

The Effects of Octopamine on the Cardiac Output of Cockroach by Using Computer-based Video Analysis on Measuring Stroke Volume

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ABSTRACT: A computer-based video analysis system has been developed for measuring the stroke volume and the heart rate of the insect. A quantitative evaluation of the effects of octopamine (OA) on the heart rate, stroke volume and cardiac output of *Periplaneta americana*, the American cockroach, was also studied. The heart rate of OA-treated cockroach significantly increased within 2 minutes, compared to that of the control groups. On the other hand, the stroke volumes of OA-treated cockroaches decreased within 2 minutes ($p < 0.05$). Furthermore, OA elicited the heart rate and the stroke volume with a dose- dependent response. Although OA increased heart rate and decreased stroke volume in the cockroach, it decreased cardiac output (mL/min) calculated based on heart rate (beats/min) and stroke volume (mL/beat).

KEY WORDS: Computer-based video analysis, *Periplaneta americana*, Stroke volume, Heart rate, Cardiac output, Octopamine.

INTRODUCTION

There are many methods for recording the cardiac potential and the mechanical force of heart in insects (Miller, 1985; Collins and Miller, 1977; Johnson *et al.*, 1997). It is easy to analyze the heart rate (the number of beats per minute) and cardiac cycle of the insects. However, during the heart contracting, it could not easily measure the volume changes, called the stroke volume (the blood volume ejected by each ventricle with each beat). Therefore, the cardiac output (the total blood volume ejected by each ventricle per minute) of an insect was also difficult to be calculated, though the cardiac output (CO) can be determined by multiplying the heart rate (HR) and the stroke volume (SV): $CO = HR \times SV$. Thus the measurement of SV is necessary for measuring CO.

Recently, because of the improvement of the technology of computer software and photography, computer-based image analysis became a very useful tool for the examination of the stroke volume and the cardiac output of small vertebrates such as chicken embryos (Faber *et al.*, 1974) and frog *Xenopus laevis* (Hou and Burggren, 1995). We have tried such method to measure the stroke volume and heart rate, then demonstrated that the cardiac output of the American cockroach, *Periplaneta americana*, increased significantly, when the insects were fed by applying glucose solution (Tsai *et al.*, 2001).

Octopamine (OA) is a potent biogenic amine of insects that affects numerous physiological functions. It is a transmitter controlling the firefly light organ (Robertson and Carlson, 1976); a neurohormone inducing mobilization of lipids to supply energy for flight (Milde *et al.*, 1995); a neuromodulator regulating flight (Stevenson and Meuser, 1997; Weisel-Eichler and Libersat, 1996); involvement in neuroendocrine functioning (Downer *et*

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al., 1984; Orchard, 1982; Roeder, 1999). These regulatory functions indicate that the role of OA in insects may be similar to the sympathetic nerve in vertebrates (Adamo *et al.*, 1995; King *et al.*, 1986; Orchard, 1982; Roeder, 1999). However, there are few data to show that whether OA can affect on the stroke volume and the cardiac output of insect heart.

Since OA is widely distributed in the insect tissues and play such important physiological roles but the effects on the heart, especially on the stroke volume and cardiac output are poorly understood, using the computer-based image analysis as a new method to determine the stroke volume and investigating the effects of OA on the stroke volume, the heart rate and cardiac output of insects is an important aspect.

In the present study we have designed a new method using a computer-based video analysis system to measure an insect's stroke volume and heart rate, then investigated the effects of OA on the heart rate, stroke volume and cardiac output of the cockroach's hearts. Our results suggest that microinjection of exogenous OA elicited the stroke volume, heart rate and cardiac output of American cockroach with a dose-dependent response.

