mine the mechanism of action of BE. The change of the endogenous AVP level in the amygdala may reflect an increased release of AVP from the terminals into the neuropil and a subsequent degradation or dissipation of the peptide. Since AVP-containing perikarya have been demonstrated in the amygdala [16], it is also possible that the change of its AVP concentration occurs as a consequence of the decreased AVP synthesis in the perikarya. It is difficult to explain the action of the s.c. treatment with Nal on the AVP content in the septum. The dose of Nal used in this study corresponded to that applied in previous experiments [1], which showed that Nal injected s.c. prevents the BE-induced plasma AVP alterations. It might be hypothesized that in the septum the AVP-ergic system is under the influence of an endogenous opiate tone, which is inhibited by Nal administration, but that BE itself in this dose is not able to alter the opiate tone. Nal has also been reported to inferfere with neurotransmitter systems [17, 18]; these effects may play a role in the observed changes of the AVP levels in the septum.

In conclusion, the present data favor the idea that BE and Nal affect the central AVP-ergic system in a regiospecific manner, without any alterations in the activity of the OXT-ergic system. However, it should also be kept in mind that brain areas toward which OXT-ergic neurons project – like the brainstem – have not been tested here.

Acknowledgments. Thanks are due to Prof. Tj. B. Van Wimersma Greidanus (Utrecht, The Netherlands) for supplying the OXT antibody, and to Dr H. M. Greven (Organon, Oss, The Netherlands) for supplying BE. The skillful technical assistance of Miss Györgyi Vorindán, Miss Klára Ádok and Miss Katalin Antal is appreciated.

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0014-4754/89/050472-03$1.50 + 0.20/0

Cycles of juvenile hormone esterase activity during the juvenile hormone-driven cycles of oxygen consumption in pupal diapause of flesh flies

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Received 23 November 1988; accepted 2 February 1989

Summary. During diapause O_2 consumption in fly pupae is a cyclic event (4-day periodicity at 25 °C) driven by cycles of juvenile hormone activity. Levels of juvenile hormone esterase activity change systematically during the cycle, with highest activity observed at the nadir of the O_2 consumption cycle.
Key words. Diapause; *Sarcophaga*; juvenile hormone esterase; O_2 consumption cycles.

Flesh flies [2] and some other insects [3] do not consume oxygen at a constant rate during pupal diapause. A cyclic pattern is observed with peak days of O_2 consumption occurring with a periodicity of about 4 days in the flesh fly *Sarcophaga crassipalpis* at 25 °C. Several lines of evidence suggest that these cycles of O_2 consumption are driven by cycles of juvenile hormone (JH): the JH titer rises progressively during the cycle [4], the cycle can be altered by application of JH analog [4], and surgical extirpation of the corpora allata (the source of JH) destroys the cyclic pattern of O_2 consumption [5]. Hemolymph JH is commonly metabolized by ester hydrolysis [6], and in this study we further examine the relationship between the O_2 consumption cycle and JH by measuring JH esterase (JHE) activity during a cycle of O_2 consumption.

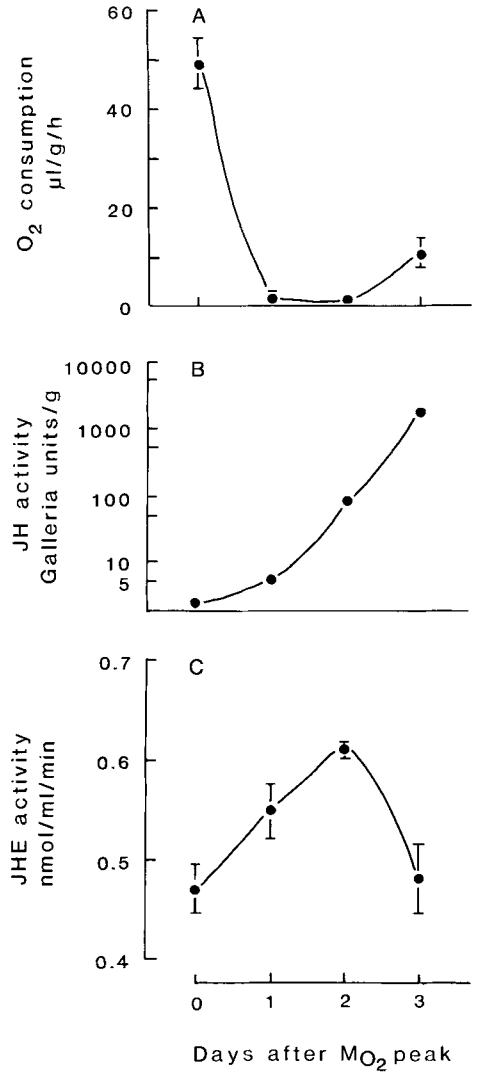
Materials and methods. Pupal diapause in *Sarcophaga crassipalpis* was induced by rearing adults at 25 ± 1 °C, 12L:12D (light:dark cycle) and their progeny at

