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THE INFLUENCE OF NEUROPEPTIDES RELATED TO PRO-OPIOMELANOCORTIN ON ACQUISITION OF HEROIN SELF-ADMINISTRATION OF RATS

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Summary

The influence of different neuropeptides related to pro-opiomelanocortin were tested on acquisition of heroin self-administration in rats. The animals were allowed to self-administer heroin intravenously on a continuous reinforcement schedule during 6 h daily sessions on 5 consecutive days. Treatment was performed subcutaneously 1 h before each daily session. It was found that the opioid peptides α-, γ- and β-endorphin hardly influenced acquisition of heroin self-administration, while the non-opioid fragments of α- and γ-endorphin modulated this behavioral response. In fact, β-endorphin (βE) 2-9 tended to facilitate the rate of acquisition, while the γ-type endorphins, des-Tyr^1-γ-endorphin (βE 2-17) and des-enkephalin-γ-endorphin (βE 6-17), decreased heroin intake. Concerning the ACTH/MSH related peptides, a decreasing effect of heroin intake was found following treatment with (D-Phe^7)-ACTH 4-10, with a high dose of the ACTH 4-9 analog Org 2766 and with γ_2-MSH, while ACTH 1-24, ACTH 4-10 and a low dose of Org 2766 did not significantly influence self-injecting behavior. It is concluded that pro-opiomelanocortin serves as a precursor molecule for peptide fragments, which modulate the acquisition of heroin self-administration in rats.

Drug self-administration by experimental animals is currently the most consistent and reliable predictor of abuse potential in man (1,2). Although the primary reinforcing properties of drugs, as assessed by drug self-administration, are the most important stimuli in this behavior, other stimuli and drug-induced changes in the homeostatic mechanisms as well as internal factors may be additionally involved in the initiation and maintenance of drug taking behavior (for references see 2). In particular, factors modulating drug-induced reinforcing activity may be of interest in this respect, since such factors may contribute to the individual variations in susceptibility to addictive drugs. Candidates for these factors are neuropeptides, which may be generated by enzymatic cleavage from large precursor molecules and which are implicated in the formation and maintenance of new behavioral patterns (3,4). Two classes of neuropeptides can be distinguished in this respect. First, neuropeptides acting as positive reinforcers and which may be involved in the physiological processes underlying reward. A striking example of this class is the opioid peptide β-endorphin, since rats work for a response contingent intracerebroventricular injection when receiving relatively low amounts of this neuropeptide (5). Also other neuropeptides have been reported to maintain self-administration under certain conditions, e.g. met^5- and leu^5-enkephalin, ACTH 1-24 and ACTH 4-10

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(6,7). Interestingly, all these peptides are related to pro-opiomelanocortin, the precursor molecule of ACTH and β-lipotropin (8).

A second class of neuropeptides is more implicated in the modulation of the consequence of drug self-administration by interfering with the complex interaction of addictive drugs with brain homeostatic mechanisms. These neuropeptides are primarily involved in the adaptation of the individual to its environment (3). Among these are peptides related to the neurohypophyseal hormones, vasopressin and oxytocin, which indeed have been reported to interfere with acquisition of heroin self-administration (9-11). The same may hold for peptide fragments derived from pro-opiomelanocortin, since these fragments have been implicated in various brain processes probably relevant for the development of opiate self-administration, such as motivation and attention (3); adequate interpretation of the environmental situation, when disturbed leading to psychopathology e.g. psychoses (12); pain perception and motivation (13,14); and the opiate withdrawal syndrome (15). Thus the present study focussed on the influence of different neuropeptides related to pro-opiomelanocortin on acquisition of opiate self-administration of rats. Heroin was selected for self-administration, because with this drug self-injection behavior develops relatively fast and is, at least under standard conditions rather reproducible (11,16). Acquisition of this behavior was chosen because it may be particularly sensitive to neuropeptides involved in adaptive processes, since rats exposed to a new environment and the availability of the addictive drug need their adaptive capacities.

