名古屋学院大学論集　人文・自然科学篇　第 57 巻　第 2 号　pp. 29–35

〔Article〕

Effects of Abietane diterpenes from *Rosmarinus officinalis* on guinea pig hearts

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Abstract

A plant in the *Lamiaceae* family, rosemary (*Rosmarinus officinalis*) is a perennial shrub native to the Mediterranean region. The current study used right atrial specimens from guinea pigs to assess the biological activity of 3 catechol diterpenes with an abietane skeleton—carnosic acid (CA–1), demethylsalvicanol (DS–1), and carnosol (CN–1)—contained in rosemary. Administration of CA–1 had a positive inotropic effect (PIE) while administration of CN–1 conversely had a negative inotropic effect (NIE). Administration of DS–1 resulted in no changes in myocardial contractility. Administration of CA–1 or DS–1 did not result in significant changes in heart rate. However, administration of CN–1 had a negative chronotropic effect (NCE), and administration of CN–1 at a final concentration of 10^{-4} M caused cardiac arrest. In addition, the PIE of CA–1 was inhibited by prior administration of the phosphodiesterase (PDE) inhibitor IBMX (5 μM).

Keywords: Abietane diterpenes, Positive inotropic effect (PIE), Negative inotropic effect (NIE), Negative chronotropic effect (NCE), heart rate (HR)

ローズマリーより得られたアビエタン系ジテルペン類のモルモット心臓に対する作用

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発行日　2021年1月31日

1. Introduction

A plant in the *Lamiaceae* family, rosemary (*Rosmarinus officinalis*) is a perennial shrub native to the Mediterranean region, and it is used as a spice and to make herb tea. This plant is reported to have various effects on conditions such as alopecia, miscarriages, gastrointestinal disorders, menorrhagia, gout, headaches, liver disorders, hypertension, and arthralgia[1]. Nevertheless, there are insufficient scientific data for most of those effects. Thus, the current study assessed the biological activity, as indicated by cardiotonic action, of 3 catechol diterpenes with an abietane skeleton - carnosic acid (CA–1), demethylsalvicanol (DS–1), and carnosol (CN–1).

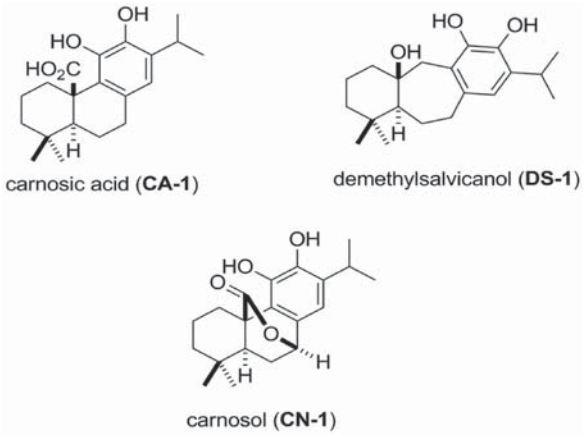

Fig. 1. Structural formulae for carnosic acid (CA–1), demethylsalvicanol (DS–1), and carnosol (CN–1), which are 3 catechol diterpenes with an abietane skeleton

2. Experimental materials and methods

2.1 Laboratory animals

Male guinea pigs (Hartley, SPF) purchased from Chubu Kagaku Shizai Co. were used in experiments. All animal experiments were conducted in accordance with the Animal Experimentation Guidelines of Kinjo Gakuin University (approval no.: 11–1).

2.2 An experiment involving right atrial specimens

Guinea pigs with a weight of around 350 g were anesthetized with ether and sacrificed. The chest was immediately opened. The beating heart was removed and temporarily stored in a Krebs-Henseleit solution (Ca^{2+}: 2 mM; 30℃) saturated with a gas mixture (95%O_2 and 5%CO_2). Right atrial

specimens were prepared. One end of the right atrial myocardium was fixed with thread to a tube for influx of the mixture in the specimen chamber, and the other end was similarly connected with thread to the end of a tensiometer (TB–612T, Nihon Kohden). Static tension of 0.5 g was applied. Tension produced by autonomic pulsations was converted to voltage via a strain gauge tensiometer and then recorded through an amplifier (AP–601G, Nihon Kohden). Heart rate was measured with a cardiotachometer (AT–601, Nihon Kohden) and recorded[2] [3].

