THE PORTACAVAL-SHUNTED RAT: A NEW MODEL FOR THE STUDY OF THE MECHANISMS CONTROLLING VOLUNTARY ETHANOL CONSUMPTION AND ETHANOL PREFERENCE?

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This work was supported by a grant from the Medical Research Council of Canada (PG 11118) (to R.F.B.). J.-P. de Wade is the recipient of a post-doctoral fellowship from the Canadian Liver Foundation.

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ABSTRACT

Portacaval anastomosis (PCA) is a surgical procedure whereby blood from the portal vein is shunted into the inferior vena cava. PCA in the rat results in a significant increase (from 0.77 ± 0.26 to 3.51 ± 0.37 g of ethanol/kg/day) in voluntary ethanol consumption in a free- choice paradigm between water and 5% ethanol solution. After PCA surgery, increased voluntary ethanol consumption starts abruptly at 6 to 7 days and is maintained for >28 weeks. Voluntary ethanol consumption in rats after PCA results in blood ethanol levels up to 158 mg%. After PCA, the ethanol preference ratio (defined as the percentage of total fluid intake constituted by ethanol) increased from 19 ± 2% to 78 ± 2% ($p < 0.001$). Administration of the nonselective opioid receptor antagonist naloxone (5 mg/kg, sc) resulted in a significant 6-fold attenuation of voluntary ethanol consumption by rats with PCA, an effect that was not mediated by an effect on locomotor activity. These findings, together with previous reports of wide- spread alterations of the p- and 6-opioid receptors in the brain after PCA, suggest that increased voluntary ethanol consumption and ethanol preference in PCA rats may result from activation of the endogenous opioid system. Preliminary studies suggest that rats with PCA manifest behavioral signs consistent with the development of dependence. The portacaval-shunted rat may provide a useful preparation for the study of mechanisms, in particular those involving the liver, implicated in the development of increased voluntary ethanol consumption and ethanol preference.

Key Words: Ethanol, Preference, Liver, Opioid System, Portacaval Anastomosis.

INTRODUCTION

CHRONIC LIVER disease of either alcoholic or non-alcoholic etiology invariably results in the formation of portal-systemic shunts as a result of the increase in portal pressure. This portal-systemic shunting frequently results in a complex neuropsychiatric syndrome known as portal- systemic encephalopathy (PSE)(1), with associated changes in brain biochemistry. Increased voluntary ethanol consumption, in a free-choice two-bottle paradigm, has been reported in rats after

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portacaval shunting(2,3) and in rats with carbon tetrachloride(CCL4)-induced cirrhosis4). This finding was surprising, because it is well known that studies of ethanol consumption in the laboratory rat are limited by the innate aversion of rats to ethanol. It is possible that the increase in voluntary ethanol consumption in rats after portacaval shunting may result from alterations in brain biochemistry; such changes include amino acids(6), monoamine(7,8), or opioid peptides.(9,10) With regard to the latter, there is substantial evidence to suggest that the endogenous opioid system of the brain is one of the major neuronal systems involved in the media- tion of increased ethanol consumption. Similarities exist between some of the physiological effects of ethanol and opiates.(11) Administration of nonselective opiate antagonists, such as naloxone(12-14) and naltrexone (12-14) and of the selective δ-opioid receptor antagonists IC1-174864 (15) and naltrindole(16) lead to reductions in voluntary ethanol consumption in rodents. Furthermore, naltrexone has been used in clinical studies for the treatment of ethanol dependence in humans (17,18). Ethanol stimulates the in vitro release of β-endorphin (β-EP) from rat pituitary(19) and hypothalamic(20) preparations, and differences in hypothalamic β-EP release have been demonstrated between ethanol- preferring and ethanol-avoiding strains and lines of animal(21-23).

