CENTRALLY ADMINISTERED " AND \( \delta \) OPIOID AGONISTS INCREASE OPERANT RESPONDING FOR SACCHARIN

BLAKE A. GOSNELL* AND CHETAN K. PATEL†

*Department of Psychiatry, University of Wisconsin-Madison, Parkway Hospital, 6001 Research Park Boulevard, Madison, WI 53719
†Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109

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IT is well established that opioid agonists increase the intake of food and palatable fluids whereas opioid antagonists cause decreases [see (5,8)]. These effects appear to be centrally mediated and can be obtained by administration of selective agonists and antagonists for \( \mu \), \( \kappa \), and \( \delta \)-opioid receptors (8). It has been suggested that these changes in intake are due to effects on brain pathways mediating palatability and/or reward [see (5,26)]. This idea is supported by findings that opioids cause an increased intake of palatable solutions in nondeprived rats, that naloxone reduces sham-drinking of sucrose solution, and that antagonists reduce saccharin preference in two-choice tests (5,9–11).

In contrast to their effects on food intake in free-feeding tests, centrally administered opioids have been reported to cause decreases in food- and water-reinforced responding in rats on fixed-ratio schedules of reinforcement (4,17); this effect is typically observed with systemic opioid administration as well [see (3)]. With fixed-interval schedules of reinforcement, both increases and decreases in responding have been observed after central administration of opioids (1,16). In addition to the type of reinforcement schedule, factors that may influence the effect of opioid administration on operant and ingestive behavior include drug dose and the value of the ratio or interval in the reinforcement schedule (25). The length of the test session also appears to be important. For example, morphine was reported to reduce or have no effect on sucrose-reinforced responding and free-drinking intake of sucrose in test sessions lasting 0.5 h or less (7,22). However, when free-drinking sessions were lengthened to 100 min morphine caused an increase in sucrose intake (7).

Another factor that may contribute to the apparent discrepancy in the effects of opioids on fixed-ratio responding vs. free feeding is the common use of food or water restriction to facilitate the operant behavior. In free-feeding tests with rats that are food deprived or tested during the dark portion of the photoperiod, baseline eating is high and opioids generally cause decreases in intake (21); in nondeprived rats tested during the light portion of the photoperiod, agonists stimulate food intake. Thus, the use of deprivation may elevate baselines to a near-maximal level such that if a drug causes any disruption of this high rate of ingestion a decrease in intake is observed.

1 To whom requests for reprints should be addressed.
In our previous studies, selective \( \mu \)- or \( \delta \)-agonists were found to stimulate the intake of saccharin and salt solutions in nondeprived rats given daily access to the solutions (9–11). The present experiments were conducted to determine whether similar effects could be observed with operant procedures. With the use of a palatable solution as the reinforcer, water or food deprivation was not necessary. Session lengths were based upon those in which positive results were obtained in free-drinking and -feeding studies (9–11) and unpublished observations. Thus, unlike the decreases in operant responding typically observed after opioid administration, we hypothesized that selective \( \mu \)- and \( \delta \)-agonists would cause increases in responding.

**METHOD**

**Subjects**

Male Sprague-Dawley rats were used in all experiments. They were obtained from Charles River Laboratories, Inc. (Wilmington, MA) for the test of \([\text{D-Ala}^2, \text{N-Me-Phe}^4, \text{Gly}^\text{\bpeptide}]\)-enkephalin (DAMGO) (initial \( n = 8 \)) and from Harlan-Sprague-Dawley (Madison, WI) for all other tests. They were individually housed in stainless steel cages in a room in which the lights were on for 12 h daily. All procedures (except initial training) were performed during the light portion of the photoperiod. Body weights at the time of surgery ranged from 407–492 g for those rats included in the data analysis. Except where noted below, all rats had ad lib access to food and water at all times.

**Apparatus**

Eight operant chambers housed in sound-attenuating cubicles were used in the study (Med Associates, East Fairfield, VT). Each chamber was equipped with a houselight, exhaust fan, two levers, and two solenoid-activated liquid dispensers. The chambers were interfaced with an IBM computer; schedules of reinforcement and data collection were controlled with MED-PC and Medstate Notation software (Med Associates).

