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Effects of the Neuropeptide DG-AVP on Morphine and Food Self-Administration by Dependent Rhesus Monkey

NANCY K. MELLO AND JACK H. MENDELSON

Alcohol and Drug Abuse Research Center
Harvard Medical School-McLean Hospital, Belmont, MA

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Morphine self-administration Food self-administration Neuropeptide pretreatment DG-AVP
Morphine dependent monkey

IT IS now well established that opiate drugs effect hormones of hypothalamic and pituitary origin in animal models [2,3] and in man [9, 10, 11]. The rapid suppression of luteinizing hormone levels and the secondary decrease in testosterone following an acute dose of heroin or morphine, have prompted the speculation that rapid changes in the hormones may comprise one aspect of drug reinforcement [8]. There is increasing evidence that pituitary peptides and gonadal steroids may act at CNS receptor sites to induce rapid changes in physiological function and behavior [6]. The corollary argument is that pretreatment with peptide hormones of hypothalamic pituitary origin may in turn influence the self-administration of opiates.

Recently, van Ree and De Wied [14] reported that pretreatment with neuropeptides related to hypothalamic-neurohypophyseal hormones significantly suppressed intravenous heroin self-administration in rat. Groups of naive rats, not dependent on opiates, were given six-hour training sessions during which intravenous injection of a heroin solution (0.25 ml over 15 sec) was available on a continuous reinforcement schedule. Control animals did not self-administer saline under these conditions, whereas 80% of the rats exposed to heroin reached the criterion of 6 injections per day or more. Some groups of rats were pretreated with a series of related neuropeptides or placebo. It was found that pretreatment with desglycinamide^9-arginine^8-vasopressin (DG-AVP), one hour before heroin availability, significantly decreased heroin self-administration in comparison to placebo controls [14]. In addition to suppressing heroin acquisition in naive animals, pretreatment with DG-AVP also attenuated heroin self-administration following three days of heroin exposure. At doses of 1 and 5 mcg, DG-AVP resulted in a dose-dependent reduction in heroin self-administration in comparison to placebo controls. A related peptide, pressinamide, the covalent ring of vasopressin, also inhibited heroin self-administration behavior in comparison to controls. However, oxytocin and its C-terminal tripeptide, prolyl-leucyl-glycinamide, slightly increased heroin self-administration in comparison to placebo controls and to animals pretreated with DG-AVP and pressinamide [14]. Pretreatment with a related pituitary peptide, desglycinamide^9-lysine^8-vasopressin (DG-AVP) has also been shown to facilitate acquisition of alcohol drinking in rat [5].

Van Ree and De Wied [14] interpret their data to suggest that DG-AVP may result in "a decrease in the reinforcing stimulus properties of this narcotic analgesic," and argue further that "neurohypophyseal hormones modulate the reinforcing properties of heroin by interfering with dopaminergic transmission" (p. 202). The present study was undertaken to evaluate the effects of DG-AVP on food and morphine self-administration in morphine dependent rhesus monkeys. Pretreatment with saline and with DG-AVP vehicle placebo were compared with two doses of DG-AVP over five consecutive weeks.

METHOD

Animals

Three morphine dependent male rhesus monkeys *(Macaca mulatta)* were studied and each animal was used as its own control. Each monkey was surgically implanted with a chronic indwelling venous catheter to permit intravenous drug self-administration (cf. [7]). Monkey A105 and B225

had a long history of morphine self-administration, 715 and 680 days, respectively. Monkey B255 had self-administered morphine for 288 days at the beginning of this study. Each monkey weighed between 6.0 and 7.0 kg.

Monkeys were maintained at ad lib weight and were 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 worked at an operant task, in a 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 could jump up and down, but did not have access to the top of his head, the point of intravenous catheter exit. The restraining apparatus was placed in a well ventilated experimental chamber, equipped with an operant response panel, a water dispenser, and banana pellet dispensers.

Apparatus

Schedules of reinforcement were programmed by silent transistor circuitry (BRS-Foringer 200 Series). A response key in the center of the operant panel 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. Completion of the response requirement under the appropriate stimulus condition resulted in delivery of one banana pellet (Noyes 1 gm) or one injection of morphine solution (0.25 mg/kg). A total volume of 0.10 ml of morphine solution was delivered in a train of 10 pulses over one second. The occurrence of drug or food delivery was signalled by a one second flash of the appropriate colored light below the response key. The apparatus dimensions and other details have been published previously [7].

