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DEPENDENCE CREATING PROPERTIES OF LIPOTROPIN C-FRAGMENT (β-ENDORPHIN): EVIDENCE FOR ITS INTERNAL CONTROL OF BEHAVIOR

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Summary

The C-fragment of lipotropin (β-endorphin) possesses reinforcing properties, in that this peptide, like heroin, induced intraventricular self-administering behavior in drug naive rats. Only mild behavioral signs reminiscent of physical dependence were present. After injection into the nucleus raphé magnus, C-Fragment appeared to act as a discriminative stimulus in rats trained to discriminate fentanyl from saline. These data indicate that naturally occurring C-Fragment exerts powerful control of behavior, which may be of significance for the understanding of the mechanisms underlying opiate dependence.

Peptides the structure of which represents some fraction of the C-terminal part of lipotropin (β-LPH; starting with amino acid residue 61) have recently been isolated from extracts of pituitary or brain (1-6). These peptides produce many pharmacological actions characteristic of narcotic drugs (7). Thus, C-Fragment (sequence 61-91) has a potent and long-lasting antinociceptive action when injected intracerebroventricularly (8,9) and chronic administration of the peptide results in tolerance to its analgesic activity (9) as well as in physical dependence (10). Related peptides shorter in sequences have also been characterized: C^1-Fragment (61-87) was obtained from pituitary extracts (1), γ-endorphin (61-77) and α-endorphin (61-76) from hypothalamic-pituitary extracts (4), and met-enkephalin (61-65) from extracts of brain (5,6). As compared with C-Fragment, these shorter peptides are about equally potent on the guinea pig ileum and the mouse vas deferens preparation (11), but are much less active in displacing specifically bound ^{3}H-naloxone from brain opiate binding sites (12), in producing analgesia (13,14) and in inducing physical dependence (10).

The presence of peptides with opiate-like activity in pituitary and brain, suggests that these endogenous substances may be involved in the functioning of physiological systems which are also affected by narcotic drugs. Due apparently to their involvement in the physiological substrate of reward, narcotics induce euphoria and other subjective effects which may be responsible for opiate dependence in humans (15). To test whether the above mentioned peptides possess dependence liability similar to that of narcotics, we have studied their reinforcing and their discriminative stimulus properties in rats. The self-

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administration method offers a direct assessment of possible reinforcing effects of drugs, a property generally considered to underlie drug dependence (16). The drug discrimination procedure is designed to determine drug class-specific stimulus properties, and may provide an animal model for drug-produced subjective effects in man (17,18).

Materials and Methods

Self-administration (experiment 1): Stainless steel guide cannulae (0.6 mm outer dia; 0.3 mm inner dia) were implanted just above the lateral brain ventricle of male Wistar rats weighing 200 - 220 g. Following a recovery period of at least 6 days, the animals were subjected to 6 h experimental sessions daily for 5 consecutive days. During these sessions, the rats were placed in sound-attenuating operant conditioning chambers and allowed to self-administer the solution being studied on a continuous reinforcement schedule. A stainless steel injection cannula was inserted via the guide cannula into the lateral ventricle, and was connected to an infusion pump through polyethylene tubing and a feed-through swivel device. Pressing the rewarded lever was programmed to deliver 2 μl of solution, infused over 15 seconds. On the first day of the experiment, the rats were given one forced injection every hour and had free access to additional injections by pressing the lever. No forced injections were given on day 2 - 5 of the experiment, and the solution was provided conditional on responding in the manner described above. Other details of the experimental conditions and procedure have been described elsewhere (16,19).

Groups of rats (n = 8 to 12) were given access to solutions of either 0.5 μg (ca $1.2.10^{-9}$ M)/μl heroin, 0.05 μg (ca $1.5.10^{-11}$ M)/μl C-Fragment, 1 μg (ca $1.7.10^{-9}$ M)/μl met-enkephalin or placebo. The substances were dissolved in artificial cerebrospinal fluid (CSF) (20) and the solutions were adjusted to pH: 7.35. C-Fragment was isolated from pig pituitary glands (2); met-enkephalin was synthetized and highly purified. Fresh solutions of all substances were prepared daily.

