feeding period also in diapause conditioned larvae indicates that induction of diapause and prolongated feeding activity is regulated by different titers of JH which, in addition, may act on different and perhaps even independent systems. The fact that both effects serve the same purpose of surviving the adverse conditions of winter does not speak against this hypothesis.

The results also indicate that prolongation of the feeding period can only be induced by JH when the insects have not yet ceased their feeding activity. Obviously a gate, limiting responsiveness of the system to JH, must exist at a certain time ahead of the end of the feeding period. After this gate has been passed neither single nor repeated application of a juvenoid can prolongate feeding. This result suggests that feeding behaviour and development in last instar larvae of *L. pomonella* are closely linked and thus unidirectional, i.e. not reversible like the spinning behaviour of prepupal *Pieris brassicae*[5].

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Prolactin and growth hormone releasing activity of [D-Met^2, Pro^5]-enkephalinamide in the rat after systemic administration

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Summary. The growth hormone (GH) and prolactin releasing (PRL) activity of [D-Met^2, Pro^5]-enkephalinamide ($EKNH_2$), an opioid peptide analog with higher opiate agonist activity that morphine, was compared in the unanesthetized male rat to those of equimolar doses of morphine upon systemic injection. $EKNH_2$ proved to be a higher PRL, but not GH, releaser than the opiate alkaloid.

Recent reports have suggested participation of morphinomimetic compounds, i.e. enkephalins and endorphins, in the control of prolactin (PRL) and growth hormone (GH) secretion in the rat[1-4].

The magnitude of the neuroendocrine and behavioral responses elicited by these peptides appears to be related to their ability to resist enzymatic destruction. Thus, the greater activity of β-endorphin than [Met^5]-enkephalin, both as GH and PRL releaser[5] and in producing analgesia[6], was ascribed to better protection of the labile Tyr-Gly bond of the enkephalin by the remaining part of the peptide chain of β-endorphin, which has the [Met^5]-enkephalin sequence at its N terminus[5]. To obtain enzyme-resistant analogs, both terminals of [Met^5]-enkephalin were blocked with N-methyl and C-amide groups[7] or the Tyr-Gly bond was protected by replacement of glycine by D-alanine in position 2[8,9].

Recently Bajusz and associates[10] have replaced in the enkephalin pentapeptide the methionine^5 moiety by proline-amide and the glycine^2 by D-methionine. The new synthetic derivative obtained [D-Met^2, Pro^5]-enkephalinamide ($EKNH_2$) showed upon systemic, as well as intracerebroventricular (i.v.t.) administration, an higher opiate agonist activity both in vitro and in vivo rat preparations than either β-endorphin or morphine[11].

In the work to be reported, the GH and PRL-releasing activity of this pentapeptide were tested in the unanesthetized male rat following systemic injection and were compared to those of equimolar doses of morphine.

Materials and methods. Male Sprague-Dawley rats (300 g b.wt) were kept at 22±2 °C and exposed to 14 h of light each day (06.00-20.00 h). Laboratory chow and water were available ad libitum. Experiments were started 3 days after the insertion of indwelling jugular cannulae into the right atrium. On the day of the experiment, in order to minimize stress effects, animals were placed in a sound-proof and temperature-controlled room, 2 h before the beginning of blood sampling.

Blood samples (0.3 ml) were taken, at different time intervals (see legend to figures) and were immediately replaced with the same amount of heparinized saline. Plasma GH and PRL levels were determined by a double antibody radioimmunoassay, using the methods of Schalch and Reichlin[12] and Niswender et al.[13] for GH and PRL, respectively. All results were expressed in ng/ml in terms of the NIH standard rat GH-RP-1, whose potency is 0.6 IU/mg and rat PRL RP-1, whose potency is 11 IU/mg. The sensitivity of the GH and PRL assays is 1.0 ng/ml; intra-assay variability was 5% for both GH and PRL while repeated assay of a reference plasma showed an interassay variation, of 12% and 6%, respectively. Doses up to 50 ng/ml of GH and PRL standard did not cross react in the reciprocal assay. To avoid possible interassay variations in each experiment all samples were assayed in a single assay. Significance of differences between groups was calculated by the Student's t-test.

[D-Met^2, Pro^5]-enkephalinamide acetate-trihydrate (mol.wt 726) was synthesized in our laboratories according to a previously described procedure[10]. Morphine hydrochloride (mol.wt 321) was obtained from Carlo Erba, Milan.

Results and discussion. As appears from figure 1, $EKNH_2$ induced a significant PRL rise at 0.2 and 1.0 mg/kg i.v. at 5 and 10 min ($p < 0.001$) and at 5 min, 10 min ($p < 0.005$), 20 min ($p < 0.001$) and 40 min ($p < 0.02$), respectively.

Only the higher dose of morphine (0.5 mg/kg i.v.) was able significantly to increase PRL levels ($p < 0.025$ at 5 and 10 min), while the lower dose (0.1 mg/kg) was completely ineffective in this sense. Analyses of the areas under each 40-min secretory profile by planimetry showed that the PRL releasing activity of each dose of $EKNH_2$ was significantly greater than that evoked by the corresponding dose of morphine (2990±297 vs 1357±410, $p < 0.01$ and

1418±183 vs 780±54, $p < 0.01$). A significant difference was present also between $EKNH_2$ 1.0 mg/kg and morphine 0.5 mg/kg at time 10 ($p < 0.05$), and between $EKNH_2$ 0.2 mg/kg and morphine 0.1 mg/kg at times 5 min and 10 min ($p < 0.001$).

Figure 2 reports the GH data. It has to be noticed that baseline GH levels were markedly different in the animals which received the higher doses of $EKNH_2$ or morphine and in those which were given the opiates at the lower doses, a finding probably attributable to the episodic pattern of GH secretion which characterizes the adult unanesthetized rat[14].

