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THE EFFECT OF 5-HTP ON THE REPRODUCTIVE TIMER IN THE MALE CRICKET*

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The post-copulatory sexually refractory stage in the male cricket *Gryllus bimaculatus* consists of the two substages: the first refractory stage (RS1, time-variable) between copulation and spermatophore preparation, and the second refractory stage (RS2, time-constant) between spermatophore preparation and the recommencement of courtship. To understand the mechanism of the timer for RS2, subcuticular or intraganglionic injection of biogenic amines (10^{-2} mol l^{-1}) was performed immediately after spermatophore preparation. RS2 was shortened by octopamine, 5-HT, 5-HTP and NA-5-HT. Among these, 5-HTP was most potent. It shortened RS2 to maximally about 38% of the control. The shortening effect continued for 4.5 h after subcuticular injection even when the hemolymph was washed out with saline at 1 hour after injection. Simultaneous injection of 5-HTP with the inhibitor (NSD-1015) of 5-HT synthesis enzyme nullified the effect of 5-HTP, indicating that the shortening effect was caused by 5-HT synthesized from extrinsic 5-HTP. Injection of the inhibitor (CHX) of protein synthesis had no effect of on RS2. These results suggest that the reproductive timer in the TAG may be controlled by 5-HT or a second messenger mediated by 5-HT.

Keywords: Male cricket – sexual refractoriness – timer – 5-HTP – 5-HT

INTRODUCTION

The reproductive cycle of the male cricket consists of the pre-copulatory mating stage (MS) and the post-copulatory sexually refractory stage (RS) (Fig. 1). The latter is further divided into the two substages: the first refractory stage (RS1) between copulation and spermatophore preparation, and the second refractory stage (RS2) between spermatophore preparation and the recommencement of courtship. During MS, the male continuously courts the female, singing a calling song and a courtship song, but he becomes aggressive and shows guarding behavior after copulation. It has been established that RS1 is time-variable (about several minutes to more than 1 h), while RS2 is time-constant (about 1 h) in *Gryllus bimaculatus* [1, 2]. We

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showed previously that the reproductive timer for RS2 was accelerated at higher temperature and decelerated at lower temperature, and stopped below 10 °C. Furthermore, the timer was found to be located in the terminal abdominal ganglion (TAG) because the functional inactivation of the TAG by local cooling (10 °C) lengthened RS2 in proportion to cooling time [3].

In this report, biogenic amines, 5-HT synthesis inhibitor and protein synthesis inhibitor were examined whether they may change RS2. Some of the data presented in this study have been already published [4].

MATERIALS AND METHODS

Animals

Crickets, Gryllus bimaculatus DeGeer. Males and females were used 10–15 days after final molt. Males were separated from females at least for 24 h before use to facilitate copulation.

Behavioral test and RS2 intervals

Males, which become sexually active, emit a calling song (CS) and show a mating response (MR) to a female model about 1 h after spermatophore preparation (SP). RS2 was measured by two methods: the interval (SPMR) between SP and MR, and the interval (SPCS) between SP and CS.

Drugs

Drugs were dissolved in saline at 10^{-2} M. Amines used were DL-octopamine (OA), 5-hydroxytryptophan (5-HTP), 5-hydroxytryptamine (5-HT), N-acetyl-5-hydroxytryptamine (NA-5-HT). 5-HT synthesis inhibitor (NSD-1015) and protein synthesis inhibitor (CHX, cycloheximide) were also used at 10^{-2} M.

Drug injection

For subcuticular injection, an amine solution (0.15 ml) was injected into the hemocoel with a syringe soon after spermatophore preparation (Fig. 1). The volume injected was about 1.5 times as much as the volume of the hemolymph. For intraganglionic injection, an amine solution (180 nl) was injected by pressure with a microinjection device into the terminal abdominal ganglion (TAG).

