Original article

Intravenous Administration of Apomorphine Does NOT Induce Long QT Syndrome: Experimental Evidence from *In Vivo* Canine Models

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Running title: Electropharmacological Effects of Apomorphine

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Abstract

Apomorphine is a nonselective dopamine D_1/D_2 receptor agonist, which has been used for patients with Parkinson's disease, and reported to induce QT-interval prolongation and cardiac arrest. In order to clarify their causal link, we assessed the cardiovascular and pharmacokinetic profile of apomorphine with the halothane-anesthetized canine model (n=4), whereas proarrhythmic potential of apomorphine was analyzed with the chronic atrioventricular block canine model (n=4). In the halothane-anesthetized model, 0.01 mg/kg, i.v. of apomorphine hydrochloride over 10 min, providing about 10 times of its therapeutic concentration, increased the heart rate and ventricular contraction; 0.1 mg/kg over 10 min, providing about 100 times of the therapeutic, prolonged the ventricular effective refractory period; and 1 mg/kg over 10 min, providing about 1,000 times of the therapeutic, decreased the ventricular contraction, mean blood pressure and cardiac output together with the intraventricular conduction delay and prolongation of the effective refractory period, whereas the left ventricular end-diastolic pressure, atrioventricular nodal conduction or ventricular repolarization were hardly affected. Meanwhile, in the atrioventricular block model, 1 mg/kg, i.v. of apomorphine hydrochloride over 10 min neither prolonged the QT interval nor induced torsade de pointes. These results suggest that apomorphine may possess wide margin of cardiovascular safety contrary to our expectations.

Keywords: Apomorphine, Monophasic action potential, QT interval, Torsade de pointes, Atrioventricular block

Introduction

Apomorphine is a nonselective dopamine D_1/D_2 receptor agonist, which has been indicated for the treatment of patients with Parkinson's disease [1]. In the interview form from the manufacturer, apomorphine was described to block the human ether-a-go-go related gene (hERG)-mediated current, whereas in a previous study using the canine ventricular muscle, apomorphine prolonged the action potential duration at 90% repolarization level [2]. In phase III studies conducted in the United States, 2 patients (one at 2 and 6 mg, the other at 6 mg) have been reported to exhibit large QTc increments of >60 ms besides prolonging QTc to >500 ms acutely after dosing [3]. Indeed, according to the postmarketing surveillance, apomorphine has been reported to develop cardiac arrest in 3 patients, and heart failure in 5 patients [4]. However, there is no direct evidence showing a causal link between apomorphine administration, QT prolongation and cardiac arrest in either humans or animals.

In order to better understand the precise mechanisms that will explain adverse events observed in the postmarketing surveillance [4], in this study we simultaneously assessed the cardiohemodynamic, electrophysiological and proarrhythmic effects of apomorphine together with pharmacokinetic profile. In experiment 1, we assessed the *in vivo* cardiovascular effects of apomorphine by using the halothane-anesthetized, closed-chest canine model, which can reflect cardiovascular responses of drugs in humans [5]. Then, in experiment 2, we examined the extent of proarrhythmic potential of apomorphine by using the chronic atrioventricular block canine model [5].

Materials and Methods

All experiments in this study were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 12-52-151, No. 13-53-152) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animals of Toho University and the Japanese Pharmacological Society. Experiments were carried out with 8 beagle dogs of either sex weighing approximately 10 kg. Animals were obtained through Kitayama Labes Co., Ltd. (Nagano, Japan).

Experiment 1: Effects of apomorphine on the halothane-anesthetized dogs

Dogs were anesthetized initially with thiopental sodium (30 mg/kg, i.v.) (n=4). After intubation with a cuffed endotracheal tube, 1.0% halothane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano, Tokyo, Japan). Tidal volume and respiratory rate were set at 20 mL/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg, i.v.) was administered.

Cardiohemodynamic parameters: A clinically available catheter-sheath set (FAST-CATH^{TM} 406108, St. Jude Medical Daig Division, Inc., Minnetonka, MN, USA) was inserted into the right femoral artery to introduce a pig-tail catheter for measuring the left ventricular pressure. The aortic blood pressure was measured at a space between inside of the sheath and outside of the catheter through a flush line. A thermodilution catheter (TC-504NH; Nihon Kohden, Tokyo, Japan) was positioned at the right side of the heart through the right femoral vein. The cardiac output was measured by using a standard thermodilution method with a cardiac output computer

(MFC-1100; Nihon Kohden). The total peripheral resistance was calculated with the basic equation: total peripheral resistance=mean blood pressure/cardiac output. The maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and the left ventricular end-diastolic pressure (LVEDP) were obtained during sinus rhythm to estimate the contractility and the preload to the left ventricle, respectively.

