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Short-term exposure to dietary Pb and/or DMSA affects dopamine and dopamine metabolite levels in the medulla, optic tectum, and cerebellum of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

Rainbow trout (*Oncorhynchus mykiss*) were randomly assigned to one of the following dietary exposure conditions: lead (Pb) solvent (2% nitric acid), *meso*-2,3-dimercaptosuccinic acid (DMSA) solvent (0.1 N NaOH), Pb, DMSA, Pb followed by Pb solvent, or Pb followed by DMSA. Medulla, cerebellum, and optic tectum homogenates were analyzed for dopamine (DA), homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC). DA levels in all brain regions tended to be highest for trout exposed to dietary Pb followed by dietary DMSA. DA levels were elevated for trout exposed to dietary DMSA and Pb followed by Pb solvent. DA levels were below control levels for trout exposed to Pb only. HVA levels varied across brain regions. However, HVA levels in all brain regions tended to be elevated for trout exposed to dietary DMSA and Pb followed by Pb solvent. DOPAC levels across all brain regions were below control levels for trout dietary exposed to DMSA, Pb only, Pb followed by Pb solvent, and Pb followed by DMSA. These data indicate that Pb and/or DMSA have the potential of altering DA, HVA, and DOPAC levels in the medulla, cerebellum, and optic tectum. The animal model of short-term dietary exposure to Pb and DMSA, both alone and sequentially, to mimic dietary exposure to Pb and the oral delivery of DMSA, that our laboratory has developed, may be useful in future studies aimed at characterizing the neurobiological mechanisms by which Pb and/or DMSA alter neurotransmitter levels and behavior.

Keywords: Lead; DMSA; Dopamine; Homovanillic acid; DOPAC; Medulla; Cerebellum; Optic tectum

1. Introduction

The role that lead (Pb) has played in developmental, neuropsychological, and behavioral deficits has been well documented. These Pb-induced disorders have been identified in children (Bellinger, 1995), adults, and various animals including mice (Cory-Slechta et al., 1987), hamsters (Deville, 1999), fish (Weber et al., 1997), and nonhuman primates (Laughlin et al., 1999b). Pb-induced hyperactivity has been observed in nonhuman primates (Rice, 1993), rats (Gong and Evans, 1997), and fish (Weber et al., 1991). Adverse effects that have been observed in humans due to Pb exposure include the following: adverse cognitive effects (e.g., abstract conceptual formation), memory and attention deficits, depression including mood and personality changes, and decreased visuomotor coordination (Bornschein and Kuang, 1990; Frumkin and Gerr, 1993). Human exposure to Pb can occur through occupational contact (Goyer, 1996) or, in the case of children, Pb-based paint and Pb-contaminated dust and soil (Lanphear and Roghmann, 1997). Wildlife exposures are generally through mine drainage, urban runoff, or bioturbation (Eisler, 1988).

Apparently, Pb-induced behavioral alterations occur in fish species within days to several weeks after the initial exposure (Alados and Weber, 1999; Tandon et al., 1994; Weber, 1993, 1996). Alterations in whole brain norepinephrine (NE) and dopamine (DA) levels measured both at single time points and multiple time points throughout a 24-h cycle in fishes have been reported (Spieler et al., 1995; Weber et al., 1991). These data seem to parallel those found in mammalian systems (Baksi and Hughes, 1982; Govoni et al., 1980; Winder, 1982), suggesting that the neurotoxic effects of Pb may act through a similar mechanism across

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species. A number of the behavioral deficits observed in fish, including visuomotor, learning, and reflexive responses, that were induced by waterborne Pb exposure require integrative functioning of the medulla, cerebellum, and optic tectum. Thus, it was important to examine potential differential effects of Pb and the Pb-chelating compound, *meso*-2,3-dimercaptosuccinic acid (DMSA), both separately and in combination on each of these three brain areas.

In addition to identifying sources of Pb exposure to facilitate their removal from the environment, a variety of drug therapies have been developed to decrease or even remove the burden of Pb from internal body stores (e.g., bone and blood). One of the most common procedures is the use of calcium disodium ethylenediaminetetraacetic acid ($CaNa_2$-EDTA) as a metal chelator. Because there are no effective oral forms, this compound must be administered either intramuscularly or intravenously (Foreman et al., 1956). Unfortunately, such therapy removes Pb from the bone and redistributes it to the brain and liver (Cory-Slechta et al., 1987). There is some evidence to suggest that long-term administration of $CaNa_2$-EDTA leads to kidney damage in humans (Foreman et al., 1956). Therefore, alternate drugs have been sought to treat Pb-exposed individuals.

