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Effect of biogenic amines on the contractile activity of visceral muscles in the beetle *Tenebrio molitor*

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Abstract

Biogenic amines are biological active compounds that play a significant role in regulation of animal physiology. In insects, the main amines are trace amines like octopamine and tyramine. Those two amines seem to be a partial counterparts of epinephrine and norepinephrine present in vertebrates. Little is known about activity of those compounds in beetles, hence we examined effects of 4 amines on the heart, hindgut and oviduct contractile activity of *Tenebrio molitor* L. (Coleoptera Tenebrionidae). The results showed that *in vivo* tyramine and octopamine caused a cardioinhibitory effect on 0-1-day old pupae and similar effects of these compounds were observed *in vitro* in adults beetles. Dopamine decreased weakly the frequency of heart rate in pupae but increased it in adult beetles at the highest concentration tested while epinephrine caused no changes in both stages. Tyramine and octopamine showed dose-dependent and organ-specific activity on *T. molitor* hindgut and oviduct. *In vitro*, they inhibited contractile activity of hindgut whereas on oviduct they acted as myostimulators. Dopamine and epinephrine caused stimulation of contractile activity of muscles of both organ studied. The pharmacological experiments suggest possible modulation of an endogenous mechanism generating muscle contractions *via* the adrenergic system and indicate the presence of adrenergic receptor(s) in the visceral organs of the beetle *T. molitor*.

Key words: biogenic amines, tyramine, octopamine, dopamine, epinephrine, insect, *Tenebrio molitor*, heart, oviduct, hindgut.

Introduction

The contractile activity of skeletal, visceral and cardiac muscles undergoes complex regulation by numerous neural agents. One of the most important, both in vertebrates and invertebrates, are biogenic amines which may act as neurotransmitters, neuromodulators and neurohormones (Monastirioti, 1999; Blenau and Baumann, 2001), thus in wide manner they control muscle activity. In invertebrate, the main role in regulation of neuromuscular system functioning have trace amines (octopamine and tyramine) and catecholamines - dopamine and serotonin (Lange, 2009), while in vertebrates the most important are catecholamines like epinephrine, norepinephrine, dopamine and serotonin.

Octopamine and tyramine are found in the nervous systems in almost all groups of animals from nematodes to mammals (Roeder, 1999). They are structurally related to epinephrine and norepinephrine and share some of the function with them, which points to an early evolutionary origin of the adrenergic/octopaminergic/tyraminergic system (Roeder, 2005). In insects, octopamine controls skeletal and visceral muscles contractility, metabolism, immune system, reproduction and development (Roeder, 1999; 2005; Farooqui, 2012). The role of tyramine is less understood. Limited data suggest its participation in neuromodulation of muscles activity, modulation of cell membrane permeability to chloride ions in the Malpighian tubules (Blumenthal, 2003), functioning of olfaction (Kutsukake *et al.*, 2000) and reproduction (Hana and Lange, 2017). It was shown that octopamine and tyramine regulate insect heart contractility, but their activity depends on species and developmental stage. In *Drosophila melanogaster* Meigen (Diptera Drosophilidae), octopamine decreases heart rate in larva and pupa and increases it in adult (Zornik *et al.*, 1999). Similarly, Johnson *et al.* (1997) showed that octopamine stimulates heart of adult *D. melanogaster*. Results obtained by Papaefthimiou and Theophilidis (2011) demonstrated that effects caused by octopamine depend on concentration. In *Apis mellifera* L. (Hymenoptera Apidae), this amine acts as cardiostimulator at high concentration (over 10^{-7} M) whereas at low concentration (below 10^{-7} M) it causes inhibition of heart contraction. Octopamine and tyramine affect also muscle contractility of insect oviduct and gut. Available data indicate species-specific and dose-dependent effects. Octopamine inhibits oviduct contractions in *Locusta migratoria* (L.) (Orthoptera Acrididae) (Lange and Orchard, 1986; Donini and Lange, 2004; Lange and Nykamp, 1996; Lange and Tsang, 1993), and flies *Stomoxys calcitrans* (L.) (Diptera Muscidae) (Cook and Wagner, 1992) and *D. melanogaster* (Rodriguez-Valentin *et al.*, 2006). This amine affects both frequency and amplitude of oviduct contractions in *Periplaneta americana* (L.) (Dictyoptera Blattidae) (Bamji and Orchard, 1995). On the other hand, it increased frequency and amplitude of *Gryllus bimaculatus* De Geer (Orthoptera Gryllidae) oviduct contractions (Tamashiro and Yoshino, 2014). Varied effects caused by octopamine were also noticed on hindgut. In *Schistocerca gregaria* (Forskal) (Orthoptera Acrididae), this amine leads to lowering of hindgut contractions frequency (Osborne *et al.*, 1990) and depression of the neurally induced contractions of *P. americana* hindgut (Brown, 1975), while in mosquito *Aedes aegypti* (L.) (Diptera Culicidae) octopamine evokes opposite effect (Messer and Brown, 1995). Myoinhibition was also indicated for tyramine in case of *L. migratoria* oviduct (Donini and Lange, 2004) and hindgut (Huddart and Oldfield, 1982).

Catecholamines are the second group of amines involved in regulation of insect muscle contraction. The dopamine's cardioactivity is not clear. Titlow *et al.* (2013) showed that this amine increases heart rate in

D. melanogaster larva while data obtained by Zornik *et al.* (1999) indicate that it is inactive in larva but inhibits pupal and accelerates adult heart rate. Whereas, experiments carried out by Johnson *et al.* (1997) showed that dopamine increases the frequency of heart contractions in pupa of *D. melanogaster*. Similarly, cardioaccelatory properties of dopamine were observed in adult cockroach *P. americana* (Collins and Miller, 1977). Dopamine modulates also oviduct muscles activity. It accelerates oviduct contraction frequency in *G. bimaculatus* (Raabe, 1989) and *Manduca sexta* (L.) (Lepidoptera Sphingidae) (Coast and Webster, 1998). The dopaminergic neurons have been found in stomatogastric nervous system innervating foregut. In *L. migratoria*, dopamine induces phasic contractions of gut (Lange and Chan, 2008). An increase of hindgut contractility caused by dopamine was noted in case of *L. migratoria* (Freeman, 1966) but in cockroach *Leucophaea maderae* (F.) (Dictyoptera Blattidae) it inhibits hindgut contractions (Holman and Cook, 1970). Socha *et al.* (2008) showed that dopamine increase locomotor activity of *Pyrrhocoris apterus* (L.) (Hemiptera Pyrrhocoridae) and injection of this amine increase adipokinetic hormone level in haemolymph. It was also shown that dopamine stimulate skeletal muscle (Klemm, 1979). Epinephrine, vertebrate "fight or flight strategy" hormone, generally has no physiological relevance in insects (Even *et al.*, 2012). There is an evidence that it induces larval metamorphosis in the clams *Venerupis pullastra* (Montagu) (Mollusca Bivalvia) and *Ruditapes philippinarum* Adams et Reeve (Mollusca Bivalvia) (Yang *et al.*, 2014) and accelerates insect heart rate in *P. americana* (Miller and Metcalf, 1968).

