Functional and Structural Similarity between Insect and Human Hearts

Electrocardiography of Insect Hearts for Screening of New Cardioactive Drugs

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Abstract: The primordial formation of insect and human hearts is orchestrated by similar sets of genes. The rhythmi city of purely myogenic, peristaltic myocardial contractions of insect heart is determined by a posterior pace-maker nodus, which is analogous to sinoatrial or atrioventricular pacemaker nodi of the human heart. Insects are very mobile animals; there are vigorous neuromuscular contractions and extracardiac pulsations in haemocoelic pressure, which may seriously interfere with recordings of the heartbeat. This problem was solved by using larvae of the waxmoth, whose neuromuscular functions were totally paralysed by proteinaceous venom of the parasitic braconid wasp. The paralysed larvae survived motionless for 3 to 4 weeks, however, the regular myogenic pulsations of the heart and intestine, regulated by depolarisation potentials of the myocardial or intestinal cells, remained fully preserved. The paralysed larvae of the waxmoth are ideal object for cardiological research. By means of a touch-free, optoelectronic method we found that the larval heart exhibited uninterrupted, forward-oriented (anterograde), peristaltic waves of systolic myocardial contractions, propagated with a rate similar to that of the human heart (at 37°C). Extensive screening of various cardioactive drugs revealed that the larval heartbeat, like the human heartbeat, was sensitive to chronotropic action of digitoxine and the nitrates or cardiomoderating action of verapamil.

1 INTRODUCTION

The recent availability of electronic recording techniques (Sláma, 2003; 2010), have revealed unexpec-ted similarities between the physiological systems of insects and mammals. For example, the autonomic, cholinergic neuroendocrine system regulating insect respiration is structurally and functionally analogous to the mammalian parasympathetic nerve system (Sláma, 2008a; 2012). The mapping of the human and insect (*Drosophila*) genome have revealed that the primordial formation and later functioning of insect and human hearts were orchestrated by identical sets of genes (review by Bodmer et al., (2005). And, as a matter of fact, the pulsations of insect and human hearts are regulated by similar, purely myogenic mechanisms based on depolarisation potentials of myocardial cells. In both cases the rhythmicity depends on a special regulatory nodi (atrioventricular and sinoatrial node of the human heart) (Hampton, 2003), or terminal regulatory node in the heart of insects (Sláma, 2008a); (Sláma and Lukáš, 2011). When genomic structures were still unknown, comparative physiological studies were justified only between the closely related groups of animals.

The mapping of the human and insect genomes, however, has created a substantially new situation, which is limited only by a serious lack of physiological data. This is true for electrocardiographic (ECG) records, which are mostly available only for the human heart.

The human heart is a compact muscular organ, which pumps blood into a closed vascular system of arteries, capillaries and veins, under increased pressure and at a constant temperature of 37°C. The main function of this mammalian circulatory system is linked with respiration; the circulating blood is carrying oxygen from the lungs to distant organs and prevents respiratory acidaemia by removing the me-

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tabolically produced carbonic acid in the form of carbon dioxide. Insects do not use a closed circulatory system of arteries, veins and capillaries. Instead of the lungs, they breathe through a segmentally arranged system of spiracles and air-filled tracheal tubes, which are ramified all over the body, thus transporting aerial oxygen directly to tissue and cells. The insect ‘‘blood’’ (haemolymph) circulates between the three major body compartments (head, thorax, abdomen), which are mutually interconnected and form an open body cavity, or haemocoelic cavity (Jones, 1977); (Miller, 1997). The dorsal vessel of adult insects consists of a narrow elastic tube called the thoracic aorta and a larger abdominal portion that is conventionally called the insect heart in the strict sense. The myocardium is segmentally prearranged, with several pairs of usually incoming ostial valves, perpendicular allatal muscles and pericardial nutritive cells. In general, the insect heart is a tubular organ, propagating waves of peristaltic contractions in the forward direction (larvae), or alternatively in both forward and backward direc-tions, which is known as the heartbeat reversal. Recent investigations show that, in comparison to the human heart, the dorsal vessel of insects is a relatively weak circulatory organ which is unable to pump blood against any large barrier of mechanical pressure (Sláma, 2000; 2012). Accordingly, the insect heart is mostly used for mixing haemolymph between the capital, thoracic and abdominal compartments of the widely open body cavity. Due to the limited, though very economic, pumping ability of their heart, insects have evolved a number of auxiliary circulatory adaptations such as the accessory pulsatile organs of the appendages, peristaltic pulsations of the intestine or strong extracardiac pulsations in haemocoelic pressure (review Sláma, 2008).

