Spinal-Supraspinal and Intrinsic μ-Opioid Receptor Agonist-Norepinephrine Reuptake Inhibitor (MOR-NRI) Synergy of Tapentadol in Diabetic Heat Hyperalgesia in Mice

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ABSTRACT

Tapentadol is a μ-opioid receptor (MOR) agonist and norepinephrine reuptake inhibitor (NRI) with established efficacy in neuropathic pain in patients and intrinsic synergistic interaction of both mechanisms as demonstrated in rodents. In diabetic mice, we analyzed the central antihyperalgesic activity, the occurrence of site-site interaction, as well as the spinal contribution of opioid and noradrenergic mechanisms in a hotplate test. Tapentadol (0.1–3.16 μg/animal) showed full efficacy after intrathecal as well as after intracerebroventricular administration (ED$_{50}$ 0.42 μg/animal i.t., 0.18 μg/animal i.c.v.). Combined administration of equianalgesic doses revealed spinal-supraspinal synergy (ED$_{50}$ 0.053 μg/animal i.t. + i.c.v.). Morphine (0.001–10 μg/animal) also showed central efficacy and synergy (ED$_{50}$ 0.547 μg/animal i.t., 0.004 μg/animal i.c.v., 0.014 μg/animal i.t. + i.c.v.). Supraspinal potencies of tapentadol and morphine correlated with the 50-fold difference in their MOR affinities. In contrast, spinal potencies of both drugs were similar and correlated with their relative systemic potencies (ED$_{50}$ 0.27 mg/kg i.p. tapentadol, 1.1 mg/kg i.p. morphine). Spinal administration of the opioid antagonist naloxone or the α_2-adrenoceptor antagonist yohimbine before systemic administration of equianalgesic doses of tapentadol (1 mg/kg i.p.) or morphine (3.16 mg/kg i.p.) revealed pronounced influence on opioidergic and noradrenergic pathways for both compounds. Tapentadol was more sensitive toward both antagonists than was morphine, with median effective dose values of 0.75 and 1.72 ng/animal i.t. naloxone and 1.56 and 2.04 ng/animal i.t. yohimbine, respectively. It is suggested that the antihyperalgesic action of systemically administered tapentadol is based on opioid spinal-supraspinal synergy, as well as intrinsic spinally mediated MOR-NRI synergy.

Introduction

Neuropathic pain is defined as pain initiated or caused by a primary lesion or dysfunction in the nervous system and occurs as a common consequence of diabetes, as 16–26% of diabetic patients suffer from pain of diabetic polyneuropathy (Jensen et al., 2006). Although drugs with a number of different pharmacological mechanisms are available for treatment, many patients still suffer from neuropathic pain, and there is a great medical need for the development of alternative, more efficacious, and more tolerable treatment options (Finnerup et al., 2010). Streptozotocin (STZ) leads to the depletion of pancreatic islet cells, rendering rodents diabetic within 1 to 2 weeks after a single treatment. Symptoms of neuropathic pain such as hyperalgesia or allodynia can be demonstrated in these animals, and clinically effective drugs, such as the $\alpha_2\delta$-selective Ca^{2+} channel subunit blocker

pregabalin (Field et al., 1999) and the norepinephrine (NE)/serotonin reuptake inhibitors duloxetine (Kuhad et al., 2009) and venlafaxine (Marchand et al., 2003), have been reported to show efficacy in this rodent model of diabetic polyneuropathic pain. The analgesic efficacy of opioids such as morphine in neuropathic pain has been demonstrated in clinical studies (Finnerup et al., 2010). Expression analysis of μ-opioid receptors (MORs) in the dorsal root ganglia of rats with mononeuropathy reveals decreased mRNA levels and reduced inhibitory function of presynaptic spinal MOR (Kohno et al., 2005), which might explain the limitations of opioid analgesia seen with mechanical stimuli in mononeuropathic rats (Bian et al., 1999). In diabetic mice, morphine shows dose-dependent efficacy against heat hyperalgesia. However, heat nociception in nondiabetic controls is reduced in the same dose range, suggesting an antinociceptive rather than a selective antihyperalgesic effect of morphine in this model of polyneuropathic pain (Christoph et al., 2010).

Coadministration of morphine by the intrathecal and intracerebroventricular routes reveals spinal-supraspinal

ABBREVIATIONS: CI, confidence interval; ED$_{50}$, median effective dose; ID$_{50}$, median infective dose; MOR, μ-opioid receptor; MPE, maximal possible effect; NE, norepinephrine; NRI, NE reuptake inhibition; SNL, spinal nerve ligation; STZ, streptozotocin; tapentadol HCl, (−)-(1*R*,2*R*)-3-(3-dimethylamino-1-ethyl-2-methyl-propyl)-phenol hydrochloride.

synergy in acute nociception, which might contribute to the high overall efficacy of opioids in moderate to severe pain states (Yeung and Rudy, 1980) and which was shown to involve bulbospinal MOR-induced spinal α_2-adrenoceptor activation (Wigdor and Wilcox, 1987). In contrast, a similar site-site synergy could not be demonstrated in mononeuropathic pain models with mechanical stimulation (Bian et al., 1995), where multisegmental elevation of spinal dynorphin was shown to be involved (Bian et al., 1999; Malan et al., 2000; Lai et al., 2006). Intrathecal administration of dynorphin antiserum restored the site-site synergy, suggesting that the spinal cord is crucially important among the different levels of pain transmission along the neuraxis (Bian et al., 1999). To our knowledge, neither polyneuropathic pain states nor heat hyperalgesia have been tested for the occurrence of opioid site-site synergy.

