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Receptor Stereospecificity in Opiate-Ethanol Interaction Using the Preexposure-Conditioned Taste Aversion (CTA) Paradigm

J. M. NG CHEONG TON AND Z. AMIT

Center for Studies in Behavioral Neurobiology, Dept of Psychology, Concordia University Montreal, Quebec, Canada H3G 1M8

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NG CHEONG TON, J. M. AND Z. AMIT. *Receptor stereospecificity in opiate-ethanol interaction using the preexposure-conditioned taste aversion (CTA) paradigm.* PHARMACOL BIOCHEM BEHAV 22(2) 255–259, 1985.—In the first experiment, rats were conditioned with different doses of levorphanol or dextrorphan to a novel tasting saccharin solution. In the second experiment, rats were either preexposed to levorphanol or dextrorphan and conditioned with either morphine or ethanol to the saccharin solution. The results showed that levorphanol, but not dextrorphan, at 1, 5 and 10 mg/kg doses effectively induced a CTA. Preexposure to the 5 mg/kg dose of levorphanol blocked both morphine- and ethanol-induced CTAs. Dextrorphan at the same dose did not affect the CTAs. These findings are discussed in terms of the involvement of the opiate receptors in opiate-ethanol interaction.

Levorphanol Dextrorphan Ethanol Morphine Opiate-alcohol Interaction
Conditioned taste aversion Preexposure effect Receptor stereospecificity

THERE have been many recent attempts to investigate the interaction between alcohol and opiates [1, 2, 6, 13, 15, 24] to delineate possible common mechanisms of actions of those two classes of drugs. It seems that while the biochemical studies have produced mostly consistent results [1, 8, 11, 14, 15, 24, 26], the behavioral work is fraught with unreliable, inconsistent data [4, 7, 12, 18]. We recently suggested that the preexposure-conditioned taste aversion paradigm can be used as a behavioral tool for the examination of ethanol-opiate interaction [20]. In the latter report, we showed that preexposure to ethanol blocked the conditioned taste aversion (CTA) induced by morphine and conversely, preexposure to morphine blocked the ethanol-induced CTA. The fact that ethanol can in some way alter the receptor binding of opiates or opioids to their binding sites [27] suggested that the interaction observed in the preexposure effect may involve the opiate receptors. To examine the role of the opiate receptor in the opiate-ethanol interaction, we compared the effects of two opiates enantiomers, the active isomer levorphanol and the nonactive isomer dextrorphan as preexposure agents on both ethanol- and morphine-induced CTAs. If the opiate receptor plays a role in the preexposure interaction between morphine and ethanol then the active isomer levorphanol should block the aversion to either morphine or ethanol. On the other hand, the nonactive isomer dextrorphan should not affect morphine- or ethanol-induced CTAs.

We initially conducted an experiment to establish a dose-response curve for the capacity of levophanol and dextrorphan to induce a conventional conditioned taste aversion. We expected to observe a U-curve relationship between doses of levorphanol and aversion to the conditioned stimulus and none or little aversion to doses of dextrorphan.

EXPERIMENT 1

METHOD

Procedure

Fourty-four male Sprague-Dawley rats (Charles River Ltd.) weighing between 272 g and 330 g at the beginning of the experiment were randomly divided into 7 groups. The animals were handled and given a few days to habituate to the animal colony prior to experimentation. They were then placed on a water deprivation schedule which restricted access to only 30-minute daily. Food (Purina Lab Chow) was made available ad lib throughout the experiment. The rats were presented with a 0.1% saccharin solution for 30 minutes immediately followed by the appropriate drug injection (first conditioning trial, baseline score). Three groups of animals received either 1, 5, or 10 mg/kg of levorphanol tartrate; another three groups were injected with either 1, 5, or 10 mg/kg of dextrorphan tartrate; and finally, saline was administered to the last group of rats. A similar pairing was performed 6 days later (second conditioning trial). Three more saccharin presentations were made without drug injection at intervals of 6 days (extinction trials). Saccharin con-

