the SNL model, the tapentadol effect is significantly above that
due to NRI agonism, a finding indicative of synergism. The effect values (with 95% CL’s) are given in Table 1.

**Interactions between the two components of tapentadol action determined from receptor occupation-effect relations in the low-intensity tail flick test**

Another view of the interactions between the two mechanisms of action of tapentadol is afforded from the use of the fractional occupation of each receptor type as determined from the values of its brain concentration in an analysis based on receptor occupation. In an additional set of experiments, tapentadol was administered IP in varying doses as the sole agent as well as following prior administration of either naloxone (1.0 mg/kg IP) or yohimbine (4.64 mg/kg IP).

Tapentadol (alone) produced potent (ED$_{50}$: 5.1 [4.4-5.8] mg/kg IP) dose- and time-dependent antinociception (treatment: $F(7,72) = 80.841$, $P < 0.001$; time: $F(2,144) = 11.817$, $P < 0.001$; interaction: $F(14,144) = 2.456$, $P < 0.001$). Full efficacy, 10 min after IP administration, was reached at 31.6 mg/kg (Figure 3, Figure 4). Naloxone significantly shifted the dose–response curve of tapentadol to the right by a factor of 5.2 (ED$_{50}$, 5.1 vs 26.3 [21.7-31.2] mg/kg, [treatment: $F(7,69) = 25.184$, $P < 0.001$; time: $F(2,138) = 0.113$, $P = 0.893$; interaction: $F(14,138) = 1.475$, $P = 0.128$], Figure 4; statistical evaluation relates to the within-group effect of tapentadol, and differences between groups were assessed based on CI overlap, see Methods). Yohimbine significantly shifted the dose–response curve of tapentadol to the right by about a factor of 3 (ED$_{50}$, 5.1 vs 15.2
[12.9-17.7] mg/kg, [treatment: $F(5,54) = 29.124$, $P < 0.001$; time: $F(2,108) = 7.023$, $P < 0.001$; interaction: $F(10,108) = 2.127$, $P = 0.028$], Figure 4). These ED$_{50}$ values are based on effects at 10 min after IP tapentadol administration. Administration of vehicle or antagonists alone did not produce antinociceptive effects (see legend to Figure 4).

**Brain concentrations and receptor occupation of tapentadol**

For use in the following analysis we show in Figure 5 the relation between each IP dose of tapentadol and the brain concentration determined 10 min after tapentadol administration. It is seen that the brain (and plasma) concentrations exhibit pronounced linearity up to doses of 46.4 mg/kg IP. Generally, brain concentrations were approximately 4.5 times higher than in plasma. Effective plasma concentrations in humans are approximately 50 to 150 ng/mL, which is similar to the concentrations found in rat plasma at IP doses of 1 to 4.64 mg/kg. Brain concentrations allow estimation of the biophase concentrations.

The fractional receptor occupation values were calculated from the biophase brain concentration values for each tapentadol dose and the previously determined dissociation constant of MOR (0.096 ± 0.009 µM) and functional inhibition constant of NAT (0.48± 0.11 µM) (Tzschentke et al., 2007).

Figure 6 shows the relation between receptor occupation and IP tapentadol dose. The receptor occupation values for each dose were coupled to the effect (here determined from the low-intensity tail flick test) thereby yielding the occupation-effect curves of Figure 7. These graphs show the occupation-
effect relation for MOR fractional occupation (using the effect data with yohimbine block) and the corresponding NAT fractional occupation that uses effects that accompany the naloxone block. For example, in figure 7A the six points shown are derived from the dose-effect data of figure 4. The doses have been transformed to MOR fractional occupancy and plotted with the observed effect and, from these, we note that the 50% effect occurs at MOR occupancy = 0.92, the value that is used in constructing the subsequent isobole of additivity. Occupation-effect relations serve the same purpose as dose-effect relations for the detection of interactions. In this case, however, the interaction is not between two agonist drugs; it is, instead, between the two receptors occupied by the same drug. Just as dose equivalence is the basis of the common isobologram, it also follows that occupation equivalence is the basis of the isobologram in this case.