MATERIALS AND METHODS

Preparation of experimental animals

The experiments were performed on adult males of the American cockroach, *P. americana*. Insects were raised in an animal house and maintained in constant temperature at 25 ± 2 °C, relative humidity of 60 ± 10 %, 12hr light / 12 hr dark periodic regime (Lin *et al.*, 1990; Lin and Wu, 1996; Tsai *et al.*, 2001). Before recording, the wings of the cockroach were removed, and the animals were immobilized with dorsal side up by sliding the animal into a box (4.5 x 3.0 x 0.3 cm) made of cardboard. A hole on top of the box was made for the observation of the cardiac performances of the insect. The hole on the bottom of the box was made for microinjecting chemicals into the body cavity of the first abdominal segment during experiment (Figs. 1a & 2a).

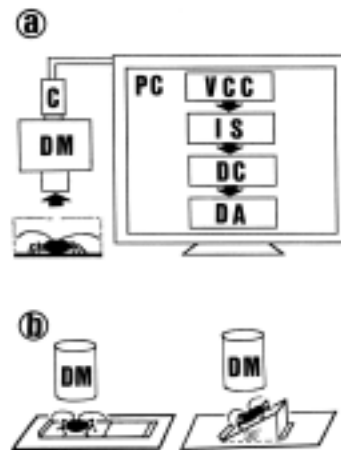


Fig. 1. Schemata of experimental equipments for study on the cardiac output of cockroach by video image analysis. (a): Diagrammatic components of experimental apparatus for video image analysis. (b): Diagram of the insects in box on the supporting plates positioned angles at 0° or 30° to microscope stage during measurement. The heart image of the first abdomen segment at the supporting plate 0° (left) or 30° (right) to horizon was picked and stored into PC software through dissecting microscope. C, camera; DA, data analysis; DC, data collection; IS, image storage; DM, dissecting microscope.

Computer-based video analysis system of cardiac activity

All experiments were carried out in the experimental apparatus (Fig. 1a) at constant temperature (22 ± 2 °C). The cockroaches were first immobilized in the cardboard box and put under a dissecting microscope. After 30 minutes of immobilization, the cardiac activities of the first heart (underneath the tergum of the first abdominal segment) were recorded and stored using a camera (JVC, TK1070U, Tokyo) and a frame grabber card (UPMOST, UPG301T, 30 frames /sec) on a computer.

Stroke volume and heart rate measurements

The images of the end-systole and end-diastole of the first heart pumping were obtained for the image analysis (Figs. 2b & 2c). After fixing a detectable point of the trachea attached on the heart (as point p in Fig. 2c), the displacement distance (D in Fig. 2c) between the point p at the end-systole and point p' at end-diastole (move D from point p) was measured. The relative values of stroke volume were obtained from the different areas (A in Fig. 2c) between end-systole and end-diastole.