**Training Procedure**

Rats were trained to press the right lever of the operant chamber to obtain 0.1 ml of a 0.1% saccharin solution (w/v) with each lever-press (fixed-ratio 1). This was accomplished by placing rats in the chambers overnight with food available ad lib and the only fluid available being that obtained by pressing the lever. Generally, rats learned the response after one or two nights in the chamber. After training, all rats were placed in the chambers for 3 h daily (two groups of four rats at a time). This procedure was repeated daily until baselines were again judged to be stable. The first test session occurred 7 days after surgery.

**Testing Procedure**

Each rat was tested for operant responding after ICV injections of 0.9% saline (vehicle) and 0.02, 0.2, and 2 nmol of DAMGO. All peptides were purchased from Sigma Chemical Co. (St. Louis, MO). Injections (5 \( \mu \)l volume) were administered with an injector that extended 1.0 mm beyond the tip of the guide cannula. Fifteen minutes after injection, rats were placed in the operant chambers and lever-pressing was measured for 3 h, as in the training sessions. This test procedure was repeated three additional times, and every rat was tested with saline and with all doses of DAMGO. Test days were 3 days apart, and normal 3-h training sessions were conducted on the intervening days (no injections). Injection orders for testing the various doses were counterbalanced, and every dose was tested in at least one rat on each test day.

Separate groups of rats were trained and tested for operant responding after injections of the selective \( \delta \)-opioid agonist \([\text{D-Thr}^2\text{leucine enkephalin-Thr}] \) (DTLET, initial \( n = 10 \)) and the selective \( \kappa \)-opioid agonist dynorphin A analog \( \kappa \) ligand (DAKLI, initial \( n = 10 \)). These drugs were tested in a manner similar to that for testing DAMGO except that the training and testing sessions were 1 h in duration and the doses tested were 0.3, 1, and 3 nmol. The shorter sessions and higher dose range were used because these agonists were not expected to be as behaviorally disruptive as DAMGO. In preliminary studies, these doses of DAKLI were observed to stimulate 1-h intake of a high-fat and/or high-carbohydrate diet (unpublished). For those rats included in the data analyses, the range of body weights at the time of surgery was 347–420 g for the DTLET trials (\( n = 5 \)) and 344–392 g for the DAKLI trials (\( n = 6 \)). Test sessions began 22 days after surgery for both groups.

**Data Analysis**

All data are expressed as the number of reinforcers. For each agonist, the number of reinforcers obtained after each of the three doses was compared to the number of reinforcers obtained in the corresponding saline condition with Dunnett’s test (one tailed). Separate tests were performed on the cumula-
The effects of DAMGO are shown in Fig. 1. The number of reinforcers obtained in the 0.2-nmol condition was significantly greater than in the control condition at the 60- through 180-min measurements. It is evident from the figure that DAMGO changed the distribution of responses within the test session. In saline-treated rats, most of the reinforcers were obtained within the first 30 min of the test session. In the 0.2-nmol condition, most reinforcers were obtained in the second 30 min, and for the 2-nmol condition most reinforcers were obtained in the second hour of the session. This delay in responding produced by DAMGO may be due to initial sedative or cataleptic effects, as we have noted in previous studies (9, 10).

When data from individual rats were compared to preinjection baselines, it was noted that one rat responded much less after saline injection than during baseline. Excluding the data from this rat, however, did not change the basic pattern of results. The 0.2-nmol dose significantly increased responding at 60 and 90 min (p < 0.05). With this exclusion, the means for the 0-, 0.02-, 0.2-, and 2-nmol conditions at 60 min were 19 ± 4, 24 ± 9, 67 ± 26, and 13 ± 12 reinforcers, respectively.

