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ELECTROPHYSIOLOGICAL SAFETY OF SERTINDOLE IN DOGS WITH NORMAL AND REMODELED HEARTS

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Running title: Cardiac electrophysiology of Sertindole

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Abbreviations: CAVB, chronic atrioventricular block; CL, cycle length; HERG, human ether-a-go-go-related gene; IC_{50}, 50% inhibiting concentration; I_{Kr}, rapidly activating delayed rectifier potassium current; LV, left ventricle; MAP, monophasic action potential; QT_c, heart rate corrected QT interval; RV, right ventricle; TAP, transmembrane action potential; TdP, torsades de pointes arrhythmias.

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Abstract

Inhibition of the potassium current I_{Kr} and QT prolongation are associated with drug-induced torsades de pointes arrhythmias (TdP) and sudden cardiac death. We investigated the cardiac electrophysiological effects of sertindole, an antipsychotic drug reported to prolong the QT interval in schizophrenic patients. In cell cultures, sertindole appeared a selective blocker of I_{HERG} over other ion currents. For I_{HERG}, the IC_{50} was 64±7 nM, whereas I_{SCN5A}, $I_{Ca,L}$, $I_{Ca,T}$, $I_{KCNQ1/KCNE1}$ and $I_{Kv4.3}$ were blocked in the micromolar range. In canine ventricular myocytes, the IC_{50} for I_{Kr} inhibition by sertindole was 107±21 nM. Action potentials in these cells prolonged in a reverse-rate and concentration-dependent manner at 10-300 nM sertindole. In vivo, sertindole was administered to anesthetized dogs at clinically relevant (0.05-0.20 mg/kg) and high doses (1.0-2.0 mg/kg) IV. At 0.05-0.20 mg/kg sertindole (plasma concentrations: 30-157 nM), QT_c was prolonged by 1-5 % in normal dogs and by 9-20% in dogs with remodeled hearts due to chronic AV block (CAVB). TdP were not induced at these doses in normal dogs or in CAVB dogs with reproducible induction of TdP by dofetilide in previous experiments. At 1.0-2.0 mg/kg sertindole (plasma concentrations: 0.5-3.1 µM), QT_c prolonged by 6-11% in normal dogs and by 22% in dofetilide-sensitive CAVB dogs. TdP occurred in 3/5 animals in the latter group. Thus, at high IV doses sertindole can pose a serious proarrhythmic risk when electrical remodeling of the ventricles is present. At clinically relevant doses, however, sertindole does not cause TdP in anesthetized dogs with normal or remodeled hearts.

An estimated 1% of the population suffers from various degrees of schizophrenia with a significant burden on the health budget due to long-term hospitalization of these patients (WHO, 2001). The average schizophrenic patient has a 10-year shorter duration of life than the rest of the population and suicidal rates are as high as 10% (WHO, 2001). Treatment of these patients is not optimal since 30% respond poorly or not at all to available drugs and non-compliance is high, in part due to neurological side effects (Oehl et al., 2000).

Sertindole (5-chloro-1-(4-fluorophenyl)-3-(1-(2-(2-imidazolidinon-1-yl)-ethyl)-4-piperidyl)-1*H*-indole) is an antipsychotic compound synthesized in the mid-1980s and introduced on the European market in 1996. Clinical phase-III trials showed therapeutic effectiveness against both positive and negative symptoms of schizophrenia (Hale et al., 2000), whereas extrapyramidal symptoms were absent (Zimbroff et al., 1997). Sertindole has a high affinity for several serotonin and dopamine receptor subtypes and α_{1A}-adrenergic receptors (Ipsen et al., 1997; Arnt, 1998; Bigliani et al., 2000). Furthermore, a high inhibitory effect on the current mediated by the potassium channel encoded by HERG has been shown in vitro (Rampe et al., 1998). HERG blocking properties with IC_{50}s ranging from low nM to low µM are common for most antipsychotic drugs (Frederiksen and Adamantidis, 2000; Haverkamp et al., 2002; Kongsamut et al., 2002) and may explain the drug-induced QT prolongation caused by some of them (Haverkamp et al., 2000).

In 1998, sertindole was withdrawn from the market due to concern about the high ratio of proven or suspected ventricular arrhythmias and sudden deaths in patients (Moore, 2002). QT_c intervals were prolonged in 4-5% of patients receiving sertindole (Kasper et al., 1998) and prolongation of action potential duration was confirmed in isolated feline (Drici et al., 1998) and rabbit hearts (Eckardt et al., 2002). Following reevaluation of the existing data, and based on new preclinical, clinical and epidemiological information, the concern about cardiac risk was outweighed by the therapeutic benefits of sertindole. This led to the reintroduction of sertindole on the European market in 2002 along with a prospective surveillance study of all patients taking the drug (Toumi, 2002).

In the present study we investigated the cardiac electrophysiological effects of sertindole in vitro and in vivo to provide an ionic basis for repolarization prolongation by the drug in relation to possible proarrhythmic actions in intact dogs.

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Methods

Animal handling was in accordance with the 'European Directive for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (86/609/EU)'. The 'Committee for Experiments on Animals' of Maastricht University approved all experiments.

Measurements on ion currents in cell cultures. CHO cells were stably transfected with human cloned HERG (I_{HERG}; GENION, Germany) and SCN5A (I_{SCN5A}; obtained from Dr. R. Kallen, University of Pennsylvania, Philadelphia) representing the rapidly activating delayed rectifier potassium current and fast inward sodium current, respectively. Maximal outward I_{HERG} and peak I_{SCN5A} were measured. KCNQ1 and KCNE1 were stably co-transfected in CHO cells ($I_{KCNQ1/KCNE1}$; GENION, Germany) representing the slowly activating delayed rectifier potassium current. HEK293 cells transiently expressing $Kv4.3_{\Delta 2\text{-}39}$ ($I_{Kv4.3}$) were used to assess the transient outward potassium current. Calcium-current experiments were performed on a NG-108-15 neuroblastoma-glioma hybrid cell line expressing endogenous L- and T-type calcium channels. Standard voltage-clamp protocols, electrodes, superfusion and internal solutions were used. Ion currents were measured at control and after 5 min incubation of sertindole at various concentrations. Each data point consisted of measurements from 3 – 8 cells. IC_{50}s were obtained by fitting the data to a two-parameter sigmoidal curve ($I = c^n/(c^n + IC_{50}{}^n)$).

Experiments in isolated canine ventricular myocytes. Twelve mongrel dogs (body weight: 29 ± 5 kg, 8 males) were sacrificed for myocyte isolation. Thoracotomy was performed under anesthesia. Heparin (10.000 IU) was administered IV to avoid intracoronary clotting. After quick excision, the heart was placed in cold oxygenated cardioplegic solution and the coronary circulation was cannulated via the aorta. The heart was mounted to a constant-pressure Langendorff-like setup and perfused for 5 min using a Tyrode's solution with nominal $[Ca^{2+}]$. Collagenase A (Roche Diagnostics GmbH, Germany) in 0.5 µM Ca^{2+} Tyrode with 0.5 mg/ml BSA perfused the heart for 30-35 min, followed by 0.2 mM Ca^{2+} Tyrode for 5 min to washout the collagenase. Midmyocardial cells were harvested from the free wall of both ventricles, gently minced, filtered, washed and stored in 0.2 mM Ca^{2+} at room temperature until use within 24 hours after isolation.

Myocytes were selected for experiments if they had sharp striations, clear contours and transparent cytoplasms without granulations or blebs. Further criteria for action-potential experiments included a stable resting membrane potential below -70 mV and a 'spike-and-dome' morphology of the action potential.

Whole-cell currents were recorded (AxoPatch 1D, Axon Instruments, Inc) using borosilicate glass patch pipettes filled with internal solution (in mM: K-asp 125, KCl 20, $MgCl_2$ 1.0, MgATP 5, HEPES 5, EGTA 10; pH

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adjusted to 7.2 with KOH) having a resistance between 1.0 and 3.0 MΩ. Cells were superfused with a standard buffer solution (in mM: NaCl 145, KCl 5.4, $MgCl_2$ 1.0, glucose 11, HEPES 10, $CaCl_2$ 1.8, nifedipine 0.005; pH adjusted to 7.4 with NaOH, 37 ºC). Sertindole was dissolved in DMSO. The rapidly activating delayed rectifier potassium current I_{Kr} was measured as the tail current fraction fully blocked by 2 µM almokalant (Carmeliet, 1993).

Transmembrane action potentials (TAP) were recorded (AxoClamp 2B, Axon Instruments, Inc) using sharp glass microelectrodes filled with 3 M KCl and with a resistance between 20 and 60 MΩ. Cells were superfused with the same solution as in the whole-cell current experiments except that nifedipine was left out. Addition of 2 µM almokalant to the superfusate was used to fully block I_{Kr}. Action potentials were recorded at each cycle length (CL) of 300, 400, 500, 1000 and 2000 ms. Action potential duration at 95% of repolarization (APD_{95}) is presented as the average of five beats > 100 beats after a change in pacing CL.

In-vivo experiments. Twenty-four anesthetized dogs (body weight: 29 ± 4 kg, 11 males) were used for these experiments. In 13 animals complete AV block was induced (van Opstal et al., 2001a). After 4 ± 1 weeks of AV block (chronic AV block; CAVB), the dogs were subjected to a TdP-susceptibility test using the I_{Kr} blocker dofetilide. Only if a dog showed reproducible TdP upon 25 µg/kg/5 min dofetilide, it was selected for the sertindole experiments. Thus, dofetilide was used as the positive reference compound. The average time between two experiments in a dog was 2 ± 1 weeks.

Anesthesia, perioperative care, signal processing, data recording and off-line analysis have been described elsewhere (van Opstal et al., 2001a). Standard and precordial ECGs were recorded. In addition, biventricular endocardial monophasic-action-potential (MAP) recordings were made (EP Technologies, Sunnyvale, CA).

RR and QT intervals in lead II, left- and right-ventricular MAP duration (LV MAPD and RV MAPD, respectively) at 100% repolarization were measured offline and averaged from five consecutive beats. The interventricular dispersion of repolarization (ΔMAPD) was calculated as the difference between the LV and RV MAPD. QT intervals were corrected for heart rate (QT_c) according to Van de Water's formula (Van de Water et al., 1989). The number of ectopic beats, defined as short-coupled beats arising from a new ventricular focus before complete repolarization of the previous beat, were counted during 10 min after administration of the drug. Both single and multiple ectopic beats were counted. The latter are considered more proarrhythmic. TdP was defined as a polymorphic ventricular tachycardia consisting of 5 or more beats twisting around the isoelectric line of the ECG in the setting of a prolonged QT interval.

