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Moderating influence of the drinking water disinfection by-product dibromoacetic acid on a dithiocarbamate-induced suppression of the luteinizing hormone surge in female rats☆

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Abstract

The disinfection by-product dibromoacetic acid (DBA) has been found in female rats to increase circulating concentrations of both estradiol (E2) and estrone (E1). This effect is apparently due, at least in part, to a suppression in hepatic catabolism. The present study investigated whether DBA, by increasing sex steroid levels, is able either to augment the hypothalamic up-regulation involved in triggering a luteinizing hormone (LH) surge, or to affect the ability of the neurotoxicant sodium dimethyldithiocarbamate (DMDC) to block the surge. Sprague–Dawley rats were gavaged for 14 days with DBA (0–150 mg/kg) and ovariectomized on dosing day 11, and at the same time implanted with an estradiol capsule to generate daily LH surges. An injection of 0.1 mM/kg DMDC was administered at 13:00 h on day 14 and blood was sampled over the afternoon. DBA induced a dose-related increase in total estrogens. For identified surges, areas under the LH curve partitioned into two groups, comprising the two lower (0 and 37.5 mg/kg DBA) and the two higher (75 and 150 mg/kg) treatment groups. Consequently, low and high DBA groups were compared and found to be significantly different. At 150 mg DBA/0.1 mM DMDC, the timing of an identifiable LH peak was comparable to non-DMDC females, unlike the 37.5 mg DBA/0.1 mM DMDC group in which the appearance of peak concentrations was delayed. A significant effect with DBA treatment alone was not present. Results indicated that this exposure to DBA induced a dose-related increase in total estrogen concentrations that paralleled a diminished DMDC blockade of the LH surge. The effect appeared to be attributable to an augmentation in the estrogen-associated up-regulation in brain mechanisms stimulating the surge.

Keywords: LH surge; Dibromoacetic acid; Estradiol; Estrone; Dimethyldithiocarbamate; Disinfection byproduct

1. Introduction

Dibromoacetic acid (DBA) is one of a number of haloacetic acids generated during the chlorination or chloramination of public drinking water. At concentrations well in excess of those present in finished water, it has been reported to have a variety of adverse effects upon reproductive functions in both male and female rodents [1–7] and in female rabbits [8]. In the female rat, a number of studies have reported alterations in circulating steroid concentrations. DBA administered during the first 8 days of pregnancy was observed to elevate serum estradiol (E2) without having any other detectable effects on early pregnancy [9]. When administered by gavage to non-pregnant rats for 14 days over a dose range of 30–270 mg/kg, DBA has also been found to increase E2 in both normally cycling animals on the day of estrus and in ovariectomized females implanted on day 11 of dosing with capsules containing amounts of estradiol benzoate that were identical across treatment groups [7]. In the latter experiments, the E2 concentrations

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Table 1
Percentage increases in serum estradiol relative to controls following gavage or drinking water exposures to DBA

2 Week gavage [7] OVX/E2 implant (mg/kg DBA)			3 Week drinking water [10] Intact-cycling (mg/kg DBA)	11 Week drinking water [10] Intact-cycling (mg/kg DBA)
30	90	270	36	36
136%	153%	214%	170%	190%

Note: Ovariectomized (OVX)/E2-implanted females were gavaged daily for 2 weeks and assayed 3 days following OVX/implant. Intact, cycling animals were assayed on the day of vaginal estrus at weeks 3 and 11 of a 20-week exposure to DBA. Dosage in drinking water was calculated from body weight and water consumption.

at 3 days post-surgery/implant showed dose-related increments over controls for DBA dosages of 30, 90 and 270 mg/kg. Moreover, such an increase with dose was present after 3 and 11 weeks in cycling Sprague–Dawley females ingesting lower concentrations (estimated as 6–36 mg/kg/day) via drinking water available ad libitum [10]. At the upper ingested dose, the increase in serum E2 over controls averaged approximately 180% (Table 1). This effect appears to be at least partially attributable to a decrease in the hepatic catabolism of estradiol [7].

These DBA-induced elevations in E2, along with additional observed increases in estrone (E1), were not found to alter estrous cyclicity over the course of 20 weeks' exposure [10], nor was there a hyperplastic effect on mammary tissue in these animals (S. Fenton, unpublished data). E2 feedback from the ovaries is known to be involved in the mechanisms of hypothalamic up-regulation underlying the marked increase in the pulsatile secretion gonadotropin-releasing hormone (GnRH) that triggers generation of the preovulatory surge of luteinizing hormone (LH) late on the afternoon of proestrus (e.g., 11–13). There also appears to be a relationship between the levels of E2 and the size of the surge, since dose-related increments in E2 concentrations from capsular implants have been shown to augment LH release during an induced surge [14,15]. The present experiments were conducted to determine whether such a DBA-associated increase in circulating E2 would augment an induced LH surge and to investigate further if the increase in E2 is able to attenuate a suppression in the surge caused by a neurotoxicant insult to the hypothalamus.

