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BRIEF COMMUNICATION

Intracerebroventricular Morphine Enhances Alcohol Consumption by Rats

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LINSEMAN, M. A. AND S. HARDING. *Intracerebroventricular morphine enhances alcohol consumption by rats.* PHARMACOL BIOCHEM BEHAV **36**(2) 405–408, 1990.—Previous experiments have shown that systemically administered low doses of opioid agonists increase subsequent alcohol consumption by rats. In this experiment, 10 micrograms of morphine were infused intracerebroventricularly (ICV) in free-feeding rats, daily for 6 days, 30 min prior to one-hour access to a 12% alcohol solution. Alcohol consumption was significantly increased in the morphine-treated group compared to that of a saline-treated control group, confirming that the locus of the effect is within the central nervous system.

Alcohol Ethanol Consumption Self-administration Morphine Opioid Intracerebroventricular (ICV)

SYSTEMIC administration of low doses of morphine has been shown to increase subsequent consumption of alcohol in rats (8, 12, 16). This effect appears to be mediated within the central nervous system since administration of the peripherally acting opioid agonist-like drug, loperamide, does not mimic the effect of morphine. Similarly, while prior administration of naltrexone antagonizes the effect, methylnaltrexone (the quaternary antagonist which does not penetrate the blood-brain barrier at low doses) fails to do so (12). This experiment confirms that the potentiation of alcohol consumption by opioid agonists is produced centrally as intracerebroventricular infusion of morphine, at a dose far below that required to produce the effect when administered systemically, increased subsequent alcohol consumption by rats using a limited access procedure.

METHOD

Subjects

Animals used in the experiment were 40 male Wistar rats weighing approximately 300 g at the beginning of the experiment. They were singly housed in hanging wire cages where food (Purina rat chow) and water were continuously available throughout the experiment. They were switched to and maintained on a reverse dark/light cycle (the dark period starting daily at 11 a.m.) immediately upon their arrival in the colony.

Surgery

Rats were anaesthetized using 60 mg/kg pentobarbital. Twenty-two gauge intracerebral guide cannulae (Plastic Products; Roanoke, VA) were chronically implanted into the right lateral ventricles (coordinates: +0.8 AP/1.5 L/3.9 mm DV, relative and perpendicular to bregma). Obturators, cut to the same length as the guide cannulae, were inserted into the cannulae when they were not in use. Injection cannulae (twenty-eight gauge) were cut so as to protrude 0.2 mm beyond the tips of the guide cannulae. Animals were allowed a period of at least one week to recover from surgery before further procedures were initiated.

Prescreening

Since a period of training is required to induce rats to drink significant quantities of alcohol, animals were prescreened so as to include in the experiment only those likely to have cannulae correctly placed within the ventricles. It is well known that an infusion of angiotensin II into the ventricles elicits robust drinking in water-sated rats (2). Accordingly, on the prescreening day, all rats were infused through the cannulae with 100 nanograms of angiotensin II, in a volume of 4 microlitres of saline immediately before being placed in a separate "drinking" cage within the colony room where water was available to drink from a Richter tube. Water consumption during the subsequent 30 min was measured to the nearest ml and animals were included in the experiment only if their water consumption during this time exceeded 10 ml/kg. The number of animals was thus reduced to 28, whose mean water consumption was 44.5 ml/kg.

Acquisition

Animals, which had passed the prescreening, were then trained

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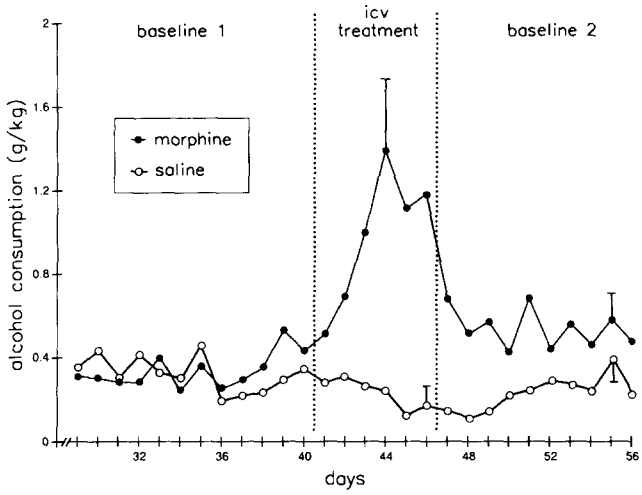

FIG. 1. Mean doses of alcohol consumed during the one-hour drinking sessions, over days, by animals infused with either morphine or saline on Days 41–47. The vertical bars represent the largest s.e.m.s for each group during that phase of the experiment.

