

Influence of Phenybut on Regulation of Daily Rhythm of Adult Eclosion in *Trichogramma embryophagum* Htg. (Hymenoptera, Trichogrammatidae)

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Abstract—Phenybut is an agonist of GABA_b receptors. Adults of *Trichogramma embryophagum* Htg. were fed with 0.5–20% solution of phenybut in 50% sugar solution. The experiments demonstrated that adult mortality during 48 h increased significantly with phenybut concentration. Thus, a dose-dependent response was revealed by enteral application of a GABA_b receptor agonist. When *T. embryophagum* females were fed with 1% solution of phenybut in 50% sugar solution, significant changes in the daily eclosion pattern in their progeny were revealed. In particular, more intensive eclosion was observed under development at the photoperiod L : D = 12 : 12 and anticipatory (after a 2-h long scotophase) light-on in the day when eclosion was recorded. The increased sensitivity to light may be due to the phenybut effect on the serotonergic system, which controls daily rhythms.

The role of beneficial insects in human life is well known. Speaking of laboratory and commercial rearing of insects, one usually means the entomophagous species, either predators or parasitoids (Monastyrskii and Gorbatovskii, 1991). At the same time, the morphological, physiological, and ecological diversity of insects combined with relatively easy and inexpensive culturing make them unique model objects for various researches. *Drosophila melanogaster* Mg. has become an irreplaceable test object for geneticists, and the cockroach *Periplaneta americana* L., for physiologists. Experimental results obtained in many other insect species, such as the blowfly *Calliphora vicina* R.-D. (Vinogradova, 1984), were used to draw conclusions concerning not only arthropods in general but other animals as well.

Since the same mediators and neurotransmitters (monoamines, γ -aminobutyric acid, acetylcholine, glycine, etc.) work in the nervous system of insects and vertebrates, including man (Hill *et al.*, 1981; Kerkut, 1985; Pitman, 1985; Neal, 1997; Bettler *et al.*, 1998; Aydar and Beadle, 1999), model insect species can be used to study a number of pharmaceutical substances affecting synaptic conduction.

This study is focused on phenybut, a phenyl derivative of γ -aminobutyric acid (GABA) which is an inhibitory transmitter in the nervous system of verte-

brates and invertebrates. GABA acts on three types of receptors: GABA_a, GABA_b, and GABA_c. In clinical practice, GABA and its derivatives are widely used as central myorelaxants, and also for treating sleep disturbance and psychostimulant dependence (*Pharmaceutical Register of Russia*, 2002).

Phenybut is an agonist of GABA_b receptors located in presynaptic terminals of excitatory and inhibitory neurons. Stimulation of GABA_b receptors in excitatory neurons with phenybut inhibits neurotransmitter release into the synaptic cleft and thus blocks the excitatory signal. In inhibitory neurons, the action of phenybut decreases the release of GABA in the synaptic cleft, reducing the inhibitory effect. Thus, phenybut has a dual influence on the inhibitory processes in the nervous system: on the one hand, it blocks the release of excitatory mediators, reducing the responses, and on the other hand, it partly blocks the inhibitory action of GABA, enhancing the same responses. The prevalence of one of the two opposite effects (i.e., the “sign” of the resulting effect) can depend on the response specificity as well as dosage (MacDemott *et al.*, 1999).

The model object for our study was the egg parasitoid *Trichogramma embryophagum* Htg. Representatives of this genus are used successfully as biological agents of pest control and also as model objects in

various experiments. Adult wasps under natural conditions have a carbohydrate diet, feeding on various plant fluids; besides this, they can consume the droplets released from the punctured chorion of the host egg, which also contain carbohydrates (Sander *et al.*, 1984). Numerous experiments of different workers (Ashley and Gonzalez, 1974; Hohmann *et al.*, 1988; Leatemala *et al.*, 1995; Hegazi and Khafagi, 1998) clearly showed that survival rates and fecundity of *Trichogramma* females in laboratory increased drastically when they were given various kinds of carbohydrate food, both natural (honey, nectar, plant sap) and artificial (solutions of sucrose, glucose, fructose, etc.). Addition of proteinic food (plant pollen, bee royal jelly, meat or yeast hydrolysate) did not increase the longevity and number of laid eggs as compared to the control individuals which were given diluted honey (Hegazi and Khafagi, 1998) or even clear water (Ashley and Gonzalez, 1974). However, since insects (unlike vertebrates) have almost no peptidases, they can absorb unmodified peptides through the intestine wall (Turunen, 1985). This allows one to use enteral application by adding the substance to the sugar food.

The process under study was the daily pattern of eclosion of adult wasps from the host egg chorion. In most *Trichogramma* species kept in a specific photoperiodic regime, the peak of eclosion is observed shortly before or after the light is switched on (Reznik *et al.*, 1998; Zaslavskii *et al.*, 1999). A considerable amount of data on the mechanisms controlling the daily eclosion pattern in *Trichogramma* has been accumulated (see Discussion).

