bits the first GlcNAc-1-P transfer to Dol·P, but not the transfer of the second GlcNAc residue. This observation has been confirmed in some other organisms, but not in insects. However, several reports have now been made on inhibition of glycoprotein synthesis by tunicamycin in insect tissues, and the authors assumed the mode of action to be interference with the transfer of the first GlcNAc residue to Dol·P[13,14].

Because we and others intend to continue investigating GlcNAc transferases and glycoprotein synthesis in insects, we decided that this mode of action must be confirmed. To determine this, we obtained unlabeled Dol·PP-GlcNAc and Dol·PP-$(GlcNAc)_2$ by scaling up the reaction decribed above (ca. 10 times) using unlabeled UDP-GlcNAc. The products were isolated following the procedure described for TLC analysis preparations above. These products were then added to the reaction mixture using [^{14}C]UDP-GlcNAc. If tunicamycin inhibits the first GlcNAc addition to Dol·P then only one labeled product, i.e. [^{14}C]Dol·PP-$(GlcNAc)_2$, should have been observed. This is indeed what happened, as can be seen in the figure. Channel 1 is the normal reaction using Dol·P only as the acceptor, while channel 2 is the same reaction with 50 ng/ml tunicamycin added. Tunicamycin effectively inhibits the transfer of carbohydrate units to Dol·P. Channel 3 is the reaction run with unlabeled Dol·PP-GlcNAc and -$(GlcNAc)_2$. As expected, two radioactive areas corresponding to Dol·PP-GlcNAc and -$(GlcNAc)_2$ are seen in channel 3. When tunicamycin (50 ng/ml) is present in the reaction only the area corresponding to Dol·PP-$(GlcNAc)_2$is observed, indicating that the second GlcNAc addition can proceed but not the first.

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D-ala^2-Metenkephalinamide blocks the synaptically elicited cortical spreading depression in rats

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Summary. Spreading depression (SD) was elicited in rats anesthetized with pentobarbital by a train of 8 electrical pulses (0.1 ms, 10 Hz) applied to parietal cortex. Local application of 50 µg of D-ala^2-metenkephalinamide (DAME) on the stimulated area evoked one or two SD waves followed by an increase of SD threshold from 40 V to 90 V. This effect could be partly prevented by naloxone (1 mg/kg i.p.) and reversed by local application of 4-aminopyridine (10^{-3} M, 2 µl), which reduced SD threshold to 5 and 20 V in normal and DAME-treated cortex, respectively. It is argued that DAME exerts an inhibitory effect on cortical neurons and that the initial SD facilitation is due to initial blockade of inhibitory neurons in the superficial cortical layers.
Key words. Rat cortex; spreading depression; D-ala^2-metenkephalinamide.

Locally applied opioid peptides elicit seizure activity[4,5] and spreading depression[6,7] (SD) in the neocortex and hippocampus, but suppress spontaneous and evoked unit activity when iontophoretically applied into the same regions[8,9]. In an attempt to resolve this apparent contradiction, the effect of a potent enkephalin analogue D-ala^2-metenkephalinamide (DAME) on the threshold of synaptically elicited cortical SD has been examined. SD is a self-propagating neurohumoral phenomenon[10] mediated by accumulation of K^+ ions in the extracellular space to a level causing depolarization of adjacent neurons and release of additional K^+ ions. It is triggered when $(K^+)_e$ exceeds the threshold level of 10–12 mM[11] in a critical volume of cortex (about 1 mm^3)[12]. The $(K^+)_e$ increase can be elicited by sudden activation of the neuronal population through afferent fibers[13]. Assuming that the $(K^+)_e$ threshold and critical volume of SD remain constant, changes of the intensity of electrical stimuli required for eliciting SD reflect the overall excitability of the stimulated region and may serve, therefore as a sensitive index of the drug induced changes of synaptic transmission in the examined network.

