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Self-Administration of an Enkephalin Analog by Rhesus Monkey

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MELLO, N. K. AND J. H. MENDELSON. *Self-administration of an enkephalin analog by rhesus monkey.* PHARMAC. BIOCHEM. BEHAV. 9(5) 579–586, 1978.—intravenous injections of a synthetic enkephalin analog, FK-33-824 were found to maintain operant responding under second-order schedule control (FR 4 [VR 16:S]) in five, morphine-dependent rhesus monkeys. All monkeys self-administered enkephalin in amounts equivalent to morphine at comparable doses per injection. Each of five doses of enkephalin (0.5, 0.25, 0.125, 0.05 and 0.01 mg/kg/inj) were substituted for morphine during 10 consecutive sessions over a 56 hr period. No monkey developed opiate withdrawal signs during enkephalin substitution except at the lowest enkephalin dose (0.01 mg/kg/inj). Although the number of enkephalin injections self-administered increased as the dose per injection progressively decreased, there was a significant linear decrease ($p<0.05$) in mg/kg/enkephalin per session at doses of 0.25 mg/kg/inj and below. Reductions in morphine dose per injection, over a range of 0.5 to 0.125 mg/kg/inj produced comparable increases in number of injections per session, but no significant changes in morphine intake. The number of food pellets earned on a second order FR 4 (VR 16:S) schedule decreased during enkephalin substitution. These decreases were significant at the highest doses of enkephalin (0.5 to 0.125 mg/kg/inj). These data attest to the reinforcing characteristics of an enkephalin analog in rhesus monkey and suggest that natural polypeptides may contribute to the reinforcing properties of opiate drugs.

Enkephalin self-administration Morphine self-administration Second order schedules Drug reinforcement

SINCE the recent discovery of the opiate receptors [11, 26, 29] and identification and sequencing of their endogenous ligands, the endorphins [12], there has been great interest in characterizing the pharmacological, biological and behavioral effects of these compounds. Although it is generally agreed that the endogenous peptides have morphine-like actions [11,17], behavioral studies have been impeded by the fact that the available endorphin preparations were rapidly degradated by peptadase enzymes in blood and the relatively large endorphin molecule could not cross the blood-brain barrier. However, direct administration of beta-endorphins and enkephalins into brain has resulted in the development of physical dependence [34]. Synthetic analogs of enkephalins have been shown to produce morphine-like analgesia which could be reversed by administration of the narcotic antagonist, naloxone [1, 5, 27, 32]. Cross-tolerance between morphine and methionine-enkephalin has also been shown in mouse [33].

In 1977, Roemer and co-workers reported the development of a synthetic pentapeptide, structurally related to met-enkephalin, which showed significant analgesic potency and could be administered parenterally or orally [28]. Details of the structure of this synthetic pentapeptide, FK-33-824 appear in the original report [28]. This compound appeared to be about 30,000 times more potent than met-enkephalin and 1,000 times more potent than morphine after ICV administration and 23 times as active as beta-endorphin, calculated on a molar basis. Substitution of FK-33-824 for morphine in morphine-dependent rhesus monkeys (1.8 mg/kg IV at 4 hr intervals over 24 hr) prevented the appearance of a morphine withdrawal syndrome. FK-33-824 also abolished the morphine withdrawal syndrome in monkeys as assessed by a single dose suppression test (1.8 to 5.6 mg/kg IV enkephalin). Three drug naive rhesus monkeys were given unlimited access to intravenous FK-33-824 for a 4 week period. Doses of 0.01 and 0.032 mg/kg/inj of FK-33-824 did not maintain self-administration behavior. However, 0.1 mg/kg/inj did maintain self-administration behavior over a 5 week period. The average daily amount of FK-33-824 self-administered after 5 weeks exposure was about 13 mg/kg and the acute administration of naloxone precipitated an opiate withdrawal syndrome. Details of the behavioral procedures and schedule of reinforcement used for self-administration studies were not described [28].

In this report, intravenous self-administration of the synthetic enkephalin analog FK-33-824 was compared with morphine self-administration in morphine-dependent monkeys, maintained under second-order schedules of reinforcement. Results obtained confirm and extend the observations of Roemer and co-workers [28] that this synthetic enkephalin analog (FK-33-824) maintains intravenous self-administration and can be directly substituted for morphine over 56 hr without disruption of operant performance for drug reinforcement or signs of morphine withdrawal.