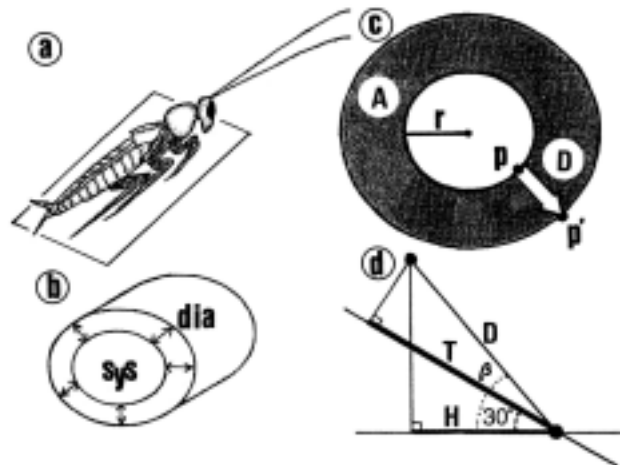


Fig. 2. Diagrams for measuring and calculating the stroke volume. (a): A cockroach cut wings in a box exposed the 12 hearts. (b): Diagram of changed volume during heart contraction (systole) and relaxation (diastole). sys: systole; dia: diastole. (c): Diagram of a cross section during heart pumping. Black area, A represents the area of cross section in the stroke volume. The radius (r) of the target heart is $90.5 \pm 0.2 \mu\text{m}$ (n = 69). Point p is a measured position on the target heart at the end of systole, then the point p moves to the point p' at the end of diastole. D is the displaced distance between the p and the p'. (d): Illustration for the relationships of D, H, and T. H is a distance of displacement from horizontal direction (0°) and T is from oblique direction (30°) between the end of systole and diastole. The relationship of D, H and T is $D = 2 \sqrt{(H^2 - \sqrt{3}HT + T^2)}$. By measuring T and H from the image analysis of heartbeat, the D value is calculated and obtained. The area of stroke volume (A) is calculated by the equation of $A = (2rD + D^2)$. Detail was described in text.

In order to accurately calculate the stroke volume, the heart pumping images can be obtained by alternating the supporting plate of the microscope from horizontal direction (0°) to an oblique direction (30°) (Fig. 1b).

As shown in Fig. 2d, H is the distance of displacement from horizontal direction (0°) and T is the distance of displacement from oblique direction (30°) between the end of systole and diastole. D is a spatial distance of the detached point moving from the end-systole (point p in Fig. 2c) to the end-diastole (point p' in Fig. 2c). By the relation of triangular function, D value was calculated, then the changing cross sectional area A and the stroke volume was determined.

From Figs. 2c and 2d.

$$\cos (\quad - 30^\circ) = T / D$$

By rearrangement, $T / \cos (\quad - 30^\circ) = D$ ----- (1)

$$\cos \quad = H / D$$

By rearrangement, $D = H / \cos \quad$ ----- (2)

On substituting this expression for D in equation 1,

$$\frac{T}{\cos(\theta - 30^\circ)} = \frac{H}{\cos(\theta)} \quad \text{----- (3)}$$

$$\cos(\theta - 30^\circ) = \cos\theta \cos 30^\circ + \sin\theta \sin 30^\circ$$

equation 3 is,

$$T \cos\theta = H \cos\theta \cos 30^\circ + H \sin\theta \sin 30^\circ$$

$$T \cos\theta = (\sqrt{3}/2)H \cos\theta + (1/2)H \sin\theta$$

By dividing by $\cos\theta$ is

$$T = (\sqrt{3}/2)H + (1/2)H \tan\theta$$

By rearrangement,

$$\tan\theta = [T - (\sqrt{3}/2)H] / [(1/2)H] = 2T/H - \sqrt{3} \quad \text{----- (4)}$$

From Fig. 2d

$$\tan\theta = \sqrt{(D^2 - H^2)} / H$$

On substituting this expression for $\tan\theta$ in equation 4,

$$2T/H - \sqrt{3} = \sqrt{(D^2 - H^2)} / H$$

By rearrangement

$$D = 2 \sqrt{(H^2 - \sqrt{3}HT + T^2)}$$

From Fig. 2c

$$A = (r + D)^2 - (r)^2 = (2rD + D^2) \quad \text{----- (5)}$$

By measuring H and T values, we obtained D and A values, and the relative stroke volume could be determined. The heart rate was defined as the heartbeat per minute, and the cardiac output was the product of the heart rate and the stroke volume. Although *P. americana* has 12 ventricles, all data were recorded from the first one to avoid the errors in different heart performance.

Chemical injections and data analysis

By using a 50 μL microinjector, 10 μL reagents were injected into the abdominal haemocoel through the suture on the first abdominal segment. The heart performance was recorded and stored in a computer within 120 min following injection. Reagents containing 10^{-6} ~ 10^{-3} M OA were freshly prepared in Ringer's solution (NaCl 214.0 mM; KCl 3.1 mM; CaCl_2 1.8 mM; Tris-HCl 10.0 mM; H_2O 1000 mL; pH = 7.2) before each experiment. In this study, cockroaches treated with Ringer's solution were used as the control groups and the OA-treated cockroaches were used as the experimental groups. Student's t-test was used to compare the data of heart rate, stroke volume, and cardiac output collected from the control and experimental groups. Regression and ANOVA test were performed for the data with various OA-treated concentrations.