Insects and mammals are phylogenetically very distant groups of animals (Prostomia and Deuterostomia) separated by millions of years of independent evolutionary pathways. In spite of this, however, there exists well substantiated genetic evidence that both insect (*Drosophila*) and human hearts share some common morphogenetic principles (review by Bodmer et al., (2005). Of particular interest in this respect is a Tinman (*Tin*) gene containing a transcriptional factor for the primordial heart in both *Drosophila* and the human body (Ocorr et al., 2007a); (Zeitouni et al., 2007). Comparisons between the two phylogenetically distant circulatory systems have been hampered for a long time by superficially different anatomical and physiological structures. Re-cently it has been found, however, that both insect and human hearts are regulated by similar, involun-tary and purely myogenic mechanisms (Sláma and Lukáš, 2011). It also appears that the rhythmicity of systolic cardiac contractions in insects depends on a special pacemaker nodus (Terminal regulatory no-dus) (Sláma, 2006; 2012), which has a similar physiological role to the atrioventricular, sinoatrial, or Hiss bundle pacemaker nodi of the human heart (Hampton, 2003). In addition to segmental, peristaltically propagated systolic contractions, certain insect species have evolved a compact, conical ventricle in the heart, characterised by a human-like atrium and synchronic, not peristaltic, mode of cardiac contractions (Sláma, 2010). Similarities between insect and human hearts prompted me to investigate the possibility of similar responses with respect to medicinal cardioactive drugs. The assays were facilitated by the development of noninvasive, touch-free, electrocardiographic methods for insects (Sláma, 2003; 2006; 2012); (Sláma and Lukáš, 2011).

It appeared that the common cardioactive drugs (noradrenaline, digitoxine, nitrates, $Ca2+$ ion blockers, indeed produced heartbeat responses in insects similar to those found in the human heart. Encouraged by the results obtained in *Drosophila* (Occor et al., 2007b); (Fink et al., 2009); (Sláma, 2010), I also looked at the hearts of some other flies. Of particular interest was a family of hoverflies (Syrphidae) including the presence of several real champions in sustained flight. One species, *Episyrphus balteatus*, revealed a quite uncommon, anatomically and functionally human-like heart, with a compact ventricle pumping the insect ‘‘blood’’ into an artery-like aorta (Sláma, 2013). Here I describe new ECG data for an insect heart and try to find possible analogies with the known facts in human cardiology.

2 RESULTS AND DISCUSSION

2.1 Electrocardiography of Pupal Hearts

The depolarization and repolarization electrical potentials created by intensive contractions of the compact human myocardium can be successfuly recorded by external electrodes located at different parts of the body. This represents the common principle of ECG recordings in medicine (Hampton, 2003). The contracting insect myocardial cells exhibit similar depolarization and repolarization potentials. However, these potentials are smaller and less convenient for recording by external electrodes, because: 1. The myocardium of an insect tubular heart is divided into metameric segmental compartments, which contract sequentially in the forward (anterograde heartbeat) or backward (retrograde heartbeat) directed waves of peristaltic contractions; 2. The rhythmicity of cardiac pulsations is determined by a pacemaker nodus located in posterior segments of the heart; 3. Each peristaltic wave of myocardial contractions can occasionally start before the previous wave arrived to its final destination (more peristaltic waves running at the same time). These data show that, in contrast to the human heart, the heart of insects creates relatively small and dispersed depoarization potentials, which are difficult to be directly recorded.