Biogenic amines act on cells by family A (rhodopsin-like) G protein-coupled receptors (GPCRs). The receptors have been divided into several groups based on their pharmacological and structural differences. The first classification for octopamine receptors was made by Evans and Maqueira (2005). They distinguished group of α-adrenergic-like octopamine receptors (OAαR), β-adrenegric-like octopamine receptors (OAβR) and tyramine receptors 1 (TAR 1) and added by Bayliss *et al.* (2013), tyramine receptors 2 and 3 (TAR 2 and TAR 3). There are no data about octopamine and tyramine receptors in *Tenebrio molitor* L. (Coleoptera Tenebrionidae), but in genome of *Tribolium castaneum* (Herbst) (Coleoptera Tenebrionidae) four octopamine receptors and one tyramine/octopamine receptor have been identified (Hauser *et al.*, 2008).

The aim of present study was to determine effects of two trace amines (octopamine and tyramine) and two catecholamines (epinephrine and dopamine) on the *T. molitor* visceral muscle contractile activity. The participation of amines tested in regulation of insect visceral muscle activity is quite well-known, but the knowledge about their role in beetles is poor. Moreover, it is interesting how epinephrine, which is thought a partial counterpart of insect trace amines, affects muscle contractions. For that we performed *in vivo* and *in vitro* bioassays on heart, oviduct and hindgut of *T. molitor* beetle.

Materials and methods

Insects

Pupae and adults of *T. molitor* beetle were breeding in the Department of Animal Physiology and Development, Adam Mickiewicz University in Poznan under the following conditions: photoperiod 12 h light and 12 h of dark; temperature 26 °C (± 1°C) and relative humidity 60% (± 2%). In experiments, newly 1-day-old pupae and 4-week-old adults were used.

Test compounds

The catecholamines (epinephrine and dopamine) and trace amines (octopamine and tyramine) were purchased from Sigma-Aldrich (USA). The chemicals were freshly dissolved on the day of the experiments in saline A (274 mM NaCl, 19 mM KCl, 9 mM $CaCl_2$, 5 mM glucose and 5 mM HEPES) for *in vitro* experiments and in saline B (274 mM NaCl, 19 mM KCl, 9 mM $CaCl_2$) for *in vivo* experiments. Working dilutions were prepared from the stock solution in saline, pH 7.0.

In vivo heart bioassay

In order to define the influence of biogenic amines tested on *T. molitor* pupal heart contractility, the non-invasive optoelectronic technique described by Slama and Rosinski (2005) was used. This technique allows to measure the heart rate of pupa up to 24 h. To avoid the influence of circadian rhythm of the heart activity the recordings for each pupa started at the same time (Ventrella *et al.*, 2015; Marciniak *et al.*, 2010). The pupae were placed on holder and the recording was carried out. After 1 h of heart rate stabilization the insects were injected with 2 µL of compounds tested in saline B at 10^{-4} M concentration (to get the final concentration in haemolymph 10^{-5} M), this corresponds to a dose of 0.2 nmols per pupa. After injection, the changes of heartbeat frequency were recorded and compared to the control rhythm (pupae injected with physiological saline B). Measurements were performed using a CMY-17 transducer (Philips, USA) connected *via* a signal amplifier (ETNA Electronics Kikiewicz, Poznań, Poland) to the computer. Acquisition of data and analysis were performed with the ADVANTECH PCI1710L A/D converter card and computer software LARWA and ANALIZA which were designed at our laboratory.

In vitro heart bioassay

To measure the *in vitro* effects of the test compounds on the heart of 4-week-old adults beetles the microdensitometric technique was used (Rosinski and Gade, 1988). Experiments were carried out on the semi-isolated heart, which preparation was as follows. After 8 minutes of anaesthesia with carbon dioxide, beetles were decapitated and then the legs and wings were cut off. The ventral cuticle was removed, leaving only narrow strips on the sides and on the last segment of the abdomen, and finally the visceral organs were carefully removed from the preparation. After isolation, the preparation was placed in superfusion chamber and installed on the microdensitometer MD-100 (Carl Zeiss,

Jena, Germany). An open-perfusion system with an application port 70 mm above the superfusion chamber was used. The flow rate of fresh saline A was 140 µL per min. The solution was continuously removed from superfusion chamber by chromatographic paper (Whatman No. 3, UK).

We performed two types of experiments: 1) Pulse applications of amines - At the beginning of experiment new-isolated heart was preincubated for 10 min with saline A. The control heart activity was recorded for 0.5 min and then the amine tested was applied and the activity was recorded for further 2 min. After recording, the heart was washed with saline A for 3 min. The sample was applied with a Hamilton syringe through the application port in volume of 10 µL and concentration range from 10^{-10} M to 10^{-2} M; 2) Continuously perfusion of amines - At the beginning of experiment new-isolated heart was preincubated for 10 min with saline A. The control heart activity was recorded for 5 min and then for 15 min the heart was continuously perfused with amine tested in concentrations of 10^{-4} M. After recording, the heart was washed with saline A for 10 min.

The heart response to tested compounds were shown as a percentage change of heart rate after sample application, as control the recording before application were used. The changes were calculated according to below equation:

$$\%\ change\ of\ heart\ rate = \frac{heart\ rate\ after\ compound\ application}{heart\ rate\ before\ compound\ application}\% - 100\%$$

The data were analysed with LARWA and ANALIZA computer software.

Oviduct and hindgut *in vitro* video-microscopy bioassays

To study the *in vitro* effects caused by compounds tested on the endogenous contractile activity of oviduct and hindgut, the video-microscopy technique and the computer-based method of data acquisition and analysis were used (Marciniak *et al.*, 2008). The oviduct with ovaries was isolated from 4-week-old females of *T. molitor*. After anaesthesia, females were decapitated and then the legs and wings were removed. In the next steps, the dorsal cuticle was cut off with microsurgical scissor and the oviduct with ovaries was isolated and placed in incubation chamber in saline. After that, accessory glands, spermathecae, rest of fat body and Malpighian tubules were removed and oviduct preparation was placed in superfusion chamber and attached to the elastomer Sylgard with Minuten pins. The hindgut was isolated in similar procedure.

Each of the preparation (oviduct or hindgut) was continuously superfused with fresh saline A at flow rate of 140 µL per min in the incubation chamber which was installed horizontally on the stage of an Olympus SZX12 stereomicroscope equipped with a Pixeling 662 camera. The preparations were stabilized for 10 min in saline A before the recording was started. The movie was recorded for 2 min. The tested compounds were applied in 30th second of registration with a Hamilton syringe in a volume of 10 µL to the application port in concentration ranged from 10^{-10} to 10^{-2} M. The time before application was used as control. The obtained data were analysed with the AnTracker (PreOptic, Warsaw, Poland) computer software. Firstly, the movies were binarized and the tracking point overlaid on video image fallowing the black/white boundary between the preparation and background. Myograms were created based on a plot of the frame-to-frame changes in the positions of the tracking points along the boundary. The myotropic activity of the amines tested is shown as a percentage change of the preparation contraction frequency after application of amines in comparison to the control (30 s before application of compound), as described above "*In vitro* heart bioassay".