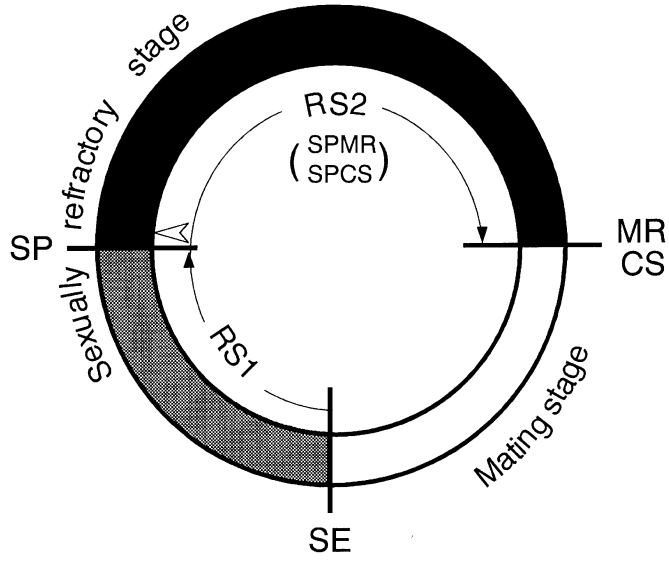

Fig. 1. The reproductive cycle of the male cricket. The white arrow head by SP indicates the timing of drug injection

Perfusion of the hemocoel

To wash out the injected drug from the hemocoel, saline (0.2 ml) was injected into the hemocoel through a syringe penetrated into the thorax and ejected through two small holes made on the abdomen.

RESULTS AND DISCUSSION

RS2 interval and temperature

RS measured by both MR and CS methods (27 ± 1°C) were comparable. The mean and standard deviations (41 animals) were 59 ± 3 min (min 49-max 72) for SPMR, and 60 ± 4 min (min 51-max 73) for SPCS. The individual variation was maximally about 20 min. Intra-individual variation of RS2 in 5 successive reproductive cycles was less than 1–2 min in 27% of the males tested. For the effect of temperature, the change rate of RS2 increased linearly at higher temperature and decreased at lower temperature (Fig. 2). The Q_{10} was nearly 2.0 for SPMR and SPCS. This is essentially different from 1.0 for the circadian rhythm.

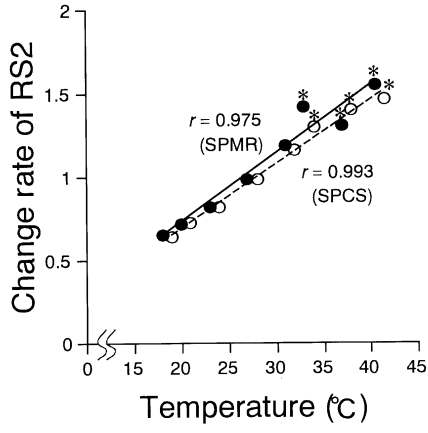

Fig. 2. Temperature dependency of RS2. Each symbol shows the change rate of the mean values of SPMR (black circle and solid line) and SPCS (white circle and dotted line) at different temperature (the rate at room temperature (27 °C) is 1). r, coefficient of correlation. Symbols with asterisk show the data in which males were exposed to higher temperature on the abdomen for the initial 30 min under restraint and then returned to the beaker containing a female. The rates with an asterisk were calculated by the following equation: [control RS2 – (RS2-30)]/30

