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Research Article

Neutral Effects of the Novel Analgesic Tapentadol on Cardiac Repolarization Due to Mixed Ion Channel Inhibitory Activities

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Strategy, Management and Health Policy				
Enabling Technology, Genomics, Proteomics	Preclinical Research	Preclinical Development Toxicology, Formulation Drug Delivery, Pharmacokinetics	Clinical Development Phases I-III Regulatory, Quality, Manufacturing	Postmarketing Phase IV

ABSTRACT Tapentadol is a novel analgesic with μ-opioid receptor agonistic and norepinephrine reuptake inhibiting activities at submicromolar concentrations. Given that inhibition of cardiac potassium currents by noncardiovascular pharmaceuticals is a critical issue in drug development, the potential effects of tapentadol on myocardial repolarization were evaluated. Tapentadol concentration-dependently inhibited hERG-related currents in CHO cells with 250 and 750 times lower potency than haloperidol and sertindole. In electrically stimulated guinea pig papillary muscles, tapentadol at 10 and 100 μM shortened the action potential duration at 30% of repolarization (APD_{30}) and APD_{90}. Maximum effects (100 μM) were more pronounced on APD_{30} than on APD_{90}. In contrast, the hERG inhibitor, *d,l*-sotalol concentration-dependently (1–100 nM) prolonged APD_{90} without affecting APD_{30}. In isolated, perfused, spontaneously beating guinea pig hearts, volume-conducted ECGs revealed that tapentadol reduced heart rate (HR) and prolonged uncorrected QT time, but did not affect HR-corrected QTc time. The hERG inhibitor, dofetilide (0.01 and 0.1 μM) reduced HR and prolonged QT and QTc time. PR time was prolonged by tapentadol but not by dofetilide. Intravenous infusion of tapentadol (3, 6, 9 mg/kg) in conscious dogs led to suprapharmacological plasma concentrations of up to 12 μM (2,531 ng/ml). QT time decreased in tapentadol-treated dogs in parallel to an increase in HR, whereas HR-corrected QTc time was not affected. In conclusion, the in vitro effects of tapentadol suggest mixed ion channel activities on potassium, calcium, and sodium channels at supra-pharmacological concentrations. These activities may be neutralizing, resulting in lack of a net effect of tapentadol on cardiac repolarization. Drug Dev Res 71:197–208, 2010

Key words: cardiac repolarization; ion channels; tapentadol

INTRODUCTION

A broad range of drugs encompassing several therapeutic classes has the ability to delay cardiac repolarization [Moss, 1999; De Ponti et al., 2000; Darpiö, 2001]. A delay in cardiac repolarization, detected as QT time prolongation in the surface electrocardiogram (ECG), favors the development of cardiac tachyarrhythmias [Sanguinetti and Mitcheson, 2005]. Ventricular repolarization is determined by the duration of the action potential. An important influence of pharmaceutical agents on the cardiac action potential may occur because of inhibition of the delayed rectifier potassium current, I_{Kr} [Yang

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et al., 2001]. This repolarizing cardiac potassium current is carried through a channel encoded by the human ether-à-go-go related gene (hERG) that is highly susceptible to blockade by drugs because of a unique binding site. This may explain the high chemical diversity of hERG blockers [Sanguinetti and Tristani-Firouzi, 2006]. However, hERG inhibition does not necessarily lead to action potential prolongation. The often poor correlation between hERG inhibition and repolarization prolongation [Martin et al., 2004] suggests that other activities in addition to hERG inhibition determine the effects of pharmaceutical agents on repolarization. Inhibitors of calcium current are able to shorten the cardiac action potential duration [Zhang et al., 1997; Noguchi et al., 1997; Bénardeau et al., 2000] and to reduce action potential prolongation induced with specific I_{Kr} blockers [Martin et al., 2004]. The reverse-frequency dependency in action potential prolongation induced by blockers of the delayed rectifier potassium current can also be reduced by co-administered calcium channel antagonists [Bril et al., 1998]. Next to a sufficient safety margin of pharmaceutical agents [Redfern et al., 2003], a mixed ion channel block, which is not unlikely to occur at high concentrations, may thus mitigate potential adverse effects on cardiac repolarization associated with hERG channel inhibition.

Tapentadol is a novel centrally acting analgesic agent with 2 mechanisms of action: μ-opioid receptor agonism and norepinephrine (NE) reuptake inhibition [Tzschentke et al., 2006, 2007]. It is effective in the treatment of moderate to severe pain of different origins [Weber et al., 2006; Rauschkolb-Loeffler et al., 2007] and has improved gastrointestinal tolerability compared with conventional opioid agonists [Oh et al., 2008]. The preclinical risk assessment [Haverkamp et al., 2000; Champeroux et al., 2000; Pollard et al., 2008] with tapentadol employed in vitro and in vivo surrogate parameters of cardiac repolarization prolongation. Although tapentadol at supra-pharmacological concentrations inhibited hERG channel activity, action potential duration in guinea pig papillary muscles was not prolonged but rather shortened. This was attributed to a concomitant calcium channel inhibitory activity of tapentadol at concentrations far higher than those active at the primary target sites. Similarly, heart rate-corrected QT time in the volume-conducted electrocardiogram of isolated, perfused guinea pig hearts or in conscious dogs was not affected by tapentadol. Different ion channel inhibitory activities of tapentadol thus may counterbalance each other to result in a neutral effect on cardiac repolarization.

METHODS AND MATERIALS

hERG Channel Activity

Chinese hamster ovary (CHO) cells were stably transfected with cDNA coding for the hERG potassium channel. Cells were grown in 50-ml flasks (Nunc, Roskilde, Denmark) in 6-ml MEM ALPHA Medium supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS), 1% (v/v) P/S/G-solution, and G-418 (all from Invitrogen, Carlsbad, CA) at 37°C in a 5% CO_2 atmosphere. Whole-cell patch-clamp experiments were performed in the voltage-clamp mode [Hamill et al., 1981]. Patch pipettes were pulled from borosilicate glass tubes GC 150 (Clark Electromedical Instruments, Pangbourne, UK) and heat-polished using a P97 Brown/Flaming Puller (Science Products GmbH, Hofheim, Germany). Current signals were amplified and digitized by an EPC-9 patch-clamp amplifier (HEKA Electronics, Lambrecht, Germany), stored, and analyzed using the Pulse/Pulsefit Software (HEKA). Experiments were conducted at room temperature (23 ± 1°C). CHO cells were clamped at a holding potential of −80 mV. They were hyperpolarized for 100 ms to −90 mV, then depolarized for 1 s to + 20 mV followed by a 1-s repolarization back to −40 mV. After 6 control stimuli, the extracellular solution was changed to a solution containing the respective test compound, and 44 additional stimuli were applied. Peaks of the inward tail-currents were analyzed. Stimulation frequency was 0.1 Hz.