Tapentadol with its MOR agonism and NE reuptake inhibitory (NRI) activity (Tzschentke, et al., 2007) is considered representative of a proposed new pharmacological class called MOR-NRI (Kress, 2010) and shows high efficacy in neuropathic and non-neuropathic chronic pain conditions (Afilalo and Morlion, 2013). The MOR affinity of tapentadol is 50-fold lower than that of morphine (Tzschentke et al., 2007), whereas the potency in the spinal nerve ligation model in rats and in diabetic hyperalgesia in mice was shown to be in the same range as that of morphine (Christoph et al., 2007, 2010; Schröder et al., 2010). This shift in potency in vivo compared with MOR affinity in vitro is thought to be attributed to the NRI component of tapentadol, which was shown to contribute more to the antihypersensitive effect in spinal nerve–ligated rats than to the antinociceptive effect in naïve rats (Schröder et al., 2010). Furthermore, isobolographic analysis suggests an intrinsic synergism of the two mechanisms of action of tapentadol in nociceptive and neuropathic pain conditions in rats (Schröder et al., 2011). This intrinsic synergy might be mediated mainly at the level of the spinal cord, where both ascending and descending pain modulatory pathways sensitive to MOR activation and NRI are integrated and where both mechanisms contribute to the systemic effect of tapentadol as demonstrated by spinal administration of opioid and noradrenergic antagonists in the spinal nerve ligation model (Bee et al., 2011).

Thus, the spinal cord is involved in the site-site synergy of opioids. The aim of this study was to test, in a model of diabetic polyneuropathic pain, whether spinal-supraspinal synergy can be demonstrated for tapentadol and to characterize the spinal contribution of MOR-NRI, which might lead to the intrinsic synergy of tapentadol.

Materials and Methods

Animals

Male C57BL/6 mice (18–20 g) (Charles River Laboratories, Sulzfeld, Germany) were housed under standard conditions (room temperature 20–24°C, 12-hour light/dark cycle, relative air humidity 45–70%, 15 air changes/hour, air movement <0.2 m/s) with food and water available ad libitum, except for the time of the experiment. There were at least 5 days between delivery of the animals and the start of the experiments. Animal testing was performed in accordance with the recommendations and policies of the International Association for the Study of Pain (Zimmermann, 1983) and the German Animal Welfare Law. All study protocols were approved by the local government committee for animal research, which is also an ethics committee.

Streptozotocin Model (Diabetic Polyneuropathy)

Diabetic heat hyperalgesia was tested as described previously (Christoph et al., 2010). Mice, randomly assigned to treatment groups (i.e., STZ, citrate), were treated intravenously with 200 mg/kg STZ or vehicle (sodium citrate, pH 5). Induction of diabetes was confirmed by blood glucose levels >25 mM 1 week after STZ treatment. One and 2 weeks after treatment, diabetic and nondiabetic control animals were randomly allocated to the different treatment groups ($n = 10$) as outlined later herein. Animals were used for a maximum of two tests, with a washout period of at least 7 days. For nociceptive and hyperalgesic testing, animals were placed on a 50°C metal plate under a transparent Plexiglas box ($13 \times 13 \times 10$ cm, $l \times w \times h$) for 2-minute periods, and the number of nocifensive reactions (licking or shaking of the hindpaws, licking of the genitals, jumping) was counted 30 minutes (baseline 1) and 15 minutes (baseline 2) before and 15, 30, 45, and 60 minutes after drug or vehicle treatment.

Experimental Design

Methods of local administrations were adapted from Hylden and Wilcox (1980) for the intrathecal route and from Haley and McCormick (1957) for the intracerebroventricular route, and they were performed with the animals under brief isoflurane anesthesia.

Systemic Administration. Dose-response curves for tapentadol and morphine were generated by assigning diabetic animals randomly to treatment groups of different doses of compound and vehicle. Nondiabetic controls were treated with vehicle only. Combinations of spinal antagonists or vehicle with systemic tapentadol, morphine, or vehicle were done by assigning diabetic animals randomly to one of the following treatment groups: vehicle, naloxone, or yohimbine intrathecally + tapentadol or vehicle intraperitoneally; vehicle, naloxone, or yohimbine intrathecally + morphine or vehicle intraperitoneally starting with intrathecal administration of vehicle or antagonist followed 15 minutes later by intraperitoneal administration of vehicle, tapentadol, or morphine. Nondiabetic control animals were treated at the same time course with vehicle (i.t. + i.p.) only. Individual dose levels are indicated in the figures.

