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Infusions of acetaldehyde into the arcuate nucleus of the hypothalamus induce motor activity in rats

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ABSTRACT

Aims: The hypothalamic arcuate nucleus (ARH) is one of the brain regions with the highest levels of catalase expression. Acetaldehyde, metabolized from ethanol in the CNS through the actions of catalase, has a role in the behavioral effects observed after ethanol administration. In previous studies acetaldehyde injected in the lateral ventricles or in the substantia nigra reticulata (SNR) mimicked the behavioral stimulant effects of centrally administered ethanol.
Main methods: In the present study we assessed the effects of acetaldehyde administered either into the ARH into a dorsal control or into the third ventricle on locomotion and rearing observed in 30 min sessions in an open field.
Key findings: Acetaldehyde injected into the ARH induced horizontal locomotion and rearing for 20 min. In contrast, administration of acetaldehyde into a control site dorsal to the ARH did not have any effect on locomotion. Although acetaldehyde administration into the third ventricle also induced locomotion, the time course for the effect in this area was different from the time course following ARH injections. Acetaldehyde in the ARH produced a long lasting induction of locomotion, while with intraventricular injections the effects disappeared after 5 min.
Significance: The present results are consistent with previous studies demonstrating that acetaldehyde is an active metabolite of ethanol, which can have locomotor stimulant properties when administered in the ventricular system of the brain or into specific brain nuclei. Some brain nuclei rich in catalase (i.e.; SNR and ARH) could be mediating some of the locomotor stimulant effects of ethanol through its conversion to acetaldehyde.

Introduction

Compelling evidence from several different laboratories supports the role of acetaldehyde (the first metabolite of ethanol) as a biologically active molecule with specific actions on the central nervous system (CNS) (Correa et al., 2003a; Hipólito et al., 2007; Israel et al., 2006; Quertemont et al., 2005). The involvement of acetaldehyde in the behavioral stimulant effects of ethanol has been a topic of study for several decades (Amit and Smith 1985; Myers and Veale 1969; Quertemont et al., 2005). The data that support this hypothesis come mainly from two different approaches: enzymatic manipulations that modulate the amount of acetaldehyde formed from ethanol, and the direct administration of acetaldehyde in the brain. Based upon the first type of studies, two main systems have been studied: cytochrome *P4502E1* (CYP2E1) and catalase. CYP2E1 seems to account for approximately 20% of the ethanol metabolized in the brain (for a recent review see Hipólito et al., 2007), and it seems to play some role in ethanol-induced motor behaviors (Correa et al., 2006). However, it has extensively been demonstrated that catalase-mediated ethanol metabolism in the brain plays a key role in some of the behavioral effects of ethanol, with the modulation of locomotion being the most solidly supported (Aragon et al., 1989, 1992; Correa et al., 1999a,b, 2001, 2004, 2005b; Pastor et al., 2002; Sanchis-Segura et al., 1999a,b). Furthermore, acetaldehyde injected in different brain regions has been shown to support operant self-administration (Rodd-Henricks et al., 2002; Rodd et al., 2005), and to induce place preference (Smith et al., 1984). In the last few years, using this type of strategy, it has been demonstrated that acetaldehyde mimics the motor stimulant properties of low doses of ethanol. Thus, acetaldehyde injected into the lateral ventricles (Arizzi et al., 2003; Correa et al., 2003a; McLaughlin et al., 2008) induced or sustained several types of motor activities in a manner similar to centrally administered ethanol. In addition, administration of acetaldehyde locally in areas that are part of the motor circuitry, such as the substantia

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nigra pars reticulata (SNR), also induces locomotion (Arizzi-LaFrance et al., 2006). Moreover, the inhibition of catalase (which is highly expressed in this nucleus) blocked the stimulation induced by local administration of ethanol into the SNR (Arizzi-LaFrance et al., 2006).

The hypothalamic arcuate nucleus (ARH), a brain region which has one of the highest levels of catalase expression (Moreno et al., 1995; Zimatkin and Lindros, 1996) and a very low level of aldehyde dehydrogenase (ALDH, the enzyme that metabolizes acetaldehyde) (Zimatkin et al., 1992), is a nucleus where acetaldehyde accumulation from ethanol metabolism could take place in substantial amounts. It has been demonstrated that ethanol-induced locomotor changes after either acute (Crabbe and Dorsa, 1986; Sanchis-Segura and Aragon, 2002; Sanchis-Segura et al., 2000) or repeated administration (Miquel et al., 2003) in rodents are clearly dependent on the integrity of the ARH. Systemic inhibition of catalase activity has been demonstrated to suppress the locomotor stimulation induced by an ethanol injection into the ARH (Pastor and Aragon, 2008), and the local inhibition of catalase specifically in the ARH suppressed the effects of peripherally administered ethanol on locomotion (Sanchis-Segura et al., 2005).

Thus, in the present study we assessed the effects of direct intra-ARH acetaldehyde administration on motor activities in rats. We also evaluated the possible effect of acetaldehyde when injected into a hypothalamic control site dorsal to the ARH. Moreover, since the administration of acetaldehyde in the lateral ventricles has been demonstrated to induce locomotion, we also studied the effect of acetaldehyde injected in a putative active site very close to the ARH, i.e., the third ventricle.