Electrophysiological parameters: The surface lead II ECG was obtained from the limb electrodes. The corrected QT interval was calculated by using Van de Water's formulas [6]: QTcV=QT–0.087 x (RR–1000). A standard 6-French quad-polar electrodes catheter (Cordis-Webster, Baldwin Park, CA, USA) was positioned at the non-coronary cusp of the aortic valve through the left femoral artery to obtain the His bundle electrogram. A bi-directional steerable monophasic action potential (MAP) recording/pacing combination catheter (1675P; EP Technologies, Inc., Sunnyvale, CA, USA) was positioned at the endocardium of the right ventricle through the left femoral vein to obtain MAP signals. The signals were amplified with a DC preamplifier (model 300; EP Technologies, Inc.). The duration of the MAP signals was measured as an interval, along a line horizontal to the diastolic baseline, from the MAP upstroke to the desired repolarization level. The interval (ms) at a 90% repolarization level was defined as MAP_{90}. The heart was electrically driven by using a cardiac stimulator (SEC-3102; Nihon Kohden) with the pacing electrodes of the combination catheter placed in the right ventricle. The stimulation pulses were rectangular in shape, 1-2 V (about twice the threshold voltage) and of 1-ms duration. The MAP_{90} was measured during sinus rhythm ($MAP_{90(sinus)}$) and at a pacing cycle length of 300 ms ($MAP_{90(CL300)}$) and 400 ms ($MAP_{90(CL400)}$). The effective refractory period (ERP) of the right ventricle was assessed with programmed electrical stimulation. The pacing protocol consisted

of 5 beats of basal stimuli in a cycle length of 400 ms followed by an extra stimulus of various coupling intervals. Starting in late diastole, the coupling interval was shortened in 5-ms decrement until refractoriness occurred. The duration of the terminal repolarization period (TRP) of the ventricle; namely, phase 3 repolarization of the action potential, was calculated by the difference between the $MAP_{90(CL400)}$ and ERP at the same site, which reflects the extent of electrical vulnerability of the ventricular muscle [5].

Experimental protocol: The aortic blood pressure, left ventricular pressure, ECG, His bundle electrogram and MAP signals were monitored with a polygraph system (RM-6000; Nihon Kohden), and analyzed with a real time full automatic data analysis system (WinVAS3 ver 1.1R24; Physio-Tech, Tokyo, Japan). Each measurement of ECG, MAP as well as atrio-His (AH) and His-ventricular (HV) intervals was the mean of three recordings of consecutive complexes. The cardiovascular variables were assessed in the following order. The ECG, His bundle electrogram, aortic and left ventricular pressures and MAP signals were recorded under sinus rhythm. Next, the cardiac output was measured three times. Then, MAP signals were recorded during the ventricular pacing at a cycle length of 400 and 300 ms. Finally, the ERP was measured. All parameters described above were usually obtained within 1 min at each time point.

After the basal assessment, a low dose of 0.01 mg/kg of apomorphine hydrochloride was intravenously infused over 10 min, and each parameter was assessed at 5, 10, 15, 20 and 30 min after the start of the infusion. Then, a middle dose of 0.1 mg/kg of apomorphine hydrochloride was intravenously infused over 10 min, and each parameter was observed in the same manner. Finally, a high dose of 1 mg/kg of

apomorphine hydrochloride was intravenously infused over 10 min, and each parameter was assessed at 5, 10, 15, 20, 30, 45 and 60 min after the start of the infusion.

Plasma drug concentration: A volume of 3 mL of blood was withdrawn from the left femoral artery to measure the plasma concentration of apomorphine. The blood samples were centrifuged at 1,500 x g for 30 min at 4°C. The plasma was stored at –80°C until the drug concentration was measured. The plasma concentration of apomorphine was determined as previously reported [7]. Briefly, 200 μL of plasma sample was extracted with 500 μL of ethyl acetate. After vortex mixing, the tubes were centrifuged at 900 x g for 10 min. Four hundred fifty μL of organic phase was collected and evaporated to dryness at 40°C. The residue was dissolved with 50 μL of a mobile phase consisting of 30% (v/v) methanol in 0.25 M sodium phosphate buffer (pH 3.3). The supernatant (30 μL) was injected onto an analytical C_{18} reversed-phase column (250 x 4.6 mm, 5 μm, Mightysil RP-18; Kanto Chemical, Tokyo, Japan) maintained at 40°C. The elution profiles of apomorphine at a flow rate of 1.5 mL/min were monitored with fluorimetric detection at an excitation wavelength of 270 nm and an emission wavelength of 450 nm.

Experiment 2: Effects of apomorphine on the chronic atrioventricular block dogs

Production of complete atrioventricular block: The catheter ablation technique for the atrioventricular node was used as previously described [8]. The dogs were anesthetized with thiopental sodium (30 mg/kg, i.v.) (n=4). After intubation with a cuffed endotracheal tube, 100% oxygen was inhaled with a volume-limited ventilator (SN-480-3; Shinano). Tidal volume and respiratory rate were set at 20 mL/kg and 15 strokes/min, respectively. To prevent blood clotting, heparin calcium (100 IU/kg, i.v.)