METHOD

Animals

Seven adolescent male rhesus monkeys *(Macaca mulatta)* were studied and each animal was used as its own control. Each monkey was physically dependent on morphine and the average history of morphine self-administration was 7 months (range 1.8 to 21.2 months).

Animals weighed between 6.0 and 7.0 kg at the beginning of the study.

Monkeys were maintained at ad lib weight throughout the study in an effort to compensate for the potentially weight-reducing effects of chronic morphine administration. Monkeys were weighed daily and given multiple vitamins, fresh fruit and vegetables to supplement a banana pellet diet. Water intake was measured twice daily. Monkeys were maintained in accordance with DHEW guidelines for the care and use of laboratory animals, and their health status was periodically monitored by a veterinarian.

Monkeys were surgically implanted with chronic indwelling venous catheters to permit intravenous drug self-administration. All surgical procedures were performed under aseptic conditions. Animals were anesthetized with 30 mg/kg pentobarbital IV and a silicon rubber catheter (I.D. 0.031 in.; O.D. 0.093 in.) was first implanted into the left internal jugular vein. Following surgery, animals were given 1 ml of longicil IM every other day for a total of 5 injections. In some instances, blockage of the catheter lumen required additional implantations. Four monkeys had jugular catheters and three monkeys had catheters implanted in the right internal iliac vein.

Monkeys worked at an operant task, in a specially designed restraining apparatus which allowed completely free movement of the arms and legs. The monkey was able to maintain a comfortable natural posture at all times, and to jump up or down, but did not have access to the top of his head, the point of the intravenous catheter exit. The restraining apparatus was placed in a well ventilated experimental chamber (interior dimensions: 28×30.5×54 in.), equipped with an operant response panel, a water dispenser, and an automatic feeder.

Apparatus

Delivery of banana pellets and drug infusions were contingent upon the operant response behavior of the monkey. Schedules of reinforcement were programmed by a silent transistor circuitry (BRS-Foringer 200 Series) located in the experimental area. Following completion of the response requirement, a single banana pellet (Noyes 1 gm) or 1 injection of drug solution was automatically dispensed. Drug solutions were delivered through a Model 1302 Lambda Pump (Harvard Apparatus Company) in a train of 10 pulses over 1 sec. Each pulse contained 10 lambda of fluid for a total volume of 100 lambda or 0.10 ml. The pump operation was audible to the monkey. Some sound attenuation was achieved by a ventilation fan located in the top of the experimental chamber.

An aluminum operant response panel (14×18 in.) contained 3 circular translucent response keys (2 in. dia.) in a horizontal row (separated by 3.5 in. center distance) at about the monkey's eye level. Only the center key was operative, and it was transluminated by colored stimulus lights (S+) associated with the conditions of food availability (red), drug availability (green) and time-out (white), when responses on the key had no programmed consequence. The occurrence of a drug or food reinforcement was signalled by a 1 sec flash of the appropriate colored light on a series of 3 quarter in. dia. circles located below the response keys, in a vertical row close to the pellet dispenser. A Plexiglas receptacle for banana pellets, located in the lower right hand corner of the response panel, was illuminated whenever a pellet was dispensed. The experimental chamber was dark except for the response panel lights.

The number and rate of responses and the occurrence of drug and food reinforcement were recorded on Gerbrands SHS-Cumulative Recorders and on electromechanical counters.

Procedures

Daily sequence of conditions. One hr of food availability was followed by 1 hr of drug availability and 2 hr of time-out periods. The first food availability session began each day at 7:00 a.m. A total of 4 periods of food availability and 4 periods of drug availability occurred in 4 hr blocks during each 24 hr period. The recurring sequences of food, drug, and time-out periods were designed to insure maximum food intake before drug intoxication as well as to increase schedule control over drug availability. Previous studies have shown that monkeys self-administer about the same amount of drug per day in temporally unrestricted and limited access conditions [10].

Experiments continued 24 hr each day, 7 days each week. Daily cleaning and weighing were completed during the morning time-out period between 9:00 a.m. and 10:00 a.m. Food and vegetable supplements were provided during the late afternoon time-out period at 5:00 p.m.

Reinforcement schedules. Food and drug self-administration were maintained under a second order schedule of reinforcement. A second order schedule is one in which the completion of each successive response requirement is reinforced with a discriminative stimulus (S+), which was previously associated with administration of the primary reinforcer, i.e., food or drug. The primary reinforcer itself is presented only intermittently according to a second reinforcement schedule. For example, under an FR 4 (VR 16:S) second-order schedule, an average of 16 responses on a variable ratio schedule produces a brief stimulus light (S+) which was formerly associated with food reinforcement. However, one banana pellet is delivered only after a fixed-ratio of 4 (FR 4) of the VR 16 response requirements have been completed. All monkeys worked on a second-order FR 4 (VR 16:S) schedule for food and an FR 4 or an FR 5 (VR 16:S) schedule for drug infusions.

