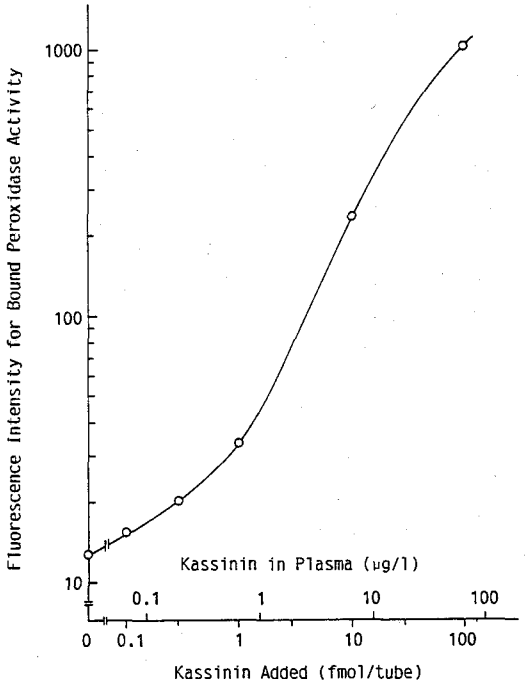

Standard curve for kassinin by the present enzyme immunoassay. Each point is the mean of 5 determinations.

15-fold lower than that obtained by competitive radioimmunoassay. Therefore, the present method appeared to be sufficiently sensitive to measure the level of plasma kassinin, when rats were intravenously injected with 0.3–3.0 µg of kassinin per 100 g of body weight to stimulate the secretion of growth hormone[2]. (The detection limit for kassinin using affinity-purified rabbit anti-kassinin IgG-coated polystyrene balls was 40 fg (30 amol)/tube or 20 ng/l using 2 µl of plasma.)

Plasma kassinin in rats. The levels of plasma kassinin in two rats, measured by the present method, were found to be 0.84 and 0.95 µg/l. However, the exact nature of the substance(s) measured by the present method remains to be investigated.

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The effects of beta-endorphin on arginine-8-vasopressin and oxytocin levels in rat brain areas

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Summary. Measurements were made of the effects of intracerebroventricular treatment with beta-endorphin (BE; 100 ng) on the arginine-8-vasopressin (AVP) and oxytocin contents of rat hypothalamic and limbic brain areas (hippocampus, amygdala and septum). The hormone concentrations were determined by radioimmunoassay. The administration of BE resulted in a significant reduction of the AVP level in the amygdala in a naloxone-reversible manner. Naloxone (Nal) administered subcutaneously significantly increased the AVP content in the septum. The results revealed that BE and Nal had regionally specific effects on the activity of the vasopressinergic system but not on that of the oxytocinergic system in the brain.
Key words. Beta-endorphin; vasopressin; oxytocin; brain regions.

The effects of opioids on the release of neurohypophyseal hormones into the peripheral circulation are well studied [1-3]. Electrophysiological studies indicate that opioid peptides may act on hypothalamic vasopressinergic (AVP-ergic) and oxytocinergic (OXT-ergic) neurons projecting either towards the pituitary or towards other brain areas [4, 5]. Investigations of the effects of endogenous opioid peptides on the vasopressin (AVP) and oxytocin (OXT) levels in the brain may be of importance, since the hypothalamus contains considerable amounts of opioid peptides [6, 7], and these peptides exert a presynaptic-like effect on the axonal endings of the neural lobe [8]. Bicknell and Leng [9] showed that endogenous opiates influence OXT but not AVP secretion from the neurohypophysis. The endogenous AVP and OXT contents of the limbic areas were altered after acute and chronic morphine treatment in mice [10]. Beta-endorphin (BE) has been reported to exert an effect on avoidance behavior [11] in rats. The hypothesis has been put forward that BE interacts with the AVP-ergic or OXT-ergic systems of the brain in mediating the behavioral effects of BE. Hypothalamic and extrahypothalamic AVP and OXT levels have therefore been measured following the intracerebroventricular (i.c.v.) administration of BE.

Materials and methods. Male rats of the CFY strain, weighing 180–220 g, were used. The animals were housed 3 per cage on ad libitum food and water. The animal house was illuminated from 07.00 h to 19.00 h. The i.c.v. treatment was performed through a permanent cannula, which had been placed into the right lateral ventricle of the rat brain under pentobarbitone anesthesia at least 5 days prior to the experiment. Animals were handled daily during the 4 days before the starting of the experiments.