Sertindole (MW: 441 g/mol) was dissolved in 0.1 M HCl and diluted in 10% hydroxypropyl cyclodextrin and 0.05 M phosphatebuffer (1:1). pH was adjusted to 7.4. The solution was filtered through a 22-µm pore filter prior to use. Sertindole was administered over 5 minutes through a cephalic vein and blood samples were taken from the contralateral cephalic vein to measure plasma concentrations.

Plasma analysis. Blood samples were obtained 5, 10 and 25 minutes after drug administration and plasma was stored at –20 ºC until analysis at H. Lundbeck A/S (Copenhagen, DK). Sertindole plasma samples were extracted by solid mixed phase extraction. The sample extracts were analyzed by a normal-phase HPLC method with a mobile phase consisting of heptane, 2% piperidine in 2-propanol and water (100/20/0.45) and quantified by fluorescence detection with excitation/emission wavelengths at 260 and 340 nm, respectively. The method had a mean recovery of 90% with a quantification limit of 0.5 ng/ml. Total plasma concentrations (free + bound) are reported.

Statistics. Electrophysiological parameters were compared to control employing (repeated-measures) ANOVA followed by Bonferroni's test. Comparisons between controls were performed with an unpaired Student's t-test. Data are reported as mean ± s.e.m. A $P < 0.05$ was considered statistical significant.

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Results

Sertindole is selective for I_{HERG}. In Fig. 1A the molecular structure of sertindole is shown. Fig. 1B shows concentration-response curves for the various cardiac ion channels expressed endogenously or by transfection in cell cultures. Sertindole inhibited I_{HERG} in a concentration-dependent manner over the range of 10-1000 nM, with 50% block at 64 ± 7 nM. $I_{KCNQ1/KCNE1}$ was inhibited by 10 ± 5 % at 300 nM sertindole (IC_{50}: 6.9 ± 2 μM), whereas other currents (I_{SCN5A}, I_{CaL}, I_{CaT}, $I_{Kv4.3}$) were only inhibited at micromolar concentrations. Representative examples of the six currents are shown in Fig. 2.

Sertindole blocks I_{Kr} in canine ventricular myocytes. Activation of I_{Kr} occurred at depolarizations to > -10 mV and showed saturation at conditioning voltages (V_{cond}) ≥ 20 mV (Fig. 3B). Maximal I_{Kr} density at control was 0.14 ± 0.07 pA/pF. Boltzmann fit to the data revealed a half-maximal activation at 11 ± 1 mV and a slope factor of 5.9 ± 0.9 pA/pF/mV. Half-time for I_{Kr} deactivation upon repolarization to –50 mV was 294 ± 23 ms.

An example of I_{Kr} recorded under control conditions and under the influence of 100 nM sertindole is shown in Fig. 3A. Sertindole inhibited I_{Kr} tails in a concentration-dependent and voltage-independent manner. At 300 nM, the maximal I_{Kr} tail density had decreased to 57 ± 4% to 0.08 ± 0.004 pA/pF ($P < 0.05$; Fig. 3B). Boltzmann fit of the remaining I_{Kr} at 300 nM sertindole showed a half-maximal activation at 8 ± 2 mV (P = NS vs. control) and a slope factor of 4.2 ± 1.4 pA/pF/mV (P = NS vs. control). Half-time for deactivation was 273 ± 48 ms (P = NS vs. control). Fig. 3C shows an example of the effects of accumulating concentrations of sertindole to illustrate the concentration dependency of the drug on I_{Kr}. Using multiple voltage protocols to analyze the properties of I_{Kr} under the influence of sertindole, a concentration-response relationship was obtained (Fig. 3D). Sertindole inhibited I_{Kr} in a concentration-dependent manner over the full range of 10-1000 nM, with 50% block at 107 ± 21 nM (n_{cells} = 10).

Sertindole prolongs the transmembrane action potential. TAP in normal canine ventricular myocytes prolonged from 166 ± 5 to 278 ± 13 ms by increasing pacing CL from 300 to 2000 ms (n_{cells} = 11). Concentration-dependent prolongation of APD_{95} was observed for 10-300 nM sertindole, reaching statistical significance at 100 nM and higher, and for CL ≥ 400 ms (Fig. 4). Under the influence of 300 nM sertindole, APD_{95} was prolonged to 197 ± 15 ms (18 %) and 345 ± 44 ms (24 %) at 300 and 2000 ms CL, respectively ($P <$ 0.05 for both CL), showing clear reverse-rate dependency. Early afterdepolarizations or abnormal automaticity were not observed.

Sertindole causes moderate prolongation of repolarization in normal hearts in vivo. Cumulative doses of 0.05, 0.10 and 0.20 mg/kg sertindole (30-min intervals) were administered to 5 dogs. Plasma concentrations

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ranged from 33 ± 1 nM after 0.05 mg/kg to 157 ± 18 nM after 0.20 mg/kg. Reported plasma concentrations after human therapeutic dosing are 22 ± 12 to 158 ± 63 nM (Wong and Granneman, 1998), hence we considered these doses in the dogs to be clinically relevant. Representative examples of the electrophysiological effects are shown in Fig. 5. QT_c interval did not prolong at 0.05 or 0.10 mg/kg sertindole. At 0.20 mg/kg QT_c prolonged from 277 ± 11 ms to 292 ± 20 ms (5%, $P < 0.05$; Fig. 6). At this dose, the RR interval increased from 465 ± 35 ms to 545 ± 47 ms (17%, $P < 0.05$) and the QT interval from 231 ± 7 ms to 252 ± 12 ms (9%, $P < 0.05$). The LV MAPD prolonged from 191 ± 8 ms to 213 ± 9 ms (10%, $P < 0.05$) whereas the RV MAPD remained unchanged (181 ± 9 ms to 197 ± 7 ms; P = NS), leaving the interventricular dispersion of repolarization unaltered.

Cumulative doses of 0.5, 1.0 and 2.0 mg/kg sertindole (30-min interval) were administered to 6 other dogs. Plasma concentrations ranged from 0.5 ± 0.2 µM after 0.5 mg/kg to 3.1 ± 0.3 µM after 2.0 mg/kg. Twenty-four hours after the high dose-range experiments the mean plasma concentration was 269 ± 31 nM. All high doses produced significant QT_c increases (Fig. 6) with a maximal QT_c prolongation from 294 ± 8 ms to 326 ± 19 ms (11%, $P < 0.05$) after 2.0 mg/kg sertindole. This involved a QT prolongation from 251 ± 10 ms to 289 ± 24 ms (15%, $P < 0.05$), whereas RR interval remained unchanged. LV MAPD increased from 214 ± 11 ms to 264 ± 28 ms (23%, $P < 0.05$) and RV MAPD from 208 ± 11 ms to 242 ± 22 ms (16%, $P < 0.05$). The interventricular dispersion of repolarization was not changed (8 ± 2 ms to 20 ± 12 ms; P = NS).

Sertindole induced no changes in the PQ interval or QRS duration. Apart from the QT prolongation, no major changes were seen in the T-wave morphology at low or high doses of administration (Fig. 5).

Sertindole carries a proarrhythmic risk in electrically remodeled hearts. Ten dofetilide-susceptible CAVB dogs received sertindole. In 5 animals 0.10 mg/kg was administered, followed by another 0.20 mg/kg after 30 min. The QT_c interval prolonged more than in normal dogs (e.g., 20% after 0.20 mg/kg in CAVB dogs vs. 5% in normal dogs). Electrophysiological data from these experiments are summarized in the Table. The 5 other dogs were tested with 1.0 mg/kg sertindole (Table). Sertindole prolonged repolarization in a dose-dependent manner, whereas the CL of the idioventricular rhythm only increased at the high dose (Table). The high dose of sertindole caused reproducible TdP in 3 of 5 dogs (Fig. 7). In these 3 animals, the first TdP was seen on average 7 ± 2 minutes after start of the 1.0 mg/kg sertindole infusion (range 6 – 9 min). The 2 dogs not responding with TdP received another 1.0 mg/kg, which caused TdP in 1 dog. During the 1-hour observation period after 1.0 mg/kg sertindole, a total of 19 TdP (6 ± 2, n_{dogs} = 3) were seen of which 4 TdP had to be cardioverted electrically. Single ectopic beats (16 ± 9) occurred in all dogs at high dosing, while multiple ectopic beats (5 ± 2) were seen

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in 4 dogs. Interventricular dispersion of repolarization tended to increase, e.g. from 45 ± 6 to 79 ± 19 ms at 1.0 mg/kg ($P = 0.09$).

Electrophysiological data on the positive reference compound dofetilide. Concentration-response studies of dofetilide on I_{Kr} in native ventricular myocytes revealed an IC_{50} of 46 ± 9 nM (Fig. 8A). Prolongation of TAP in the myocytes was reverse-rate dependent (Fig. 8B). In normal anesthetized dogs, IV doses of 12.5, 25 and 50 µg/kg dofetilide (van Opstal et al., 2001a) caused significant QT_c prolongation (19% - 25%; $P < 0.05$ vs. control; Fig. 8C). RR also increased, e.g., by 13% after 12.5 µg/kg ($P < 0.05$ vs. control). Plasma concentrations of dofetilide are given in Fig. 8C. Dofetilide (25 µg/kg) induced TdP in 10/13 anesthetized CAVB dogs (Fig. 8D for $n_{dogs} = 10$ used for sertindole testing in vivo).

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Discussion

The present study investigates the electrophysiological properties of sertindole from cloned cardiac ion channels to anesthetized dogs with normal and remodeled hearts. The results can be summarized as follows: 1: Sertindole is a selective blocker of I_{HERG} over other ion currents expressed in cell cultures. 2: Sertindole causes concentration-dependent block of native I_{Kr} and this translates into reverse-rate dependent lengthening of myocyte action potentials. 3: In anesthetized dogs, dose-dependent prolongation of in-vivo repolarization by sertindole is observed. 4: Clinically relevant doses of sertindole do not cause TdP in anesthetized normal dogs or in CAVB animals with reproducible induction of TdP by dofetilide in previous experiments. 5: High doses of sertindole induce multiple ectopic beats and TdP in the majority of these CAVB dogs.

