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Research Report

Intermittent high-dose ethanol exposures increase motivation for operant ethanol self-administration: Possible neurochemical mechanism

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ABSTRACT

We investigated the neurochemical mechanism of how high-dose ethanol exposure may increase motivation for ethanol consumption. First, we developed an animal model of increased motivation for ethanol using a progressive ratio (PR) schedule. Sprague–Dawley rats were trained to administer 10% ethanol-containing gelatin or plain gelatin (on alternate weeks) in daily 30-min sessions under different fixed ratio (FR) and PR schedules. During FR schedules, rats self-administered about 1 g/kg ethanol, which was decreased to 0.4±0.03 g/kg under PR10. Rats then received four pairs of either 3 g/kg ethanol or saline injections during the weeks when the reinforcer was plain gelatin. During subsequent ethanol gel sessions, breakpoints and ethanol consumption rose 40% in the high-dose ethanol group by the fourth set of injections with no change in plain gel responding. Alterations in amino acids in the ventral striatum (VS) during PR10 responding for 10% ethanol gelatin and plain gelatin were measured using microdialysis sampling coupled with capillary electrophoresis and laser-induced fluorescence detection. There was greater release of taurine, glycine and glutamate in the NAC of the high-dose ethanol rats during 10% ethanol-containing gelatin responding, compared to the control rats or during plain gel responding. An increase in the release of glycine in this same brain region has recently been shown to be involved with anticipation of a reward. Thus, it appears that intermittent high-dose ethanol exposure not only increases motivation for ethanol responding but may also change neurotransmitter release that mediates anticipation of reinforcement, which may play a key role in the development of alcoholism.

1. Introduction

Dopamine (DA) release in the nucleus accumbens (NAC) has been proposed as a neurobiological substrate of reward (Koob, 1998; Tupala and Tiihonen, 2004). Consistent with this hypothesis, ethanol administration increases release of DA (Imperato and Di Chiara, 1986; Yoshimoto et al., 1991, 1992; Diana et al., 1992; Tanda and Di Chiara, 1998; Gonzales et al., 2002; Lominac et al., 2006) in NAC. However, other neurotransmitters in NAC, particularly amino acids, are affected by

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ethanol and so may play a role in alcohol addiction. For example, taurine levels in NAC are selectively and dose-dependently increased by acute ethanol exposure, and this escalation is enhanced after repeated ethanol treatment (Dahchour et al., 1994; Dahchour and De Witte, 1996, 2000a; Smith et al., 2004; Li et al., 2008). Similarly, although acute ethanol exposure has small and inconsistent effects on glutamate levels in NAC, repeated ethanol exposure increases glutamate levels (Moghaddam and Bolinao, 1994; Dahchour et al., 1994, 1996, 2000; Selim and Bradberry, 1996; Piepponen et al., 2002; Szumlinski et al., 2005, 2007; Lominac et al., 2006). Finally, increases of glycine in NAC are related to the amount of anticipation of reward (Li et al., 2008) so may be altered if repeated exposure to ethanol changes the reward value of ethanol.

One of the objectives of the present study was to develop a rat model of alcoholism to investigate the neurobiological consequences of forced intermittent ethanol (FIE) exposures that result in loss of consciousness. Recently an ethanol-containing gelatin (10% ethanol, 10% Polycose®, 0.25% gelatin, w/v) has been used to induce reliable and robust self-administration without food or water restriction (Rowland et al., 2005). The ethanol gel produces pharmacologically relevant concentrations of ethanol in the brains of rats (about 8 mM) that are comparable to the concentrations observed after voluntary ethanol drinking (Peris et al., 2006). In the present study, we used this procedure to develop a rat model with increased motivation for alcohol consumption (IMAC) using operant self-administration of ethanol. We chose fixed ratio (FR) schedules to establish baseline responding for ethanol reinforcement and progressive ratio (PR) schedules to assess motivation. We then used the IMAC model to determine whether a history of repeated high-dose FIE influences neurotransmitter response to ethanol self-administration or to a 3 g/kg ethanol test injection. We hypothesized that the high-dose ethanol group would exhibit sensitized release of glutamate, glycine and taurine in the NAC.