We are reasonably thinking, however, that due to recent progress in electronic recording techniques, it will be soon possible to monitor electrical depolarisation potentials of insect myocardial segments, just like in ECG of the human heart. Recently we have developed several ECG methods, which enable accurate monitoring of myocardial contractions in the insect heart. The methods cannot record the depolarisation potentials directly, but reveal precisely the myocardial contractions in one or multiple segments of the body. So far, we have obtained the best results with two, previously described ECG methods suitable for insect heart (Sláma, 2003); (Sláma and Miller, 2001), i. e. thermocardiographic and optocardiographic methods. In principle, the thermocardiographic method is based on the use of miniature thermistor sensors positioned externally above the pericardial sinus of the heart. The sensors are gently warmed to create a temperature gradient around their bodies.

Figure 1: Miniature thermographic sensors positioned externally over the heart of a diapausing pupa of Manduca sexta (from Sláma, 2003).

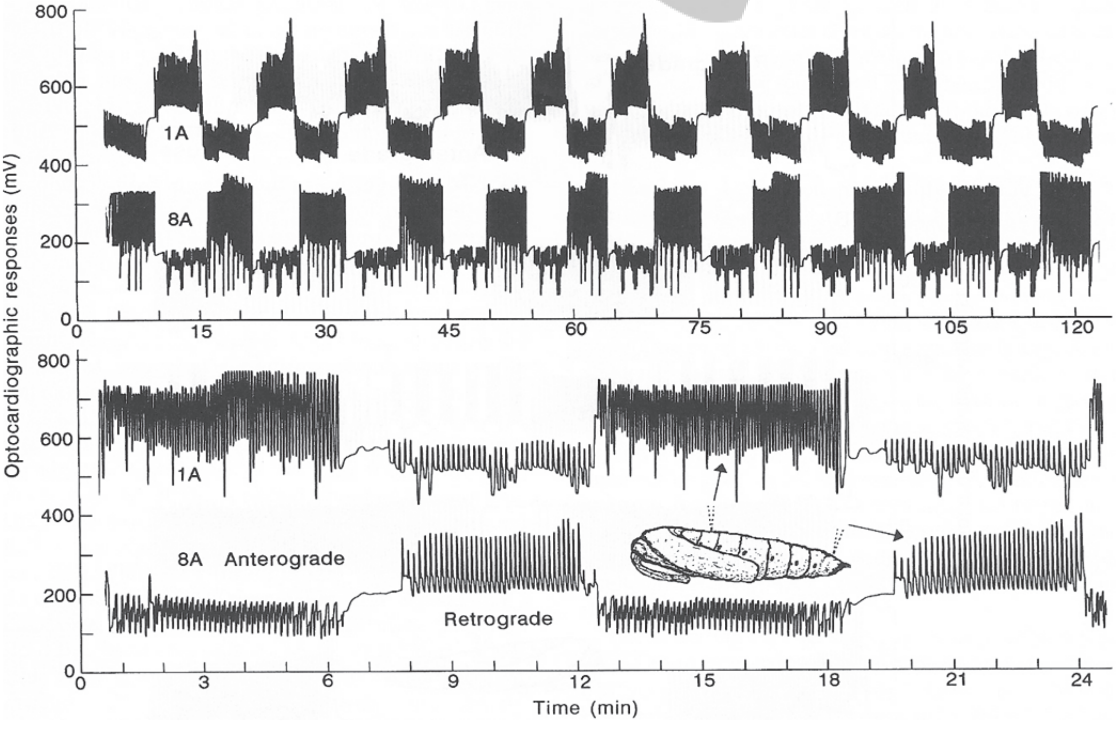

Figure 2: Heartbeat reversal recorded by two thermographic sensors from the first (1A) and eight (8A) body segments of diapausing pupa of Manduca sexta. Lower portion shows the detail with expanded time scale (From Sláma, 2003).