Previous studies have revealed alterations of the endogenous opioid system in patients with liver disease (24,25) and in an animal model of PSE (26) as well as in CC14,-induced cirrhosis(27). Recently, we reported that portacaval anastomosis (PCA) in the rat induces alterations in the β-EP system of the brain, as well as in μ- and δ-opioid receptors(10). Four weeks after PCA surgery, quantitative autoradiographic studies using selective radioligands revealed that ethanol-naive PCA rats manifest significant changes in the densities of opioid binding sites in nuclei of the limbic system known to modulate the rewarding and the positive- reinforcing effects of many drugs of abuse, including ethanol(18). The aim of the present study was to characterize further the increase in voluntary ethanol consumption by rats after PCA. Ethanol consumption, in a free-choice paradigm be- tween 5% ethanol and water, was monitored before and after either PCA or sham operation (control). The time come of the development of ethanol preference with respect to PCA surgery time and with respect to day time and night time activity patterns was also assessed. To evaluate the implication of the endogenous opioid system in the increased voluntary ethanol consumption by PCA rats, effects of the opioid antagonist naloxone was studied on both ethanol intake and locomotor activity. Preliminary assessment of dependence on ethanol was made by monitoring relapse to ethanol consumption after episodes of withdrawal.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 250 to 300 g (Charles River, St.-Constant, Québec) were housed singly under a 12-hr light/dark cycle (lights on at 06:00 AM and lights off at 06:00 PM), with food (Purina Lab Chow) and water available ad libitum. After a 24-hr acclimatization period, the animals were given the choice between two identical drinking bottles: one containing 100 ml of tap water and the other containing 100 mI of a 5% ethanol solution, made up of 95% ethanol diluted with tap water. The two drinking bottles were surrounded by food pellets to avoid any food-related preference bias. For similar reasons, the position of the two drinking bottles in the home cage was changed every day. The volume of liquid consumed and the animals' body weight were monitored daily.

After 2 weeks of ethanol exposure, rats underwent a laparotomy under halothane anesthesia, and an end-to-side portacaval anastomosis was performed as previously decribed.(29). Surgery time was under 15 min. For sham operation, after laparotomy, the portal vein and vena cava were clamped for 15 min. Animals were returned to their home cage with food available ad libitum and the choice between water and the 5% ethanol solution. Body weight, as well as water and ethanol consumption, were monitored daily for a period of 5 weeks postsurgery. Six weeks after PCA surgery, the concentration of the ethanol solution was increased to 10% for the PCA rats.

ACUTE EFFECTS OF NALOXONE ON ETHANOL CONSUMPTION

The effects of naloxone were studied after 5 weeks of voluntary 5% ethanol consumption by PCA rats versus sham-operated controls. Ethanol and water were presented in a two-bottle free-choice paradigm, with food and liquid available ad libitum.

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Before the administration of either saline or naloxone, ethanol consumption was monitored on consecutive days between 06:00 and 09:00 PM and between 09:00 and 11:00 PM (lights-off period). NaIoxone-HCI (Sigma Chemical Co., St. Louis, MO) was diluted in sterile saline to afford a stock solution of 50 mg naloxone/ml. The stock solution was diluted with saline and administered subcutaneously at a final dose of 5 mg of naloxone/kg of body weight. The day before naloxone administration, animals were administered an equivalent volume of sterile saline subcutaneously to control for the effects of injection stress. Ethanol consumption was monitored for 180 and 300 min after the administration of saline or naloxone, and compared with ethanol consumption measured in the absence of either saline or naloxone.

Behavioral Testing

Animals were transferred to an isolated room equipped with an Opto-Varimex activity monitor coupled to an Auto-Track system (Columbus Instruments, Columbus, OH) for a period of five consecutive days, under a 12-hr light cycle (lights on at 06:00 AM and lights off at 06:00 PM), with food, water, and 5% ethanol available ad libitum. Locomotor activity of the animals was monitored during the lights-off period on each day. The activity of the animals measured on days 2 and 3 was used to define the normal (baseline) of the animals. On day 4, just before the onset of the lights-off period, rats were administered a single injection of saline, whereas on day 5, they received a single injection of naloxone (5 mg/kg sc).

Determination of Blood Ethanol Levels

Tail blood (20 μL) was collected at various time intervals and was immediately deproteinized in 180 μl of 6.25% trichloroacetic acid. Ethanol content was determined enzymatically by the oxidation of ethanol to acetaldehyde coupled to the reduction of NAD to NADH (Sigma Diagnostics Kit 332-UV, Sigma).