Cells were superfused with a solution containing (in mM): NaCl 130, KCl 5.4, $MgCl_2$ 1, $CaCl_2$ 1, glucose 5, and HEPES 10. The pH was adjusted to 7.4. Patch pipettes were filled with the following solution (in mM): KCl 130, HEPES 10, EGTA 1, ATP-Mg 2, and glucose 5. The pH was adjusted to 7.2. Current amplitudes were measured before and after administration of the test compounds.

The relative remaining current was calculated as the ratio of the initial current amplitude and the current amplitude in the presence of the respective test compound. A 100-ms pre-pulse to −90 mV was given to determine the linear leak current. The current amplitude measured during this pre-pulse was multiplied by 4 to estimate the leak current amplitude during the test pulse to −40 mV. This value was then subtracted from the estimated value for the hERG current amplitude. Effects were evaluated from 3–6 experiments per concentration of each test compound and were presented as means ± standard deviation (SD). The half-maximum inhibiting concentration (IC_{50}) and Hill coefficient were calculated by nonlinear least-squares fit to the individual data.

Action Potential Measurement in Guinea Pig Papillary Muscles

Right ventricular papillary muscles were taken from female guinea pigs and mounted horizontally in a 6-ml tissue chamber (FMI GmbH, Seeheim, Germany). The distal end was attached with the remaining chordae tendinae to a tensile hook, while the proximal end was fixed by a suction tube (suction: −60 kPa). The papillary muscles were continuously superfused with pre-warmed and oxygenated (5% CO_2, 95% O_2) modified Tyrode's solution (in mM: NaCl 119.75, KCl 4.75, $MgSO_4$ 0.60, $CaCl_2$ 2.38, $NaHCO_3$ 25.00, KH_2PO_4 0.60, glucose 5.55; pH 7.50) delivered to the tissue chamber by means of a roller pump at a rate of 10 ml/min. Temperature in the chamber was held constant at 36 ± 0.2°C. The muscles were electrically stimulated by square wave pulses (3-ms duration; 50% above threshold voltage) at rates of 1 Hz and 0.5 Hz applied via a platinum electrode located near the preparation inside the tissue chamber. As the second electrode, the silver-coated suction tube was used. Pulses were generated by the Stimulus Isolator A360 combined with the Accupulser A310 (WPI, Berlin, Germany).

Transmembrane potentials were recorded by conventional sharp microelectrodes made from borosilicate glass capillaries (outer diameter 1.5 mm, inner diameter 0.87 mm; Hilgenfeld, Malsfeld, Germany) using a horizontal microelectrode puller DMZ (Zeitz-Instrumente GmbH, München, Germany). The microelectrodes were filled with 3 M KCl solution yielding tip resistances between 10 and 20 MΩ. Microelectrodes were connected to a capacitance-compensated high impedance amplifier in current-clamp mode. Voltage signals were digitized at a sampling rate of 50 kHz by a 12-bit (± 5 V) AD converter NI-DAQ PC-LPM-16 (National Instruments, Austin, TX). Data acquisition of action potentials was performed using IOX software version 1.57 (EMKA Technologies, Paris, France). The software was also used to trigger the pulse generators.

Test compounds were applied consecutively at increasing concentration steps. Baseline values were recorded following an equilibration period of 60 min. Different concentrations of test compounds or vehicle were applied for 30 min each. Preparations were electrically stimulated at 1 Hz throughout the experiment, with the exception of the last 5 min of each concentration step, when stimulation frequency was reduced to 0.5 Hz. Action potentials were recorded during minute 25 and minute 30 of each concentration step. As action potential parameters, the action potential duration at 30% and 90% repolarization (APD_{30}, APD_{90}) and the maximum upstroke velocity (V_{max}) were analyzed. Data were presented as mean values ± standard error of the mean (SEM) of 5–6 individual experiments. Differences between values in vehicle- and test compound-treated groups were tested for statistical significance by means of the unpaired, 2-tailed Student's *t*-test ($P < 0.05$).

Volume-Conducted Electrocardiogram in Isolated Guinea Pig Hearts

Female guinea pigs were deeply anesthetized by pentobarbital. After in situ cannulation, the thoracic aorta was retrogradely perfused with pre-warmed, oxygenated (5% CO_2, 95% O_2) modified Tyrode's solution. The heart was excised and finally mounted to a custom-made vertical Langendorff apparatus. During this procedure, the time of anoxia was kept below 20 s. The spontaneously beating hearts were perfused at a constant perfusion pressure of 48 mmHg and a temperature of 36 ± 1°C. Recording of the volume-conducted ECG was carried out using a method adapted from that described by Franz et al. [1992] for rabbit hearts. Hearts were completely immersed in an organ bath filled with warmed Tyrode's solution that was thermally equilibrated with the coronary perfusion fluid. Three Ag–AgCl pellets were positioned as recording electrodes in a triangular arrangement, 1 at the bottom and 2 on the side walls of the organ bath. A fourth Ag–AgCl pellet at the bottom was used as ground electrode. The inner diameter of the bath was 35 mm, and the distance from the bottom ("foot") electrode to the side ("arm") electrodes was 40 mm. The 2 side electrodes were located beside the left and right atria, slightly above the plane of the atrioventricular valves. Signals from the electrodes were recorded by ECG amplifiers ECG100 (Biopac Systems, Goleta, CA) as standard 3-lead ECGs, closely resembling body surface ECG. Voltage signals were digitized at a sampling rate of 2 kHz by means of a 16-bit (± 10 V) AD converter MP100WS (Biopac Systems). Data acquisition was performed using the AcqKnowledge III software, version 3.5.3 (Biopac Systems).

