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# Effects of Opiate Antagonists and Putative Mu- and Kappa-Agonists on Milk Intake in Rat and Squirrel Monkey

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LOCKE, K. W., D. R. BROWN AND S. G. HOLTZMAN. *Effects of opiate antagonists and putative mu- and kappa-agonists on milk intake in rat and squirrel monkey.* PHARMAC. BIOCHEM. BEHAV. 17(6) 1275–1279, 1982.—The effects of a number of relatively pure opiate antagonists (naloxone, naltrexone, diprenorphine), and putative mu- (morphine, etorphine) and kappa- (ketocyclazocine, ethylketocyclazocine) receptor agonists on sweetened condensed milk intake were examined over a broad range of doses in non-deprived rats and squirrel monkeys. The antagonists consistently decreased milk intake in both the rat and squirrel monkey. There were, however, species differences: diprenorphine was 30 times more potent than either naloxone or naltrexone in the squirrel monkey, but was of similar potency in the rat. The effects of the opiate agonists were more variable than those of the antagonists. In both species, all agonists decreased milk intake at high doses that also produced behavioral depression. Significant increases in drinking were produced only by low doses of ketocyclazocine and ethylketocyclazocine in the rat. The suppression of milk intake by the antagonists supports a modulatory role of opiate receptors in the control of drinking behavior, however, the effects of the agonists on drinking are less easily interpreted within this conceptual framework.

Opiate agonists  Opiate antagonists  Drinking  Milk intake  Endorphins  Primates  Opiate receptors

EVIDENCE has accumulated suggesting a role for the endogenous opiates in the control of ingestive behavior. Most of the evidence for this proposal has come from the consistent observation that naloxone, as well as other "pure" opiate antagonists, reduce both feeding and drinking in a number of species of animals under various experimental conditions [2–8, 10, 12–14, 18–20, 22, 23, 26–28, 32]. Water intake appears to be more sensitive to the suppressant effects of naloxone than is food intake [3], and is relatively independent of the deprivation state of the animal [10]. Naloxone reduces drinking induced by both intracellular (i.e., hypertonic saline) and extracellular (i.e., polyethylene glycol, isoproterenol, and angiotensin II) thirst stimuli [4, 6, 8, 10, 27] in a dose-dependent manner. Furthermore, the effects of the opiate antagonists on food and water intake are both stereospecific [4, 22, 27] and centrally-mediated [7,27]. These findings, taken together, suggest that the narcotic antagonists suppress ingestive behavior through an opiate receptor-mediated mechanism [28].

If the endogenous opioids are involved in the normal regulation of ingestive behavior [11, 25, 29, 30], the administration of exogenous opiate agonists might be expected to have an effect opposite that of the antagonists, and enhance food and water intake. However, in contrast to the well-documented suppression of intake caused by the opiate antagonists, exogenous opiates do not consistently produce the expected increase in food and water consumption. In several studies, certain doses of morphine and other opioids were found to increase fluid intake [1, 11, 16, 19, 22, 23, 29, 30]. The effects were biphasic, with stimulation of food and water intake at low doses and decreases in intake at higher doses. The increase in eating and drinking often occurred only after a delay of several hours from the time of drug administration [11, 29, 30]. On the other hand, some investigators have only been able to demonstrate a decrease in ingestive behavior following the administration of morphine [13, 19, 21, 26]. Thus opiate-induced increases in eating and drinking are not as robust phenomena as antagonist-induced decreases, and appear to be both dose- and situation-dependent.

The purpose of this investigation was to systematically characterize the effects of opiate agonists and antagonists on fluid intake. In an attempt to determine the extent to which endogenous opioids regulate ingestive behavior in the non-deprived animal, all drugs were tested in a sweetened condensed milk intake paradigm. Non-deprived animals readily consume sweetened condensed milk. Various antagonists (naloxone, naltrexone, diprenorphine) and agonists of the mu-(morphine, etorphine) and kappa-(ketocyclazocine, ethylketocyclazocine) opiate receptor subtypes [15], as well as the partial agonist buprenorphine, were tested. Diazepam, which is known to enhance appetite [9,31], was also evalu-

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ated as a control. In view of the well known species-dependency of the behavioral effects of the opiates, a second objective of this study was to compare the drug effects in two species, the rat and the squirrel monkey.