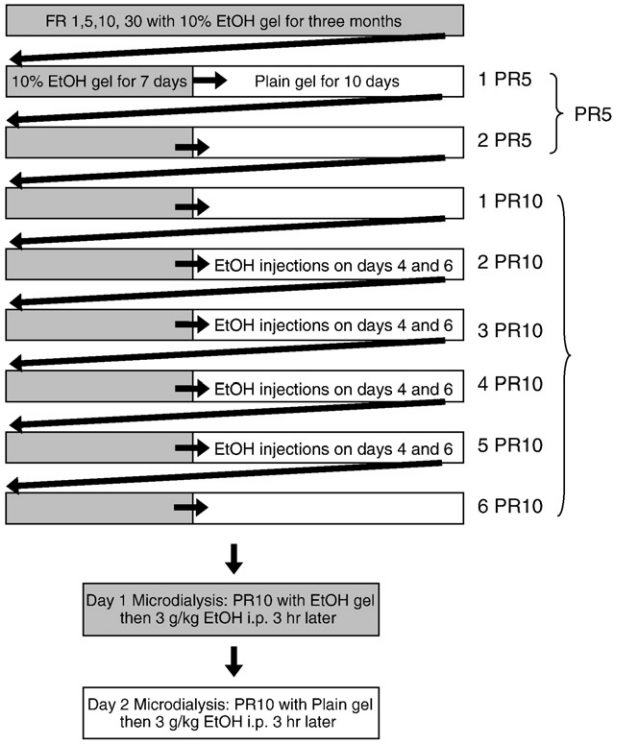

Fig. 1 – Summary of fixed and progressive ratio training regimens. The rats were first trained under an FR1 schedule using 10% ethanol-containing gelatin as the reinforcer, which was then gradually increased to FR30 over the course of 3 months. Rats were next tested using PR5 schedules for two cycles of training followed by PR10 schedules for six cycles of training. These cycles each consisted of 7 daily 30-min sessions using 10% ethanol-containing gelatin as the reinforcer followed by 10 daily sessions using plain gelatin as the reinforcer. During the last four cycles of PR10 responding for plain gel, rats received either a 3 g/kg ethanol injection (FIE treatment) or saline injection (control) on Days 4 and 6 after the end of the operant session. After the last PR10 period of ethanol reinforcement, rats were readied for microdialysis testing.

2. Results

Rats were initially trained on FR schedules of reinforcement, increasing from FR1 to FR30 over a 3-month period (Fig. 1). Using an FR1 schedule, the average ethanol consumption during the 30-min sessions was 1.3±0.05 g/kg (Fig. 2A). During the last 10 days of FR5 responding, average consumption was 1.2±0.05 g/kg (Fig. 2B). This decreased during the 10 days of FR30 responding to 0.7±0.05 g/kg ethanol per session (Fig. 2C). Blood ethanol concentrations were determined immediately after the 30-min session on Day 11 of the FR1 schedule by sampling tail blood from a subset of these rats (Fig. 2D). There was a significant correlation between ethanol dose and blood ethanol concentration.

When rats were moved to daily PR5 sessions (see Fig. 1), ethanol consumption was 0.6±0.03 g/kg. There was no difference in breakpoints for ethanol gel (34.5±1.3) versus plain gel (36.2±1.8) on the PR5 schedule. Similar results were found for a PR10 schedule with ethanol consumption of 0.5±0.02 g/kg and breakpoints of 51.8±3.1 for ethanol gel and 52.2±3.8 for plain gel. There was no change in ethanol intake when rats returned to ethanol reinforcement after the 10-day plain gel reinforcement periods for either PR schedule (data not shown).

When the number of responses on the non-reinforced lever were tabulated, there was initially very little responding (2.7±0.5 per FR1 session) and this decreased further over days (FR5=2.2±0.3; FR30=0.7±0.1; PR5=0.7±0.1; PR10=0.4±0.1). The average numbers of responses per session on the reinforced lever were: FR1=22.5±1.2; FR5=80.6±3.1; FR30=263.4±17.1; PR5=325.9±19.6; PR10=264.0±21.5.

In order to determine if high doses of ethanol (similar to what a human might experience when they drink enough ethanol to pass out) could alter motivation for ethanol responding as well as ethanol-induced changes in neurotransmitter levels, it was necessary to give a dose high enough to ensure loss of righting in 100% of subjects. Since Sprague–Dawley rats do not voluntarily consume enough ethanol to cause the loss of righting reflex, we examined whether forced