Procedures

Daily sequence of conditions. Beginning at 7:00 a.m. each day, one hour of food availability was followed by one hour of drug availability and two hours of time-out. Four periods of food availability (7:00 a.m., 11:00 a.m., 3:00 p.m., 7:00 p.m.) and four periods of drug availability (8:00 a.m., 12:00 noon, 4:00 p.m., 8:00 p.m.) occurred during each 24 hr period. Experiments continued 24 hr each day, seven 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. Fruit and vegetable supplements were provided during the late afternoon time-out period at 5:00 p.m.

All monkeys worked on a second-order schedule for food [FR 4 (VR16:S)] and for drug infusion [FR 5 (VR16:S)]. Completion of each successive response requirement (VR 16) was 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 was presented only after a fixed ratio of four (FR 4) or five (FR 5) of the VR 16 response requirements were completed.

Access to the period of drug availability was also contingent upon completion of a chaining procedure under control of a blue light (S+) on the response key. Monkeys were required to make 100 responses on a chain [FR 10 (FR10:S)] schedule in order to turn on the drug availability session. These procedures were in effect for all animals throughout the course of the study.

Pre-treatment conditions. In order to evaluate the effect of DG-AVP on morphine and food self-administration behavior in morphine dependent monkeys, animals were pretreated with the active compound, a placebo vehicle, and saline. Each pretreatment condition was in effect for five consecutive days. The sequence of pretreatment conditions was as follows: (1) saline; (2) DG-AVP vehicle placebo; (3) DG-AVP (25 mcg/kg/inj); (4) DG-AVP (125 mcg/kg/inj). The design of this study, and the number of pretreatment sessions observed under DG-AVP and vehicle placebo conditions were dictated in part by the limited supply of these compounds.

Each drug or control solution was administered subcutaneously at 10:45 a.m. and at 2:45 p.m., immediately before the morning and afternoon food and drug sessions. Consequently a total of ten food and ten drug pretreatment sessions occurred in each five day condition. Control (nonpretreatment) food and drug sessions occurred 4 hr before and 4 hr after the experimental sessions each day beginning at 7:00 a.m. and 7:00 p.m.

Monkeys were observed twice daily for evidence of possible DG-AVP side effects indicated by changes in food and water intake, stool composition, or general activity.

Drug solution. 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. Forty morphine injections (0.25 mg/kg/inj) were potentially available in each of the four daily 1 hr sessions.

Sterile ampules of DG-AVP and DG-AVP vehicle placebo were provided by the Organon Scientific Development Group. Sterile ampules were kept under refrigeration and fresh solutions were prepared immediately prior to each pretreatment dose. Equivalent volumes of DG-AVP, DG-AVP vehicle placebo, and saline were administered subcutaneously.

RESULTS

Morphine Intake During Pre-Treatment and Control Sessions

Morphine (mg/kg) self-administered during the two daily DG-AVP pretreatment sessions over 5 days, was compared with morphine (mg/kg) taken during the equivalent saline and DG-AVP placebo pretreatment sessions for individual monkeys. There were no differences in morphine self-administration during DG-AVP pretreatment sessions and DG-AVP vehicle placebo and saline pretreatment sessions as evaluated by *t*-tests. Moreover, morphine self-administration following pretreatment with the high dose of DG-AVP (125 mcg/kg) was not significantly different from morphine intake following pretreatment with the low dose of DG-AVP (25 mcg/kg). Finally, morphine intake during the two pretreatment sessions (12:00 noon and 4:00 p.m.) in each of the pretreatment conditions (saline, DG-AVP vehicle, and DG-AVP 25 and 125 mcg/kg) also did not differ significantly from morphine intake during the noon and 4:00 p.m. sessions over a 20 day baseline period.