TABLE 1

Trial	Measure
1st Conditioning Trial	Baseline
2nd Conditioning Trial	P1 (Score for 1st Conditioning Trial)
1st Extinction Trial	P2 (Score for 2nd Conditioning Trial)
2nd Extinction Trial	T1 (Score for 1st Extinction Trial)
3rd Extinction Trial	T2 (Score for 2nd Extinction Trial)

sumption was measured on conditioning trials and extinction trials. Saccharin consumption on the first conditioning trial was used as a baseline level. It is important to note that the assessment of the conditioning or extinction trials is made by examining change in saccharin intake at the next presentation following the given trial. Scores are expressed in terms of percent change from baseline intake. For example, to assess the effect of the first conditioning trial, the saccharin intake measured on the second conditioning trial was expressed in term of percent change from the baseline score. Thus, P1, and P2 refer to the percent change scores for the first and second conditioning trials, and T1 and T2 to the first and second extinction trials respectively (see Table 1).

Drugs

Both levorphanol tartrate and dextrorphan tartrate (Hoffman-LaRoche Ltd) were dissolved in saline solution to a concentration of 1 mg/ml, 5 mg/ml, or 10 mg/ml and injected in a volume of 1 ml/kg.

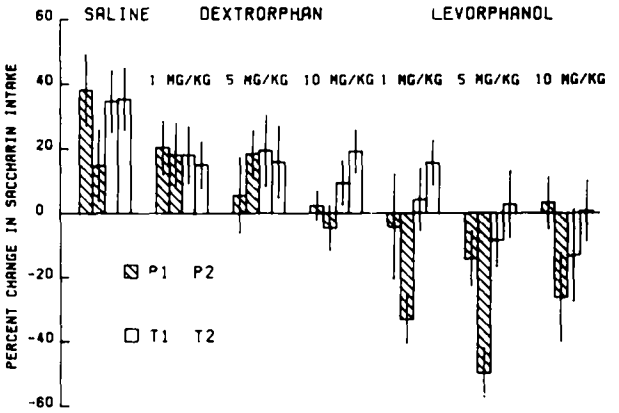

FIG. 1. Percent change in saccharin intake for the groups conditioned with either dextrorphan (1, 5, or 10 mg/kg) or levorphanol (1, 5, or 10 mg/kg) for conditioning (hatched bars) and extinction trials (open bars).

RESULTS

There were no significant differences in saccharin intake in all 6 groups on the first conditioning trial (baseline values). The means and standard errors for the baseline values are: saline—19.0±1.88, dextrorphan 1 mg/kg—22.3±1.69, dextrorphan 5 mg/kg—20.2±1.56, dextrorphan 10 mg/kg—23.2±0.95, levorphanol 1 mg/kg—20.2±0.98, levorphanol 5 mg/kg—21.8±0.79, and levorphanol 10 mg/kg—22.3±1.04. Percent change scores for the experimental groups are illustrated in Fig. 1. A priori t-tests were performed to test for significant departures from the baseline level within each group. The saline group showed a significant increase in saccharin intake over the conditioning and extinction trials, $p<0.05$. In the groups that received dextrorphan as conditioning drug no decrease in saccharin consumption was observed except for the 10 mg/kg dose dextrorphan where a non-significant decrease was obtained for the second conditioning trial. Levorphanol produced significant decreases in saccharin intake at all three doses tested for the second conditioning trial ($p<0.05$). The largest decrease in saccharin consumption was seen at the 5 mg/kg dose. When compared to the saline group, dextrorphan at all doses caused a decrease in saccharin consumption, particularly at the highest dose ($p<0.05$).

EXPERIMENT 2

The results of experiment 1 demonstrate that levorphanol can produce a conditioned taste aversion in the dose range of 1–10 mg-kg. The dose-response of this levorphanol effect displays a U-curve function which was also shown to be the case for morphine [9]. In the second experiment, we examined the role of the opiate receptor in the preexposure interaction between morphine and ethanol. The approach used was to compare levorphanol and dextrorphan in terms of their effectiveness in blocking morphine- and ethanol-induced CTAs. The dose of levorphanol and dextrorphan selected was 5 mg/kg, the dose at which levorphanol produced the strongest CTA.