Statistical analysis

All data are presented as the mean values ± SD or ± SEM of *n* number of replicates. The statistical significance of differences between control and testing values was determined using Student's *t*-test or, if there was not a normal distribution, the nonparametric Mann-Whitney U test was used. The statistical analyses were performed using GraphPad Prism software. Differences were considered statistically significant if $p \leq 0.05$ (*), $p \leq 0.01$ (**), or $p \leq 0.001$ (***).

Results

Effects of biogenic amines on *T. molitor* heart *in vivo*

The obtained data showed that action of pupa heart can be divided into three phases: 1) an orthodromic phase with a high frequency and an anteriorly directed propagation wave of heart contractions 2) an antidromic phase with a low-frequency and an posteriorly directed propagation wave of heart contraction, and 3) more or less prolonged periods of diastasis. The average frequency of heartbeats during orthodromic phase was 20.9 (± 3.4 SD) contractions per min, and during antidromic phase it was 12.2 (± 2.4 SD) (figure 1). Moreover, in the heart work it can be noted a circadian rhythm of frequency of heart contractions (periodic changes of contractions frequency). It is particularly marked in case of antidromic phase. We can observe oscillation between maximal value of frequency (12.2 contractions per min) to minimal value (8.9 contraction per min) during 24 h. This oscillation is also observed in non-injected pupae.

Injection of tyramine in concentration 10^{-4} M caused a slight cardioinhibitory effect. This biogenic amine decreased the contractions frequency both in antidromic and orthodromic phase. In case of antidromic phase, a significant reduction of heart rate was observed only in 8th hour after injection and it was 10% lower compared to control (figure 1A). The cardioinhibitory effect was considerably stronger in orthodromic phase. For example, in 18th hour after injection average value of heart rate was 31% lower than in the control pupae. In this phase, the observed effect was also more prolonged and maintained until 22nd hour after injection. The second trace amine, octopamine showed similar effect to tyramine. Nevertheless, the significant negative chronotropic effect of octopamine in antidromic phases appeared between 8th and 12th hour and the highest reduction of heart rate (32%) was observed 12 hours after in-

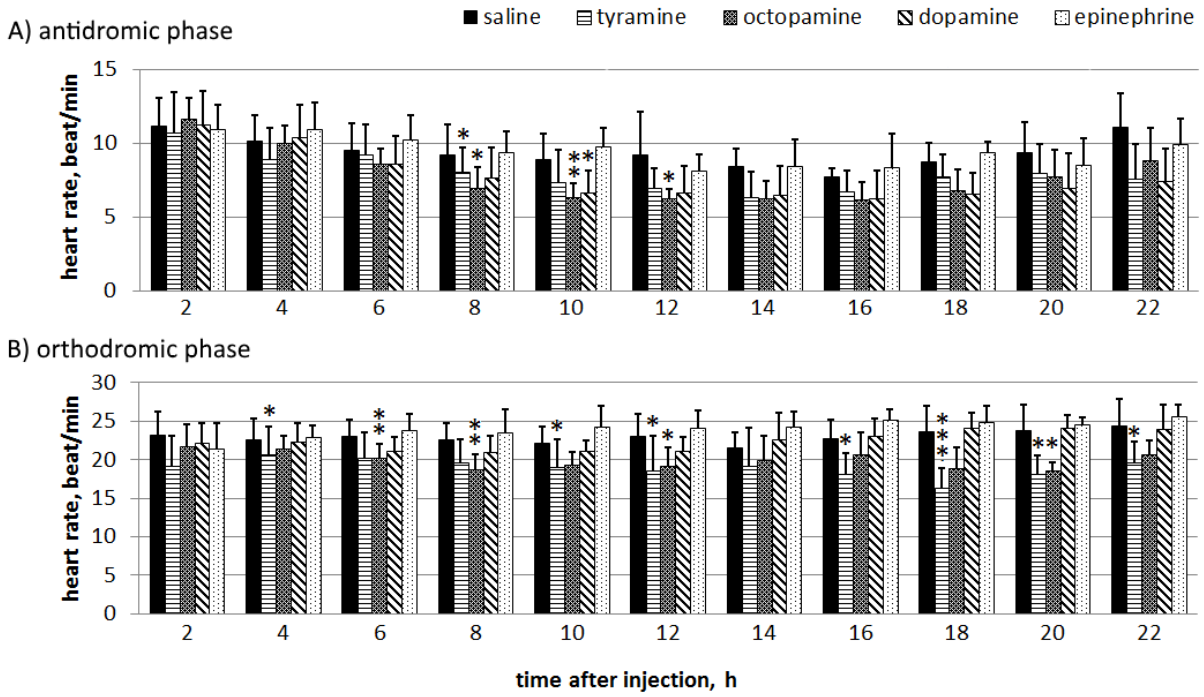

Figure 1. The *in vivo* heart rate contraction frequency in antidromic (**A**) and orthodromic (**B**) phases of 1-day-old pupae of *T. molitor* after injection of saline and tested compounds at 10^{-4} M concentration. Results are expressed as mean of $n \geq 21$ replicates ± SD. Significant differences $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) are indicated by asterisks (Mann-Whitney test).

jection (figure 1A). The significant decrease of heart rate in orthodromic phases appeared at 6th hour after injection and maintained almost the entire period of registration with lesser or stronger intensity up to 20th hour after injection with the strongest effect at 20th hour (figure 1B). Dopamine also caused decrease of heart rate, but the negative chronotropic effect was slight and not statistically significant. In case of epinephrine, generally there were no substantial changes observed in heart activity but mainly in the orthodromic phase a slight acceleration of heart was noticed (figure 1B).

Effects of biogenic amines on *T. molitor* heart *in vitro*

The obtained result showed that in the *in vitro* experiments the average heart rate frequency of adult *T. molitor* beetle is 107.4 (± 15.2 SD) contractions per min, and the heart work is stable at this level for several hours. In pulse application, both trace amines caused a strong cardioinhibitory effect which corresponds with *in vivo* results. Nevertheless, tyramine showed stronger impact on heart. The significant decrease of heart rate in presence of this compound was observed in concentration range 10^{-6}-10^{-2} M and the strongest slowdown was noticed at 10^{-3} M. At this concentration tyramine led to reduction in the number of heart contractions per minute by an average of approximately 53% (± 31% SD) (figure 2A). The value of tyramine half maximal inhibitory concentration (IC_{50}) was 2.9×10^{-5} M. Moreover, at two of the highest concentrations tyramine caused a reversible cardiac arrest for several seconds in case of some preparations (figure 3B-C). The effect evoked by octopamine was lesser with the strongest inhibition at 10^{-3} M; the frequency of heart contractions after octopamine application was by an average of approximately 42% (± 29% SD) lower than in control (figure 2B). The IC_{50} value for this compound was 4.3×10^{-5} M and it was 1.5-times higher than IC_{50} for tyramine which reflects with higher potency of tyramine to inhibition of heart contractility.

