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Comparison of dehydroepiandrosterone (DHEA) and pregnanolone with existing pharmacotherapies for alcohol abuse on ethanol- and food-maintained responding in male rats

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ABSTRACT

The present study compared two putative pharmacotherapies for alcohol abuse and dependence, dehydroepiandrosterone (DHEA) and pregnanolone, with two Food and Drug Administration (FDA)-approved pharmacotherapies, naltrexone and acamprosate. Experiment 1 assessed the effects of different doses of DHEA, pregnanolone, naltrexone, and acamprosate on both ethanol- and food-maintained responding under a multiple fixed-ratio (FR)-10 FR-20 schedule, respectively. Experiment 2 assessed the effects of different mean intervals of food presentation on responding for ethanol under a FR-10 variable-interval (VI) schedule, whereas Experiment 3 assessed the effects of a single dose of each drug under a FR-10 VI-80 schedule. In Experiment 1, all four drugs dose-dependently decreased response rate for both food and ethanol, although differences in the rate-decreasing effects were apparent among the drugs. DHEA and pregnanolone decreased ethanol-maintained responding more potently than food-maintained responding, whereas the reverse was true for naltrexone. Acamprosate decreased responding for both reinforcers with equal potency. In Experiment 2, different mean intervals of food presentation significantly affected the number of food reinforcers obtained per session; however, changes in the number of food reinforcements did not significantly affect responding for ethanol. Under the FR-10 VI-80 schedule in Experiment 3, only naltrexone significantly decreased both the dose of alcohol presented and blood ethanol concentration (BEC). Acamprosate and pregnanolone had no significant effects on any of the dependent measures, whereas DHEA significantly decreased BEC, but did not significantly decrease response rate or the dose presented. In summary, DHEA and pregnanolone decreased ethanol-maintained responding more potently than food-maintained responding under a multiple FR-10 FR-20 schedule, and were more selective for decreasing ethanol self-administration than either naltrexone or acamprosate under that schedule. Experiment 2 showed that ethanol intake was relatively independent of the interval of reinforcement in the food-maintained component, and Experiment 3 showed that naltrexone was the most effective drug at the doses tested when the interval for food reinforcement was low and maintained under a variable-interval schedule.

Introduction

In 2010, 7% of the U.S. population age 12 or older, or an estimated 17.9 million persons, met the criteria listed in the Diagnostic and Statistical Manual of Mental Disorders for alcohol dependence or abuse (American Psychiatric Association, 2000; Substance Abuse and Mental Health Services Administration, 2011). Compounding this social and economic problem is the lack of suitable pharmacotherapeutic options for treatment. Naltrexone (Depade®, ReVia®) and acamprosate (Campral®) are two FDA-approved compounds for alcohol abuse and dependence, but both have substantial limitations clinically (Ross & Peselow, 2009). For instance, the opioid antagonist naltrexone has limited efficacy except in a subset of individuals, such as those with: 1) a family history of alcohol dependence, 2) an enhanced opioid response to ingestion of alcohol, 3) enhanced alcohol cravings, or 4) a specific μ-opioid receptor polymorphism response (Oslin, Berrettini, & O'Brien, 2006; Pettinati & Rabinowitz, 2006; Ross & Peselow, 2009). Likewise,

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experiments involving the synthetic homotaurine derivative acamprosate suggest that it is only fully effective in highly motivated subjects with a "goal of abstinence" (Mason & Ownby, 2000), and that the combined experience of acamprosate with ethanol is necessary for decreasing ethanol intake (Hölter, Landgraf, Zieglgänsberger, & Spanagel, 1997). In addition, the mechanism by which acamprosate modulates ethanol intake is poorly understood.

Due to the well-documented limitations of currently available therapies, effective treatments for alcohol abuse and dependence remain an active area of investigation in the alcohol research field. Two promising compounds are the neurosteroids dehydroepiandrosterone (DHEA) and pregnanolone, which have been shown to decrease voluntary home-cage ethanol intake and operant responding for ethanol reinforcement in male rats, likely via negative and positive modulation of the $GABA_A$ receptor, respectively (Amato, Hulin, & Winsauer, 2012; Gurkovskaya, Leonard, Lewis, & Winsauer, 2009; Hulin, Amato, & Winsauer, 2012). For these substances to be viable clinical options for the treatment of alcohol abuse, however, they should be at least as effective as current treatment options, and ideally, demonstrate selectivity for ethanol-maintained behavior relative to other behaviors (Haney & Spealman, 2008; Wojnicki, Rothman, Rice, & Glowa, 1999). One pre-clinical strategy for assessing the selectivity of potential treatments is to compare their effects on operant responding for more than one type of reinforcer within the same experimental session under a multiple schedule. This strategy has been effective for comparing cocaine and food reinforcement (Kleven & Woolverton, 1990; Mello, Kamien, Lukas, Drieze, & Mendelson, 1993; Woolverton & Virus, 1989), ethanol and food reinforcement (Amato et al., 2012), and ethanol and alternative liquid reinforcers (Czachowski, Samson, & Denning, 1999; Shelton & Grant, 2001; Slawecki, Hodge, & Samson, 1997). These types of studies are also valuable for studying behavioral variables that can affect drug and ethanol self-administration, such as response rate, reinforcement density, availability of non-drug reinforcers, or deprivation level. For example, increased food deprivation has been consistently shown to increase drug and ethanol self-administration (Carr, 1996; Carroll & Boe, 1984; Carroll, Stotz, Kliner, & Meisch, 1984; Kliner & Meisch, 1989; Meisch & Lemaire, 1991). Unlike food deprivation, the availability of alternative non-drug reinforcements such as sucrose or food can either increase (Shahan & Burke, 2004; Weatherly, Bishop, & Borowiak, 2004; Winsauer & Thompson, 1991) or decrease drug self-administration, particularly under schedules of reinforcement that operate concurrently (Campbell & Carroll, 2000; Carroll, Rodefer, & Rawleigh, 1995; Cosgrove & Carroll, 2003; Samson, Roehrs, & Tolliver, 1982).

