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Cardioinhibitory Properties of Potato Glycoalkaloids in Beetles

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Abstract The semi-isolated heart bioassay was used to evaluate the effect of glycoalkaloids extracted from potato leaves on the heart contractile activity of three beetle species *Zophobas atratus*, *Tenebrio molitor* and *Leptinotarsa decemlineata*. The dose–response curves indicated species specific action of tested substances. Application of glycoalkaloids on the continuously perfused *Z. atratus* heart inhibited progressively frequency contractions; higher concentrations exerted short and reversible cardiac arrests. In the rest two beetle species tested glycoalkaloids caused no cardiotropic effect. In vivo bioassay with 1 day old *Z. atratus* pupae showed that the extract induces a negative inotropic effect on the heart.

Keywords Potato · Glycoalkaloids · Beetles · Pupae

Many plant secondary metabolites serve as natural pesticides which can be widely utilized for commercial use (Fenwick et al. 1990). Potatoes, as well as other Solanaceae plants contain glycoalkaloids, a family of steroidal compounds that can act as cellular membrane disrupting factors or inhibit acetylcholinesterase activity (Friedman and Mc Donald 1997). In potato (*Solanum tuberosum* L.), glycoalkaloids function as natural defense substances against pathogens and insects (Lachman et al. 2001). Because naturally occurring pesticides often are synthesized when plants are under stress, injured plant tissues instigate synthesis of higher concentrations of these compounds. Hlywka et al. (Hlywka et al. 1994) found that tubers from plants subjected to Colorado potato beetle (*Leptinotarsa decemlineata* Say) defoliation contained higher glycoalkaloid concentrations than tubers from control plants.

Physiological activities of potato glycoalkaloids in insect are limited. To date only few investigations were made. α-chaconine, one of the most abundant glycoalkaloids in potato, was shown to inhibit acetylcholinesterase activity in various insect species (Wierenga and Hollingworth 1992). Moreover, differential neurosensory responses to solanine, tomatine and leptine were shown in *L. decemlineata* adults (Hollister et al. 2001). Recently we observed that glycoalkaloid extract from potato leaves influences hatching success in *Spodoptera exigua* (Adamski et al. 2009).

In a screen for a novel physiological activity of secondary plant metabolites in insects, we report here for the first time cardiotropic properties of potato glycoalkaloids in several beetle species including common potato pest *L. decemlineata*.

Materials and Methods

Tenebrio molitor L. adults were obtained from a culture maintained as described previously (Rosinski et al. 1979).

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Zophobas atratus were reared according to the Quennedy et al. (1995) procedure. *L. decemlineata* adults were obtained from Institute of Plant Protection in Poznań.

Freshly harvested leaves of potato plant were freeze-dried immediately to arrest maturation. Leaves' samples were stored at −20°C until extraction. For each sample, 5 g of dried leaves were placed in 20 mL of 5% acetic acid aqueous solution, extracted and analyzed as in Cataldi et al. (2005). The two major glycoalkaloids ascertained in freeze-dried leaves were α-chaconine and α-solanine at rate of 2 mg g^{-1} and 1 mg g^{-1}, respectively. Minor compound were also found as dehydro α-chaconine, dehydro α-solanine and a hydroxy-derivative of α-chaconine. For the determination of bioactivity, the liquid phase was eliminated by lyophilization.

Lyophilized samples were re-suspended in saline (274 mM NaCl, 19 mM KCl, 9 mM $CaCl_2$, 5 mM glucose, and 5 mM HEPES, pH 7.0) to obtain 1×10^{-1}% concentration and stored at −30°C. Working dilutions were made from the stock solution in saline and assayed in vitro in a semi-isolated heart prepared according to Gäde and Rosinski (1990). In the bioassay, the video microscopy technique and the computer-based method of data acquisition and analysis were used to study the action of the samples on continuously perfused heart preparations as described previously (Marciniak et al. 2008). Many pulse applications (10 μL) of samples could be sequentially assayed in a single preparation. After the initial 15 min stabilization, the activity of the isolated heart was recorded for 2 min. Next the sample was applied and the heart activity was recorded for further 2 min. The activities of tested glycoalkaloids are presented as percentage changes in the control frequency of the heart contractions. The heart responses were considered to be significant when the average changes in contractile activity were higher than 10%.

