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〔Article〕

Effects of *ent*-Kaurene diterpenes from *Rabdosia excisa* on the cardiac function of guinea pigs

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Abstract

The medicinal plant *Rabdosia excisa* in the *Lamiaceae* family is found in Northeastern China and is used to treat a fever due to a cold, mastitis, arthralgia, and bruises[1]. A previous study reported that an aqueous extract from that plant yielded *ent*-kaurene diterpenes with antitumor action. In addition, diterpenes have been used to synthesize analogs in relatively large quantities, and their structure-activity relationship in terms of cytotoxic activity[2] and inhibition of the activation of the intracellular transcription factor NF-κB[3] has been examined. Various studies have also examined *ent*-kaurene diterpenes[4]–[7]. Three diterpenes—kamebakaurin (1), kamebanin (2), and excisanin A (3)—can be obtained in relatively large quantities from *R. excisa*. The current study examined the effects of those diterpenes on cardiac function in guinea pigs. Some of those findings are reported here.

Keywords: *Rabdosia excisa* (Labiatae), *ent*-Kaurene diterpenoids, Positive inotropic effect (PIE), Positive chronotropic effect (PCE), Heart rate (HR)

狗日草（コウジツソウ）ジテルペン類のモルモットにおける心機能作用

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1. Introduction

The medicinal plant *Rabdosia excisa* is a member of the *Lamiaceae* family that is found in Northeastern China, and it is source of 3 *ent*-kaurene diterpenes – kamebakaurin (1), kamebanin (2), and excisanin A (3). The current study examined the effect of those *ent*-kaurene diterpenes on cardiac function in guinea pigs.

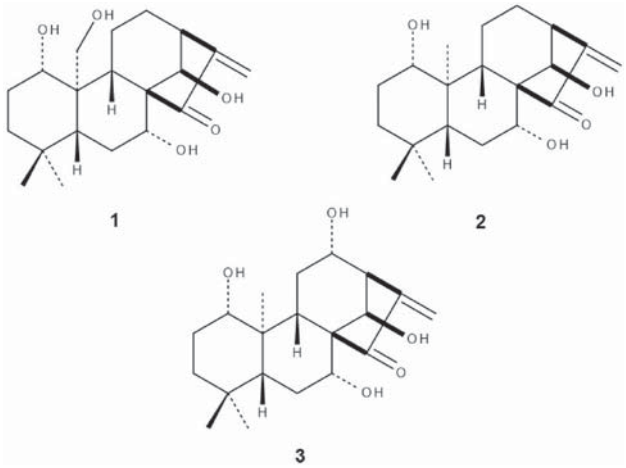

Fig. 1. Chemical structures of kamebakaurin (1), kamebanin (2), and excisanin A (3).

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 between the two ends. 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[8] [9].

The sensitivity of a specimen to each compound was expressed as the pD_2 value (negative log of EC_{50}), which was derived from the dose-response curve using a non-linear regression calculation.

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

Three *ent*-kaurene diterpenes—kamebakaurin (1), kamebanin (2), and excisanin A (3)—can be obtained from the medicinal plant *R. excisa* in the *Lamiaceae* family. The effects of those diterpenes was examined using right atrial specimens from guinea pigs. Kamebanin (2) significantly increased cardiac contractility at a final concentration of 1×10^{-6} M or higher compared to the control (drug-free), and it had a positive inotropic effect (PIE) in a concentration-dependent manner (Figs. 2, 3a). Kamebakaurin (1) at a final concentration of 3×10^{-6} M or higher and excisanin A (3) at a final concentration of 3×10^{-5} M or higher significantly increased cardiac contractility, and both had a PIE in a concentration-dependent manner (Fig. 3a).

