passing currents across the membrane and by changing the concentration of potassium in the external solution. As shown in figure 2, the light-evoked potential became larger with depolarizing shifts, and smaller with hyperpolarizing shifts of the membrane potential. In normal (10 mM K^+) saline, the polarity of the light response was reversed at membrane potentials more negative than about −72 mV (fig. 2B). This reversal potential (−72 mV) in the normal saline was increased to approximately −86 mV when the external potassium concentration was halved (fig. 2A), and was decreased to approximately −62 mV when it was increased to 15 mM (fig. 2C). In figure 3, the results obtained from five experiments of this type are presented in graphic form, and the slope of shifts in reversal potential for a 4-fold change in external potassium shows 27 mV. This value reached roughly 80% of the theoretical slope value, 35 mV/4-fold change in external K^+, predicted by the Nernst equation.

Various ionic substitutions were made to test the contribution of other ions to the light response. Substitution of isethionate for external Cl^- had little effect on either the resting potential or the light response. Reduction of external Na^+ to 50 mM caused the resting potential to be hyperpolarized by 10–15 mV and reduced the amplitude of the light response. However, membrane polarization to the original resting potential by passing depolarizing currents returned the light response to the original level, indicating that reduction of the response by low Na^+ is due to passive hyperpolarization of the membrane. In addition, replacement of 10 mM Ca^{2+} with 20 mM Mg^{2+} did not effect the response.

These results suggest that the depolarizing receptor potential of A-P-1 is mainly produced by a decrease in K^+ conductance. In *Aplysia* extraocular photoreceptors, the light-evoked increase in K^+ conductance is elicited most effectively with 470–490 nm light[6,8]. Thus, it is interesting that a similar spectral sensitivity to light is associated with changes in K^+ conductance of opposite sign between *Onchidium* and *Aplysia* photoreceptors. In conclusion, this study is the first demonstration that a sensory receptor potential can be produced by a selective decrease in membrane K^+ conductance. This also shows that the photoresponse associated with a decrease in membrane conductance is not unique to the vertebrate photoreceptor.

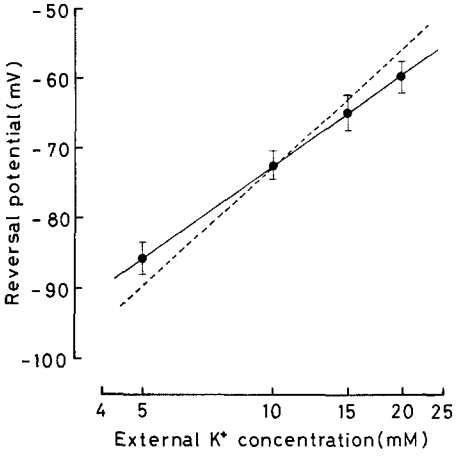

Figure 3. Relationship between the log of external potassium concentration and the reversal potential for the photoresponse of A-P-1. Each point was averaged from five A-P-1 neurones, with the SE of the mean shown by bars. The solid line drawn through each point shows 27 mV slope for 4-fold change in external potassium, and this slope value reached roughly 80% of the theoretical slope value (35 mV, dotted line) predicted by the Nernst equation.

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Cholinergic control of extracardiac pulsations in insects

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Summary. The autonomic nerve system which controls extracardiac pulsations of hemolymph pressure appears to be quite susceptible to cholinesterase inhibitors (physostigmine, organophosphorus insecticides). In pupae of *Tenebrio molitor* L. and *Galleria mellonella* L., the treatment induced large peaks in hemolymph pressure (over 4 kPa). The peaks were repeated at more-or-less constant, 30-s intervals for several hours or days. Removal of the pupal brain neither affected extracardiac pulsations nor influenced the specific responses to the investigated drugs or insecticides.