Analysis of the ECG from the original traces (usually "lead III" was chosen for the analysis) was performed offline using the AcqKnowledge III software. As parameters, heart rate (HR), PR, QRS, and QT times were determined. An individual correction formula was used to correct the QT times for changes in HR. To vary the HR over a wide range, in 9 preparations the sinoatrial and the atrioventricular nodes were dissected to slow the intrinsic HR. Then the heart was paced by an external stimulation electrode placed on the ventricle near the base of the aorta at frequencies from 100–260 beats per minute (bpm) in steps of 20 bpm. The resulting relation between HR and QT time was fitted by means of a linear regression function from which the equation for QT correction was derived as

$QTc = QT - 0.4 \times (200 - HR)$. For each recording sequence, the last 5 completely acquired ECG complexes were evaluated by means of manual cursor measurements, and the median was calculated for each parameter. The values were imported into a custom-built Excel spreadsheet for further computation. Test compounds were applied consecutively at increasing concentration steps following an equilibration period of 60 min and the determination of baseline values. Alternatively, vehicle (0.1% dimethylsulfoxide in Tyrode's solution) was applied as a control. Each concentration or vehicle was applied for 30 min. Electrocardiogram recordings of 60 s each were carried out during minutes 1, 2, 5, 10, 20, and 30. Data were presented as mean values ± SEM of 4–6 individual experiments. Differences between values in vehicle- and test compound-treated groups were tested for statistical significance by means of the unpaired, 2-tailed Student's *t*-test ($P < 0.05$).

QT Time Measurement in Conscious Dogs

Six male beagles (10.5–11.9 kg) were used. The dogs were placed under local anesthesia (lidocaine 2%), and a catheter was inserted via the femoral artery into the abdominal aorta for measurement of blood pressure. Another catheter was inserted into the femoral vein to obtain blood for toxicokinetics. An indwelling catheter was inserted into the left cephalic antebrachial vein to administer vehicle or tapentadol via a short-term infusion. Electrodes were placed for electrocardiographic recording using the standard limb leads I, II, and III. For blood pressure measurement, an indwelling intra-arterial catheter (0.8 × 1.4 mm; Braun, Melsungen, Germany) was connected to a transducer (DT-X, Pfrimmer-Viggo, Erlangen, Germany). The signal was boosted by a Hellige Servomed amplifier and recorded by Cardiognost EK 512 P (Hellige, Freiburg, Germany) at a paper speed of 2.5 mm/s. Electrographic recordings were made on a Schiller Cardiovit AT 1 (Schiller Medizintechnik, Ottobrunn, Germany) with a paper speed of 50 mm/s. HR was determined from the ECG. The QT time (in ms) of the ECG was corrected for changes in HR using the Fridericia formula $QTc = QT/\sqrt[3]{RR}$ [Funck-Brentano and Jaillon, 1993].

Following these preparations, vehicle solution (saline) and 3 escalating doses of tapentadol (3, 6, 9 mg/kg) were administered as intravenous infusions over 5 min. There were 70-min intervals between the infusions. Blood pressure and electrographic parameters were evaluated before, during, and at regular intervals after the infusions. Blood samples for the determination of serum concentrations of tapentadol were drawn before (pre-dose) and at 5, 12, and 30 min following the start of each infusion. Whole blood was processed for serum. Tapentadol was determined by high-performance liquid chromatography with fluorometric detection. Data are presented as means ± SEM. The statistical significance of the differences between infusions was evaluated by means of analysis of variance followed by the Dunnett 2-sided test ($P < 0.05$).

Animals

All experiments followed the Guidelines of the European Council Directive 86/609/EEC and the German Animal Welfare Law. Additionally, the experiments were approved by the Ethics Committee of the local government authority.

Data Presentation and Analysis

Graphical presentation of data was performed using GraphPad Prism software (GraphPad Software, La Jolla, CA). Systat software (Systat Software, San José, CA) was used for statistical analyses.

RESULTS

Inhibition of hERG Potassium Currents

Tapentadol, sertindole, and haloperidol concentration-dependently inhibited hERG potassium currents in CHO cells (Fig. 1). The IC_{50} value of tapentadol was 36 ± 2 μM. Tapentadol inhibited hERG channel activity with 250 and 750 times lower potency than sertindole and haloperidol (Table 1). Tapentadol-*O*-glucuronide, the main metabolite of tapentadol in humans and dogs [Terlinden et al., 2007], did not affect hERG potassium currents up to the maximum test concentration of 300 μM (data not shown).

Effects on Action Potential in Guinea Pig Papillary Muscles

In electrically stimulated (1 Hz) guinea pig papillary muscles, tapentadol had no effect on action

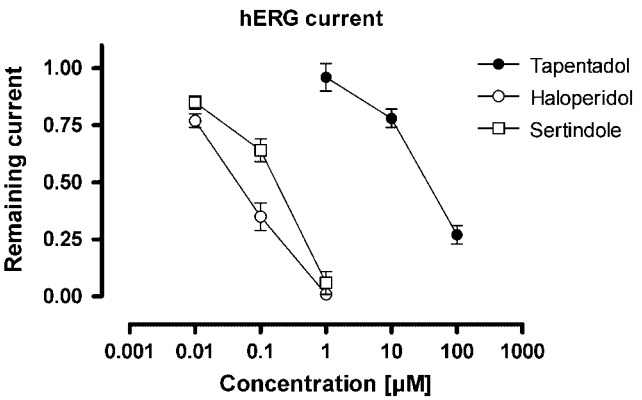

Fig. 1. Inhibition of hERG potassium currents in CHO cells. Data indicate the remaining current in the presence of increasing concentrations of tapentadol, haloperidol, and sertindole as means ± SD of $n = 3$–6 experiments.

TABLE 1. Half-Maximum Inhibiting Concentration (IC_{50}) and Corresponding Hill Coefficient (nH) for hERG Inhibition in CHO Cells by Tapentadol, Sertindole, and Haloperidol*

Compound	IC_{50} (μM)	nH
Tapentadol	36.1±1.51	0.97±0.03
Sertindole	0.145±0.06	1.08±0.48
Haloperidol	0.048±0.01	0.90±0.14

*Values are expressed as means±SEM.

potential duration or upstroke velocity at the concentration of 1 μM. The action potential duration at 90% of repolarization (APD_{90}) was concentration-dependently shortened by tapentadol at concentrations of 10 and 100 μM. The action potential duration at 30% of repolarization (APD_{30}) was also shortened by tapentadol. This effect became apparent at the concentration of 100 μM. The maximum changes induced by tapentadol 100 μM were more pronounced for APD_{30} (−19±4%) than for APD_{90} (−9±3%). The upstroke velocity (V_{max}) of the action potential of guinea pig papillary muscles was insignificantly reduced by tapentadol 100 μM (Fig. 2A,B). Tapentadol-*O*-glucuronide was free of any effect on action-potential duration or V_{max} up to the maximum test concentration of 300 μM (data not shown).

