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Involvement of μ-Opioid Receptors in Alcohol Drinking by Alcohol-Preferring AA Rats

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HYYTIÄ, P. *Involvement of μ-opioid receptors in alcohol drinking by alcohol-preferring AA rats.* PHARMACOL BIOCHEM BEHAV 45(3) 697–701, 1993.—The present study examined the role of μ- and δ-opioid receptors in alcohol drinking using antagonists selective for these receptor types. Food- and water-sated male and female AA (Alko, alcohol) rats consistently drank 10% alcohol during daily 30-min access periods in their home cages in the middle of the 12-h light phase. On 3 consecutive days, the animals received the μ-opioid receptor antagonist D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP, 1 μg ICv), the δ-receptor antagonist N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864, 3 μg ICV), or saline 15 min before the alcohol access period. Relative to saline, the μ-antagonist CTOP decreased alcohol drinking both by males and females progressively over the 3 treatment days, with a continued suppression on the first days after the termination of the administration. Treatment with the δ-antagonist ICI 174,864 had no effect on alcohol drinking in males, and produced transient hind limb dysfunction and barrel rolling in over half of the females. These results suggest that selective blockage of μ-opioid receptors is sufficient to suppress alcohol drinking in AA rats.

Alcohol drinking Alcohol-preferring rats μ-Opioid receptor δ-Opioid receptor
Opioid receptor antagonists Reinforcement

EVIDENCE for the involvement of the endogenous opioid system in the regulation of alcohol drinking has been growing. Agonists at opioid receptors, such as morphine, were first shown to alter alcohol consumption (28,33). Subsequently, opioid antagonists have been found to suppress intravenous and intragastric alcohol self-administration (1,35), as well as alcohol drinking in a number of experimental paradigms (8, 23,28,32,40). These compounds are now being applied with success clinically (39).

There is also evidence that the genetic factors controlling alcohol drinking include opioidergic mechanisms. The AA (Alko, alcohol) and ANA (Alko, nonalcohol) rat lines, developed by bidirectional selection for differential voluntary alcohol consumption (7), differ greatly in their voluntary intake of etonitazene, an opioid agonist (19). Differences between the AA and ANA rats have also been detected in the hypothalamic content of proopiomelanocortin mRNA, the precursor of β-endorphin, and the content of β-endorphin-like immunoreactivity in distinct brain areas (10). Consequently, the AA rats appear to be a useful tool for studying the opioidergic control of alcohol drinking.

At least three distinct opioid receptor types exist in the CNS, referred to as μ, δ, and κ (26). Naloxone and naltrexone, the opioid antagonists most commonly used in alcohol self-administration studies, have highest affinities for μ-receptors but bind readily to other types of opioid receptors (25). Therefore, the nonselective antagonists do not very well differentiate the involvement of the three opioid receptor types in alcohol consumption.

The purpose of the present study was to compare the roles of μ- and δ-opioid receptors in alcohol drinking by the alcohol-preferring AA rats by using a selective μ-receptor antagonist, D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP) (16), and a selective δ-receptor antagonist, N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864) (5). It has been reported previously that systemically administered ICI 174,864 decreases alcohol consumption by the high-drinking HAD rats as efficiently as naloxone (9). Because the μ-antagonist CTOP has been suggested to penetrate the blood–brain barrier with difficulty (15), we chose to administer both antagonists intracerebroventricularly (ICV).

Previous studies of opioid antagonists in AA rats (32) have examined only males, but we now tested both males and females. We used a limited access procedure that does not involve food or fluid deprivation (34), with alcohol access eventually restricted to 30 min daily.

METHOD

Animals

AA male rats of the F_{61} generation and female rats of the F_{62} generation were used. At the age of 3 months, the animals

were placed in individual stainless steel cages in a room with controlled temperature (22 ± 2°C) and humidity (55%) on a 12L : 12D cycle (0600–1800 lights on). Animals were given a continual free choice between tap water and 10% (v/v) ethanol, both in graduated Richter tubes. Standard powdered Ewos R3 rat food (Södertälje, Sweden) was freely available.