Fifteen minutes after the day 5 experimental session, the animals were intraperitoneally injected with 10 mg/kg naloxone. Before as well as 30 and 120 min after this injection, body weight was recorded; rectal temperature was measured by means of a thermistor probe, both before and 30 min after naloxone. Immediately following injection, the rats were placed in a 2.5 l clear glass beaker, and their behavior was observed during the subsequent 15 min. The following items were recorded: exploring (standing on the hindlegs against the wall), jumps (attempt to jump - standing against the wall on one hindleg -, or actual jump with leaping on the brim of the beaker), wet shakes (rigorous body movements, similar to those observed in wet rats), and teeth chattering.

Upon completion of the experiment, the functioning and localization of the cannulae were inspected by means of Evans Blue injections. Data from animals with failing function or improper localization were deleted.

Drug discrimination (experiment 2): Food-deprived male Wistar rats weighing 220 ± 20 g at the beginning of the experiment, were trained to press either of 2 levels for food on a fixed-ratio: 10 schedule of reinforcement. Thirty min before the daily 15 min session, the animals were injected subcutaneously with either 0.04 mg/kg fentanyl (citrate) or saline (volume: 1 ml/100 g body weight). According to whether the rat was pretreated with either fentanyl or saline, it was required to press either the drug or the saline lever to obtain food. The first lever on which 10 responses were totalized was considered as the selected lever. Fentanyl and saline sessions were run 5 days a week, according to sequences specified elsewhere (21). Discrimination training was continued till the animals reached the criterion of 20 consecutive sessions in which lever selection was errorless (17). The median number of session-to-criterion was 43.

Using materials similar to those applied in the first experiment; cannulae were implanted at the level of the nucleus raphé magnus (NRM) according to the stereotaxic co-ordinates: posterior 2.5 mm, lateral 0.0 mm, horizontal 1 mm below the interaural line (22). Following a 5 to 8 days recovery period during which food was freely available, the food-deprivation schedule was reinstigated for another 2 days, after which a serie of 6 experimental sessions was scheduled. The first two of the test sessions served to verify whether the rats would show discriminative responding appropriate to the training conditions when being injected with either 2 µg fentanyl or placebo into the NRM. The sequence of these control tests was fentanyl-placebo in half of the animals, and placebo-fentanyl in the other half. During the subsequent 4 days, 2 µg (ca 5.9×10^{-10} M) C-Fragment, 40 µg (ca 7.10^{-8} M) met-enkephalin, 2 µg (ca 6.10^{-9} M), or placebo (4 µl) were tested in a sequence which was randomized for each rat. During all 6 post-operative test sessions, the animals were free to select any one of the 2 levels. Test sessions were started 5 min following injection.

Results and Discussion

The data of the self-administration experiment (experiment 1) are presented in Figure 1.

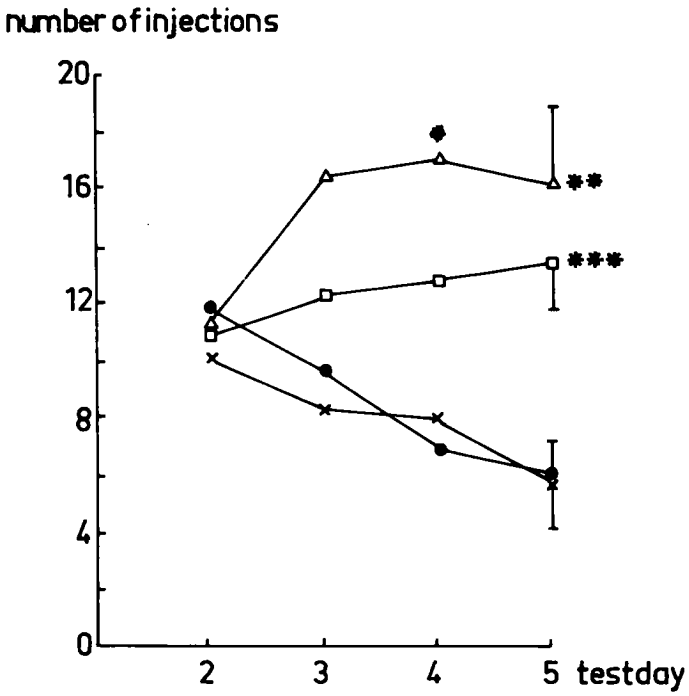

FIG. 1

Intracerebroventricular self-administration behavior of rats. Pressing the rewarded lever was followed by an intraventricular injection of 2 µl solution, containing heroin (Δ-Δ; 0.5 µg/µl; n = 12), C-Fragment (□-□; 0.05 µg/µl; n = 9) met-enkephalin (x-x, 1 µg/µl; n = 8) or placebo (o-o, artificial CSF; n = 12). The mean number of self-injections per group are plotted versus the day of testing. Vertical bars indicate S.E.M. *significantly different from placebo animals (* $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$; Student t-test).