DMSA (succimer) was first proposed 35 years ago in China as an antidote for toxic metal exposure (Ting et al., 1965; Wang et al., 1965). Although its mechanism of action and pharmacokinetics are only now being elucidated (Asiedu et al., 1995; Maiorino et al., 1989; Pigman et al., 1999), it has been shown to be an effective weapon in the war on childhood Pb toxicity (Graziano et al., 1992), as well as the mobilization of other toxic metals, including cadmium (Srivastava et al., 1996), arsenic (As) (Aposhian et al., 1984; Graziano et al., 1978), mercury (Hg) (Aaseth et al., 1982; Ewan and Pamphlett, 1996; Magos, 1976; Planas-Bohne, 1981), methylmercury (Aaseth and Friedheim, 1978; Magos, 1976), and nickel (Xie et al., 1995). DMSA has been shown to be effective in lowering bone and brain Pb levels without causing major redistributions of Pb to other organs, especially the brain (Cory-Slechta et al., 1987; Jones et al., 1994; Tandon et al., 1994). Recently, there have been indications that DMSA may also be able to reverse specific Pb-induced alterations of locomotor activity in fish (Weber et al., 1997) and mice (Stewart et al., 1996). Whether this is due to Pb–DMSA binding rendering Pb unavailable for toxic effects or direct DMSA–neuron interactions is not yet clear. Pb–DMSA complexes have been identified and analyzed (Rivera et al., 1989).

It has been reported that monkeys exposed to dietary sources of DMSA tested in delayed response tasks required more sessions to reach criterion. Similar results occurred during Pb exposure, although probably due to different mechanisms (Laughlin et al., 1999a). Increased locomotor activity was observed in mice during DMSA exposure (Stewart et al., 1995). Other behavioral deficits in animals orally exposed to DMSA have been reported (see Stewart et al., 1996). These data would suggest that brain neurochemistry has been altered by the Pb chelator alone, although to date there have been no studies to confirm this.

To our knowledge, all of the studies to date examining the interaction of DMSA with brain neurochemistry have involved DMSA in conjunction with the metal it was chelating. The neurochemical effect of sodium arsenate in male rats with and without DMSA treatment had been examined. Intraperitoneal injections of DMSA diesters produced both an increase in As burden in the brain as well as altered levels of whole brain neurotransmitters (i.e., acetylcholine, biogenic amines) and specific enzymes (i.e., monoamine oxidase). In addition, dimethyl DMSA reduced As burden yet altered brain neurochemistry (Tripathi et al., 1997). Depressed levels of whole brain NE and one of its metabolites, vanillylmandellic acid (VMA), have been observed after Pb exposure. Following DMSA treatment, NE levels rebounded, but not to control levels, while VMA levels were greater than control values (Weber et al., 1997). Pb-induced depression in workers has been reported to respond positively to DMSA treatment (Frumkin and Gerr, 1993). This, too, would suggest that whether it was due to Pb removal or DMSA–neuron interactions, DMSA had an effect on brain neurochemistry. Post-Pb exposure to oral DMSA treatment resulted in recoveries in brain biogenic amine levels (Flora et al., 1997). However, in all of the above studies, the effects of DMSA alone on brain neurochemistry were not examined. One of the objectives of the present study was to deal with that omission.

A model of dietary Pb exposure was created by using intraperitoneally injected fathead minnows (*Pimephales promelas*) that were then fed to rainbow trout (*Oncorhynchus mykiss*) to mimic dietary exposure to Pb and the oral delivery of the chelating drug, DMSA. The goals of the present study were to: (a) create an animal model of short-term (i.e., 2 weeks) dietary exposure to Pb and DMSA both alone and sequentially; (b) test the hypothesis that short-term Pb exposure would result in altered levels of DA, homovanillic acid (HVA), and 3,4-dihydroxyphenylacetic acid (DOPAC); (c) test the hypothesis that Pb exposure followed by DMSA exposure would result in restoring DA, HVA, and DOPAC to control levels; (d) identify differences in DA, HVA, and DOPAC in the medulla, cerebellum, and optic tectum, all of which seem to be involved in previously observed behavioral deficits due to short-term exposures to Pb and DMSA; and (e) compare the efficacy of short-term DMSA administration to removal of Pb from the diet.