Preliminary Assessment of Ethanol Dependence

Two approaches were used to determine whether PCA rats developed signs of ethanol dependence. First, ethanol was withdrawn for periods of 1,3, or 8 days, with the ethanol solution being replaced by water; there was 1 week of ethanol availability between each withdrawal session. Voluntary ethanol consumption was monitored during the lights-on period, between 10:00 AM and noon, before ethanol withdrawal, and at the same time period after re-exposure to ethanol. The behavior of the animals was monitored for signs of ethanol withdrawal. In a second experiment, the effect of 3 days ethanol withdrawal was monitored 2 hr after onset of the lights-off period.

Statistical Analysis

For statistical evaluation of the data, one- or two-way repeated measures ANOVAs, followed by the Newman-Keuls or Dunnet post-hoc tests, were used for multiple group comparisons. The unpaired Student's t test for two group comparisons was also used; $p \leq 0.05$ was considered to be statistically significant. The nonparametric Mann Whitney U test was used in the naloxone experiment, with $p \leq 0.05$ considered to be statistically significant.

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RESULTS

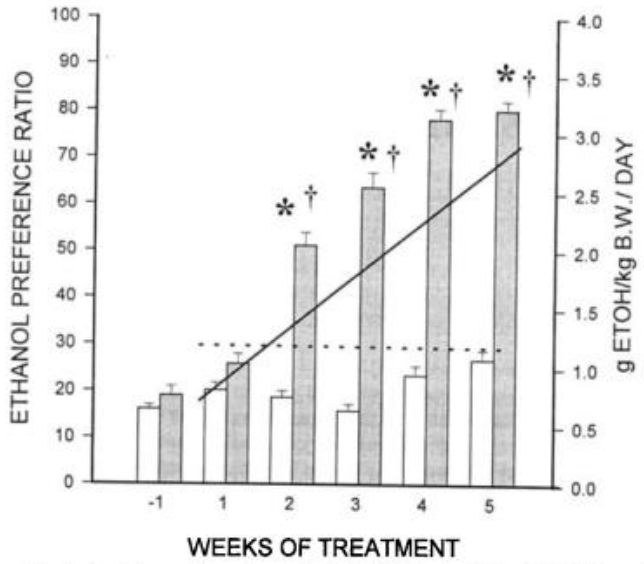

Fig. 1. Development of preference for the 5% ethanol solution in PCA (closed bars) but not sham-operated control (open bars) rats. Data represent the ratio of 5% ethanol solution to water as total liquid consumption and indicate the mean ± SE of daily liquid intakes for 7 PCA and 6 sham-operated control rats, calculated for a consecutive 7-day period. *Significant difference from control ($p < 0.01$). †Significant difference from presurgery levels ($p < 0.05$). Lines represent the slope of the changes in the amount of ethanol consumed voluntarily after surgery in PCA (solid line) and sham-operated control (dotted line) rats. ETOH, ethanol; B.W., body weight.