Acquisition of Ethanol Drinking Under Limited Access

The male AAs had 52 days and the females 35 days of free-choice alcohol drinking before alcohol availability was restricted to one 60-min access period daily. The details of our limited access procedure have been described elsewhere (34). Briefly, the animals were weighed daily at noon, and after an interval of about 15 min, alcohol was returned by attaching a 20-ml Richter tube filled with ethanol solution on the home cage. After the 60-min presentation, the amount of alcohol drunk was recorded to the nearest 0.1 ml. The availability of water and food was not restricted. Consequently, the consumption of water during the alcohol access hour was negligible, and only the 24-h consumption was recorded. After the animals had reliably learned to drink during the daily access hour, the availability of ethanol was further restricted to 30 min. This did not change the level of consumption, because rats on limited access normally drink almost all of their alcohol during the first minutes that it is available each day. The AA males were on limited access for 54 days and the females for 37 days before they were operated. The mean alcohol consumption (±SEM) of the males (mean weight 354 g) and females (227 g) during the 30-min access during the last 4 days preceding surgery was 0.70 ± 0.06 and 0.91 ± 0.07 g/kg/30 min, respectively.

Intraventricular Injection of Opioid Peptides

Under halothane anesthesia, a 23-gauge stainless steel guide cannula was implanted into the right lateral ventricle stereotaxically. The coordinates of the cannula (relative to bregma) were: A, −0.9; L, −1.5; V, −3.5, according to the stereotaxic atlas of Paxinos and Watson (27). The cannula was secured on the skull by anchor screws with dental acrylic. A dummy cannula, cut to the same length as the guide cannula, was inserted into the guide cannula when it was not in use. The 30-gauge injection cannula was cut to extend 0.5 mm beyond the tips of the guide cannula. Animals were allowed to recover from surgery for at least 3 days before any further procedures. Cannula placement was verified behaviorally by an ICV injection of angiotensin II (150 ng): all rats drank 5 ml or more of water during the 30 min following the injection.

Following recovery from surgery, the rats were returned to the daily 30-min alcohol access. The level of alcohol intake remained essentially unaffected by the surgery. The limited access procedure continued for a week before drug injections started. During this period, the animals were handled daily with the dummy cannulae temporarily removed. The rats were divided into three groups of males and three groups of females matched on the basis of their alcohol consumption and body weight on the preceding 4 days on limited access.

The following opioid peptides were dissolved in sterile saline and administered ICV: D-Pen-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH2 (CTOP; Peninsula Laboratories, Belmont, USA), at a dose of 1 μg in a volume of 3.0 μl; N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH (ICI 174,864; Cambridge Research Biochemicals, Cambridge, UK), at a dose of 3 μg in 3.3 μl.

For microinjections, the dummy cannula was removed, and an injection cannula attached to a Hamilton microsyringe with a polyethylene tube was inserted into the guide cannula. The solution was infused over a 60 s period while the animals were gently restrained in one experimenter's hand, and the cannula was left in place for another 60 s to allow complete solution delivery. The injections were given 15 min before the daily 30-min alcohol access on 3 consecutive days. Each individual received the same drug on all 3 days. After the series of injections, alcohol drinking sessions were continued for at least 3 days to observe the return to the baseline.

Each AA male received a single series of three consecutive ICV injections. With females, a within-subjects design was used. One group was first injected with saline, and then with CTOP; the second group got CTOP and saline. The third group was first injected with ICI 174,864. Because of side effects, injections with ICI 174,864 were terminated after the first day; subsequently, this group received saline and CTOP. After drug injections, the animals were allowed at least 5 days to return reliably to the baseline before any new injections.

Data Analysis

Alcohol and food consumption are expressed as g per kg body weight, and water consumption as ml per kg body weight. For statistical analysis, the difference between consumption on the drug treatment days and the mean consumption on the last 4 baseline days was used. Data were analyzed by two-way (treatment, time) analyses of variance (ANOVA) with repeated measures on one factor (time). After the ANOVAs, multiple comparisons between saline- and drug-treated groups on individual treatment days were performed by unpaired Student's *t*-tests. On the 3 posttreatment days, the saline- and drug-treated groups were also compared with their pretreatment baseline with matched-pair *t*-tests. The accepted level of significance for all tests was $p < 0.05$.