On the first day of testing (day 2), operant response rate was similar for all groups; the mean number of injections varied between 10 and 12 injections. From day 3 onwards, the response rate in the placebo group progressively decreased to a mean level of about 6 injections on day 5. In contrast, the rate of animals on heroin increased to approximately 16 injections on day 3, and remained at that level on consecutive testdays. A similar pattern of responding was observed in the animals on C-Fragment, though the ceiling level was at about 13 injections per day in this group. The behavior of the met-enkephalin group was similar to that of the placebo group (Fig. 1). As a result, the mean total number of self-administered injections on days 3, 4 and 5 significantly exceeded that of control (23 ± 5) in the groups on heroin (50 ± 7; $p < 0.001$; Student t-test) or C-Fragment (39 ± 6; $p < 0.05$), but not in the group on met-enkephalin (22 ± 4; $p > 0.05$). The increase of behavior on subsequent testdays in the group tested on C-fragment was rather small, which may be the result of either a slow or a rapid acquisition. In the last case the ceiling level of intake, which apparently occurs in self-administration (16), may already be reached by the first days of testing. The response rate on the second (non-reinforced) lever of the 4 tested groups was not significantly different from each other on any of the testdays. These data indicate that, at the doses applied, C-Fragment, but not met-enkephalin possesses reinforcing properties and hence acts on the physiological substrate of reward in rats. The seeming discrepancy between met-enkephalin data and those reported by Belluzi and Stein (23) may be explained by the fact that these authors used a Ringer's solution of pH 5.8 as a vehicle to the peptide. This vehicle suppresses operant responding, and a peptide-induced counteraction of this suppression may have yielded a misleading result.

Body weight loss, hypothermia, and behavioral phenomena such as exploring, jumps, wet shakes and teeth chattering are reliably induced by narcotic antagonists in organisms chronically treated with narcotics, and are widely employed to quantify physical dependence in laboratory animals (10,24). However, naloxone injected just after the termination of the experimental session on day 5, failed to decrease body weight and to induce wet shakes in any of the experimental groups, and had no (heroin group, met-enkephalin group) or only a slight effect (C-Fragment group) on rectal temperature (Table 1). Some mild albeit statistically significant behavioral signs reminiscent of physical dependence were observed in rats that had self-administered heroin (exploring, jumps, teeth chattering) or C-Fragment (exploring, teeth chattering), but not in the met-enkephalin group. Thus, degree of physical dependence of animals that have experiences with self-administration of heroin and of C-Fragment is low and it is therefore highly improbable that this property should contribute to the induction of self-administering behavior. This is consistent with the conclusion reached from previous experiments with heroin, in that tolerance and physical dependence are not prerequisite for heroin self-administration (16).

Experiment 2 was designed to determine the possible equivalence of the discriminative stimulus properties of C-Fragment and met-enkephalin to those of the narcotic analgesic fentanyl (25). The experiment was carried out in rats trained to discriminate fentanyl from saline. In these animals, stimulus generalization of novel (test) compounds with fentanyl occurs with narcotics only, non-narcotic drugs inducing responding appropriate to the saline condition (26). Considering the role of the nucleus raphé magnus (NRM) in morphine produced analgesia (27), and that the discriminative stimulus and analgetic potency of narcotics correlate very closely (28), the NRM was choosen as the intracerebral injection site for the entities being studied.

