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PHARMACOLOGICAL ANALYSIS OF LOCOMOTION AND HEART CONTRACTION DURING THE DEVELOPMENT OF *HELISOMA* (MOLLUSCA: GASTROPODA)*

SHORT COMMUNICATION

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We investigated involvement of different 5-HT receptors in regulation of ciliary rotation, gliding locomotion and heartbeat of *Helisoma* embryo at pre- and post-metamorphic stages. Pharmacological analysis suggested that activation of 5-HT_1 receptor enhance ciliary rotation but do not affect gliding locomotion. Activation of 5-HT_4 receptor depresses both types of locomotion. Before metamorphosis heart contraction is depressed by activation of 5-HT_4 and enhanced by activation of 5-HT_7 receptor. However, the heart became insensitive to all agonists by hatching. We hypothesized that alterations in affinity or expression of particular 5-HT receptors can underlie the well-coordinated character of serotonin-dependent larval behavior.

Keywords: 5-HT agonists – ciliary rotation – gliding locomotion – heartbeat

We demonstrated earlier that serotonin (5-HT) released from the apical sensory neurons modulated the development of molluscan larvae [4]. The effect of apical cells activation was found to be bidirectional in the freshwater pulmonate snail, *Lymnaea* and *Helisoma*, and both developmental speed and behaviors such as locomotion, buccal rhythm and heart contraction were affected. While at larval stages prior to metamorphosis these embryonic behaviors were slowed down by 5-HT, at postmetamorphic stages the same programs were enhanced [5], suggesting the activation of different 5-HT receptors at various developmental stages. Nevertheless, the type of the involved receptors cannot be even hypothesized. At the present work we applied pharmacological analysis to investigate the involvement of 5-HT_1, 5-HT_4 and 5-HT_7 receptors in the modulation of two distinct types of locomotion (ciliary rotation and gliding) and the heart contraction during three crucial phases of embryogenesis: before metamorphosis, during metamorphosis and after metamorphosis.

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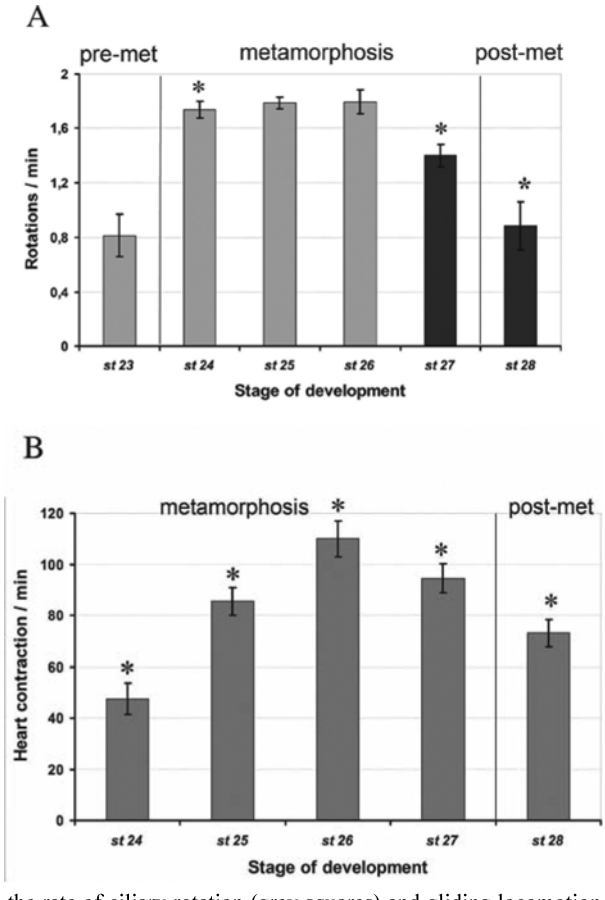

Fig. 1. Changes in the rate of ciliary rotation (grey squares) and gliding locomotion (black squares) (A) and in the heart beat frequency (B) during normal *Helisoma* development. Pre-met – premetamorphic developmental period, post-met – postmetamorphic developmental period. * Statistically significant differences from data at previous stage, $p < 0.005$. See full descriptions in the text