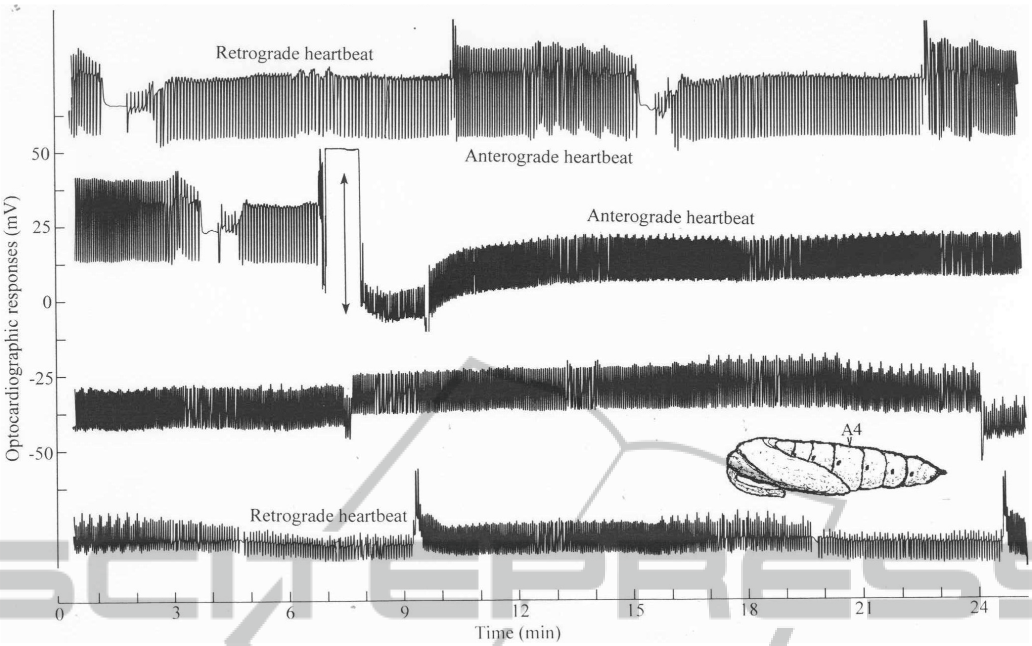

Figure 3: Effect of digitoxine injection (arrow; 20 mg/kg of body mass) on pupal heartbeat in Manduca sexta.

The subintegumental movements of haemolymph caused by pulsations of the heart disturb the established temperature gradients around the sensors, which are converted into the corresponding electrical sisgnals that are finally recorded by the recording devices (see Figure 1 and 2).

The recording of insect heartbeat is often frustrated by movements of somatic muscles and by special extracardiac pulsations in haemocoelic pressure, used for inspiration and expiration of air through spiracles. Suitable developmental stages for recording the heartbeat of insects are immobile pupae (Figure 1). The records in Figure 2 show that the peristaltically propagated waves of myocardial contractions move alternatively forwards and backwards. The rhythmicity is determined by a pacemaker nodus located in posterior region of the heart. After sectioning in the middle of the heart, posterior section preserved rhythmicity and periodicity of heartbeat reversal, whereas anterior section lacking the pacemaker nodus exhibited only some slow, indifferent contractions similar to human heart deprived from the ventricular nodus (Sláma, 2006). The forward oriented, anterograde heartbeat, which pumps abdominal haemolymph into the head, represents the most important circulatory function. There are numerous insect larvae which show only this unidirectional, forward oriented anterograde cardiac pulsation. In Figure 2, the thermographic sensors located at the base of pupal abdomen reveal increased amplitude of haemolymph circulation during anterograde heartbeat. During the reciprocal, retrograde heartbeat the relationships are reversed. Diapausing pupae of *Manduca sexta* (Figure 1) are very convenient for recording of insect heartbeat. They can be stored in refrigerator for several months before use. Figure 3 shows an example of chronotropic effect of digitoxine injection on pupal heartbeat of *Manduca.* Using this model system, we tested a number of cardio-stimulatory or inhibitory pharmacological preparations and found similar structure-activity relationships known from the human medicine (Sláma, 2008b).