METHOD

Procedure

Fifty male Sprague-Dawley rats (Charles River Ltd.), weighing between 285 g and 326 g, were individually housed in stainless steel cages in the animal colony room. The rats were handled four times on different days before the start of the experiment. The animals were first adapted to a water-deprivation schedule consisting of a daily access to water for a period of 30 minutes. Food (Purina Laboratory Chow) was made available ad lib throughout the experiment. The animals were preexposed to 5 mg/kg of levorphanol or dextrorphan IP (dose was selected from the first experiment as being the most effective dose in inducing a CTA) once a day for three alternate days following the 30-minute access to water. On the second day following the last preexposure treatment, the rats were presented with a novel 0.1% saccharin solution for 30 minutes and immediately after injected intraperitoneally with either 1.2 g/kg of ethanol or 12 mg/kg

of morphine HCl or saline (first conditioning trial). Preexposure treatment with levorphanol and dextrorphan resumed for another three sessions two days after the first conditioning trial. Another similar conditioning trial and three extinction trials, consisting only of saccharin presentations, were carried out at intervals of 8 days. Saccharin consumption on the first conditioning trial was taken as a baseline intake to compute the values for saccharin intake on subsequent conditioning and extinction trials in terms of percentage change. The same labelling notation is used as in the first experiment. Again, P1, P2 refer to the percent change scores from baseline values for the first and second conditioning trials; T1, T2 refer to the percent change scores for the first and second extinction trials.

Drugs

Levorphanol tartrate and dextrorphan tartrate (Hoffman-LaRoche Ltd) were prepared in saline solution to a concentration of 5 mg/ml and the volume of injection was 1 ml/kg for all the drugs. Morphine hydrochloride (BDH Chemical Canada Ltd) was also dissolved in saline to a concentration of 12 mg/ml and injected in a volume of 1 ml/kg. Ethanol was diluted from 95% ethanol in distilled water to a concentration of 20% and injected in a volume of 7.5 ml/kg.

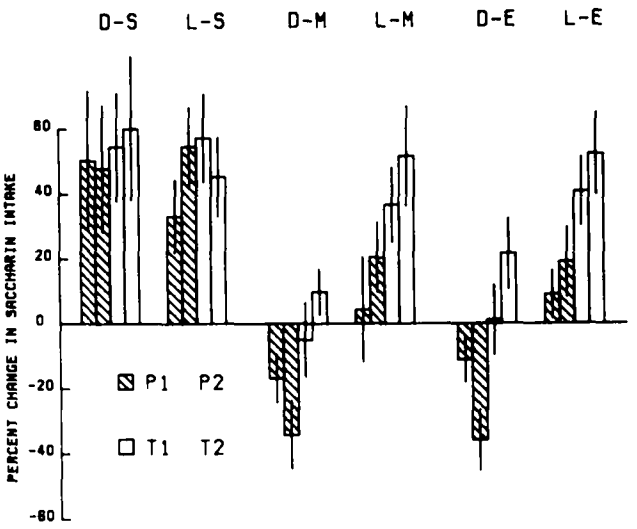

FIG. 2. Percent change in saccharin intake for the groups preexposed with either dextrorphan (D) or levorphanol (D) and conditioned with either morphine (M), ethanol (E), or saline (S) for conditioning (hatched bars) and extinction trials (open bars).