Figure 1 shows the effects of PCA on voluntary ethanol consumption in the rat. PCA had no effect on the total volume of liquid consumed by the animals, which was maintained in the 72 to 81 ml/kg of body weight/day range for the duration of the experimental period. PCA induced an 11% decrease in body weight during the first 2 weeks postsurgery, which was followed by a gradual increase in body weight (data not shown), so that 2 weeks postsurgery, weight gain curves for the animals in the shunted group were not significantly different from sham-operated controls. Results of ethanol consumption are expressed as the ethanol preference ratio (volume of ethanol solution/total volume of fluid consumed) to assess the development of ethanol preference in PCA rats. This ratio was not significantly modified by sham operation. Before PCA surgery, 19 ± 2% of the rats' total fluid intake constitued ethanol solution (1.93 ± 0.25 ml/kg of body weight/day). PCA led to an increase in this ratio to 78 ± 2% (6.5 ± 0.34 ml/kg of body weight/day), 4 weeks after surgery. A two-way ANOVA with treatment and time as independent variables demonstrated a significant effect of treatment [$F(1,44) = 50.12$; $p < 0.001$] and time [$F(4,44) = 45$;$p < 0.001$]. These significant increases in ethanol preference ratio in PCA rats were manifested at weeks 2, 3, and 4 after PCA ($p < 0.05$, Newman-Keuls test). There was no difference in ethanol consumption with time in sham-operated control rats. The level of ethanol consumption after PCA resulted in mean blood ethanol levels, measured 3 hr after the onset of the lights-off period, of 40 mg% (range: 3 to 158 mg%). In parallel with the change in ethanol preference ratio, there was a significant increase in the total amount of ethanol consumed daily by PCA rats, which showed a positive correlation between volume of ethanol consumed and day of treatment (Spearman's $r = 0.648$; $p < 0.001$). Sham- operated control rats maintained a low but stable consumption of ethanol throughout the duration of the study (Spearman's $r = 0.017$; $p = 0.789$) (Fig. 1).

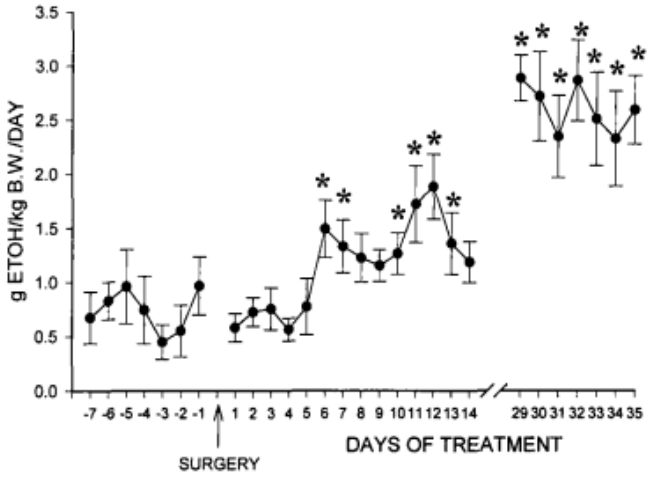

Fig. 2. Changes in the mean daily consumption of ethanol in seven PCA rats before PCA, during the first 2 weeks after surgery and in the fifth week after surgery. *Significant difference from presurgery levels ($p < 0.05$). ETOH, ethanol; B.W., body weight.

The increase in ethanol consumption in rats after PCA took 6 to 7 days to develop, as demonstrated in Fig. 2. After a sharp increase, ethanol consumption increased steadily for three consecutive weeks, as shown in Fig. 1. A one-way repeated-measures ANOVA followed by the Dunnet's test demonstrated a significant effect of time [$F(22,160) = 6.94$; $p < 0.001$]. Daily ethanol consumption by PCA rats then stabilized 4 weeks after surgery and remained at comparable levels for up to

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28 weeks. Similar levels of ethanol consumption were observed when ethanol concentration was doubled to 10% (3.51 ± 0.37 g/kg of body weight/day). At this time, the PCA rats consumed 59% of their ethanol during the lights-off period.