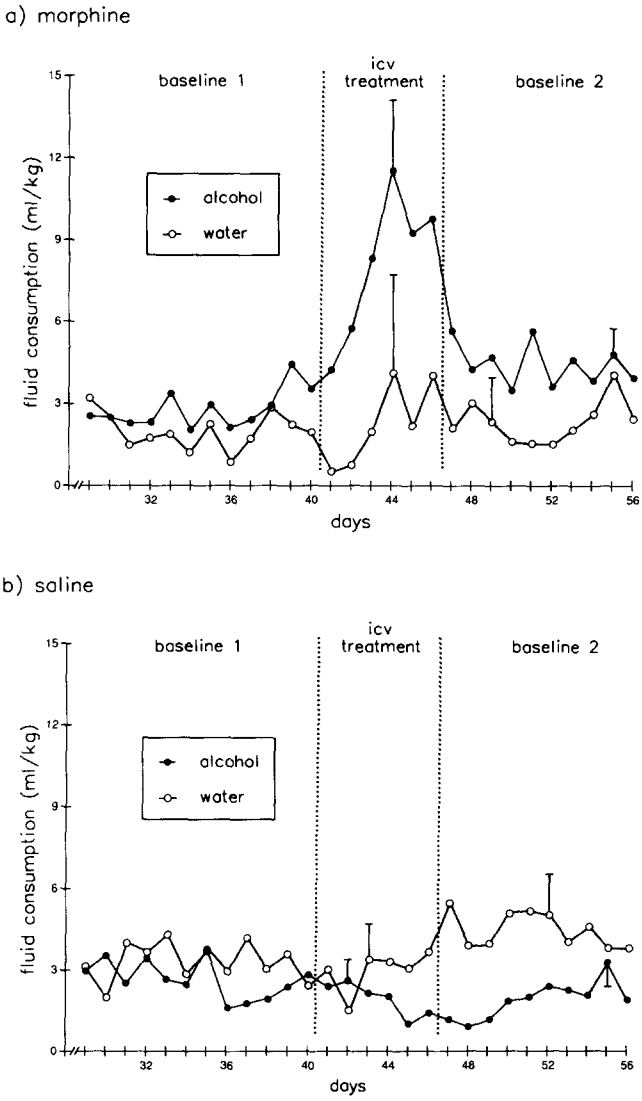

FIG. 2. Mean volumes of alcohol and water consumed during the one-hour drinking sessions by a) the morphine- and b) the saline-treated animals across days of the experiment. The vertical bars represent the largest s.e.m.s for each group during that phase of the experiment.

to drink alcohol using a limited access procedure similar to that described previously (10,11). Drinking using this paradigm has been shown to result in the attainment of pharmacologically detectable and behaviorally significant blood alcohol levels by rats (4,10). It also allows the *E* to schedule drinking during the period when the pretreatment drug is maximally effective. Briefly, animals were weighed each day before being transferred to separate individual "drinking" cages, also within the colony room, where two modified Richter tubes, one containing increasing concentrations of alcohol and the other, water, were made available after an interval of about 20 min. The concentration of alcohol was 3% w/v (in tap water) over the first 10 days, was increased to 6% on Days 11 to 28, and then to 12% for the duration of the experiment. One hour following the presentation of the fluids, amounts of alcohol and water drunk were recorded to the nearest 0.1 ml, corrected for spillage, and the animals were returned to their home cages. Fresh alcohol and water solutions were provided daily, and the positions of the alcohol and water tubes were alternated daily to control for possible position preferences. Drinking sessions were scheduled daily beginning at 2:30 p.m.

ICV Treatment Period

Following the acquisition period, animals were divided into two groups matched on the basis of their alcohol consumption over Days 29 to 40 (when consumption was asymptotic at an alcohol concentration of 12%). One group was randomly selected to be the morphine-treated group; the other became the saline-treated group. For Days 41 to 46, the daily procedure continued as before except that, following weighing and prior to being placed in the drinking cages, animals were infused via their ICV cannulae with either 4 microlitres of a morphine sulphate solution (2.5 micrograms/microlitre of saline; total dose per animal = 10 micrograms) or an equivalent volume of physiological saline, according to their group assignment. Solutions were infused slowly over a period of 20 sec and injection cannulae remained in place an additional 15 sec to allow for diffusion of the solution from the tips of the cannulae. Alcohol and water tubes were presented to the rats 30 min following their infusion and remained in place for a period of one hour. Infusions were administered daily for a period of 6 consecutive days. Following the series of infusions, drinking sessions continued as before for an additional 10 days to observe the return to baseline.

