A sensory input inhibiting heart rate in an insect, *Rhodnius prolixus*

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Abstract. The dorsal vessel of the blood feeding insect, *Rhodnius prolixus*, was found to increase or decrease its rate of contraction in response to a number of different stimuli. Handling increased contraction rates whereas tactile stimulation of the ventral abdominal cuticle inhibited contraction. Injection of very low concentrations of serotonin or of high concentrations of octopamine enhanced the inhibitory effect, apparently by acting via the nervous system. Higher concentrations of serotonin increased heart rate by acting directly on the myocardium. The inhibitory response is suggested to be one facet of a generalised thigmotactic response.
Key words. Dorsal vessel; heart; insect; serotonin; octopamine; inhibition.

The physiology and pharmacology of the dorsal vessel, the major circulatory organ in insects, has been examined in a variety of insects, and as a result a good deal is known about the internal controls on the rate of beating of the heart in a number of species[1-3]. There are several endocrine factors which affect heart rate in insects[2], and in many insects the dorsal vessel receives direct innervation which may also control the rate of beating[2]. However, there is relatively little information available concerning those inputs from the environment which might alter heart rate. General excitation or stress often leads to an endocrine mediated increase in heart rate[4], and in one instance, a specific peripheral sense organ has been implicated in the release of a cardiotropin[5]. We have been investigating the control of heart rate in the blood feeding hemipteran, *Rhodnius prolixus*, in which an intact dorsal vessel is required in order to sustain the endocrine system[6,7], and report here some novel observations on the effect of external stimuli on the heart.

Materials and methods

A colony of *Rhodnius prolixus* was fed on rabbits, and maintained at 28 °C in humid incubators. Insects examined were unfed adult females within one month after emergence. To view the dorsal vessel in an intact animal, the animal was secured, dorsal side up, with modelling clay placed over its legs. Wings were held anteriorly with a small piece of modelling clay allowing the dorsal vessel to be seen through the dorsal cuticle under the dissecting microscope. The dorsal vessel appeared as a slender greenish vessel with alary muscles attached to it in the last two abdominal segments (for further details of structure, see Chiang et al.[8]). The time required for 5 or 10 contractions of the dorsal vessel in intact animals was measured with a stopwatch, and rates were expressed as the number of contractions per minute.

Dorsal vessels were exposed to different concentrations of serotonin (5-hydroxytryptamine, Sigma Chemical Co., St. Louis) or octopamine (octopamine hydrochloride, Aldrich Chemical Co. Inc., Milwaukee) made up in *Rhodnius* saline consisting of 129 mM NaCl, 8.6 mM KCl, 2.0 mM $CaCl \cdot 2\ H_2O$, 8.2 mM $MgCl_2 \cdot 6\ H_2O$, 34 mM D-glucose, 15 mM Tris-HCl at pH 7.4[9]. Test saline was administered into intact animals through a calibrated glass capillary pulled to a fine tip by an electrode puller. The capillary was inserted through the inside margin of the lateral pleat at the level of the last full size abdominal segment, and 1 µl of test saline was pressure injected into the abdomen. For each animal examined, the first injection contained the lowest concentration of serotonin or octopamine. Effects of each injection were noted over a 5–10-min period following injection, then the next higher concentration of serotonin or octopamine was tested.

In semi-isolated preparations in which the dorsal vessel was completely exposed to the test saline, the animals were pinned dorsal side down on a Sylgard covered dish. The head and thorax were left intact, while the ventral cuticle, abdominal nerves, and all internal viscera in the abdomen were removed leaving the dorsal vessel and its attached alary muscles.

Semi-isolated preparations were first subjected to 4–5 changes of normal saline, then to a series of test salines containing increasing concentrations of serotonin or octopamine. Between changes, the saline was continuously perfused with a small pipette during which time measurements of the rate of the heart beat were recorded. All experiments were performed at room temperature.

Results and discussion

The dorsal vessel in the abdomen is divided into two distinct regions: the heart, located in the last abdominal segment, and the aorta extending from the heart to the thorax. In normal, restrained animals, a complete contraction of the dorsal vessel consists of a beat of the heart followed by the twisting action of the aorta which forces haemolymph into the thorax and head[8]. In some instances, the heart may produce a weak contraction that is not followed by a contraction of the aorta, and haemolymph is not carried forward to the head. All measurements in this report refer to the stronger contractions of the heart which are followed by contractions of the aorta.