Spinal Supraspinal Interaction. Diabetic animals were randomly assigned to treatment groups. Each animal received two simultaneous administrations (i.t. + i.c.v.). Dose-response curves for intracerebroventricular tapentadol and intracerebroventricular morphine were generated in animals that received an administration of intrathecal vehicle. Dose-response curves for intrathecal tapentadol and intrathecal morphine were generated in animals that received an administration of intracerebroventricular vehicle. The interaction studies were done by combining increasing dose combinations (i.c.v. + i.t.) based on equianalgesic ratios (tapentadol i.t.:i.c.v. = 1:2; morphine i.t.:i.c.v. = 1:150). Each dose-response curve was accompanied by a group of nondiabetic controls treated with intracerebroventricular + intrathecal vehicle only. Individual dose levels are indicated in the figures.

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 differences between drugs.

Data Analysis. Primary data were the number of nocifensive reactions occurring within 2 minutes. Using baseline 2 of diabetic and of nondiabetic controls as 0 and 100% MPE, respectively, the percent of maximal possible effects (MPE) was calculated. Antihyperalgesic ED_{50} as well as antagonistic median infective dose ID_{50} values [with 95% confidence intervals (CIs)] were calculated by linear regression based on percent MPE. Data were analyzed by means of a repeated measures analysis of variance with post hoc Bonferroni's test (level of significance $P < 0.05$).

The isobolographic analysis was done as described by Tallarida et al. (1989). The isobologram, introduced by Loewe and Muschiinek

(1926), provides a graphical display of the respective dose by using rectangular coordinates (x, y) to represent equieffective pairs of doses of drugs. In the calculation according to Tallarida et al. (1989), the ED_{50} values of both components and their combination are used for comparison of equieffective doses. The isobologram was constructed by connecting the ED_{50} of intrathecal tapentadol plotted on the ordinate with the ED_{50} of intracerebroventricular tapentadol plotted on the abscissa to obtain the line of additivity. The ED_{50} value of the combination of both routes of administration can be represented as point on the X-Y isobole. The ED_{50} of the combination was calculated based on the experimental data and statistically compared with the theoretically additive ED_{50} that would be expected if the combination was additive. Points on the isobole that are significantly lower than the line of additivity indicate synergistic (or superadditive) interaction. Combinations that are significantly above this line indicate subadditivity, and those that do not differ significantly from the line of additivity represent additive interaction. Synergism is indicated by an interaction index $ED_{50experimental}/ED_{50theoretical} < 1$.

Note that our definition of ED_{50} value differs from the original strict mathematical definition in that our ED_{50} values refer to the calculated dose that would yield 50% of the MPE in the test population rather than to the dose that would yield a given effect in 50% of the test population. Although this modified definition deviates from the formal mathematical definition, it is commonly used for the analysis of behavioral data.

Drugs and Chemicals. The following drugs were used: tapentadol HCl (mol. wt. 257.8; Grünenthal GmbH, Aachen, Germany), morphine HCl (mol. wt. 321.8; Merck AG, Darmstadt, Germany), naloxone HCl (Sigma-Aldrich Laborchemikalien, Seelze, Germany), yohimbine HCl (Sigma-Aldrich Laborchemikalien), and streptozotocin (Sigma-Aldrich Laborchemikalien).

Central administration of drugs or vehicle was performed intrathecally and intracerebroventricularly with the animals under brief ether anesthesia. All drugs were dissolved in saline and injected at 5 μl/animal i.t. or i.c.v. and at 10 ml/kg i.p.

For all drugs, the description of salt form was omitted from the text. All doses indicated refer to the respective salt form as indicated in this section.

Results

The ED_{50} values, E_{max} values, and slopes of dose-response curves (with 95% confidence limits) from all experiments are summarized in Table 1. The mean baseline 2 values (number of nocifensive reactions within 2 minutes) were in the range of 15.4–16.9 for nondiabetic controls and 35.3–37.5 for diabetic animals (Table 2).

Supraspinal Administration. When given by the intracerebroventricular route, tapentadol $[F(4,45) = 198.46, P < 0.001]$ and morphine $[F(4,45) = 147.75, P < 0.001]$ induced dose-dependent antihyperalgesic effects and reached efficacies of 95 and 77% MPE, respectively. Potencies differed by a factor of 45 as seen by comparison of the ED_{50} values (95% CI) of 0.180 (0.138–0.233) and 0.004 (0.001–0.009) μg/animal, respectively (Fig. 1A).

Spinal Administration. After intrathecal administration, tapentadol $[F(4,45) = 218.22, P < 0.001]$ and morphine $[F(4,45) = 83.61, P < 0.001]$ induced dose-dependent antihyperalgesia with efficacies of 92 and 80% MPE, respectively, and comparable potency, with ED_{50} values (95% CI) of 0.420 (0.258–0.580) and 0.547 (0.239–1.057) μg/animal, respectively (Fig. 1B).