was administered. The surface lead II ECG was continuously monitored with a polygraph system (RM-6000; Nihon-Kohden). A quadpolar electrodes catheter with a large tip of 4 mm (D7-DL-252; Cordis-Webster) was inserted through the right femoral vein by using the standard percutaneous technique under the sterile condition, and positioned around the tricuspid valve, watching the bipolar electrograms from the distal electrodes pair. The optimal site for the atrioventricular node ablation was based on the intracardiac electrogram, of which a very small His deflection was recorded and atrial/ventricular voltage ratio was >2. The site was usually found at 1-2 cm proximal from the position where the largest His bundle electrogram was recorded. The power source for atrioventricular node ablation was obtained from an electrosurgical generator (MS-1500; Mera, Tokyo, Japan), which delivers continuous unmodulated radiofrequency energy at a frequency of 500 kHz. After proper positioning, the radiofrequency energy of 20 W was delivered for 10 s from the tip electrode to an indifferent patch electrode positioned on the animal's back, which continued for 30 s if junctional rhythm was induced. The end point of this procedure was the development of the complete atrioventricular block with an onset of stable idioventricular escaped rhythm.

Holter ECG recording: A Holter recording and analysis system (QR2100 and HS1000, Fukuda ME Kogyo, Tokyo, Japan) was used to record and analyze ECG over 24 h. The effects of apomorphine on the ventricular rate, QT interval and corrected QT calculated with the Fridericia's formula [9]: $QTcF=QT/(RR/1000)^{1/3}$ in addition to their proarrhythmic effects were assessed without anesthesia. The ventricular rate, QT interval and QTcF were expressed as the mean of ten consecutive complexes. In this study, torsade de pointes was defined as a polymorphic ventricular tachycardia

associated with QT-interval prolongation, consisting of 5 beats or more twisting QRS complexes around the baseline [10].

Experimental protocol: Experiments were conducted at least 4 weeks after the induction of complete atrioventricular block. We have assessed proarrhythmic effects of many drugs with the group size of 4-6, which has enough sensitivity and reliability to detect the drug-induced torsade de pointes [5]. About 2 h after the start of Holter ECG recording, 1 mg/kg of apomorphine hydrochloride was intravenously infused over 10 min without anesthesia. The p.o. administration of drugs has been used in order to maintain the plasma drug concentration for more than several hours. Since apomorphine has been known to induce vomiting in conscious beagle dogs [11], in this study we administered it intravenously to obtain effective levels of the plasma drug concentration with high reproducibility. The ECG parameters at 1 h before the drug administration were defined as the control and the ECG was recorded for >20 h when lethal arrhythmia was not induced.

Beat to beat analysis: ECG of 51 consecutive beats under the stable idioventricular automaticity without ectopic activity was adopted before and at 1-1.7 h after the drug administration. When the QT interval was obscured by P wave, we estimated the end of T wave by cancelling the component of the P wave from the ECG waveform on screen. Poincaré plots with QT_n versus QT_{n+1} were prepared for each of two analysis time points. The mean orthogonal distance from the diagonal to the points of the Poincaré plot was determined as short-term variability ($=\Sigma|QT_{n+1}–QT_n|/[50 \times \sqrt{2}]$). On the other hand, the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot was determined as long-term variability ($=\Sigma|QT_{n+1}+ QT_n–2QT_{mean}|/[50 \times \sqrt{2}]$). These nomenclatures are adopted from investigations of

heart rate variability in humans [12], which have been applied to the QT-interval analysis of normal dogs and chronic atrioventricular block dogs [13].

Drugs

The following drugs were purchased: apomorphine hydrochloride hydrate (Apokyn® subcutaneous injection, Kyowa Hakko Kirin Co., Ltd., Tokyo, Japan), pentobarbital sodium (Tokyo Kasei, Tokyo, Japan), thiopental sodium (Ravonal® 0.5 g for Injection, Mitsubishi-Tanabe Pharma, Osaka), halothane (Fluothane®, Takeda Pharmaceutical Company, Osaka) and heparin calcium (Caprocin®, Sawai Pharmaceutical Co., Ltd., Osaka). Apomorphine was diluted with saline in concentrations of 0.01, 0.1 and 1 mg/mL as hydrochloride form.

Statistics

Data are presented as the mean±SE. The statistical significances within a parameter were evaluated with one-way repeated-measures analysis of variance (ANOVA) followed by Contrasts for mean values comparison or paired *t*-test. A *p*-value <0.05 was considered statistically significant.

Results

Experiment 1: Effects of apomorphine on the halothane-anesthetized dogs

No animal died from lethal ventricular arrhythmias or cardiohemodynamic collapse during the experimental period.

Plasma drug concentration: The time course of the plasma drug concentration of apomorphine is summarized in Fig. 1 (n=4). The decrease in the plasma concentration of apomorphine followed a pattern that could be predicted by the two-compartment theory of pharmacokinetics. The peak plasma concentrations after 0.01, 0.1 and 1 mg/kg infusion were 47±9, 452±121 and 3,528±791 ng/mL, respectively.