At a stimulation frequency of 0.5 Hz, comparable effects of tapentadol on action potential to those at 1 Hz were observed, indicating the absence of any frequency-dependent effects. The reference compound *d,l*-sotalol concentration-dependently prolonged APD_{90} at concentrations of 10 and 100 μM. In contrast to its effects on APD_{90}, *d,l*-sotalol had no effect on APD_{30}. No effect on V_{max} was seen in *d,l*-sotalol-treated preparations (Fig. 2A,B).

Effects on Volume-Conducted Electrocardiogram in Isolated Guinea Pig Hearts

The spontaneous sinus rate in isolated, perfused, guinea pig hearts was 229±5 beats per min. Tapentadol concentration-dependently decreased HR with a maximum decrease at 30 μM of 30±2% versus baseline. As HR decreased, uncorrected QT time was increased by tapentadol, with a maximum effect of +25±3% at 30 μM. HR-corrected QTc time was not affected by any concentration of tapentadol. PR and QRS times were increased by tapentadol, with maximum effects at 30 μM of +22±6% and +31±5%, respectively (Fig. 3).

The reference compound dofetilide reduced HR and prolonged QT time (maximum effects at 0.1 μM of −20±3% and +34±2%, respectively). QTc time was prolonged by 0.01 and 0.1 μM dofetilide by +14±3% and +21±2%. Dofetilide had no effect on PR time and prolonged QRS time (+18±4%) at a concentration of 0.1 μM (Fig. 3).

Effects in Conscious Dogs

Mean arterial blood pressure, HR, QT, and QTc times did not differ before infusions of vehicle solution (saline) or escalating doses of tapentadol (Table 2). Parameters remained stable during and up to 60 min after the start of the saline infusion. The infusions of tapentadol induced a sharp increase in HR at 5 min at all three dose levels. The maximum increase was observed with the intermediate dose (+113%). Increases following the low and high doses were similar. Tachycardia was transient. HR returned to pre-dose levels at 10 min (low dose) or 15 min (intermediate and high dose) after the start of the infusion (Fig. 4, upper panel). Mean arterial blood pressure increased dose-dependently during the infusion of tapentadol, up to a maximum change of +43%. This increase was significant at 2 and 5 min following the start of the infusion and was reversed within 30–60 min (data not shown).

Uncorrected QT time was shortened by all three doses of tapentadol at 5 min after the start of the infusions, i.e., when a marked increase in HR was observed. The maximum degree of QT shortening was similar for all dose levels of tapentadol. The effects on QT time disappeared when HR returned toward pre-dose values (Fig. 4, intermediate panel).

The HR-corrected QTc time was not changed by any dose of tapentadol (Fig. 4, lower panel). For the time points of 5 and 10 min after the start of the infusions, i.e., during maximum tachycardia, a QT/RR plot was performed with all individual values obtained following treatment with saline (vehicle) and the 3 doses of tapentadol. With the exception of 3 individual measurements (i.e., one for the intermediate and one for the high dose level at 5 min and one for the low dose level at 10 min), the QT/RR plot fitted well using linear regression analysis (Fig. 5). In the case of the 5-min values, linear regression resulted in a regression coefficient of $r^2 = 0.7598$ ($P < 0.0001$).

The infusion of escalating doses of tapentadol led to dose-dependent serum concentrations. The highest concentrations were achieved at the time after completion of the 5-min infusion (Table 3).

Exposure Ratios

Concentrations of in vitro and in vivo repolarization tests with tapentadol were compared to the serum maximum concentration (C_{max}) value obtained with a clinically relevant dose schedule of tapentadol [Tzschentke et al., 2006]. The serum C_{max} value of tapentadol in conscious dogs, where no QTc effect was observed, was 33 times higher than the clinical C_{max} value. Even larger ratios were found between the clinically relevant exposure and the in vitro concentration