Handling of the insects increased contractions of the dorsal vessel above the resting level. Immediately after securing insects with modelling clay, the contraction rate ranged from 28.0 to 41.4 beats/min with a mean of 33.3 ± 4.2 (N = 8). A similar maximum value (40 beats/min) was reported by Baehr and Baudry[10] who also measured the wave of contraction associated with the aorta in intact *Rhodnius*. After a 15–20-min period in which the animals were left undisturbed, the rate of contractions was reduced to a range of 16.7–29.9, and a mean of 22.2 ± 4.19 (N = 8). Some animals were left undisturbed in this position for at least 1–2 h, and contraction rates at the end of this time were similar to that at 15–20 min.

In addition to this response to stress, *Rhodnius* also displayed a nervous reflex that caused contractions to stop. When rates had stabilized following initial handling of the insect, gentle stroking of the ventral lateral sides of the abdomen with a small probe caused an immediate inhibition, or marked reduction in the rate, of contractions which persisted as long as the stimulus continued. Contractions remained inhibited, or greatly reduced in frequency, while the cuticle was being stroked, and resumed at a normal rate within 1 min after tactile stimulation had ceased. This inhibition was elicited in animals only after their contraction rates had stabilized following handling: stroking the ventral lateral side of the abdomen of insects immediately following their immobilisation did not result in inhibition.

The sensory receptors initiating this nervous inhibition were localized on the ventral cuticle since tactile stimulation of the dorsal surface of the abdomen failed to elicit this response. There are many more sensory hairs on the ventral abdomen than on the dorsal abdomen.

Some potential clues to the pathway involved in the nervous inhibition of the heart emerged from experiments with serotonin and octopamine. Another biogenic amine, dopamine, was also tested but it had no effect on the rate of contractions of the heart. The act of holding the cuticle with forceps in order to insert the capillary for injection of test solutions elicited the nervous inhibition associated with tactile stimulation. The period of inhibition resulting from injection of insect saline was altered when the saline contained either serotonin or octopamine. Injection of 10^{-10} M serotonin led to a prolonged inhibition, whereas higher concentrations reduced the period of inhibition in a dose-dependent fashion (fig. 1, A). For octopamine, higher concentrations were required to have an effect, and concentrations of 10^{-5} or 10^{-3} M prolonged the period of inhibition (fig. 1, B).

Since the effect of these drugs mimics the effect of tactile stimulation, serotonin and octopamine may be stimulating nerve cells which, in turn, inhibit the pacemaker mechanism of the dorsal vessel. In support of this hypothesis, both serotonin and octopamine are known to have an excitatory effect on insect tissue[11–14], and nerve terminals have been found on the dorsal vessel of *Rhodnius*[8]. These terminals possess dense core vesicles typically observed in peptide containing nerves[15,16] and may be candidates for the source of inhibition that affects the pacemaker of the dorsal vessel. Moreover, these terminals are located in the posterior region of the dorsal vessel where the normal heartbeat is initiated. Although this hypothesis requires the existence of a cardioinhibitory factor in *Rhodnius*, such a factor does exist in insects. A cardioinhibitory factor has been isolated from the corpora cardiaca of the mealworm *Tenebrio molitor*[17].