Materials and Methods

Male rats of Wistar strain, weighing 200 - 240 g were surgically equipped with chronic jugular cannulae of silicone rubber tubing prepared in such a way that infusion fluid could be injected into the vein, but that blood could not enter the cannula system (16). Following the operation, the animals were housed individually (home cages) at 24°C and with a 12 h/day and 12 h/night reversed schedule of illumination for the duration of the experiments. The rats were allowed to recover from the operation procedure for five days and were then placed in operant conditioning chambers (experimental cages) in sound attenuated rooms. They were tested on five consecutive days for 6 h per day during the dark period of the illumination cycle. The rest of the day the rats were housed in the home cages. Food and water were available ad libitum in the home cages only.

During testing the intravenous cannula was connected via a swivel with an infusion pump. The swivel permits the animals to move relatively freely. Due to the construction of the jugular cannula contact between infusion fluid and blood was only present during the time of infusion, which permits disconnection of the infusion system and prevents blood coagulation without using anticoagulants. Pressing one of the two levers available in the experimental cage, marked by an illuminated light placed just above the lever, was programmed to deliver an injection of 0.25 ml of a heroin solution (0.15 mg/kg/infusion) on a continuous reinforcement schedule. During the time of infusion (15 sec) the stimulus light was switched off and pressing the lever was not reinforced by an additional injection.

Peptide or placebo was subcutaneously administered daily 1 h before the rats were placed in the experimental cage. On the first day of testing, the rats received every hour an infusion of heroin given by the experimenter and two infusions were given every day at the start of the experimental session.

Heroin (diacetylmorphine hydrochloride, OPG, Utrecht, The Netherlands) was dissolved in saline and the pH of the solution was adjusted to 7.3. The

following peptides (donated by Organon International B.V., Oss, The Netherlands) were used: ACTH 1-24 (H-Ser-Tyr-Ser-Met-Glu-His-Phe-Arg-Trp-Gly-Lys-Pro-Val-Gly-Lys-Lys-Arg-Arg-Pro-Val-Lys-Val-Tyr-Pro-OH), ACTH 4-10, (D-Phe^{7})ACTH 4-10, Org 2766 (((Met/O_2)^{4},D-Lys^{8},Phe^{9})ACTH 4-9), β-endorphin (βE) (H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-Phe-Lys-Asn-Ala-Ile-Val-Lys-Asn-His-Lys-Lys-Gly-Gly-Glu-OH), γ-endorphin (βE 1-17), α-endorphin (βE 1-16), des-Tyr^{1}-γ-endorphin (DTγE, βE 2-17), β-endorphin 2-9 (βE 2-9), des-enkephalin-γ-endorphin (DEγE, βE 6-17), des-enkephalin-α-endorphin (DEαE, βE 6-16) and γ_2-MSH (H-Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly-OH). Peptides were dissolved in saline and subcutaneously injected in a volume of 0.5 ml per animal.

Animals which took more than 6 injections on the second test day were considered to demonstrate self-administration behavior. This criterion serves as a useful baseline for defining self-administration under the experimental conditions described (9,11). Approximately 80% of the rats tested exhibited self-administration. Effect of drug or peptide treatment was statistically analysed using ANOVA testing and Student's t-tests.

Results

In the course of the experiments 4 control groups, each consisting of 9-13 animals treated with placebo, were run. Since the self-injecting behavior of these groups was not significantly different from each other, they were combined. The mean number of self-injections in the various groups of rats, treated with placebo and peptide during the 6 h test session on days 2 and 5 are presented in Table I. In rats pretreated with placebo, self-administering behavior was readily acquired and increased during the first test days reaching a ceiling level on the fourth and fifth test day. The mean number of self-injections (± S.E.M.) of all placebo pretreated rats (n=41) was 18.9 ± 1.2; 22.2 ± 1.6; 27.3 ± 2.9; and 28.4 ± 2.4 on day 2, 3, 4 and 5 of testing respectively (see for example the group of the placebo pretreated rats in fig. 1).