As seen in Figure 8, the isobole of additivity (50% effect) is horizontal. This occurs because for all fractional occupancy values of the NAT that are less than 0.54, that occupied receptor yields no detectable effect in this low-intensity tail flick test. (This is analogous to the situation in which one of the two drugs is devoid of efficacy, in which case the isobole of additivity is horizontal.) Thus, NAT occupation in the range 0 to 0.54 is negligible, and therefore the expected additive isobole is horizontal at the 0.92 level (over the NAT domain up to 0.54) of the expected MOR fractional occupation for the half-maximal effect of the combined action (see also Figure 7A). The experimentally determined occupation pair for this 50% effect, obtained from the tapentadol ED50 dose 5.1 mg/kg, is at a lower MOR occupancy value (0.67) with only 0.32 occupancy of NAT (X on
Figure 8), thereby showing synergism because occupancy 0.32 yields no effect. In other words, since this experimentally derived point is significantly below the additive isobole a synergistic interaction between these occupied receptors is indicated.
Discussion

Tapentadol exerts its antinociceptive action through two mechanisms, MOR agonism and NRI, that have been well documented (Tzschentke, et al., 2006; Tzschentke, et al., 2007; Tzschentke, et al., 2009). The current set of antinociceptive tests and accompanying analysis further confirm this dual mechanism and provide a quantitative analysis that shows that these two mechanisms interact in a synergistic way. This is very likely the first demonstration of a synergistic interaction between the occupied receptors for a single compound with a dual mechanism of action. This synergistic interaction was derived from our two pronged analysis that included (1) an examination based on occupation isoboles, and (2) the observed and predicted effect levels.

Viewed from the effect scale, the predicted (additive) effect of a drug dose combination uses the concept of dose equivalence, i.e., adding the drug B-equivalent of drug A to the dose of drug B, in order to calculate the combination effect when there is no interaction. This same principle applies to isobolographic analysis but that method derives its conclusion from comparisons of observed and expected doses (or receptor fractional occupations) that give the specified effect magnitude. In this study the isobolographic analysis employed occupation isoboles and is conceptually identical to that used in traditional isobolographic analysis with doses. When using occupation isoboles it is not dose-equivalence; instead it is occupation equivalence that underlies the analysis. All other aspects of the traditional isobole apply to the occupation isobole in quantitatively
characterizing the interaction which, as applied to tapentadol, is between the two occupied receptors (MOR and NAT). The synergistic interaction between the two mechanisms of action of tapentadol may well explain two remarkable observations. Tapentadol has a 50-fold lower affinity for the (rat) MOR than morphine (\(K_i = 0.096 \mu M\) versus \(0.002 \mu M\)), yet tapentadol is only 2-3-fold less potent than morphine across a variety of preclinical pain models (Tzschentke, et al., 2006), strongly suggesting that the NRI component of tapentadol contributes to its analgesic effect, and that it does so in a synergistic manner. Since the NRI activity of tapentadol is also only relatively moderate (\(K_i = 0.48 \mu M\) for rat synaptosomal uptake inhibition), a simple additive effect cannot explain the potent analgesia observed for tapentadol. Thus, through this synergistic interaction, two moderate pharmacological activities are sufficient to produce powerful analgesia, along with reduced MOR-related and without relevant NRI-related side effects.

The fact that the noradrenergic component contributes in a synergistic way may also explain why tapentadol produces potent analgesia in acute as well as in various chronic pain states. In acute pain, monoaminergic compounds are generally relatively ineffective (see Tzschentke, 2002), and in chronic pain, pure opioids, while still effective, are relatively less potent than in acute pain, necessitating dose escalation to obtain satisfactory analgesia. These high doses often cause intolerable opioid-typical side-effects, limiting the usefulness of pure opioids in chronic pain (Portenoy, 1996; Kalso, et al., 2004). Interestingly, while in acute pain models, the potency of tapentadol is (only) 2-3 times lower than that
of morphine, in rat and mouse models of chronic (mono- and polyneuropathic) pain, the potency difference between tapentadol and morphine is even smaller, or tapentadol is even more potent than morphine (Tzschentke, et al., 2009; Christoph, et al., 2010; Schröder, et al., 2010). This is probably related to the fact that noradrenergic mechanisms play a more relevant role in chronic as in acute pain states (Fishbain, 2000; Tzschentke, et al., 2007; Schröder, et al., 2010), such that the (synergistic) contribution of this mechanism is even more pronounced in chronic pain, leading to an even more pronounced potency advantage over pure opioids (relative to the MOR affinity).