Key words. Autonomic nerve system; anticholinesterase; physostigmine; epinephrine; acetylcholine; hemolymph pressure; *Tenebrio molitor; Galleria mellonella.*

The immobile developmental stages of insects exhibit occasionally rhythmic pulsations in mechanical pressure of the hemolymph[1]. The pulsations are produced by telescopic contractions of flexible abdominal segments. The control over frequency and amplitude of these extracardiac pulsations is achieved by an autonomic nerve system located in the thoracic ganglia of the ventral nerve cord[2]. According to certain physiological criteria (involvement in osmotic regulations and water balance, increased ventilation of respiratory gases, enhanced circulation of hemolymph through the appendages), the system can be considered analogous with the autonomic parasympathetic nerve system of the vertebrates. In this work I have used the extracardiac pulsations in pupae of the mealworm (*Tenebrio molitor* L.) and of greater wax-moth (*Galleria mellonella* L.) as a model system

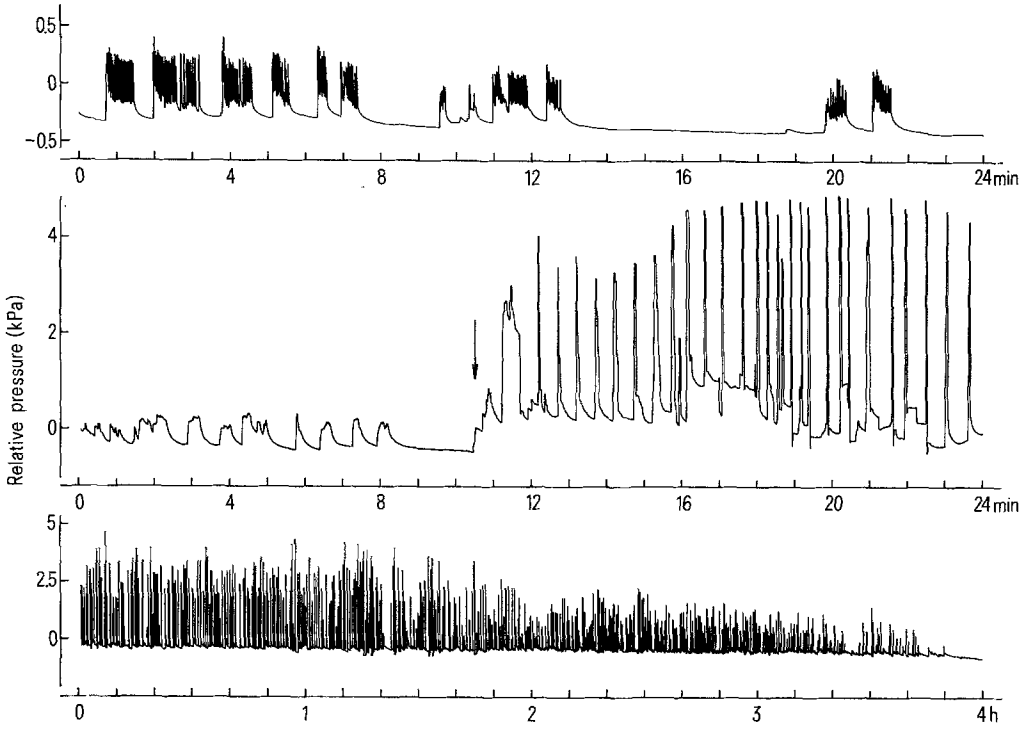

Figure 1. The effect of physostigmine on hemolymph pressure pulsations in a 4-day-old pupa of *Tenebrio molitor*, at 24 °C. Upper record shows one broken series of the pulsations, approx. 140–120 min. before injection of 1 $\mu g \cdot g^{-1}$ of the drug (see arrow). Lower record shows large peaks in hemolymph pressure, 16–20 h after the injection, followed by death.

for investigations concerning the action of some sympathomimetic or sympatholytic drugs on this autonomic nerve system of insects.

The mechanical pressure of the hemocoel was recorded by the previously described methods[3,4]. The solutions to be tested (2-µl amounts) were sucked into the measuring needle of the hydraulic transducer before insertion of the needle into the hemocoel. The solution was isolated on both sides by a 1 µl droplet of an inert mineral oil. After the initial 2 or 3 h of control recording of hemolymph pressure, the solution was expelled into the body cavity together with the outer droplet of oil. The recordings then continued for 5 h (in the case of physostigmine and organophosphates up to several days). Each concentration was tested on at least five specimens. Epinephrine (Spofa, Czechoslovakia) was used in the doses of 1 µg and 10 $\mu g \cdot g^{-1}$ of b.wt; acetylcholine (VEB, Berlin Chemie) at 10 µg and 100 $\mu g \cdot g^{-1}$ and physostigmine (Merck, Darmstadt) at 0.1 µg, 1.0 µg and 10 $\mu g \cdot g^{-1}$, unless otherwise stated. The values for hemolymph pressure are