Spinal Supraspinal Interaction. Comparing the ED_{50} values of tapentadol resulted in a intrathecal/intracerebroventricular ratio of 2:1 that was used for the analysis of combined

equianalgesic intrathecal + intracerebroventricular administration. Simultaneous intrathecal + intracerebroventricular administration of tapentadol induced dose-dependent antihyperalgesia $[F(4,45) = 168.98, P < 0.001]$ with an efficacy of 83% MPE and an ED_{50} value (95% CI) of 0.053 (0.032–0.074) μg/animal. Isobolographic analysis of intrathecal, intracerebroventricular, and combined intrathecal + intracerebroventricular dose-response curves suggested site-site synergistic interaction of tapentadol with an interaction index of 0.181 (Fig. 2; Table 1). Morphine was used in an equianalgesic intrathecal/intracerebroventricular ratio of 1:150 for simultaneous intrathecal + intracerebroventricular administration and resulted in dose-dependent antihyperalgesia $[F(4,45) = 166.04, P < 0.001]$, with an efficacy of 84% MPE and an ED_{50} value (95% CI) of 0.014 (0.003–0.026) μg/animal. Isobolographic analysis revealed site-site synergistic interaction of morphine with an interaction index of 0.0486 (Table 1).

Systemic Administration. When given by the intraperitoneal route, tapentadol $[F(3,45) = 137.454, P < 0.001]$ and morphine $[F(3,56) = 40.07, P < 0.001]$ induced dose-dependent antihyperalgesia with efficacies of 82 and 84% MPE, respectively. Potencies differed by a factor of 0.25 as seen by comparison of the ED_{50} values (95% CI) of 0.274 (0.215–0.343) and 1.1 (0.83–1.51) mg/kg, respectively (Fig. 1C).

Spinal Mechanisms of Tapentadol. Although the potency ratio of intracerebroventricularly administered tapentadol and morphine (1:45) correlates well with the MOR affinity ratio of both compounds in vitro (1:50), the potency ratio of tapentadol and morphine after intrathecal administration (1:1) suggested the spinal cord to be crucial for the analgesic potency of tapentadol. Hence, we combined systemic intraperitoneal administration of tapentadol with intrathecal administration of naloxone and yohimbine.

Equianalgesic doses of tapentadol (1 mg/kg i.p.) and morphine (3.16 mg/kg i.p.) were combined with intrathecal pretreatment of vehicle or antagonist and analyzed at the time of maximal efficacy (15 minutes after intraperitoneal administration). ID_{50} values were calculated by taking the mean (±S.E.M.) of the control groups (tapentadol 87.1 ± 1.8% MPE; morphine 77.0 ± 2.4% MPE) as maximal effect. Dose-dependent antagonism was seen with naloxone (0.3–10 ng/animal i.t.) for tapentadol (Fig. 3A) and morphine (Fig. 3B), resulting in an ID_{50} (95% CI) of 0.75 (0.61–0.90) and 1.72 (1.22–2.22) ng/animal, respectively. The minimal effective dose (MED) was 1 ng/animal for tapentadol and 3.16 ng/animal for morphine. Likewise, yohimbine (0.3–10 ng/animal i.t.) dose-dependently reduced the antihyperalgesic effects of tapentadol (Fig. 3C) and morphine (Fig. 3D) with ID_{50} values (95% CI) of 1.56 (1.33–1.82) and 2.04 (1.47–2.64) ng/animal, respectively. MEDs were identified at 1 ng/animal for tapentadol and 3.16 ng/animal for morphine.

Discussion

The present data characterize tapentadol as centrally acting analgesic in a mouse model of diabetic polyneuropathic pain. Dose-dependent antihyperalgesia was shown after intracerebroventricular administration of tapentadol and morphine (Fig. 1A), with a potency difference of a factor 45, which is close to the difference in affinity (factor 50) of both

TABLE 1

ED_{50} values (microgram per animal) (95% Cl), slopes (95% Cl), and E_{max} (mean % MPE) (95% Cl) values of tapentadol (Tap) and morphine (Mor) in diabetic mice

Drug Route			Tapentadol	Morphine	**Tap/Mor** Ratio
i.t.	i.c.v.	i.p.			
Drug	Vehicle	—	0.420 (0.258–0.580)	0.547 (0.239–1.057)	1
			48.69 (34.25–61.13)	22.75 (14.75–30.69)	
			91.72 (86.50–96.94)	79.58 (72.36–86.80)	
Vehicle	Drug	—	0.180 (0.138–0.233)	0.004 (0.001–0.009)	45
			59.91 (47.12–72.69)	19.86 (11.51–28.21)	
			95.17 (89.46–100.87)	76.75 (64.31–89.18)	
Drug	Drug	—	0.053 (0.032–0.074)[a]	0.014 (0.003–0.026)[a]	4
			43.46 (28.16–58.75)	28.51 (13.61–43.87)	
			82.92 (71.61–94.23)	80.94 (74.04–87.84)	
Drug	Drug	—	0.291 (0.229–0.353)[b]	0.288 (0.101–0.556)[b]	n.a.
			n.a.	n.a.	
			n.a.	n.a.	
—	—	Drug	0.274 (0.215–0.343)[c]	1.107 (0.830–1.506)[c]	0.25
			54.81 (41.78–67.84)	65.19 (45.62–84.77)	
			82.44 (75.63–89.26)	83.61 (70.30–96.92)	

—, not done; MPE, maximal possible effect; n.a., not applicable.
[a] Experimental data.
[b] Theoretical additive data.
[c] ED_{50} value (mg/kg).