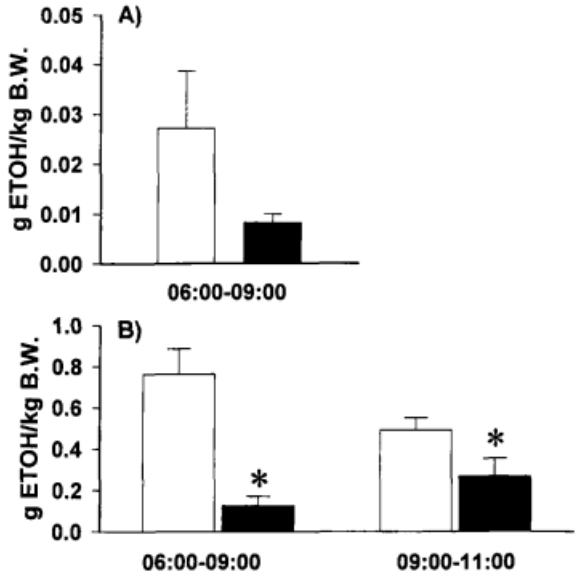

Fig. 3. Effect of naloxone administration (5 mg/kg, sc) on the voluntary consumption of a 5% ethanol solution by sham-operated (A) and PCA (B) rats. Data represent the amount of ethanol consumed (mean ± SE for seven rats for each group) in the absence (open bars) and presence (closed bars) of naloxone over a 3-hr period, from the onset of the lights-off period, for the sham-operated rats and 5 hr for the PCA rats. *Significant difference from saline treatment ($p < 0.01$). ETOH, ethanol; B.W., body weight.

The effects of saline or naloxone administration (5 mg/kg sc) on voluntary ethanol consumption in rats 5 weeks after PCA or sham operation are presented in Fig. 3. Administration of naloxone resulted in a significant ($p < 0.01$, Student's t test) decrease in voluntary ethanol consumption in PCA rats, but not in sham-operated control rats ($p > 0.1$, Student's t test or $p > 0.08$, Mann Whitney nonparametric U test). This decrease in voluntary ethanol consumption in rats with PCA was still present 5 hr after administration of naloxone, a period that represents approximately twice the biological half-life of the antagonist.

The effects of saline and naloxone on locomotor activity in PCA rats are presented in Table 1. Locomotor activity increased in the hours after the onset of the dark period as expected. Although the animals were more active after saline injection than in basal conditions, this difference was not statistically significant ($p > 0.1$, Student's t test). Administration of naloxone (5 mg/kg sc) however, had no effect on the locomotor activity of PCA rats.

Table 1. Effect of the Injection of Saline and Naloxone (5 mg/kg, sc) on Locomotor Activity in PCA Rats

	Total activity counts/hour		
Time	Control	Saline	Naloxone
Hour 1	409 ± 85	723 ± 268	407 ± 140
Hour 2	466 ± 87	666 ± 185	437 ± 115
Hour 3	557 ± 225	1113 ± 251	629 ± 230

Locomotor activity of PCA rats was measured using an Animex activity monitor. Results are expressed as the number of beams crossed in 1 hr under normal conditions and after the subcutaneous injection of saline or naloxone (5 mg/kg body weight) on two consecutive days after 2 days of determination of normal (baseline) activity. Each value represents the mean ± SE for six different animals. Neither saline nor naloxone had a significant effect on locomotor activity in PCA rats.

During withdrawal from ethanol, PCA rats showed signs of withdrawal such as teeth chattering, piloerection, vocalization, and aggressivity when handled, even though the animals had been handled daily for 7 weeks. After periods of withdrawal, there was an immediate relapse to increased voluntary ethanol consumption (Fig. 4A). When access to ethanol was resumed by replacing the water of one of the two drinking bottles by ethanol, all PCA rats resumed drinking within 1 min, after a minimum of exploratory, mainly