RESULTS

There were no significant differences in baseline scores for all groups. The baseline means and standard errors values were 19.0±1.07 for the D-S group, 18.4±1.24 for the L-S group, 20.9±1.58 for the D-M, 18.0±1.21 for L-M, 20.3±1.89 for D-E, and 18.0±1.31 for L-E. The data presented in Fig. 2 illustrate the mean change in saccharin consumption for the two conditioning trials and the two extinction trials. Using Dunnett's test, the following comparisons were made. First, we found that the two groups that received either dextrorphan or levorphanol as the preexposure drug and saline on conditioning trials showed significant increases in saccharin consumption for all conditioning and extinction trials ($p<0.01$, see Fig. 2). The group of animals that were injected with dextrorphan as preexposure drug and with morphine as conditioning drug displayed substantial decreases in saccharin intake following the two conditioning trials. The reduction in saccharin intake was significant after the second conditioning trial, P2 ($p<0.05$). The rats that were injected with levorphanol, the active isomer, showed increases in saccharin intake at all trials following morphine-saccharin pairings. Significant increases in saccharin consumption were observed in the latter group after the two extinction trials ($p<0.01$). Similarly, the dextrorphan-preexposed group that received ethanol on conditioning trials demonstrated decreases in saccharin intake which was significant after the second pairing, P2 ($p<0.01$). It can also be seen that the levorphanol preexposed animals showed increases in saccharin intake following the two ethanol-saccharin pairings which was significant after the second pairing trial, $p<0.05$.

GENERAL DISCUSSION

As hypothesized, the results showed that levorphanol can induce conditioned taste aversion. The nonactive isomer, dextrorphan was not potent in inducing the aversion at all three doses tested. It is unlikely that the differential effects of dextrorphan and levorphanol relate to differences in their pharmacokinetic properties since optical enantiomers possess highly similar physicochemical properties. It has been shown, for instance, that the optical isomers of pentazocine have comparable half-lives and that the differential analgesic potencies of l-pentozocine and d-pentozocine cannot be accounted for by differences in regional distribution [3]. We thus concluded that conditioned taste aversion induced by opiates was mediated through activity at a stereospecific opiate receptor. Earlier studies have reported that morphine CTA can be attenuated or blocked when naloxone is administered within about an hour of the morphine injection [16,28]. This contrasts with the preexposure effect of opiate agonists on morphine CTA since the interval between the last preexposure injection and the morphine injection is usually two days or longer. The interpretation that the opiate receptor is involved in the CTA induced by opiates is, however, not convincing because of the fact that naloxone itself can produce CTA [28]. In particular, it is difficult to conceive of a CTA induced by the antagonist naloxone as being mediated through the opiate receptor since activation of the opiate receptor characterized by agonists as opposed to only binding by antagonists seems to be required for the induction of CTA. Therefore, if naloxone CTA involves a non-opiate mechanism, it is also possible that its antagonism of morphine CTA is effected through that same non-opiate mechanism. The present study clarified this issue by demonstrating that the major action of opiates in inducing a CTA is through the opiate receptor.

One should also note the U-curve relation between the magnitude of the CTA and levorphanol doses. Such dose-response curve has also been observed by other investigators for morphine [9]. One also observes that dextrorphan, while not causing a decrease in saccharin intake from baseline, produced a decrease in saccharin consumption when compared to the saline group. This might suggest that opiate CTA may at least in part be mediated through a nonopiate-receptor mechanism.

More importantly, however, the present study demonstrated that levorphanol but not dextrorphan blocked with

equal effectiveness both morphine and ethanol CTAs. This finding confirmed our previous report that preexposure to morphine and ethanol will block the CTAs induced by both these drugs in a symmetrical fashion [18]. Thus, we concluded that the preexposure effect of opiates on ethanol CTA involves the opiate receptor. In another study, we have also shown that both the ethanol and morphine CTAs can be reversed by pretreatment with the long-acting opiate antagonist, naloxazone [21]. In contrast to naloxone, naloxazone by itself did not show a CTA. Thus, a stronger statement about the involvement of the opiate receptor in opiate CTA can be made. We therefore suggest that a stereospecific opiate receptor plays an important role both in the CTAs associated with both opiates and ethanol, and in the preexposure effect between opiates and ethanol.