Dopamine evoked a slight positive chronotropic effect but it was observed only at the highest concentrations tested 10^{-3} and 10^{-2} M where heart contraction frequency was by an average of approximately 25% higher compared to control (figure 2C). What interesting, this effect was opposite to that caused by this monoamine *in vivo*. Epinephrine like in *in vivo* experiments did not affect heart rate (figure 2D and 3E). In presence of all monoamines tested no changes in the amplitude of the myocardium contractions were observed ($p > 0.05$).

Experiments with continuous perfusion showed that only in case of epinephrine a slight cardiostimulatory effect (17%) was observed. During perfusion with remaining amines, a weak cardioinhibitory effect was observed. After application of tyramine, dopamine or octopamine mean inhibition was about 14.3%, 7.4% and 2.5%, respectively.

Influence of monoamines on oviduct contractility

In vitro bioassays, endogenous contractile activity of oviduct of *T. molitor* was remained irregular by an average of approximately 6.8 (± 3.9 SD) contractions per 1 min. All biogenic amines studied in the experiments showed myotropic effects on oviduct which were dose-dependent. Nevertheless, differences in maximal response evoked by particular amines were observed (figure 4A-D).

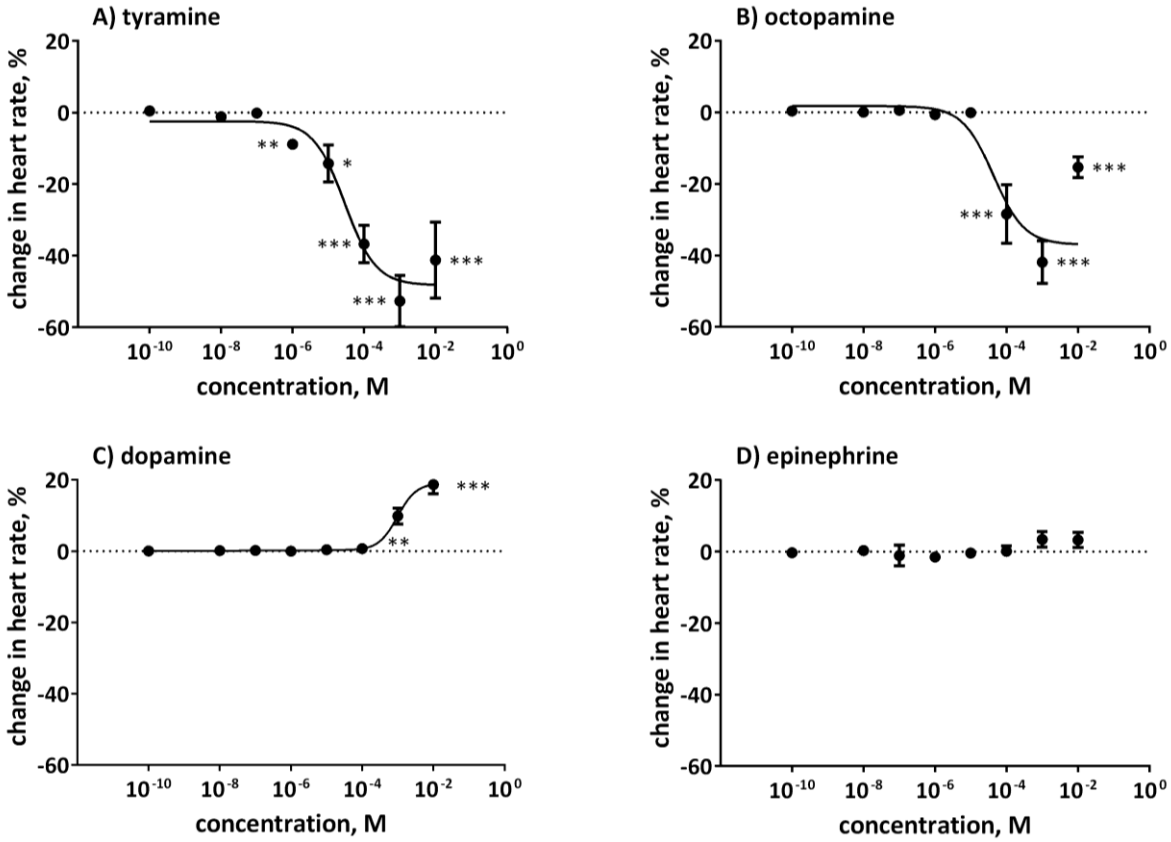

Figure 2. Dose-response curves for the effect of tyramine (**A**), octopamine (**B**), dopamine (**C**) and epinephrine (**D**) on the contractions frequency of *T. molitor* hearts *in vitro*. Results are expressed as mean of $n \geq 16$ replicates ± SD. Significant differences $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) from control (saline buffer) are indicated by asterisks (Student's *t*-test).

Tyramine and octopamine act as myoinhibitors on oviduct. The response evoked by these compounds was dose-dependent and the contraction activity of oviduct was progressively inhibited with an increase in amines concentration. Nevertheless, tyramine showed a higher activity than octopamine. It inhibited oviduct contractions in almost whole tested concentration range (10^{-8} to 10^{-2} M) with $IC_{50} = 6.54 \times 10^{-6}$ M and caused the strongest decrease of oviduct contraction frequency by 78% at 10^{-3} M (figure 4A). While octopamine lowered the number of oviduct contractions in concentration range 10^{-6}-10^{-2} M with the higher activity at 10^{-3} M. At this concentration the contraction frequency was lower by 69% (figure 4B) than in control. The value of IC_{50} determined for this amine was 2.41×10^{-4} M and thus 36-times higher than IC_{50} for tyramine what indicates that octopamine are definitely slighter inhibitor of oviduct muscle contractility. Moreover, both amines at the two highest concentrations evoked reversible arresting of oviduct contraction for 30-40 s (figure 5B-C).

Dopamine and epinephrine exhibited less unequivocal activity but generally they act as myostimulators. Dopamine increased frequency of oviduct contraction but interestingly at the lowest tested concentration and at 10^{-7} M it slightly decreased the contraction frequency by 10-20% (figure 4C). In case of epinephrine, also myostimulatory activity was observed. Response in oviduct contraction frequency to this amine maintained at a similar level in the whole range of concentrations of tested and only at 10^{-8} and 10^{-4}-10^{-3} M the number of oviduct contractions per min increased significantly ($p \leq 0.05$) (figure 4D).