A mechanistic/anatomical basis for this synergistic interaction may lie in the intricate interplay between the opioid system and monoaminergic systems (in particular the descending inhibitory noradrenergic system). Opioids act at several levels of the pain transmitting system. At the spinal level, opioids reduce the transmission of the pain signal from the primary afferents to the fibers of the spinothalamic tract via pre- and postsynaptic mechanisms (Millan, 1999). At the supraspinal level, besides various other effects, opioids activate the descending inhibitory pathways to the spinal cord. Within these pathways, noradrenaline is an important transmitter (Millan, 2002). Thus, a NRI mechanism of action contributes to analgesia by increasing noradrenergic activity at the spinal level by augmenting the influence of the descending inhibitory projection. By combining MOR and NRI mechanisms of action, analgesic potency is enhanced, not only through a summation of the individual effects at the supraspinal and the spinal level, but also through a mutual interaction of supraspinal and spinal effects: the
effect of the opioid-induced supraspinal activation of the descending inhibitory noradrenergic pathways is further enhanced through the action of the NRI component at the spinal level. In other words, the MOR-agonistic component increases spinal levels of NA that in turn acts on spinal α2 adrenoceptors, and the NRI component also blocks the reuptake of this additionally released NA. Previously, it was shown that noradrenaline reuptake inhibitors are antinociceptive on their own and also potentiate the analgesic effect of both systemic and intrathecal morphine when administered spinally (Hwang and Wilcox, 1987). Furthermore, the complex supraspinal-spinal interaction between MOR and α2 adrenoceptors as described above was shown to underlie the antinociceptive synergism obtained with concurrent intrathecal and intracerebroventricular morphine administration in the mouse tail flick test (Wigdor and Wilcox, 1987). Likewise, we were able to demonstrate a pronounced supraspinal-spinal synergism for tapentadol in heat hyperalgesia in a mouse model of streptozotocin-induced diabetic polyneuropathy and showed that this site-site synergism is predominantly mediated at the spinal level (T. Christoph, personal communication).

Because of this intricate interaction and mutual augmentation of the individual effects, relatively moderate pharmacological activities are sufficient for both mechanisms of action of tapentadol to achieve a powerful analgesic effect. This, in turn, translates into a broad efficacy profile and clearly improved clinical tolerability (Hale, et al., 2009; Hartrick, et al., 2009; Hartrick, 2009).
In conclusion, a quantitative analysis based on additive isoboles of occupation and/or additive effects can be applied to study the interaction between two mechanisms of action located within a single molecule. The data presented here for tapentadol show that these mechanisms interact in a highly synergistic way. This may well explain why tapentadol is only 2-3-fold less potent than morphine across a variety of preclinical pain models despite a 50-fold lower affinity for the MOR. This is very likely the first demonstration of a synergistic interaction between the occupied receptors for a single compound with a dual mechanism of action.
Acknowledgments

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Authorship Contributions

Participated in research design: Schröder, Tzschentke, Terlinden, De Vry, Jahnel, Christoph, Tallarida

Conducted experiments: Schröder, Christoph

Performed data analysis: Schröder, Jahnel, Tallarida

Wrote or contributed to the writing of the manuscript: Schröder, Tzschentke, Terlinden, Jahnel, Christoph, Tallarida
References


Footnotes

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Legends for Figures

Figure 1. The common isobole (which may be nonlinear or linear) is a decreasing curve (such as curve #1) when both drugs contribute based on their individual dose-effect curves. When both drugs achieve the desired effect (e.g., 50% level) then there are two intercepts representing the dose of drug A (denoted $A$) and the dose of drug B (denoted $B$) that individually give the specified effect level. When one of the drugs (e.g., drug A) does not contribute to the effect, then the isobole is horizontal (such as curve #2).

Figure 2. Dose-effect relations for tapentadol and its modifications due to selective block of each of its two mechanisms of action are shown for two pain models at 30 min after IV tapentadol administration. Data are presented as % MPE (mean ± SEM). $^* P<0.05$ vs corresponding vehicle. % MPE, percentage of maximum possible effect; SEM, standard error of the mean. Data from Schröder et al., 2010.

Figure 3. Dose- and time-dependent antinociceptive effect of tapentadol in the low-intensity tail flick test in rats. All injections were made IP. Data are presented as % MPE (mean ± SEM). $^* P<0.05$ vs corresponding vehicle. % MPE, percentage of maximum possible effect; SEM, standard error of the mean. Corresponding brain concentrations of tapentadol were determined in satellite groups 10 min after IP administration of tapentadol (Figure 5).
Figure 4. Naloxone shifted the dose–response curve of tapentadol farther to the right than yohimbine in the low-intensity tail flick test in rats. Data are presented as % MPE (mean ± SEM) 10 min after IP administration of tapentadol. *P < 0.05 vs corresponding vehicle. % MPE, percentage of maximum possible effect; SEM, standard error of the mean.

Administration of vehicle and antagonists alone did not produce antinociceptive effects. The respective % MPE (mean ± SEM) 10 min after the second IP administration were as follows: saline IP + saline IP, 0.2 ± 4.0; naloxone 1 mg/kg IP + saline IP, 2.1 ± 2.9; yohimbine 4.64 mg/kg IP + saline IP, −4.2 ± 3.0.

Figure 5. The brain (slope = 406.3) and plasma (slope = 89.4) concentration of tapentadol determined 10 min after IP administration is seen to be linearly related to the IP tapentadol dose over the main range of doses (up to 46.4 mg/kg). The error bars are standard deviation based on n = 5.

Figure 6. Fractional receptor occupation for MOR (solid curve) and NAT (broken curve) based on the published values of \( K_i \) for increasing IP doses of tapentadol.

