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Research report

Pharmacological effects of ethanol on ingestive behavior of the preweanling rat

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ABSTRACT

The present study was designed to test the hypothesis that sensitivity of ingestive behavior of infant rat to the pharmacological effects of ethanol changes between postnatal (P) days 9 and 12. The intake of 0.1% saccharin and water, general motor activity, and myoclonic twitching activity were assessed following administration of three doses of ethanol (0, 0.25, and 0.5 g/kg) while fluids were free available to the animals. The 0.5 g/kg dose of ethanol attenuated saccharin intake in P9 pups and enhanced saccharin intake in P12 rats. On P12 some sex-related differences emerged at 0.5 g/kg of ethanol, with saccharin intake being higher in females than in their male counterparts. Taste reactivity probe revealed that 0.5 g/kg of ethanol increased taste responsiveness to saccharin on P12 but only to infusions presented at a high rate. The results of the present study indicate that ontogenetic changes in sensitivity to the effects of ethanol on ingestive behavior occur during the second postnatal week, with P9 animals being more sensitive to the inhibitory (sedative) effects on saccharin intake and P12 rats being more sensitive to the stimulatory effects of ethanol. We suggest that acute ethanol enhanced saccharin intake via sensitization of oral response to appetitive taste stimulation.

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1. Introduction

Infancy is a unique period during which rat pups consume large quantities of ethanol without any initiating procedures. High ethanol consumption during this age has been seen either in the context of suckling behavior [1] or independent feeding [2–6]. When independent ingestion of ethanol was tested during infancy, intake of ethanol in low and moderate concentrations (5–15%) gradually increased from postnatal day 4 to day 12 with the peak of consumption occurring near the end of the second postnatal week [2,3,5].

Taste plays a substantial role in initiation of ethanol ingestion. Although several taste components determine the flavor of alcohol, the palatability of ethanol is thought to be an important predictor of high ethanol consumption in early infancy [7,8]. Palatability of various tastants, including ethanol, has been measured in adults by analysis of short latency motor and affective reactions to intraoral infusions of small amounts of flavored fluid [9,10]. However, assessment of taste responsiveness in the neonatal rat has usually been based on intake measured in relatively long procedures (10–20 min) when the animal consumed a substantial amount of ethanol or other fluids (2.5% of body weight) [1,11–16]. With such tests it is difficult to distinguish between changes in orosensory properties of ethanol and postingestive consequences of ethanol.

When separate tests for assessment of intake and palatability were employed in our recent study, a clear dissociation between developmental changes of ethanol acceptance and responsiveness to ethanol taste was revealed [17]. It was found that ethanol intake increased while intake of saccharin decreased between postnatal (P) days 9 and 12, whereas no age-related differences in taste responsiveness to saccharin and ethanol were seen. The dissociation between ontogenetic patterns of ethanol intake and taste responsiveness suggests that increased ethanol acceptance between P9 and P12 may not be solely related to developmental changes in the pups perception of the drugs orosensory properties. It is possible that the increased intake is a consequence of changes in sensitivity of ingestive behavior to the pharmacological effects of ethanol that occur between P9 and P12.

The aim of the present study was to compare acute effects of three doses of ethanol (0, 0.25 and 0.5 g/kg) on intake and taste responsiveness to a freely available palatable fluid in P9 and P12 pups. During the active (waking) phase of the ethanol intake test conducted in our previous study [17] pups ingested about 0.2 g/kg on P9 and about 0.5 g/kg on P12. Given that intake of ethanol in a range of 0.25–0.5 g/kg resulted in detectable (15–25 mg/dl) blood ethanol concentrations (BEC) and reinforcement from pharmacological effects of ethanol occurred in neonatal rats at comparable BECs [12,18,19], the doses of 0.25 and 0.5 g/kg were used in the present study to reproduce pharmacological consequences of ethanol consumption when it is freely available to the animal.