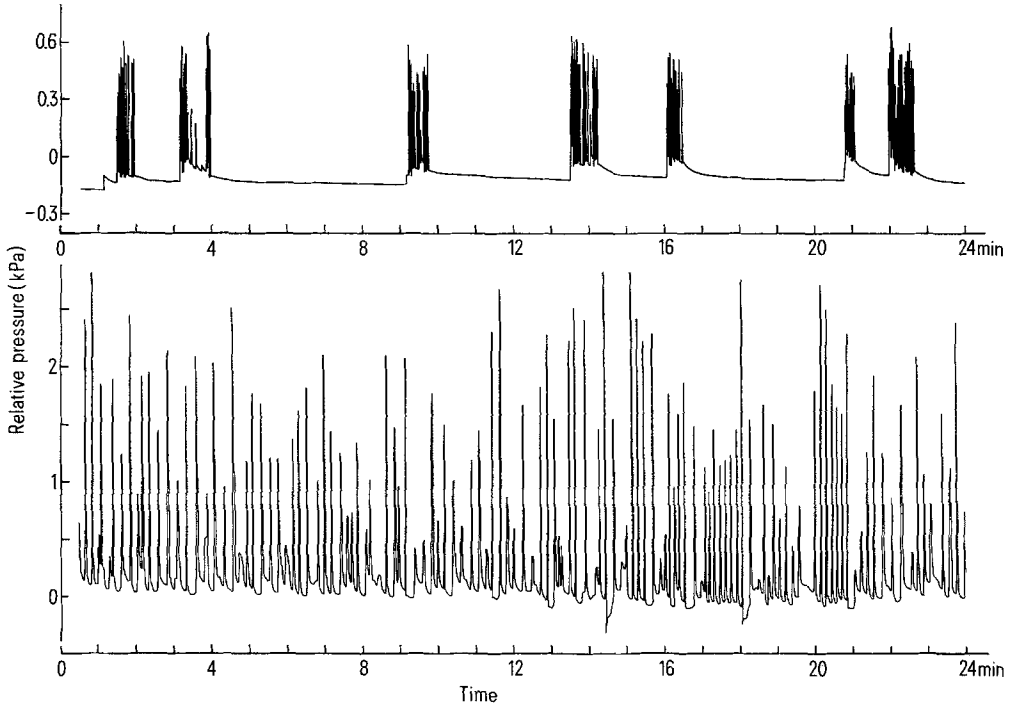

Figure 2. Hemolymph pressure changes in the decerebrated, 4-day-old pupa of *Tenebrio*, approx. 1 h before (upper trace) or after (lower trace) the injection of 1 µg·g−1 of physostigmine. Decerebration was made shortly after pupal ecdysis, followed by 4 days of storage at 27.5 °C; recordings made at 25 °C.

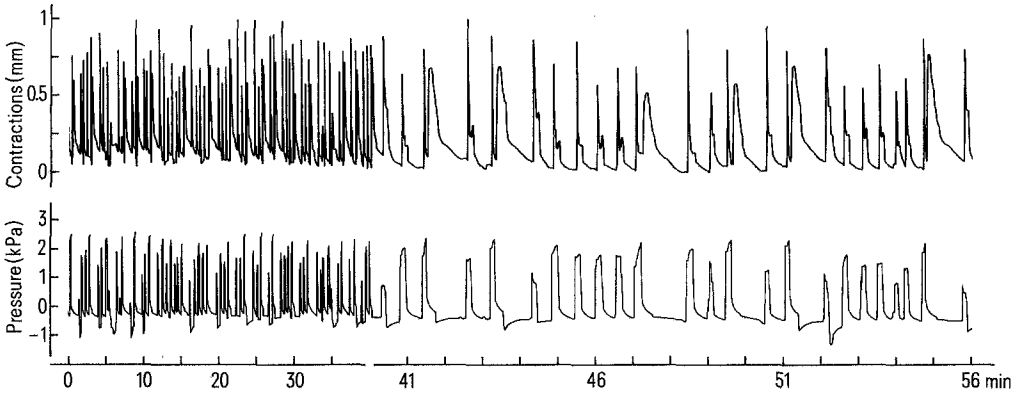

Figure 3. Large peaks in hemolymph pressure and convulsive contractions of the abdomen after injection of 0.2 $\mu g \cdot g^{-1}$ of physostigmine into 5-day-old pupa of *Galleria mellonella*, at 25 °C. The relative recording time corresponds to the period between 5 and 6 h after the injection. The abdominal contractions (upper trace) were recorded by the indirect contact method simultaneously with the signal from the hydraulic transducer (lower trace).

expressed in Pascal (Pa) units relative to the local atmospheric (zero) pressure. The data obtained by the recently invented indirect method[4] denote mechanical contractions of the distal abdominal segment.

