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Data

Cardiovascular Effects of Mongolian Medical Plant *Aconitum barbatum* in Rats

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ABSTRACT: *Aconitum barbatum* Pers. (*A. barbatum*) is a perennial herbaceous plant in Mongolian traditional medicine, which is used for treating palpitation but has occasionally induced bradycardia and/or cardiac arrest. We assessed the cardiovascular effects of the aqueous extract of the flower of *A. barbatum* on rats. The extract of *A. barbatum* was intravenously administered in doses of 0.1, 1, and 10 mg/kg; 10, 100, and 1,000 times greater than its clinically recommended dose, respectively. No animals exerted any lethal ventricular arrhythmias or hemodynamic collapse. *A. barbatum* showed negative chronotropic, inotropic, and dromotropic effects, along with hypotensive action *in vivo* (n = 4). *A. barbatum* hardly altered the isoproterenol-stimulated adenylyl cyclase activity of membrane preparations (n = 4) made from the left ventricle of two rats. Thus, *A. barbatum* has a wide range of safety margin, and its cardiovascular effects could be explained by a ß-adrenoceptor independent mechanism, including Ca^{2+} channel blockade.

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KEYWORDS: *Aconitum barbatum*, cardiovascular effects, adenylyl cyclase, safety margin

Introduction

Aconitum barbatum Pers. *(A. barbatum)* is a perennial herbaceous plant. It grows naturally in limited areas of Khubsugul, Khangai, Khentei, Mongol Daurian, Mongolian Altai, and Gobi Altai in Mongolia. In Mongolian traditional medicine, the flowers of *A. barbatum* have been empirically used as a remedy for palpitation. Its clinically recommended daily dose has been 5-10 mg/body as dried powder, which is orally administered with some medicinal plants and mineral powder, whereas its overdoses are known to induce bradycardia and/or cardiac arrest. Fifty-six kinds of constituents have been identified in alcoholic or organic solvent extraction from *A. barbatum* (Table 1), in which lappaconitine, denudatine, luciculine, 1-acetylluciculine, songorine, and delcosine can modify the cardiohemodynamic function.[1–5]

For example, in anesthetized dogs, lappaconitine in a dose of 150 μg/kg (i.v.) and denudatine of 2.5 mg/kg (i.v.) decreased the blood pressure, heart rate, and maximum upstroke velocity of the left ventricular pressure (peak + dP/dt).[1,2] Luciculine, in a dose of 20 mg/kg (i.v.), and 1-

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Table 1 Constituents identified in the extract of *Aconitum barbatum* by 2022

Classification	Constituents	References
Alkaloid	barpuberudine barpubesines A-D barpubenines A-B	Ablajan N, et al., Phytochemistry. 2021; doi: 10.1016/j.phytochem.2020.112567.
	alkaloids I-III	Batbayar N, et al., Chem Nat Compd. 1992; 28: 388-9.
	ranaconitine lappaconitine septentriodine septentrionine lycaconitine puberanine puberanidine puberaconitine puberaconitidine	De-Quan Y and Das BC., Planta Med. 1983; 49: 85-9.
	delcosine lycoctonine songorine bataconine leucostinine A	Gromova AS, et al., Chem Nat Compd. 2007; 43: 119-20.
	puberunine puberudine monticamine puberumines A-D	Mu ZQ, et al., Org Lett. 2012; 14: 2758-61.
	puberulines A-F luciculine lucidusculine 12-acetylluciculine 1-acetylluciculine lepenine denudatine 11-acetyllepenine 12-epidehydronapelline 11-acetyl-1, 19-epoxydenudatine dehydroluciduscuIine dehydroacosanine demethylenedelcorine 6-O-acetyldemethylenedelcorine 14-O-acetulvirescenine flavamine	Sun LM et al., Helv Chim Acta. 2009; 92: 1126-33.
	corydine tuguaconitine	Sun LM, et al., Chem Nat Compd. 2009; 45: 934-5.
Flavonoid	barbaside A	Pogodaeva NN, et al., Russ Chem Bull. 2000; 49: 1905-9.
Other	aconitic acid	Sun LM, et al., Chem Nat Compd. 2009; 45: 934-5.