β-Endorphin related neuropeptides. Although pretreatment with β-endorphin did not significantly influence acquisition of heroin self-administration, fragments of this opioid peptide differentially affected this behavioral response. γ-Endorphin slightly decreased self-injection behavior, but not significantly. C-terminal fragments of γ-endorphin i.e. DTγE and DEγE in general suppressed the responding of the rats, especially during the second phase of testing (Table I, fig. 1). DTγE was somewhat more potent in this respect than DEγE. The low dose of DTγE increased the rate of responding, but only on the first two days of testing; thereafter responding gradually decreased to a relative low level on day 5. α-Endorphin and DEαE did not significantly affect self-injecting behavior. βE 2-9 tended to facilitate acquisition of heroin self-administration (fig. 1), although on none of the test days did this increase reach statistical significance. The influence of βE 2-9 was most pronounced with the 1 and 5 μg dose and less pronounced following the 25 μg dose.

ACTH/MSH related neuropeptides. Neither ACTH 1-24 nor ACTH 4-10 significantly affected the acquisition of heroin self-administration, although a slight increase in responding was noted on the first 2 days in rats pretreated with ACTH 4-10. Such an effect was, however, not present on the following three days of testing. The ACTH 4-10 analog in which the amino acid phenylalanine in position 7 is replaced by its D-enantiomer decreased self-injecting behavior. This decrease was present on day 3, 4 and 5 (mean number of self-injections ± S.E.M. on day 3 and 4: 14.9 ± 2.3 ($p < 0.05$, as compared to placebo treated rats) and 17.6 ± 3.0 ($p < 0.05$) respectively). A low dose of the highly potent ACTH 4-9 analog Org 2766 mimics ACTH 4-10 in that a slight, but nonsignificant increase of heroin intake was observed. In contrast, a relatively high dose

TABLE I

Influence of Various Peptides Related to Pro-Opiomelanocortin on Acquisition of Heroin Self-Administration

Treatment	dose	number of animals	self-injections (mean ± SEM) day 2	day 5
placebo (saline	0.5 ml	41	18.9 ± 1.2	28.4 ± 2.4
β-endorphin (βE)	10 µg	8	18.1 ± 1.7	24.5 ± 4.1
γ-endorphin (βE 1-17)	5 µg	11	17.5 ± 2.4	21.7 ± 4.7
	25 µg	8	19.9 ± 3.7	22.4 ± 5.1
DTγE (βE 2-17)	5 µg	10	26.6 ± 4.4*	15.6 ± 3.4**
	25 µg	11	8.9 ± 1.1**	16.9 ± 2.5**
DEγE (βE 6-17)	5 µg	13	16.4 ± 2.2	23.7 ± 4.9
	25 µg	12	16.9 ± 2.5	16.3 ± 3.0**
α-endorphin (βE 1-16)	5 µg	8	19.9 ± 3.0	25.9 ± 4.6
	25 µg	9	22.9 ± 3.6+	24.6 ± 2.5
DEαE (βE 6-16)	25 µg	5	12.8 ± 2.6	32.5 ± 9.9
βE 2-9	1 µg	7	22.1 ± 3.5	32.4 ± 7.4
	5 µg	7	25.1 ± 6.0	29.3 ± 3.5
	25 µg	8	21.9 ± 1.8+	21.3 ± 3.5
ACTH 1-24	50 µg	7	18.0 ± 2.9	24.0 ± 5.7
ACTH 4-10	50 µg	10	22.9 ± 3.4	23.4 ± 5.4
ACTH 4-10 (D-Phe^7)	50 µg	13	18.0 ± 2.9	19.1 ± 2.5*
Org 2766	.01 µg	6	22.2 ± 7.2	32.2 ± 7.2
	1 µg	7	19.4 ± 2.4	28.0 ± 6.6
	50 µg	5	12.6 ± 1.5	11.6 ± 5.2*
γ_2-MSH	5 µg	14	23.2 ± 2.1	20.2 ± 5.1
	50 µg	13	18.1 ± 2.3	10.7 ± 2.1**