Effects on the cardiohemodynamic variables: The time courses of changes in the heart rate, mean blood pressure, cardiac output, total peripheral resistance, $LVdP/dt_{max}$ and LVEDP are summarized in Fig. 1 (n=4), and typical tracings of the aortic and left ventricular blood pressures are depicted in Fig. 2. The pre-drug control values (C) were 103±14 beats/min, 97±9 mmHg, 1.33±0.27 L/min, 80±12 mmHg/(L/min), 1,721±263 mmHg/s and 10.7±2.3 mmHg, respectively. After the administration of the low dose of 0.01 mg/kg of apomorphine hydrochloride infusion, the heart rate and $LVdP/dt_{max}$ increased for 10-20 min and at 20 min, respectively, whereas no significant change was detected in the other variables. After the middle dose of 0.1 mg/kg, no significant change was detected in them. After the high dose of 1 mg/kg, the mean blood pressure, cardiac output and $LVdP/dt_{max}$ decreased at 20 min, for 10-60 min and for 10-60 min, respectively, whereas no significant change was detected in the other variables.

Effects on the electrophysiological variables during sinus rhythm: Typical tracings of the ECG, His bundle electrogram and MAP during sinus rhythm are depicted in Fig. 2, and the time courses of changes in the ECG variables, AH and HV intervals, and $MAP_{90(sinus)}$ are summarized in Fig. 3 (n=4). The pre-drug control values (C) of the PR interval, QRS width, QT interval, QTcV, AH and HV intervals, and $MAP_{90(sinus)}$ were 100±1, 60±2, 311±43, 344±35, 76±4, 29±4 and 271±47 ms, respectively. After the low and middle doses, no significant change was detected in them. After the high dose, the QT and HV intervals were prolonged for 10-15 min; and at 20, 45 and 60 min, respectively, whereas no significant change was detected in the other variables. No ventricular arrhythmia was detected during the observation period of this study.

Effects on the MAP_{90}, ERP and TRP during the ventricular pacing: The time courses of changes in the $MAP_{90(CL300)}$, $MAP_{90(CL400)}$, ERP and TRP are summarized in Fig. 4 (n=4), of which pre-drug control values (C) were 226±18, 257±33, 210±26 and 47±13 ms, respectively. After the low dose, no significant change was detected in them. After the middle dose, the ERP was prolonged at 10 and 20 min, whereas no significant change was detected in the other variables. After the high dose, the ERP was prolonged for 5-20 min and at 45 min, whereas no significant change was detected in the other variables.

Experiment 2: Effects of apomorphine on the chronic atrioventricular block dogs

After the administration of apomorphine, each animal vomited several times.

Effects on the ECG: The time courses of the changes in the ECG variables and the number of surviving animals are summarized in Fig. 5. The pre-drug control values (C) of the ventricular rate, QT interval and QTcF were 24±2 beats/min, 342±2

ms and 250±6, respectively. After the administration of apomorphine, no significant change was detected in them. Typical tracings of the ECG showing the effects of apomorphine are depicted in Fig. 6. After the administration of apomorphine, neither QT-interval prolongation nor onset of torsade de pointes was observed.

Beat-to-beat analysis: Beat-to-beat analysis was used for each animal to assess the extent of torsadogenic potential of the drugs, as depicted in Fig. 7. The QT interval of ECG of 51 consecutive beats under stable idioventricular rhythm was measured in each animal before and at 1-1.7 h after the administration of apomorphine. The basal control values of short-term variability and long-term variability were 3.8±0.2 and 4.6±0.1 ms, respectively. After the administration of apomorphine, these values tended to increase to 4.7±0.4 and 6.5±0.6 ms, respectively, which did not achieve a statistical significance.

Discussion

Given the limited information on the cardiovascular profile of apomorphine, we assessed it by using the well-established halothane-anesthetized *in vivo* canine model in three escalating i.v. doses of 0.01, 0.1 and 1 mg/kg. Moreover, the proarrhythmic effect of apomorphine was assessed with atrioventricular block model. We found that apomorphine at approximately 1,000 times of therapeutic concentration neither delayed the repolarization nor induced torsade de pointes.

Dose of apomorphine: The EC_{50} of apomorphine was described to be 19.5 nmol/L (5.2 ng/mL) for dopamine D_2 receptor in the assessment report from Pharmaceuticals and Medical Devices Agency. The therapeutic dose of apomorphine in humans was described to be 1-6 mg, subcutaneously per day; and the peak plasma concentration (C_{max}) after the single subcutaneously-administered doses of 1, 2 and 3 mg to healthy subjects was shown to be 3.3±1.2, 7.8±2.3 and 12.0±3.7 ng/mL, respectively, in the interview form from the manufacturer. Therefore, the doses of apomorphine used in this study can be considered to be supratherapeutic level; namely, approximately 10-1,000 times of therapeutic ones, as shown in Fig. 1.

Apomorphine-induced vomiting: After the administration of apomorphine, each chronic atrioventricular block dog vomited several times in the experiment 2, which was not observed at all in the halothane-anesthetized dogs in the experiment 1. Apomorphine-induced vomiting has been reported in conscious beagle dogs [11], but it was not observed in α-chloralose-anesthetized dogs [2,14]. Apomorphine has been reported to act on the chemoreceptor trigger zone, namely, a chemosensitive region where dopamine receptors play important roles [15,16]. Indeed, apomorphine-induced

vomiting has been considered to be mediated by activation of dopamine D_2 receptors, since this effect was abolished by pretreatment with a D_2 receptor antagonist haloperidol or domperidone [17] but not with an opioid antagonist naloxone [18]. Precise mechanisms that vomiting was not observed in halothane-anesthetized dogs in this study remained unknown, although halothane-induced marked muscle relaxation might have in part contributed to the inhibition of the emetic response [19]. Since vomiting will modify the drug-induced cardiovascular responses, the halothane-anesthetized canine model may be an effective way to better analyze the cardiovascular effects of drugs with emetic action.