The in vivo heart bioassay was conduced on 1 day old *Z. atratus* pupae by means of noninvasive optocardiographic technique of Slama and Rosinski (2005). Samples were re-suspended in saline (274 mM NaCl, 19 mM KCl, 9 mM $CaCl_2$) to obtain 1×10^{-1}% concentration and stored at –30°C. Tested extract (4 μL) was injected with Hamilton syringe through the ventral membrane between the second and third abdominal segments toward the head to avoid injuries. After initial hour of stabilization, the sample was injected and the heart activity was recored for further five hours.

Results and Discussion

The semi-isolated hearts rhythm remained regular during superfusion with physiological saline for 5 h and showed 31 ± 7 for *Z. atratus*, 89 ± 6 for *T. molitor* and 37 ± 7 for *L. decemlineata* contractions per minute. Tested glycoalkaloid extract showed species specific cardioactivity in beetles. In two of examined beetle species, *T. molitor* and *L. decemlineata*, glycoalkaloids exerted no effects on semi-isolated heart preparations (Figs. 1 and 2b, c). Application of glycoalkaloid extract on *Z. atratus* heart caused dose-dependent cardioinhibitory effects. The extract decreased the frequency of the heart contractions (negative chronotropic effect) with a threshold for observable effect between 1×10^{-3}% and 1×10^{-1}% (Fig. 1). In the two highest concentrations, the extract caused fast and reversible cardiac arrests (Fig. 2a). The strongest observable inhibition at the concentration 1×10^{-1}% gained a level of 30%.

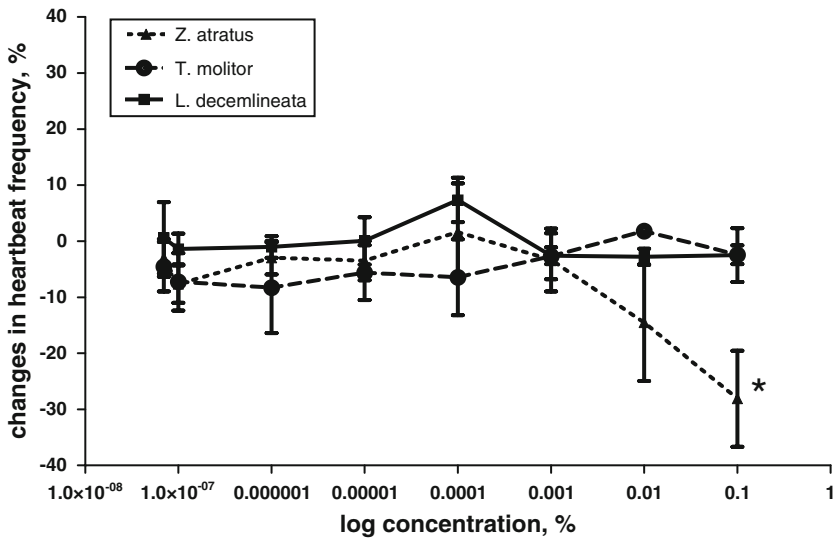

Fig. 1 Dose-response curves for the effect of glycoalkaloid extract on the frequency contractions of *Z. atratus* (*filled triangle*), *T. molitor* (*filled circle*) and *L. decemlineata* (*filled square*) hearts. Mean ± SEM are given from 5 to 12 determinations. Significant differences ($p < 0.05$) from controls (Ringer saline) are indicated by asterisk (Wilcoxon test)

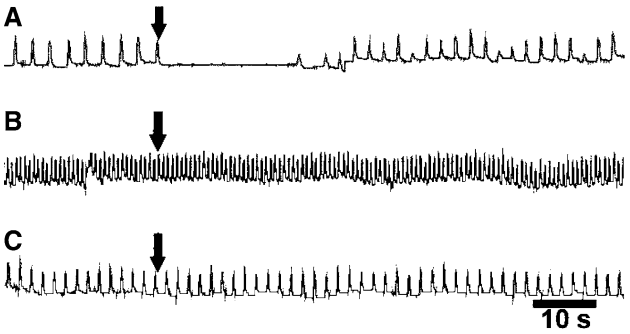

Fig. 2 Myograms displaying typical responses on the spontaneous activity of the *Z. atratus* **(a)**, *T. molitor* **(b)** and *L. decemlineata* **(c)** hearts to 0.01% of glycoalkaloid extract. Sample application is indicated by an arrow