Experiment 1 assessed ethanol effects on intake and temporal patterns of ingestion in rat pups allowed to freely extract fluid from

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an intraoral cannula. The fluid flow during this extraction was monitored using the flow tracking system described in our recent paper [17]. The same flow recording technique was used for assessment of taste reactivity in Experiment 2. Infant rats show prominent sensitization and habituation of taste-elicited oral behavior [20–22], with the temporal course of habituation or sensitization determined mainly by rate of taste stimulation. To control for these processes we conducted two tests in which taste probes were presented either with low (Experiment 2A) or high frequency (Experiment 2B). To control for pharmacological effects of ethanol independently from its orosensory properties we replaced the 10% ethanol used in our previous study with 0.1% saccharin—a sweet tastant devoid of ethanol's pharmacological effects but similar to 10% ethanol in terms of palatability measured in a previous taste reactivity probe [17]. Experiment 3 examined whether BEC for a given ethanol dose differ as function of age and post-administration time. Given that ethanol effects on independent ingestive behavior may depend on baseline motor activity and behavioral state of the infant rat in terms of the time spent in sleep/wakefulness, overall motor activity and twitching activity, which represents a reliable index of active sleep, were recorded and analyzed along with changes of fluid flow.

2. Methods

2.1. Subjects

A total of 472 pups, derived from 96 dams, served as subjects for all experiments. Experimental subjects were Sprague–Dawley rats born in our colony at Binghamton University. Pregnant female rats were housed individually in plastic maternity cages (47 cm long × 20 cm high × 36 cm wide) containing pine shavings as bedding material. They were checked for birth daily between 15:00 and 18:00 h, and the day of birth was considered P0. Litters were culled to 10 pups, with an equal sex ratio whenever possible. All animals were housed in a temperature-controlled (22 °C) vivarium maintained on a 14-/10-h light/dark illumination cycle (lights on at 07:00 h), with ad libitum access to food (Purina Rat Chow, Lowell, MA, USA) and water. In all respects, maintenance and treatment of the animals were in accord with guidelines for animal care established by the National Institutes of Health, using protocols approved by the Binghamton University Institutional Animal Care and Use Committee.

2.2. Cannulation

Pups were separated from the dam about 1.5 h prior to testing and placed into an incubator in group of 10 subjects at 33 °C and 75% humidity. Following a 15–20 min acclimation period an intraoral cannula was inserted through the pup's cheek. For flow recording during continuous fluid availability (Experiment 1) experimental subjects were implanted with a one-channel cannula. When taste reactivity was being assessed (Experiments 2A and 2B) animals were implanted with a two-channel intraoral cannula. The cannula preparation and implantation procedure were described extensively in our previous studies [17,23]. In brief, cannulas were made from 6-cm sections of PE-10 polyethylene tubing (Clay Adams, Parsippany, NJ, USA). For preparation of double-channeled cannula two pieces of tubing were fastened together and simultaneously heated at one end until a single flange linking both tubing was formed. The flange diameter was 2.0–2.5 mm. The non-flanged end of each of the two channels of the cannula was tightly fit onto one end of a separate carved stainless-steel wire (0.28 mm diameter, approximately 4 cm in length). The two wires were placed together and inserted simultaneously into the cheek 3–4 mm caudal to the mystacial pad. Both pieces of tubing were then drawn through the cheek, leaving the flanged end in the oral cavity. A drop of a liquid bandage was used to secure the position of the cannula. The implantation of a one-channeled cannula was done in a similar way using a single wire to pull the cannula through the cheek. The cannulation procedures were accomplished within 6–12 s with no bleeding and minimal stress for the animal.

2.3. Experiment 1: intake test

2.3.1. Procedure

Testing procedure occurred in a plastic circular arena with transparent walls (diameter 10 cm, wall height 2 cm). Absorbent paper covered the plastic floor of the arena. The floor of the test arena was heated to a temperature of 32–34 °C during experiments using 9-day-old pups and at 30–32 °C during experiments using P12 animals. Throughout the experiment ambient room temperature was maintained at 23 ± 0.2 °C.

During the intake test (20 min) animals were allowed to voluntarily ingest 0.1% saccharin or water from an intraoral cannula. Each subject was exposed to only one fluid and this fluid was continuously available during the entire test. The intake test began 5 min after administration of ethanol or saline. Ethanol (190-proof Ethanol, Pharmaco, Brookfield) was administered i.p. as a 12.6% (v/v) solution in physiologic saline. The solutions were warmed to 34 °C for injection. All experimental subjects were voided, weighed to the nearest 0.01 g prior to testing and weighed again immediately after the test session.