Cardiohemodynamic effects: The low dose of 0.01 mg/kg of apomorphine exerted the positive chronotropic and inotropic effects. The chronotropic effect of apomorphine was in accordance with those reported in previous studies using the α-chloralose-anesthetized dog after a single intravenous administration of 0.05-0.5 mg/kg [2,14,20]. Meanwhile, the positive inotropic effect of apomorphine was not detected in a previous report assessed with the α-chloralose-anesthetized dogs [2]. Since a blockade of muscarinic receptors by atropine suppressed apomorphine-induced tachycardia [14], the positive chronotropic effect observed by the low dose may be at least in part mediated through a cholinergic inhibition. The positive inotropic effect in this study might be explained by a framework of the positive stairway-case phenomenon; namely, the contractile force increases as the beating rate increases [21].

In contrast to the low and middle doses, the high dose of apomorphine exerted the negative inotropic effect, leading to the decrease in the cardiac output followed by the decrease in the mean blood pressure. Apomorphine has been reported to stimulate the peripheral presynaptic D_2 dopamine receptors [14,22], leading to the decrease in

noradrenaline release from the sympathetic nerve endings [23], which might be at least in part related to the negative inotropic effect of apomorphine in this study. More importantly, the negative inotropic effect could be induced by direct Na^+ channel inhibition by apomorphine, which was discussed below. In addition, the decrease in cardiac output can be explained by the negative inotropic effect and slight decrease in the heart rate. Meanwhile, similar hypotensive effect has been reported in previous studies with conscious beagle dogs [11], anesthetized dogs [2,11,20] and the clinical trial described in the interview form from the manufacturer. The total peripheral resistance tended to increase in the present study, possibly due to an increase of reflex-mediated sympathetic tone, although apomorphine-induced vasodilator action has been reported previously with α-chloralose-anesthetized dogs [2]. The discrepancy of the results between the previous study [2] and the present one might be in part explained by the difference in the experimental conditions; for example, α-chloralose-anesthesia versus halothane-anesthesia; and bolus i.v. injection versus 10 min of infusion.

Electrophysiological effects: Apomorphine at the low or middle dose did not affect any of the electrophysiological variables except that the ERP was prolonged after the middle dose, whereas after the high dose, the QT and HV intervals together with the ERP were prolonged. PR or AH interval was hardly affected by any of the doses of apomorphine, suggesting a lack of inhibitory effect on Ca^{2+} channels *in vivo* [5], which has not been reported. Meanwhile, the HV interval was prolonged by the high dose, suggesting the presence of inhibitory action on Na^+ channels [5], which has not been reported so far. The QTc or MAP duration under sinus rhythm and during the ventricular pacing were not changed, suggesting a lack of inhibitory effect on the repolarization currents *in vivo* [5], although apomorphine has been shown to block the

hERG-mediated current with IC_{50} values of 127 nM (33.9 ng/mL) in the interview form from the manufacturer, which was sufficiently attained in this study. While precise mechanisms that explain this discrepancy are unknown, one can speculate that relatively small amount of the drug might be distributed to the K^+ channels in the heart and/or influence from the central nervous system might have modified the electrophysiological responses of apomorphine.

Proarrhythmic effect: In the halothane-anesthetized model, TRP, a marker of electrical vulnerability of the ventricular muscle [5], tended to decrease in a dose-related manner, although it did not achieve a statistical significance, suggesting a lack of potential inducing torsade de pointes. In order to confirm this hypothesis, we assessed the torsadogenic potential of apomorphine by using atrioventricular block dogs. After the administration of apomorphine, no significant change was detected in QTc, short-term variability or long-term variability of repolarization, moreover, torsade de pointes was not induced at all, indicating a lack of torsadogenic potential for apomorphine.

Conclusions

The present studies suggest that apomorphine at approximately 10-1,000 times of therapeutic concentration will not induce repolarization delay or torsade de pointes. Moreover, apomorphine only at 1,000 times of therapeutic concentration exerted the negative inotropic effect possibly through Na^+ channel inhibition, followed by the decrease in the cardiac output, leading to the decrease in the mean blood pressure, whereas the left ventricular end-diastolic pressure, atrioventricular nodal conduction or ventricular repolarization were hardly affected. Thus, contrary to our expectation

based on previous knowledge, apomorphine can be considered to possess a wide margin of cardiovascular safety.

Conflict of interest statement:

The authors declare no conflicts of interest.

Acknowledgments:

This study was supported in part by Grant-in-aid from the Ministry of Education, Culture, Sports, Science, and Technology in Japan (#22590237, #25460344, #S1101016), Japan Science and Technology Agency (#AS2116907E), and Toho University Joint Research Fund. The authors thank Ms. Misako Nakatani for her technical assistance.