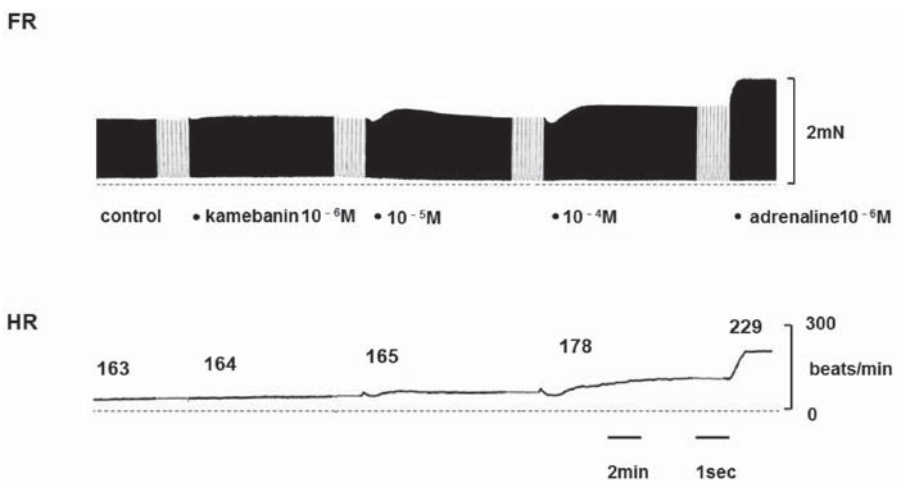

Fig. 2. Positive inotropic effects (PIE) and positive chronotropic effects (PCE) of kamebanin (2) on isolated guinea pig atria.

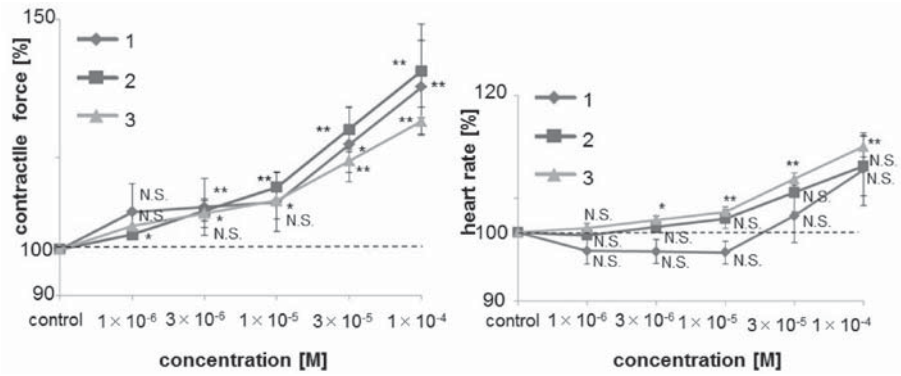

Fig. 3a　　Fig. 3b

Fig. 3a. Positive inotropic effects (PIE) of kamebakaurin (1), kamebanin (2), and excisanin A (3) on isolated guinea pig atria.

Fig. 3b. Positive chronotropic effects (PCE) of kamebakaurin (1), kamebanin (2), and excisanin A (3) on isolated guinea pig atria.

Data are expressed as the mean for 5 specimens ± S.E.M; N. S.; $^{*}p<0.05$; $^{**}p<0.01$;

Only excisanin A (3) significantly increased heart rate (HR) at a final concentration of 3×10^{-6} M or higher compared to the control (drug-free), and it had a positive chronotropic effect (PCE) in a concentration-dependent manner. Kamebakaurin (1) and kamebanin (2) did not have a PCE at any final concentration (Fig. 3b).

pD_2 was 4.78±0.19 for kamebakaurin (1), 4.95±0.17 for kamebanin (2), and 4.99±0.21 for excisanin A (3), so the 3 compounds had similar pD_2 values (Table 1).

