Pharmacology Biochemistry & Behavior, Vol. 35, pp. 493–495. © Pergamon Press plc, 1990. Printed in the U.S.A. 0091-3057/90 $3.00 + .00

BRIEF COMMUNICATION

Cholecystokinin Octapeptide Reduces Ethanol Intake in Food- and Water-Sated Rats

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Received 24 May 1989

TOTH, P., C. SHAW, E. PERLANSKI AND L. A. GRUPP. *Cholecystokinin octapeptide reduces ethanol intake in food- and water-sated rats.* PHARMACOL BIOCHEM BEHAV **35**(2) 493–495, 1990.—The putative satiety peptide cholecystokinin octapeptide (CCK-8) has been shown to reduce ethanol intake induced by prior fluid deprivation. Since fluid-deprived animals tend to reduce their food intake and consequently become hungry, the ability of CCK-8 to reduce ethanol intake might be limited to conditions where the motivation for food and fluid are accentuated. The present study assessed this possibility by examining the effect of peripheral injections of CCK-8 on voluntary ethanol intake fostered by the limited access procedure which uses food- and water-sated rats. Under these conditions CCK-8 still produced a dose-dependent decrease in ethanol intake. These results demonstrate that CCK-8 reduces ethanol intake even in the absence of hunger and thirst drives.

Ethanol intake Cholecystokinin octapeptide CCK-8 Satiety peptide Food intake

A great deal of experimental evidence suggests a role for cholecystokinin octapeptide (CCK-8) in the short-term limitation of food intake (3, 9, 10). Recently, Kulkosky *et al.* have shown that CCK-8 can also reduce the consumption of ethanol (4–6). In the Kulkosky *et al.* experiments fluid deprivation-induced thirst was employed to motivate ethanol consumption. Fluid deprivation, however, not only alters fluid balance to make animals thirsty, but also reduces food intake and, therefore, makes animals hungry (1). Ethanol is a rich nonprotein source of calories (7.1 kcal/g) as well as a psychoactive drug and under conditions of food restriction ethanol intake can increase in order to replace calories unavailable from food (11). It is, therefore, possible that CCK-8 may be effective in reducing ethanol intake only under conditions of water restriction and the consequent reduced food intake. The present experiment, therefore, examined the effect of three doses of CCK-8 on voluntary ethanol intake fostered by a procedure which does not employ fluid or food deprivation to induce the consumption of pharmacologically relevant doses of ethanol (7) and, therefore, does not carry with it the motivation for food and water. This procedure, termed the limited access procedure, was first described by MacDonall and Marcucella (8) and promotes ethanol intake by restricting its availability to a relatively brief (40 min) daily access period. A reduction in ethanol intake by the CCK-8 peptide under limited access conditions would suggest that thirst and/or hunger are not necessary preconditions for CCK-8 to exert its effect.

METHOD

Subjects

The subjects were 35 naive male Wistar rats (Charles River, Montreal) weighing 250–300 g. Animals were individually housed and maintained on a reverse 12-hr/12-hr light/dark cycle with lights off at 7 a.m. Purina rat chow and tap water were available in home cages ad lib.

Procedure

A limited access drinking procedure was used to promote ethanol consumption (7,8). On weekdays at approximately 10 a.m. (i.e., during the dark cycle) rats were weighed and then transferred from their standard hanging wire home cages to

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individual "drinking cages" where an ethanol solution was available in one graduated tube and tap water in another. The home cages and drinking cages were standard hanging wire cages identical in size (30×24×21 cm) and construction and were located in the same colony room. The position of the tubes was alternated daily and no food was available in the drinking cages. After 40 min had elapsed, the animals were returned to their home cages and ethanol and water consumption were measured to the nearest 0.1 ml.

The limited access procedure employs a gradual exposure of the animals to the taste and effects of ethanol. The animals were initially offered a choice between a relatively low ethanol concentration (3% w/v) and water for 9 days. This was followed by a baseline phase lasting 8 days during which animals were offered a choice between 6% (w/v) ethanol and water. At the end of this phase animals were divided into 4 groups matched for ethanol intake each designated to receive either the saline vehicle (n=9), 4 μg/kg CCK-8 (n=8), 8 μg/kg CCK-8 (n=8) or 16 μg/kg CCK-8 (n=8) during the subsequent treatment phase which lasted 7 days. Each group received its respective dose of CCK-8 or saline by the intraperitoneal route on a daily basis 10 min prior to the ethanol access period.