RESULTS

In order to realize the heart rate and stroke volume of the cockroach under normal conditions, the insects of control group were microinjected with saline, then the cardiac performance was continuously observed and recorded by the computer-based video analysis for 120 mins. In this study we found that the average heart rate of American cockroach is 130

± 10 beats/min, and the average stroke volume is $1.92 \pm 0.31 \mu\text{L}/\text{beat}$ ($n = 10$). Results showed that saline-microinjection could not significantly change both the stroke volume and the heart rate through the recording period. In the experiment group various concentrations of OA solution were microinjected to test the effects of OA on the stroke volume and the heart rate of American cockroach.

The heart rate increased $12.47 \pm 3.70\%$ in two minutes when 10^{-4} M OA solution was injected compared to that of the saline treated and it increased $20.25 \pm 4.52\%$ when 10^{-3} M OA solution was injected. When the OA concentrations were low (10^{-6} M), no significant increase of heart rate was observed (Fig. 3a). The effects of OA on the heart rate of cockroach were rapid and obvious, the most effective response occurred within two minutes of OA microinjection with dose-dependent response ($R^2 = 0.99$, $p < 0.001$), however, there were no further effects at 20 or 40 minutes after microinjection (Fig. 3b).

The stroke volume of cockroaches treated with 10^{-5} M OA solution decreased $15.76 \pm 1.71\%$ ($p < 0.05$) within two minutes, compared to that of the control group. However, no significant effect of OA on stroke volume was found when the OA concentration was lower than 10^{-6} M (Fig. 4a). The effects of OA on the stroke volume were also obvious and last for a long time. The significant effects of OA on the stroke volume persisted for 40 mins after OA-microinjection in the concentration of 10^{-4} M and showed dose-dependent response (Fig. 4b).

In this study the cardiac output (mL/min) was calculated by multiplying the heart rate (beats/min) and the stroke volume (mL/beat). The increase in heart rate was insufficient to compensate for the decline in stroke volume. As a result the cardiac output of American cockroach was reduced significantly under the effects of OA and decreased significantly by OA treated in the concentration of 10^{-5} M (Fig. 5).

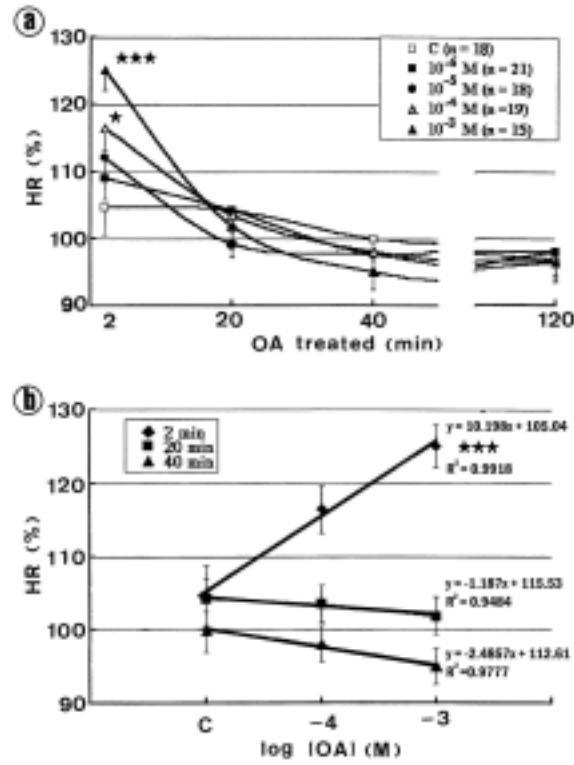


Fig. 3. Time course of the dose effects of OA on the heart rate. (a): Relative changes of the heart rate following microinjection of OA in various concentrations. Each point represents mean (the test number in each parentheses) and bar at each point represents standard error. The heart rate before treated with OA or saline was 100%; c: control group treated with saline; *: $p < 0.05$; ***: $p < 0.005$ (one-way *t*-test). (b): Linear regressive analysis of OA affected at 10^{-4} M and 10^{-3} M on the heart rate for 2, 20 and 40 min after microinjection. (ANOVA).