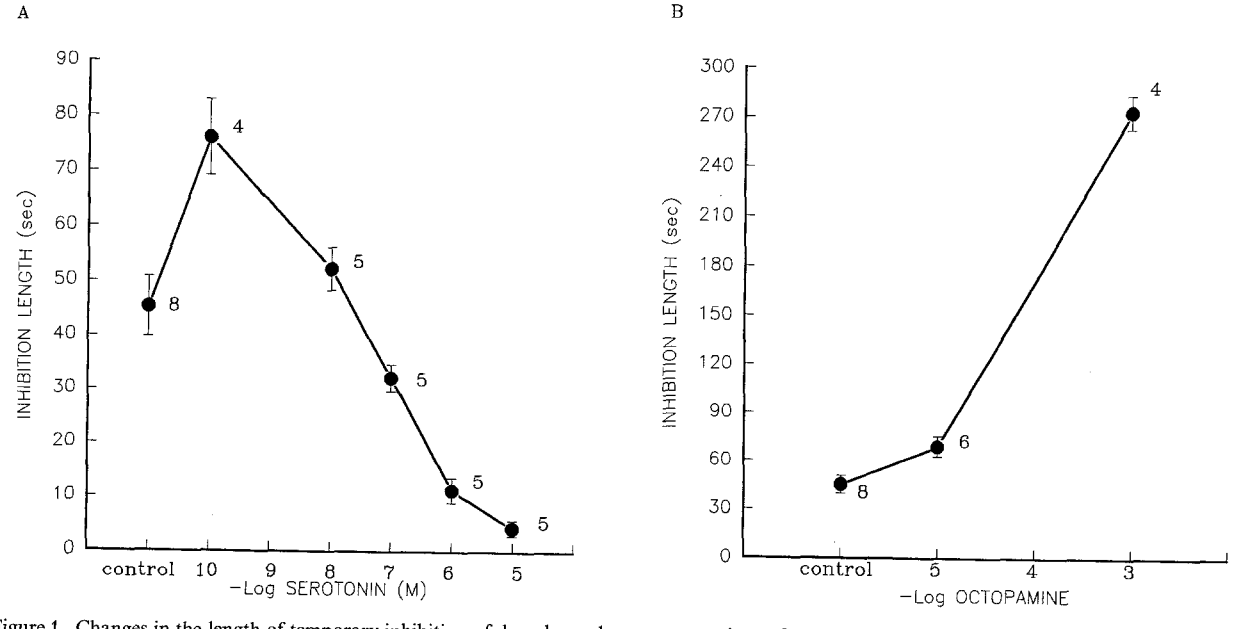

Figure 1. Changes in the length of temporary inhibition of dorsal vessel contractions following injection of 1 µl of test saline containing differing concentrations of serotonin (A) or octopamine (B). Sample points represent means ± standard deviations; numbers represent N.

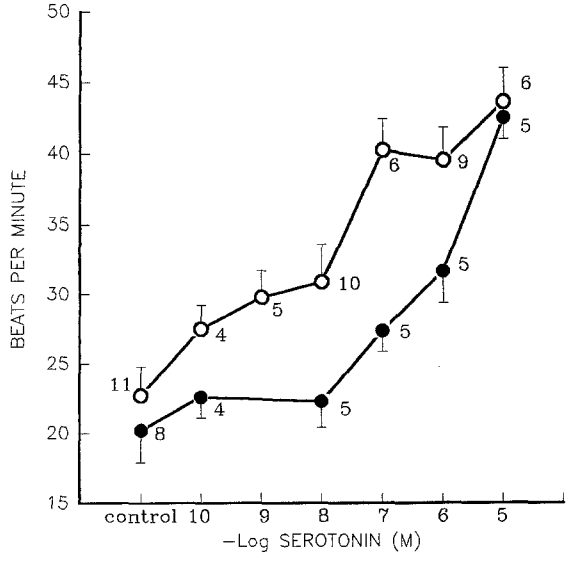

Figure 2. Changes in the rate of dorsal vessel contractions following injection of differing concentrations of serotonin into intact animals (filled circles) or exposure of semi-isolated dorsal vessels to test salines containing differing concentrations of serotonin (open circles). Sample points represent means ± standard deviations; numbers represent N.

The action of serotonin, however, is clearly more complex. As noted, the injection of 1 µl of saline containing concentrations of serotonin above 10^{-8} M reduced the period of inhibition. These higher concentrations also increased the heart rate in intact insects (fig. 2). In semi-isolated preparations, the threshold of effect was somewhat lower, but it is important to remember that with a haemolymph volume of approximately 20 µl [18], the effective concentration of injected serotonin is at least one order of magnitude lower. Even allowing for this difference, however, it is clear that the effect of injecting low concentrations into intact insects is qualitatively different from applying the same concentration to semi-isolated hearts which are not connected to the nervous system. These observations imply that injection of lower concentrations of serotonin have their effect through the nervous system, and thus cannot inhibit the semi-isolated heart. Higher concentrations appear to act directly on the heart muscle.

On the other hand, octopamine as high as 10^{-3} M had no effect on the rate of contractions in semi-isolated preparations suggesting that an intact nervous system is required for the inhibitory response of octopamine. Although detailed data are not presented here, it has been possible to completely isolate the heart from all other tissues: such preparations continue to contract rhythmically, and the rate is increased by serotonin and unaffected by octopamine.

The low maximal rate of contraction and the relatively simple structure of the dorsal vessel of *Rhodnius* has facilitated the interpretation of the results of the present study. Clearly, the heart of *Rhodnius* is myogenic. The dorsal vessel contains no intrinsic nerve cells [8], yet the semi-isolated dorsal vessel with its extrinsic nervous input removed, or isolated portions of the dorsal vessel, are capable of rhythmic contractions. These results suggest that the myogenic mechanism is inherent in the myocardium, and is stimulated by serotonin which directly affects the myocardium. This stimulation by serotonin is not surprising since serotonin has been shown to stimulate the rate of contractions of several other insect visceral muscles [2, 13, 19]. Furthermore, changes in the rate of contraction of the dorsal vessel after handling the insect may actually reflect the amount of serotonin in the abdomen. Similar rates of contraction were obtained in semi-isolated dorsal vessels when they were exposed to 10^{-8} M serotonin, a concentration of serotonin detected in the haemolymph of unfed larva *Rhodnius* in response to handling [20]. Whether or not serotonin has a role to play in the inhibition of heart rate which results from stroking the abdomen is not clear: it is, however, apparently capable of stimulating the inhibitory pathway. It is possible that octopamine may be involved as a transmitter or modulator in the same inhibitory pathway.