Methods. Experiments were performed in 28 male hooded rats of the Druckray strain weighing 200–250 g. The animals were anesthetized with pentobarbital (50 mg/kg) and two adjacent trephine openings 4 mm in diameter were made over the frontoparietal cortex. A pair of spring mounted ball tipped silver wire electrodes with a 2 mm interelectrode distance was placed into the caudal trephine opening. Glass capillary electrodes with a tip diameter of 10–20 µm filled with the physiological saline were introduced 1 mm below the cortical surface, one close to the stimulating electrodes, the other at a point 4–5 mm distant in the rostral trephine opening. The fluid in the capillaries was connected by salt bridges with calomel half cells. Another wick calomel cell electrode applied on the neck muscles served as reference. The potential differences were amplified with high impedance input instrumentation amplifiers and recorded with a conventional polygraph. The electrodes

were connected through an optically coupled stimulus isolation unit to a gated stimulator which delivered a train of eight pulses (0.1 ms, 10 Hz), the amplitude of which could be varied from 0 to 100 V. The stimulus trains were applied at 3-min intervals which were prolonged to 10 min after an SD wave had been elicited. DAME (25 µg/µl) and 4-aminopyridine (10^{-3} M) were applied in a volume of 2 µl of a saline onto the exposed cortex around the stimulating electrodes. 10 min after application the brain surface was dried with a cotton pledget and threshold estimation continued for 60 min.

Results. Estimation of the SD threshold is illustrated in figure 1. Stimulus amplitude was increased in 10-V steps until the 45-V pulse train elicited SD. After 10 min stimulus intensity was decreased and the procedure was repeated. With stable experimental conditions, SD threshold remained during several hours within the range of ± 10 V. Application of DAME elicited after the latency of 3–5 min an SD wave in 60% of rats (n = 10). Later, SD threshold increased and 30–45 min after DAME application exceeded 80 V. In seven rats SD could not be elicited even by the strongest stimuli (100 V). In six rats treatment of the cerebral cortex with 4-aminopyridine (10^{-3} M, 2 µl, 10 min) reversed the DAME effect and decreased the SD threshold below the resting level (fig. 1). The results are summarized in figure 2. DAME elicited a significant threshold increase ($t(9) = 9.8$, $p < 0.001$, paired comparison) and 4-aminopyridine a significant threshold decrease ($t(5) = 4.6$, $p < 0.01$, paired comparison).

Further decrease of SD threshold was induced by 4-aminopyridine in rats (n = 10) not pretreated by DAME (fig. 2). The difference between the SD thresholds established 30–45 min after 4-aminopyridine application in the two groups was significant at the $p < 0.05$ level (Mann-Whitney).

In another group of eight rats SD threshold was estimated before and after i.p. injection of naloxone (1 mg/kg). DAME (50 µg/2 µl) applied 5 min after naloxone administration elicited SD in two rats (25%) and caused only a low increase of SD threshold (fig. 2). Comparison of the DAME induced threshold increments in control and naloxone treated rats reveals a significant difference ($t(16) = 4.2$, $p < 0.01$).

Discussion. The present study confirms the reports[7] that opioid peptides can elicit SD, but indicates at the same time that the increased SD susceptibility rapidly changes into a blockade of the synaptically induced SD. This biphasic effect is probably due to the differential influence of DAME on the superficial and deep cortical layers. According to Palmer et al.[9], DAME elicits excitatory reactions in the upper 500 µm of cerebral cortex but mainly inhibitory reactions below this level. It is conceivable that SD is elicited during the initial phase of DAME effect before the drug has penetrated into deeper cortical layers. The transient excitation can also be due to differential effect of DAME on various types of neurons. As pointed out by Zieglgänsberger et al.[8] opioid peptides may excite hippocampal pyramidal neurons by inhibiting adjacent inhibitory interneurones. This explanation is supported by intracellular recordings from hippocampal neurons[14,15] showing reduction of inhibitory postsynaptic potentials after application of low concentrations of enkephalins[16]. Seizures activity elicited by i.c.v. application of methionin enkephalin (100 µg) is only shortlasting (about 6 min)[4] and the subsequent SD susceptibility decrease cannot be due, therefore, to enhanced potassium clearance, which accounts for the SD threshold increase in active epileptic foci[17]. This conclusion is supported by the reversal of the DAME effect by 4-aminopyrimidine, a drug which enormously increases evoked presynaptic transmitter release[18] by blockade of voltage-dependent potassium conductance. Since excitation induced blockade of SD is enhanced rather than reduced by 4-aminopyridine[19] the present results suggest