2. Method

2.1. Aquarium design

There were two rows of aquaria with each row consisting of twelve 20-l glass aquaria (i.e., a total of 24 aquaria) that were set up as flow-through systems (12.0 ± 1.0°C, with an approximate flow rate 250 ml/min, and a high aeration

level). A 14 light/10 dark photoperiod was used. One female rainbow trout (*O. mykiss*; Hide Away Springs Hatchery, Kewaskum, WI) that weighed from 350 to 450 g was placed into each aquarium and allowed to acclimate overnight.

2.2. Experimental design

At noon each day for 1 week, one live fathead minnow (*P. promelas*; Aquatic Resources, Sebastopol, CA) that was 4 to 5 cm in length was fed to each trout. Experimental exposures began after 1 week of feeding to ensure that the trout had successfully switched from relying on trout chow to fathead minnows as their food source.

All solutions were administered intraperitoneally (0.15-ml aliquots) into the minnows. The dose of Pb selected (1.5 ppm) was based on previous research suggesting that this dose was sublethal yet sufficient to cause neurotransmitter and neurotransmitter metabolite level alterations (Weber et al., 1997). Previous research demonstrated that the aliquot is a volume that can be held without any leakage in the minnow peritoneum (Weber et al., 1992). Following the injection, each minnow was immediately placed into the aquarium with the trout. Within approximately 5 s, the trout consumed the prey item. This ensured that the entire volume of the injected solution was ingested by the trout. Stock solutions consisted of: Pb (Fisher Scientific, Pittsburgh, PA) as 1.00 g/l $Pb(NO_3)_2$ in 2% nitric acid; 2% nitric acid; DMSA (Sigma, St. Louis, MO) as 21.84 g/l, to equal a 25 DMSA/1 Pb molar ratio, in 0.1 N NaOH (Sigma); and 0.1 N NaOH. DMSA was stored in a freezer at -20°C while the DMSA stock solution was stored in a refrigerator at 4°C to prevent oxidation of the sulfhydral groups.

The feeding regime was used for 2 weeks for each of the following treatment groups ($N=8$ per group): 2% nitric acid, 0.1 N NaOH, Pb, and DMSA. Note that an untreated control group was not used in the present study because, based on pilot data, DA, HVA, and DOPAC levels for an untreated control group were not significantly different when compared to DA, HVA, and DOPAC levels in either of the solvent control groups, regardless of brain region (unpublished observations). Two additional Pb-treated groups were fed for two more weeks either 2% nitric acid- or DMSA-treated minnows. These were the only food items received by the trout during the experiment. All feeding was done at the same time of day.

Upon completion of the feeding regime, each fish was sacrificed individually by a blow to the head followed by decapitation. The medulla, cerebellum, and optic tecta were dissected and immediately placed in a tared 1.5-ml Eppendorf tube containing 200 μl of 0.05 M perchloric acid. The medulla, cerebellum, and optic tecta were weighed and placed on dry ice. Eight fish were sacrificed each day (i.e., the number that could be completed within 1 h) at the anticipated feeding time so that changes in the circadian rhythms of neurotransmitters would be minimized (see Spieler et al., 1995). Note that the feeding regime starting day was offset to ensure that each group of fish ($N=8$ per group) within each block (i.e., 24 fish per block) received an identical number of exposure days. All samples were maintained at -80°C prior to neurochemical analysis.