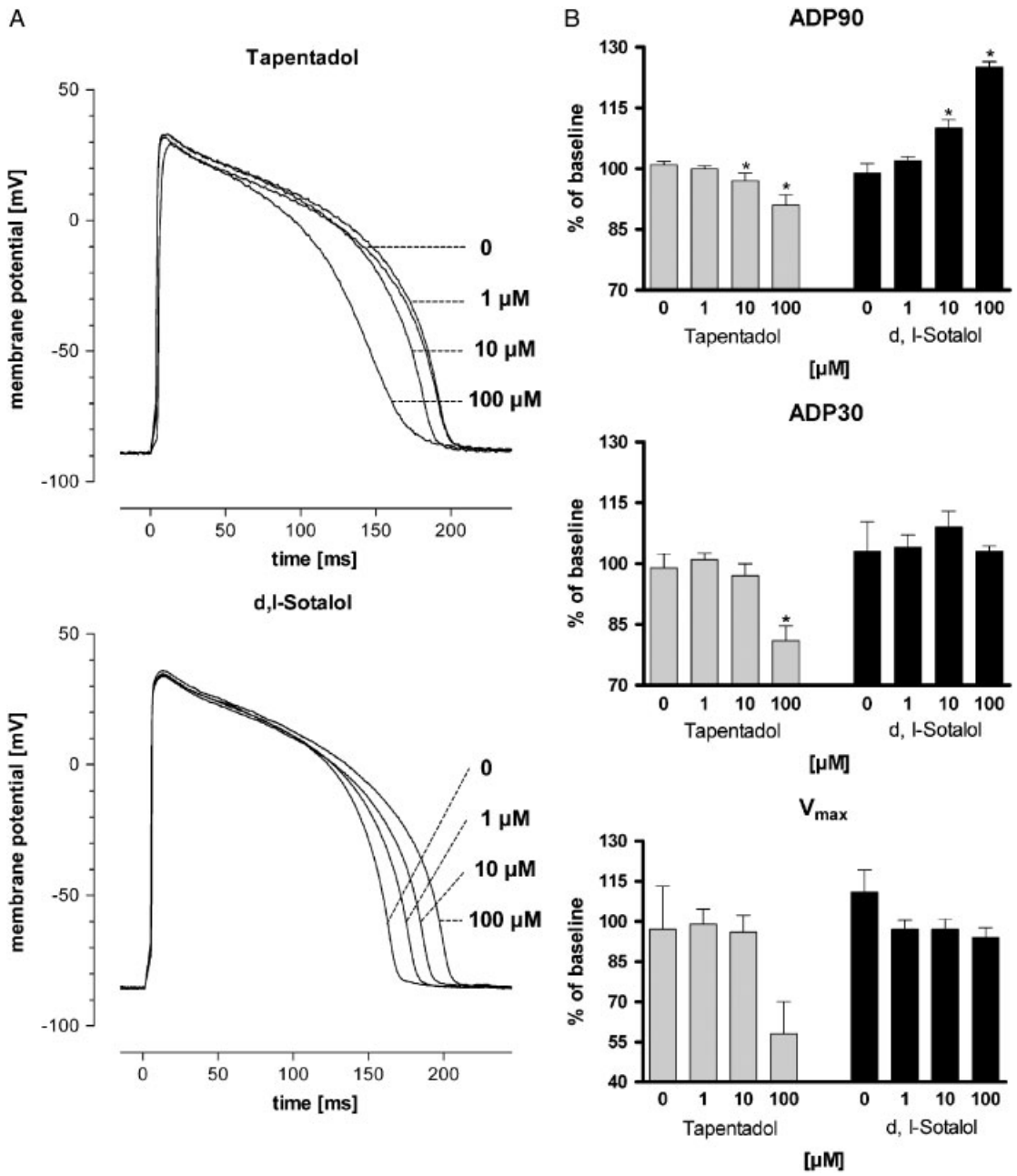

Fig. 2. **A**: Representative action potential recordings in guinea pig papillary muscle. Concentration-dependent effects of tapentadol and *d,l*-sotalol. **B**: Concentration-dependent effects of tapentadol and *d,l*-sotalol on APD_{90}, APD_{30}, and V_{max} in guinea pig papillary muscles (electrically stimulated at 1 Hz). Data are means ± SEM of $n = 5–6$ individual experiments. Asterisks indicate statistical significance ($P < 0.05$) versus time-matched vehicle controls.

in the guinea pig isolated perfused heart test, where no signs of repolarization prolongation were detected (85 times), or the concentration with half-maximum inhibitory effect on hERG-related currents (102 times; Table 4).

DISCUSSION

In line with common strategy in the pharmaceutical industry [Pollard et al., 2008], risk evaluation for potential QT time prolongation by the novel analgesic tapentadol started with a test on hERG channel activity. The antipsychotics haloperidol and sertindole which block hERG channels at nanomolar concentrations [Lacerda et al., 2001; Katchman et al., 2006] were used as reference compounds. Tapentadol did not affect the hERG channel except at micromolar concentrations. It was thus 250 and 750 times less potent than haloperidol and sertindole. Tapentadol