On the day of the experiment, the cannulated animals were first treated with a s.c. injection of naloxone hydrochloride (Nal, Endo Labs., Du Pont de Nemours; USA, 1 mg/kg b.wt) or physiological saline, and 15 min later BE (Organon); 100 ng/µl artificial cerebrospinal fluid (CSF) or artificial CSF was given i.c.v. The animals were decapitated 30 min after the i.c.v. treatment, the brains were quickly removed and the hypothalamus, the hippocampus (ventral + dorsal of both sides), the septum and amygdala were dissected [12]. Tissue samples were sonicated in ice-cold 1.0 M HCl. Subsequently, phosphate buffer was added to adjust the pH to 4.0. An aliquot of the homogenate was kept for the determination of protein content [13]. The remainder was centrifuged and supernatants were taken for the extraction of AVP and OXT, using thermally activated Vycor glass powder [14]. The evaporated residue from the extraction was dissolved in radioimmunoassay (RIA) buffer and subjected to RIA analysis [15].

The antisera were highly specific; the cross-reactivity of the anti-OXT serum with AVP was less than 0.1%, and with arginine-vasotocin was less than 0.01%, while the cross-reactivity of the anti-AVP serum with OXT was less than 0.01%, and with arginine vasotocin was 0.03%. The detection limits were 2 pg/tube OXT and 1 pg/tube AVP.

Results are expressed in pg/mg protein (albumin equivalent) ± SEM. Results were calculated with one-way ANOVA, followed by Scheffe's test for multiple comparison. A probability level of 0.05 was accepted as indicating a significant difference.

Results and discussion. The changes of the endogenous AVP and OXT levels in various brain areas are shown in the table. Significant differences in the AVP levels were observed in the septum ($F = 4.16$, $df = 3$, $p < 0.05$) and in the amygdala ($F = 4.39$, $df = 3$, $p < 0.01$). In the amygdala BE reduced the AVP content in a Nal-reversible manner. In the septum, Nal significantly increased the AVP level. The AVP contents in the hypothalamus and hippocampus and the OXT levels in all brain regions were not significantly altered by the treatment.

The present data indicate that i.c.v. treatment with a behaviorally active dose of BE reduced the amygdala AVP content in a Nal-reversible manner. The findings demonstrated that the components of the brain AVP-ergic and OXT-ergic systems respond differently to BE administration, and the effect on the AVP-ergic system was regionally specific. At present it is difficult to deter-

The effect of beta-endorphin (BE) (100 ng, i.c.v.) on the arginine-8-vasopressin (AVP) and oxytocin (OXT) contents of various brain regions

Hormone contents (pg/mg protein)	Controls		BE		Nal ± BE		Nal	
Hypothalamus								
AVP	4471 ± 909[a]	(20)[b]	4844 ± 1125	(10)	3734 ± 1355	(10)	3981 ± 1887	(10)
OXT	1349 ± 96	(21)	1372 ± 193	(10)	1226 ± 75	(11)	1145 ± 150	(9)
Septum								
AVP	24.2 ± 2.7	(21)	29.2 ± 3.8	(9)	33.4 ± 5.9	(10)	69.9 ± 24.7	(9)*
OXT	20.4 ± 1.9	(20)	30.3 ± 5.1	(11)	24.8 ± 3.6	(10)	17.4 ± 3.5	(9)
Amygdala								
AVP	22.6 ± 2.6	(22)	7.0 ± 0.8	(9)**	28.5 ± 7.3	(11)	17.1 ± 4.4	(10)
OXT	12.7 ± 1.1	(22)	12.7 ± 2.7	(10)	11.8 ± 2.3	(8)	7.1 ± 1.0	(10)
Hippocampus								
AVP	8.6 ± 1.1	(17)	7.3 ± 1.3	(10)	7.5 ± 1.7	(11)	7.4 ± 1.8	(9)
OXT	5.5 ± 0.7	(19)	3.9 ± 0.6	(10)	4.6 ± 0.5	(10)	3.9 ± 0.6	(10)

[a] means ± SEM; [b] number of animals; * significantly different from controls (* $p < 0.05$, ** $p < 0.01$).

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.

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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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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