drugs for MOR in vitro (Tzschentke et al., 2007). Thus, the suspraspinal analgesia of tapentadol seems driven mainly by MOR, and suspraspinal tapentadol is clearly less potent compared with suspraspinal morphine. Similar data on both drugs reported for rodent models of acute somatic and visceral nociception after systemic and intracerebroventricular administration (Tzschentke et al., 2006) are in line with this finding. As with the suspraspinal route, spinal administration resulted in similar efficacy for tapentadol and morphine. However, the difference in potency seen on the suspraspinal level was completely absent at the spinal level (Fig. 1B). When given systemically via the intraperitoneal route, tapentadol even showed a higher potency than morphine (Fig. 1C). This corroborates data reported in the same model after intravenous administration, demonstrating similar potency and efficacy for tapentadol and morphine (Christoph et al., 2010). Both morphine and tapentadol have good central availability, and to the best of our knowledge, there is no reported evidence suggesting region-specific distribution

within the central nervous system. Therefore, comparison of potencies based on administered local and systemic dose rather than on measured central and peripheral drug concentrations seems justified. The latter would not be feasible in the current experimental setting with combined spinal-suspraspinal or systemic-spinal administrations in a pathologic model with a complex behavioral readout. Loss of peripheral analgesic potency using heat stimulation has been demonstrated for morphine in a mouse model of mononeuropathic pain, and it was hypothesized that loss of peripheral MOR expression is the main reason for this opioid insensitivity (Rashid et al., 2004). A clear rightward shift in morphine potency was reported (Rashid et al., 2004) when comparing the intracerebroventricular and the intrathecal route, which correlates well with our morphine data. In contrast, the spinal potency of tapentadol was shifted to a much smaller extent, suggesting that the nonopioid mechanism of tapentadol contributes significantly to its analgesic potency at the spinal level. Furthermore, since

TABLE 2

Baseline 2 number of nocifensive reactions within 2 minutes of tapentadol (Tap) and morphine (Mor) in diabetic and nondiabetic mice

Drug Route			Baseline 2 (Mean ± S.E.M.)		
i.t.	i.c.v.	i.p.	Nondiabetic Control Group	Diabetic Tapentadol Group	Diabetic Morphine Group
Drug	Vehicle	—	16.2 ± 0.3	36.8 ± 0.4	36.6 ± 0.5
Vehicle	Drug	—		35.5 ± 0.6	36.6 ± 0.5
Drug	Drug	—	16.4 ± 0.4 (Tap)	36.3 ± 0.5	37.5 ± 0.5
			16.7 ± 0.4 (Mor)		
—	—	Drug	16.0 ± 0.1 (Tap)	36.1 ± 0.3	35.5 ± 0.4
			15.4 ± 0.3 (Mor)		
Nx	—	Drug	16.3 ± 0.5 (Tap + 1, Mor + 1)	36.9 ± 0.3 (Tap + 0.3)	36.4 ± 0.4 (Mor +1)
			16.9 ± 0.3 (Tap + 0.3, 3)	36.9 ± 0.3 (Tap + 1)	36.8 ± 0.3 (Mor + 3)
			16.9 ± 0.4 (Mor + 3)	36.7 ± 0.3 (Tap + 3)	37.2 ± 0.4 (Mor + 10)
			16.2 ± 0.3 (Mor + 10)		
Yoh	—	Drug	16.3 ± 0.5 (Tap + 1, Mor + 1)	37.0 ± 0.3 (Tap + 0.3)	36.1 ± 0.5 (Mor + 1)
			16.9 ± 0.3 (Tap + 0.3, 3, 10)	36.7 ± 0.4 (Tap + 1)	37.4 ± 0.3 (Mor + 3)
			16.9 ± 0.4 (Mor + 3)	37.2 ± 0.2 (Tap + 3)	37.5 ± 0.3 (Mor + 3)
			16.2 ± 0.2 (Mor + 10)	36.6 ± 0.3 (Tap + 10)	