Influence of monoamines on hindgut contractility

The hindgut of the *T. molitor* adults, superfused with physiological saline showed irregular contractions activity with an average of 5.5 (± 4.1 SD) contractions per minute (figure 5A). All amines tested stimulated the hindgut contractile activity with different efficiencies (figure 6A-D). In case of tyramine and octopamine, the obtained data indicate opposite myotropic properties according to these obtained in the experiments on oviduct, where those amines inhibited muscles contractility. Tyramine and octopamine stimulated the hindgut contractility approximately by 40-120% and 50-90%, respectively. The response evoked by tyramine showed dose-dependence with the highest maximal response at 10^{-4} M (120% increase of hindgut contraction frequency) and EC_{50} equal to 2.30×10^{-7} M. The changes induced in hindgut contractility by octopamine maintained at a similar level across the whole concentrations range tested of this amine. The strongest effect was caused by application of octopamine at concentration 10^{-8} and 10^{-7}-10^{-6} M (figure 6B).

Dopamine and epinephrine also acted as myostimulators on *T. molitor* hindgut muscles. The intensity of re-

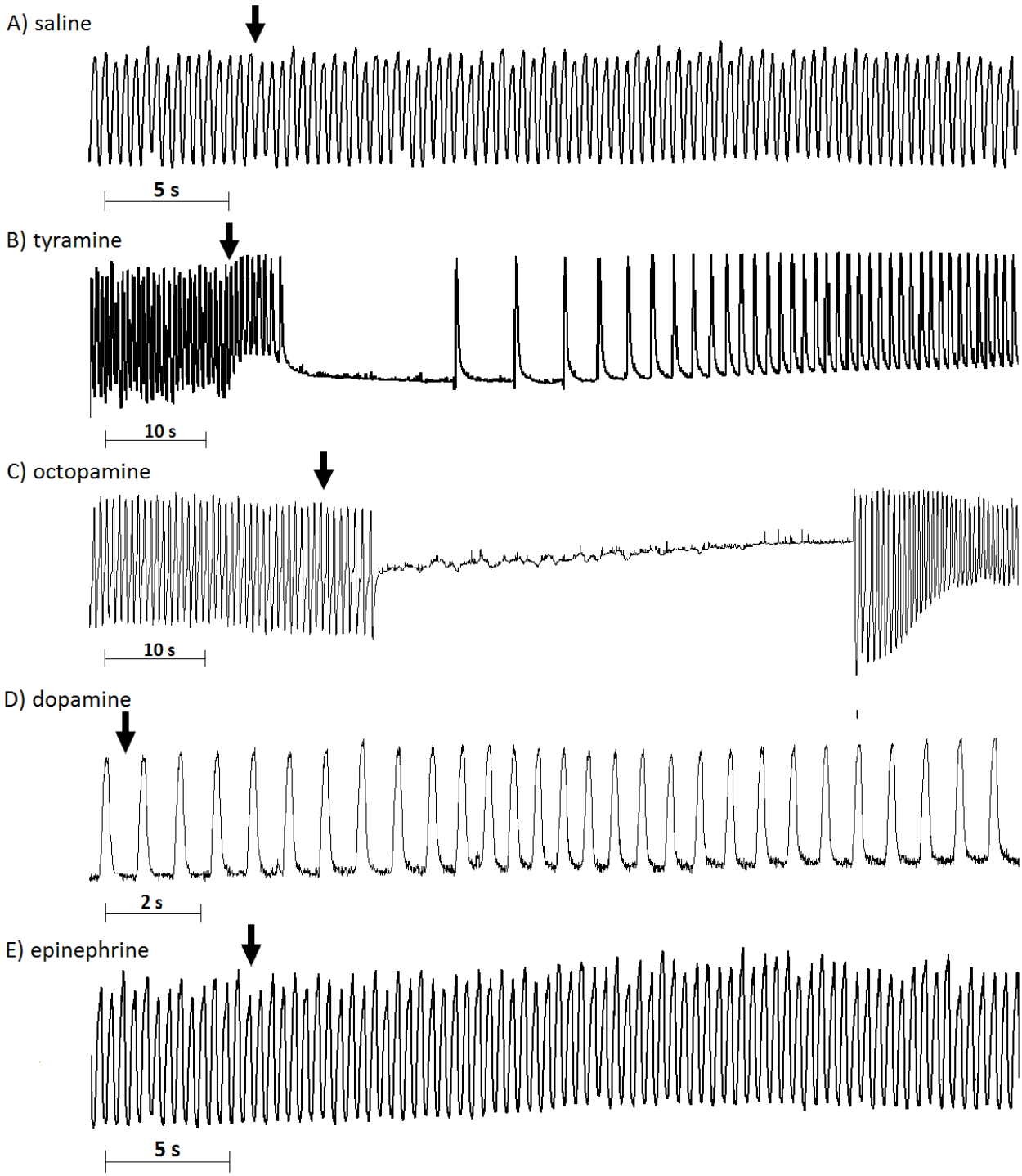

Figure 3. Cardiomyograms presenting the effects of the application of saline (**A**), tyramine (**B**), octopamine (**C**), dopamine (**D**), and epinephrine (**E**) at a concentration 10^{-4} M on the semi-isolated myocardium of *T. molitor* adults. The arrows indicate the application of the compounds.

sponse increased with the dose of applied compounds. The value of EC_{50} for dopamine was 2.11×10^{-3} M and for epinephrine 3.02×10^{-8} M which is comparable to EC_{50} for tyramine. In presence of dopamine, the highest increase of hindgut contractility was observed at the highest concentration tested (10^{-2} M) and the frequency increased by 80%. In case of epinephrine, the maximal response was observed at 10^{-3} M and reached similar level to this caused by dopamine.

Discussion

The insect visceral muscles are striated, however, they possess similar properties to vertebrate smooth muscles with the mode of contraction. They can contract slowly and rhythmic and are able to generate peristaltic wave which is characteristic for visceral muscles. Moreover, they maintain the possibility to contract also after isolation for relatively long time. The endogenic contractile