DISCUSSION

Insect hearts are generally composed of only one thin layer of myocardial cells (Edwards and Challice, 1960; Sanger and McCann, 1968). Therefore, it was difficult to measure the stroke volume of insect heart due to its thin ventricle and small volume by the methods used for the hearts of vertebrates. In this study we first developed the new method of a

computer-based video system for measuring the stroke volume, heart rate and analyzing the effects of exogenous OA on the stroke volume, heart rate and cardiac output of the American cockroach. We found that OA increased quickly and sharply the heart rate but decreased the stroke volume for a longer time, and decreased the cardiac output calculated based on the data of the heart rate and the stroke volume.

Because OA increased rapidly, effectively the heart rate, several reports suggested that the physiological function of OA in insects might be similar to that of noradrenaline (from the sympathetic nerve) in vertebrates (Orchard, 1982; King *et al.*, 1986; Adamo *et al.*, 1995; Roeder, 1999). Although we found that OA increased rapidly the heart rate and exhibited a dose-dependent response significantly in present study, we also found that microinjection of OA decreased significantly the stroke volume compared to that of the control group in the low concentration and exhibited a dose-response. These results revealed that it is close, but not identical, to that of noradrenaline described in vertebrate hearts.

The primary function of the heart in cockroaches, as in other insects, is to transport nutrients from the absorptive sites to the tissues of the body, waste products to excretory organs, and hormones from glands to target cells. Needs for oxygen in insects is supplied through the tracheal system, therefore gaseous transport is not important to the heart (Fox, 1982). Probably, the response of heart to analogous neurohormones in insects (OA is the monohydroxyphenolic analogue of nora-drenaline) is very different from that of vertebrates.

A cockroach has well-developed circulatory system with a main dorsal vessel that carries blood from the tiny hearts. Filling of the hearts with blood is achieved by suction, provided by a set of muscles (the aliform muscles radiate from the hearts) that pull externally on the walls of the hearts. When the muscles contracted, the blood flew through ostia into the lumen of the hearts. Therefore, the activities of the aliform muscles control the cardiac performance (Fox, 1982). A few reports (Huddart and Oldfield, 1982) showed that OA accelerated the contraction frequency and diminished the contraction amplitude of the visceral muscles in the locust *Locusta migratoria*. OA, as a neurohormone increased the heart rate but decreased the stroke volume, might affect directly on the alary muscle to regulate the contractile frequency not the contractile force as response in the visceral muscles.