2.3 Statistical analysis

All values are expressed as the mean ± S.E.M unless otherwise specified. The unpaired t-test or one-way analysis of variance (ANOVA) followed by Dunnett's method was used to compare means between groups. Statistical significance was defined as $p < 0.05$.

3. Results and discussion

The ionotropic effects (myocardial contractility) and chronotropic effects (heart rate) in right atrial myocardium from guinea pigs when CA–1 was administered are shown in Fig. 2(A). When CA–1 was cumulatively administered at a final concentration of 10^{-6}M, 10^{-5}M, and 10^{-4}M, contractility (Fc) increased significantly compared to the control (prior to administration), and a positive inotropic effect (PIE) was evident. However, significant differences in the heart rate (HR) were not noted at any of the final concentrations.

The ionotropic effects and chronotropic effects in right atrial myocardium from guinea pigs when DS–1 was administered are shown in Fig. 2(B). When DS–1 was cumulatively administered at a final concentration of 10^{-6}M, 10^{-5}M, and 10^{-4}M, significant differences in contractility (Fc) and HR compared to the control (prior to administration) were not noted at any of the concentrations.

The ionotropic effects and chronotropic effects in right atrial myocardium from guinea pigs when CN–1 was administered are shown in Fig. 2(C). When CN–1 was cumulatively administered at a final concentration of 10^{-6}M, 10^{-5}M, and 10^{-4}M, contractility (Fc) decreased significantly in a concentration-dependent manner compared to the control (prior to administration) at a final concentration of 3×10^{-6}M–3×10^{-5}M, and a negative inotropic effect (NIE) was evident. HR also decreased significantly in a concentration-dependent manner at a final concentration of 3×10^{-5}M–10^{-4}M, and a negative chronotropic effect (NCE) was evident. Moreover, administration of CN–1 at a final concentration of 10^{-4}M caused cardiac arrest. When adrenaline was administered at a concentration of 10^{-6}M, cardiac contractility and HR temporarily recovered, but cardiac arrest occurred again and cardiac contractility and HR did not recover.

Changes in cardiac contractility and HR in isolated right atria from guinea pigs as a result of CA–1, DS–1, and CN–1 are shown in Fig. 3(A) and 3(B) for comparison. In a *t*-test, the status prior to