Table 1. Comparison of pD_2 values for *ent*-kaurene diterpenoids, the amplitude of PIE, Δ mN (%), and heart rate (%) in isolated right atria of guinea pigs.

	pD_2value	Δ mN［%］	HR［%］	n
1	4.78 ± 0.19	1.16 ± 0.16 ** (135.25 ± 10.19)	206.00 ± 3.37 $^{N.S.}$ (109.24 ± 5.34)	5
2	4.95 ± 0.17	1.40 ± 0.30 ** (138.79 ± 10.13)	204.67 ± 9.46 $^{N.S.}$ (109.71 ± 4.37)	5
3	4.99 ± 0.21	1.16 ± 0.18 ** (127.84 ± 3.06)	209.40 ± 9.22 ** (112.62 ± 1.58)	5

1: kamebakaurin　2: kamebanin　3: excisanin A
Mean ± S.E.M.; $^{**}p<0.01$; Δ mN (%), with the control (drug-free) serving as 100%.

The PIE and PCE of kamebakaurin (1) and excisanin A (3) were mostly inhibited by prior administration of 5 μM 3-isobutyl-1-methylxantine (IBMX), a phosphodiesterase (PDE) inhibitor

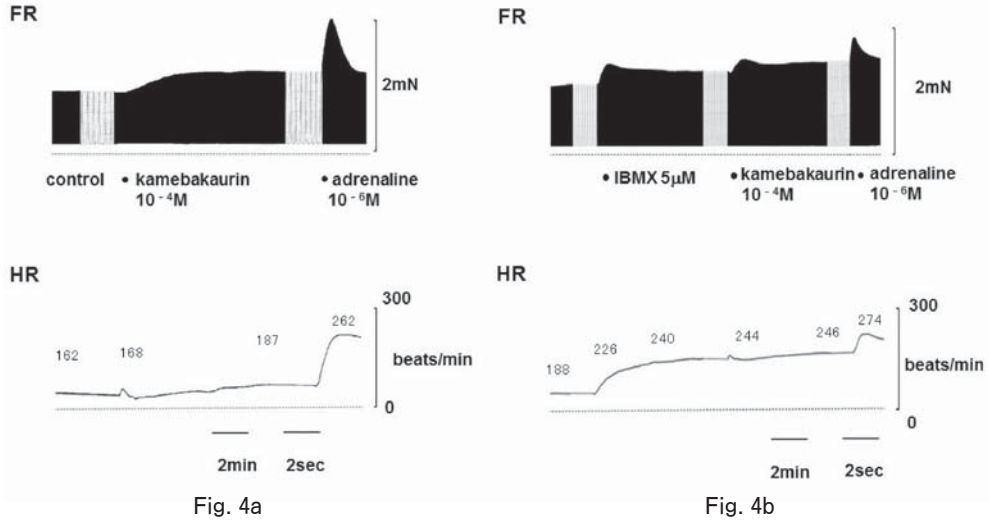

Fig. 4a Fig. 4b

Fig. 4a. Positive inotropic effects (PIE) and positive chronotropic effects (PCE) of 10^{-4}M kamebakaurin (1) on isolated right atria of guinea pigs.

Fig. 4b. Positive inotropic effects (PIE) and positive chronotropic effects (PCE) of 5 μM IBMX + 10^{-4} M kamebakaunin (1) on isolated right atria of guinea pigs.