Second-order schedules were used to minimize the possible disruptive effect of drug infusions on response rate. Since the monkey works to produce a discriminative stimulus previously associated with reinforcement, until completion of the specified response requirement yields a drug infusion, the interval between successive drug infusions is lengthened. The possible suppressant effects of a drug infusion on subsequent response behavior and/or the satiation effects of food reinforcement on subsequent food maintained responding are attenuated. Second-order schedules are effective in generating stable and sustained response rates of drug self-administration and are preferable to simple schedules in our experience. Second-order schedules have been shown to maintain drug self-administration behavior for cocaine, methahexitol and morphine [7, 8, 9, 15]. Moreover, it has been possible to maintain responding for a period of up to 1 hr under second-order schedules following which the monkey received only a single dose of cocaine (1.5 mg/kg) or morphine (5 mg/kg) [7, 9, 15].

Access to the period of drug availability was also contingent upon completion of a chaining procedure under control of a distinctive discriminative stimulus (S+), a blue light to the response key. Monkeys were required to emit 100 re-

sponses on a chain FR 10 (FR 10:S) schedule in order to turn on the drug availability session. These schedule procedures were in effect for all animals throughout the course of this study.

Substitution procedures. In order to evaluate the capacity of FK-33-824 to maintain self-administration behavior in morphine dependent monkeys, and to determine if the enkephalin was effective in preventing morphine withdrawal signs, a standard substitution procedure was employed [37]. Enkephalin was substituted for morphine for 10 consecutive drug sessions over a 56 hr period. However, data for the first substitution session was not included because the drug injections earned during that session emptied the catheter of morphine solution and only a few enkephalin infusions in fact reached the monkey. The problem of catheter dead space in brief substitution procedures is rarely discussed. However, we estimate that monkeys with jugular catheters in our particular set-up require infusion of approximately 0.5 ml to move the fluid column to the catheter tip whereas, monkeys implanted with iliac catheters require infusion of approximately 0.8 ml to move the column of fluid to the catheter tip. It is important to note that flushing the catheter with saline in no way obviates this problem, but rather insures that the first drug availability session resulted in several saline injections. Our solution to the catheter dead space problem was to discard the first session after enkephalin was substituted for morphine and the first session after morphine was substituted for enkephalin. In every instance, the number of infusions earned during these transition sessions were sufficient to clear the catheter of the previous drug solution.

Ten sessions of enkephalin substitution were followed by 18 consecutive sessions of morphine availability over four days. This sequence was followed over five consecutive weeks during which each of the five doses of FK-33-824 were studied. Control studies compared the effects of morphine dose reduction per infusion over 3 dose levels (0.5, 0.25 and 0.125 mg/kg) with saline. Three days or 12 sessions of morphine dose reduction were followed by 4 days or 16 sessions at the usual dose of morphine per injection.

Monkeys were observed for evidence of withdrawal signs following enkephalin substitution, during each 2 hr time-out period which followed the 8:00 a.m. and 12 noon drug availability sessions.

Drug solutions and dosage. Morphine sulphate was dissolved in sterile saline (0.9%) and diluted to the appropriate concentration for individual monkeys. Drug doses are expressed in terms of salts. Three monkeys (8525, A105, B285) worked for morphine injections at a dose of 0.25 mg/kg per infusion. Two monkeys (B225, B255) worked for morphine injections of 0.5 mg/kg per infusion. The number of morphine infusions available in each 1 hr session was limited to 40.

The enkephalin analog was provided by the Sandoz Pharmaceutical Co. in lyophilised form, in sealed sterile ampules. Ampules were kept under refrigeration and fresh solutions were prepared for each substitution dose. Enkephalin