RESULTS

The μ-antagonist, CTOP, produced a progressive decrease in alcohol drinking in both males and females (Fig. 1). The two-way ANOVAs across the 3 drug-treatment days showed a significant difference between the three treatments for males, $F(2, 12) = 5.76, p = 0.0176$, and between the two treatments for females, $F(1, 37) = 4.47, p = 0.0413$. Similarly, significant differences between the treatments both for males, $F(2, 12) = 7.95, p = 0.0063$, and females, $F(1, 37) = 8.80, p = 0.0052$, were found when the 3 posttreatment days were included in the ANOVAs.

The suppression produced by CTOP persisted after the treatment. Alcohol drinking by the males 24 h after the last CTOP administration ("POST1") was significantly reduced relative to both the controls' intake at this time, $t(8) = 2.61$, $p = 0.0311$, and their own baseline ($p = 0.0052$). For females, the suppression persisted significantly not only on POST1 but also on POST2 and POST3 days (relative to controls, $p = 0.0035$, $p = 0.0225$, and $p = 0.0101$; relative to baseline, $p < 0.0001$, $p = 0.0002$, and $p = 0.0039$).

In order to see whether the progressive decline in alcohol drinking produced by CTOP treatment could be due to any adverse effects, the 24-h water and food intake by the AA females was also recorded. The injections both with saline and with CTOP on the first day were accompanied by decreases in body weight, and water, and food intake following the injections (Fig. 2), but two-way ANOVAs revealed no significant differences between the groups over the 3-day treatment.

The δ-antagonist, ICI 174,864, produced no significant changes in alcohol drinking in male AA rats on any treatment

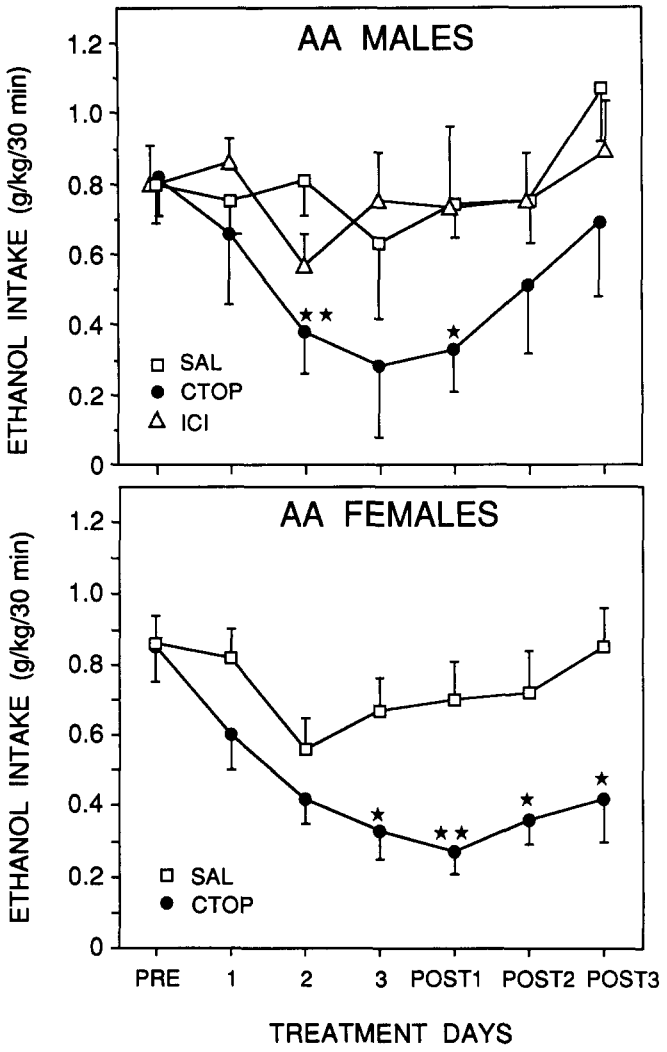

FIG. 1. Upper panel: mean ethanol consumption (±SEM) by AA males during the daily 30-min alcohol access periods after ICV injections of saline (n = 5), CTOP (n = 5), and ICI 174,864 (n = 5). The "pre" level represents the mean ethanol intake on the preceding 4 days on limited access. During the "pre" and "post" sessions, alcohol was presented without injections. Asterisks indicate significant differences between saline- and drug-treated groups: $*p < 0.05$, $**p < 0.01$, unpaired t-tests. Lower panel: mean ethanol consumption (±SEM) by AA females during the 30-min access periods after injections of saline (n = 19) and CTOP (n = 20). Asterisks show significant differences between saline and CTOP groups: $*p < 0.05$, $**p < 0.01$, unpaired t-tests.