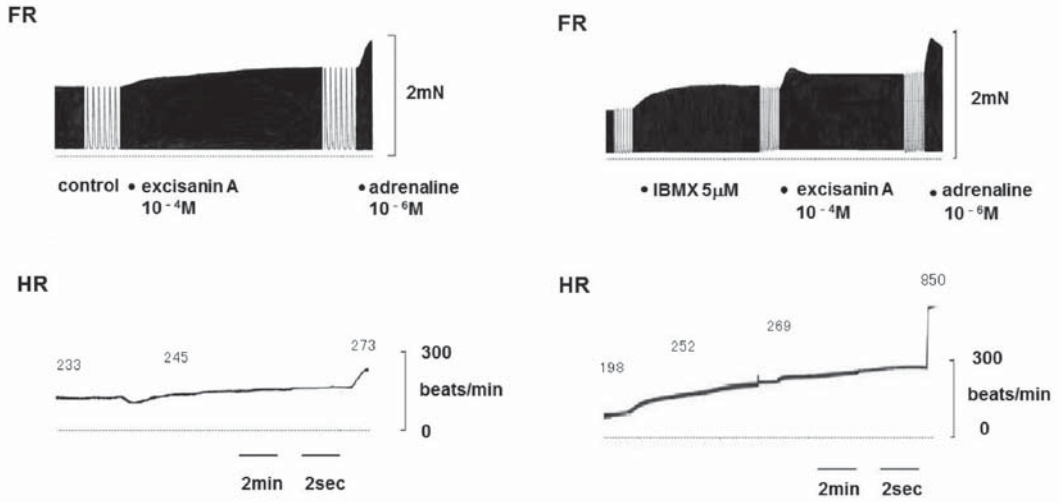

Fig. 5a Fig. 5b

Fig. 5a. Positive inotropic effects (PIE) and positive chronotropic effects (PCE) of 10^{-4} M excisanin A (3) on isolated right atria of guinea pigs.

Fig. 5b. Positive inotropic effects (PIE) and positive chronotropic effects (PCE) of 5 μM IBMX + 10^{-4} M excisanin A (3) on isolated right atria of guinea pigs.

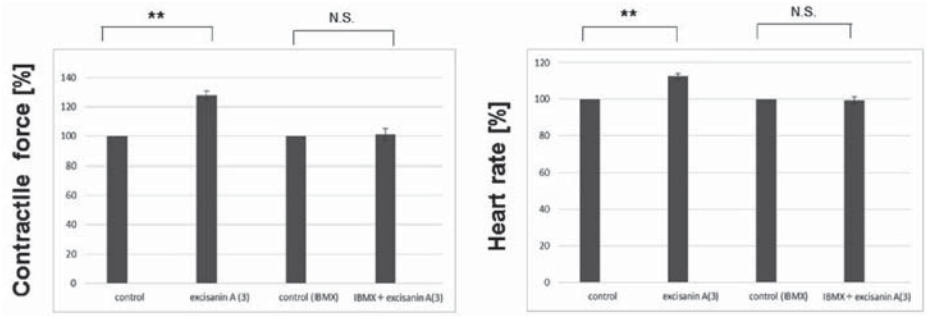

Fig. 6a　　Fig. 6b

Fig. 6a. Comparison of the contractile force (%) induced by 10^{-4} M excisanin A (3) in guinea pig atria following administration or no administration of 5 μM IBMX. ΔmN (%), with the control (drug-free) serving as 100%. Data are expressed as the mean for 5 specimens$\pm$ S.E.M; N. S.; $^{*}p < 0.05$; $^{**}p < 0.01$;

Fig. 6b. Comparison of the heart rate (%) induced by 10^{-4} M excisanin A (3) in guinea pig atria following administration or no administration of 5 μM. Data are expressed as the mean for 5 specimens$\pm$S.E.M; N. S.; $^{*}p<0.05$; $^{**}p<0.01$;

(Fig. 4a, 4b, 5a, 5b).

When 5 μM IBMX was not administered beforehand, the control (drug-free) served as 100%. After administration of excisanin A (3) at a concentration of 10^{-4} M, cardiac contractility increased 128$\pm$3.06% and HR increased 113$\pm$1.58% (Fig. 6a, 6b). When excisanin A (3) was administered at a concentration of 10^{-4} M after prior administration of 5 μM IBMX, cardiac contractility was 101$\pm$ 4.05% and HR was 99.4$\pm$2.03%. The control when only 5 μM IBMX was administered beforehand served as 100%, and significant differences were not evident (Fig. 6a, 6b). However, the quantity of kamebanin (2) was too small, so the experiment involving prior administration of 5 μM IBMX could not be performed. Based on this experiment, the PIE and PCE of 10^{-4} M excisanin A (3) were almost entirely inhibited by prior administration of 5 μM IBMX. This suggests that a PDE inhibitor plays an important role in inhibiting the effects of these *ent*-kaurene diterpenes on cardiac function.