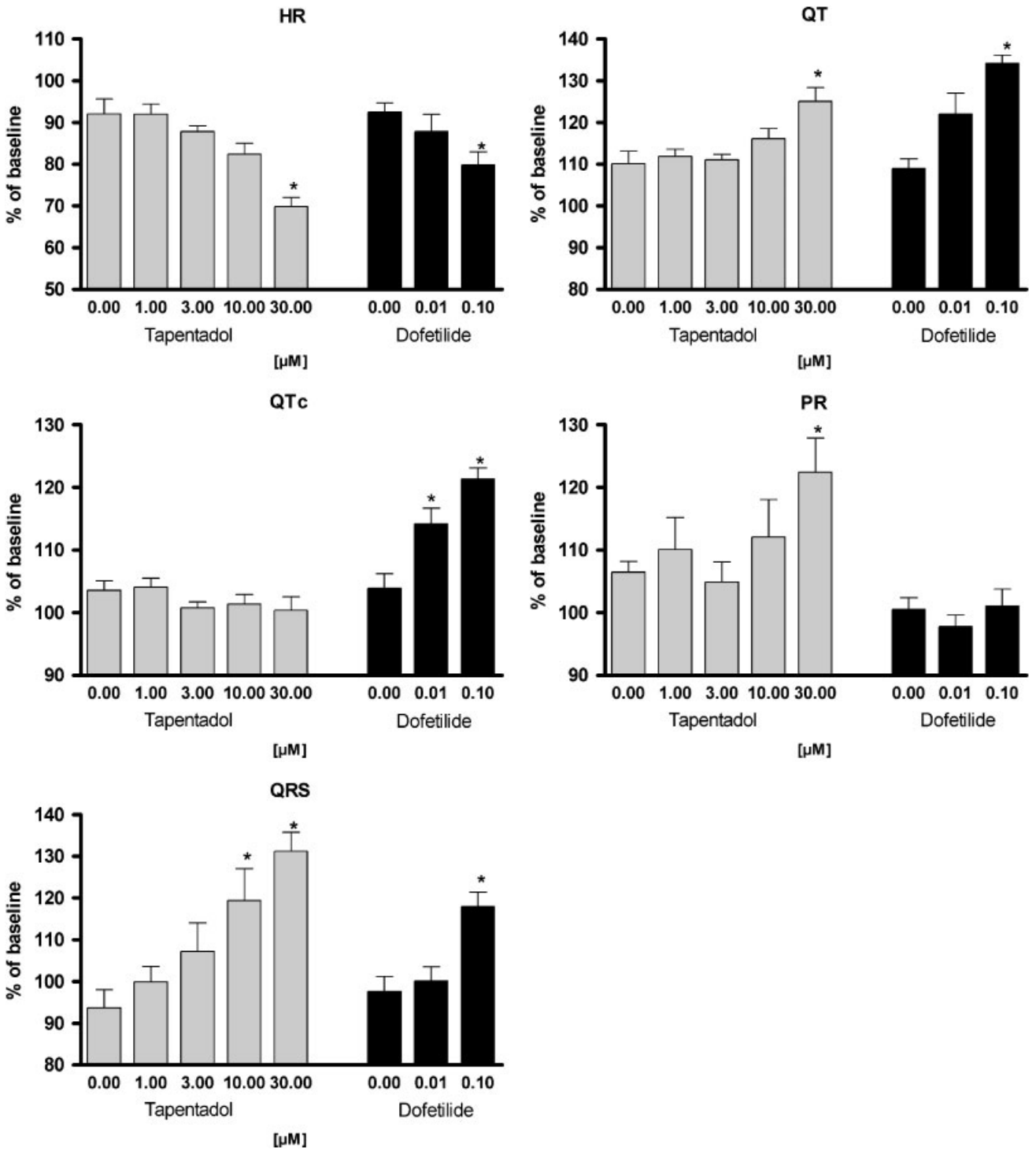

Fig. 3. Concentration-dependent effects of tapentadol and dofetilide on ECG parameters in spontaneously beating, isolated, perfused guinea pig hearts. Data are means ± SEM of $n = 4$–6 individual experiments. Asterisks indicate statistical significance ($P < 0.05$) versus time-matched vehicle controls.

reaches its primary targets in the submicromolar range [Tzschentke et al., 2007]. There was no activity of tapentadol on cardiac potassium channels at these low concentrations. The half-maximum concentration (IC_{50}) for hERG inhibition in CHO cells was 36.1 μM, corresponding to 7,978 ng/ml tapentadol. This is a more than 100 times higher concentration than the maximum exposure (C_{max}) to tapentadol (78 ng/ml) in

TABLE 2. Pre-dose Values of Mean Arterial Blood Pressure, Heart Rate, and QT Time (Uncorrected and Heart Rate-Corrected According to Fridericia) in Conscious Dogs*

	MABP (mmHg)	HR (n/min)	QT (ms)	QTc (F) (ms)
Vehicle	111±13	101±4	222±8	250±8
Tapentadol				
3 mg/kg	118±10	98±6	298±8	255±13
6 mg/kg	116±10	90±3	236±7	266±18
9 mg/kg	107±8	88±3	236±4	261±5

*Values are expressed as means±SEM ($n = 6$).

human subjects [Tzschentke et al., 2006]. According to existing experience [Redfern et al., 2003], this large exposure ratio constitutes a sufficient safety margin for tapentadol regarding the hERG-related risk of repolarization disturbances.

Despite inhibition of the hERG channel, i.e., the carrier of the delayed rectifier potassium current, I_{Kr} [Yang et al., 2001], at high concentrations, tapentadol did not prolong the APD in guinea pig papillary muscles. Rather, APD_{90}, representing the repolarization phase of the action potential, was shortened by tapentadol at concentrations of 10 and 100 μM. At these concentrations, tapentadol induced moderate to marked inhibition of hERG-related currents. Moreover, at the concentration of 100 μM, tapentadol also shortened APD_{30}. The maximum effect of tapentadol was even more pronounced on APD_{30} (−19±4%) than on APD_{90} (−9±3%). This indicates an additional effect at high concentrations on inward currents during the action-potential plateau, which are carried by L-type calcium channels or slowly inactivating sodium channels [Bénardeau et al., 2000]. Indeed, tapentadol binds to L-type calcium channels in the higher micromolar range (data not shown).The inhibition of inward currents during the plateau phase may counterbalance any action potential-prolonging effect that could be expected because of the inhibition of the repolarizing potassium current, as evidenced from the hERG blocking activity. As a net result of these mixed ion channel activities of tapentadol, there was no prolongation but a shortening of the action potential.

The activity of tapentadol in electrically stimulated guinea pig papillary muscles clearly contrasted to that of *d,l*-sotalol. The hERG inhibitor *d,l*-sotalol [Numaguchi et al., 2000] expectedly prolonged the action potential in guinea pig papillary muscles in a strictly concentration-dependent (1–100 μM) manner. This effect was demonstrated only on APD_{90}, whereas no effect of *d,l*-sotalol was observed on APD_{30}. The activity of *d,l*-sotalol is thus restricted to the late repolarization phase that is mainly carried by I_{Kr}. Tapentadol at the concentration of 100 μM also reduced the upstroke velocity, albeit in a nonsignificant manner, indicating that there is a tendency for attenuation of the fast sodium inward current in guinea pig papillary muscles at very high concentrations.

The cardiac electrophysiological effects of high concentrations (1–30 μM) of tapentadol were studied at the organ level in isolated, perfused, spontaneously beating guinea pig hearts. The most prominent effect at concentrations higher than 3 μM tapentadol was a decrease in HR. The bradycardia induced by tapentadol in perfused guinea pig hearts may be attributed to a calcium channel antagonistic effect, as delineated from the APD_{30} shortening effect in papillary muscles. Calcium antagonists produce negative chronotropy in guinea pig myocardial preparations [Noguchi et al., 1997]. Bradycardia might also be an indirect effect of tapentadol induced by an opioid receptor-mediated regulation of calcium channels in myocytes of the sinoatrial node [Saeki et al., 1995]. However, in contrast to the high opioid receptor affinity of tapentadol in the submicromolar range [Tzschentke et al., 2007], bradycardia started at concentrations of tapentadol at least one order of magnitude higher than those needed for its primary activity. A specific receptor-mediated effect of tapentadol underlying the decrease in spontaneous sinus rate in isolated guinea pig hearts is thus unlikely. In parallel to the decrease in HR, QT time increased in guinea pig hearts treated with tapentadol at concentrations higher than 10 μM. Following correction of QT values for changes in HR, no effect of tapentadol was detectable on the repolarization process. This differed from the results obtained with the selective hERG current inhibitor dofetilide [Snyders and Chaudhary, 1996]. As reported for isolated, paced guinea pig hearts [Cheng et al., 2006], dofetilide induced dose-dependent QT prolongation also in the present study employing spontaneously beating hearts. Dofetilide not only affected QT time in the presence of a reduction in HR, but also prolonged HR-corrected QTc time, indicating a true delay of repolarization. This clearly separated the activity of tapentadol from that of dofetilide. The profile of tapentadol was also clearly different from that of dofetilide regarding the effect on PR time as a measure of atrioventricular conduction time. Tapentadol (30 μM) prolonged PR time, whereas dofetilide did not. The PR time can be prolonged by calcium channel antagonists in guinea pig myocardial preparations [Cheng et al., 2006]. The effect of tapentadol on this ECG parameter provides further evidence for an inhibitory activity in myocardial calcium channels. The concentration-dependent QRS prolongation induced by tapentadol indicates an increase in cardiac conduction time. Such an effect points to an additional