The aim of this study was to test the technique for enteral phenybut application and to study its effect on the daily eclosion pattern of *T. embryophagum*.

MATERIALS AND METHODS

In the preliminary experiment, we determined the mortality of *Trichogramma* in relation to the concentration of phenybut in the sugar food. Freshly eclosed adults were randomly divided into vials on the walls of which were placed several drops of phenybut solution in 50% sugar solution; the control individuals were offered 50% sugar solution without phenybut. The experiments were conducted under constant photoperiodic conditions L:D = 18:6 and at 20°. Adult mortality was determined 48 h after the beginning of experiment. Each concentration of phenybut was tested in two trials including no less than 100 individuals each. Since a certain level of mortality (about 10%) was

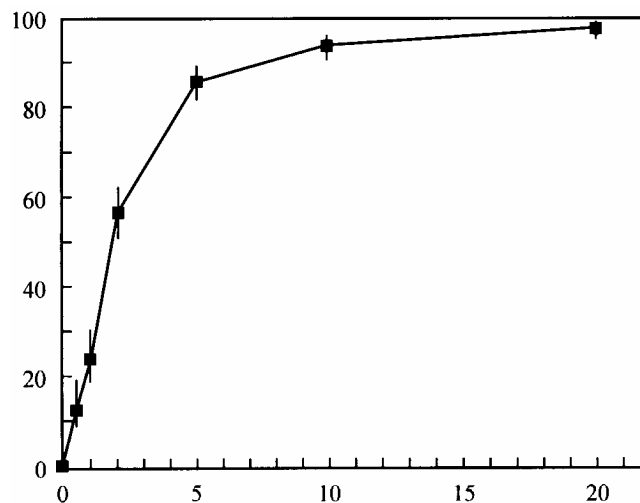


Fig. 1. Effect on phenybut concentration in the sugar food on adult mortality in *Trichogramma embryophagum*. Abscissa, phenybut concentration (%); ordinate, fraction of individuals that died during 48 h since the beginning of experiment (% and 0.95 confidence intervals).

observed in the control as well, the data were transformed by the formula $Y = (X - C)/(1 - C)$, where X is adult mortality during 48 h in the presence of phenybut, and C is mortality in the control batches. The transformed data (percentages and confidence intervals) are shown in Fig. 1.

To characterize the effect of phenybut on the daily eclosion pattern, *Trichogramma* females during the first 24 h after eclosion were offered 1% phenybut solution in 50% sugar solution; the control individuals were offered 50% sugar solution. The adult eclosion pattern was studied in the progeny of the experimental and control insects, which developed under standardized conditions L:D = 12:12 and 20°. In the first variant, these conditions were constant during the whole experiment, and in the second variant light was switched on after a 2-h scotophase. The experiment comprised a total of 16 trials. The eclosed adults were counted every 2 hours. The relative amounts of insects which eclosed by the end of each 2-h period are shown in Fig. 2. The significance of differences was evaluated using Fisher test for f-transformed data (Ivanter and Korosov, 1992).

RESULTS

As can be seen from Fig. 1, adult mortality depends on the phenybut concentration in the sugar food. The LD₅₀ with the above experimental design was about 2%.