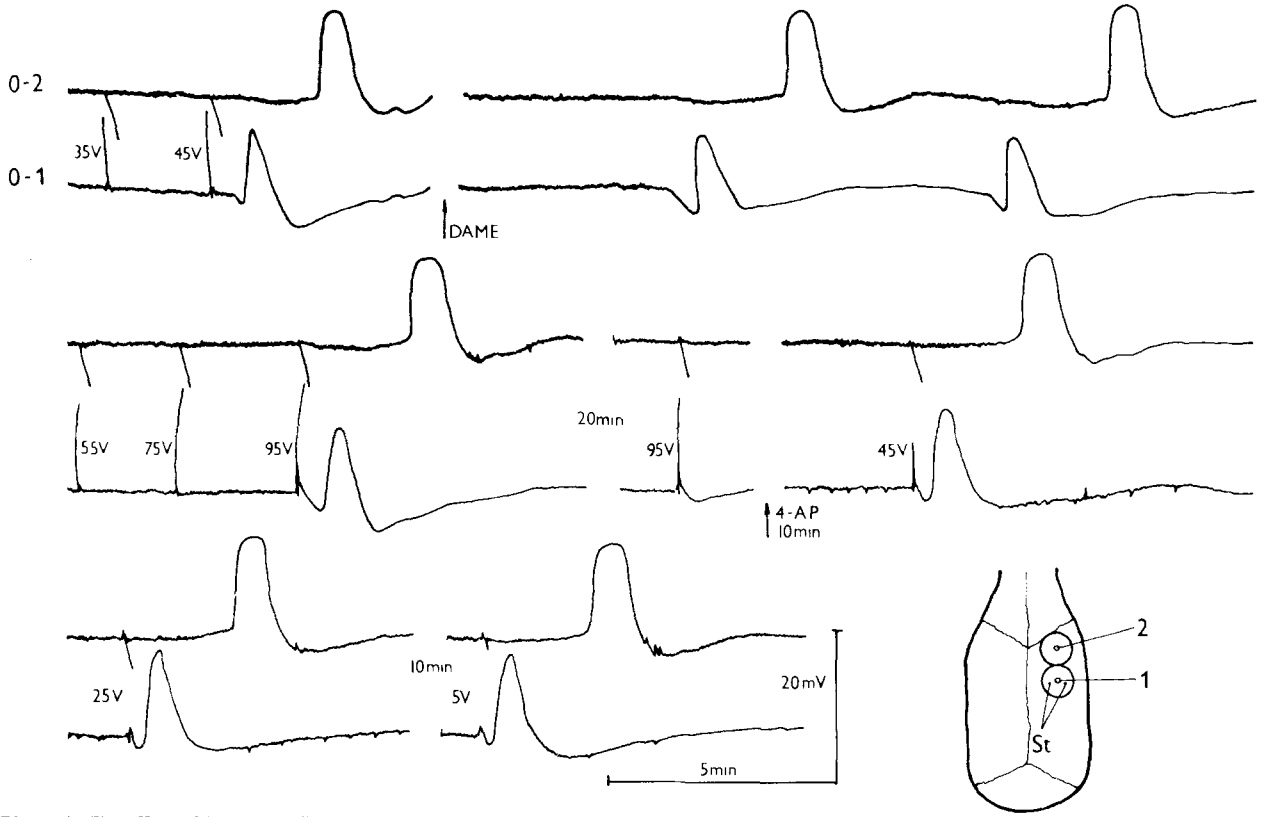

Figure 1. The effect of locally applied DAME and 4-aminopyridine on the SD threshold of cerebral cortex. Rat under pentobarbital anesthesia. Position of the stimulating (St) and recording (1, 2) electrodes is shown in the inset diagram. 0–1, 0–2: slow potential recordings. The voltage values at the stimulus artefacts denote stimulus strength. The arrows denote application of DAME or of 4-aminopyridine into the caudal trephine opening. Interruptions of the otherwise continuous recordings are indicated in minutes.