The stimulation of the heart beat that occurs as a result of handling may be part of the mechanism that prepares the animal to respond suddenly to an external stimulus. Inhibition, however, does not have an apparent physiological role and inhibition of the heart rate has yet to be given a detailed discussion in other systems. We suggest that the tactile stimulation which inhibits beating of the dorsal vessel may be part of a thigmotactic or stereokinetic response, whereby insects are rendered less sensitive to a range of stimuli by stimulation of their sensory hairs [3]. *Rhodnius* is a nocturnal animal, and spends the daylight hours wedged into crevasses out of sight of potential hosts or predators. The inhibition of the beating of the dorsal vessel of *Rhodnius* by gentle stroking of the sensory hairs on the abdomen may be part of the general mechanism that makes the animal less responsive to other stimuli once a hiding place is found.

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Phosphocreatine and ATP concentrations increase during flow-stimulated metabolism in a non-contracting muscle

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Abstract. The gracilis muscle was excised from cold-acclimated rats, placed in vitro, and simultaneously perfused via its artery by high pO_2 medium and superfused by low pO_2 medium. With a doubling of the perfusion rate (from 50 to 100 µl/min) phosphocreatine and ATP increased by 39% and 44%, respectively.
Key words. Adenosine-triphosphate; phosphocreatine; nuclear magnetic resonance; muscle respiratory control.

The mechanisms by which cytosolic factors control cytochrome turnover and hence respiration in mitochondria remain controversial[1] at a cellular level. Even more at the organ level it remains unclear how some naturally-occurring factors stimulate the respiration of a skeletal muscle as a whole. One of these factors is blood flow, which under some conditions stimulates metabolism in a non-contracting skeletal muscle. A useful model for flow-stimulated metabolism is the rat gracilis muscle, simultaneously superfused and perfused through its nutrient artery with medium in vitro[2]. In this preparation, as the medium flow rate increases, the oxygen consumption and heat production rates increase in a flow-rate dependent manner. However, the metabolic energy flux (expressed as the product of oxygen consumption and the mean energetic equivalent for oxygen) is higher than the heat production[2]. These experimental results and a formulation of non-linear thermodynamics[3] offer a hypothesis that the difference between the two steady-state fluxes is due to an increase in steady phosphocreatine (PCr) and ATP concentration. Therefore the aim of this pilot study was to measure the concentration of both phosphates in the flow-dependent non-contracting rat gracilis.

Materials and methods

Gracilis cranialis muscles weighing 207 mg ± 31 SEM (n = 5) from male rats of the Sprague-Dawley strain were used for the preparation which was simultaneously perfused and superfused in vitro. The method used was published recently[2]. There were several differences in the present work compared with that described in the former paper. The rats were kept at 5.5 °C for 17 days prior to the experiment, which brought about a lesser metabolic response of the muscle to increased perfusion flow rate (about 70% of the previous response). The muscle was placed in a chamber with a total volume of 980 µl (the chamber volume within the radio frequency coil was 610 µl), and superfused and perfused with an inorganic phosphate (P_i) free MOPS-Ringer medium[4]. The medium was filter-sterilized, and contained 10 mg/l gentomycin and 5 mM glucose. The average superfusion flow rate was 1.1 ml/min; a low pO_2 of 5 kPa was achieved by equilibration of the medium with a gas mixture, consisting of 5% carbon dioxide and 4.9% oxygen, balanced by nitrogen. The superfusion medium was kept at a constant temperature of 28 °C by a glass heat exchanger placed in the close vicinity of the chamber containing the muscle preparation. Two flow rates (50 and 100 µl/min) were used for perfusion; a high pO_2 of 97 kPa was achieved by equilibration with a gas mixture (5% carbon dioxide, 95% oxygen). The perfusion medium reached the nutrient artery by a thin stainless steel tube which maintained gas content and partial pressures.

Oxygen consumption was monitored polarographically, using an oxygen electrode (Radiometer). Phosphates were measured using ^{31}P NMR spectroscopy. The preparation was fixed in the glass chamber, slipped into a solenoidal radiofrequency coil and placed in a superconducting magnet operating at a field of 2T. The rapid multi-pulsing technique was used with the following parameters: pre-delay 488 ms, mutation angle 47°, number of acquisitions 1024, total pulse sequence repetition time (τ) 1 s. The 47° pulse (width = 2 µs) was selected experimentally as the pulse giving an optimal signal-to-noise ratio for the PCr peak calculated from the Ernst