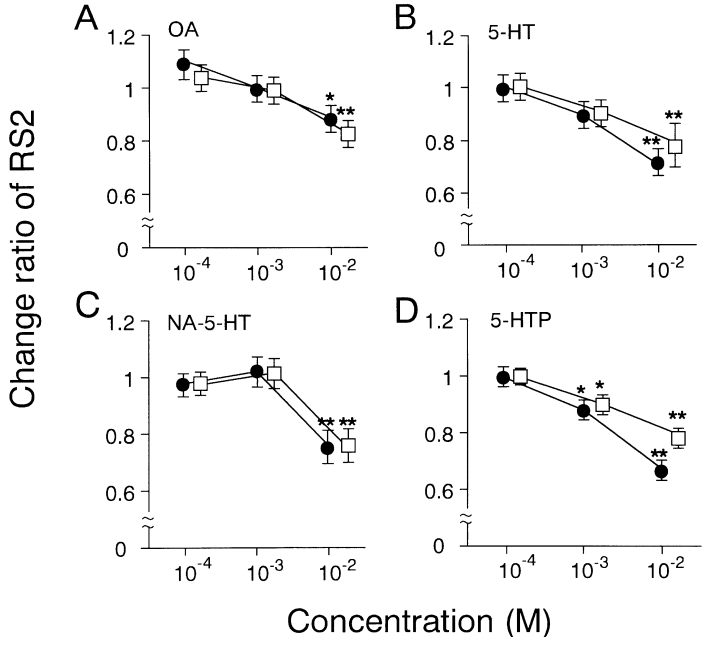

Fig. 3. Effects of amines at different concentrations on RS2. Abscissa, concentration; ordinate, the change ratio of RS2 by four amines (A–D). Dark circle shows SPMR and white square shows SPCS. Vertical bar, 95% confidence interval. Significance level indicated by asterisks: *P < 0.05; **P < 0.01

Effects of amines at different concentrations on RS2

To show the effect of amines on RS2, the change ratio was calculated by dividing a treatment RS2 by the RS2 in the non-injected cycle. The change ratios of OA, 5-HT, NA-5-HT and 5-HTP were 0.88, 0.67, 0.75 and 0.67 for SPMR, and 0.83, 0.80, 0.79 and 0.79 for SPCS (Fig. 3). These four amines significantly decreased RS2 by approximately 20%.

Duration of amine effects

To examine the duration of effectiveness of amines, RS2 was measured at least two cycles after injection. The shortening effect of amines (OA, 5-HT and NA-5HT) disappeared in the next cycle (about 1 h later) following the injected cycle. In contrast, 5-HTP caused a prolonged shortening of both SPMR and SPCS over 5 reproductive cycles after injection (nearly 4.5 h). The shortest RS2 occurred in the next cycle following the injected cycle in which the SPMR was 38% (23 min) of the control (Fig. 4A). Perfusion of the hemolymph with saline did not eliminate the effect of 5-HTP which was injected 1 hour before perfusion (Fig. 4B).

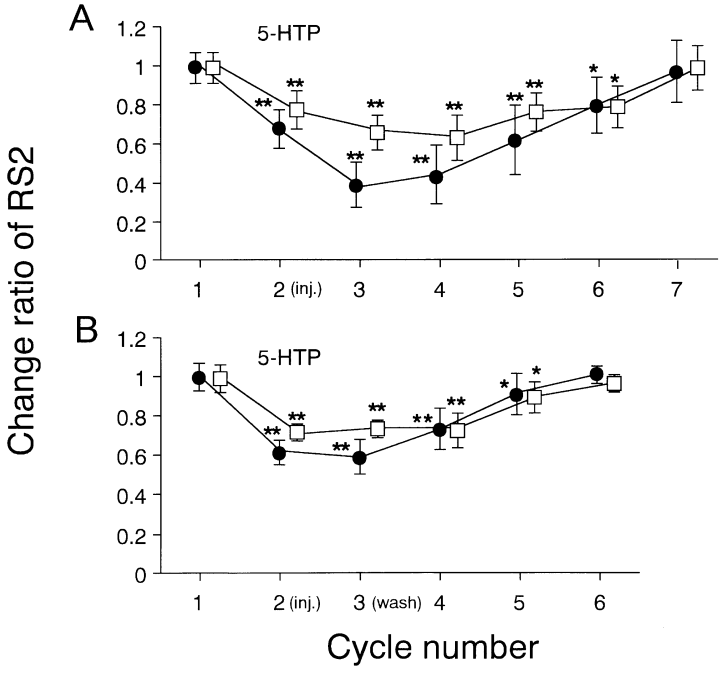

Fig. 4. Duration of the effect of 5-HTP on RS2. A: Subcuticular injection of 5-HTP soon after spermatophore preparation in the second cycle. B: The same as A but the hemolymph was washed out soon after spermatophore preparation in the third cycle