Rats were subjected to a 6 h experimental session daily for 5 consecutive days. During these sessions the animals were allowed to self-administer heroin (0.15 mg/kg/injection) intravenously. Treatment was performed subcutaneously 1 h prior to each session. Statistical analysis was performed by comparing the data of the peptide-treated rats with those of rats simultaneously treated with placebo (+ $p < 0.1$, * $p < 0.05$, ** $p < 0.01$).

of this peptide (50 µg) significantly suppressed self-injecting behavior (table I). Also on day 3 and 4 an attenuation of responding was observed (day 3: 10.4 ± 2.5, $p < 0.05$ as compared to placebo treated rats; day 4: 15.6 ± 3.4). γ_2-MSH, a peptide fragment of the N-terminal part of pro-opiomelanocortin molecule and structurally related to ACTH 4-10, decreased the acquisition of heroin self-administration, as reported before (15). The inhibitory effect of this peptide was hardly present on day 2 and 3 of testing, but after that period self-injecting behavior was markedly suppressed.

Discussion

The present data show that the opioid peptides α-, β- and γ-endorphin did not significantly affect the acquisition of heroin self-administration, but that the non-opioid fragments of α- and γ-endorphin modulated this behavioral

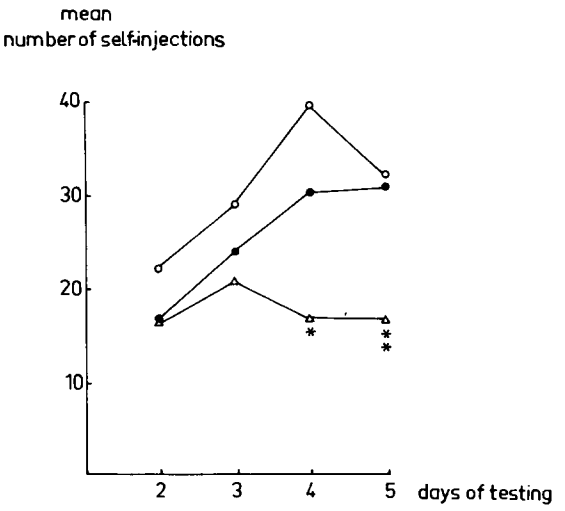

FIG. 1

The influence of the β-endorphin fragments βE 2-9 and DEγE on acquisition of heroin self-administration. Rats were subjected to a 6 h experimental session daily for 5 consecutive days. During these sessions the animals were allowed to self-administer heroin (0.15 mg/kg/injection) intravenously. Groups of rats were treated with placebo (0.5 ml saline, n=11, ●—●), βE 2-9 (1 µg, n=7, o—o) or DEγE (25 µg, n=12, Δ—Δ). Treatment was performed subcutaneously 1 h prior to each session. Mean number of self-injections versus days of testing is depicted. * different from placebo treated rats (* $p < 0.05$, ** $p < 0.01$, Student's t-tests).

response. Thus, βE 2-9 tended to enhance the rate of acquisition, while the γ-type endorphins, DTγE and DEγE, decreased heroin intake. The effect of βE 2-9 was, however, not marked, which may be related to the nearly maximal rate of acquisition under the present conditions. Opposite effects of α-type endorphins, including βE 2-9, and γ-type endorphins have also been observed in other behavioral test procedures, e.g. extinction of active avoidance behavior, passive avoidance behavior, intracranial electrical self-stimulation and apomorphine-induced behavioral changes (17-24). The effects of α-type and γ-type endorphins resemble in certain aspects those of psychostimulants and neuroleptics respectively (17,20). Accordingly, subcutaneous treatment with 100 µg amphetamine tended to facilitate acquisition of heroin self-administration, whereas 25 - 225 µg haloperidol dose dependently decreased heroin intake under the conditions described in this paper (data not shown).