In Experiment 1 of the present study, responding for both ethanol and food pellets was maintained under two separate fixed-ratio (FR) schedules with different stimuli that alternated throughout an experimental session. This "multiple" schedule (Ferster & Skinner, 1957) was used to compare the selectivity of DHEA and pregnanolone with the selectivity of naltrexone and acamprosate. Both naltrexone and acamprosate have been shown to decrease voluntary ethanol intake in rats (Boismare et al., 1984; Le Magnen, Tran, Durlach, & Martin, 1987; Stromberg, Mackler, Volpicelli, & O'Brien, 2001), but to our knowledge, no studies have compared them directly with multiple neurosteroids. Of the studies examining the effects of naltrexone and acamprosate on operant responding for ethanol in rats (Hölter et al., 1997; Hyytia & Sinclair, 1993), only a few have tested the specificity of these compounds by determining their effects on reinforcers other than ethanol (Bienkowski, Kostowski, & Koros, 1999; Heyser, Schulteis, Durbin, & Koob, 1998; Middaugh, Kelley, Cuison, & Groseclose, 1999). Moreover, the results of those studies have been mixed. For example, Samson and Doyle (1985) reported that doses up to 20 mg/kg of naloxone (an opioid antagonist similar to naltrexone) did not affect operant responding for sucrose, while Schwarz-Stevens, Files, and Samson (1992) found that doses of naloxone as low as 1 mg/kg reduced responding for sucrose. Studies investigating the selectivity of acamprosate on operant behavior are also scarce in the literature, as a majority of the animal studies have focused on the effect of acamprosate on the alcohol-deprivation effect (Heyser, Moc, & Koob, 2003; Spanagel, Hölter, Allingham, Landgraf, & Zieglgänsberger, 1996) or "drug-seeking" behavior (Czachowski, Legg, & Samson, 2001). When Czachowski and Delory (2009) did compare the effect of acamprosate on operant responding for ethanol and sucrose, they found that acamprosate decreased both ethanol- and sucrose-maintained responding non-selectively.

Experiment 2 was designed to probe the role of food reinforcement in maintaining ethanol self-administration under a multiple schedule, whereas Experiment 3 examined the effects of all of the drugs on ethanol self-administration when the number of food reinforcements was maintained at a low level under a VI-80 schedule. As mentioned above, reinforcement rate is one of the many behavioral variables that can mediate self-administration behavior as well as mediate the effects of another drug upon schedule-controlled behavior (cf. Lucki & DeLong, 1983; MacPhail & Gollub, 1975). Given that the response and reinforcement rate have generally differed for each reinforcer in previous experiments using a multiple FR FR schedule (Amato et al., 2012; Ginsburg, Koek, Javors, & Lamb, 2005), showing that these variables were not responsible for the observed selectivity of DHEA seemed essential to supporting its potential as a pharmacotherapy for alcohol abuse and dependence. For these reasons, a variable-interval (VI) schedule of food presentation that produced comparable numbers of reinforcements between components was selected from Experiment 2, and behaviorally effective doses of all of the drugs were retested.

Materials and methods

Subjects

Twenty-five male Long-Evans hooded rats served as subjects. Sixteen of these were used for Experiment 1, whereas 9 naïve rats were purchased and trained for Experiments 2 and 3. Males were used exclusively to limit the differential influence of ovarian hormones on self-administration behavior (Anker & Carroll, 2010, 2011). Eight of the subjects serving in Experiment 1 were involved in a previous experiment during which they received subchronic administration of lorazepam ($n = 3$), DHEA ($n = 1$), or vehicle ($n = 3$) during adolescence (postnatal days 35–64) (Hulin et al., 2012). These adolescent treatments were found to alter their preference for ethanol or saccharin, but following behavioral training under the operant procedure detailed below, the effects of adolescent exposure were no longer evident. More specifically, responding for ethanol under the multiple schedule was fairly homogenous for the group (i.e., comparable to other groups trained in the laboratory) and the effects of the drugs were similar across subjects within the group. The remaining 9 subjects serving in Experiment 1 had a history of responding under a multiple FR-10 FR-20 schedule of ethanol and food reinforcement, respectively, and were tested acutely with several drugs (Amato et al., 2012).

Subjects were housed in polypropylene cages with hardwood chip bedding, and the colony room was maintained at 21 ± 2 °C with 50 ± 10% relative humidity on a 14:10 light/dark cycle, respectively. Water was provided *ad libitum*. Subjects were maintained at 90% of their free-feeding weight by food pellets obtained