2.2 Optocardiography of Larval and Pupal Hearts

Thermographic sensors need to be firmly attached to integumental surface over the pericardial region. This condition can be satisfied in immobile pupae, but not in the mobile larvae with smooth and elastic cuticle. These obstacles were restrained by development of absolutely touch free, optoelectronic techniques. The device shown in Figure 4 uses four independent channels of red pulselight, focused to a small (0.3 mm^2) area over the heart through optic fibers and lenses from the distance of 7 mm. The beam of constant, stabilised pulse-light is applied to the measured epidermal area by one optic fiber. Changes in optical density, which are caused by contractions of the heart, are dispatched to photomultipliers of the device by incoming optic fibers.

After multiplication and decoding, the heartbeat pulsations are converted into output voltage and are recorded. Figure 4 shows immobile pupa of *Zophobas atratus* during touch-free recording of heartbeat. Optocardiographic recordings could be executed in all stages with reasonably transparent cuticle and in specimens which do not move for a short time. The dorsal vessel of insects (insect heart) is located just under the integumental cover. Many insects are resting immobile for shorter or longer periods of time. Their heartbeat can be easily recorded by optocardiographic methods, simply by focusing the pulse-light beam over the pericardial region, such as in case of *M. sexta* caterpillar during feding (see Figure 5).

Figure 4: Four-channel optoelectronic device used for pulse-light optocardiographic recording of heartbeat.

Figure 5: Caterpillar of *M.sexta* during the pulse- light, optoelectronic recording of heartbeat by Sláma (2003).

2.3 Heartbeat during Neuromuscular Paralysis

Larvae of the greater waxmoth (*G. mellonella*) are perhaps the best experimental model of insects. They are adjusted to live in 37°C of the bee hives. In the laboratory, they can be reproduced in large numbers at any time of the year. The larvae exhibit unidirectional, purely anterograde heartbeat with the rates comparable to that of the human heart (at 37°C). The problem is that these larvae are extremely mobile and have rather fast developmental rate. Under these conditions, recording of heartbeat is very difficult. A great progress in this respect has been achieved few years ago when Sláma and Lukáš (2011) found that larvae, which were subjected to neuromuscular paralysis induced by venom of the parasitic braconid wasp, exhibited apparently normal heartbeat. The profound neuromuscular paralysis, which could last for several weeks, affected all somatic muscles innervated through neuromuscular transmission. The peristaltic contractions of the heart and intestine, however, which were regulated by depolarisation potentials of the myocardium, remained unaffected and fully functional. The heartbeat patterns of these motionless, paralysed larvae can be easily monitored by all types of the recording methods. Electrocardiographic investigations on these larvae revealed the autonomic (brain independent) nature of heartbeat regulation. Further advantage of *Galleria* depends on the prolonged survival when deprived of the brain hormone source by ligatures made behind the head. The ligatured larvae with arrested development (Figure 6 (A)) can be stored for many weeks for later use. In combination with additional paralysis by the braconid venom (Figure 6 (B)), these motionless larvae represent the best experimental material for routine screenings of various cardio-active materials on insects. Due to total absence of the somatic movements, it was possible to use the pralysed larvae for prolonged optocardiographic recordings of the heartbeat. The use of multiple optocardiographic sensors (see Figure 7), enabled determination of the propagation velocity of individual systolic contraction waves.

Earlier recordings (Sláma and Lukáš, 2011) revealed more or less constant cardiac pulsations in the paralysed larvae, characterised by 20–25 systolic contractions per minute. The contractions were peristaltically propagated in the forward (anterograde) direction with a more or less constant speed of 10 mm per second (23–25°C). Sectioning performed in the middle of the heart (4th abdominal segment) seriously impaired the pacemaker rhythmicity and slowed down the rate of heartbeat in the anterior sections. By contrast, the functions of the posterior compartment of the disconnected heart remained unaffected.