It has been suggested that brain dopamine plays an essential role in brain reward and the reinforcing effects of opiates (25,26). Accordingly, it has recently been shown that rats self-administered opiates (fentanyl, heroin) via cannulae implanted in the ventral tegmental-medial substantia nigra area, where the bodies of the mesolimbic and nigro-striatal dopaminergic systems are located (27,28). Thus it might be that βE 2-9 and the γ-type endorphins modulate acquisition of heroin self-administration by interfering with mesolimbic, nigro-striatal and/or interdiencephalic dopaminergic systems. More direct evidence for such a mode of action was obtained from experiments in which intracranial electrical selfstimulating behavior (ICSS) was used as test procedure. ICSS

elicited via electrodes implanted in the ventral tegmental-medial substantia nigra area, was attenuated by DTγE and facilitated by α-endorphin especially when threshold currents were delivered (21). ICSS elicited via electrodes implanted in the nucleus accumbens area was similarly decreased by DTγE but not affected by α-endorphin (22). These effects may be in accordance with the differential effects of α-type endorphins including βE 2-9 and γ-type endorphins on apomorphine-induced behavioral responses mediated by the nucleus caudatus and accumbens respectively (24,29). Although these data taken together favour the mesolimbic and nigrostriatal dopaminergic systems as mediators of the modulatory action of the peptides on acquisition of heroin self-administration, it should be kept in mind that α-type and γ-type endorphins have also opposite effects on transmission of intradiencephalic dopaminergic systems (30,31) and that the significance of the different dopaminergic systems in the brain for opiate self-administration should be studied in more detail before definite conclusions can be drawn.

Concerning the ACTH related peptides, a decreasing effect on heroin intake was observed following treatment with (D-Phe^7)ACTH 4-10 and with a high dose of Org 2766, while ACTH 4-10 and a low dose of Org 2766 slightly increased self-injecting behavior. Considering the above mentioned relationship between peptide and drug effects on heroin self-administration and ICSS elicited via electrodes implanted in the ventral tegmental-medial substantia nigra area, unfortunately studies on the influence of ACTH-related peptides on ICSS from this area are lacking. It has, however, been shown that ACTH 4-10 and a low dose of the highly potent ACTH 4-9 analog Org 2766 increased, while (D-Phe^7)ACTH 4-10 and a high dose of Org 2766 decreased ICSS elicited from the medial septal area under threshold conditions (32,33). Similar increasing and decreasing effects were observed on acquisition of heroin self-administration. However, the increasing effects were not marked and statistically nonsignificant probably due to the already mentioned high rate of acquisition under the described conditions. Nevertheless, these data together with the effects of β-endorphin fragments on ICSS favour the relationship between ICSS and acquisition of heroin self-administration.

γ_2-MSH decreased the acquisition of heroin self-injecting behavior. Extensive studies have shown that the behavioral profile of γ_2-MSH resembles that of naloxone in several aspects, suggesting that γ_2-MSH may act as a functional antagonist of endorphins (15). Accordingly, the opiate antagonist naltrexone, like γ_2-MSH, decreased acquisition of heroin self-administration (data not shown). Although γ_2-MSH injected into the periaqueductal gray matter of the brain stem of opiate naive rats elicited symptoms reminiscent of those seen after opiate withdrawal (15), it is rather unlikely that this effect of γ_2-MSH underlies the decreasing effect on heroin intake, since physical dependence does not play a primary role in the acquisition of heroin self-administration (2,16).

In conclusion, the present data suggest that fragments of pro-opiomelanocortin specifically modulate acquisition of heroin self-administration, in addition to the positive reinforcing effects of some fragments e.g. β-endorphin, which interestingly have little if any effect on this acquisition. Thus, pro-opiomelanocortin serves as a precursor molecule for peptide fragments, which may either directly or indirectly be critically involved in the initiation of heroin self-administration.

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