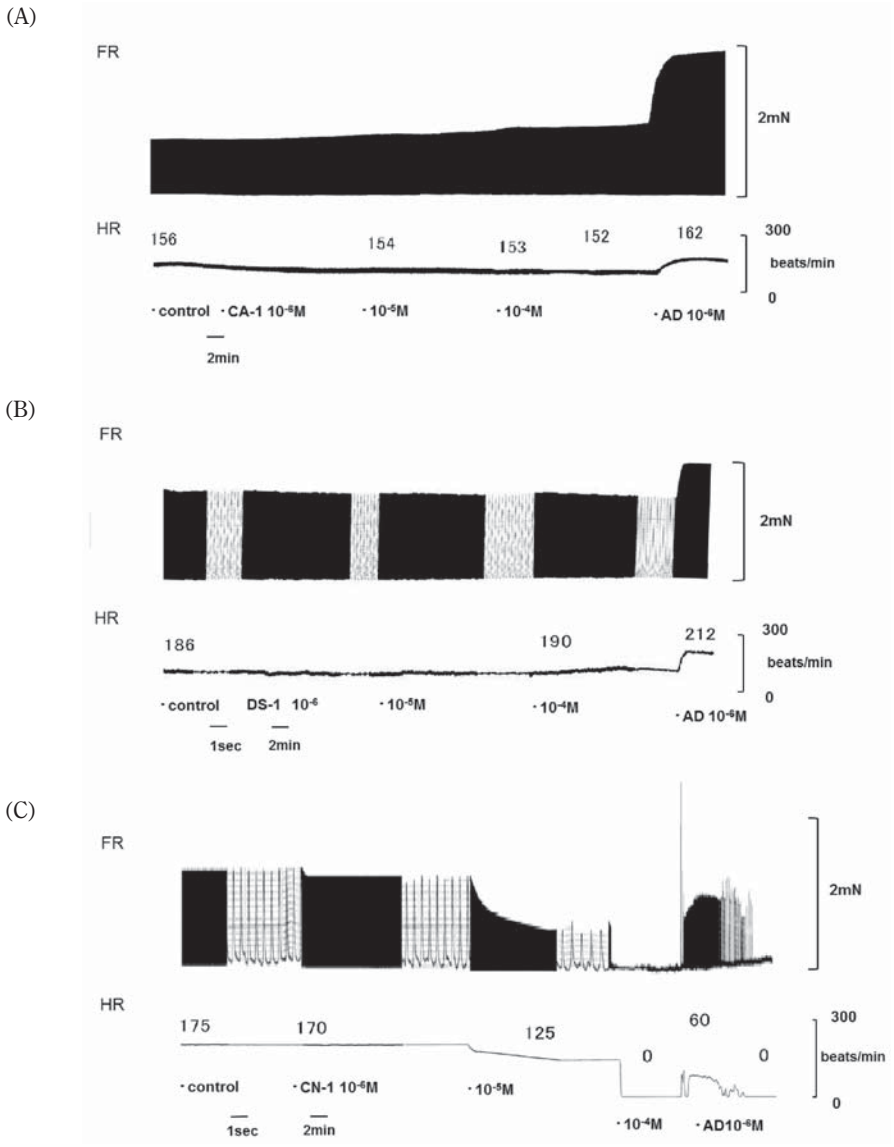

Fig. 2. Changes in cardiac contractility and heart rate in the right atrium of guinea pigs as a result of CA−1 (A), DS−1 (B), and CN−1 (C).

administration of each drug (control) served as 100%. When cardiac contractility and HR as a result of CA−1, DS−1, and CN−1 were compared to the control, administration of CA−1 significantly increased cardiac contractility in a concentration-dependent manner compared to the control, and $p<0.01$ for all final concentrations from 10^{-6}M−1×10^{-4}M. In contrast, administration of CN−1 significantly decreased cardiac contractility in a concentration-dependent manner compared to the control, and $p<0.01$ for final concentrations from 3×10^{-6}M−1×10^{-4}M. With administration of DS−1,

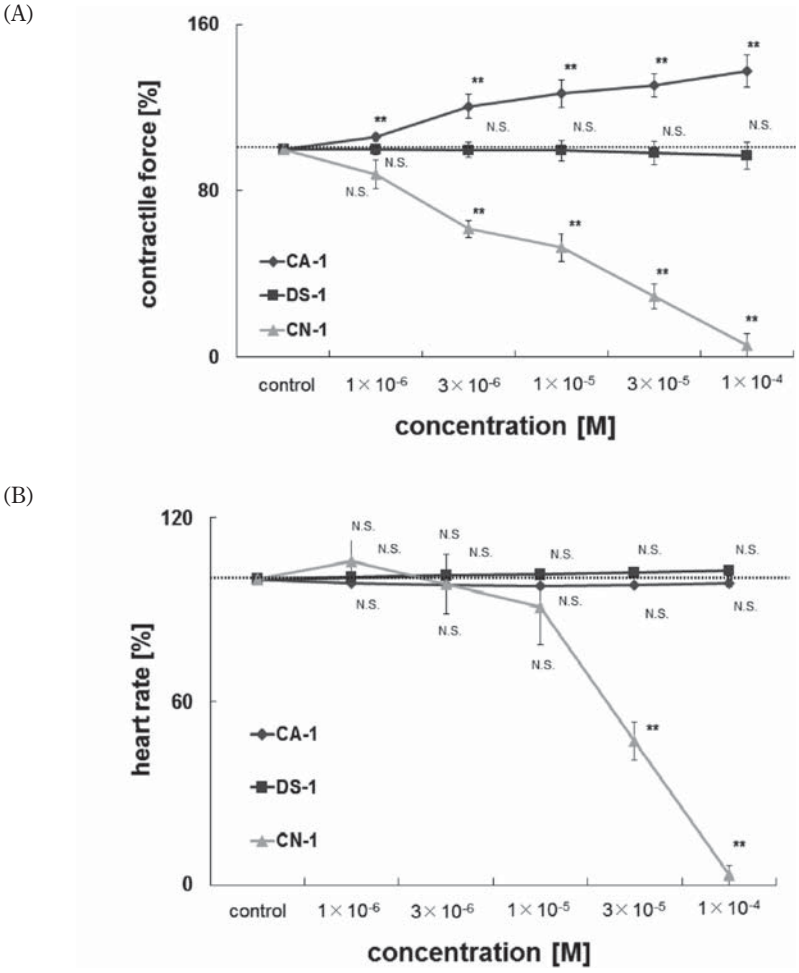

Fig. 3. Comparison of the effects of CA–1 (n＝7), DS–1 (n＝3), and CN–1 (n＝4) on cardiac contractility (A) and heart rate (B) in right atrial specimens from guinea pigs. Data are expressed as the mean ± S.E.M; N.S.; $^{*}p<0.05$; $^{**}p<0.01$ (significant difference vs. control).