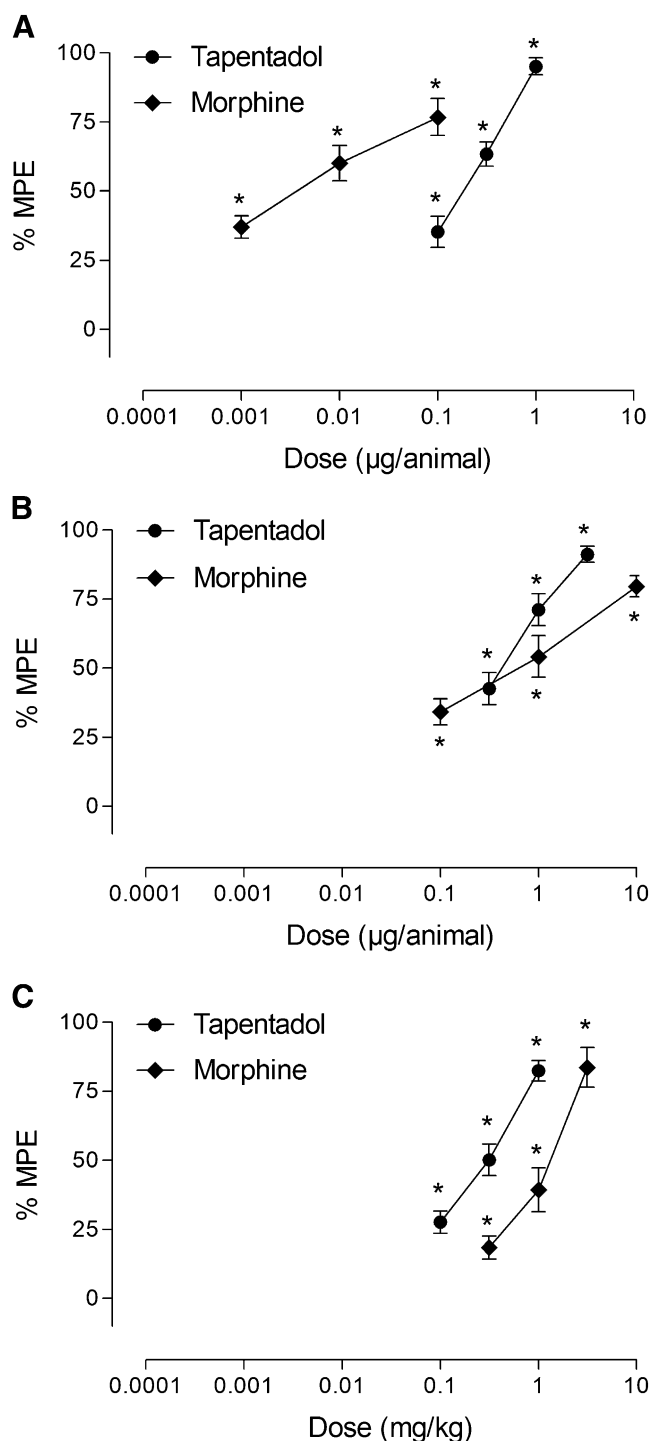—, n.d., not done; Nx, naloxone; Yoh, yohimbine.

Fig. 1. Effect of tapentadol and morphine on heat hyperalgesia in diabetic mice (A) after intracerebroventricular administration, (B) after intrathecal administration, and (C) after intraperitoneal administration.

Combined intracerebroventricular and intrathecal administration of tapentadol resulted in an experimentally determined ED_{50} value that was significantly below the theoretical additive ED_{50} value (Fig. 2; Table 1), suggesting spinal-supraspinal synergy. This kind of site-site synergy is well known for opioids such as morphine in acute heat nociception (Yeung and Rudy, 1980; Wigdor and Wilcox, 1987; Roerig and Fujimoto, 1989), although it could not be demonstrated in mononeuropathic rats using tactile allodynia because of the complete loss of spinal efficacy after nerve ligation (Bian et al., 1999). In light of these data, it may seem surprising that site-site synergism could be demonstrated for morphine in this study (Table 1). However, thermal hyperalgesia and tactile allodynia are mechanistically distinct. Noxious thermal stimuli are believed to be transmitted primarily through high-threshold, thin, unmyelinated primary afferent C fibers, whereas non-noxious tactile stimuli are thought to be transmitted mainly through low-threshold, large-diameter, myelinated $A\beta$ fibers (Ossipov et al., 2000). Whereas morphine does not show site-site synergy in $A\beta$ fiber mediated tactile allodynia, it is feasible that C fiber–mediated heat hyperalgesia supports this kind of interaction. In line with this argument, MOR were shown to be localized mainly on presynaptic C fibers (Besse et al., 1990; Scherrer et al., 2009). Furthermore, mononeuropathic and polyneuropathic pain states might differ in terms of receptor regulation. Mononeuropathic lesions lead to decreased MOR expression on both A and C fibers (Kohno et al., 2005). In diabetic polyneuropathic rats, the number and affinity of spinal MOR as measured by [^{3}H]DAMGO binding were not altered, and their function as measured by DAMGO-stimulated [^{35}S]guanosine 5[prime]-O-(3-thio)triphosphate binding was reduced compared with control rats (Chen and Pan, 2003; Chen et al., 2002). In addition, comparison of the present data measuring heat hyperalgesia in diabetic polyneuropathic mice, where morphine was much more potent after intracerebroventricular compared with intrathecal administration (Fig. 1; Table 1), with published data in rodent models of acute heat nociception demonstrating stronger potencies of spinal versus supraspinal morphine (Yeung and Rudy, 1980; Wigdor and Wilcox, 1987; Roerig and Fujimoto, 1989) suggests loss of spinal MOR activity also in diabetic mice. Indeed, the antihyperalgesic effect of intrathecal morphine as measured by the paw pressure test was not completely lost, but its potency was reduced 2-fold in diabetic polyneuropathic rats compared with control rats (Chen and Pan, 2003). On the other hand, the potency of supraspinal morphine was markedly increased in this study compared with acute nociception (Wigdor and Wilcox, 1987). This difference might be explained both by changes in MOR sensitivity in diabetic compared with nondiabetic mice and by differences in the nociceptive test reflecting a spinal reflex in the tail-flick test and contribution of supraspinal input in the hotplate test. Nevertheless, as demonstrated in the current study, spinal MOR appears to be sufficiently available to maintain the opioidergic spinal-supraspinal synergy. In contrast to morphine, tapentadol contributes not only MOR agonism but also NRI activity, which is able to amplify spinal α_2-adrenoceptor–mediated inhibition of pain transmission (Tzschentke et al., 2007), and thus amplifies the opioid-induced norepinephrine-based site-site synergy at the spinal level. Taken together, the findings from local administration of

similar potency ratios were found for tapentadol and morphine after systemic and spinal but not after supraspinal administration, it is hypothesized that the synergistic interaction of MOR activation and NRI as demonstrated with tapentadol in rat models of acute nociceptive and chronic neuropathic pain (Schröder et al., 2011) is predominantly mediated at the spinal level.