20 ± 0.5 °C, 12L:12D [7]. Oxygen consumption of individual pupae was monitored at 25 °C using a Scholander respirometer (Mark Co., Brockton, MA). Diapausing pupae, 10–30 days after pupariation, were transferred from 20 °C to 25 °C at least two days before making the initial recording. Pupae were kept in the respirometer continuously and manometric measurements were recorded at 24-h intervals. Each pupa was monitored for 1 complete 4-day cycle before sampling JHE activity at different days of the infradian cycle.

Hemolymph-esterase activity was determined by the partition method of Hammock and Sparks [8]. Hemolymph was collected from the heads of diapausing pupae by first centrifuging the pupae (heads up) in a clinical centrifuge for 3 min at 5000 rpm to facilitate collection of a hemolymph sample free of fat body. 10 µl hemolymph from each pupa was mixed with an equal volume of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.01% phenylthiourea, and stored at −70 °C until used. The sample was thawed, centrifuged at 10,000 rpm for 2 min (Eppendorf, model 5414), and aliquots were removed for assay. The supernatant was further diluted with 0.1 M potassium phosphate buffer. Labelled (^{3}H-JH III, 12 Ci/mmol, New England Nuclear) plus unlabelled hormone (JH III, Sigma Chemical) in ethanol (1 µl 5×10^{-4} M) was added as a substrate to 100 µl of hemolymph dilution. 1 µl of the substrate contained about 18,000 dpm. Plasma dilutions and incubation time were chosen so that the initial enzyme activity was in the linear portion and the specific activity of the enzyme could be determined. Reactions were allowed to continue for 10 min at 30 °C and terminated with 50 µl ammoniacal methanol followed by 250 µl of isooctane. The mixture was vortexed and centrifuged, and 50 µl of the aqueous layer was removed and mixed with scintillation cocktail for counting. Each sample was assayed in triplicate.

Results and discussion. The infradian cycles of O_2 consumption during pupal diapause in flesh flies (fig. A) have a periodicity of about 4 days at 25 °C. As described [2], pupae consume O_2 at a rate of about 50 µl/g/h on the peak day, during the next 2 days O_2 consumption is barely detectable, and then on the third day O_2 consumption again increases as the next peak day is approached. During this 4-day interval the JH titer progressively increases (fig. B), and we have suggested that this rise and fall in JH is the hormonal signal that drives the cycles of O_2 consumption [4, 5].

The rise and rapid decline of JH activity implies that the JH titer is precisely regulated, probably resulting from the additive effects of regulation of JH biosynthesis, JH metabolism in the hemolymph, and the uptake and metabolism by body tissues. Though JH metabolism has not been extensively studied in flies, results from several species indicate that JH can be metabolized by an esterase, epoxide hydrase, and microsomal oxidase [9], as well documented for the Lepidoptera [6]. Activity of JH-metabolizing enzymes during the fly's pupal stage is characteristically low [9], and the levels of JH esterase activity we find in the hemolymph during pupal diapause (fig. C) are likewise much lower than can be detected in other life stages.



The relationship between *A* phases of the oxygen consumption cycle in diapausing pupae of *Sarcophaga crassipalpis* at 25 °C, *B* juvenile hormone activity (from Denlinger et al.[4]), and *C* juvenile hormone esterase activity. Pupae used for determination of O_2 consumption rates were used for determination of juvenile hormone esterase activity, mean ± SE, each N = 5.