## METHOD

### *Subjects*

The subjects were male Sprague-Dawley derived rats (Holtzman Co., Madison, WI) and 10 adult squirrel monkeys *(Saimiri sciureus),* 1 male and 9 females. Rats and monkeys weighed between 300–350 and 500–950 g, respectively, at the beginning of the experiments. Rats were housed 2–3 per cage, and monkeys were housed in individual cages. Rats and monkeys were maintained in separate colony rooms illuminated from 0700 to 1900 hr, and at average temperatures of 72°F and 80°F, respectively. Food (Rodent Laboratory Chow No. 5008 and High Protein Monkey Chow No. 5045, Ralston Purina Co., St. Louis, MO) and water were available ad lib in the home cages.

### *Milk Intake Determinations*

A group of 8 rats was weighed and injected subcutaneously with drug or vehicle 20–30 min prior to each test session. Following the drug pretreatment period, rats were placed in small individual cages with wire tops and given access to graduated cylinders fitted with metal drinking spouts and containing the drinking solution, Borden's Eagle Brand sweetened condensed milk and water in a 1:2 ratio. At the end of the 30-min test session, milk intake was measured to the nearest 0.2 ml. Rats were returned to their home cages immediately following the test session. All animals underwent at least three familiarization trials with injections of vehicle before drug testing began. Drugs were administered on Tuesdays and Fridays with control sessions being conducted on Mondays and Thursdays. Doses of all drugs were administered in random order.

Six squirrel monkeys were weighed and injected with drug or vehicle in the thigh muscle 15–20 min prior to the test session. Following the drug pretreatment period, monkeys were presented with 100 ml graduated cylinders (No. LC-274, Wahmann Manufacturing Co., Timonium, MD) fitted with ball-point drinking tubes (No. LC-213) which contained the drinking solution (sweetened condensed milk and water in a 1:5 ratio). Milk consumption was measured to the nearest 1.0 ml 30 min after presentation. Monkeys were given a second 30-min test session 4 hr post-injection. Food was present throughout the test sessions, but water was removed during the two 30-min milk presentations. All subjects underwent at least three familiarization trials before drug testing began. Doses of all drugs were administered in random order on Wednesdays and Fridays. Control (vehicle) sessions were performed on the preceding days.

### *Drugs*

The hydrochloride salts of etorphine, buprenorphine, naloxone, naltrexone, and diprenorphine were generously provided by the National Institute on Drug Abuse (Rockville, MD). These drugs and morphine sulfate (Penick Corp., Newark, NJ) were dissolved in 0.9% saline. Ketocyclazocine and ethylketocyclazocine base (Sterling-Winthrop Research Institute, Rensselaer, NY) were dissolved in a vehicle of 8.5% lactic acid and 1.0 N sodium hydroxide in a 3:2 ratio.

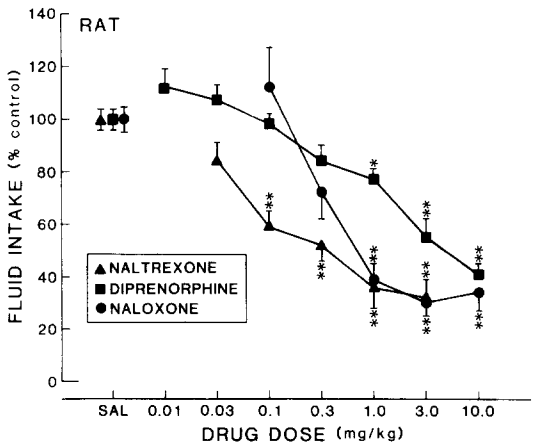


FIG. 1. Suppression of sweetened condensed milk intake by narcotic antagonists in the rat. Each point represents the mean and SE of 8 observations in each experimental group. The absolute values obtained in tests with isotonic saline (points above Sal) are: 30.0±2.0, 35.0±3.0, and 25.0±2.0 ml/kg of body weight in the groups that received naltrexone, naloxone, and diprenorphine, respectively. Significant differences between control and treatment means are indicated as: $*p<0.05$ and $**p<0.01$.

The vehicle for diazepam (Roche Laboratories, Division of Hoffman-LaRoche, Nutley, NJ) was 40% ethylene glycol, 10% 95% ethanol, and 50% 0.9% saline. Drug doses and vehicles were administered in random sequence, subcutaneously in rats and intramuscularly in monkeys. Injections in rats and monkeys were in volumes of 1.0 and 0.5 ml per kg of body weight, respectively. All drug doses are expressed in terms of the free base.