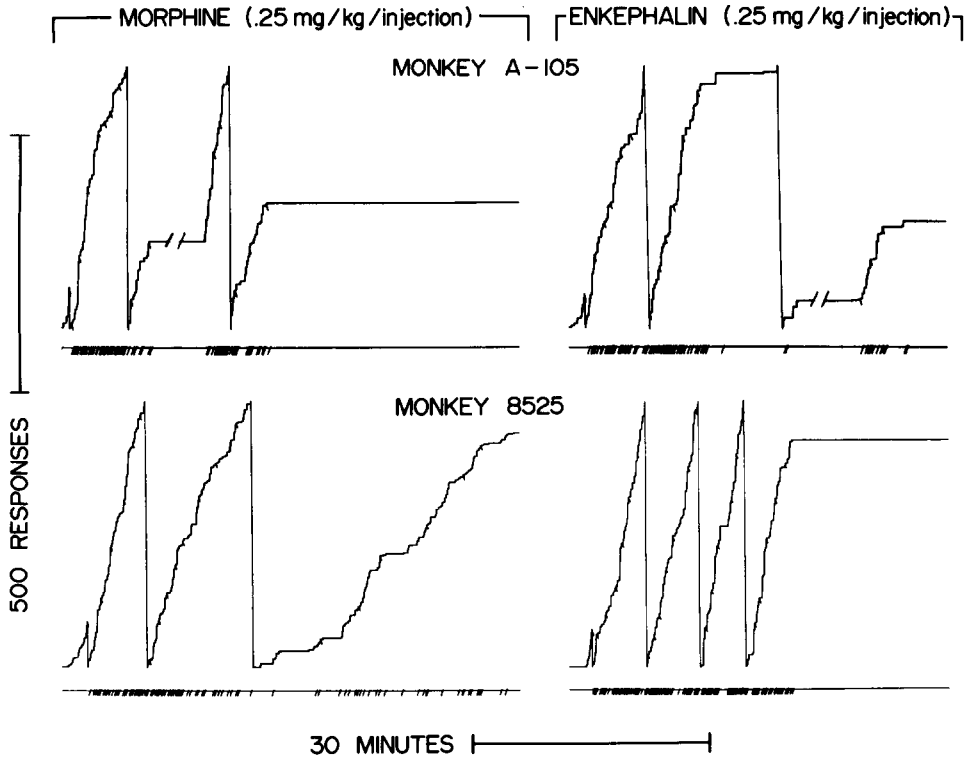

FIG. 1. Cumulative records of responding for equivalent doses of morphine and enkephalin. Responding for morphine or enkephalin was maintained on a second-order FR 4 (VR 16:S) reinforcement schedule. Each session was initiated following completion of a chaining procedure on a second-order schedule [FR 10 (FR 10:S)]. Each downward deflection of the response pen indicates the occurrence of a drug injection. Each downward deflection of the baseline pen denotes the occurrence of a secondary reinforcing stimulus (S+). Broken lines in the response record indicate pauses which exceed 10 min.

was dissolved in sterile Ringers solution diluted to the appropriate concentration for individual monkeys. The design of this study, and the number of enkephalin doses examined were dictated by the limited supply of enkephalin available. Each of 5 doses of enkephalin (0.5, 125, 0.125, 0.05 and 0.01 mg/kg/per infusion) were substituted for morphine during 10 consecutive sessions over a period of 5 weeks. All other experimental conditions were identical during the enkephalin substitution period.

RESULTS

All monkeys self-administered the synthetic enkephalin analog FK-33-824 when it was substituted for morphine over 10 sessions or 56 hr. Typical cumulative records of responses for morphine infusions and for enkephalin infusions are shown in Fig. 1. These cumulative records were selected from sessions at the same time of day, since there is session to session variability in response patterns within individual monkeys. Monkey A105 showed comparable patterns of responding for equivalent doses of morphine and enkephalin (0.25 mg/kg/inj). Monkey 8525 responded at a higher rate for enkephalin than for morphine injections at equivalent doses per injection.

Each individual monkey's actual daily intake of morphine during baseline and enkephalin during five substitution periods is shown in Table 1. The 12 day morphine baseline (Column 2) can be compared with the daily dose of enkephalin administered at the same dose per injection as the accustomed maintenance dose (italicized values in Columns 3 and 4). One monkey (8525) self-administered an almost identical amount of enkephalin at the usual maintenance dose per drug infusion. Two monkeys (B225 and A105) administered less enkephalin than morphine, and two monkeys (B255 and B285) self-administered more enkephalin than morphine at the usual maintenance dose per infusion.