The enkephalin analog induced at 1.0 mg/kg i.v. a significant increase in GH levels at 10 min ($p < 0.001$), 20 min ($p < 0.001$) and 40 min ($p < 0.05$); the GH increase induced by morphine (0.5 mg/kg i.v.) was statistically significant at 20 min ($p < 0.05$) and 40 min ($p < 0.05$). The lower doses of the 2 compounds did not elicit any significant GH rise.

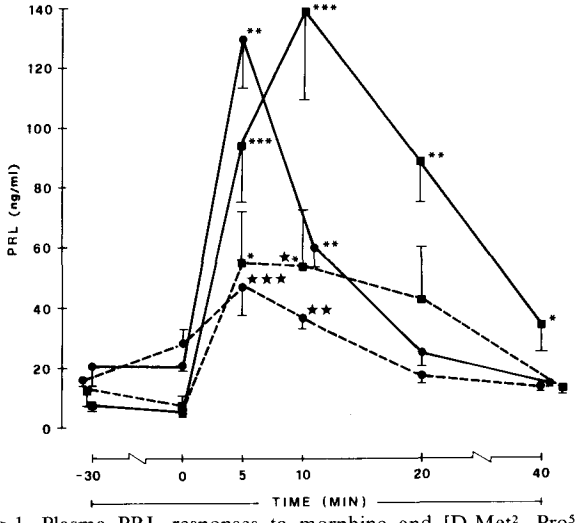

Fig. 1. Plasma PRL responses to morphine and [D-Met², Pro⁵]-enkephalinamide injected i.v. at equimolar doses. Drugs were given at time 0. Values are the mean with SE for each group of 6 animals. *p <0.02; **p <0.001; ***p <0.005 vs baseline. ★p <0.05; ★★p <0.02; ★★★ <0.005 vs corresponding time of the equimolar dose of [D-Met², Pro⁵]-enkephalinamide. ●——● (D-Met², Pro⁵)-enkephalin-NH_2, 0.2 mg/kg; ■——■ (D-Met², Pro⁵)enkephalin-NH_2, 1.0 mg/kg; ●----● Morphine, 0.1 mg/kg; ■----■ Morphine, 0.5 mg/kg.

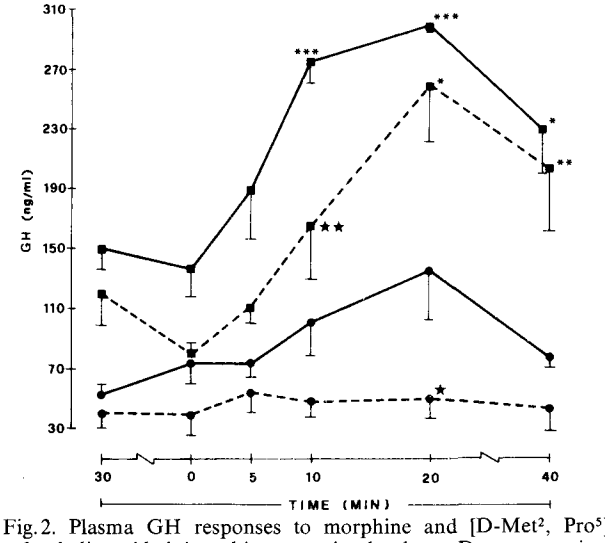

Fig. 2. Plasma GH responses to morphine and [D-Met², Pro⁵]-enkephalinamide injected i.v. at equimolar doses. Drugs were given at time 0. Values are the mean with SE for each group of 6 animals. *p <0.05; **p <0.02; ***p <0.001 vs baseline. ★p <0.05; ★★p <0.02 vs corresponding time of the equimolar dose of [D-Met², Pro⁵]-enkephalinamide.

Evaluation by planimetry of the area under each rGH secretory profile did not reveal the presence of significant differences between equimolar doses of the opiate compounds; a significant difference was present instead between $EKNH_2$ 0.2 mg/kg and morphine 0.1 mg/kg at time 20 min ($p < 0.05$) and between $EKNH_2$ 1.0 mg/kg and morphine 0.5 mg/kg at time 10 ($p < 0.02$).

Collectively, these data demonstrated that the greater analgesic activity of [D-Met², Pro⁵]-enkephalinamide than morphine[10] is coupled with a higher potency of the opiate receptor activator than morphine as PRL releaser. Even a dose as low as 0.2 mg/kg of the enkephalin analog induced by systemic injection a clear-cut plasma PRL rise; interestingly, this dose represents also the ED_{50} of the compound in the tailflick test[11]. An important stimulation of plasma PRL levels has been obtained by Labrie et al.[15] in the rat following i.v. injection of 1.0 mg of D-Ala²-Met⁵-enkephalinamide and by Shaar et al.[16], after injecting 10 mg/kg s.c. of the compound.

Although a trend towards higher GH levels was present in the animals treated with the higher dose of the analog with respect to the rat injected with equimolar doses of morphine, the differences obtained were not statistically significant. In addition, both compounds at the lower dose failed to raise plasma GH levels, while the lower dose of the enkephalin analog increased plasma PRL levels (see above). Thus, in accordance with similar findings of Chihara et al.[5], who injected endorphins, [Met⁵]-enkephalin and morphine into the lateral ventricle of urethane-anesthetized male rats, it appears that the stimulatory threshold for PRL release via activation of opiate receptors is lower than that for GH release. Probably the occurrence and magnitude of the rise in plasma PRL by the opiates is a more reliable indicator of their analgesic and cataleptogenic properties than the rise in plasma GH.

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