Materials and methods

Animals

Sprague Dawley male rats (250–300 g; total $n=35$) (Harlan Sprague-Dawley, Indianapolis, IN) were singly housed in a colony maintained at 23 °C on a 12:12 h light:dark cycle (lights off at 08:00 h) with food and water available ad libitum. The animal protocols were approved by the institutional animal care and use committee, and the methods were in accord with the Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press (1996).

Drugs

Acetaldehyde (Fisher Scientific) was dissolved in artificial cerebrospinal fluid (aCSF: 147.2 mM NaCl, 1.2 mM $CaCl_2$, 4.0 mM KCl). For the surgery, rats were anesthetized with a solution (1.0 ml/kg, IP) that contained ketamine (100 mg/ml) and xylazine (20 mg/ml) (Phoenix Pharmaceutical, Inc. St. Joseph, Mo).

Selection of doses and coordinates

Pilot studies were used to determine time course, doses, and placements for these experiments. Unilateral cannula implantation (10 mm length, 23 ga) was the method selected because the target area is located near the midline, and also because unilateral injection did not produced abnormal or asymmetrical motor activity. In pilot studies (total $n=11$), there were no significant differences in rotation (measured by direct observation in a circular chamber: 30 cm in diameter) between groups injected with acetaldehyde and the group injected with vehicle in the 10 first min [$F(2,8)=3.51$, n.s.]. Average rotation counts in the rotometer for vehicle in the first 10 min was 21.7 ± 1.6, for 0.70 μmol acetaldehyde was 22.8 ± 1.3, and for 1.4 μmol acetaldehyde was 16.7 ± 2.0.

Because the purpose of these experiments was to find doses of acetaldehyde that have activating effects, the final doses of acetaldehyde chosen were: 0.0, 0.35 or 0.70 μmol (i.e., 0, 15.41 or 30.83 μg). Although relatively high in concentration, these doses were selected based upon the present pilot results, and also upon what was observed to be the low range of the motor stimulating doses used in previously published papers (Arizzi-LaFrance et al., 2006; Correa et al., 2003a) that also injected acetaldehyde into discrete brain nuclei or into the ventricles.

Based upon these studies, the final stereotaxic coordinates for the cannulation into the ARH were: AP −0.2 mm (from bregma), ML ± 2.0 mm lateral (from midline), and DV −9.7 mm ventral (from the surface of the skull), setting the incisor bar at 5.0 mm above the interaural line (Pellegrino and Cushman, 1967), and the cannula holder 10° from the perpendicular axis. The coordinates for the dorsal control and the third ventricle sites were the same except for the DV dimension (dorsal control, −8.7 mm; ventricle, −10.0 mm). The animals with dorsal control coordinates (near the ventromedial nucleus of the hypothalamus; VHM) provided information about the specificity of the effects inside the hypothalamus. Since in previous studies acetaldehyde injected in the lateral ventricles increased locomotion in the open field (Correa et al., 2003a), we decided to compare the group that received the dose of acetaldehyde (0.35 μmol), which in the ARH was more effective across time, with injections of this dose in a new group that received the acetaldehyde in the third ventricle, a structure adjacent to the ARH. Different groups of animals were used for the different brain areas and for the different doses ($n=5$).

Surgical procedure

Rats were anesthetized and unilateral guide cannulae (23 ga stainless steel tubing, Small Parts) were chronically implanted to be 1.5 mm dorsal to the target structures. The cannulae were attached to the scalp with steel screws and cranioplastic cement (Small Parts). All animals were single-housed following surgery and were allowed to recover for 7–10 days before behavioral testing. This postsurgical recovery period was chosen to minimize the impact of the surgery procedure on the animal's behavior. Stainless steel stylets were kept in the guide cannulae to maintain their integrity.

Behavioral procedure

On the day prior to the test session, the animals were habituated to the open field for 15 min. The vehicle control procedure consisted of injections of 1.0 μl of aCSF. Injections were made via 30 ga stainless steel injectors extending 1.5 mm below the guide cannulae. The injectors were attached to 10.0 μl Hamilton syringes by PE-10 tubing, and driven by a syringe pump (Harvard Apparatus) at a rate of 0.5 μl/min, for a total volume of 1.0 μl. Following infusion of the drug, the injectors were left in place for 1 min to allow time for diffusion, and after that time the animal was introduced in the open field. Rats were then assessed for horizontal locomotion and rearing during 30 min sessions in an open field (113 cm × 113 cm × 44 cm), which was placed in a room illuminated by a soft light. Behavior was recorded in blocks of 5 min. The behavior of each animal was recorded by a trained observer, who was unaware of the experimental conditions. For horizontal locomotion, a single activity count was defined as a complete crossing from one black square (22.5 cm × 22.5 cm) to another, at the end of which the animal had both hindpaws completely over the white line. Rearing was recorded simultaneously; a rearing score was counted every time the animal stood up with both forepaws in the air or against the wall. Separate studies of inter-rater reliability for locomotor activity counts demonstrated a high degree of agreement between observers ($r>0.90$, $p<0.05$). The open field chamber was completely wiped down between animals.