acetylluciculine, in a dose of 20 mg/kg (i.v.), rapidly increased the cutaneous blood flow in the hind foot of mice.[3] In a study using the isolated rat cardiomyocytes, songorine at 4 μg/mL (10 μM) blocked ryanodine-induced Ca^{2+} liberation from the sarcoplasmic reticulum.[4] Delcosine at 4.5 μg/mL, lycoctonine at 4.7 μg/mL, and songorine at 3.6 μg/mL blocked G protein-coupled inwardly-rectifying potassium (GIRK) channel.[5]

Given that these previous *in vivo* and *in vitro* information may not fully explain the mechanisms of clinical efficacy and adverse events of *A. barbatum*, we initially assessed the electropharmacological effects of its water soluble ex-

Fig. 1 The photo of wild *Aconitum barbatum* blooming at Jargalant, Töw province, Mongolia.

tract on the anesthetized rats. Next, we analyzed underlying molecular mechanisms of cardiac effects of *A. barbatum*, including its β-adrenoceptor blocking effects *in vitro*.

Methods

Preparation of the extract of *A. barbatum*

The flowers of *A. barbatum* were collected at Jargalant, Töw province, Mongolia (48° 52' N, 105° 85' E) on the 5th of August, 2016 (Fig. 1). The voucher specimen (No. 20160801 TUAB) was deposited in the Department of Pharmacology, Faculty of Medicine, Toho University, Tokyo, Japan. The plant materials were dried at room temperature in darkness for 2 weeks, which were then kept desiccated in a cool place at 4°C. The dried flowers were grinded into powder in a mortar and pestle. One gram of the powder was immersed with 30 mL of boiling purified water for 30 min, amounting to a 33.3 mg/mL solution. Then, the suspension was filtered twice; initially with a filter paper (Grade No. 2, circle 150 mm; Advantec Toyo Kaisha, Ltd., Tokyo, Japan), followed by EB-DISK 25 (Cat. No. 96924-01, pore size 0.45 μm; KANTO CHEMICAL CO., INC., Tokyo, Japan), and the filtrate was dispensed into 1-2 mL aliquots and was frozen at −20°C until the *in vivo* experimentation. We diluted the extract using purified water for infusion. *A. barbatum* has been empirically used in a p.o. dose of 5 mg/body, twice a day. Supposing that its bioavailability is 1%, 10%, and 50%,[6–9] and the body weight is 50 kg, clinical daily dose would correspond to 0.001, 0.01, and 0.05 mg/kg, i.v.; thus, the intravenous doses of 0.1, 1, and 10 mg/kg could be 100, 1,000, and 10,000 times; 10, 100, and 1,000 times; and 2, 20, and 200 times higher than the clinical value, respectively.

Experiment 1: Assessment of the *in vivo* electropharmacological effects

Experiments were performed in male Sprague-Dawley rats, weighing 440-540 g (n = 4) (Japan SLC, Inc., Shizuoka, Japan). Animals were kept at 23°C ± 1°C under a 12-h light-dark cycle and provided with standard chow and water ad libitum. The animals were anesthetized initially with thiopental sodium (50 mg/kg, i.p.). After tracheal intubation, 1.5% isoflurane vaporized with 100% oxygen was inhaled with a volume-limited ventilator (SN-480-7; Shinano Manufacturing Co., Ltd., Tokyo, Japan). The tidal volume and respiratory rate were set at 10 mL/kg and 60 breaths/min, respectively, whereas the body temperature was maintained at 37°C using a heating pad (BWT-100A; Bio Research Center Co., Ltd., Nagoya, Japan). The surface lead II electrocardiogram was recorded from the limb electrodes. Three heparinized catheters were used. The first one was inserted into the left external jugular vein for the drug administration. The second one was inserted into the aorta through the left common carotid artery to measure the blood pressure. The third one was inserted into the left ventricle through the right common carotid artery to obtain the left ventricular pressure. The maximum upstroke velocity of the left ventricular pressure (peak + dP/dt) and the left ventricular end-diastolic pressure were used to estimate the inotropic status and the preload to the left ventricle, respectively, as previously described.[10] The aortic and left ventricular pressures and electrocardiogram were monitored with a polygraph system (RM-6000; Nihon Kohden Corporation, Tokyo, Japan), which were analyzed with a real-time, fully automatic data analysis system (WinVAS3 ver. 1.1R24; Physio-Tech Co., Ltd., Tokyo, Japan). Each measurement of the cardiohemodynamic and electrocardiographic variables adopted the mean of three consecutive recordings. We infused 0.3 mL/kg of the solution for each dose, wherein such a small volume of 0.3 mL/kg by itself would hardly alter the cardiohemodynamic variables. After the basal assessment, *A. barbatum* in a dose of 0.1 mg/kg was intravenously infused over 10 min, and each variable was assessed at 5, 10, 15, 20, and 30 min after the start of infusion. Then, *A. barbatum* in a dose of 1