Histology

At the conclusion of the experiment, all animals were given an overdose of pentobarbital and subsequently perfused intracardially with physiological saline, followed by a 10% formalin solution. Brains were removed and later sliced into 50 micron sections which were mounted for further examination. All animals in the experiment were found to have cannula tips located within the ventricle or on the border of it so all of the data were retained for further analysis.

Statistical Analysis

A one-way analysis of variance for matched groups was used to

evaluate overall average differences in alcohol and water consumption, as well as possible linear and quadratic trends during and following days of treatment.

RESULTS

The effects of ICV morphine and saline on doses of alcohol consumed during the one-hour daily sessions on Days 41 to 46 are illustrated in Fig. 1, and on the relative volumes of alcohol and water consumed, in Fig. 2. Morphine significantly increased alcohol consumption across the period of treatment compared to the saline group, $F(1,13) = 32.89$, $p = 0.000$. The effect increased gradually over days as evidenced by a significant linear trend between the two groups, $F(1,13) = 6.91$, $p = 0.021$. Finally, alcohol consumption by the morphine group remained slightly but significantly elevated above that of the saline group during the 10 days after the infusions were discontinued, $F(1,13) = 19.38$, $p = 0.001$. There were no significant effects of morphine on water consumption.

DISCUSSION

Intracerebroventricular infusion of a low dose of morphine enhanced subsequent alcohol consumption by rats in a manner similar to that seen earlier following systemic administration of the drug (12). Since this dose, 10 micrograms per rat, is far below the threshold dose needed when the drug is given systemically (9), the effect must be produced within the central nervous system.

Amounts of alcohol drunk during baseline by rats in this experiment were below those we have usually seen using the limited access paradigm (10,11). Although no unoperated control group was included to allow a direct comparison, it is possible that the surgery itself somewhat suppressed alcohol consumption. A similar observation has been made earlier in regard to rats that had been implanted with stimulating electrodes into the hypothalamus (18). Nevertheless, morphine-treated rats increased their alcohol consumption irrespective of the amount of baseline drinking. Even those which had been drinking none, began drinking alcohol after ICV morphine and often continued to drink beyond the period of treatment. The increased consumption by the morphine-treated group when morphine was no longer administered, though significantly greater than the saline-treated group, was small. However, it is interesting to note that there are now several studies which report increased alcohol consumption following some form of chronic treatment with opioids: via osmotic minipumps (7), chronic systemic injections (5), as well as the chronic ICV infusion of opioid-like byproducts of alcohol metabolism (14).

In contrast to the results of this experiment, there is a previous report in the literature that ICV Met-enkephalin caused a decrease in alcohol consumption by rats (6). This was a high dose effect (200 micrograms), and a significant effect of treatment was not seen until several days following a single infusion of the drug in spite of its very short duration of action. Similarly, a single injection of a high dose of morphine, administered systemically, has also been reported to suppress alcohol consumption in rats, and this suppression far outlasts the duration of the sedating effects of the drug (17). High doses of opioids administered ICV (1,3) and systemically (13) have been shown to induce electrophysiological seizure activity in rats. Alcohol consumption is suppressed in animals which have recently experienced seizures induced by electroconvulsive shock (15) and kindling (unpublished data from this laboratory). It is possible, therefore, that the high dose effects of opioids on alcohol consumption are an indirect effect of the seizures they produce, rather than reflecting an effect of opioids within a lower, more physiological dose range. Further studies, in which the effects of a range of doses of ICV morphine on subsequent alcohol consumption are studied, are needed to test this hypothesis.

In summary, this study confirms that the increased alcohol consumption following administration of opioid agonists is centrally mediated and is further evidence for a possible etiological role of opioids in excessive alcohol consumption. This paradigm described should, in addition, be useful for the study of the effects of certain endogenous opioids—β-endorphin, delta receptor agonists, and enkephalins (as increased by enkephalinase inhibitors)—that can only be administered ICV, on alcohol consumption. Ultimately, to the degree that brain tissue can sustain several infusions of drug, it should also be possible to localize the effect to specific sites within the brain, and thereby deduce a possible mechanism for the effect, e.g., whether the effect is upon systems related to ingestive behavior or to reinforcement.

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