Normal and remodeled hearts. To elucidate whether a drug is devoid of proarrhythmic properties, a reproducible animal model is essential. Testing drugs in normal hearts is necessary, but is not sufficient for the recognition of proarrhythmic effects in the diseased heart. We used the canine model with CAVB, known to have acquired QT prolongation. Creation of CAVB results in a bradycardia-induced volume overload. Hypertrophy is observed in ventricular myocytes (Volders et al., 1998) as well as in the whole heart (Vos et al., 1998; Verduyn et al., 2001a). Contractile remodeling in vivo restores initially-depressed cardiac output (compensated function), which is associated with an increased cytosolic Ca^{2+} transient in vitro (de Groot et al., 2000; Sipido et al., 2000). Downregulation of I_{Ks} and I_{Kr} (Volders et al., 1999; Ramakers et al., 2003) and upregulation of the sodium-calcium exchanger (Sipido et al., 2000) contribute to the electrical alterations in remodeled CAVB hearts. This ventricular remodeling predisposes to TdP and sudden cardiac death (van Opstal et al., 2001c).

Whereas most class-III-antiarrhythmic drugs cause TdP in 2 - 5% of patients (Haverkamp et al., 2000), an incidence in the order of 56 - 67% is encountered in anesthetized CAVB dogs, making the model very sensitive (Verduyn et al., 1997; van Opstal et al., 2001a). In the present study, the non-cardiovascular drug sertindole was tested in a number of different ways and using a broad dose regimen. Based on earlier clinical reports of low proarrhythmia of sertindole in patients (0.3% cardiac mortality rate or ~10% of anti-arrhythmic drugs (Kasper, 2002)), we anticipated a low TdP incidence in the CAVB dog. Serial testing in this model has shown reproducible induction of TdP (Verduyn et al., 2001b). Therefore, we chose to increase the sensitivity of the model and to evaluate the proarrhythmia of sertindole only in dogs that showed reproducible TdP after administration of 25 µg/kg dofetilide.

Cardiac safety of sertindole. This is the first report on sertindole, in which both in-vitro and in-vivo investigations are combined. Sertindole caused prolongation of repolarization in both normal and CAVB dogs, although at variable degree. E.g., at 0.20 mg/kg, QT_c interval increased by up to 5% in normal hearts and up to 20% in CAVB dogs. The plasma concentrations measured in dogs in this study at the low doses were comparable with plasma concentrations from human volunteers (4 to 20 mg/day sertindole PO range from 22 ± 12 nM to 158 ± 63 nM (Wong and Granneman, 1998)). These doses did not cause TdP in dofetilide-sensitive CAVB dogs. Administration of 25 µg/kg dofetilide led to a plasma concentration of 79 ± 11 nM. Reported plasma concentrations from human volunteers receiving dofetilide ranged from 5 - 23 nM (Pfizer, 1999).

Eckardt et al. reported a low torsadogenic potential of sertindole in isolated rabbit hearts (Eckardt et al., 2002). They showed a 15-17% prolongation of the QT interval at a perfusion concentration of 1.5 µM sertindole without induction of TdP. In the present investigation in anesthetized dogs with normal hearts, 9% prolongation of the QT_c interval was observed at 1.3 ± 0.1 µM. No TdP was observed, confirming the results from Eckardt et al. Plasma protein binding in vivo and unknown levels of accumulation in cardiac tissue complicate comparisons between these models.

Relating plasma concentrations to concentrations employed in the in vitro setting can only be done with great caution. Among the factors to be taken into account are plasma protein binding, tissue accumulation and the distance between the plasma protein and the receptor on the cardiomyocyte in situ. Plasma protein binding of sertindole in humans is high (> 99% (Ereshefsky, 1996)), indicating a free plasma concentration of maximally 1 – 2 nM after therapeutic administration, based on the plasma concentrations in human volunteers (Wong and Granneman, 1998). The level of accumulation in cardiac tissue is unknown, but a rather large volume of distribution is reported (20 - 40 L/kg (Ereshefsky, 1996)), indicating accumulation of sertindole in various tissues. In our dogs, maximal QT prolongation was already seen 5-10 minutes after the start of infusion of sertindole, suggesting a rapid inhibition of I_{Kr} once the drug is present in the circulation. The relative I_{Kr} block induced by sertindole in vivo or in the clinic could be underestimated when plasma concentrations are compared to in vitro concentrations.

Apart from an inhibition of I_{Kr}, sertindole has also been reported to block the human dopamine D_2 and the 5-HT_{2A} receptors (Arnt, 1998). It does also show α_{1A}-blocking properties in rat mesenteric arteries (Ipsen et al., 1997). New studies are required to test possible additional electrophysiological properties of sertindole in the heart under conditions when physiological levels of these agonists are present.

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Pharmacological implications. Earlier studies using chronic amiodarone administration have shown that TdP can be absent in CAVB dogs despite prolongation of the QT_c interval by 21% (van Opstal et al., 2001b). The present study indicates again a poor association between the degree of QT_c prolongation and the incidence of TdP (Table): at a comparable QT_c after 0.2 and 1.0 mg/kg sertindole, TdP were only induced after the higher dose. This stresses the importance of testing several doses when assessing the proarrhythmic potential of a drug, but also the relevance of addressing other proarrhythmic factors like ectopic beats and dispersion.

If our in-vitro data from cell cultures and isolated canine myocytes would have determined the future for sertindole, the drug would have likely been abandoned from further development (e.g., based on the recommendations of the 'Policy Conference of the European Society of Cardiology' (Haverkamp et al., 2000)). The expansion of our study to in-vivo testing showed a discrepancy between the in-vitro finding of I_{Kr} inhibition and prolonged cellular repolarization and the absence of arrhythmias in normal anesthetized dogs. Our data strongly advocate the use of pathological animal models when testing for proarrhythmic properties of cardiovascular and non-cardiovascular drugs.

A recent risk-benefit analysis of the preclinical and clinical data on sertindole by the European 'Committee for Proprietary Medicinal Products' led to the reintroduction of sertindole to the European market in 2002.

Limitations. Steady-state plasma concentrations were not obtained in this study, as opposed to previous clinical studies, and the pharmacokinetic difference between acute IV and repeated oral dosing should be considered when extrapolating our data to humans. Differences in accumulated tissue concentrations after acute IV versus chronic oral administration will likely exist. Furthermore, species differences between dogs and patients should be taken into account.

In conclusion, in-vitro studies clearly show sertindole's selective inhibition of I_{HERG} over other ion currents. Block of native I_{Kr} forms the ionic basis for action-potential prolongation in canine ventricular myocytes and QT prolongation in vivo. At high IV doses, sertindole can pose a serious proarrhythmic risk when electrical remodeling of the ventricles is present, as in dogs with CAVB. At clinically relevant doses, sertindole does not cause TdP in anesthetized dogs with normal or remodeled hearts.

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Footnotes

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Legends for figures

Fig. 1. Selective block of I_{HERG} by sertindole. *A:* Chemical structure of sertindole (MW: 441 g/mol). *B:* Composite data on blocking effects of sertindole on I_{HERG}, I_{SCN5A}, $I_{Ca,L}$, $I_{Ca,T}$, $I_{KCNQ1/KCNE1}$ and $I_{Kv4.3}$ in heterologous and endogenous expression systems. Data points represent the means for 3 to 8 cells. Shown are the concentration-response curves, where the remaining current is plotted relative to its control level. IC_{50}s for the different currents are given in the legend. Hill coefficients are 1.16 for I_{HERG}, 1.27 for I_{SCN5A}, 1.15 for $I_{Ca,L}$, 3.98 for $I_{Ca,T}$, 0.99 for $I_{KCNQ1/KCNE1}$ and 2.05 for $I_{Kv4.3}$.

Fig. 2. Representative tracings of I_{HERG}, I_{SCN5A}, $I_{Ca,L}$, $I_{Ca,T}$, $I_{KCNQ1/KCNE1}$ and $I_{Kv4.3}$ during treatment with sertindole. Concentrations of sertindole were chosen to indicate selectivity for I_{HERG} block. Insets, voltage-clamp protocols.

Fig. 3. Effects of sertindole on I_{Kr} in normal canine ventricular myocytes. *A:* Representative current recordings at control (arrows) and during 100 nM sertindole (C_m= 207 pF). Left horizontal bar is at 600 pA. Below are illustrated second-order exponential fits of the tail currents at control (black arrow), full I_{Kr} block (white arrow) and for 100 nM sertindole. Current axis is enlarged, but time axis is identical to current trace above. *B:* I_{Kr}-tail densities (difference currents at control and during 300 nM sertindole minus full block by almokalant; n_{cells} = 6). * $P < 0.05$ vs. control. Inset: voltage-clamp protocol. *C:* Tail-current amplitudes in a representative cell during increasing concentrations of sertindole (C_m = 191 pF). Almokalant (2 µM) provides full I_{Kr} block. Voltage-clamp protocol as in A. *D:* Concentration-response curve of I_{Kr} inhibition by sertindole. IC_{50} is 107 ± 21 nM (Hill coefficient = 0.76, n_{cells} = 10).

Fig. 4. Transmembrane APD_{95} during sertindole treatment. Concentrations used were 10, 30, 100 and 300 nM. Pacing CL of 300, 400, 500, 1000 and 2000 ms were applied. Left and right vertical axes are identical. * $P <$ 0.05, 100 and 300 nM vs. control, n_{cells} = 9. Inset: representative action potentials at control and during 300 nM sertindole, CL = 2000 ms (0 mV level indicated, scale bar: 100 ms at –80 mV).

Fig. 5. Electrophysiological effects of cumulative doses of sertindole in an anesthetized normal dog. Three ECG leads, LV and RV MAP recordings in each panel. V6 refers to a precordial lead placed in the sixth intercostal space on the left lateral side of the thorax. RR intervals are above and QT times below lead II.