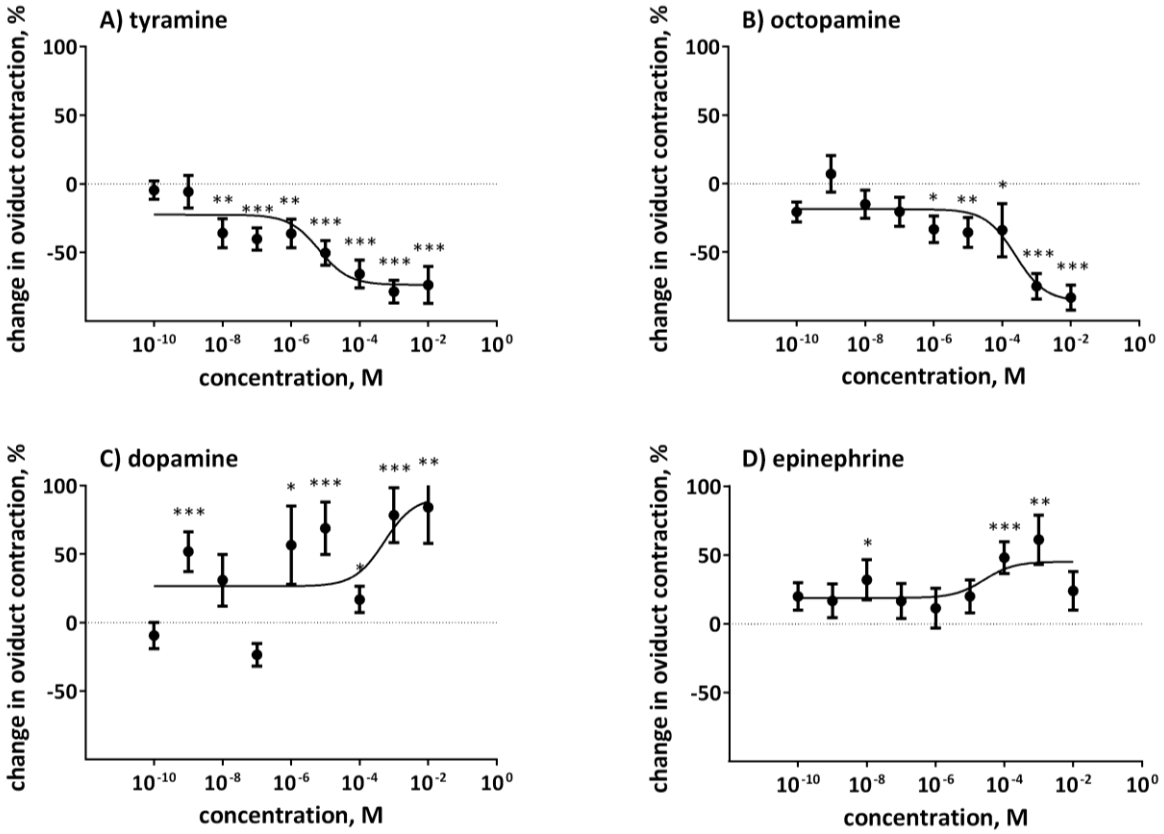

Figure 4. Dose-response curves for the effect of tyramine (**A**), octopamine (**B**), dopamine (**C**) and epinephrine (**D**) on the contractions frequency of *T. molitor* oviduct *in vitro*. Results are expressed as mean of $n \geq 12$ replicates ± SEM. Significant differences $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) from control (saline buffer) are indicated by asterisks (Mann-Whitney test).

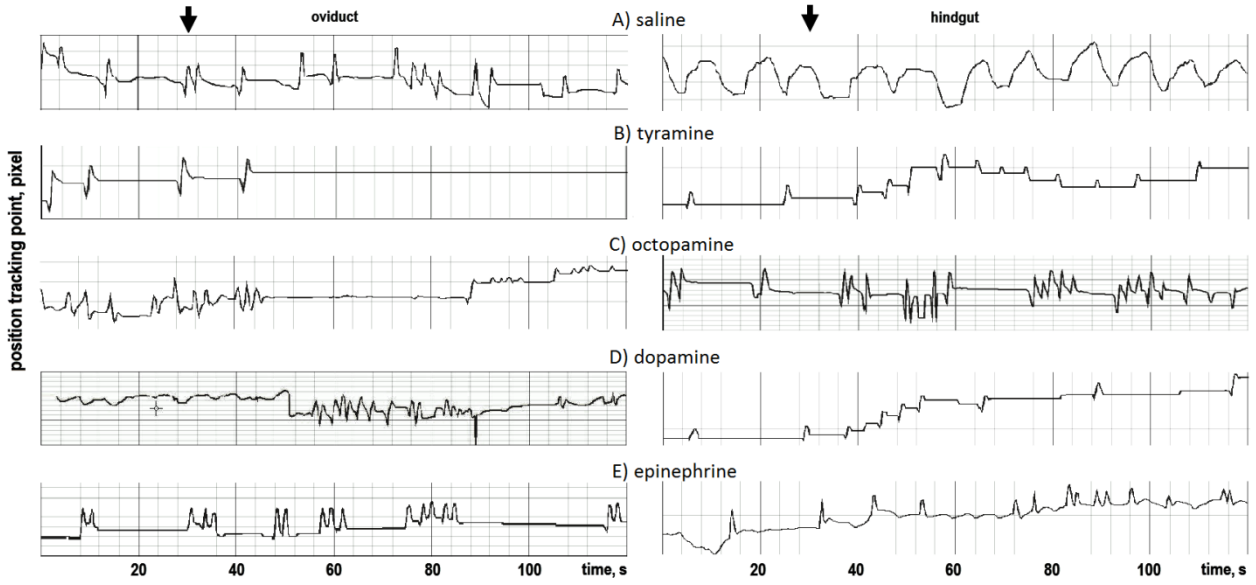

Figure 5. Myograms presenting the effects of the application of saline (**A**), tyramine (**B**), octopamine (**C**), dopamine (**D**), and epinephrine (**E**) at a concentration 10^{-5} M on the oviduct and hindgut of *T. molitor* adults. The arrows indicate the application of the compounds.