In vivo studies on 1 day pupae of *Z. atratus* using previously described optocardiographic technique (Slama and Rosinski 2005) showed different effect of the extract on heart contractile activity. A constant pattern of heartbeat reversal in these pupae is manifested by regular alternations of the forward orientated (anterograde) and the backward orientated (retrograde) cardiac pulsations. Fig. 3a shows an example of typical heartbeat record in which retrograde phase of peristaltic waves of the heart pulsations, clearly distinguished by the lower frequency of the systolic contractions (12–14 beats/min), and fast anterograde phase of contractile activity (23–25 beats/min) are presented. Injection of tested extract (1×10^{-1}%) caused serious but reversible changes in the pupae heart contractile cycle. The heartbeat reversal recorded in 3rd hour after injection showed that the extract caused a decrease in the amplitude contractions (a negative inotropic effect) in both retrograde and anterograde phases of the contractile activity with a level 40% and 72%, respectively. Moreover, changes in duration of both phases in the heartbeat were observed (Fig. 3b). The retrograde phase was four times shorter and anterograde phase seven times shorter when comparing to the control heart activity. All effects were completely reversible in 4–5 h after injection.

In conclusion, we found a new cardioinhibitory activity of potato glycoalkaloids in *Z. atratus*. Lack of inhibitory action of the extract on *L. decemlineata* heart indicates evolutionary adaptation of this beetle to potato glycoalkaloids. Interestingly, from two closely related species *T. molitor* and *Z. atratus*, only *Z. atratus* appeared to be sensitive to potato glycoalkaloids. In vivo studies showed that glycoalkaloids induce completely different heart response from in vitro bioassay. Further experiments are needed to determine which of the alkaloids in the potato leaves extract cause cardioinhibition and to gain more details about effects of alkaloids on whole insect organism.

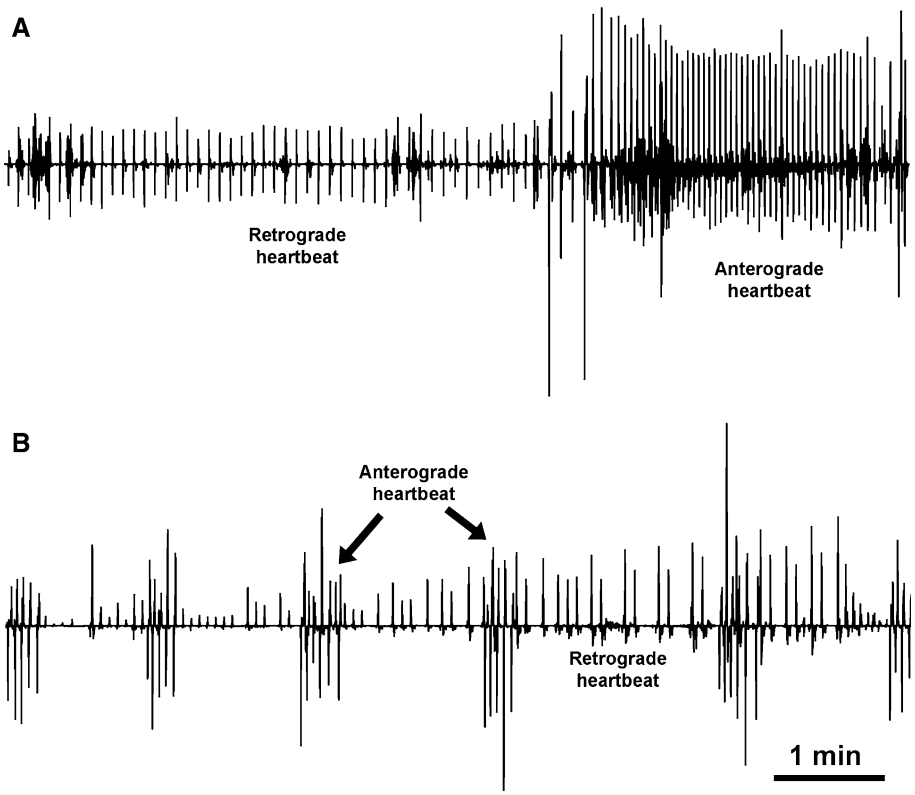

Fig. 3 Optoelectronical recordings of 1 day old *Z. atratus* pupae heartbeat before injection **(a)** and in 3rd hour after injection of 0.1% glycoalkaloid extract **(b)**

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