2. Materials and methods

2.1. Animals

Upon arrival, 60 day-old Sprague–Dawley female rats (Charles River Labs, Raleigh, NC) were provided with ad libitum food/water and acclimated to their new surroundings. They were housed under a 14-h light:10-h dark photoperiod (lights on 05:00 h) with controlled temperature (22 ± 2 °C) and humidity (40–50%). Vaginal lavages were taken for 2 weeks to determine cycling status, and any non- or irregular cycling animals were eliminated from the study. All experimental procedures conformed to NIH standards for laboratory animal research and were approved by the Animal Care and Use Committee at the National Health and Environmental Effects Research Laboratory.

2.2. Treatment

As indicated above, DBA administered either by gavage to ovariectomized (OVX) rats (both unpublished data and results reported in ref. [7]) or in the drinking water to intact female rats [10], caused progressive dose-related elevations in serum estradiol. Based upon these findings, DBA (Aldrich Chemical, 97% purity) was administered daily (150 mg/kg kg in water @ 5 ml/kg, pH adjusted to 6.5–6.8) for 14 days by gavage between 13:00 and 13:30 h in order to evaluate the influence of this haloacetic acid on the afternoon rise in LH. Gavage was chosen as the route of exposure to provide better control over the concentration administered. The presence of a statistically significant difference at this dose would then trigger a full dose–response evaluation.

On day 11 of dosing, all rats were bilaterally OVX and implanted with a 6 mm silastic capsule containing estradiol benzoate (4 mg/ml in sesame oil) to induce a daily surge of LH [16]. Three days after surgery [day 14], 220 μl aliquots of blood were gently expressed from a nick in a lateral tail vein into small serum separation tubes at 14:00, 16:00 and 18:00 h. The animals were then killed at 20:00 h and trunk blood taken. Sera from all time points were analyzed for LH in order to evaluate an effect of DBA exposure alone on the surge peak and area under the curve (AUC). Assays for serum E2 and E1 were then performed for the final sampling time (20:00 h).

Using a second approach, preliminary evidence with 60 mg/kg DBA showed a modest, but suggestive, ability to oppose a reduction in a steroid-induced LH surge caused by a single administration of a centrally acting pesticidal dithiocarbamate. Consequently, a subsequent experiment was conducted to elaborate more fully the effectiveness of a DBA-associated elevation in circulating estrogens in attenuating the effectiveness of the neurotoxicant sodium dimethyldithiocarbamate (DMDC) in suppressing the surge. DMDC (Aldrich Chemical, 98% purity), a metabolite of the fungicide thiram, is known to promptly decrease brain norepinephrine (NE) concentrations by inhibiting the activity of dopamine-β-hydroxylase (DβH), an effect found both in rodents [17–19] and humans [20]. As alpha-noradrenergic receptor activation is closely tied to the increase in GnRH pulsatile secretion triggering the rodent surge (for review, see ref. [21]), compounds in this class of dithiocarbamates are effectively able to suppress the rise in LH and block ovulation [22–24].

An initial component of this work focused on selection of an appropriate DMDC dosage that was able to suppress, but not entirely eliminate, the surge in LH. Rats were gavaged with DBA (150 mg/kg/day) for 2 weeks then OVX and implanted as above. On day 14 of dosing, DMDC was injected i.p. (0, 0.05, 0.1 or 0.2 mM/kg [7.1, 14.2 and 28.4 mg/kg, respectively]) at 13:00 h to block the LH surge. This expression of DMDC concentrations in mM/kg is intended to eliminate any confusion between the DBA and DMDC treatments. As in Experiment 1, blood was sampled at 14:00, 16:00, 18:00 and 20:00 h for analyses of serum LH (all times) and E2 (20:00 h only).

A second cohort of animals was gavaged with DBA (0, 37.5, 75, or 150 mg/kg) for 2 weeks, with ovariectomies and E2 implants performed on day 11. On day 14, DMDC was injected i.p. (13:00 h) at a dose (0.1 mM/kg [14.2 mg/kg]), determined in the above work to cause a marked suppression of the surge. Blood was again sampled at 14:00, 16:00, and 18:00 h, before the animals were killed at 20:00 h. LH was determined for all sampling times for assessments of the surge peak and AUC. E2 and E1 were also measured from serum separated from blood taken at 20:00 h. At this time, pituitaries were taken out to be weighed, since an increase in wet weight is an indication of a response to an elevation in serum E2. Brains were also removed and quickly frozen for later analysis of hypothalamic NE in order to assess the effectiveness of the selected dose of DMDC on NE synthesis.

2.3. Norepinephrine determination

For NE analysis, anterior hypothalamic tissue was dissected out on a thermoelectric cold plate by cuts at the anterior and posterior margins of the optic chiasm and a horizontal cut at the ventral margin of the anterior com-