2.3. Neurochemical analyses

The general neurochemical analytical procedures have been described elsewhere (Zhou et al., 1999). Briefly, each brain region (medulla, optic tectum, and cerebellum) sample was homogenized, centrifuged at 5°C at 5000 rpm for 15 min. Exactly 40 μl of the supernatant was removed with a 100-μl Hamilton syringe (Bioanalytical Systems, West Lafayette, IN) and injected into a high-performance liquid chromatography (HPLC) sample injection valve (Rheodyne, Cotati, CA). Each tissue sample was analyzed by a C_{18} reverse phase (150×1 mm^2 internal diameter, 5-μm particle size) HPLC system using a Hewlett Packard HP 3365 Series II Chemstation, a Waters 510 solvent delivery system (Millipore, Milford, MA) and a LC-4C electrochemical detector (Bioanalytical Systems) with the working electrode set at +750 mV versus reference for the detection of DA, HVA, and DOPAC. The mobile phase consisted of 235 mM Na_2HPO_4, 47 mM sodium citrate, 2.23 mM 1-octanesulfonic acid, 0.07% vol/vol EDTA, 3.3% vol/vol methanol, and 2.8% vol/vol *N*,*N*-dimethylacetamide adjusted to pH 3.01–3.02 with phosphoric acid. The mobile phase was filtered through a 0.45-μm nylon membrane filter (Nalge, Rochester, NY), degassed under vacuum, and delivered at a flow rate of 100 μl/min. All reagents used were HPLC grade and were obtained from Sigma. The chromatographic standard consisted of 10^{-7}-M concentrations of DA, HVA, and DOPAC. The chromatographic standard was mixed daily from stock solutions. The stock solutions consisted of 10^{-3}-M concentrations of DA, HVA, and DOPAC. All standard chemicals were purchased from Sigma. Each 10^{-3}-M stock solution was prepared separately and in the same manner. Based on the molecular weight of each compound, exactly 18.96 mg DA, 18.22 mg HVA, and 16.81 mg DOPAC were weighed and dissolved in 100 ml of 0.05 M perchloric acid, yielding a 10^{-3}-M solution of DA, HVA, and DOPAC. A 10^{-5}-M solution of DA, HVA, and DOPAC was yielded by combining 100 μl of each 10^{-3}-M solution with 10 ml of 0.05 M perchloric acid. Finally, a 10^{-7}-M solution of DA, HVA, and DOPAC was yielded by combining 100 μl of the 10^{-5}-M solution with 10 ml of 0.05 M perchloric acid.

2.4. Statistical analysis

Data (pg/μl) were transformed to nanograms per gram of wet tissue and analyzed by one-way analysis of variance (ANOVA). An α level of .05 was used for all statistical tests. Post hoc comparisons of the means were conducted using Tukey's HSD tests. The relevant comparisons were: (a) 2% nitric acid (Pb solvent) versus 0.1 N NaOH (DMSA

solvent); (b) 2% nitric acid versus Pb versus Pb followed by 2% nitric acid versus Pb followed by DMSA; (c) 0.1 N NaOH versus DMSA versus Pb followed by DMSA; and (d) Pb followed by 2% nitric acid versus Pb followed by DMSA. All data were analyzed using the SPSS Base 9.0 statistical package (Prentice-Hall, Upper Saddle River, NJ).

2.5. Animal care and hazardous chemical handling and storage

All experimental and holding protocols, including methods of handling and disposing of hazardous chemicals and contaminated carcasses, were preapproved by the University of Wisconsin-Milwaukee Animal Care and Use Committee.

3. Results

3.1. Dopamine

Separate one-way ANOVAs were conducted for each neurotransmitter/neurotransmitter metabolite for each brain region. Significant effects were found for the DA in the medulla [$F(5,44) = 6.941$, $P < .001$], optic tectum [$F(5,44) = 5.141$, $P < .01$], and cerebellum [$F(5,44) = 31.320$, $P < .001$]. Post hoc Tukey's HSD comparisons revealed that there were no differences in DA levels between the two solvent control groups in the medulla ($P > .05$), optic tectum ($P > .05$), and cerebellum ($P > .05$).

In the medulla, DA levels were greater in the condition in which trout were exposed to dietary Pb followed by dietary DMSA compared to both solvent controls (P's $< .01$), dietary Pb followed by no Pb in the diet ($P < .01$), dietary DMSA ($P < .01$), and dietary Pb ($P < .001$). In the optic tectum, DA levels were greater in the condition in which trout were exposed to dietary Pb followed by dietary DMSA compared to the DMSA solvent control (0.1 N NaOH; $P < .01$) but not the Pb solvent control (2% nitric acid; $P > .05$) and dietary Pb ($P < .01$). In the cerebellum, DA levels were greater in the condition in which trout were exposed to dietary Pb followed by dietary DMSA compared to both solvent controls (P's $< .001$), dietary DMSA ($P < .05$), and dietary Pb ($P < .001$). DA levels were greater in the condition in which trout were exposed to dietary Pb followed by no Pb in the diet compared to both solvent controls (P's $< .001$) and dietary Pb ($P < .001$). DA levels were greater in the condition in which trout were exposed to dietary DMSA compared to both solvent controls (P's $< .001$) and dietary Pb ($P < .001$). These data are illustrated in Fig. 1A–C.