The progressive decrease in daily enkephalin intake as a function of successive reductions in the dose of enkephalin per injection shown by individual monkeys in Table 1 (Column 3 to 7) is shown for the entire group in Fig. 2. Data are presented as mg/kg/session of enkephalin (mean ± SE) for 9 sessions at each of the five doses of enkephalin studied. The

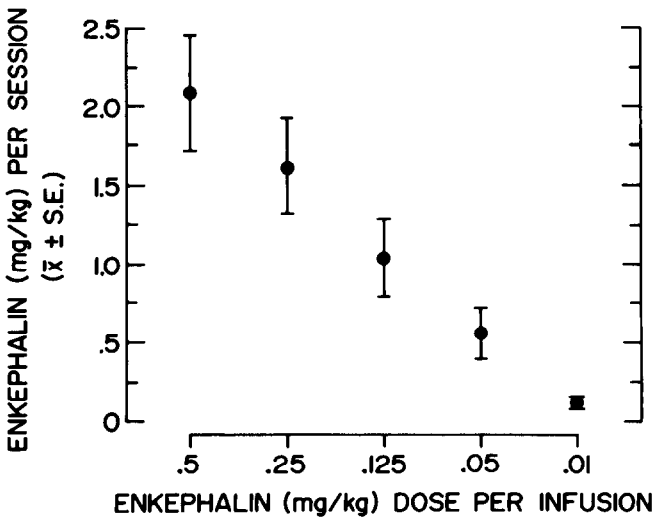

FIG. 2. Average enkephalin intake per session as a function of dose per infusion. Each data point represents an average of 9 sessions for 5 monkeys (mean ± SE). Enekphalin self-administration decreased significantly over a dosage range of 0.25 to 0.01 mg/kg/inj ($p<0.05$).

average number of enkephalin injections self-administered per session decreased linearly as the dose per injection decreased over the range of doses studied (0.5 to 0.01 mg/kg/inj). At doses of 0.25 mg/kg/inj and below, each successive dose reduction resulted in a significant decrease in average drug intake from the preceding dose as evaluated by t tests ($p<0.05$). Since monkeys could earn a maximum of 40 injections in each drug availability session, it would have been possible to maintain the usual level of drug intake except at the lowest enkephalin dose (0.01 mg/kg/inj).

Analysis of the average amount of morphine (mg/kg) taken during the sessions which intervened between each enkephalin substitution showed that monkeys took significantly more morphine following 0.5 mg/kg/inj of enkephalin than during the baseline ($p<0.05$). However, there were no significant changes in morphine self-administration after substitution of enkephalin over a dose range of 0.25 to 0.01

TABLE 1

COMPARISON OF DAILY ENKEPHALIN + MORPHINE INTAKE BY INDIVIDUAL MONKEYS

Monkey Number and Usual Morphine Dose per Injection	Baseline Morphine Intake for 12 Days (mg/kg/day) Mean ± S.E.	Average Enkephalin Intake as a Function of Dose per Injection (mg/kg/day)				
		Dose 0.5	0.25	0.125	0.05	0.01
B255 (0.5 mg/kg/inj)	4.3 ± 0.22	*5.56*	3.32	1.28	0.72	0.28
B225 (0.5 mg/kg/inj)	6.2 ± 0.46	*4.24*	5.0	2.68	0.88	0.24
A105 (0.25 mg/kg/inj)	11.81 ± 0.63	10.24	*6.12*	5.80	2.72	0.40
B285 (0.25 mg/kg/inj)	7.35 ± 0.72	12.44	*10.76*	6.68	4.24	1.0
8525 (0.25 mg/kg/inj)	7.48 ± 0.54	9.12	*7.12*	4.60	2.80	0.52

mg/kg/inj as evaluated by t tests. Monkeys maintained an average morphine intake of 1.68 mg/kg/session throughout this period.

Morphine and enkephalin appeared to be approximately equivalent in their capacity to maintain morphine dependence, since no monkey showed evidence of withdrawal signs during enkephalin substitution, except at the lowest enkephalin dose. Some mild tremors were observed following substitution of an enkephalin dose of 0.01 mg/kg.

Comparison of Morphine and Enkephalin Injections

It is of some interest to compare these data on substitution of decreasing doses of enkephalin with the effects of reduction in the dose of morphine per injection, under comparable conditions. Figure 3 compares the average number of drug infusions self-administered per session by 5 monkeys studied in the enkephalin dose-reduction series and by 4 monkeys studied in the morphine dose-reduction series. Two monkeys were studied in both series. Morphine data are based on an average of 12 sessions, and enkephalin data are based on an average of 9 sessions at each dose point. When the dose of morphine per injection was decreased from 0.5 to 0.125 mg/kg, morphine dependent monkeys increased the number of drug injections in an arithmetic progression. Saline substitution usually resulted in an initial self-administration of a large number of infusions, however, saline infusions decreased with repeated exposure over 12 sessions or three days.