One of the proposed mechanisms of opiate-alcohol interaction suggests that ethanol may influence the synthesis and release of endogenous ligands of the opiate receptors [26]. Some support for this idea has begun to accumulate. For instance, opioid activity level was reported to increase 4-fold in plasma of normal volunteers following acute ethanol administration [19]. In rats, acute ethanol treatment increases beta-endorphin and met-enkephalin levels whereas chronic ethanol treatment decreased the levels of these opioid peptides in selective regions of the brain [25,26]. A reduction in leu-enkephalin level was also observed in basal ganglia following chronic ethanol exposure in hamsters [5]. However, whether these changes in levels of opioid peptides reflect changes in release, and/or changes in synthesis of these peptides still remains to be determined. Interestingly, chronic morphine administration has also been reported to decrease the level of met-enkephalin in the rat striatum [23] in a similar manner to ethanol [26]. Although different mechanisms may be involved in this effect, one can speculate that the actions of both ethanol and opiates on the opioids systems and opiate receptor dynamics are in some way similar, hence, possible explaining the observed preexposure interaction of these two drugs in the present experiment.

Recent evidence has supported the notion of an inhibitory action of ethanol on opioid binding to specific delta opiate receptors [10,22]. However, it is very unlikely that this action of ethanol is responsible for the preexposure interaction for two reasons. First, the effect of ethanol in this paradigm is similar to an opiate agonist, and not to an antagonist. The idea is that opiate agonists produce CTAs but not opiate antagonists or compounds that act like antagonists by interfering with binding of opioids to their receptors. Second, both levorphanol and morphine which have been shown to attenuate or block the CTA to ethanol are relatively selective for the mu receptor [17] as opposed to the delta or kappa receptors.

In summary, the present study demonstrates that the opiate receptor plays an important role in both morphine-and ethanol-induced CTAs. It is evident that more work is needed to examine the roles of specific opiate receptor subtypes in CTA and in the preexposure paradigm between the same drugs and across different drugs. When this information will be available it may become possible to characterize the selectivity of the opiate-ethanol interaction in terms of specific opiate receptor subtypes.

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REFERENCES

1. Barbaccia, M. L., A. Reggiani, P. R. Spano and M. Trabucchi. Ethanol effects on dopaminergic function: Modulation by the endogenous opioid system. *Pharmacol Biochem Behav* **13**: 303–306, 1980.
2. Bass, M. B., H. J. Friedman and D. Lester. Antagonism of naloxone hyperalgesia by ethanol. *Life Sci* **22**:1939–1946, 1978.
3. Berkowitz, B. A. and E. L. Way. Analgesic activity and central nervous system distribution of the optical isomers of pentazocine in the rat. *J Pharmacol Exp Ther* **177**: 500–508, 1971.
4. Bird, K. D., G. B. Chesher, J. Perl and G. A. Starmer. Naloxone has no effect on ethanol-induced impairment of psychomotor performance in man. *Psychopharmacology (Berlin)* **76**: 193–197, 1982.
5. Blum, K., A. H. Briggs, L. Elston, L. De Lallo and P. J. Sheridan. Reduced leucine-enkephalin-like immunoreactive substance in hamster basal ganglia after long-term ethanol exposure. *Science* **216**: 1425–1427, 1982.
6. Blum, K., M. G. Hamilton and J. E. Wallace. Alcohol and opiates: A review of common neurochemical and behavioral mechanisms. In: *Alcohol and Opiates–Neurochemical and Behavioral Mechanisms*, edited by K. Blum. New York: Academic Press, 1977, p. 203.
7. Boada, J., M. Feria and E. Sanz. Inhibitory effect of naloxone on the ethanol-induced antinociception in mice. *Pharmacol Res Commun* **13**: 673–678, 1981.
8. Davis, V. E. and M. J. Walsh. Alcohol, amines, and alkaloids: A possible biochemical basis for alcohol addiction. *Science* **167**: 1005–1007, 1970.
9. Farber, P. H., S. Gorman and L. D. Reid. Morphine injections in the taste aversion paradigm. *Physiol Psychol* **4**: 365–368, 1976.
10. Hiller, J. M., L. M. Angel and E. J. Simon. Multiple opiate receptors: Alcohol selectively inhibits binding to delta receptors. *Science* **214**: 468–469, 1981.
11. Hoffman, P. L., S. Urwyler and B. Tabakoff. Alterations in opiate receptor function after chronic ethanol exposure. *J Pharmacol Exp Ther* **222**: 182–189, 1982.
12. Hymson, D. L. and M. D. Hynes. Evidence that ethanol-induced impairment of roto-rod performance is not mediated by opioid mechanisms. *Prog Neuropsychopharmacol* **6**: 159–165, 1982.
13. Jeffcoate, W. J., M. H. Cullen, M. Herbert, A. G. Hastings and C. P. Walder. Prevention of effects of alcohol intoxication by naloxone. *Lancet* **2**: 1157–1158, 1979.
14. Jorgensen, H. A. and K. Hole. Does ethanol stimulate brain opiate receptors? Studies on receptor binding and naloxone inhibition of ethanol-induced effects. *Eur J Pharmacol* **75**: 223–229, 1981.
15. Khanna, J. M., A. D. Le, H. Kalant and A. E. LeBlanc. Cross tolerance between ethanol and morphine with respect to their hypothermic effects. *Eur J Pharmacol* **59**: 145–147, 1979.
16. LeBlanc, A. E. and H. Cappell. Antagonism of morphine-induced aversive conditioning by naloxone. *Pharmacol Biochem Behav* **3**: 185–188, 1975.