The effects of DTLET and DAKLI are shown in Fig. 2. As with DAMGO, DTLET increased the number of responses and changed the distribution of responses within the session. At 30 min, the 0.3-nmol dose slightly increased the number of reinforcers obtained, whereas the 3-nmol dose slightly decreased this number; there were no significant differences from the control condition. At 60 min, the 1- and 3-nmol doses of DTLET caused significant increases in the number of reinforcers obtained; the increase in the 0.3-nmol condition fell just short of statistical significance. As with the results with DAMGO, this delayed effect may be due to initial sedative or catalepsy.

There were no significant effects of DAKLI at any dose or time point. An inspection of the data for individual rats indicated that at the 3-nmol dose responding was completely suppressed in some rats and increased in others such that the means for the various conditions were similar. Barrel-rolling and postural asymmetry were observed in some rats when given the 1- and 3-nmol doses of DAKLI; this behavior usually subsided in the 15-min interval between injection and the start of the test session.

**DISCUSSION**

These experiments demonstrate that μ- and δ-agonists facilitate operant responding for saccharin in nondeprived rats. The results are similar to those observed when no response was required to obtain saccharin (9). The use of a reinforcer that rats will consume in the absence of deprivation may account for the inconsistency between the increases in behavior observed in the present report and the decreases typically caused by opioids in rats when fixed-ratio responding is facilitated by food or water deprivation. It is possible, however, that the discrepancy is due to the use of a different reinforcer and not to the state of deprivation. As noted in the introductory section, several factors may influence the effects of opioids on operant behavior.

The α-agonist DAKLI caused some motor disturbances initially, and it is possible that these effects may have interfered with lever-pressing and saccharin consumption. In a longer test session, the impairment may have subsided such that an increase in lever-pressing would be observed in the latter part of the session, as was the case for DAMGO. While the present data cannot directly address these possibilities, it should be pointed out that the selective α-agonist U-50,488H also had no significant effect on saccharin intake and no behavioral depression was apparent (9). It is still possible, however, that these α-agonists caused some disruption of behavior that was not detectable by casual inspection.

The lack of effect of α-agonists on the intake of saccharin [(9) and present results] contrasts with reports that α-agonists increase the intake of lab chow, palatable food, sweetened milk, and sucrose solutions (14, 18, 19, 21). While this difference may be due to differences in procedures, route of drug administration, and the specific agonists and doses used, it is
also possible that the mechanisms controlling saccharin intake may be different from those involved in other forms of ingestion. There are some indications that access to saccharin and sucrose solutions have opposite effects (compared to control) on pain sensitivity, morphine analgesia, and opiate receptor binding affinity (6,20). Beczkowska et al. (2) recently reported evidence of a role for $\mu$- and $\kappa$-receptors in controlling the intake of a sucrose solution, but only a role for $\delta$-receptors in controlling the intake of a saccharin solution. While this report differs from the present study in terms of the involvement of $\mu$-receptors in saccharin intake, the two reports are in agreement about the apparent lack of involvement of $\kappa$-receptors. Beczkowska et al. (2) suggested that the differential involvement of various receptor types in saccharin vs. sucrose intake may be related to the differing postgestional consequences of the fluids. Our previous observation that $\kappa$-agonists had no effect on the intake of a palatable salt solution (11) may therefore be due to the fact that sodium chloride, like sodium saccharin, is noncaloric.

The mesolimbic dopaminergic system has been implicated in mediating the reinforcing effects of opioids, brain self-stimulation, and various other types of reward [see (23,26)].

In conditioned place preference studies, which are presumed to reflect the reinforcing properties of drugs, $\mu$- and $\delta$-agonists generally have been shown to produce place preferences whereas $\kappa$-agonists have been reported to produce place preferences and aversions (12,13,23). $\mu$- and $\delta$-Agonists, but not $\kappa$-agonists, have been shown to facilitate lateral hypothalamic self-stimulation (15). Further, $\mu$- and $\delta$-agonists cause dopamine release in the nucleus accumbens whereas $\kappa$-agonists decrease dopamine release (24). The pattern of positive results in the present experiment (i.e., increased responding with $\mu$- and $\delta$- but not $\kappa$-agonists) and in previous reports (9-11) is generally consistent with the possibility that opioids increase the intake of saccharin via enhancement of activity in the mesolimbic dopaminergic system.

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