MAPDs are below each signal. ECG calibrated to 1 mV/cm. Scale bar to the right indicates 20 mV on the MAP signals. Horizontal scale bar is 1 s. In this example, the plasma concentrations measured at the time of these tracings (10 min after start of infusion) are 31, 77 and 121 nM after 0.05, 0.10 and 0.20 mg/kg, respectively.

Fig. 6. Relation between QT_c intervals and plasma concentrations of sertindole in anesthetized normal dogs. Cumulative doses of sertindole were administered IV as indicated ($n_{low\ doses}$ = 5 dogs, $n_{high\ doses}$ = 6 dogs). Plasma samples were obtained 10 min after start of drug infusion at each dose. * $P < 0.05$ vs. respective QT_c at control.

Fig. 7. Proarrhythmia by sertindole at high doses in an anesthetized CAVB dog. TdP (right-most panel) occurred at 6 minutes after infusion of 1.0 mg/kg sertindole. Proarrhythmia was not observed at the low dose of 0.2 mg/kg (second-left panel, 10 min) in this or all other CAVB dogs. Three ECG leads, LV and RV MAP recordings in each panel. RR intervals are above and QT time below lead II. MAPDs are below each signal. ECG calibrated to 1 mV/cm. Scale bar to the right indicates 20 mV on the MAP signals. Horizontal scale bar is 1 s.

Fig. 8. Electrophysiological data on the positive reference compound dofetilide. *A:* Concentration-response curve of the inhibition of I_{Kr} tails by dofetilide in normal canine ventricular myocytes (IC_{50}: 46 ± 9, Hill coefficient: 0.76, mean C_m = 180 ± 11 pF, n_{cells} = 9). 2 µM almokalant (Almo) was used for full block of I_{Kr}. *B:* Transmembrane APD_{95} upon increasing concentrations of dofetilide. * $P < 0.05$, 100 and 300 nM vs. control, n_{cells} = 5. Vertical axes are identical. Inset shows two representative action potentials at control and during 300 nM dofetilide, CL = 2000 ms (scale bar: 100 ms at –80 mV, 0 mV level indicated). *C:* QT_c prolongation by increasing doses of dofetilide IV to 6 anesthetized normal dogs and corresponding plasma concentrations. * $P <$ 0.05 vs. control QT_c. For comparison, plasma concentrations after oral administration to humans are 5 - 23 nM (plasma protein binding: 60 – 70%; volume of distribution: 3 L/kg (Pfizer, 1999)). *D:* Electrophysiological effects of 25 µg/kg dofetilide IV to the 10 anesthetized dogs with CAVB that were TdP-inducible and used for sertindole testing. Values in ms and percentage increases in brackets. * $P < 0.05$ vs. control. Singles and multiples, numbers of single and multiple ventricular ectopic beats. TdP and shocks, number of TdP and electrical cardioversions. Times after start of dofetilide infusion.

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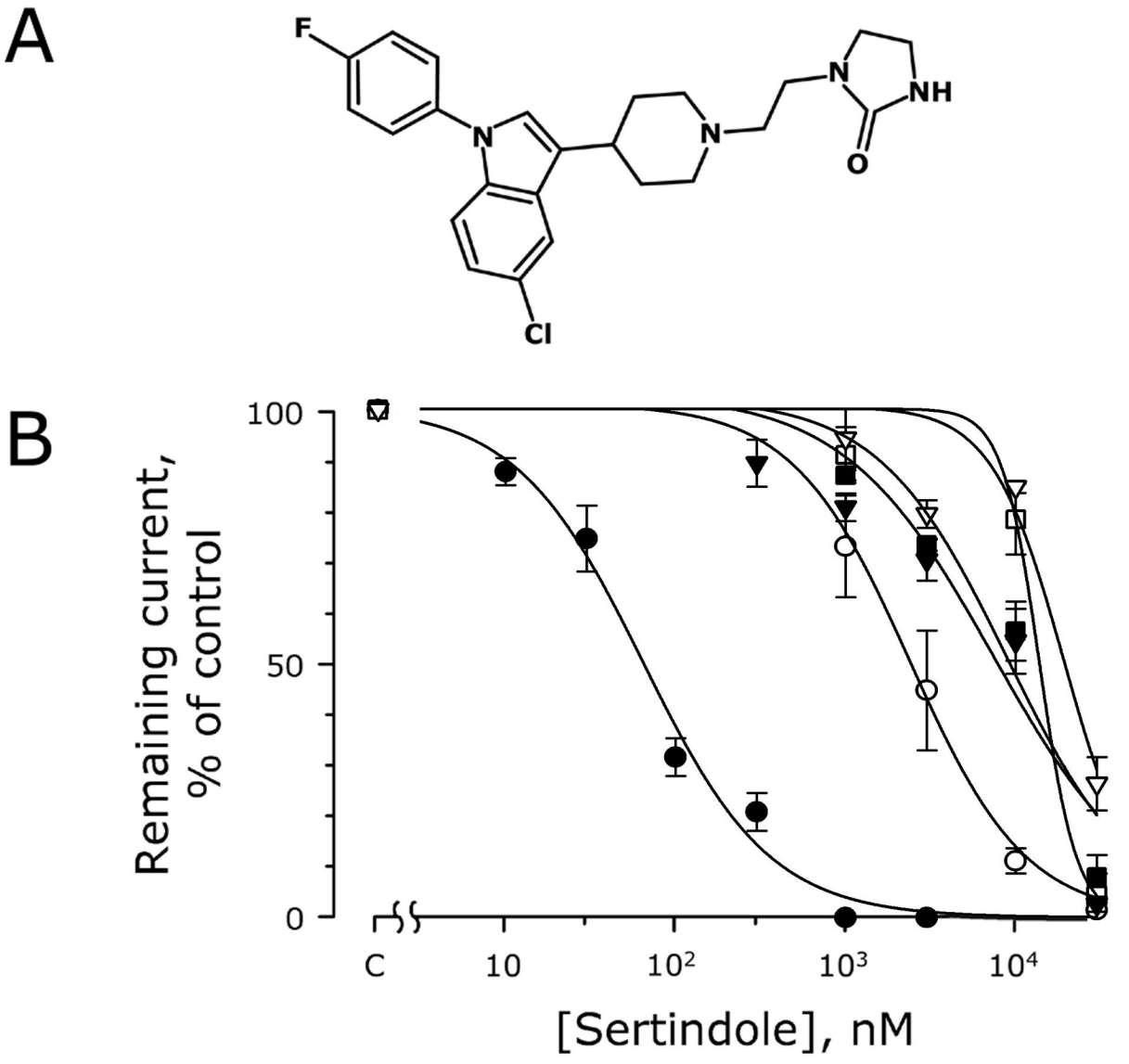

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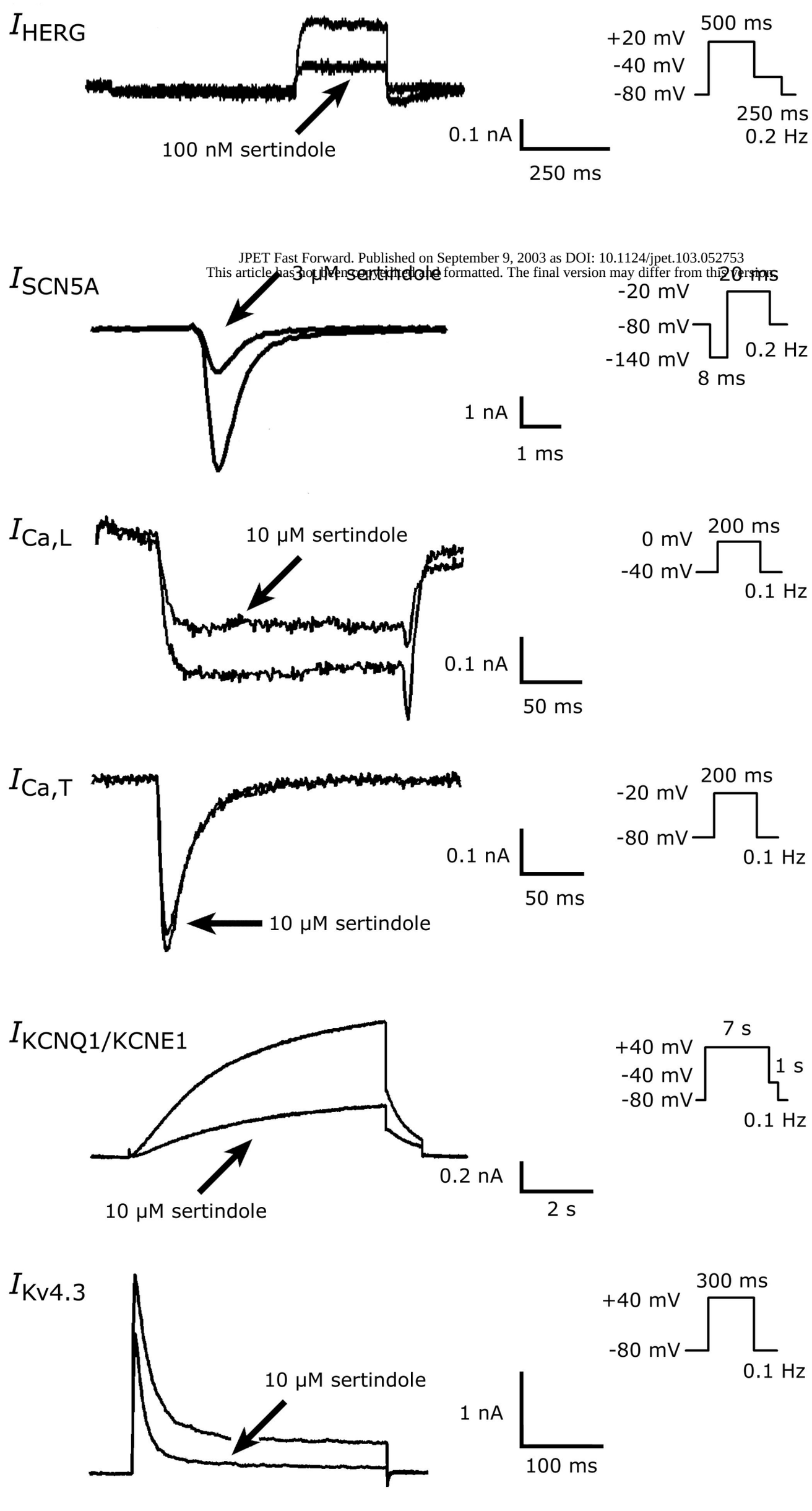