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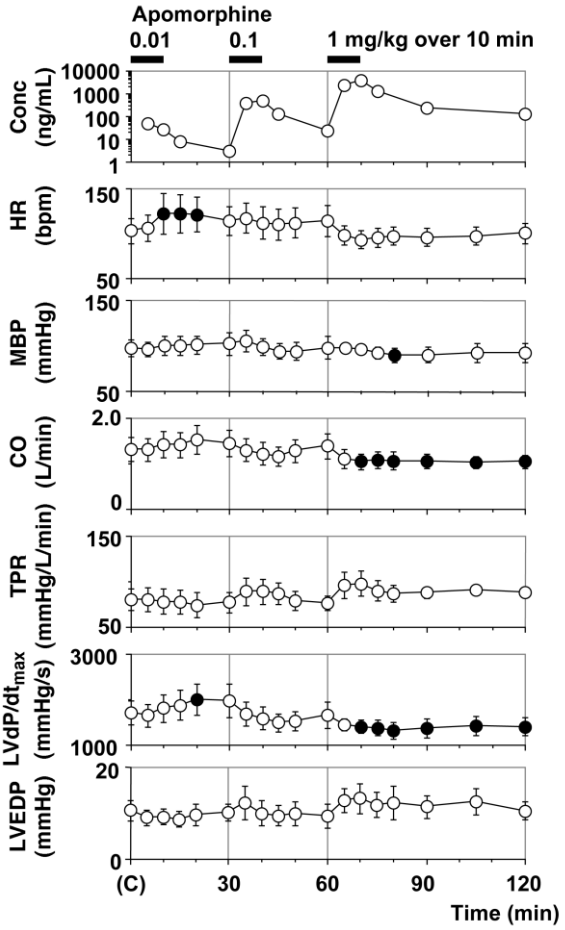

Fig. 1

Time courses of the plasma drug concentration (Conc), heart rate (HR), mean blood pressure (MBP), cardiac output (CO), total peripheral resistance (TPR), maximum upstroke velocity of the left ventricular pressure ($LVdP/dt_{max}$) and left ventricular end-diastolic pressure (LVEDP) in the halothane-anesthetized dogs. Data are presented as mean±SE (n=4). The closed symbols represent significant differences from each control (C) by $p<0.05$.

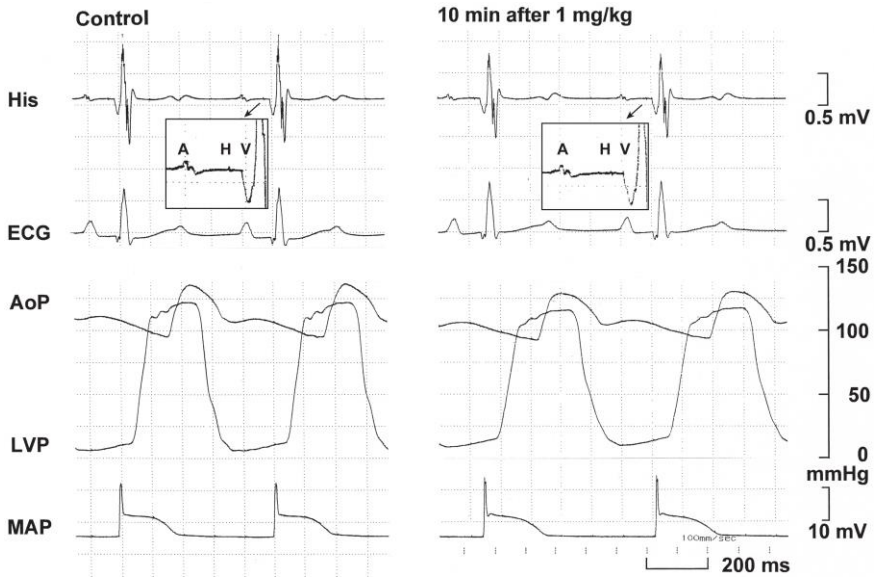

Fig. 2

Typical tracings showing His bundle electrogram (His), surface lead II electrocardiogram (ECG), aortic blood pressure (AoP), left ventricular pressure (LVP) and monophasic action potential (MAP) during sinus rhythm at pre-drug control (Control) and 10 min after the start of intravenous infusion of 1 mg/kg of apomorphine hydrochloride in the halothane-anesthetized dog. Inserted are enlarged views of His-bundle electrogram, showing atrial (A), Hisian (H) and ventricular (V) waves.