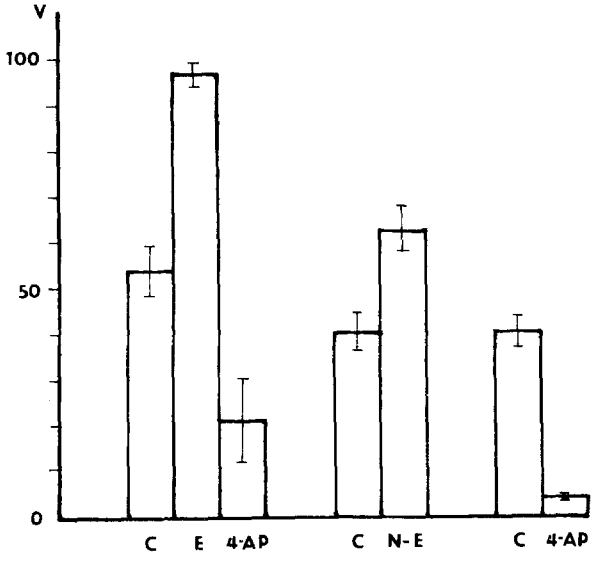

Figure 2. Changes of the average SD threshold (ordinate) elicited by DAME (E), 4-aminopyridine (4-AP) and naloxone (N). C: untreated cortex. E: 30–60 min after local application of DAME. 4-AP: 30 min after application of 4-aminopyridine to the DAME treated cortex. N–E: 30–60 min after application of DAME to naloxone (1 mg/kg) pretreated rats. 4-AP: 30–60 min after 10-min treatment of intact cortex with 4-aminopyridine.

that the 4-aminopyridine counteracts the DAME induced inhibition.

The specificity of the DAME effect, is confirmed by its attenuation in the naloxone-treated rats. Partial suppression of the DAME elicited cortical SD by naloxone was reported by Sprick et al.[7] but the reduction of the DAME induced increase of SD threshold seems to be a more consistent manifestation of the DAME-naloxone antagonism.

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Naloxone prevents the analgesic action of α-MSH in mice

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Summary. α-MSH (0.1, 1, 10 μg) was administered intracerebroventricularly and its action on pain sensitivity was investigated by the hot-plate method in mice. α-MSH produced dose-dependent analgesia and this analgesic effect was prevented by naloxone (1 mg/kg, s.c.). It is possible that α-MSH may play a role in the mechanism of pain through endogeneous opioid systems.
Key words. α-MSH; analgesia; naloxone.

α-MSH, one of the neuropeptides, is released, stored by the intermediate lobe of the pituitary[1] and widely distributed in the central nervous system[2]. Various studies concerning the effects of MSH on learning and memory processes have been reported[3,4]. On the other hand several interactions between opiates and MSH have been reported[5,6]. Morphine and β-endorphin induce an increase of plasma α-MSH levels[7]. Furthermore, it is reported that α-MSH elicits excessive grooming[8] and that the peptide-induced behavior could perhaps reflect the agonistic properties on the opiate recepter[9]. The present study was carried out to examine the involvement of α-MSH in the development of analgesia.

Male ddY strain mice weighing 20–22 g were used after acclimatization in a breeding room for one week. To test the analgesic action of α-MSH, the hot-plate method as described by Woolfe and Macdonald[10] was used. Mice were placed on the hot-plate (55 ± 0.5 °C) and the latency to the response (licking the feet, jumping or rapidly stamping the feet) was recorded. α-MSH dissolved in 0.9% NaCl solution was injected intracerebroventricularly (i.c.v.) in a volume of 5 μl. I.c.v. injection was made at the coordinates described by Haley and McCormick[11]. A microsyringe equipped with a 27 gauge needle was used. 5 min after the i.c.v. injection of MSH, pain sensitivity was measured. In order to study the interaction of MSH analgesia with opioid peptides, naloxone was administered s.c. 5 min before the MSH injection.

As can be seen in the accompanying figure, central administration of α-MSH had a dose-related analgesic action. It is proposed that α-MSH acts either directly or indirectly to activate the release of endogeneous agents which produce analgesia. Since α-MSH analgesia is prevented by naloxone, this released endogeneous substance probably interacts with opioid receptors. It is reported that MSH is metabolized very rapidly, i.e. in approximately 2 min[12]. So, in the present study, experi-