Of the 8 rats which survived surgery and were in satisfactory physical condition thereafter, 1 animal failed to show discriminative responding appropriate to intracerebrally administered fentanyl and placebo, and its data were

TABLE 1

Assessment of Physical Dependence in Animals which had 5 Days of Experience with Intraventricular Self-Administration of Heroin, C-Fragment, Met-Enkephalin or Placebo Solution

	ΔT (30 min) (°C)	Δb.w. (30 min) (g)	Δb.w. (2 h) (g)	rearings	jumps	teeth chattering %
placebo	0.0 ± 0.1	-0.2 ± 0.2	-0.7 ± 0.5	1.5 ± 0.8	0.2 ± 0.1	0
heroin	-0.1 ± 0.2	0.5 ± 0.5	3.0 ± 1.4*	5.6 ± 1.0††	2.0 ± 0.8†	60°
C-Fragment	-0.5 ± 0.1*	-0.1 ± 0.2	0.1 ± 0.2	8.0 ± 1.5††	0.5 ± 0.2	100°
met-enkephalin	-0.2 ± 0.2	-0.5 ± 0.2	-0.2 ± 0.2	1.7 ± 0.6	0.3 ± 0.2	16

Changes in rectal temperature (ΔT) and body weight (Δb.w.) 30 min resp. 30 min and 2 hours after naloxone HCl (10 mg/kg, i.p.) treatment were calculated. The number of rearings to the wall of the beaker, the number of jumps and the percentage of animals exhibiting teeth chattering were determined during 15 min after naloxone. Results are given as mean ± S.E.M.

*$p < 0.02$ (Student t-test), †$p < 0.05$, ††$p < 0.001$ (Mann-Whitney U-test), °$p < 0.001$ (X^2-test), as compared to placebo controls.

deleted from the results. The data obtained with the remaining 7 animals are summarized in Table 2. All animals reliably selected the drug lever upon 2 μg fentanyl injection and the saline lever after placebo injection, indicating that they had retained their pre-operative ability to detect the discriminative stimulus associated with narcotic drugs. In these animals 2 μg C-Fragment induced drug lever selection, indicating that the peptide possesses discriminative stimulus properties similar to those of narcotics.

TABLE 2

Stimulus Generalization Experiments on C-Fragment and Met-Enkephalin in Rats Trained to Discriminate Fentanyl from Saline

solution	dose/rat	drug lever selected	median number of responses
fentanyl	2 μg	7/7*	280
placebo	4 μl	0/7	277
C-Fragment	2 μg	7/7*	193
met-enkephalin	40 μg	0/7	290

Test sessions were conducted (5 min) following injection of compounds into the nucleus raphé magnus of rats (n = 7) previously trained on subcutaneously administered fentanyl and saline. Results are expressed as the number of rats selecting the drug lever, and are based on 2 determinations with fentanyl and saline and on 1 determination with C-Fragment and met-enkephalin.
* different from placebo ($p < 0.001$, X^2-test).

Met-enkephalin however induced saline lever selection, indicating that this peptide is devoid of narcotic cuing properties at the dose level being studied (40 μg). Statistical analysis of the C-Fragment data reveals a highly significant difference from placebo, but not from fentanyl performance; the inverse was true for met-enkephalin. Between different test conditions, there were no statistically appreciable differences ($p > .05$; Wilcoxon test) as regards the total number of responses emitted during test sessions, although a slight decrease of responding was observed after treatment with C-Fragment. These data constitute unprecedented evidence that an endogenous peptide (i.c. C-Fragment) possesses discriminative stimulus properties similar to those of narcotic drugs. The ability of narcotics and other drugs of abuse to induce characteristic subjective effects has long been considered as an important factor in narcotic dependence (15). Considering that the discriminative stimulus properties of narcotics have initially been established to serve as a model for opiate-like subjective effects in man (17), the present data appear to imply that C-Fragment has a dependence potential comparable to that of narcotic drugs.

The self-administration and the drug discrimination procedure serve to investigate the ability of drugs to produce internal (as opposed to environmental) stimuli capable of controlling the behavioral output of organisms. The first of the present studies showed that the naturally occurring C-Fragment of lipotropin can act as a positive reinforcer, thus suggesting that the peptide may be involved in the physiological processes underlying reward. The second study showed that C-Fragment can act as a discriminative stimulus, i.e. as an internal signal, indicating which out of several response alternatives is more

adequate in a given set of environmental conditions. Both of these stimulus properties of C-Fragment suggest that the peptide may exert powerful control of behavior. Narcotic drugs appear to mimick central nervous system actions of this peptide, giving rise to similar internal stimuli. The present studies provide evidence that it are these internal stimuli which control behavior to the point that the integrity fo the organism becomes conditional upon the substances, a phenomenon generally referred to as opiate dependence. These findings could be important for the understanding of the mechanisms underlying drug dependence, including disturbances in brain processes through which C-Fragment exerts its control of behavior.

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