mg/kg was intravenously infused over 10 min, and each variable was assessed in the same manner. Finally, *A. barbatum* in a dose of 10 mg/kg was intravenously infused over 10 min, and each variable was assessed at 5, 10, 15, 20, 30, 45, and 60 min after the start of infusion.

Experiment 2: Analysis of the *in vitro* molecular mechanisms of cardiac effects

The adenylyl cyclase activities of the ventricular membrane preparations were measured as previously described.[11] Briefly, the left ventricles were obtained from two male Sprague-Dawley rats, weighing 387 and 400 g (Japan SLC, Inc., Shizuoka, Japan). The animals were anesthetized with an intraperitoneal injection of thiopental sodium 250 mg/kg. Ventricles were excised and placed in ice-cold SET buffer (0.25 M sucrose, 0.1 mM EDTA, 5.0 mM Tris-HCl, pH 7.4). The ventricles were minced and homogenized in five volumes of ice-cold SET buffer. The homogenate was centrifuged at 10,000 × g for 10 min at 4℃. The pellet was resuspended in the 500 μL of SET buffer, and the mixture was centrifuged at 10,000 × g under the same setting. After this procedure was repeated three times in total, the pellet was finally suspended with SET buffer to a concentration of about 3 mg protein/mL and was stored at −80℃ until the adenylyl cyclase activities were measured. Protein quantitation was performed using bicinchoninic acid (BCA) method (TaKaRa BCA Protein Assay Kit, T9300A, Takara Bio Inc., Shiga, Japan). In the measurement of adenylyl cyclase activity, 30 μL of assay medium (100 mM Tris-acetate, pH 7.4, 20 mM KCl, 10 mM $MgCl_2$, 20 mM phosphoenolpyruvate, 2 mM ATP, 2 mM GTP, 2 mM dithiothreitol, 0.04% bovine serum albumin, 0.1 mM 3-isobutyl-1-methylxanthine [IBMX], and 100 μg/mL pyruvate kinase) with or without either of *A. barbatum* (20 μg/mL), isoproterenol (20 μM), *A. barbatum* (20 μg/mL) with propranolol (2 mM), or isoproterenol (20 μM) with propranolol (2 mM) was added to microcentrifugation tubes in duplicate at 4℃. Next, the membrane suspension in a volume of 30 μL was added to each of the tubes at 4℃. The mixture was incubated at 37℃ for 30 min and heated at 95℃ for 5 min to terminate the reaction. Then, the mixture was centrifuged at 2,000 × g for 5 min. A volume of 5 μL of the supernatant was transferred to a 10 × 75 mm disposable glass tube (Iwaki Lab Ware, Tokyo, Japan) in triplicate. The cyclic AMP concentration was measured with the enzymatic fluorometric assay as previously described.[12] The assay protocol was repeated four times for each of the pharmacological interventions.