### *Data Analysis*

Milk intake data were converted to ml of milk consumed per kg of body weight for both rats and monkeys. The data were then further normalized to a percentage of the vehicle control values for each subject in each drug series in order to facilitate comparisons among the different groups. The transformed data were evaluated using an analysis of variance for randomized block designs; comparison of a treatment mean with the mean of its respective vehicle control was made by two-tailed Dunnett's and paired $t$-tests. A $p$ value of less than 0.05 was considered statistically significant.

## RESULTS

### *Antagonists*

Naltrexone was the most potent of the narcotic antagonists in suppressing sweetened condensed milk intake in the rat. As little as 0.1 mg/kg reduced milk consumption to 59% of the saline control value (Fig. 1). Naloxone produced a statistically significant decrease in milk intake only at doses of 1.0 mg/kg or greater. Diprenorphine was the least potent of the narcotic antagonists in attenuating milk consumption in the rat. Ten mg/kg of diprenorphine was required to decrease drinking by 59%. The highest dose of each antagonist tested suppressed milk intake to approximately 30–40% of the respective saline control values.

Sweetened condensed milk intake was also attenuated in

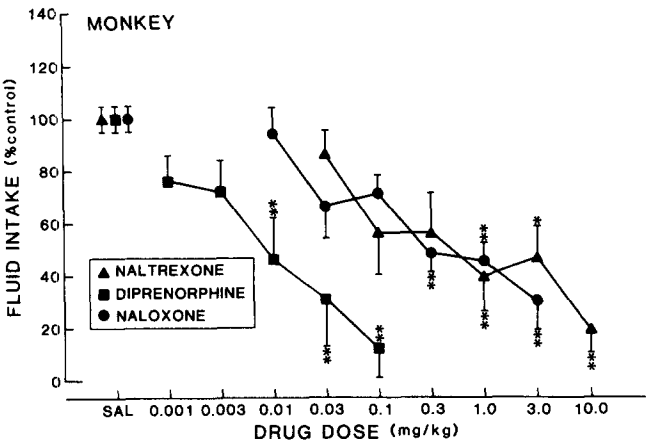


FIG. 2. Suppression of sweetened condensed milk intake by narcotic antagonists in the squirrel monkey. Each point represents the mean and SE of 6 observations in each experimental group. The absolute values obtained in tests with isotonic saline (points above Sal) are: 58.4±5.3, 53.2±4.4, 55.5±5.2 ml/kg of body weight in the groups that received naltrexone, naloxone, and diprenorphine, respectively. Significant differences between control and treatment means are indicated as: $*p<0.05$ and $**p<0.01$.

the squirrel monkey by the narcotic antagonists. However, a different order of potency for these drugs was observed in this species as compared to the rat: diprenorphine was approximately 30 times more potent than either naloxone or naltrexone (Fig. 2). Milk intake was reduced to 46% of saline control values by a dose of diprenorphine as low as 0.01 mg/kg. A dose of 0.1 mg/kg diprenorphine further suppressed drinking to 12% of control without producing any overt behavioral changes. Naloxone and naltrexone were approximately equipotent in attenuating milk intake in the squirrel monkey, reducing milk consumption to 48 and 56% of saline control values, respectively, at a dose of 0.3 mg/kg. The highest dose of naltrexone (10 mg/kg) and naloxone (3.0 mg/kg) invariably caused salivation and vomiting in the monkey during the first 30-min test session. The effects of all the narcotic antagonists declined considerably over the 4.5 hr session. Only the highest dose of each antagonist reduced milk consumption, by 30–40%, during the second 30-min test session given 4 hr post-injection (data not shown).

*Agonists*

In the rat, ethylketocyclazocine and ketocyclazocine were the only opiates to produce statistically significant increases in milk consumption. A dose of 0.03 mg/kg ethylketocyclazocine increased drinking to approximately 45% above control levels (Fig. 3). Ketocyclazocine produced an orderly dose-related increase in milk intake at doses from 0.1 to 1.0 mg/kg. At the latter dose, drinking was elevated to 66% above control values. Although several doses of morphine and etorphine caused small increases in drinking (Fig. 3), due to a large interanimal variability these increases were not statistically significant. The highest doses of morphine (10 mg/kg) and etorphine (0.01 mg/kg) produced a large decrease in milk intake which was associated with behavioral depression. Buprenorphine had no effect on fluid intake at doses up to 10 mg/kg (Fig. 3). Diazepam increased sweetened condensed milk intake over a broad range of doses (Fig. 3). At 3.0 mg/kg, diazepam increased drinking to nearly 125% above control levels. Dose-response curves for morphine and buprenorphine were also determined in test sessions conducted 3 hr after drug administration. Although dose-related decreases in milk intake were observed, no significant increases were produced by these drugs (data not shown).