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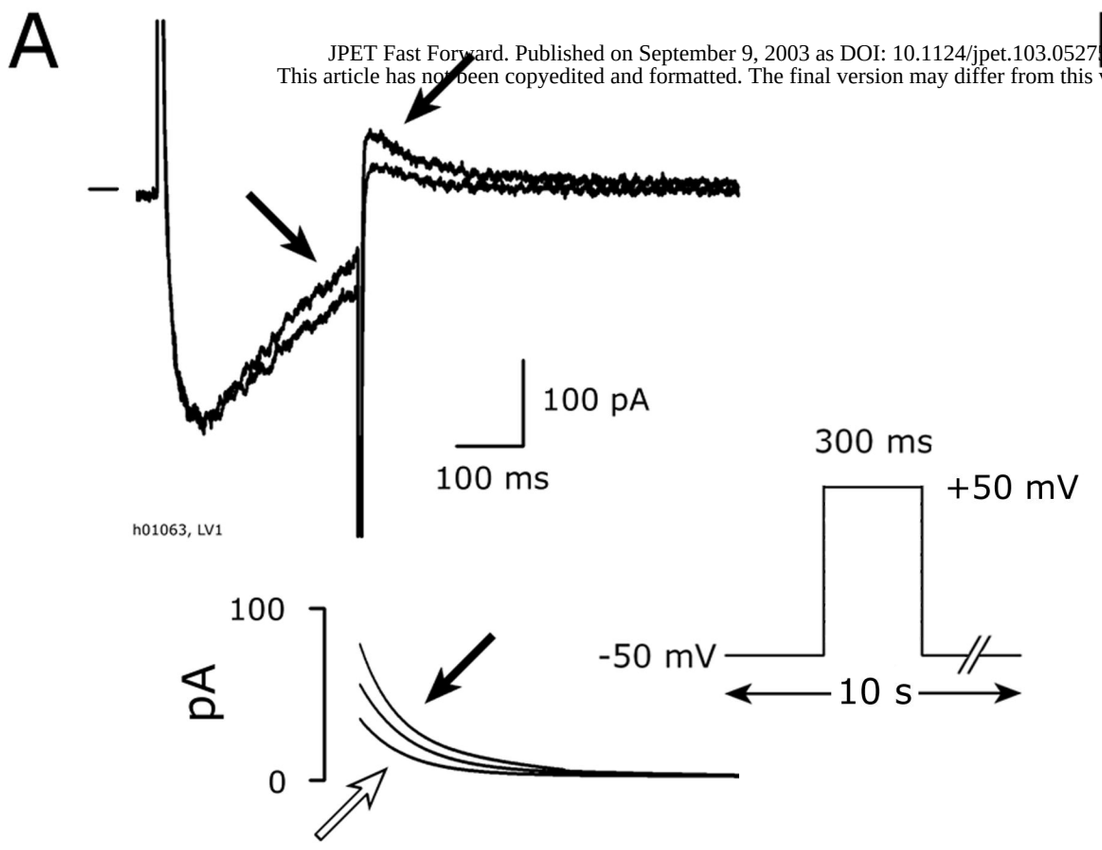