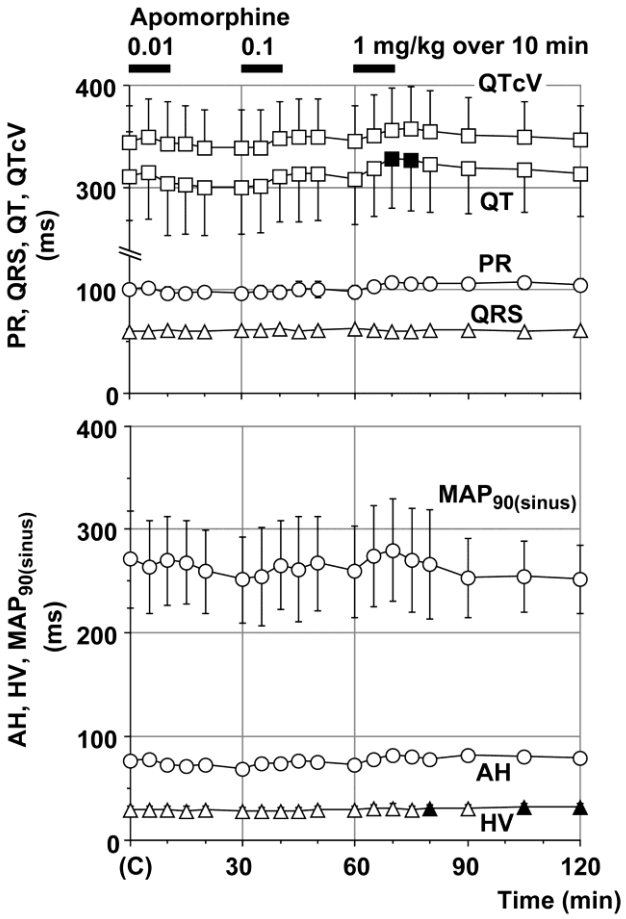

Fig. 3

Time courses of the PR interval, QRS width, QT interval, and QTcV; atrio-His (AH) and His-ventricular (HV) intervals; and monophasic action potential at 90 % repolarization level during sinus rhythm ($MAP_{90(sinus)}$) in the halothane-anesthetized dogs. Data are presented as mean±SE (n=4). The closed symbols represent significant differences from each control (C) by $p<0.05$.

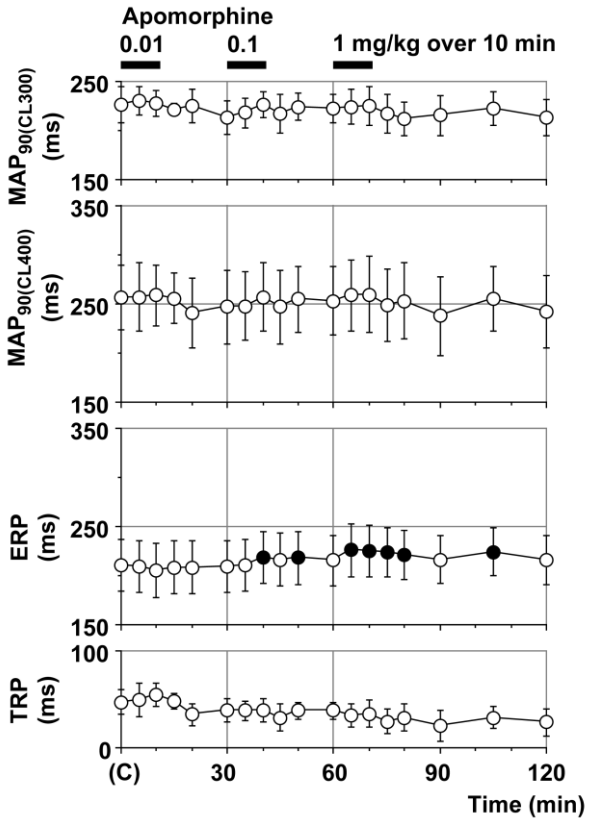

Fig. 4

Time courses of the MAP_{90} during the electrical pacing at a cycle length of 300 ms ($MAP_{90(CL300)}$) and 400 ms ($MAP_{90(CL400)}$); effective refractory period of the right ventricle (ERP); and terminal repolarization period (TRP=$MAP_{90(CL400)}$–ERP) in the halothane-anesthetized dogs. Data are presented as mean±SE (n=4). The closed symbols represent significant differences from each control (C) by $p<0.05$.