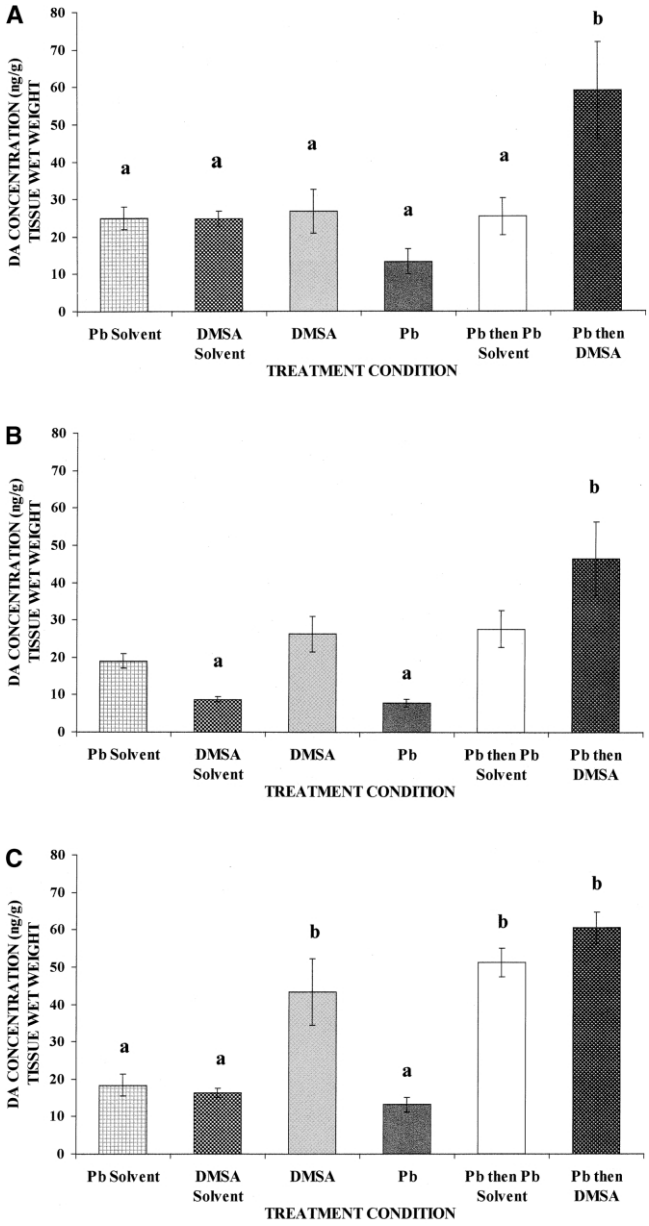

Fig. 1. (A) Mean (±S.E.M.) concentration of DA in the medulla grouped by treatment condition. (B) Mean (±S.E.M.) concentration of DA in the optic tectum grouped by treatment condition. (C) Mean (±S.E.M.) concentration of DA in the cerebellum grouped by treatment condition. Bars labeled as "b" indicate a statistically significant difference when compared to the bars labeled as "a."

3.2. Homovanillic acid

Significant effects were found for the HVA in the medulla [$F(5,44) = 20.297$, $P < .001$], optic tectum [$F(5,44) = 9.915$, $P < .001$], and cerebellum [$F(5,44) = 9.915$, $P < .001$]. Post hoc Tukey's HSD comparisons revealed that there were no differences in HVA levels between the two solvent control groups in the medulla ($P > .05$), optic tectum ($P > .05$), and cerebellum ($P > .05$).