Drugs and chemicals

Thiopental sodium (Ravonal® 0.5 g for Injection, Mitsubishi Tanabe Pharma Co., Osaka, Japan), isoflurane (ISOFLU®, DS pharma Animal Health Co., Ltd., Osaka, Japan), heparin calcium (Caprocin®, Sawai Pharmaceutical Co., Ltd., Osaka, Japan), propranolol (Sigma-Aldrich Co., LLC., St. Louis MO, USA), isoproterenol (Sigma-Aldrich Co., LLC.), alkaline phosphatase (ORIENTAL YEAST Co., Ltd., Tokyo, Japan), hexokinase (ORIENTAL YEAST Co., Ltd.), myokinase (ORIENTAL YEAST Co., Ltd.), pyruvate kinase (Sigma-Aldrich Co., LLC.), adenosine deaminase (Roche Diagnostics GmbH., Mannheim, Germany), apyrase (Sigma-Aldrich Co., LLC.), phosphodiesterase (Sigma-Aldrich Co., LLC.), phosphoglucose isomerase (Roche Diagnostics GmbH.), glucose 6-phospate dehydrogenase (FUJIFILM Wako Pure Chemical Corporation, Tokyo, Japan), EDTA (Sigma-Aldrich Co., LLC.), Tris-HCl (Sigma-Aldrich Co., LLC.), Tris-asetate (Sigma-Aldrich Co., LLC.), KCl (FUJIFILM Wako Pure Chemical Corporation), $MgCl_2$ (FUJIFILM Wako Pure Chemical Corporation), phosphoenolpyruvic acid monopotassium salt (FUJIFILM Wako Pure Chemical Corporation), sucrose (FUJIFILM Wako Pure Chemical Corporation), ATP (Roche Diagnostics GmbH.), GTP (Roche Diagnostics GmbH.), dithiothreitol (Sigma-Aldrich Co., LLC.), bovine serum albumin (Sigma-Aldrich Co., LLC.), and IBMX (Sigma-Aldrich Co., LLC.) were purchased.

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). In Experiment 1, differences within a parameter were evaluated with one-way, repeated-measures analysis of variance (ANOVA), followed by Contrasts as a post-hoc test for the comparison of the mean values. In Experiment 2, differences among the treatments were evaluated with one-way factorial ANOVA, followed by Tukey's post-hoc multiple comparison test. A P-value of <0.05 was considered to be significant.

Results

Experiment 1: Effects on the cardiohemodynamic and electrocardiographic variables

No animals exerted any lethal ventricular arrhythmias or hemodynamic collapse, leading to the animals' death during the experiment. Typical tracings of the electrocardiogram, left ventricular pressure, and aortic pressure are depicted in Fig. 2, and the time courses of changes in the cardiohemodynamic and electrocardiographic variables

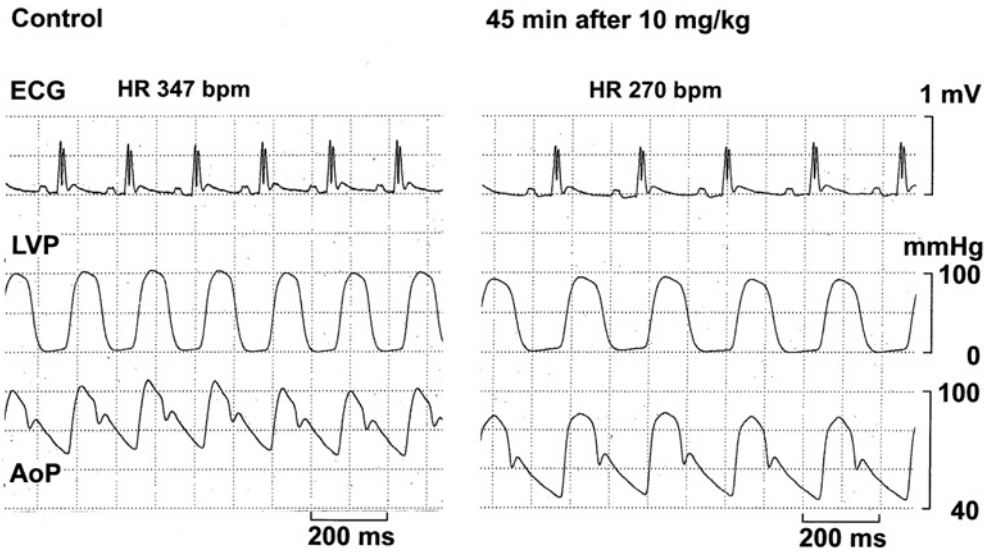

Fig. 2 Typical tracings showing the lead II electrocardiogram (ECG), left ventricular pressure (LVP), and aortic pressure (AoP) during sinus rhythm at pre-drug basal control (Control, left) and 45 min after the start of 10 mg/kg of *Aconitum barbatum* infusion (45 min after 10 mg/kg, right).

are summarized in Fig. 3. The pre-drug basal control values (C) of the heart rate, mean blood pressure, peak+dP/dt, left ventricular end-diastolic pressure, PR interval, and QRS width were 302±27 beats/min, 67±10 mmHg, 3,759±778 mmHg/s, 5.0±1.3 mmHg, 64±8 ms, and 35±4 ms, respectively. The low dose decreased the heart rate, mean blood pressure, and peak+dP/dt but prolonged the PR interval at 30 min after the start of infusion, whereas no significant change was detected in the other variables. The middle dose decreased the heart rate for 5-30 min, the mean blood pressure for 10-30 min, and the peak+dP/dt for 5-30 min and prolonged the PR interval for 5-30 min, whereas no significant change was detected in the other variables. The high dose further decreased the heart rate, mean blood pressure, and peak+dP/dt for 5-60 min and prolonged the PR interval for 5-60 min, whereas no significant change was detected in the other variables.