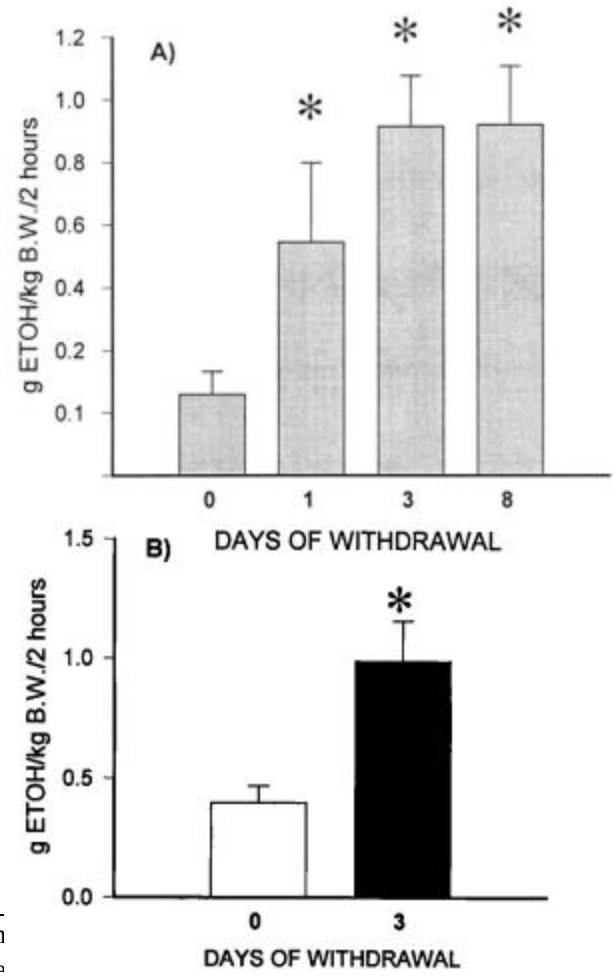

Fig. 4. (A) Effects of periods of ethanol deprivation on the amount of ethanol consumed by PCA rats in the 2 hr after reinstatement of the 10% ethanol bottle during the lights-on period. (B) Effects of 3 days of ethanol deprivation on the amount of ethanol consumed by PCA rats in the 2 hr after reinstatement of the 10% ethanol bottle during the lights-off period. Each bar represents the mean ± SE for seven PCA rats. *Significant difference from control ($p < 0.01$). ETOH, ethanol; B.W., body weight.

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olfactory, behavior. A one-way ANOVA shows a significant effect of time of withdrawal [$F(3,40) = 9.844$, $p < 0.001$]. The effects of 3 and 8 days of withdrawal on ethanol consumption were similar ($p > 0.1$, Newman-Keuls), and blood ethanol levels reached 30 ± 10 mg% at the end of the experimental period. Similar results were observed when ethanol consumption was measured during the first 2 hr of the lights-off period (Fig. 4B). However, in both cases, there was no significant difference in the total volume of ethanol consumed in 24 hr after withdrawal, compared with control values.

DISCUSSION

Results of the present study demonstrate that rats develop increased ethanol preference and increased consumption within 7 days of PCA, and that this increased voluntary ethanol consumption is sustained for several months. PCA rats orally self-administer pharmacologically significant amounts of ethanol in a free-choice paradigm, producing blood ethanol levels up to 158 mg%. They develop a preference for ethanol solution over drinking water and show behavioral signs suggestive of ethanol dependence. These results confirm and extend previous observations of increased ethanol consumption by rats after PCA.(2,3) Because, in the present studies, the positions of ethanol and water bottles were alternated every day, development of a position preference bias in either the PCA or sham-operated control rats was eliminated. Both the ethanol preference ratio (defined as the percentage of total fluids consumed in the form of ethanol) and total ethanol consumption remained at low values in the sham-operated control group. In contrast, the volume of ethanol consumed by the PCA rats increased 2-fold (from 0.77 ± 0.26 to 1.49 ± 0.26 g/kg of body weight/day), and the ethanol preference ratio increased 4-fold within 7 days of surgery. Mean voluntary ethanol consumption, with free access to water and food, by the selectively bred alcohol-preferring P and AA lines of rats, are reportedly in the 5 to 7 g of ethanol/kg of body weight/day range, a pattern of consumption that produces blood ethanol levels as high as 100 mg%.(30,31). PCA rats consume less ethanol than these selectively bred ethanol-preferring rats, an observation that may result from the fact that genetic selection, either by in-breeding or by selective breeding, could induce an exacerbation of the selected trait (32), thus resulting in an exceedingly high amount of ethanol consumed by these animals. However, preliminary findings in the present study suggest that PCA rats do develop neurobehavioral signs consistent with physical dependence on ethanol suggest that PCA rats do develop neurobehavioral signs consistent with physical dependence on ethanol. For example, signs of withdrawal, corresponding to stages I1 to IV of withdrawal,(33) were observed when the ethanol solution was removed from PCA rats after 5 weeks of continuous ethanol exposure. Other indications of the possible development of physical dependence is the observation that PCA rats respond to a period of ethanol deprivation by a significant increase in ethanol consumption when unrestricted access is reinstated, a behavior previously reported both in ethanol-dependent animals(34) and in humans.(35) In the present study, the volume of ethanol consumed was monitored both between 10:00 AM and noon, when normal animals are inactive and when ethanol consumption would normally be minimal, and also during the 2 hr after the onset of the lights-off period. The volume of ethanol consumed during the 2-hr period of lights-on, after reinstatement of ethanol exposure after 3 or 8 days of withdrawal, represents a 5- to 10-fold increase in the amount of ethanol normally consumed during the 10:00 AM to noon period. Similarly, the PCA rats respond to periods of withdrawal by a significantly higher level of ethanol consumption during the 2-hr period after reinstatement of ethanol, at the onset of the lights-off period after 3 days of withdrawal (Fig. 4B). In both cases, the increased ethanol consumption is restricted to the first 2 hr after ethanol reinstatement, whereas no significant difference is observed in the total volume of ethanol consumed during the 24-hr period after reinstatement of ethanol, compared with the total ethanol intake for 24 hr before withdrawal episodes.