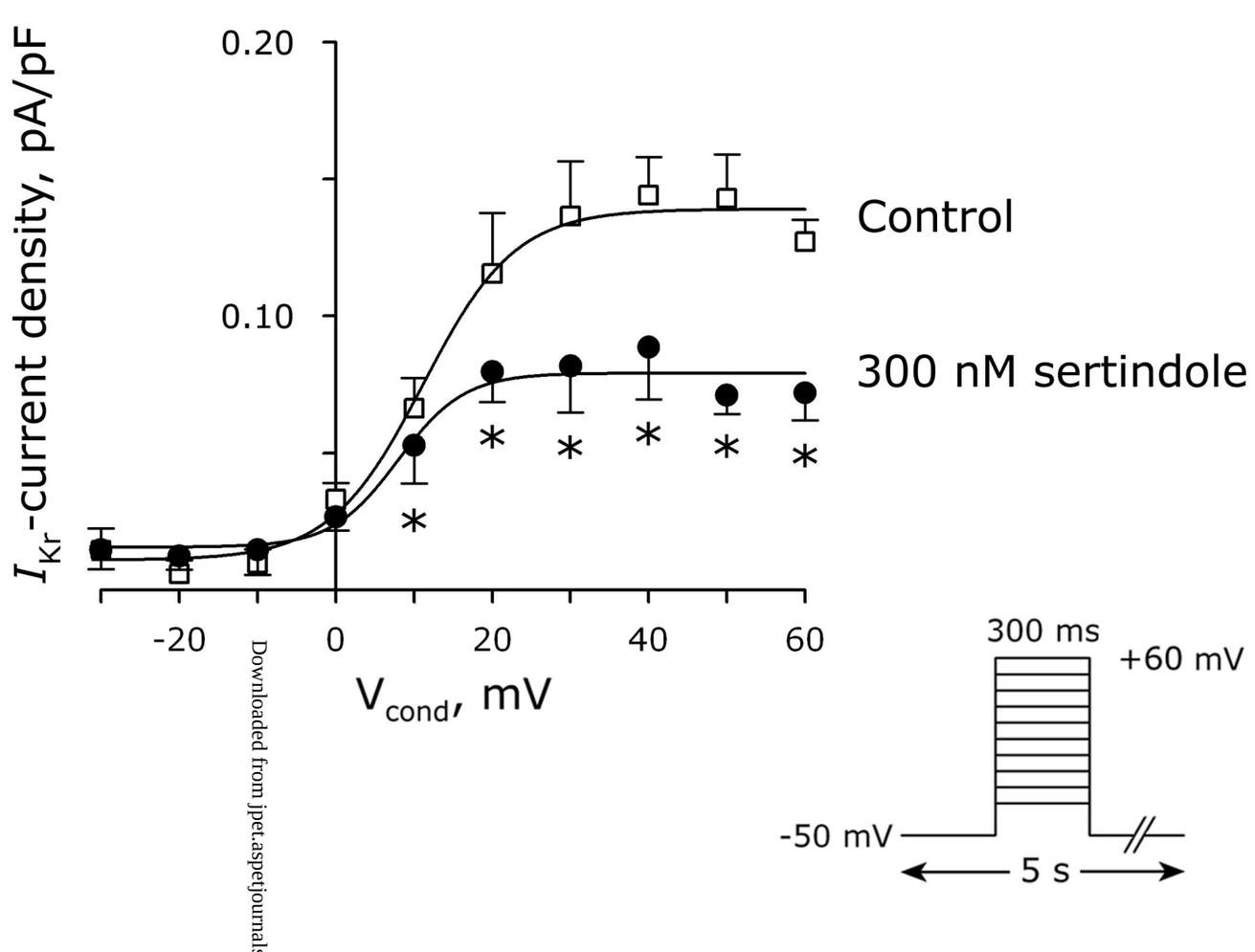

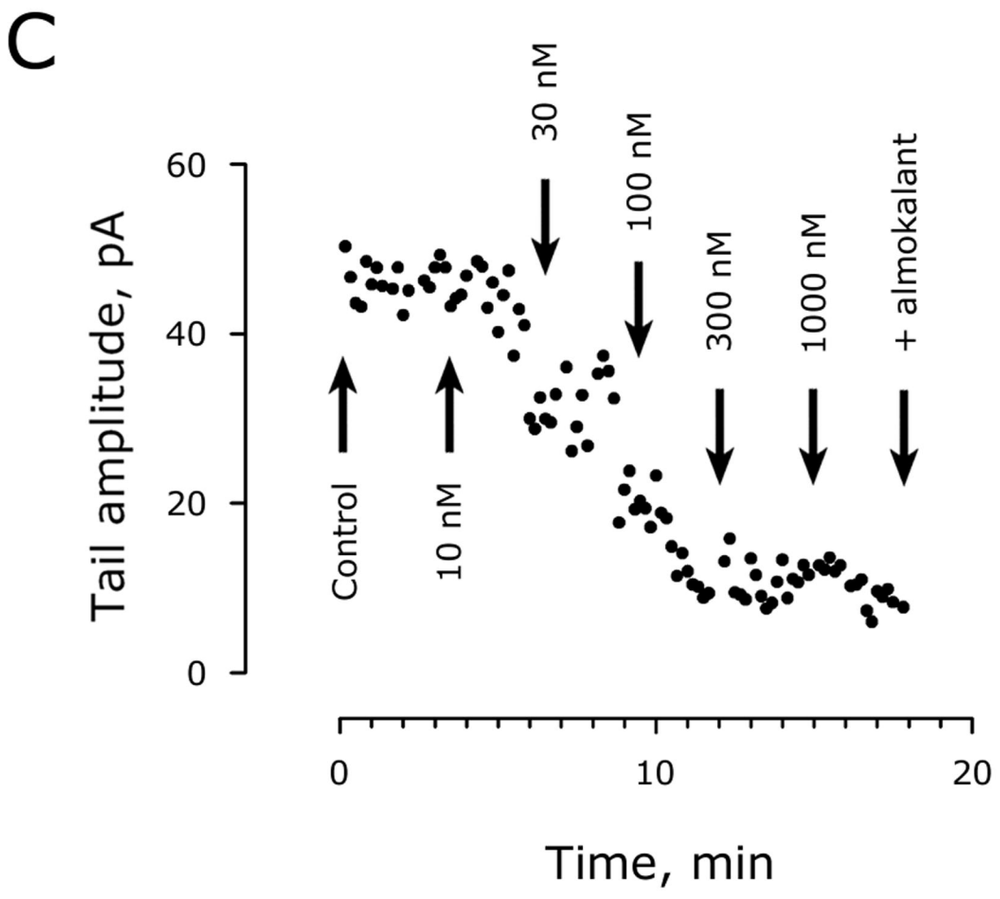

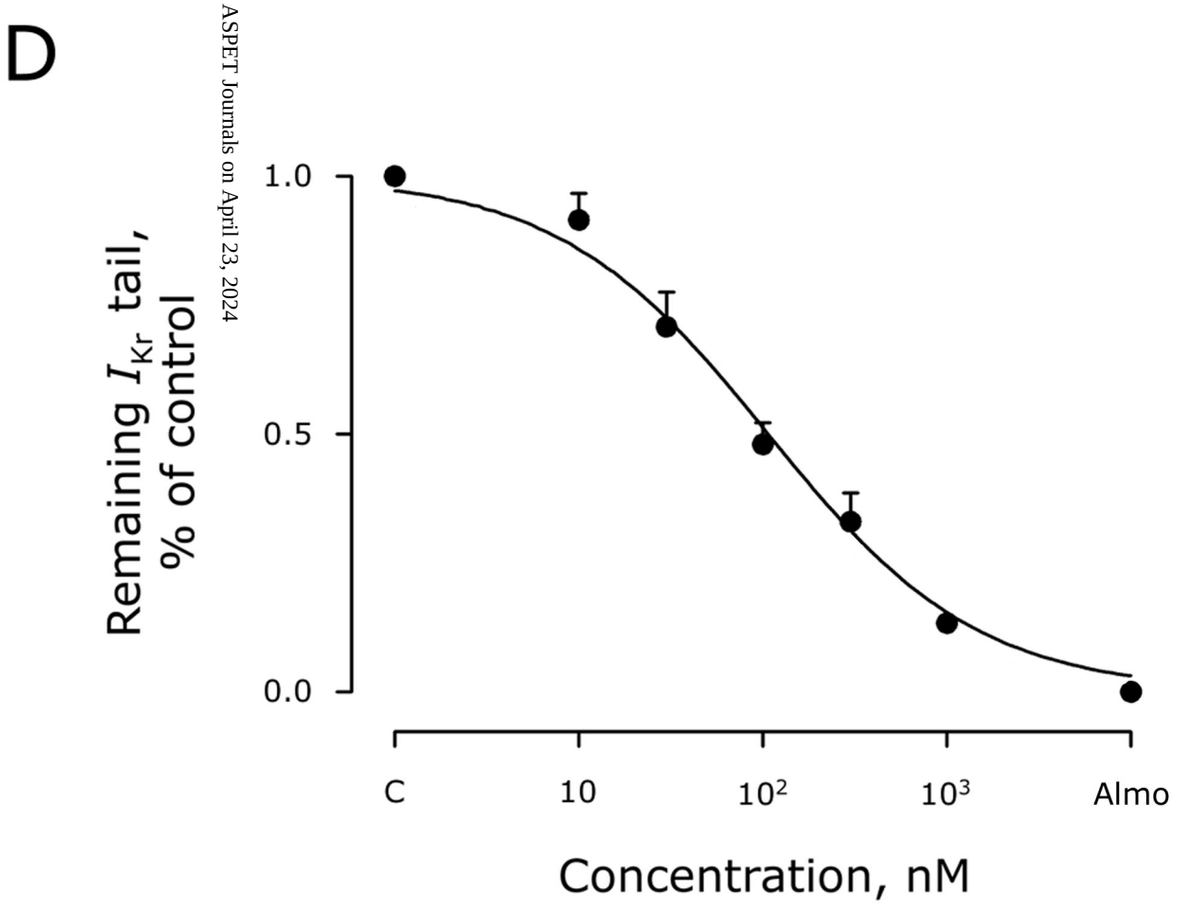

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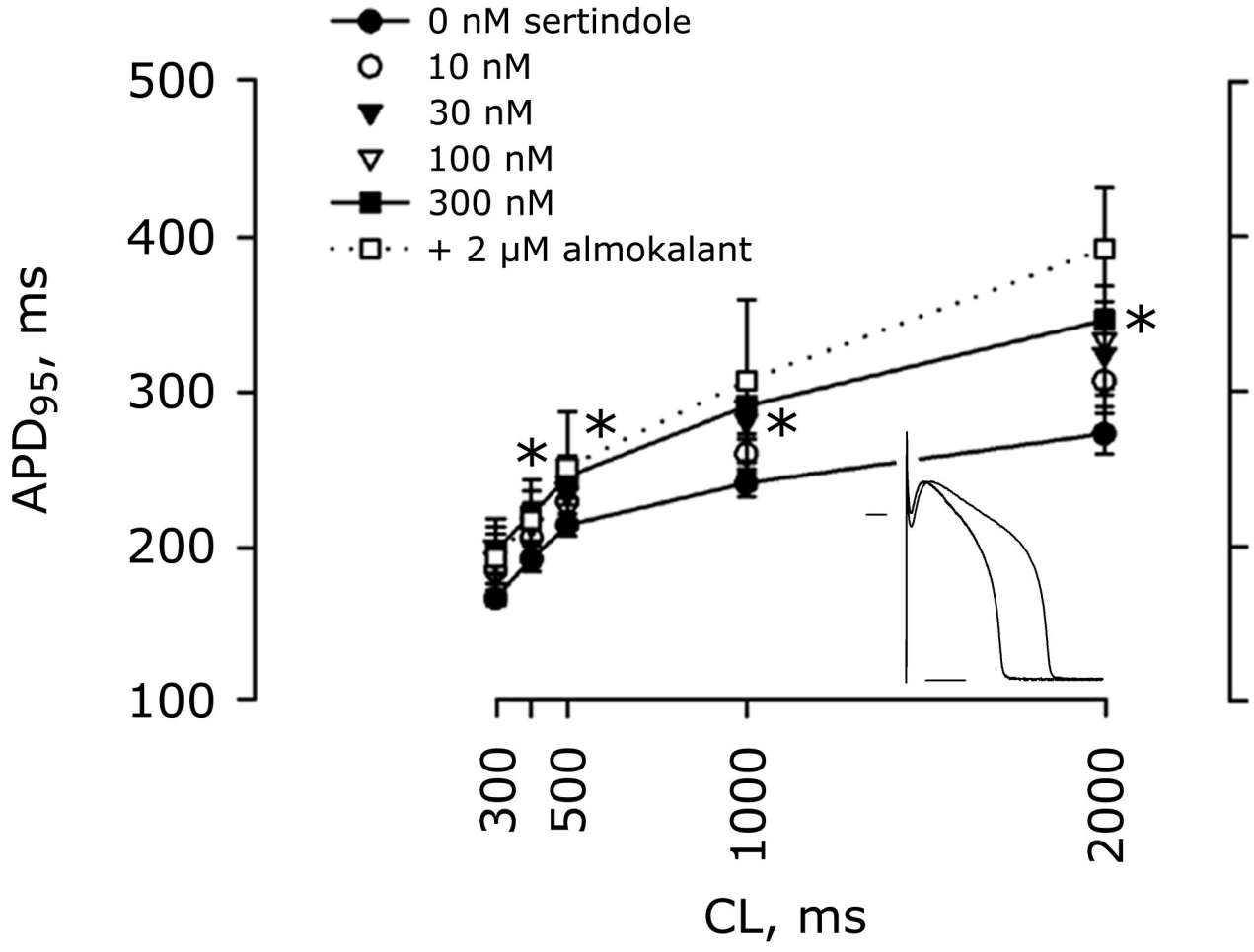

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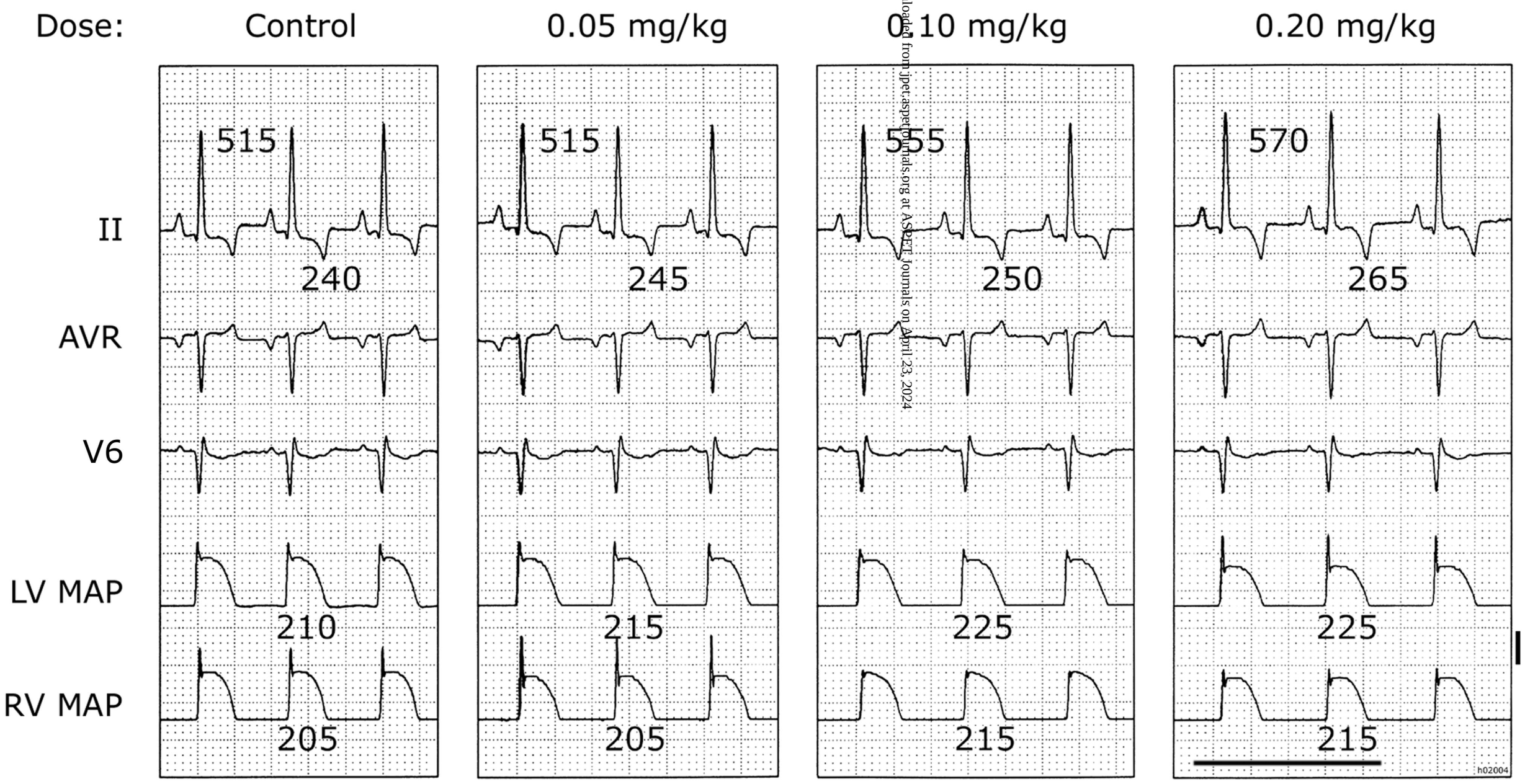