Experiment 2: Effects on the adenylyl cyclase activity

The effects of the pharmacological interventions on the adenylyl cyclase activity in the membrane preparations are summarized in Fig. 4. The adenylyl cyclase activities in the basal condition, isoproterenol (10 μM), isoproterenol (10 μM) with propranolol (1 mM), *A. barbatum* (10 μg/mL), and *A. barbatum* (10 μg/mL) with isoproterenol (10 μM) were 36.5±1.7, 47.7±2.3, 32.5±0.9, 33.1±1.2, and 42.9±2.5 (cyclic AMP pmol/min/mg protein, n = 4), respectively. The adenylyl cyclase activity was elevated by isoproterenol, of which response was suppressed by propranolol. *A. barbatum* did not alter basal adenylyl cyclase activity or isoproterenol-stimulated activity.

Discussion

We analyzed pharmacological action of *A. barbatum* using the isoflurane-anesthetized rats, along with the membrane preparations obtained from the left ventricles of rats. The obtained results provided a clue to better understand the clinical utility and adverse events of *A. barbatum* as discussed below.

In Experiment 1, we used approximately 10, 100, and 1,000 times larger doses than the clinical daily dose to estimate the efficacy and safety margins of *A. barbatum*. Given that *A. barbatum* did not induce lethal cardiovascular adverse event *in vivo*, it may have a wide range of safety margin. *A. barbatum* induced the negative chronotropic, inotropic, and dromotropic effects, along with hypotensive action. These results are in accordance with previous studies of anesthetized dogs showing that lappaconitine and denudatine decreased the heart rate and ventricular contraction.[1,2] Also, *A. barbatum* tended to decrease the left ventricular end-diastolic pressure, although it did not achieve a statistical significance. *A. barbatum* hardly altered the QRS width, suggesting that it would not block the Na^+ channel.