17. Magnan, J., S. J. Paterson. A. Tavani and H. W. Kosterlitz. The binding spectrum of narcotic analgesic drugs with different agonist and antagonist properties. *Naunyn Schmiedebergs Arch Pharmacol* **319:** 197–205, 1982.
18. Middaugh, L. D., E. Read and W. P. Boggan. Effects of naloxone on ethanol-induced alterations of locomotor activity in C57B1/mice. *Pharmacol Biochem Behav* **9:** 157–160, 1978.
19. Naber, D., M. G. Soble and D. Pickar. Ethanol increases opioid activity in plasma of normal volunteers. *Pharmacopsychiatry* **14:** 160–161, 1981.
20. Ng Cheong Ton, J. M. and Z. Amit. Symmetrical effect of pre-exposure between alcohol and morphine on conditioned taste aversion. *Life Sci* **33:** 665–670, 1983.
21. Ng Cheong Ton, J. M. and Z. Amit. Attenuation of ethanol-induced conditioned taste aversion by naloxazone—Behavioral evidence for an opiate receptor-mediated morphine-ethanol interaction. *Neurosci Lett* **48:** 127–132, 1984.
22. Pfeiffer, A., B. R. Seizinger and A. Herz. Chronic ethanol imbibition interferes with delta-, but not with mu-opiate receptors. *Neuropharmacology* **20:** 1229–1232, 1981.
23. Przewolocki, R., V. Hollt, Th. Duka, G. Kleber, Ch. Gramsch, I. Haarmaann and A. Herz. Long-term morphine treatment decreases endorphin levels in rat brain and pituitary. *Brain Res* **174:** 357–361, 1979.
24. Ross, D. H., M. A. Medina and H. L. Cardenas. Morphine and ethanol: Selective depletion of regional brain calcium. *Science* **186:** 63–64, 1974.
25. Schulz, R., W Wuster, Th. Duka and A. Herz. Acute and chronic ethanol treatment changes endorphin levels in brain and pituitary. *Psychopharmacology (Berlin)* **68:** 221–227, 1980.
26. Seizinger, K., D. Bovermann, D. Maysinger, V. Hollt and A. Herz. Differential effects of acute and chronic ethanol treatment on particular opioid peptide systems in discrete regions of rat brain and pituitary. *Pharmacol Biochem Behav* **18:** 361–369, 1983.
27. Tabakoff, B. and P. L. Hoffman. Alcohol interactions with brain opiate receptors. *Life Sci* **32:** 197–204, 1983.
28. Van Der Kooy, D. and A. G. Phillips. Temporal analysis of naloxone attenuation of morhpine-induced taste aversion. *Pharmacol Biochem Behav* **6:** 637–641, 1977.