The JH esterase activity we detect during diapause clearly changes during the 4-day O_2 consumption cycle (fig. C): activity at mid-cycle is higher than activity during the O_2 consumption peak (Duncan's multiple range test, day 2 > days 0 and 3, $p > 0.05$). The activity pattern is thus a mirror image of the O_2 consumption cycle. Certain events, such as protein synthesis, are restricted to days of peak O_2 consumption [10], but in the case of JHE highest activity is recorded at midcycle. The differences in pattern profiles underscore the dynamic nature of the O_2 cycles and indicate that not all biochemical events are at their lowest level during the nadir of the O_2 cycle. The

drop in JHE activity prior to the O_2 peak (3 days after the previous peak) may contribute to the rise of the JH titer at that time, but changes in JHE activity can not explain the major drop in JH activity on the peak day of cycle. Regulation of JH biosynthesis is a more likely scenario, or possibly other degradative mechanisms involving epoxide hydrase or microsomal oxidase are more important in metabolizing JH in this system.

Why O_2 is consumed in a cyclic fashion during diapause remains unresolved. Possibly periodic episodes of high metabolism are more economic than a sustained lower metabolic rate. That such an episodic event is regulated by a hormone is not unusual, but this appears to represent a novel role for JH, a hormone best known for its roles in regulating growth and reproduction.

1 Supported in part by grant 88-37153-3473 from the USDA Competitive Grants Office and grant 23-088 from the USDA Forest Service. Thanks to Dr Ming Tu Chang for her helpful advice.

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0014-4754/89/050474-03$1.50 + 0.20/0

Temperature compensation in an ultradian rhythm of tyrosine aminotransferase activity in *Euglena gracilis* Klebs

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Received 18 October 1988; accepted 13 January 1989

Summary. Tyrosine aminotransferase activity of *Euglena* oscillates with an ultradian period of approximately 4–5 h. The oscillation frequency in the time series was determined by cosine fitting. Experiments which were performed between 16 and 31.5 °C revealed temperature compensation.
Key words. Euglena; rhythm; temperature compensation; tyrosine aminotransferase; ultradian.

Temperature compensation denotes the ability of biological rhythms to re-adjust period length upon changes of temperature. Therefore, comparisons of the frequency of oscillation which are made at different steady state temperatures reveal Q_{10} values close to 1. This phenomenon is usually regarded as a typical feature of circadian rhythms[1,2]. However, this important property of biological oscillations has occasionally also been detected in infradian and ultradian periodicities[2–5]. With regard to the ultradian cycles, there appear to exist two different classes[2], one of which is highly temperature-dependent, such as the leaf movement rhythm in *Desmodium gyrans*[6], and another which is temperature-compensated. The few examples of the latter class which are known up to now have been documented most clearly in unicellular organisms[2]. Almost all of these cycles exhibit periods in the range of 0.5 to 1 h. In this study, we demonstrate temperature compensation in a longer ultradian oscillation of about 4–5 h.

Material and methods. Euglena gracilis Klebs, strain Z (No. 1224-5/25) was grown autotrophically at 23 °C, in a light-dark cycle (LD 12:12), as described earlier[7]. Stationary cultures were transferred at CT ('circadian time') 23 h to the experimental temperature and were investigated, if not stated otherwise, in constant light (LL). Aliquots of 4 ml were removed after gently agitating the cultures. Cells were homogenized directly in the medium, using a KLN ultrasound generator 281/101, equipped with the sonatrode TU 157/1; samples were sonicated in an ice bath, applying 5 pulses of 10 s at intensity 7, with intermissions of 10 s. Activity of tyrosine aminotransferase (L-tyrosine:2-oxoglutarate aminotransferase; EC 2.6.1.5) was determined in the 18,000 × g supernatant by the method of Diamondstone[8], at an incubation temperature of 32 °C. Protein as reference value was measured according to Lowry et al.[9]. In order to eliminate circadian trends[10] from the time series, data were detrended by means of a moving average. Period length was computed by determining the best-fitting cosine, using a program for least square fitting run on an Atari 520+.

Results and discussion. In figure 1, three examples of ultradian rhythms in tyrosine aminotransferase activity are shown. The curves were obtained at different experimental temperatures covering a range of 13.5 °C. The