When the 3 *ent*-kaurene diterpenes were administered at a final concentration of 10^{-4} M and adrenaline was then administered at a concentration of 10^{-6} M, the PIE of the control (administration of adrenaline alone) served as 100%. There were no significant differences in the PIE of adrenaline (Fig. 2, 4a, 5a). In other words, prior administration of compounds 1–3 did not affect the subsequent increase in cardiac contractility caused by adrenaline. When kamebakaurin (1) or kamebanin (2) was administered at a final concentration of 10^{-4} M and adrenaline was then administered at a concentration of 10^{-6} M, each significantly reduced the PCE of adrenaline compared to the control (administration of adrenaline alone) (Fig. 2, 4b). When excisanin A (3) was administered at a concentration of 10^{-4} M, however, it had almost on effect on the subsequent PCE of adrenaline (Fig.

5b).

4. Conclusion

Three *ent*-kaurene diterpenes – kamebakaurin (1), kamebanin (2), and excisanin A (3) – can be obtained from the medicinal plant *R. excisa* in the *Lamiaceae* family. An examination of their effect on cardiac function in guinea pigs yielded several findings. Kamebanin (2) at a final concentration of 1×10^{-6} M or higher, kamebakaurin (1) at a final concentration of 3×10^{-6} M or higher, and excisanin A (3) at a final concentration of 3×10^{-5} M or higher significantly increased cardiac contractility, and all 3 had a PIE. Only excisanin A (3) at a final concentration of 3×10^{-6} M or higher significantly increased HR, and it had a PCE. However, neither kamebakaurin (1) nor kamebanin (2) had a PCE when administered at a final concentration from 10^{-6}–10^{-4} M.

Prior administration of 5 μM IBMX mostly inhibited the PIE and PCE of kamebakaurin (1) and excisanin A (3) subsequently administered at a concentration of 10^{-4} M. This suggests that increased cardiac contractility caused by kamebakaurin (1) and excisanin A (3) in the right atrial myocardium from guinea pigs involved inhibition of PDE.

Administration of compounds 1–3 did not affect the PIE of adrenaline. Nevertheless, the effects of diterpenes on the increased HR as a result of adrenaline differ depending on the type of diterpene. Administration of excisanin A (3) did not affect the increase in HR caused by subsequently administered adrenaline, but administration of kamebakaurin (1) and kamebanin (2) significantly reduced the increase in HR caused by subsequently administered adrenaline.

PDE inhibitors are compounds synthesized as an alternative to cardiac glycosides, and drugs such as milrinone and pimobendan are used clinically to treat heart failure. In an exploratory study of heart failure treatments, the current authors conducted an experiment with compounds that have a PIE. One was (–)-epigallocathechin–3–gallate (EGCg), which is a catechin isolated from green tea[10]. Among the catechins found in green tea, EGCg did not significantly increase HR. EGCg modulates Ca signaling[11] in the same manner as pimobendan, a Ca sensitizer that activates contractile proteins[12]. In an experiment involving heart failure (an ischemia and reperfusion experiment), EGCg restored cardiac contractility after ischemia and reperfusion[10]. Compounds 1–3 that were used in the current experiment all have an *ent*-kaurene skeleton and they differ only in terms of the number and position of hydroxyl groups. This may affect the pharmacological activity of these compounds. An experiment with Ca fluorometry (fura–2) needs to be conducted to determine whether these *ent*-kaurene diterpenes activate contractile proteins like EGCg does. In the current experiment involving the right atrial myocardium, the compounds kamebakaurin (1) and kamebanin (2) did not have a significant PCE like catechins found in green tea have. An experiment involving ischemia and reperfusion needs to be conducted to determine whether kamebakaurin (1) and kamebanin (2) restore

contractions after ischemia and reperfusion[13] [14].

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