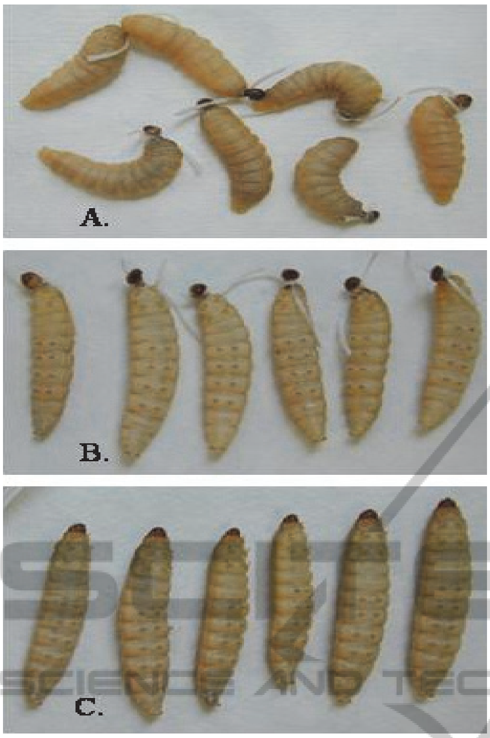

Figure 6: The fully grown larvae of *Galleria*, which have been ligatured behind the head (A), ligatured and injected with braconid venom (B) or injected without ligaturing.

An example of simultaneous, 4 channel optocardio-graphic, ECG-like record with the paralysed larva (as shown in Figure 7), can be found in Figure 8. The multichannel records of this type reveal the details of systolic contractions of the heart. In addition to the rate of heartbeat, these records reveal the propagation velocity of individual, systolic contraction waves.

Figure 7: Paralysed larva of Galleria during multiple optocardiographic recordings.

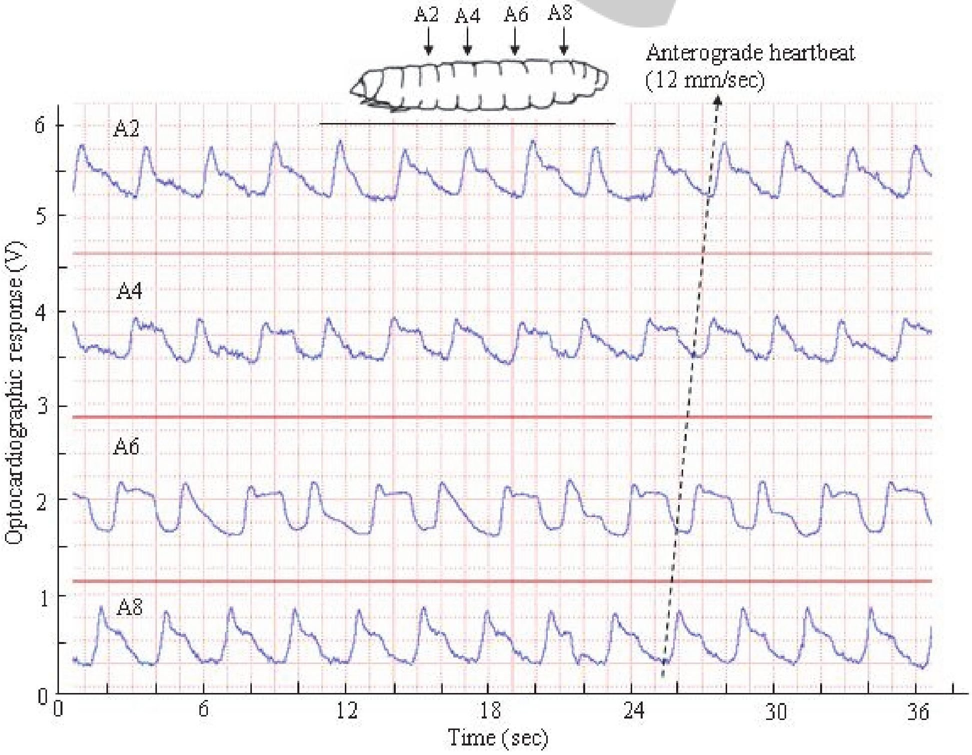

Figure 8: Example of simultaneous optocardiographic record of heartbeat in paralysed larva of Gallera, obtained by means of the multichannel setup shown in Figure 7 (from Sláma, 2011).