Morphine dependent monkeys maintained on enkaphalin also increased the number of enkephalin infusions per session as a function of reduction in the enkephalin dose per injection. However, it is apparent from Fig. 3 that the rate of increase was slightly less than for the morphine group. When the dose per injection of morphine and enkephalin were 0.5 to 0.25 mg/kg, the number of infusions earned per session were virtually identical. The enkephalin group self-administered about the same number of injections at enkephalin doses of 0.05 and 0.01 mg/kg/inj as the morphine group self-administered at a dose of 0.125 mg/kg/inj. Statistical evaluation indicated there were no differences in the number of morphine and enkephalin injections taken at equivalent doses per injection.

Although monkeys had an opportunity to self-administer 40 drug injections during each drug availability session, no monkey persisted in taking all the enkephalin potentially available at the lowest doses per injection (0.05 and 0.01 mg/kg). Rather each monkey self-administered some enkephalin in each session and took significantly more injections at doses of 0.25, 0.125, 0.05 and 0.01 mg/kg/inj than at 0.5 mg/kg/inj as evaluated by t-tests $p<0.05$). However, the number of enkephalin injections administered per session at doses between 0.25 and 0.01 mg/kg/inj did not differ significantly from each other. Comparison of sessions 3 through 6 with Sessions 7 through 10 revealed no significant changes in number of injections through time as a function of repeated exposure at any dose of enkephalin.

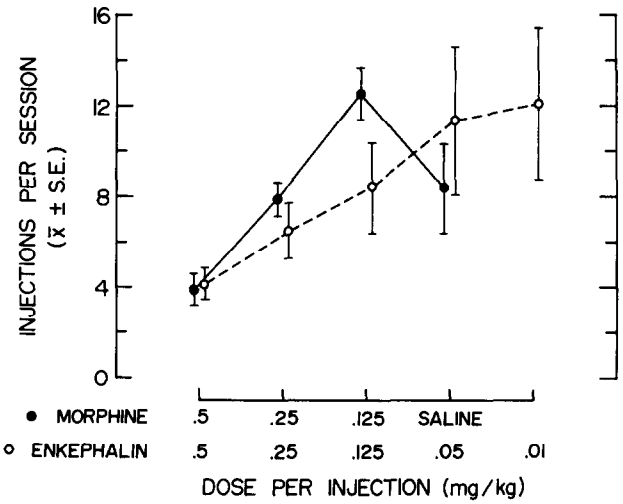

FIG. 3. Comparison of morphine and enkephalin self-administration during progressive reductions in the dose per injection. The dose per injection is shown on the abscissa and the average number of injections per session on the ordinate. Each morphine data point represents 12 consecutive sessions and 4 monkeys. Each enkephalin data point represents 9 sessions and 5 monkeys.

Table 2 presents the actual amount of morphine (mg/kg/session) taken by each of the 4 individual monkeys shown in Fig. 3. Comparison of Tables 1 and 2 shows that morphine intake was more consistent than enkephalin intake across each dose reduction series. However, individual monkeys maintained on morphine were less consistent with the group average than individuals maintained on

TABLE 2

EFFECTS OF MORPHINE DOSE REDUCTION PER INJECTON ON MORPHINE INTAKE BY INDIVIDUAL MONKEYS OVER 12 SESSIONS

Monkey (Maintenance Morphine Dose per Injection = 0.5 mg/kg)	Dose	Morphine Intake as a Function of Dose per Drug Injection (mg/kg/session)		
		0.5 mg/kg/inj	0.25 mg/kg/inj	0.125 mg/kg/inj
B245		1.44	1.64	1.77
B225		1.34	1.91	1.23
8525		2.98	2.48	1.77
8520		1.88	1.54	1.26
	Mean	1.91	1.89	1.51
	S.E.	0.38	0.21	0.15

enkephalin. As morphine dose per injection was decreased from 0.5 to 0.125 mg/kg, two monkeys (8525 and 8520) showed a linear decrease in morphine intake per session. One monkey (B245) showed an increase in morphine intake per session and the other monkey (B225) was somewhat variable. In contrast, although monkeys self-administered equivalent amounts of enkephalin (mg/kg) at 0.5 and 0.25 mg/kg/inj, subsequent reductions in the dose available per injection were accompanied by significant decreases in the amount of enkephalin actually taken ($p<0.05$) (Table 1, Fig. 2). Consequently, despite the fact that monkeys increased the number of enkephalin injections, they did not maintain stable levels of enkephalin intake across all doses per injection.