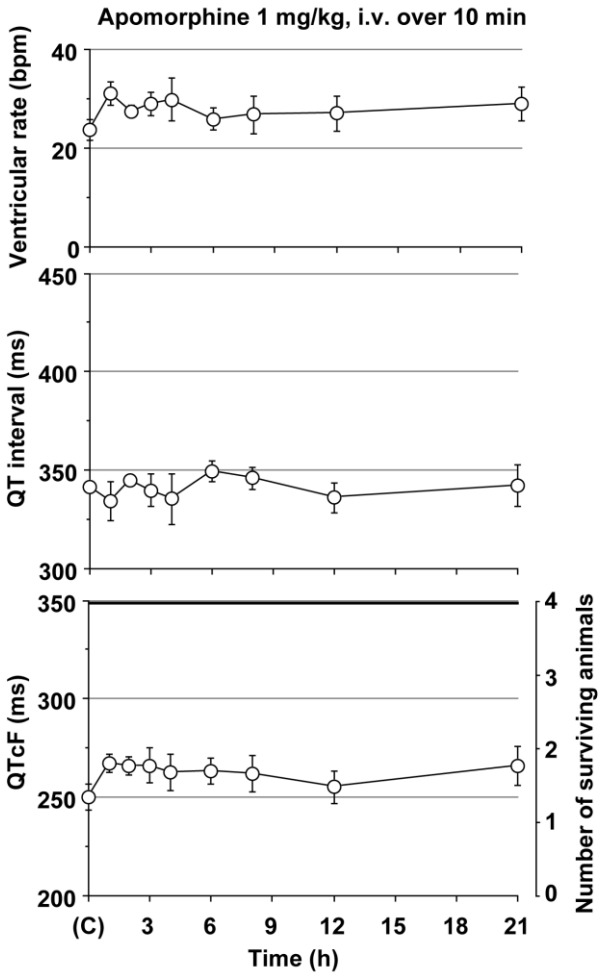

Fig. 5

Effects of 1 mg/kg, i.v. of apomorphine on the ECG variables in the chronic atrioventricular block dogs. Time courses of the effects on the ventricular rate, QT interval, QTcF and the number of surviving atrioventricular block animals. Data are presented as mean±SE (n=4).

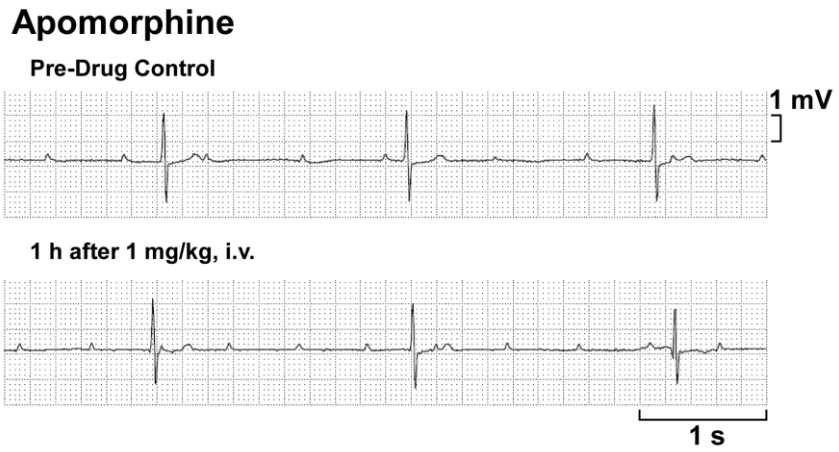

Fig. 6

Typical tracings of ECG in the chronic atrioventricular block dogs before and after the administration of apomorphine. Torsade de pointes was not induced by the intravenous administration of 1 mg/kg of apomorphine hydrochloride.

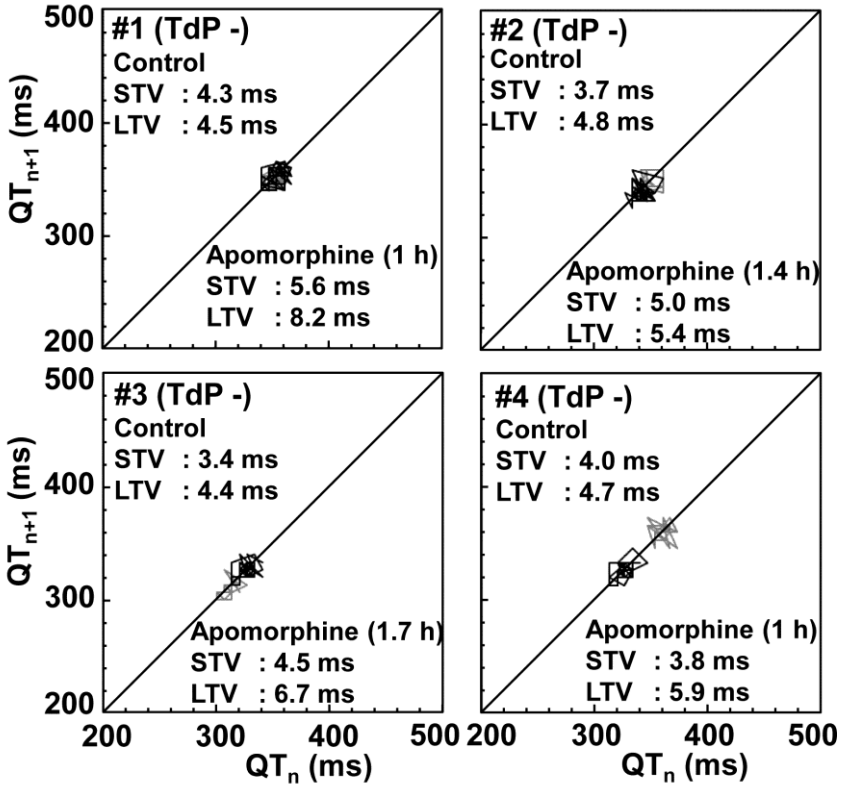

Fig. 7

Poincaré plots of the QT interval obtained in each atrioventricular block animal. A total of 51 beats were plotted for each of the two analysis time points; at pre-drug control (grey) and after (black) 1 mg/kg, i.v. of apomorphine hydrochloride. STV: short-term variability; and LTV: long-term variability.