The effect of NSD-1015

To examine whether RS2 was due to the effect of 5-HTP itself or to its metabolite (5-HT) synthesized after being uptaken into serotonergic neurons, NSD-1015 was injected into the hemocoel with 5-HTP (Fig. 5). The results indicated that NSD-1015 significantly reduced the effect of 5-HTP on the RS2 shortening. This suggests that the shortening is due to 5-HT newly synthesized from extrinsic 5-HTP.

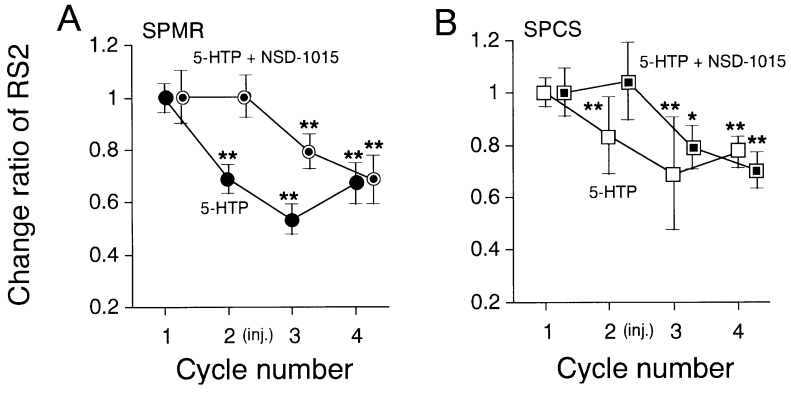

Fig. 5. The effect of 5-HT synthesis enzyme inhibitor NSD-1015 on RS2 shortening by 5-HTP. A: RS2 (SPMR) measured by MR. Filled circles, 5-HTP alone; double circles, 5-HTP with NSD-1015. B: RS2 (SPCS) measured by CS. Open squares, 5-HTP alone; double squares, 5-HTP with NSD-1015

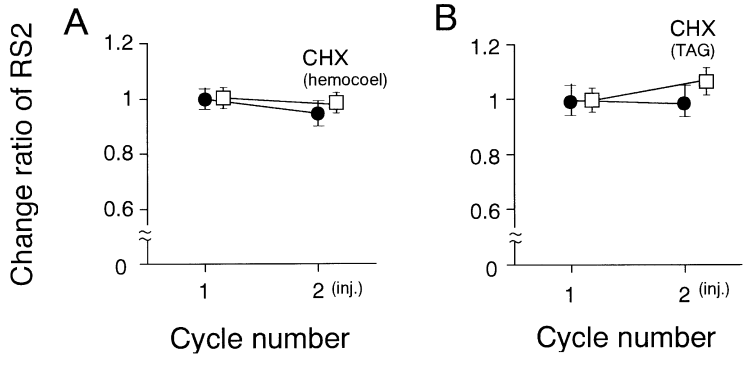

Fig. 6. The effect of cycloheximide (CHX) on RS2. Subcuticular injection (A) and intraganglionic injection (B) of CHX. Note that neither SPMR nor SPCS were significantly changed

The effect of CHX

To examine whether the shortening of RS2 results from proteins synthesized via stimulation of 5-HT or from 5-HTP itself, CHX was injected into the hemocoel soon after spermatophore preparation. The change ratios of RS2 were 0.95 for SPMR and 0.99 for SPCS, which were not significantly different from those in the first non-injected cycle (Fig. 6). For intraganglionic injection of CHX, SPMR was 0.98 and SPCS was 1.07. No difference was present between those and controls. These results revealed that protein synthesis is not required for the reproductive timer to keep time.

These results suggest that the reproductive timer may be regulated by 5-HT and a second messenger in the TAG without protein synthesis during post-copulatory time-fixed sexually refractory stage.

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