Food and Water Self-Administration

There were no significant changes in average water intake per day across this series of alternating morphine sessions and enkephalin substitution sessions. However, the average number of food pellets earned were significantly lower during enkephalin substitution than during the immediately preceding morphine availability period. Enkephalin substitution at doses of 0.5 and 0.25 mg/kg resulted in an average daily reduction of 17 and 10 food pellets, respectively. This decrease was significant as evaluated by matched t-tests ($p<0.01$). An enkephalin dose of 0.125 mg/kg/inj was associated with an average daily increase of 11 food pellets which was also statistically significant ($p<0.05$). The lowest doses of enkephalin (0.05 and 0.01 mg/kg/inj) were associated with an average reduction of 9 and 8 food pellets but these changes were not statistically significant.

In order to determine if food intake changed during the 10 sessions of enkephalin substitution, average food intake during Sessions 2 through 5 was compared with Sessions 7 through 10 at each enkephalin dose. There were no significant differences in average food intake at the beginning and end of each enkephalin substitution period, rather food intake remained depressed throughout. A qualitative examination of the cumulative records of responding for food revealed no consistent changes in response patterns either within or across animals. The statistically significant depression in food intake was not accompanied by any change in body weight over the 10 enkephalin substitution sessions, a period of two-and-one-half days.

DISCUSSION

These data clearly indicate that the synthetic enkephalin analog FK-33-824 is reinforcing and maintains response behavior leading to its administration in monkeys. The orderly dose-dependent decrease in the amount of enkephalin self-administered as a function of reduction in enkephalin dose per infusion (Fig. 2) suggests that the enkephalin analog, rather than any unspecified variable was in fact controlling the response behavior. These data confirm and extend the behavioral observations of Roemer and coworkers [28] that this enkephalin analog is self-administered and will prevent the appearance of opiate withdrawal signs when equivalent doses are substituted for morphine dependent monkeys. These data are also consistent with the observations of Stein [30] that rats will self-administer leucine and methionine enkephalin intraventricularly. Leucine enkephalin was slightly preferred to morphine and methionine enkephalin in that preparation [30].

FK-33-824 does not appear to be readily discriminated from morphine by morphine dependent monkeys. Qualitative examinations of cumulative records (Fig. 1) and quantitative examination of injections earned at equivalent dose levels (Fig. 3) indicates that there is no disruption of customary response patterns as a function of substitution of enkephalin for morphine at equivalent doses per injection. Some monkeys self-administered approximately equivalent amounts of morphine and enkephalin (Table 1, Fig. 3).

When morphine dependent monkeys are exposed to a reduction in dose per injection or challenged by a narcotic antagonist, it has been consistently observed that monkeys will self-administer additional drug injections [10, 20, 35, 36]. This same phenomenon was observed in the enkephalin dose reduction series (Fig. 3). Since monkeys did not increase the number of enkephalin injections enough to maintain the usual level of drug intake per session, it can be assumed that the immediate reinforcing quality of the drug infusion controlled the response behavior.

The basis for the significant suppression of food maintained responding at the high doses of enkephalin is unclear. Food maintained responding remained depressed throughout the 10 enkephalin substitution sessions and there was no recovery towards the end of the series. Since food sessions preceded drug sessions, it is unlikely that this reflects any suppressive effects of enkephalin intoxication on responding.

In commenting on the possible physiological significance of the opiate peptides, Kosterlitz and Hughes [17] suggest these have much broader functions than analgesia and the so-called side effects of opiates may be in fact exaggerated physiological responses. Opiates are known to suppress intestinal motility and electrically induced contractions of guinea pig ileum are inhibited by enkephalins and by opiates [17]. Consequently, it is possible that enkephalin produces side effects in monkeys similar to those of morphine in the naive animal, i.e., nausea, and decreases in intestinal motility which affect food consumpiton.

Alternatively, this enkephalin analog may have increased gastrointestinal motility which was associated with decreased food consumption. This explanation is somewhat more compelling, since administration of FK-33-824 to healthy adult males resulted in "an impressive increase in bowel sounds" in 50 to 60% of the subjects studied [31]. Bowel sounds increased within 3 to 10 min following intramuscular injection of FK-33-824 (0.1 to 1.2 mg) and persisted for 30 to 60 min [31]. Von Graffenried and coworkers [31] did not comment on the possible mechanism by which FK-33-824 induced the increased gastrointestinal motility observed. This effect of an enkephalin analog is the opposite of that of morphine, even though the data of Roemer and coworkers [28] indicate cross tolerance and dependence between morphine and enkephalin.