In *Tenebrio*, most of the records were made with 4-day-old pupae (reared at 27 °C). This stage is characterized by subatmospheric hemolymph pressure, commonly −200 to −500 Pa[1,2]. The extracardiac pulsations of these pupae are grouped into special series, which are interrupted by more or less constant (30–40 min at 25 °C) periods of relative rest. One of these pulsation series is shown by the upper record in figure 1. The injections of epinephrine and acetylcholine were quite ineffective. They did not disturb the established pattern of extracardiac pulsations, at least during the initial 5 h of the continuously monitored post-treatment period. However, this lack of any remarkable response in hemolymph pressure does not prove unequivocally that the nervous system of the extracardiac pulsations is indeed insensitive to epinephrine and acetylcholine. The general problem with exogenous application of these neurochemical compounds is that they might be broken down, or cannot penetrate to the intracellular sites (for more details see Jones[5]; Bořkovec and Kelly[6]). In contrast to the above data, the injections of 0.1 $\mu g \cdot g^{-1}$ or more of physostigmine (eserine) induced almost immediate and quite dramatic responses in hemolymph pressure. The records in figure 1 show rapid paralysis of all coordinated extracardiac pulsations. Visual observations revealed apparent symptoms of intoxication manifested by irritation-like movements, vibrations of the abdomen and convulsive spasms of all flexible abdominal segments. Each convulsion was associated with a large, positive peak in hemolymph pressure, which occasionally surpassed 4 kPa (fig. 1). The peaks continued at rather regular 30-s intervals for several hours or days, depending on the dose. The physiological consequences of such extreme oscillations in hemolymph pressure can best be described as hyperventilation and desiccation of the body, progressive muscular exhaustion and death of the animal. Additional studies revealed that virtually identical responses of the hemolymph pressure of these immobile stages could be obtained by topical treatments with 10 µg of various organophosphate (paraoxon, malathion, phosphothion) or carbamate (lannate) insecticides. This suggests that the pharmacological nature of all these changes in hemolymph pressure depends on an inhibition of cholinesterase in the responsible nerve system.

The autonomic nerve system of the extracardiac pulsations in *Tenebrio* appears to be the sole somatic neuromuscular system that is fully functional throughout the immobile metamorphosis stage[1,3]. The oscillations in mechanical pressure of the body are caused by genuine contractions of the intersegmental muscles between the flexible abdominal segments[4]. The nervous command does not come from the brain but from the thoracic ganglia of the ventral nerve cord[2]. According to these data, such an autonomic nerve system should obviously function unimpaired even in complete absence of the central nervous system. Thus, experimental evidence that the statement is true has been provided in figure 2, which shows one of the well-coordinated series of the extracardiac pulsations in a decerebrated pupa. In addition, the lower record of figure 2 documents quite clearly that the effects of physostigmine (as well as those of the other inhibitors of cholinesterase) can also occur in the absence of the brain.

Further studies have revealed that the described action of physostigmine on hemolymph pressure may be widely distributed among the pupal stages of other, taxonomically unrelated insect species. This may be documented, for instance, by the records obtained in *Galleria* (fig. 3). Here, just as in *Tenebrio*, the injection of physostigmine induced large convulsive peaks in hemolymph pressure, with 30-s rest intervals and a duration of several days. It appears, therefore, that the common cholinergic transmission of the nerve impulses may be added to the number of similarities between the autonomic nerve system of insects and the autonomic parasympathetic nerve system of the vertebrates. It is also hoped that the demonstration of enormous changes in hemolymph pressure (associated with hyperventilation and increased water loss) after anticholinesterase treatment might perhaps contribute to the final elucidation of the still incompletely known[7] mode of action of these drugs and insecticides.

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