RESULTS

Ethanol Intake

Ethanol intake for each animal was averaged across the 8-day baseline and 7-day treatment phases and the group grand means for each phase are depicted in Fig. 1a. The average dose of ethanol consumed for all four groups during baseline was approximately 0.6 g/kg. Given that animals can metabolize 0.3–0.35 g/kg/hr, and that most of the ethanol tends to be consumed in a bout that lasts for the first 15–20 min of the daily drinking session, it is clear that the animals were experiencing a pharmacological effect of the drug (7). A one-way analysis of variance performed on the treatment phase data yielded a significant effect of Drug, $F(3, 31)=10.41$, $p<0.001$, indicating that CCK-8 did indeed reduce ethanol intake fostered by the limited access procedure. Post hoc Duncan's test ($p<0.05$) revealed a differential effect of dose indicating a dose-dependent effect of CCK-8 on ethanol intake.

Water Intake

Water intake for each animal was averaged across the 8-day baseline and 7-day treatment phases and the group means are depicted in Fig. 1b. A one-way analysis of variance of intake during the treatment phase yielded a significant effect of Drug, $F(3,31)=2.98$, $p<0.05$, indicating that CCK-8 increased water intake and a post hoc Duncan's test revealed that both the 8 μg/kg and 16 μg/kg doses of CCK-8 increased water intake compared to the saline control group.

DISCUSSION

Previous work by Kulkosky *et al.* has shown that CCK-8, a brain-gut peptide known to reduce food intake, can also reduce ethanol intake (4–6). Under conditions of fluid deprivation such as existed in those experiments, animals naturally reduce their food intake and become hungry as well as thirsty. Therefore, an interpretation of those findings in terms of an effect of the peptide on the motivation to consume ethanol (presumably for its pharmacological properties) is difficult in view of the fact that the peptide-mediated reduction in ethanol intake was demonstrated only in a situation where the motivation for food and water was also present. The present study attempted to address this problem by reinvestigating the effect of CCK-8 on ethanol intake using the limited access procedure which fosters *voluntary* intake without recourse to fluid deprivation or food restriction. The present experiment confirmed that CCK-8 could produce a dose-dependent reduction in ethanol intake even in animals who were food- and water-sated indicating that hunger and thirst are not necessary for the effect on ethanol intake to be expressed. This in turn suggests that CCK-8 may reduce ethanol intake, in part, by altering its pharmacological or psychoactive effects.