Carney and Rosecrans [3] have observed that intraventricular injection of morphine and enkephalin produced dose-related decreases in water maintained responding in water deprived rats. Data were interpreted as consistent with an extensive literature which shows that opiates produce dose-related decreases in schedule controlled responding [3]. In the present study, water was freely available and there were no significant changes in consumption as a function of enkephalin or morphine availability.

The discovery of opiate receptors and endorphins have prompted many speculations about the biological and behavioral functions of the endogenous ligands. Among the many

provocative suggestions offered, several seem potentially relevant to these findings that a synthetic enkephalin analog is reinforcing for rhesus monkey. Since opiate receptors are highly concentrated in the limbic system, Goldstein [11] has suggested that endorphin may play a central role in the control of affective states and possibly in appetitive drives for food, water, and sex which are known to be associated with limbic system function. Byck [2] has suggested that "enkephalin is a controlling neurotransmitter and its binding to opiate receptors determines mood state as well as influencing respiratory and sleep patterns". Byck suggests that the endogenous ligands with morphine like activity regulate feelings of euphoria in a manner similar to the action of acute doses of morphine [2]. Kosterlitz and Hughes [17] have cautioned against "speculation for which the experimental basis is insecure". However, they also indicate that "in principal, it is likely that the peptides will mimic the actions of morphine, such as limitation of experience of pain, depression of respiration, constipation, changes in the extrapyramidal motor system and euphoric changes of mood. It is further likely that the various peptides, the long chain endorphins and the short chain enkephalins, will have physiological patterns with subtle differences in action."

The first report of the effects of acute administration of the enkephalin analog FK-33-824 in man indicated there were several unexpected differences between it and morphine [31]. In addition to the increased gastrointestinal motility described earlier, "Classic symptoms of morphine, such as changes in emotional behavior or mental alertness, formication and nausea were not observed" [31]. Moreover, the clinical effects were not prevented by pretreatment with 10 mg of the narcotic antagonist nalorphine. Subjects reported "a feeling of heaviness in all of the muscles of the body, often combined with a feeling of oppression on the chest or tightness in the throat which induced a certain amount of anxiety" ([31], p. 729). These symptoms occurred within 3 to 5 min of FK-33-824 injection, reached peak intensity within 5 to 15 min and diminished within 15 to 30 min. Other effects of this enkephalin analog included whole body flush, redness of the face, conjunctival injection, excessive edema of the ocular conjunctiva, and vasomotor rhinitis [31]. In effect, these signs and symptoms appear to resemble opiate withdrawal more than opiate intoxication. Differences between the effects of enkephalin analogs and morphine on behavior have also been reported in studies of rodents [13,14].

Although the reinforcing properties of this synthetic enkephalin analog, FK-33-824 and morphine appear comparable in both the drug-naive [28] and morphine-dependent rhesus monkey, the studies of Von Graffenried and coworkers [31] indicate that there are significant unexplained differences in the behavior effects of FK-33-824 and morphine in drug-naive humans. The acute effects described in man [31] appear to be rather aversive and not consistent with the hypothetical characteristics of a compound likely to be sought after and abused. However, many abused drugs, including opiates, have highly aversive properties in naive users (cf. [18,19]). The way in which aversive drug effects in a naive user eventually come to maintain repetitive drug use and abuse in the same individual is not understood. The possibility that aversive consequences of drug use may be an important aspect of the process of drug reinforcement has rarely been considered seriously [18,19], despite a large literature which testifies to the capacity of seemingly aversive events to maintain behavior leading to their administration (cf. [24,25]). Self-administration of a previously avoided electric shock is only one example of this more general phenomenon [16]. Consequently, one implication of the apparent discrepancies in the behavioral effects of the enkephalin analog FK-33-824 in man and monkey, is to illustrate how little is known about the critical factors which account for the reinforcing properties of drugs.

The finding that synthetic polypeptides are reinforcing has potential implications for analysis of the biological aspects of the process of drug reinforcement. There is now considerable evidence that both opiates and opiate antagonists directly effect hypothalamic and pituitary hormones [4, 6, 11, 21, 22, 23]. Insofar as certain polypeptides (e.g., luteinizing hormone) may be directly effected by opiate drugs, it is not unreasonable to ask if these substances in turn modulate biological and subjective responsivity to drug effects, and are one component of the process which we call reinforcement. Further examination of the behavioral pharmacology of these synthetic polypeptide analogs of enkephalin should clarify the possible contribution of the natural polypeptides to the reinforcing characteristics of opiate drugs.

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