Figure 7. (A) The occupation-effect relation for MOR fractional occupation was obtained from dose-effect data of tapentadol in the presence of yohimbine. The ED50 for MOR fractional occupation is 0.92 (corresponding to dose 15.3 mg/kg) with 95% CL’s (0.86 – 0.94). (B) Occupation- effect relation for NAT (obtained
with naloxone block) is plotted here. This component of tapentadol’s action is not evident for NAT fractional occupation less than 0.54.

Figure 8. Isobologram for 50% of the maximum possible effect based on receptor occupancy showing synergism between the two components that contribute to tapentadol action in this test. Because NAT activity is not apparent up to fractional occupancy 0.54, the additive isobole (solid line) is horizontal (with 95% CL’s shown as broken lines) and represents the occupation of MOR for this effect level. The experimental occupation pair is shown (X) with 95% confidence limits and its position below the isobole indicates synergism.
Table 1. Effects of tapentadol and its dual component in two pain models.

Effect values are presented as % MPE (with 95% confidence limits) at 30 min after IV tapentadol administration. In each pain model the tapentadol effect is expected to equal that of the indicated component if the interaction is simply additive. These significantly greater effects indicate synergism.

<table>
<thead>
<tr>
<th>Tapentadol Dose [mg/kg] IV</th>
<th>Low-intensity Tail Flick</th>
<th>SNL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tapentadol effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>MOR component effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(yohimbine block)</td>
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<tr>
<td></td>
<td>Tapentadol effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NRI component effect</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(naloxone block)</td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>21.8</td>
<td>21.8</td>
</tr>
<tr>
<td></td>
<td>(4.6 – 29.0)</td>
<td>(4.6 – 29.0)</td>
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<tr>
<td>2.15</td>
<td>27.4</td>
<td>54.3</td>
</tr>
<tr>
<td></td>
<td>(17.5 – 37.3)</td>
<td>(44.4 – 64.2)</td>
</tr>
<tr>
<td></td>
<td>5.47</td>
<td>6.6</td>
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<tr>
<td></td>
<td>(0 – 14.5)</td>
<td>(0 – 15.8)</td>
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<tr>
<td>4.64</td>
<td>71.7</td>
<td>37.5</td>
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<tr>
<td></td>
<td>(55.9 – 87.5)</td>
<td>(22.8 – 52.2)</td>
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<tr>
<td></td>
<td>37.5</td>
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</tr>
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</table>

Data from Schröder et al., 2010
Figure 1

ED50\textsuperscript{B} (B)

dose B

dose A

curve #1

curve #2

Dose A
Figure 2

A

Low-Intensity Tail Flick

- saline IP + tapentadol
- yohimbine 2.15 mg/kg IP + tapentadol
- naloxone 1 mg/kg IP + tapentadol

MPE [%]

<table>
<thead>
<tr>
<th>tapentadol dose [mg/kg] IV</th>
<th>2.15</th>
<th>4.64</th>
<th>10.00</th>
<th>21.50</th>
<th>31.60</th>
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<td>20</td>
<td>40</td>
<td>80</td>
<td>*</td>
<td>20</td>
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<tr>
<td>yohimbine 2.15 mg/kg IP + tapentadol</td>
<td>80</td>
<td>80</td>
<td>80</td>
<td>*</td>
<td>80</td>
</tr>
<tr>
<td>naloxone 1 mg/kg IP + tapentadol</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>*</td>
<td>20</td>
</tr>
</tbody>
</table>

B

Spinal Nerve Ligation

- saline IP + tapentadol
- yohimbine 2.15 mg/kg IP + tapentadol
- naloxone 1 mg/kg IP + tapentadol

MPE [%]

<table>
<thead>
<tr>
<th>tapentadol dose [mg/kg] IV</th>
<th>1.00</th>
<th>2.15</th>
<th>4.64</th>
<th>10.00</th>
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<tr>
<td>saline IP + tapentadol</td>
<td>20</td>
<td>40</td>
<td>*</td>
<td>*</td>
<td>20</td>
</tr>
<tr>
<td>yohimbine 2.15 mg/kg IP + tapentadol</td>
<td>80</td>
<td>80</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>naloxone 1 mg/kg IP + tapentadol</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>*</td>
<td>*</td>
</tr>
</tbody>
</table>
Figure 3
**Low-Intensity Tail Flick**

![Graph showing the effect of different treatments on MPE (%) in relation to tapentadol dose (mg/kg) IP.](image-url)

- **saline IP + tapentadol**
- **yohimbine 4.64 mg/kg IP + tapentadol**
- **naloxone 1 mg/kg IP + tapentadol**

**Figure 4**
Figure 6
Figure 7
Figure 8