ED$_{50}$ (95% CI) mg/kg i.t. / i.c.v

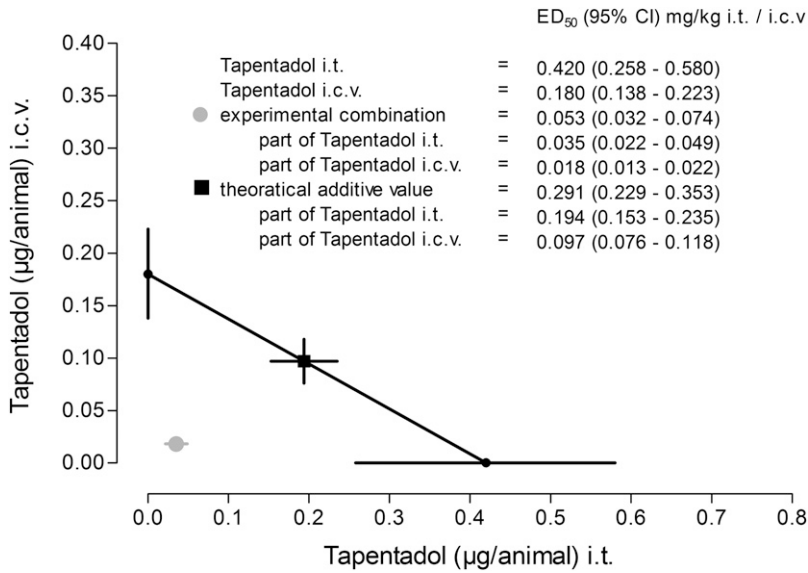|		
Tapentadol i.t.	=	0.420 (0.258 - 0.580)
Tapentadol i.c.v.	=	0.180 (0.138 - 0.223)
● experimental combination	=	0.053 (0.032 - 0.074)
part of Tapentadol i.t.	=	0.035 (0.022 - 0.049)
part of Tapentadol i.c.v.	=	0.018 (0.013 - 0.022)
■ theoratical additive value	=	0.291 (0.229 - 0.353)
part of Tapentadol i.t.	=	0.194 (0.153 - 0.235)
part of Tapentadol i.c.v.	=	0.097 (0.076 - 0.118)

Fig. 2. Isobolographic analysis of the effect of tapentadol on heat hyperalgesia in diabetic mice after intrathecal, intracerebroventricular, and combined administration.

tapentadol and morphine suggest that the spinal cord has a key role in the analgesic activity of tapentadol. Therefore, spinal administration of the MOR antagonist naloxone and the α_2-adrenergic antagonist yohimbine was used to analyze more completely the spinal contribution to the antihyperalgesic effects of systemic tapentadol in diabetic mice. Both antagonists are able block completely the antihyperalgesic effect of tapentadol and morphine in a dose-dependent manner. Whereas ID$_{50}$ values tend to be smaller for naloxone compared with yohimbine, MEDs are identical (1 ng/animal tapentadol; 3.16 ng/animal morphine), suggesting comparable contribution of opioid and noradrenergic components for both
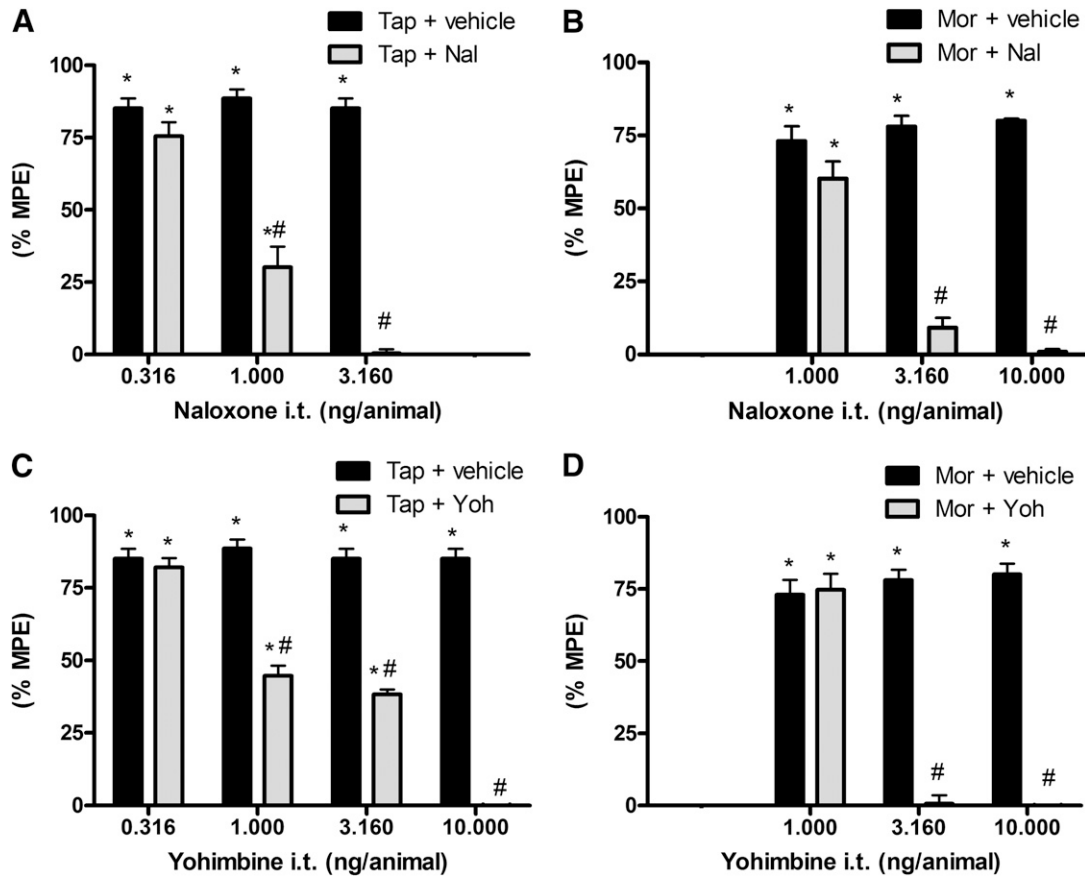