In contrast to their effect in the rat, the opiate agonists produced only decreases in milk consumption in the squirrel monkey during the first 30-min test session (Fig. 4). With every drug, the dose-related suppression of drinking was associated with ataxia and signs of behavioral depression. Milk intake returned to control levels by the test session 4 hr post-injection, except at the highest dose of morphine tested (1.0 mg/kg) (data not shown).

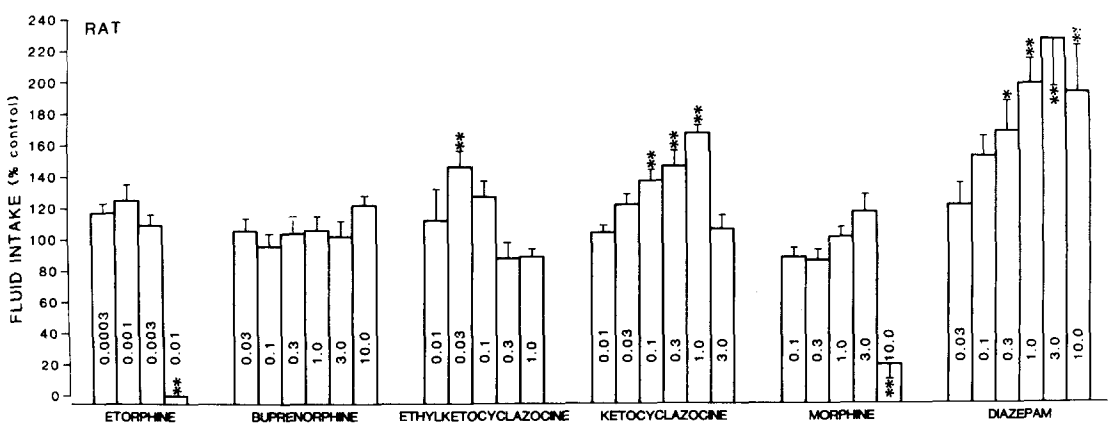


FIG. 3. Effects of several narcotic agonists and diazepam on sweetened condensed milk intake in the rat. Each bar represents the mean and SE of 8 observations in each experimental group. The absolute values obtained in tests with isotonic saline or vehicle were within the range of those obtained in control experiments with the narcotic antagonists (see Fig. 1 legend). Significant differences between control and treatment means are indicated as: $*p<0.05$ and $**p<0.01$.

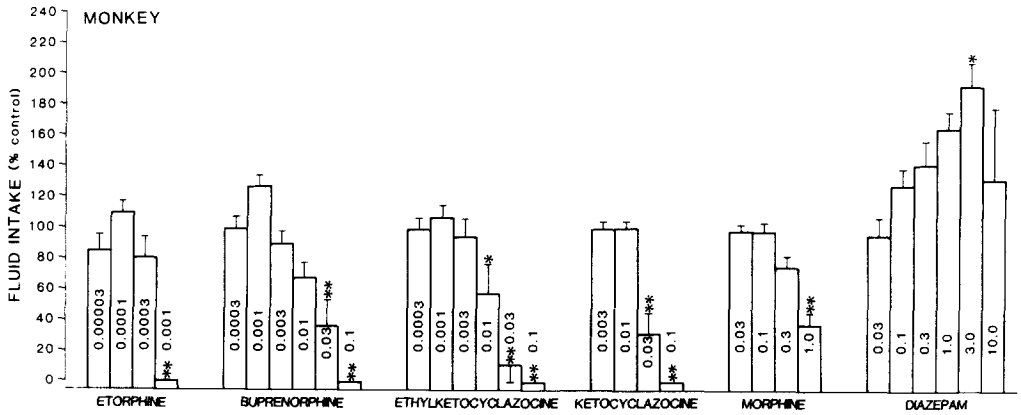


FIG. 4. Effects of several narcotic agonists and diazepam on sweetened condensed milk intake in the squirrel monkey. Each bar represents the mean and SE of 6 observations in each experimental group. The absolute values obtained in tests with isotonic saline or vehicle (55.5±6.9–74.3±5.1) were slightly higher than those obtained in control experiments with the narcotic antagonists (see Fig. 2 legend). Significant differences between control and treatment means are indicated as: $*p<0.05$ and $**p<0.01$.