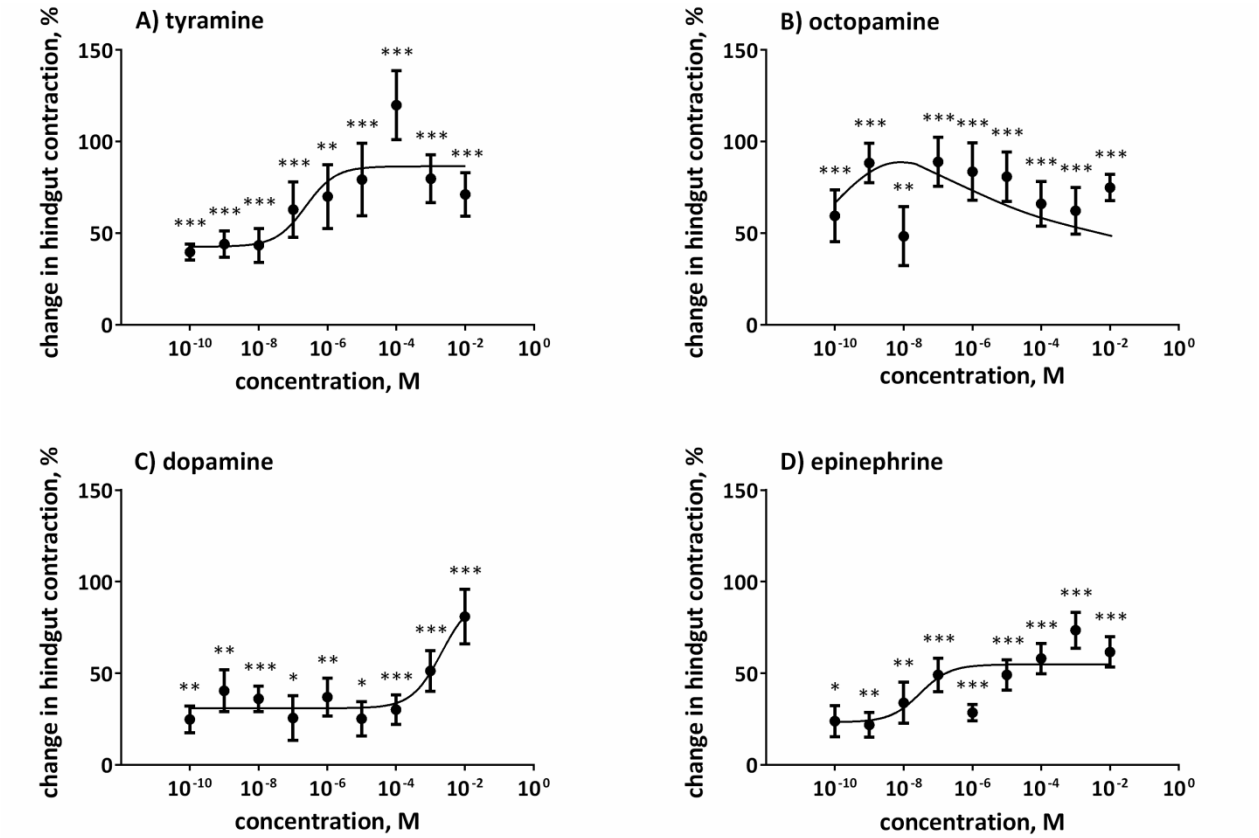

Figure 6. Dose-response curves for the effect of tyramine (**A**), octopamine (**B**), dopamine (**C**) and epinephrine (**D**) on the contractions frequency of *T. molitor* hindgut *in vitro*. Results are expressed as mean of $n \geq 14$ replicates ± SEM. Significant differences $p \leq 0.05$ (*), $p \leq 0.01$ (**), $p \leq 0.001$ (***) from control (saline buffer) are indicated by asterisks (Mann-Whitney test).

activity of visceral organs is regulated in a complex manner by nervous and neurohormonal system (Orchard and Lange, 1988).

The studies carried out by our group characterized the myotropic properties of four biogenic amines, two trace amines (tyramine and octopamine) and two catecholamines (dopamine and epinephrine) in *T. molitor* beetle. The results showed that tested compounds have differentiated and organ-specific myotropic properties and modulate in varied mode the endogenic muscle contractions of heart, oviduct and hindgut.

Cardiotropic action of biogenic amines

This study was mainly focused on determination of influence of amines tested on *T. molitor* beetle heart and comparison of their activity *in vitro* and *in vivo* conditions. Our experiments demonstrated cardioinhibitory properties of octopamine and tyramine in studied beetle, both *in vivo* and *in vitro* assays. *In vivo* these amines caused decreasing of pupa heart rate both in anti-and orthodromic phase, what consists with results obtained by Zornik *et al.* (1999). The difference in time of maximal response after application of amines in ortho- and antidromic phase may be a result of difference in innervation of posterior and anterior heart segments, and regulation of heart activity in both phases partially by various nerves (Miller, 1997). The effects caused by trace amines on heart may be a result of activation of octopaminergic/tyraminergic receptors present in beetle heart. El-Kholy *et al.* (2015) confirmed expression of tyramine receptor (TyrR) in *D. melanogaster* heart. Nevertheless, there is no evidence of octopamine receptors in insect heart, but their presence was confirmed in heart of other invertebrate for example in bivalve *Crassostrea virginica* Gmelin (Mollusca Bivalvia) (Bess *et al.*, 2015). Direct influence of amines on heart muscles seems to be confirmed by the results obtained *in vitro* experiments in which we also observed strong cardioinhibitory properties of octopamine and tyramine. Interesting is the prolonged negative chronotropic effect noted *in vivo* in case of these two amines which maintained up to 22nd hour after injection. Nevertheless, it still may be the direct influence of amines on myocardium cells. The half-life of octopamine in insect haemolymph is about 15 min (Adamo and Baker, 2011), so after 6 h after injection the concentration in haemolymph is still on level over 10^{-13} M (when larvae are injected with concentration 10^{-4} M). On the other hand, it may be indirect effect by influence on neurosecretory structures. For example, excitatory effect of cardioacceleratory peptide 2 (CAP2) was markedly enhanced by the presence of subthreshold concentrations of octopamine (Prier *et al.*, 1994). Moreover, octopamine mediates release of neurohormones from retrocerebral complex. For example, it stimulates the secretion of adipokinetic hormone from *corpora cardiaca in vitro* in

locust (Pannabecker and Orchard, 1986) and stimulates juvenile hormone biosynthesis in *corpora allata* of honey bee (Kaatz *et al.*, 1994). Socha *et al.* (2008) showed, that injection of dopamine increase the level of adipokinetic hormone in *P. apterus* haemolymph. What is more, cardioactivity of adipokinetic hormone was shown in such insect species like *T. molitor*, *P. americana*, *D. melanogaster* and *Baculum extradentatum* (Brunner von Wattenwyl) (Phasmatodea Phasmatidae) (Chowański *et al.*, 2016). Moreover, Johnstone and Cooper (2006) suggest that the activity of octopamine as well as other biogenic amines can be a result of changes in neurons innervated insect heart.

The negative chronotropic effect in case of tyramine is definitely stronger than effect caused by octopamine. The IC_{50} for octopamine is 1.5-times higher than IC_{50} value for tyramine which reflecting higher potency of tyramine to inhibition of heart contractility. Bayliss *et al.* (2013) summarized that tyramine can activate both tyramine and octopamine receptors but these second with lower efficiency. This suggests that inhibition of muscle contraction by tyramine is enhanced by activation also the octopamine receptors (if both classes are presented in myocardium). In *T. molitor*, we observed only a depressive effect of octopamine on contractions frequency but not on amplitude. However, at the highest concentration tested (10^{-2} M) the response of heart to octopamine and tyramine was definitely lesser than changes evoked by lower concentration. This effect was especially strong in case of tyramine. The reason of that can be an internalization of tyramine/octopamine receptor(s) and/or presence in myocardium receptors with different sensitivity to these amines. As showed Bayliss *et al*. (2013), tyramine can cause an internalization of tyramine receptors. So during the test, application of increasing concentration of tyramine could cause changes in populations of particular receptors in myocardium. Moreover, Papaefthimiou and Theophilidis (2011) have shown a biphasic effect of octopamine on heart activity of honey bee *in vitro* depending on the concentration of this compound. A high concentration of it increased the heart contractions frequency, but low concentration caused opposite effect. Similar to our results, this amine affected only frequency of heart contractions but did not influence on amplitude.