2.3.2. Recording of fluid flow

A technique of on-line monitoring of fluid flow was designed to measure the amount of fluid ingested through an intraoral cannula. We have described this technique in detail elsewhere [17,23]. In short a piece of translucent PE-90 tubing (Clay Adams, Parsippany, NJ), 90–100 cm long, connected through a valve and adapter to intraoral cannula was used as a reservoir for the fluid to be presented to the subject. A custom built fluid tracker consisted of a photocell unit and a stepped motor rotating the wheel around which the tubing containing the fluid was twisted. The piece of PE-90 tubing was pulled through the photocell unit of the fluid tracker, with the outer end of tubing being mounted on the wheel. The photocell system was aimed through the translucent tubing to detect the border between filled and unfilled parts of the tube. An output signal from the photo sensor controlled a stepper motor that rotated the wheel and moved the tube through the photocell assembly until the light beam "hit" the filled part of the tubing. When the experimental subject consumed the test fluid, the fluid moved down the tube. The number of steps the motor performed pulling the tube through the photocell assembly in order to locate the fluid edge against the light beam was directly proportional to the amount of fluid ingested. The resolution of flow measurement was about 0.2 μl/step

The current modification of this technique includes two units set up for simultaneous flow recording in two subjects. The controller unit built on a PIC16F874 microprocessor was used to program movements of step motors and to record data from 2 flow inputs and 4 switch inputs used to mark behavioral events. The data sampled at a rate of 10 Hz were transferred to a personal computer via a RS232 input. The system was designed and programmed by W. Kashinsky at Binghamton University.

2.3.3. Behavioral observations

The selected categories (see below) of behavioral activity were scored online by an experimenter blind to treatment condition. The behavioral scores were recorded using a flow tracking device and stored in one file with flow data. The behavior of all subjects was also continuously monitored by a video camera (Panasonic Model AF-X8) and recorded on DVD (LiteOn 5002 DVD recorder, Milpitas, CA, USA). The data acquisition module included a video overlay unit that provided simultaneous display of video images, flow counts and markers of behavioral events.

The behavioral analysis included three categories of behavioral activity: appetitive/ingestive reactions, overall motor activity and myoclonic twitching. The appetitive/ingestive reactions included probing (activity mimicking licking or picking food from floor) and paw licking. The behavior was scored as overall motor activity if it was not ingestion-related behavior (i.e. mouthing, probing, paw licking) or aversive behavior (such as gapes, chin scraping, wall climbing). Twitching activity, which represents a highly reliable measure of active sleep in the infant rat [24,25], was defined as phasic, rapid, and independent movements of any part of the hind limbs and the tail. Twitches were recorded as event data; for further analysis we computed the number of 5-s intervals during which at least 2 twitches were observed without any coordinated movements of the body.

2.3.4. Data analysis

To avoid possible confounding of litter with treatment, no more than two subjects per litter (one male and one female) were assigned to the same treatment group. Order of testing for the different treatment groups was counterbalanced across the experiment. Numbers of males and females in each group were equated.

Body weight gain (BWG) was measured as the difference (in mg) between body weights assessed immediately before and immediately after the intake test. Fluid flow through the cannula was measured as the volume (μl) of fluid extracted from cannula. This measure was used to evaluate total fluid intake and per minute intake (μl/min). The level of motor activity was indexed as time spent in this behavior. Twitching activity as a measure of active sleep yielded two indices: (a) duration of initial waking period (time from start of the test to the first occurrence of twitching activity); and (b) time spent with myoclonic twitching, which indexed duration of active sleep.

Total flow intake, body weight gain and behavioral indexes (total time spent in motor and twitching activities as well as latency of active sleep onset) were analyzed via separate 2(fluid) × 2(age) × 3(dose) × 2(sex) between-groups ANOVA procedures (Statistica 6.0, StatSoft, Inc., Tulsa, OK, USA). All behavioral scores were converted to percent of time spent in particular activity and were arc-sin transformed before being submitted to ANOVA. Post hoc comparisons were conducted using Fisher's PLSD test. The type 1 error level (alpha) was set at 0.05, and for multiple comparisons the critical alpha level was corrected using the Bonferroni adjustment. Unless otherwise specified population estimates are presented in the form of means ± S.E.M.

The temporal changes of per minute flow rate and per minute scores of behavioral activities were analyzed using a MANOVA with successive 1-min intervals of the intake test treated as repeated measure. When a temporal trend was observed, linear and non-linear regression analysis (Graph Pad Prism 5.0, Graph Pad Software,

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