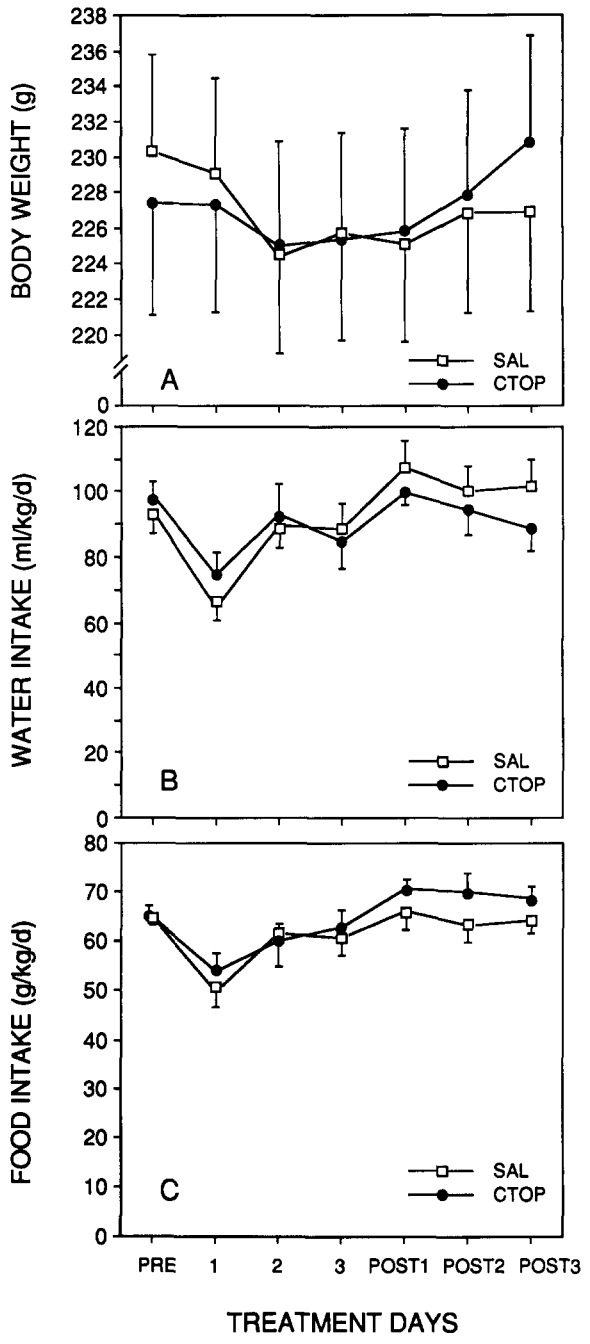

FIG. 2. Mean (±SEM) body weight (A), water consumption (B), and food consumption (C) by AA females following ICV injections of saline (n = 19) and CTOP (n = 20). The "pre" level of body weight shows the weight of the rats on the last day prior to the first injection, and the baselines of water and food intake illustrate the mean of the preceding 4 days. The body weights were recorded before injections; the measurements of water and food intake represent the consumption during the 24 h following injections.

or posttreatment day (Fig. 1). On the first day that the compound was given to females, hindlimb dysfunction was seen in three of the nine rats, and vigorous ipsilateral rotation ("barrel rolling") in three animals. These symptoms appeared approximately 2 min after the injections and lasted for 2–5 min. During and after this period, the rats drank little alcohol. The remaining three females with no side effects from the drug did not decrease their alcohol drinking.

Because of the side effects, injections with the dose of 3 μg were not continued in females after the first day. When the dose of ICI 174,864 was decreased to 1.5 μg in a separate small experiment using an identical procedure, neither motor dysfunction nor differences in alcohol drinking between female AAs treated with saline (n = 5) and ICI 174,864 (n = 5) were observed, $F(1, 8) = 0.42$, $p = 0.53$.