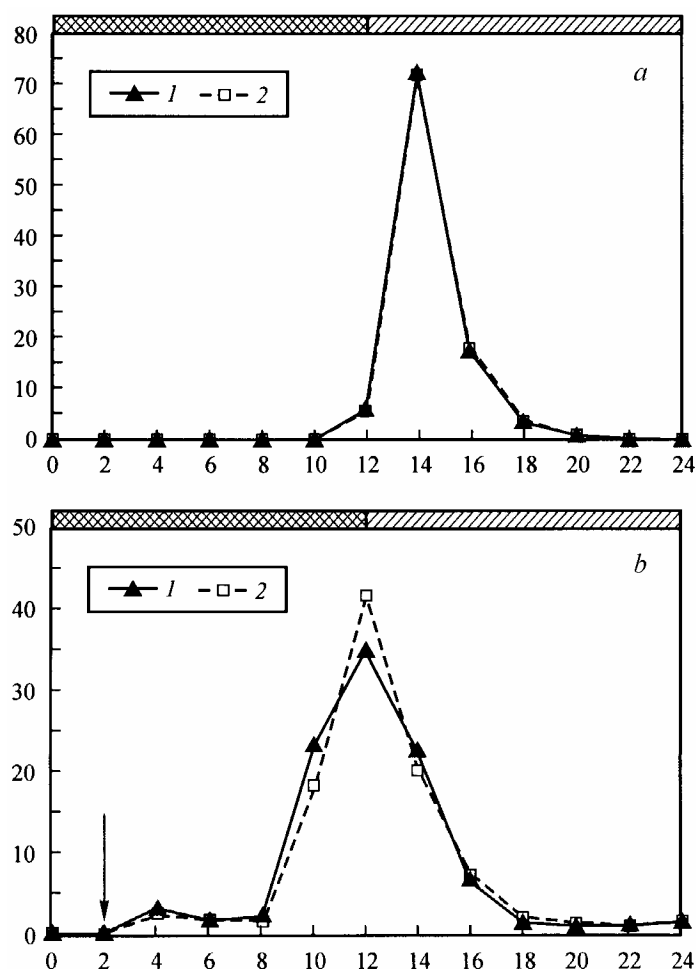


Fig. 2. Pattern of eclosion of *Trichogramma embryophagum* adults from the host egg chorion. Abscissa, time (h); ordinate, fraction of adults which emerged during the corresponding 2-h interval. In variant (a) the photoperiod L:D = 12:12 was constant during the whole experiment (the position of scoto- and photophase is marked with dark and light bars above the graph, respectively); in variant (b) on the day of recording the light was switched on prematurely (arrow). The maternal generation was fed with 1% phenybut solution in 50% sugar (1) or with 50% sugar (control) (2).

As concerns the daily eclosion pattern, in the first variant of the experiment (when both larval development and adult eclosion proceeded at L : D = 12 : 12) the eclosion peak was observed 2 h after switching on the light, with practically no difference from the control (Fig. 2a). In the variant with premature "light-on" (Fig. 2b) there was some difference between the experimental and control individuals. The progeny of females treated with phenybut showed more intensive early eclosion: the fraction of adults which emerged during the first 2 h of the peak eclosion period (from 8 till 10 h through the experiment) was significantly greater ($F = 11.2$, $p < 0.01$), and the peak intensity (from 10 till 12 h), significantly lower ($F = 14.2$, $p < 0.01$) than the corresponding parameters for the progeny of control females. Thus, phenybut modified the daily eclosion pattern in the filial generation but

did not change the position of the peak and the general rhythm.

DISCUSSION

The curve shown in Fig. 1 demonstrates a dosage effect of enteral phenybut application. Therefore, intestinal absorption of phenybut (and possibly other amino acids and monoamines) depends on its concentration in food, and its effect may thus be quantified.

Our experiment (Fig. 2) revealed a significant influence of phenybut on the daily eclosion pattern in the progeny of experimental females. The fact that the effect was observed only in the variant with premature "light-on" may be explained by the following traits of the rhythm control in insects, and particularly in *Trichogramma*.

The daily activity pattern in insects is known to be controlled by endogenous rhythmic processes, usually termed the "biological clock." In addition, changes in illumination or temperature can directly increase or reduce activity, producing the so-called "exogenous effects" (Saunders, 1982). For example, switching on the light can stimulate eclosion of *Trichogramma* from host eggs at almost any time of day (Zaslavskii *et al.*, 1999).

Readiness for eclosion in *T. embryophagum* was previously shown to change in a 24-h cycle under the control of an endogenous circadian rhythm (Karpova and Reznik, 2002). This parameter is the lowest during early "night" and reaches its maximum by the end of scotophase (i.e., by the 12th hour at L : D = 12 : 12). Switching on the light at this time triggers mass emergence of adult wasps from host eggs. The high degree of endogenous readiness for eclosion obviously leveled the fine difference between the experimental and control individuals in the variant with a 12-h scotophase (Fig. 2a).

In order to study the response to this factor against the background of a lower sensitivity, experiments with premature switching on the light were conducted (Fig. 2b). After a short period of darkness, light causes a very weak response with an 8-h delay, allowing a significant difference between the experimental and control individuals to be detected. In our opinion, the more intensive eclosion within the interval from 8 till 10 h in the progeny of females treated with phenybut indicates an increase in photosensitivity induced by this neurotransmitter.

It is well known that the effect of light on the mechanisms controlling the daily rhythms in both invertebrates and vertebrates is to a considerable extent regulated by the serotonergic system (Takahashi *et al.*, 1989; Morin and Blanchard, 1991; Proseer *et al.*, 1993). Injection of serotonin in insects reduces photosensitivity of neurons in the visual system (Tomioka *et al.*, 1993; Kloppenburg and Erber, 1995; Tomioka, 1999). The action of methergoline (a non-specific serotonin antagonist) on the optic lobes of crickets caused an opposite effect, i.e., increased neuronal reactivity (Saifullah and Tomioka, 2002). Blocking of serotonin synthesis in crickets with a neurotoxin was shown to enhance their behavioral response to switching on the light (Germ and Tomioka, 1998).

The increased light sensitivity of *Trichogramma* under the influence of phenybut may also be explained

by inhibition of serotonergic conduction, since stimulation of GABA_b receptors is known to inhibit monoaminergic conduction in neuronal nets, including those employing serotonin as a postsynaptic mediator (Neal, 1997).

Of special interest, in our opinion, is the very possibility of using insects to study the fine mechanisms of action of a number of psychotropic drugs (agonists and antagonists of dopamine, serotonin, catecholamines, and GABA receptors) by enteral application.

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