The partial agonist buprenorphine produced similar dose-response curves for both 30-min test sessions in the squirrel monkey. Buprenorphine slightly increased milk intake at a dose of 0.001 mg/kg (26% (Fig. 4) and 28% (data not shown), respectively) and depressed drinking at higher doses. Drinking was still significantly reduced by 0.1 mg/kg of buprenorphine after 4 hr (data not shown), and usually remained attenuated 24 hr later.

As in the rat, diazepam caused a significant increase in milk consumption in the squirrel monkey over a broad range of doses. Three mg/kg of diazepam produced a maximal increase in drinking of 92% above control levels (Fig. 4). Increases in drinking were seen with 3.0 and 10 mg/kg of diazepam despite the production of ataxia by these doses. The increase in intake caused by diazepam did not persist to the test session 4 hr post-injection.

## DISCUSSION

The results of these experiments confirm the observations of other investigators that the narcotic antagonists consistently reduce fluid intake in the rat and squirrel monkey, while the narcotic agonists produce variable effects. Although the narcotic antagonists (naloxone, naltrexone, diprenorphine) all decreased fluid intake, there were marked species differences. Diprenorphine was 30 times more potent than either naloxone or naltrexone in the squirrel monkey, and produced a greater maximal effect and a dose-response curve having a steeper slope than the other two antagonists. A similar potency of diprenorphine relative to naloxone and naltrexone in suppressing water consumption in squirrel monkeys deprived of water for 18 hr has been noted previously [6]. The other two oripavine derivates examined, etorphine and buprenorphine, were also substantially more potent in the monkey than in the rat. A dose of buprenorphine (10 mg/kg) 1000 times higher than the dose required to produce behavioral depression in the monkey (0.1 mg/kg) had no effect on the milk intake or overt behavior of the rat. The reason for the striking potency of the oripavines in the squirrel monkey as compared to the rat is obscure.

In general, there was more interanimal variability in the effects of the opiate agonists on milk intake than with the antagonists. Furthermore, the effects of the opiate agonists were usually biphasic. A small increase in milk consumption, usually not statistically reliable, was observed at low doses, followed by a dose-related suppression of milk intake at higher doses, which was invariably associated with behavioral depression. Despite these generalities, there were some notable species differences.

Agonists of the $\mu$-opiate receptor subtype, morphine and etorphine [15], were ineffective in increasing milk consumption in both the rat and the squirrel monkey. In contrast, several doses of ketocyclazocine and ethylketocyclazocine, agonists of the proposed $\kappa$-opiate receptor subtype [15], produced significant increases in drinking in the rat. The observation that the $\kappa$-receptor agonists increase milk intake in the rat, but not the squirrel monkey, is consistent with the observed species differences in discriminative stimulus properties of these drugs [17].

There are several possible explanations for the absence of a consistent increase in drinking caused by the opiate agonists. One explanation may be that the paradigm employed was not conducive to showing increases in drinking. While the intake of sweetened condensed milk was ideal for showing decreases in consumption, the high baseline intake may have prevented the demonstration of small, but physiologically significant, increases in intake by the opiate agonists. However, the observation that the $\kappa$-agonists in the rat and diazepam in both the rat and the squirrel monkey increased milk intake suggests that deficiencies in the paradigm cannot be the entire explanation. Furthermore, doses of diazepam (1.0–10 mg/kg) which produced ataxia in the squirrel monkey did not prevent increases in milk intake well above control levels, suggesting that motor depression was not the reason for the lack of an agonist-mediated increase in drinking.

An alternative explanation for the inability of the opiate agonists to increase sweetened condensed milk intake may be that once the animal is motivated to drink, the controlling neuronal systems are maximally activated so that further stimulation of the systems by the administration of receptor agonists does not result in additional increases in drinking.

Narcotic antagonists, on the other hand, would be capable of producing significant decreases from the high baseline of milk intake. It is also possible that the proper opiate agonist for stimulating drinking has not yet been examined. The opiate alkaloids may not be capable of fully mimicking the actions of endogenous opiate peptides which may mediate increases in drinking. The opiate alkaloids may not be selective for the appropriate opiate receptor subtype, or once bound to the receptor may not be in the proper configuration for stimulating the drinking response.

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