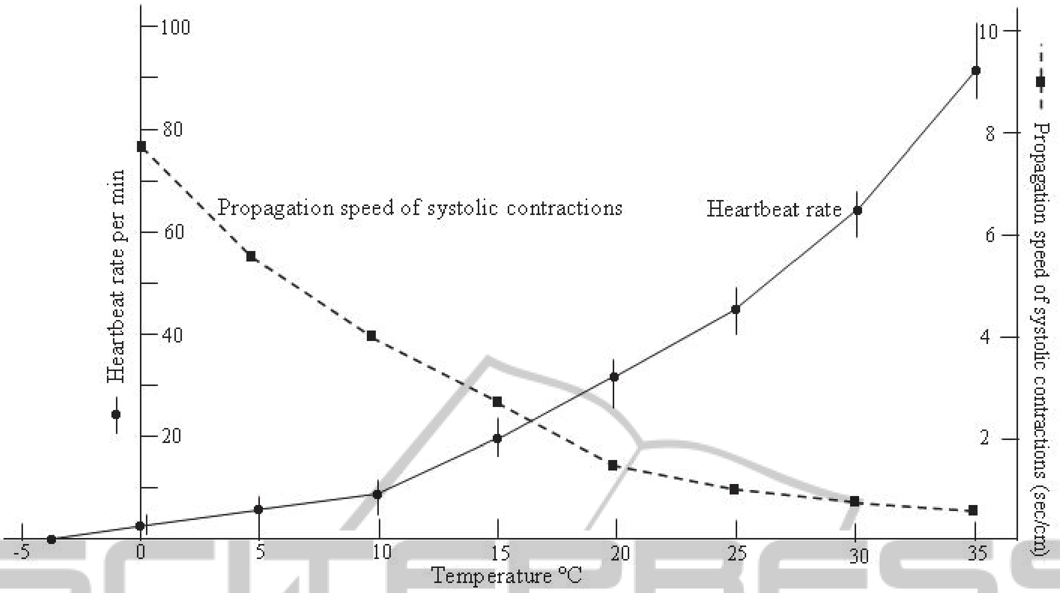

Figure 9: Effect of different temperatures on heartbeat rate and velocity of peristaltic myocardial contractions in the paralysed larva of *Galleria* (from Sláma, 2012a).

Figure 9 gives detailed acount of the effect of different temperatures on the rate of heartbeat in the paralysed larva of *Galleria*. The larvae are paralysed by purified extract prepared from the venom glands of the tiny braconid wasp, *Habrobracon hebetor* (10 glands per ml of Ringer, 20 µl injection).The results show that the rate of heartbeat pulsation is between 80 and 90 pulses per min and the velocity of peristaltic myocardial contractions is less than one second, at 35°C.

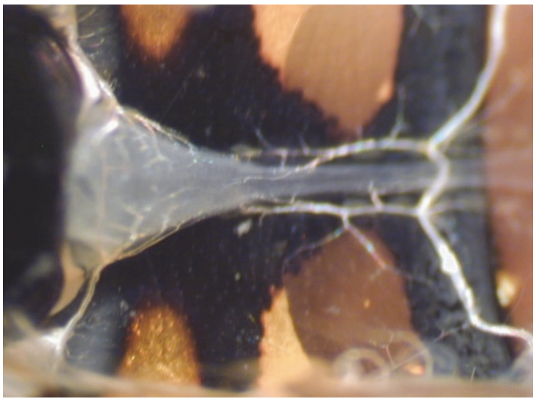

Figure 10: Compact, human-like heart ventricle seen from the ventral side of abdomen in an adult hover fly, *Episyrphus balteatus*. The ventricle is stretched within the body cavitly by lateral ligaments (left), with a narrow posterior heart (right) (From Sláma, 2013).

The compact ventricle found in the hoverfly, shown in Figure 10, represents a new and enigmatic feature in comparative animal physiology. The heartbeat rate shows real animal recod (frequency over 10 Hz) and, what is most important, the mechanism is not peristaltic, but synchronic, like in the human heart (the whole ventricle composed of several metameric segments contracts unisono). The compact muscular ventricle and synchronic contraction mechanism are apparently the physiological adaptations to increasing demands for the forward oriented, anterograde pumping of haemolymph to extensively developed, thoracic flight musculature.