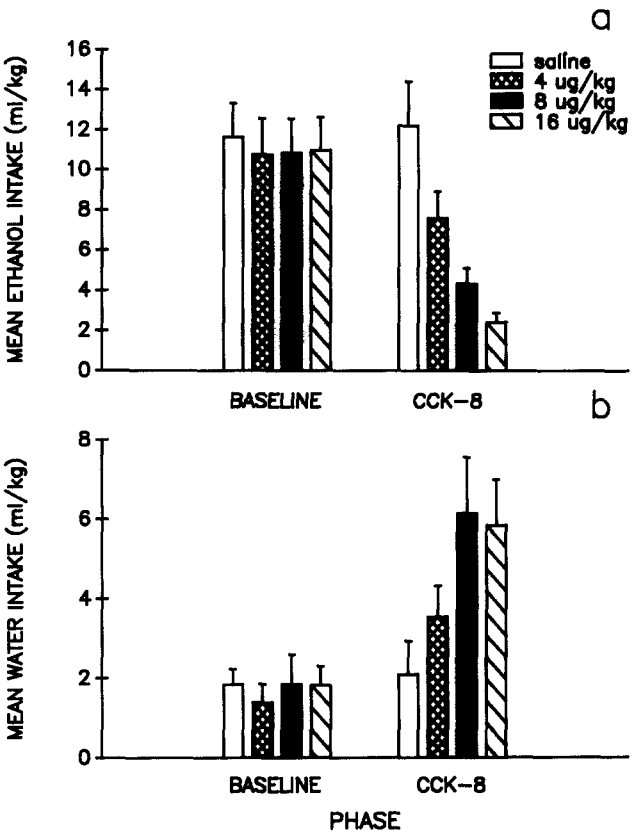

FIG. 1. (a) Mean 6% (w/v) ethanol intake (ml/kg) for the three cholecystokinin groups and the saline control group before (baseline) and after (CCK-8) drug treatment. Vertical lines represent standard error of the mean. (b) Mean water intake (ml/kg) for the three cholecystokinin groups and the saline control group before (baseline) and after (CCK-8) drug treatment. Vertical lines represent standard error of the mean.

Doses of CCK-8 that suppressed ethanol intake also stimulated water intake. However, it is unlikely that CCK-8 exerted its effect on ethanol intake by simply making animals "thirstier" for water, since Kulkosky (4) demonstrated that CCK-8 is able to reduce ethanol without altering water intake. Additionally, since CCK-8 suppressed ethanol intake specifically, and not all drinking behavior, it is unlikely that CCK-8 reduced ethanol intake by making animals feel ill.

Ethanol contains calories and is consumed orally, therefore, ethanol consumption can be viewed as the intake of a *drug* and a *calorically rich foodstuff* (4). In the present study, the relevance of ethanol as a source of calories was minimized by testing animals who were not hungry and yet CCK-8, a peptide known to participate in the regulation of feeding, was still able to modify ethanol intake. These findings support a role for CCK-8 in the control of ethanol intake that is based on its ability to alter some aspect of the pharmacological properties of the drug and independent of its effect on food intake.

ACKNOWLEDGEMENTS

The authors thank Dr. J. Gibbs and Dr. M. A. Linseman for the critical reading of the manuscript and E. R. Squibb & Sons for supplying the cholecystokinin octapeptide. This research was supported by the Addiction Research Foundation of Ontario and P. Toth is a recipient of the Ontario Graduate Scholarship.

REFERENCES

1. Di Bella, L.; Rossi, M. T.; Scalera, G. A contribution to a correlation between drinking and feeding behaviour. Appetite 4:198; 1983.
2. Fedorchak, P. M.; Bolles, R. C. Nutritive expectancies mediate cholecystokinin's suppression-of-intake effect. Behav. Neurosci. 102: 451–455; 1988.
3. Gibbs, J.; Young, R. C.; Smith, G. P. Cholecystokinin decreases food intake in rats. J. Comp. Physiol. Psychol. 84:488–495; 1973.
4. Kulkosky, P. J. Effect of cholecystokinin octapeptide on ethanol intake in the rat. Alcohol 1:125–128; 1984.
5. Kulkosky, P. J.; Chavez, M. R. Sulphated cholecystokinin octapeptide inhibits ethanol consumption in the rat. Alcohol 1:409–412; 1984.
6. Kulkosky, P. J. Brain-gut neuropeptides and the limitation of ethanol consumption. Neurosci. Biobehav. Rev. 9:179–190; 1985.
7. Linseman, M. A. Alcohol consumption in free-feeding rats: Procedural, genetic and pharmacokinetic factors. Psychopharmacology (Berlin) 92:254–261; 1987.
8. MacDonall, J. S.; Marcucella, H. Increasing the rate of ethanol consumption in food- and water-sated rats. Pharmacol. Biochem. Behav. 10:211–216; 1979.
9. Pi-Sunyer, X.; Kissileff, H. R.; Thornton, J.; Smith, G. P. C-terminal octapeptide of cholecystokinin decreases food intake in obese men. Physiol. Behav. 29:627–630; 1982.
10. Smith, G. P.; Gibbs, J. The effect of gut peptides on hunger, satiety, and food intake in humans. In: Wurtman, R. J.; Wurtman, J. J., eds. Annals of the New York Academy of Sciences: Human obesity. vol. 499. New York: The New York Academy of Sciences; 1987:132–136.
11. Stiglick, A.; Woodworth, I. Increase in ethanol consumption in rats due to caloric deficit. Alcohol 1:413–415; 1984.