Egg masses were obtained from laboratory reared population of *Helisoma trivolvis*. Adult animals and egg masses were maintained as previously described [4]. Embryos were staged according to Mescheriakov [3] and Voronezhskaya et al. [4]. Videorecordings at premetamorphic (st 23), metamorphic (st 24–27) and postmetamorphic (st 28) stages were made during normal development and after incubations with various agonists. The following drugs (all from Sigma) were used: RS 67 333 – potent and highly selective 5-HT4 agonist; 8-OH-DPAT – the standard selective 5-HT_{1A} agonist, also has moderate affinity for 5-HT_7 receptors; 5-CT – 5-HT_1 agonist with high affinity at 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_5 and 5-HT_7 receptors; S-WAY 100135 – potent, selective 5-HT_{1A} receptor antagonist. At least 30 animals were examined for each treatment.

During normal development ciliary rotation gradually increased from the trochophore to metamorphic stage and gliding locomotion slightly gradually decreased after metamorphosis (Fig. 1A). 5-CT (5 μM) significantly (up to 2 folds) increased ciliary rotation but had no affect on gliding locomotion after metamorphosis. 8-OH-DPAT (5 μM) had no effect on locomotion at all stages examined. RS 67333 (2.5 μM) dramatically (by 2–3 folds) repressed both ciliary rotation and gliding locomotion.

Heart started to contract at the beginning of metamorphosis (st 24) during normal *Helisoma* development. The frequency of the rhythm increased by twofolds by the end of metamorphosis and then it gradually decreased (Fig. 1B). Effects of chronic application of RS 67333 and 8-OH-DPAT were concentration-dependent. While 5 μM progressively depressed the frequency, 1 μM had no effect and 0.1 μM enhanced frequency. Thus, 1 μM was selected for accurate applications at chosen developmental stages. The results obtained are shown in Table 1.

Our pharmacological analysis demonstrated that at least three types of 5-HT receptors: 5-HT_1, 5-HT_4 and 5-HT_7 are involved in the regulation of embryonic behavior in *Helisoma*. Previously, two 5-HT receptors (5-HT_{1Hel} and 5-HT_{7Hel}) have been cloned by Mapara et al., and their expression has been demonstrated in *Helisoma* embryo [2], however their function remained unclear. Goldberg et al. [1] hypothesized that *Helisoma* receptors underlying cilio-excitation are most similar to those of the 5-HT_1 and 5-$HT_{2/1C}$ receptor families. Based on the obtained results we suggest that 5-HT_1 receptor is involved in the activation of ciliary rotation but not in the regulation of gliding locomotion. Activation of 5-HT_4 receptor depresses both type of ciliary locomotion throughout entire development. At the initial stages of differentiation heart contraction is depressed by activation of 5-HT_4 receptor and enhanced by activation of 5-HT_7 receptor. However, the heart became insensitive to activation of all three tested receptors by hatching. Altogether our results indicated that either the affinity or the expression of a particular 5-HT receptor is changed at different developmental stage. Such alterations can underlay the well-coordinated character of 5-HT-dependent behavior throughout the embryonic development of freshwater molluscs.

Table 1

Effects of 5-HT agonists on heart contraction frequency at different developmental stages

5-HT agonist-antagonist	Stage of development				
	Metamorphosis				Post-Met
	st 24	st 25	st 26	st 27	st 28
RS67333 1 μM	68±6*	47±7*	70±7*	95±8	99±7
8-OH-DPAT 1 μM	94±9	92±9	88±9*	94±8	103±6
(S)-WAY 1 μM	108±7	107±7	112±6*	116±6*	104±6
8-OH-DPAT+(S)-WAY	111±9	128±7*	125±6*	108±9	109±7

Expressed as percentage from control ± standart deviation.

*Statistically significant difference from control, $p<0.005$.

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