3 CONCLUSIONS

We are reasonably thinking that due to the described functional similarities between insect and human hearts, the new cardioactive or inhibitory substances can be tested on the hearts of insects. The biassays, performed by the described, touch-free cardiogical methods on the paralysed larvae of *Galleria*, can potentially serve as a convenient and inexpensive way how to avoid the use of large animals for biological testing of cardiologically important chemicals.

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REFERENCES

Bodmer, R., Wessells, R. J., Johnson, E. C., Dowse, H. B., 2005. Heart development and function. In:Gilbert, L. I., Iatrou, K., Gill, S. (Eds.), *Comprehensive Molecular Insect Science, V*ol. 2, 199–250.

Fink, M., Callol-Massot, C., Chu, A., Ruiz-Lozano, P., Belmonte, J. C. I., 2009. A new method for detection and quantification of heartbeat parameters in *Drosophila*, zebrafish and embryonic mouse hearts. *Bio Techniques* 46, 101-113.

Hampton, J. R., 2003. *The ECG in Practice,* Fourth ed. Elsevier Limited, Oxford.

Jones, J. C., 1977. *The circulatory system of insects*. Charles, C.Thomas. (Ed.) Springfield, Ill.

Miller, T. A., 1997. Control of circulation in insects. *Gen. Pharmacol*. 29, 23–38.

Ocorr, K., Perrin, L., Lim, H.-Y., Qian, L., Wu, X., Bodmer, R., 2007a. Genetic control of heart function and aging in *Drosophila*. *Trends in Cardiovascular Medicine* 17, 177–182.

Ocorr, K., Reeves, N. L., Wessels, R. J., Fink, M., Chen, H. S., 2007b. KCNQ potassium channels mutations cause cardiac arrhythmias in *Drosophila* that mimic the effectřs of aging. *Proc. Natl. Acad. Sci. USA*, 104, 3943-3948.

Sláma, K., 2000. Extracardiac versus cardiac haemocoelic pulsations in pupae of the mealworm (*Tenebrio molitor* L.). *J. Insect Physiol.* 46, 977–992.

Sláma, K., 2003. Mechanical aspects of heartbeat reversal in pupae of *Manduca sexta J. Insect Physiol.* 49, 645–657.

Sláma, K., 2006. Heartbeat reversal after sectioning of the dorsal vessel and removal of the brain in diapausing pupae of *Manduca sexta,* Lepidoptera: Sphingidae). *Eur. J. Entomol.* 103, 17–26.

Sláma, K., 2008a. Extracardiac haemocoelic pulsations and the autonomic neuroendocrine system (coelopulse) of terrestrial insects. *Terrestrial Arthropod Reviews* 1, 39–80.

Sláma, K., 2008b. Method of testing cardioactive preparations on insects. *Czech Patent Application* PV 2008–671.

Sláma, K., 2010. Physiology of heartbeat reversal in adult Drosophila melanogaster (*Diptera: Droso-philidae*). *Eur. J. Entomol.* 107, 13–31.

Sláma, K., 2012. Neuromuscular paralysis induced in insect larvae by the proteinic venom of a parasitic wasp. In: *Paralysis: Causes Classification and Treatment* , Nova Science Publishers Inc., New York, pp. 1–24.

Sláma, K., 2013. A new look at the comparative physiology of insect and human hearts. *J. Insect Physiol.* 58, 1072-1081.

Sláma, K., Lukáš, J., 2011. Myogenic nature of insect heartbeat revealed by neuromuscular paralysis caused by the sting of a braconid wasp. *J. Insect Physiol*. 57, 251–259.

Sláma, K., Miller, T. A., 2001. Physiology of heartbeat reversal in diapausing pupae of the tobacco hornworm, *Manduca sexta* (*Lepidoptera: Sphin-gidae*). *Eur. J. Entomol.* 98, 415–431.

Zeitouni, B., Senatore, S., Severac, D., Aknin, C., Semeriva, M., Perrin, L., 2007. Signalling pathways involved in adult heart formation revealed by gene expression profiling in *Drosophila*. *PLoS Genetics* 3, 1907–1921.