The dopamine cardioactivity is not clear. Our experiments indicate that dopamine has a weak cardioactivity in *T. molitor.* It slightly depressed pupa myocardium activity *in vivo* whereas in adult beetle caused slight acceleration of heart rate but only at high concentrations (10^{-3}-10^{-2} M). Titlow *et al*. (2013) showed that this amine increases heart rate in *D. melanogaster* larva while data obtained by Zornik *et al*. (1999) indicate that it is inactive in larva but inhibits pupal heart rate and accelerates the heart in adult what confirms our results. On the other hand, experiments carried out by Johnson *et al*. (1997) showed that dopamine increases the frequency of heart contractions in pupa of *D. melanogaster*. Cardioaccelatory properties of dopamine were observed also in adult cockroach *P. americana* (Collins and Miller, 1977). The opposite effect *in vivo* and *in vitro* condition may result from the interference of dopamine with other cardioregualtory agents *in vivo*. Only epinephrine did not change heart activity significantly, what suggests lack of receptors sensitive for epinephrine in *T. molitor* myocardium.

Effects of biogenic amines on oviduct contractility

Insect oviduct after isolation remains capable to spontaneous and rhythmic contractions, what indicates the myogenic source of contractile activity (Lange and Orchard, 1983; Orchard and Lange, 1988). The activity of this organ is controlled both *via* neural and neurohormonal manner. In case of locust *L. migratoria*, the oviduct is innervated by neurons from seventh ganglion of ventral cord which play an important role in generating the pattern and coordination of oviduct contractions (Kalogianni, 1992). These neurons contain neuroactive chemicals such as proctolin, SchistoFLRFamide, octopamine and glutamate (Lange, 2002). Moreover, the oviduct of *P. americana* (Orchard and Lange, 1987a) and *L. migratoria* contains octopamine (Orchard and Lange, 1987b) and tyramine (Donini and Lange, 2004) what indicates that these compounds may also act as a local modulators. These two biogenic amines seem to be one of the crucial agents involved in regulation of oviduct contractile pattern.

Our data showed that octopamine and tyramine decrease the frequency of *T. molitor* oviduct contraction what is consistent with effect caused by these amines in *L. migratoria* (Lange and Orchard, 1986; Donini and Lange, 2004; Lange and Nykamp, 1996; Lange and Tsang, 1993) and fly *S. calcitrans* (Cook and Wagner, 1992). It was confirmed by Lim *et al*. (2014) that *D. melanogaster* oviduct epithelial cells express the octopamine receptors (OAβ2R). The expression of various classes of octopamine and octopamine/tyramine receptors was also confirmed in *Nicrophorus vespilloides* Herbst (Coleoptera Silphidae) (Cunningham *et al.*, 2015), *Trichoplusia ni* (Hubner) (Lepidoptera Noctuidae), *Pseudaletia unipuncta* (Haworth) (Lepidoptera Noctuidae) and *Pieris rapae* (L.) (Lepidoptera Pieridae) (Lam *et al.*, 2013). It suggests that observed effects are results of activation the aminergic receptors. Similar to myocardium, tyramine shows higher potency to inhibit muscle contraction than octopamine. Our experiments showed that effects caused by both trace amines are dose-dependent and in the threshold for tyramine is lower (10^{-8} M) than for octopamine (10^{-6} M) but the maximal responses evoked by both amines are similar (in concentrations tested). Additionally, the IC_{50} for octopamine is approximately 37-times higher than for tyramine. As was shown by Donini and Lange (2004), tyramine, except increasing the frequency of contractions, also increases the amplitude of excitatory junction potentials (EJPs) of locust oviduct muscle cells at low concentrations (10^{-9}-10^{-8} M), therefore, in the oviducts tyramine at low concentrations may lead to increased excitability of the muscle cells. The explanation of higher activity of tyramine may be, as was said previously, activation by tyramine both, tyramine and octopamine receptors.

Participation in regulation of oviduct contractility was also showed for dopamine and epinephrine. However,

these catecholamines influence on oviduct contractility in the opposite way like octopamine and tyramine. Similar effects induced by dopamine was shown in *G. bimaculatus* (Raabe, 1989) and in *M. sexta* (Coast and Webster, 1998). Interesting is fact that epinephrine, which is analogue of trace amines (Roeder, 2005), causes opposite effect to them. It would be expected that epinephrine similar to octopamine and tyramine will act as myoinhibitor on oviduct muscles. Nevertheless, the activity of epinephrine is definitely weaker than in case of octopamine and tyramine. In the literature, there is no evidence how epinephrine influences on oviduct contractility in other insect species and no information about presence in insects receptors for epinephrine, what suggests that effects caused by epinephrine are nonspecific.

Myotropic action of biogenic amines on hindgut

The nature of insect gut contractility is myogenic. Nevertheless, the functioning of this organ is regulated by stomatogastric nervous system and by the lateral nerves from caudal ganglion of ventral cord (Copenhaver, 2007).

All amines tested showed strong myostimulatory properties on *T. molitor* hindgut and increased the frequency of hindgut contractions. Tyramine and octopamine evoked the opposite effects on hindgut to these observed on heart and oviduct and thereby indicate tissue-specific activity. The reason for that may be presence of different types of aminergic-like receptors in studied tissues or different ratio between particular receptors. Presence of β-adrenergic octopamine receptors in midgut was confirmed in *Bactrocera dorsalis* (Hendel) (Diptera Tephritidae) (Li *et al.*, 2016) and *Chilo suppressalis* (Walker) (Lepidoptera Crambidae) (Wu *et al.*, 2014). Interesting is the fact that epinephrine which had no activity on heart and affected oviduct in very slight manner acts on hindgut with high potency. Acceleration of hindgut contraction by this amine suggests presence of receptors sensitive to this amine in the muscle cells, however there are no available data about expression of epinephrine receptors in insects. Nevertheless, it cannot be excluded that receptors for octopamine and tyramine are activated by epinephrine, similarly like octopamine receptors can be activated by tyramine but with low efficiency (Bayliss *et al.*, 2013). Generally, effects caused by amines tested on insect hindgut are not equivocal. In *L. migratoria*, octopamine at low concentration increased slightly tonus, while at high concentration (10^{-5} M) it increased contraction amplitude at the same time decreasing frequency (Huddart and Oldfield, 1982). In *S. gregaria*, this amine leads to lowering of hindgut contraction frequency (Osborne *et al.*, 1990) and depresses the neurally induced contractions of *P. americana* hindgut (Brown, 1975) which is an opposite effect to this observed in *T. molitor*, but in mosquito *A. aegypti* octopamine (Messer and Brown, 1995) acts in the same way as in the studied beetle species. Differences are also observed in case of tyramine, which decreases frequency of hindgut contractions in *L. migratoria* (Huddart and Oldfield, 1982) but increases it in *T. molitor.*

In conclusion, biogenic amines modulate in different organ-specific mode the endogenic contractile activity of heart, oviduct and hindgut in *T. molitor*. The results suggest the presence of adrenergic receptors in these organs for amine tested and indicate the potential ability for these amines to regulate an endogenous mechanism generating muscle contraction. The new knowledge about participation of aminergic system in regulation of insect physiology may be useful in designing new agents for pest control.

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