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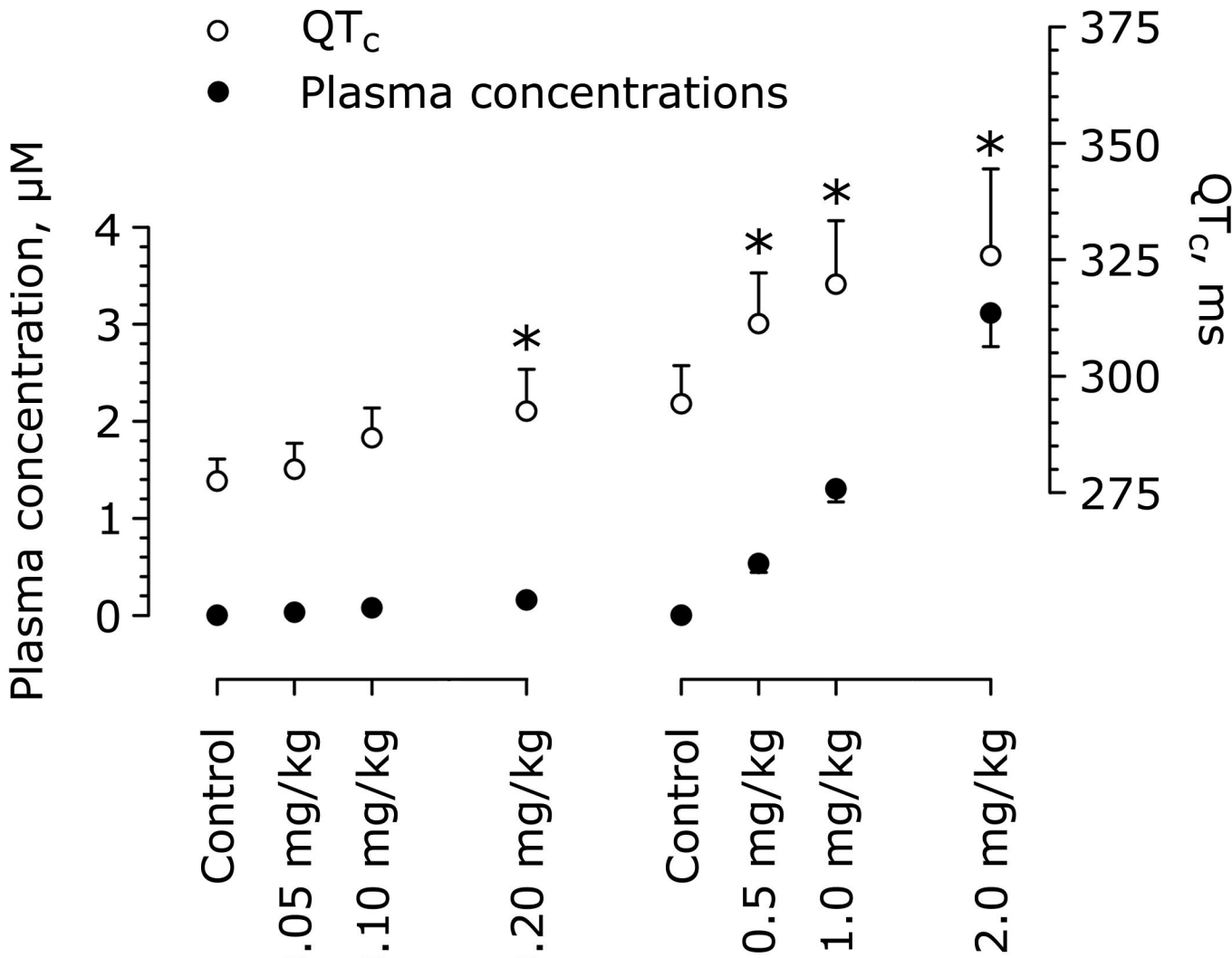

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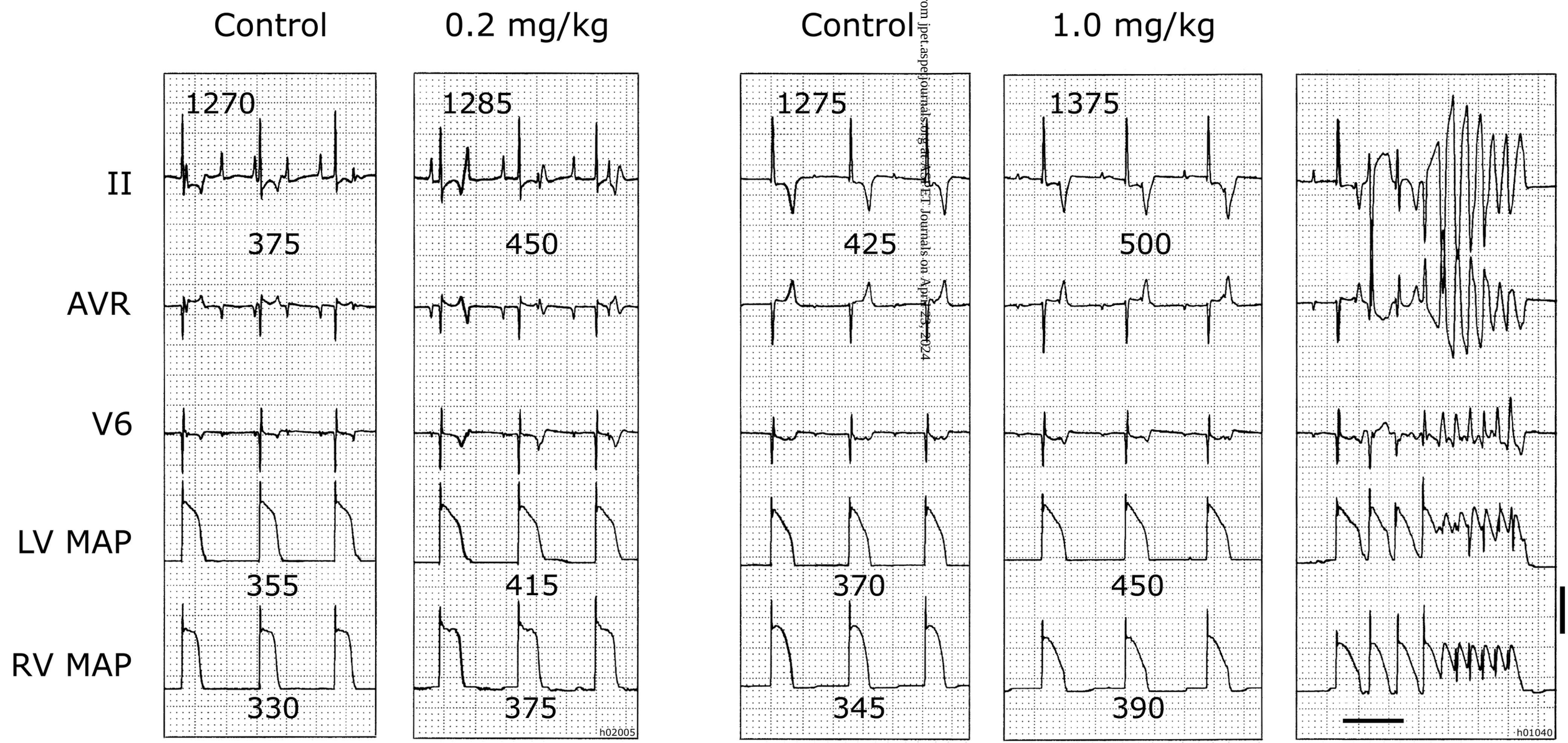

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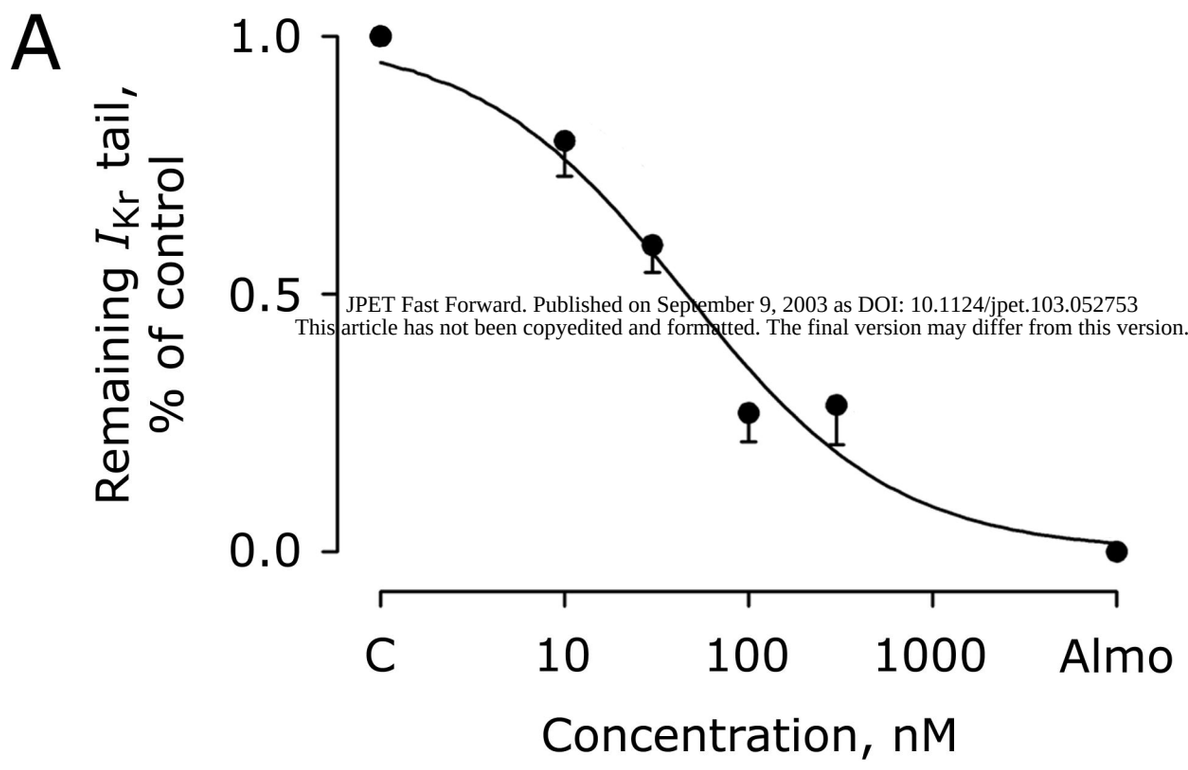