cardiac contractility did not change at any of the final concentrations (Fig. 3A). Administration of CN–1 at a concentration of 3×10^{-5}M or higher significantly reduced HR, and $p<0.01$. Moreover, administration of CN–1 at a concentration of 10^{-4}M caused cardiac arrest. This was temporarily alleviated by administration of adrenaline at a concentration of 10^{-6}M, but cardiac arrest occurred again and HR did not recover. Administration of CA–1 or DS–1 did not change HR at any concentration (Fig. 3B). Furthermore, it is necessary to add an experiment for a elucidation of the mechanism of NCE or NIE.

The ionotropic effects (myocardial contractility) and chronotropic effects (heart rate) of CA–1 in right atrial myocardium from guinea pigs were compared when CA–1 (10^{-4}M) was administered alone

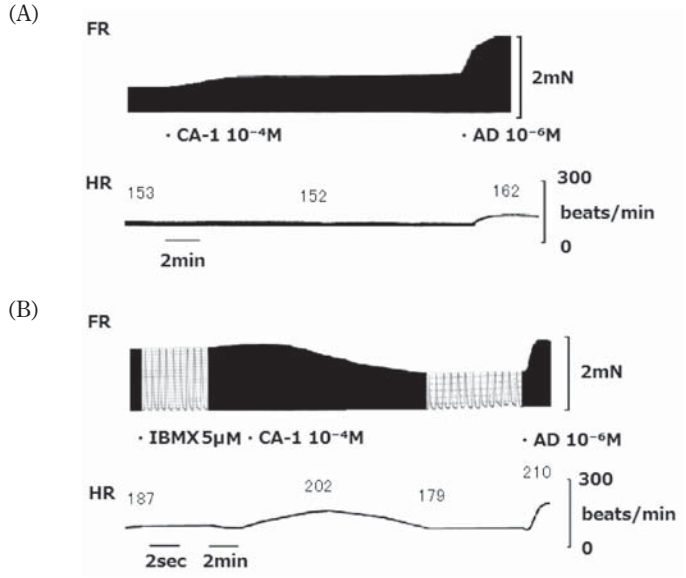

Fig. 4. Comparison of the ionotropic effects (myocardial contractility) and chronotropic effects (heart rate) in right atrial myocardium from guinea pigs when CA−1 was administered alone (A) and when IBMX＋CA−1 were administered (B).

(A) and when CA−1 (10^{-4}M) was administered after administration of IBMX (5 μM). The PIE of CA−1 was inhibited by prior administration of the phosphodiesterase (PDE) inhibitor IBMX (5 μM) (Fig. 4).

4. Conclusion

CA−1 has a normal abietane skeleton and PIE. In contrast, CN−1 has an ether bond and NIE. DS−1 has a 7−membered B ring and caused no changes in myocardial contractility whatsoever. There were no significant changes in HR as a result of administration of CA−1 or DS−1. However, CN−1 had NCE and caused cardiac arrest at a concentration of 10^{-4}M. The PIE of CA−1 was inhibited by prior administration of the PDE inhibitor IBMX (5 μM), suggesting that inhibition of PDE in involved in this mechanism of action. Based on the above findings, the effects of these drugs differ substantially due to differences in the skeleton indicated in the structural formula for each. In order to specifically determine the PIE, NIE, NCE, and the mechanism of cardiac arrest of these compounds, compounds with different skeletons need to be synthesized and examined along with changes in Ca dynamics[3][4].

Acknowledgements

The authors wish to sincerely thank Ms. Tomoyo Kawane, Ms. Ayane Sumikawa, and Ms. Mai Yorizane of the Department of Pharmacy, Kinjo Gakuin University for all of their assistance with this study.

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