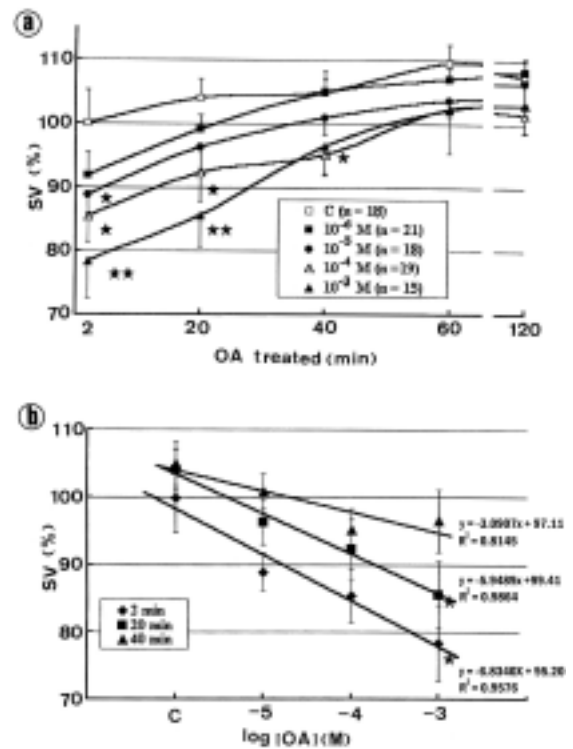


Fig. 4. Time course of the dose effect of OA on the stroke volume. (a): Changes of the stroke volume following microinjection of various OA concentrations. Each point represents mean (the test number in each parentheses) and bar at each point represents standard error. The stroke volume before microinjection of OA or saline was 100%; c, Ringer's solution-treated control group; *: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$ (one-way t -test). (b): Linear regressive analysis of OA affected at 10^{-5} , 10^{-4} and 10^{-3} M on the stroke volume for 2, 20 and 40 min after OA microinjection. (ANOVA).

Although *P. americana* has 12 ventricles, all data in present study were obtained from the first one to avoid the errors in different heart performance. In fact, we do not know whether the first ventricular performances is different from that of others. Further studies on other ventricles are necessary in future.

Even less is known about the modulatory role of OA at visceral muscles in insects. OA in higher concentrations reduced the frequency and the amplitude of the spontaneous contractions of the oviduct and diminished the beat rate of the antennal heart in *P. Americana* (Hertel *et al.*, 1988; Penzlin, 1994). Control mechanisms for the insect heart are not fully elucidated but several works indicated that aminergic and peptidergic hormones have effects both on the isolated heart and the heart in the whole animal (Collins and Miller, 1977; Fox, 1982; Johnson *et al.*, 1997; Miller, 1985; Tsai *et al.*, 2001). In fact, the control factors of the cardiac output in arthropods are complex. Heart rate and stroke volume can be controlled independently (McMahon, 2001). Further studies in the OA effects on the stroke volume and the cardiac output are necessary for explaining the mechanism of OA more in detail and realizing the physiological role of OA on insect hearts

To summarize, the results presented in this paper suggested that an exogenous OA increased the heart rate, decreased the stroke volume, and decreased the cardiac output of the American cockroach.

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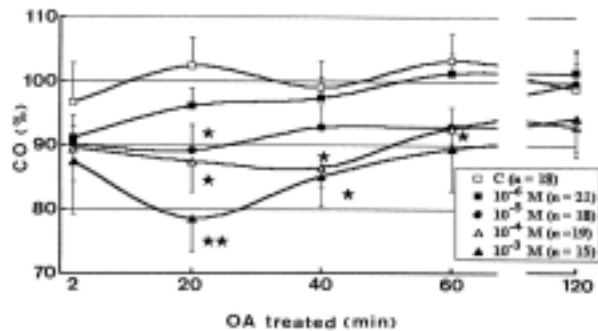


Fig. 5. Time courses of dose effect of OA on the cardiac output. Changes of the cardiac output following microinjection of various OA concentrations. The cardiac output was directly calculated from the production of the heart rate and the stroke volume. Each point represents mean (test number in each parentheses) and bar at each point represents standard error. The cardiac output before injection was as 100%; c: Ringer's solution-treated control; *: $p < 0.05$; **: $p < 0.01$ (one-way t -test).

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電腦錄影分析法測定蟑螂的心搏量並探討章涎胺對其心輸出量的效應

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摘 要

本研究設計一套結合電腦錄影分析的新方法，用於測定昆蟲的心搏量(stroke volume)和心跳率(heart rate)，並定量探討章涎胺(octopamine)對美洲蟑螂(*Periplaneta americana*)心搏量、心跳率和心輸出量(cardiac output)的效應。章涎胺處理兩分鐘內，實驗組昆蟲會增加心跳率，但降低心搏量，二者均具顯著性差異($p < 0.05$)，而且對不同劑量的處理結果，都具有劑量效應。利用計算(心搏量 \times 心跳率 = 心輸出量)方法，得知章涎胺增加心跳率和降低心搏量的結果，會降低心輸出量。

關鍵詞：電腦錄影分析、美洲蟑螂、心搏量、心跳率、心輸出量、章涎胺。

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