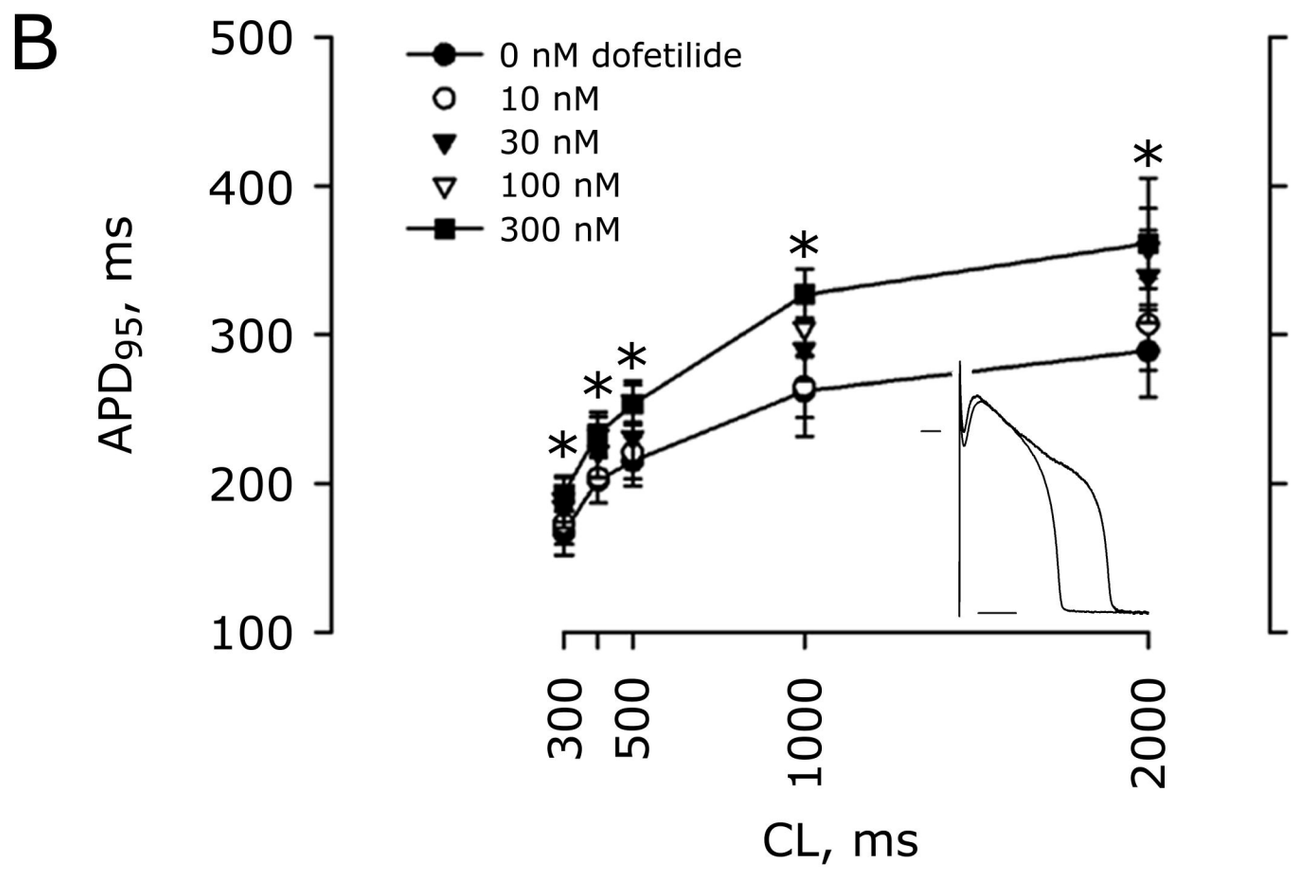

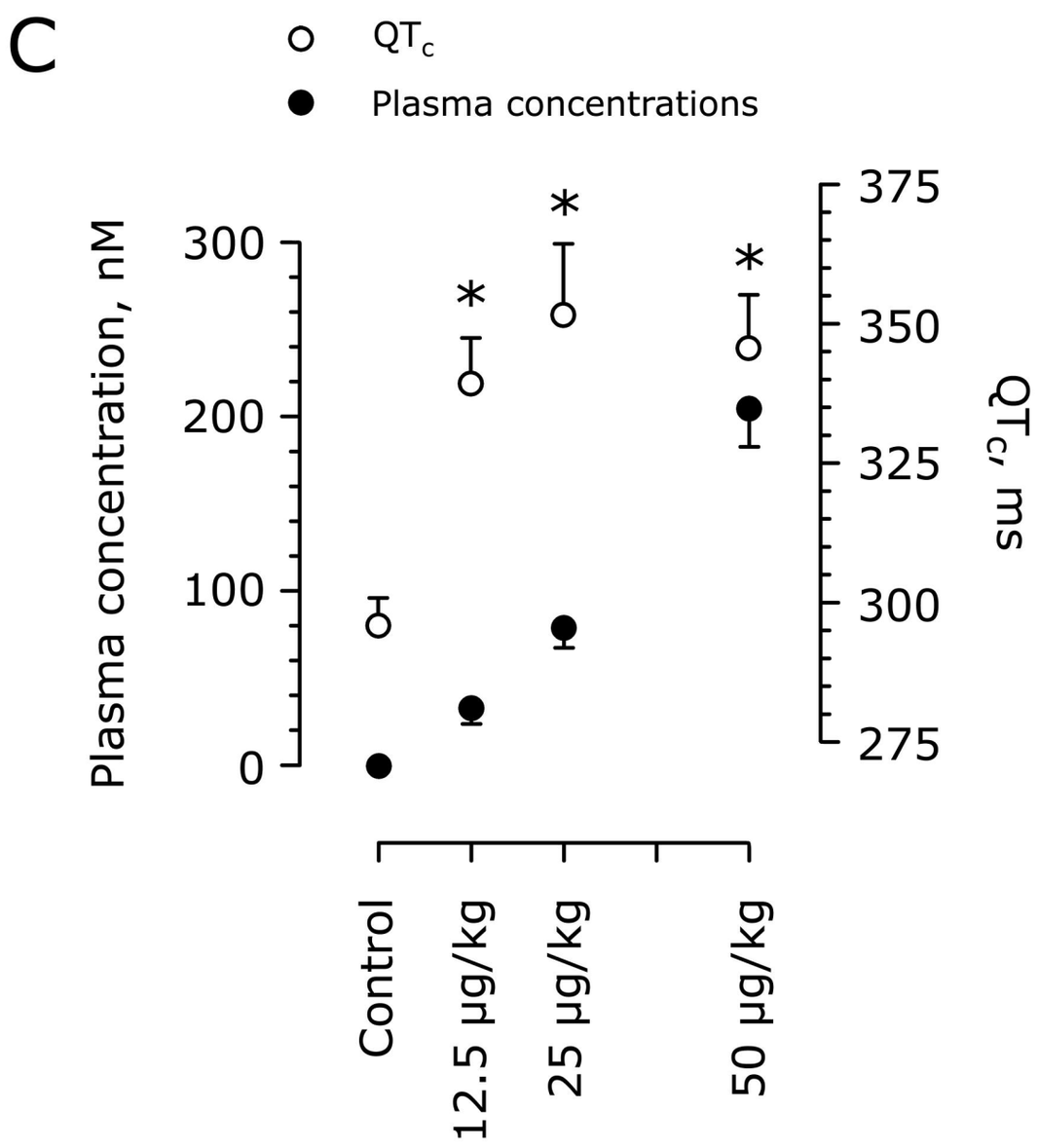

D

	Control	25 µg/kg dofetilide	%
RR	1259 ± 164	1443 ± 204*	(15)
QT	414 ± 41	523 ± 52*	(26)
QT_c	391 ± 37	485 ± 54*	(24)
LV	357 ± 37	462 ± 46*	(29)
RV	306 ± 37	382 ± 60*	(25)
ΔMAPD	51 ± 28	80 ± 41*	(56)
Singles		38 ± 42	
Multiples		22 ± 15	
TdP		12 ± 4	
Shocks		2 ± 1	
Time to 1st TdP, min		4 ± 1	
Time to intervention, min		16 ± 5	

Tables

TABLE

Electrophysiological data from anesthetized dogs with CAVB during treatment with sertindole (n_{dogs} = 5 in each group.). Percentage increases from control to drug are depicted in brackets. Measurements were performed at 10 min after start of drug infusion or just prior to first TdP. * $P < 0.05$ vs. control. There was no differences in control values for any parameter.

	Control	0.10 mg/kg	%	0.20 mg/kg	%	Control	1.0 mg/kg	%
RR (ms)	1240 ± 136	1271 ± 127	(2)	1235 ± 116	(0)	1442 ± 87	1562 ± 110*	(8)
QT (ms)	385 ± 26	420 ± 22	(9)	458 ± 45*	(19)	408 ± 26	500 ± 39*	(23)
QT_c (ms)	364 ± 17	397 ± 15	(9)	438 ± 38*	(20)	370 ± 24	451 ± 34*	(22)
LV MAPD (ms)	337 ± 20	372 ± 24	(10)	402 ± 43*	(19)	356 ± 19	447 ± 32*	(26)
RV MAPD (ms)	309 ± 15	326 ± 19	(5)	354 ± 30*	(14)	311 ± 19	368 ± 27*	(18)
ΔMAPD (ms)	28 ± 9	46 ± 6	(64)	48 ± 19	(72)	45 ± 6	79 ± 19	(76)
Reproducible TdP induction		0 / 5		0 / 5			3 / 5	