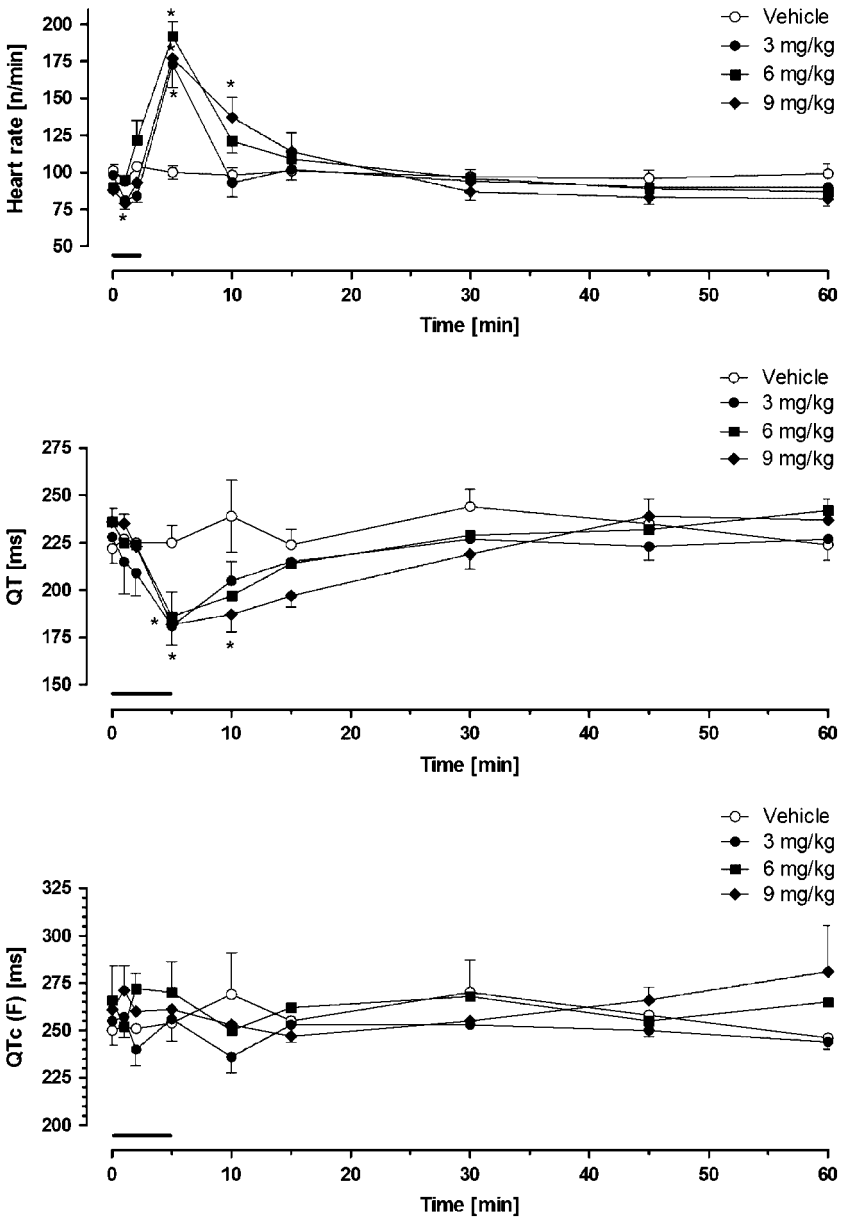

Fig. 4. Time course of effects on HR, QT time, and HR-corrected QTc (F) time (correction according to Fridericia formula) following sequential infusions of saline (as vehicle; open circles), or tapentadol 3 mg/kg (filled circles), 6 mg/kg (filled squares) and 9 mg/kg (filled diamonds). The 5-min infusion period is indicated by a horizontal bar over the x-axis. All data are given as means ± SEM of $n = 6$ experiments. Asterisks indicate significant differences ($P < 0.05$) versus vehicle infusions.

inhibitory activity on sodium channels that was also suggested by the tendency of tapentadol for a reduction of the upstroke velocity in guinea pig papillary muscles.

The potential outcome of any hERG-blocking activity of high concentrations of tapentadol was finally studied under in vivo conditions. Tapentadol was infused intravenously in conscious dogs up to the highest feasible dose (9 mg/kg), resulting in a manifold of clinically relevant systemic exposure [Terlinden et al., 2007]. The most remarkable effect of tapentadol in conscious dogs was a sharp increase in HR observed during the infusion of all three doses. A vagolytic effect due to an antimuscarinic activity of tapentadol (unpublished data) might be responsible for this tachycardic response. In addition, NE reuptake inhibition, one of the main features of the analgesic action of tapentadol [Tzschentke et al., 2007], might increase sympathetic nerve activity and contribute to the elevations in HR and blood pressure. Blockade of neuronal reuptake of NE has been shown to cause