DISCUSSION

The present results suggest that μ-opioid receptors are involved in the alcohol drinking by the alcohol-preferring AA rats and in its suppression previously seen with nonselective antagonists. The ICV administration of the highly selective μ-antagonist CTOP had little effect on the first day of testing; on subsequent days, however, alcohol drinking declined progressively, and remained suppressed after the termination of the treatment. Similar changes in alcohol intake after repeated administrations of nonselective opiate antagonists (naloxone, naltrexone) on consecutive days have been found in previous studies with AA rats (32), Long-Evans rats (18), and monkeys self-administering alcohol intravenously (1).

The progressive decrease has previously been explained as extinction caused by the attenuation of the reinforcing actions of alcohol by opiate antagonists (1,32). The present results are consistent with this hypothesis. The decreases produced by CTOP cannot be attributed to motor impairment or sedation because the initiation of drinking was not disturbed by the drug. The ICV administered CTOP has been reported to produce conditioned place aversion but only at higher doses than the one used in the present study (3). Therefore, it seems unlikely that a CTOP-induced malaise or conditioned taste aversion could account for the results.

Unlike the situation with naloxone, cumulative drug effects cannot be totally excluded as an explanation for the progressive decrease in alcohol drinking by CTOP because the half life for the members of the octapeptide μ-antagonist family is probably much longer that of naloxone (about 40 min) (37). Nevertheless, if CTOP has a half life similar to CTP (another μ-antagonist differing from CTOP by one amino acid, and having a half life of 270 min and a duration of biological action in vivo of 300 min) (31), only about 3% would remain after 24 h, which probably is not sufficient to account for the progressive increase in effect across days nor for the continued suppression on the posttreatment days. Further evidence that CTOP did not accumulate comes from the fact that the CTOP and saline groups did not differ in 24-h food and water intake. If large amounts of CTOP had been present during the dark phase (6 to 18 h after drug administration) when most food is consumed, CTOP-treated animals should have shown lower food intake than the saline group because eating has been reported to be suppressed by μ-antagonists (2,17,38).

The μ-antagonist CTOP has also been reported to decrease stress-induced eating in rats (17). Thus, the suppressive effect of CTOP on alcohol drinking, like that of the nonselective opioid antagonists, is not specific to alcohol. However, the rats in the present study had free access to water and food, and took very little water or food at the time of their daily alcohol access. Consequently, alcohol drinking was the only ingestive response emitted frequently when high CTOP levels were present and could, therefore, be selectively extinguished.

Single systemic injections of the selective δ-antagonist ICI 174,864 have been reported to reduce alcohol drinking by the high-drinking HAD rats, thus suggesting a role for δ-receptors in alcohol drinking (9). Similarly, a systemically given δ-antagonist, naltrindole, suppressed alcohol drinking in C57BL mice (22). Contrary to these findings, we observed no significant changes in alcohol drinking by the male AA rats with the ICV- administered ICI 174,864; in females, this compound produced postural abnormalities and barrel rolling.

Because different routes of administration and animal lines have been employed in these studies, a direct comparison is difficult. However, it is unlikely that the present results could be explained by an insufficient drug dosage: the dose given ICV has been shown, for example, to antagonize analgesia (30) and the release of dopamine in the nucleus accumbens (36) induced by β-endorphin, and to abolish conditioned place preference induced by a δ-agonist DPDPE (4). The side effects of ICI 174,864 now seen in the female AAs have been previously reported in rats, with an ED_{50} of 2.8 nmol ICV (24). The reason for the higher sensitivity of the AA females than males to these effects with the 3 μg (4.3 nmol) dose is not known; one contributing factor could be the smaller body weight of the females.

The present results with an extinction-like decline in alcohol drinking produced by the μ-receptor antagonist CTOP suggest that μ-opioid receptors are implicated in the mediation of alcohol reinforcement. Although our findings do not give support for the involvement of δ-receptors, their role cannot be dismissed: there are data that the endogenous ligands of both μ- and δ-receptors are implicated in ethanol's actions. For example, acute alcohol doses cause the release of both β-endorphin and Met-enkephalin (6,29). Furthermore, both μ- and δ-receptors have been shown to be involved in feeding and drinking (2,11,21,38), as well as other ingestive behaviors, such as saccharin (12), sodium chloride (13,14), and high-fat diet intake (20). While these behaviors may be regulated by partly the same opioidergic systems as alcohol drinking, further experiments are needed to elucidate the role and the relationship of different opioid receptor types in alcohol reward.

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