Since the duration of action of DG-AVP is estimated to be 5 hr (H. Van Riezen, personal communication, 1978), it seemed possible that the maximal cumulative effect of DG-AVP pretreatment might occur in the 8:00 p.m. drug session. Alternatively, monkeys might have concentrated morphine self-administration in the 8:00 a.m. drug session

TABLE 1

DAILY MORPHINE (MG/KG) SELF-ADMINISTRATION (MEAN ± SE) BEFORE AND AFTER DG-AVP AND PLACEBO PRE-TREATMENT

		Pre-Treatment Conditions			
Animals	Morphine Baseline (20 Days)	Saline	DG-AVP Vehicle Placebo	DG-AVP 25 mcg/kg	DG-AVP 125 mcg/kg
B255	5.5 ± 0.2	4.3 ± 0.1†	4.0 ± 0.2‡	4.3 ± 0.6*	5.2 ± 0.6
B225	8.9 ± 0.6	10.7 ± 1.2	8.9 ± 1.1	9.9 ± 0.9	9.7 ± 0.7
A105	11.8 ± 0.4	12.1 ± 0.5	14.0 ± 0.5¶	12.5 ± 0.9	14.1 ± 0.9§

Morphine intake lower than 20 day baseline (t-tests)
*$p<0.02$.
†$p<0.01$.
‡$p<0.001$.

Morphine intake higher than 20 day baseline (t-tests)
§$p<0.02$.
¶$p<0.01$.

TABLE 2

DAILY FOOD SELF-ADMINISTRATION (MEAN ± SE BANANA PELLETS) BEFORE AND AFTER DG-AVP AND PLACEBO PRE-TREATMENT

		Pre-Treatment Conditions			
Animals	Food Baseline (20 Days)	Saline	DG-AVP Vehicle Placebo	DG-AVP 25 mcg/kg	DG-AVP 125 mcg/kg
B255	122.0 ± 4.9	123.6 ± 4.4	97.4 ± 6.8*	105.6 ± 9.2	110.2 ± 7.8
B225	123.6 ± 8.9	100.4 ± 26.7	97.8 ± 8.7	89.8 ± 17.5	110.2 ± 16.2
A105	155.8 ± 4.8	132.6 ± *	124.8 ± 7.9*	143.6 ± 10.0	138.4 ± 9.7

*A significant decrease from baseline as evaluated by t-tests ($p<0.05$).

before daily DG-AVP or placebo pretreatment. If so, the lack of DG-AVP effect observed during the pretreatment drug sessions at 12:00 noon and 4:00 p.m. could be an artifact of an aberrant morphine self-administration pattern. However, neither of these possibilities were substantiated when the 8:00 a.m. and 8:00 p.m. drug sessions were compared across baseline, saline, DG-AVP vehicle placebo, and high and low dose DG-AVP pretreatment conditions. Morphine self-administration during these sessions did not differ significantly after the low or high dose of DG-AVP or DG-AVP vehicle placebo. The effect of the lowest dose of DG-AVP (25 mcg/kg) was also indistinguishable from saline pretreatment and from baseline for all animals. However, the highest dose of DG-AVP (125 mcg/kg) was associated with a significant increase in morphine intake, in comparison to the 20 day baseline, during the 8:00 a.m. session ($p<0.05$) in two monkeys (A105 and B255). Monkey B255 also took significantly more morphine ($p<0.02$) during the 8:00 a.m. drug session in the high dose DG-AVP condition than during the saline pretreatment condition.

Daily Morphine Intake Across Pre-Treatment Conditions

The average total daily intake (mean ± SE) of morphine (mg/kg) across baseline and pretreatment conditions is shown for individual monkeys in Table 1. These data are consistent with the analysis of morphine intake by sessions. Daily morphine intake following low and high dose DG-AVP pretreatment did not differ from saline or vehicle placebo pretreatment for any monkey, as evaluated with t-tests. Monkey B255 had the shortest morphine self-administration history and had stabilized at the lowest daily dose of morphine prior to these studies. His daily morphine intake decreased significantly from baseline during saline, vehicle placebo and low dose of DG-AVP pretreatment conditions. However, during pretreatment with the highest dose of DG-AVP, morphine intake increased to a level which was not significantly different from baseline. Monkey B225 stabilized at a higher daily dose of morphine and was unaffected by the control or drug pretreatment conditions. Monkey A105 took more than twice the amount of morphine than

Monkey B255 during baseline. Both DG-AVP vehicle placebo and the high dose of DG-AVP were associated with a significant ($p<0.05$) increase in daily morphine intake in comparison to the 20 day baseline. This change is in the opposite direction from observations reported by van Ree and De Wied [14].