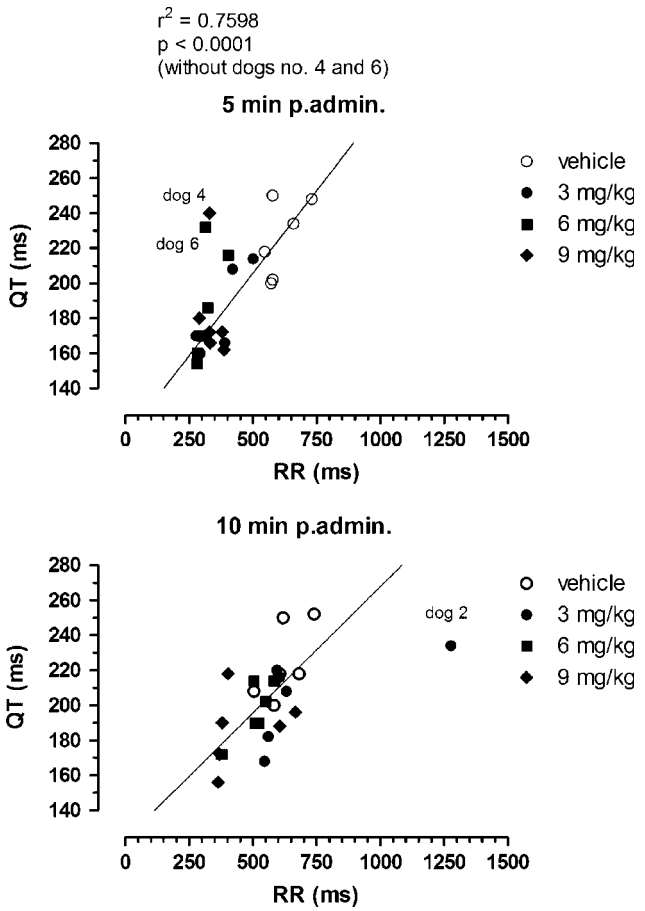

Fig. 5. Individual QT values obtained at 5 min (upper panel) and at 10 min (lower panel) after the start of the infusions plotted versus the corresponding RR values. Data obtained following the vehicle (saline) treatment (open circles) and data obtained following tapentadol infusions (filled symbols) were fitted by linear regression analysis. The following individual data were excluded from regression analysis: dog no. 6 at the 6-mg/kg dose level at 5 min, dog no. 4 at the 9-mg/kg dose level at 5 min, and dog no. 2 at the 3-mg/kg dose level at 10 min.

centrally mediated cardiovascular stimulation in conscious dogs [Wilkerson, 1988]. It must be noted that the infusion of tapentadol, even at the lowest dose (3 mg/kg), led to maximum plasma concentrations that were approximately 5 times higher than clinically relevant plasma exposure [Terlinden et al., 2007]. Accordingly, cardiovascular events have not been reported during clinical use of tapentadol [Rauschkolb-Loeffler et al., 2007; Daniels et al., 2009].

HR is a major determinant of the QT time [Ahnve and Vallin, 1982]. Accordingly, QT time decreased during and following tapentadol infusion when HR increased. The maximum shortening of the QT time was coincident with the peak tachycardia. Following cessation of tapentadol infusion, QT times returned toward baseline mainly in parallel to the decline of the HR. A prolongation of QT time following infusion of

TABLE 3. Serum Concentrations in Conscious Dogs Following Sequential Infusions of Tapentadol*

Dose (mg/kg)	Time after start of 5-min infusions (min)	Serum concentration (ng/ml)
3	6	655±93
	12	662±152
	30	190±43
6	6	1105±160
	12	1074±185
	30	407±76
9	6	2531±538
	12	1897±229
	30	580±134

*Values are expressed as means±SEM ($n = 6$).

tapentadol was not observed. The QT time shortening effect due to the increase in HR could be completely eliminated by correction of QT values for changes in HR using Fridericia's formula. Tapentadol had no effect on the HR-corrected QTc values at any dose. This implies that tapentadol has no effect on cardiac repolarization in conscious dogs. The usefulness of HR correction formulas, however, has limitations, and marked differences exist between QTc values when different calculation methods are used [Funck-Brentano and Jaillon, 1993]. The HR correction was especially critical because of the sharp increase in HR shortly after the start of the tapentadol intravenous infusion. Therefore, in addition to the HR correction of QT values, the relationship of uncorrected QT times to the RR intervals was analyzed. The correlation was done for individual values obtained at 5 min and at 10 min after the start of the infusions, i.e., during the maximum increase in HR. Correlation of QT times against the corresponding RR intervals comprised individual values from animals under treatment with saline or with tapentadol at the low, intermediate, and high dose. The QT/RR correlation was highly significant when fitted by linear regression analysis. Tapentadol thus did not interfere at any tested dose with the QT/RR relationship at the time of maximum elevation of HR. This confirms, in addition to the evaluation of HR-corrected QTc data, the absence of an effect of tapentadol on cardiac repolarization in conscious dogs. At 5 min after the start of the infusion, two individual QT/RR pairs, one evaluated in a dog infused with the intermediate dose and one in a dog infused with the high dose of tapentadol, were excluded from the QT/RR correlation. Their values obviously did not fit well with the other QT/RR pairs because QT times were longer than expected according to the corresponding RR intervals. At 10 min after the start of the infusion, the QT times of these individuals were back within the normal range. This points toward

TABLE 4. Comparison of Concentrations of Preclinical Surrogate Markers Regarding Cardiac Repolarization to Clinically Relevant Exposure of Tapentadol

Marker	System	Concentration, ng/ml (μM)	Effect
IC_{50} hERG	CHO cells	7,978 (36.1)	hERG inhibition
APD_{90}	Guinea pig papillary muscles	22,100 (100)[a]	No prolongation, shortening effect
QTc	Guinea pig isolated perfused heart	6,630 (30)[a]	No prolongation
QTc	Conscious dog	2,531 (12)[b]	No prolongation
C_{max}	Human volunteers	78 (0.4)	Tzschentke et al. [2006]

[a]Highest concentration tested.
[b]Maximum serum concentration (mean value) after highest test dose.

a delay of adaptation of QT time to rapidly changing RR intervals during the tachycardia. Hysteresis can take 2–3 min [Lau et al., 1988] during rapid changes in HR. Therefore, the 2 outliers represent a hysteresis phenomenon rather than an indication of a QT prolonging effect of tapentadol.

In conclusion, effects of tapentadol on in vitro and in vivo electrophysiological parameters occur at concentrations far above clinically relevant systemic exposure (Table 4). These concentrations are at least 2 orders of magnitude higher than the concentrations needed to activate μ-opioid receptors ($K_i = 0.1\,\mu M$) and to inhibit neuronal NE reuptake ($K_i = 0.5\,\mu M$), which are the primary activities of tapentadol [Tzschentke et al., 2007]. The observed effects thus represent unspecific activities at supratherapeutic doses or concentrations. The large separation between these doses or concentrations and the clinically relevant exposure of tapentadol indicates a sufficient safety margin. In addition to that, any inhibition of repolarizing cardiac potassium current is counterbalanced by inhibition of calcium and probably also sodium channels by tapentadol. These mixed ion channel activities at high tapentadol concentrations finally result in a neutral effect on cardiac repolarization, evident as a lack of any changes in QTc time, as demonstrated in guinea pig isolated hearts and in conscious dogs.

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