The intravenous dose of 10 mg/kg of *A. barbatum* used

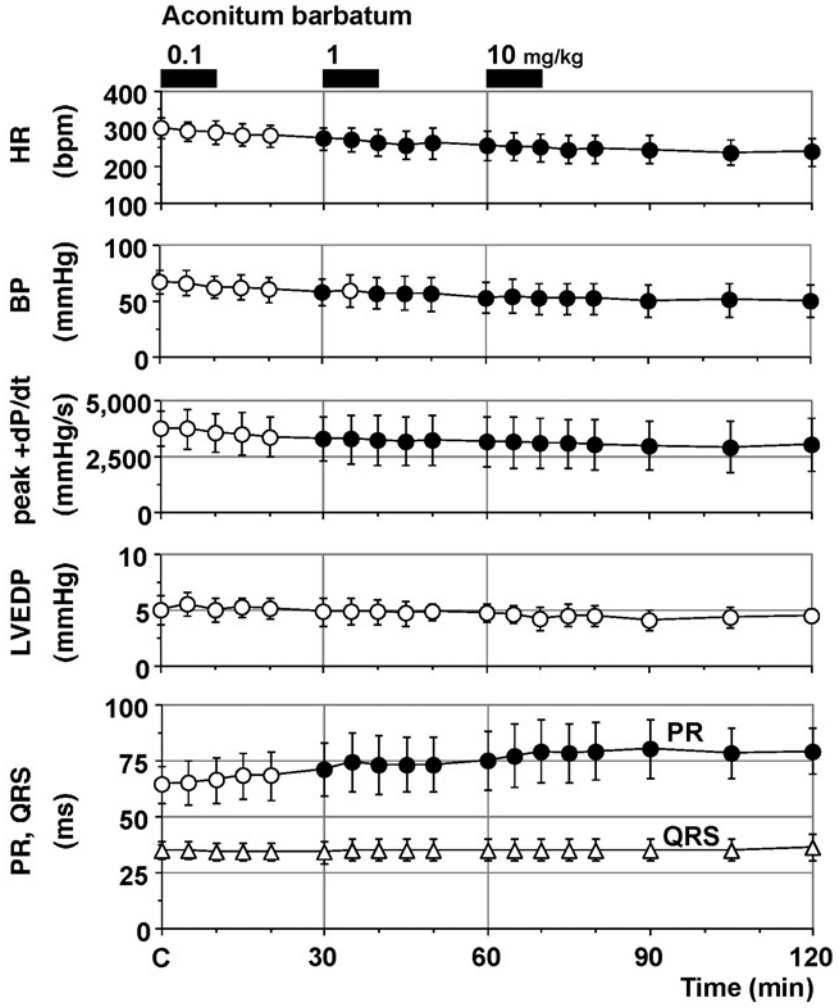

Fig. 3 Time courses of changes in the heart rate (HR), mean blood pressure (BP), maximum upstroke velocity of the left ventricular pressure (peak + dP/dt), left ventricular end-diastolic pressure (LVEDP), PR interval, and QRS width after the administration of *Aconitum barbatum* (n = 4). Data are presented as mean ± standard error of the mean (SEM). Filled symbols represent significant differences from the corresponding pre-drug basal control value (C) by $P < 0.05$.

in Experiment 1 would provide approximately 10 μg/mL of plasma concentration, based on our previous *in vivo* experiments.[13] In Experiment 2, we assessed whether *A. barbatum* may inhibit the isoproterenol-stimulated adenylyl cyclase activity. *A. barbatum* hardly altered the adenylyl cyclase activity, indicating a presence of ß-adrenoceptor-independent mechanism for explaining its cardiovascular effects, including Ca^{2+} channel blockade.

In conclusion, the aqueous extract of the flower of *A. barbatum* has little toxic effect even at a high concentration. The results also showed the negative chronotropic, inotropic, and dromotropic effects of the extract, along with hypotensive action *in vivo*, possibly due to ß-adrenoceptor-independent mechanism, which may partly explain the clinical efficacy of the *A. barbatum* extract.

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Authors' contribution: Koki Chiba and Atsushi Sugiyama conceived and designed the research. Koki Chiba conducted the experiments. Koki Chiba, Ryuichi Kambayashi, and Ai Goto analyzed the data. Koki Chiba and Atsushi Sugiyama wrote the manuscript. All

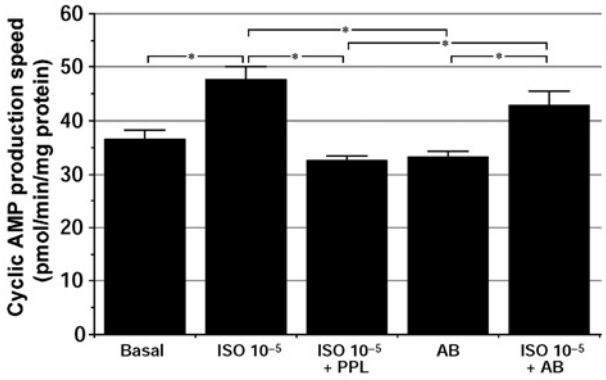

Fig. 4 Adenylyl cyclase activities assessed as cyclic AMP production speed in the basal condition and in the presence of isoproterenol (10 μM), isoproterenol (10 μM) with propranolol (1 mM), *A. barbatum* (10 μg/mL), or *A. barbatum* (10 μg/mL) with isoproterenol (10 μM) in the membrane preparations made from the left ventricle of rats. Data are presented as mean ± standard error of the mean (SEM; n = 4). ISO 10^{-5}: isoproterenol (10 μM), PPL: propranolol (1 mM), and AB: *Aconitum barbatum* (10 μg/mL). $*P < 0.05$. No significant difference was detected between the basal and ISO 10^{-5} + PPL, basal and AB, or ISO 10^{-5} and ISO 10^{-5} + AB.

authors read, discussed, and approved the manuscript. The authors declare that all data were generated in-house and that no paper mill was used.

Ethical statement: All experiments were approved by the Animal Research Committee for Animal Experimentation of Toho University (No. 17-55-251, 18-51-391) and performed in accordance with the Guidelines for the Care and Use of Laboratory Animal of Toho University.

Conflicts of interest: None declared.

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