Daily Food Intake Across Pre-Treatment Conditions

Table 2 shows the number of banana pellets (mean ± SE) acquired each day by individual monkeys before and after DG-AVP and placebo pretreatment. Since variations in food intake are often an indication of a monkeys' general health, these data were collected to ascertain if DG-AVP had any discernible side-effects. Pretreatment with DG-AVP at the doses studied did not result in a significant change in food self-administered in comparison to saline or DG-AVP vehicle placebo pretreatment, or the 20-day baseline. Monkey B255 and Monkey B225 were closely matched in baseline and subsequent food intake. Monkey B225 maintained stable food self-administration across conditions and there were no significant differences between baseline, placebo control, and drug pretreatment conditions. Variations in food intake did not appear to be systematically related to the introduction of pretreatment conditions. However, pretreatment with the DG-AVP placebo vehicle resulted in a significant decrease in food self-administration by Monkey B255 in comparison to the 20 day food baseline and to saline pretreatment. Although drug pretreatment did not affect food self-administration by Monkey A105 (Row 2, Table 2), pretreatment with saline and with DG-AVP vehicle placebo were both associated with a significant reduction in daily food intake in comparison to the 20-day baseline ($p<0.05$). An analysis by individual sessions, identical to that described for individual drug sessions, did not reveal any significant or orderly relationship between the number of banana pellets acquired per session and pretreatment conditions.

There were no obvious changes in general activity, water intake, body weight, or stool composition suggestive of major side effects from DG-AVP administration.

DISCUSSION

Pretreatment with the neuropeptide DG-AVP did not decrease morphine self-administration by morphine dependent monkeys, in comparison to pretreatment with saline and DG-AVP vehicle placebo. The total daily morphine intake (mg/kg) and morphine intake during pretreatment sessions were not significantly affected by either a low (25 mcg/kg) or a high (125 mcg/kg) dose of DG-AVP. Food self-administration, studied under identical conditions, was also unaffected by DG-AVP pretreatment in comparison to control conditions. These findings are at variance with the report of van Ree and De Wied [14] that DG-AVP suppressed heroin self-administration in rats, in a dose dependent manner.

In addition to the obvious species differences, a number of other procedural differences may have contributed to the difference between the findings of van Ree and De Wied [14] and the present study. Experimentally naive rats with one or two days of heroin self-administration experience are not comparable to experienced monkeys, physically dependent upon morphine. An intermittent schedule of reinforcement, [FR 5 (VR:16 S)] controlled drug self-administration in the present study, whereas van Ree and De Wied [14] used a continuous reinforcement schedule. Although heroin effects the central nervous system somewhat more rapidly than morphine [12,13] and is alleged to be a somewhat more potent reinforcer, this difference probably cannot explain the current results. It would be expected that DG-AVP might exert a more suppressive effect on morphine than on heroin self-administration if morphine is in fact less potent.

The doses of DG-AVP (25 and 125 mcg/kg) used in the present study were equivalent to or higher than those found to be behaviorally active in rat. Doses of 1–5 mcg per 200 to 230 gram rats were effective in suppressing heroin self-administration [14]. A dose of 5 mcg is approximately equivalent to the lowest dose used in the present study (25 mcg/kg). Consequently, differences in relative dose probably cannot account for the differences in findings between rat and monkey. It is difficult to evaluate the effect of possible differences in session duration and duration of drug infusions between the two studies.

On the basis of their findings in rat, van Ree and De Wied concluded "the long lasting activities of the neuropeptide on drug reinforced behavior may have important consequences for the treatment of narcotic drug dependence" (p. 202). Since the human heroin abuser is usually addicted to heroin, physically dependent monkeys would appear to be an appropriate model to test the van Ree and De Wied hypothesis. The complete absence of suppressive effects of DG-AVP over a five-fold dose range indicates that this compound is not effective in modifying morphine self-administration by monkeys under the conditions studied.

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