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The increase in voluntary ethanol consumption by rats after PCA may result from alterations in the activity of the endogenous opioid system(10). In favor of such a mechanism, previous reports describe significant increases in the density of δ-opioid receptors in the nucleus accumbens of ethanol-naive PCA rats, compared with sham-operated controls (10), and increased densities of δ-opioid receptors have been described in the nucleus accumbens of ethanol-naive alcohol-preferring AA rats, compared with alcohol-avoiding as well as in preliminary studies of the P rats compared with NP rats(37) and for the C57BL/6 (alcohol-preferring) compared with the DBN2 (alcohol-avoiding) inbred strains of mice.(38) The rewarding effects of ethanol and other drugs of abuse are mediated by the release of dopamine from the terminal fields of the A10 dopaminergic system located in the nucleus accumbens(39,40) and ethanol-induced release of dopamine in the nucleus accumbens of Sprague-Dawley rats has been demonstrated in vivo.(28,41) Microdialysis studies demonstrate that focal injection into the nucleus accumbens of [D-Ala-2] deltorphin II, a δ-receptor (42) blocked the ethanol-induced release of dopamine in the nucleus accumbens, suggesting that δ-receptors in the nucleus accumbens play a major role in the mediation of the positive-reinforcing effects of ethanol and thus in the control of voluntary ethanol consumption. These observations suggest that increased ethanol preference in PCA rats may be mediated, at least in part, by the endogenous opioid system, a suggestion that is further supported by the observation, in the present studies, of a significant reduction in ethanol intake after the administration of the nonspecific opioid receptor antagonist naloxone to these animals (Fig. 3). Previous studies suggest that naloxone induces a reduction in motor and one could argue that this may have been responsible for the decrease in ethanol consumption in the present study. However, this does not seem to be the case, because there were no differences in locomotor activity of PCA rats in the absence and presence of naloxone (Table l), although there was a transitory increase in locomotor activity after the injection of saline alone, which probably resulted from injection stress. Thus, the observed decrease in voluntary ethanol consumption in PCA rats could be attributable to the blockade of opioid receptors, preventing the mediation of the positive-reinforcing effects of ethanol in these animals. Further studies using selective antagonists to both p- and S-opioid receptor subtypes are required to elucidate further the precise nature of the role of the opioid system in the modulation of increased ethanol consumption and preference by PCA rats.

The PCA rat model may facilitate the study of molecular mechanisms responsible for the initiation and development of ethanol preference and consumption in the same animal, irrespective of genetic predisposition. Furthermore, a careful examination of the biochemical changes occurring during the first 2 weeks after PCA, in relation to the development of increased voluntary ethanol consumption, may provide new insights into the role of neurotransmitter and/or neuropeptide systems involved in the development of alcohol preference and increased ethanol consumption. The observation that PCA induces an increase in voluntary ethanol consumption raises the possibility that some forms of excessive ethanol consumption may be the result of liver dysfunction, and that individuals with alcoholic or nonalcoholic liver disease may be susceptible to the development of hazardous drinking.

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