Fig. 3. Effect of naloxone (0.316–10 ng/animal i.t.) on the effect of (A) tapentadol (1 mg/kg i.p.) or (B) morphine (3.16 mg/kg i.p.) on heat hyperalgesia in diabetic mice. Effect of yohimbine (0.316–10 ng/animal i.t.) on the effect of (C) tapentadol (1 mg/kg i.p.) or (D) morphine (3.16 mg/kg i.p.) on heat hyperalgesia in diabetic mice.

drugs at the spinal level. Interestingly, there is a higher sensitivity of tapentadol for both antagonists. This finding supports the concept of intrinisic synergism of tapentadol on the spinal level, as reported earlier (Schröder et al., 2011). Synergistic interaction depends on two independent mechanisms that are able to potentiate each other. If one of the contributing mechanisms is blocked, the remaining mechanism can still work by itself, although only with a clearly weaker potency compared with the synergistic effect. Regarding the effect of systemic morphine, the strong spinal noradrenergic component as shown by the potent ID_{50} and full antagonistic efficacy of yohimbine was unexpected. Several aspects can be considered. First, systemic morphine very likely will have a strong supraspinal action as suggested by the comparison of local efficacies (Table 1). MORs are located in the brainstem and are able to activate descending inhibitory pathways, resulting in antinociception mediated by spinal release of norepinephrine (Millan, 2002). Since the potency of intracerebroventricular morphine is more than 100-fold higher compared with intrathecal morphine, a potent noradrenergic effect of systemic morphine can be expected. Second, animals with neuropathic pain show a tendency toward increased noradrenergic sensitivity. For example, intrathecal injection of norepinephrine or clonidine produced stronger antinociception in diabetic mice compared with nondiabetic controls (Omiya et al., 2005, 2008). Furthermore, an increased density of α_2-adrenoceptors was found in diabetic mice (Omiya et al., 2008), and increased noradrenergic innervation was demonstrated in animals with mononeuropathic lesions (Ma and Eisenach, 2003).

Opioids are not the only class of analgesics known to activate descending inhibitory mechanisms. For example, gabapentin and ketamine have also been shown to increase spinal release of norepinephrine (Kawamata et al., 2000; Hayashida et al., 2008). However, unlike pure opioids, gabapentin or ketamine, tapentadol not only activates descending inhibitory projections by a (presumably) supraspinal mechanism, its NRI activity also blocks the reuptake of NE at the spinal level, thus greatly amplifying the effect of the supraspinally mediated increase in NE release. As outlined herein, this may be of particular relevance under neuropathic conditions.

In summary, the present study demonstrates, in diabetic heat hyperalgesia, site-site synergy of MOR agonists, with a strong supraspinal, MOR-dependent descending inhibitory drive projecting to the spinal cord. Furthermore, the intrinsic synergism of the combined MOR-NRI mechanism of tapentadol, which has been reported in mononeuropathic rats (Schröder et al., 2011), appears to arise mainly from the spinal cord level, as suggested by comparison of systemic, spinal, and supraspinal antihyperalgesic potencies. Both spinal-supraspinal site-site synergy and local intrinsic MOR-NRI synergy in the spinal cord can contribute to the high analgesic potency and efficacy of systemic tapentadol in patients with neuropathic pain.

Authorship Contributions

Participated in research design: Christoph, Schröder, De Vry, Tzschentke.

Conducted experiments: Christoph.
Performed data analysis: Christoph, Tallarida.
Wrote or contributed to the writing of the manuscript: Christoph, Schröder, Tallarida, Tzschentke.

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