In the medulla, HVA levels were greater in the condition in which trout were exposed to dietary Pb followed by dietary DMSA compared to both solvent controls (P's $< .001$) and dietary Pb ($P < .001$). HVA levels were greater in the condition in which trout were exposed to dietary Pb followed by no Pb in the diet compared to both solvent controls (P's $< .001$) and dietary Pb ($P < .001$). HVA levels were greater in the condition in which trout were exposed

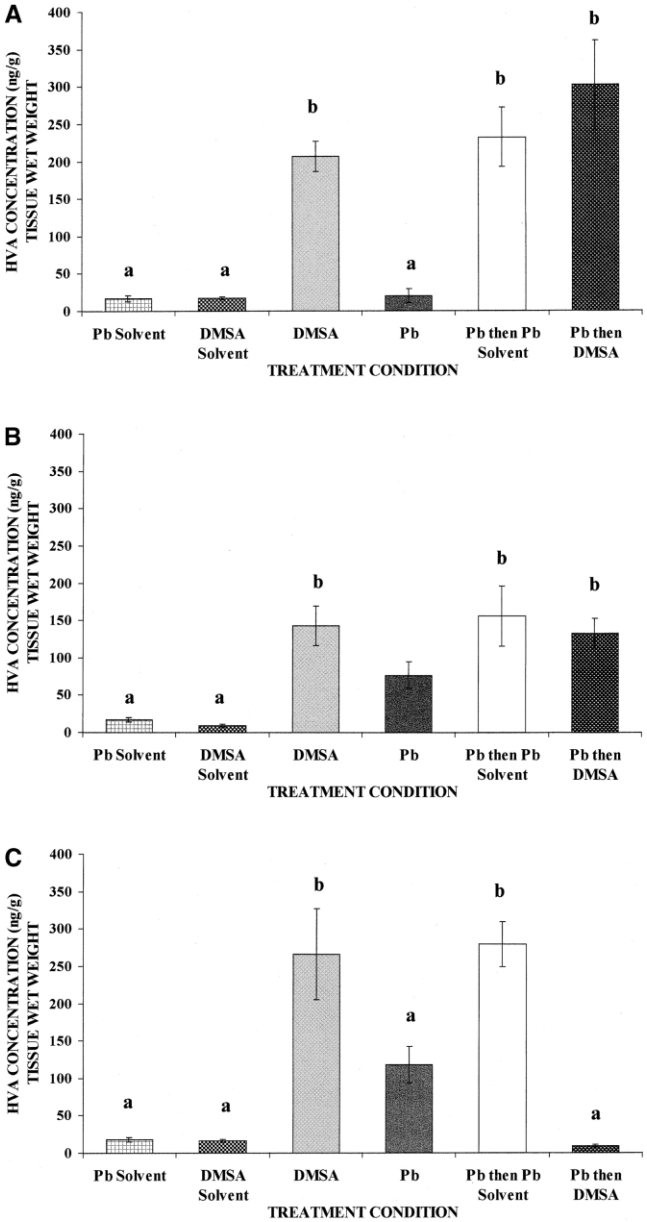

Fig. 2. (A) Mean (±S.E.M.) concentration of HVA in the medulla grouped by treatment condition. (B) Mean (±S.E.M.) concentration of HVA in the optic tectum grouped by treatment condition. (C) Mean (±S.E.M.) concentration of HVA in the cerebellum grouped by treatment condition. Bars labeled as "b" indicate a statistically significant difference when compared to the bars labeled as "a."

to dietary DMSA compared to both solvent controls (P's < .001) and dietary Pb (P < .001). In the optic tectum, HVA levels were greater in the condition in which trout were exposed to dietary Pb followed by dietary DMSA compared to the DMSA solvent control (0.1 N NaOH; P < .01) and the Pb solvent control (2% nitric acid; P < .05). HVA levels were greater in the condition in which trout were exposed to dietary Pb followed by no Pb in the diet compared to both solvent controls (P's < .001). HVA levels were greater in the condition in which trout were exposed to dietary DMSA compared to both solvent controls (P's < .01). In the cerebellum, HVA levels were greater in the condition in which trout were exposed to dietary Pb followed by no Pb in the diet compared to both solvent controls (P's < .001), dietary Pb (P < .01), and dietary Pb followed by dietary DMSA (P < .001). HVA levels were greater in the condition in which trout were exposed to dietary DMSA compared to both solvent controls (P's < .001), dietary Pb (P < .01), and dietary Pb followed by dietary DMSA (P < .001). These data are illustrated in Fig. 2A–C.

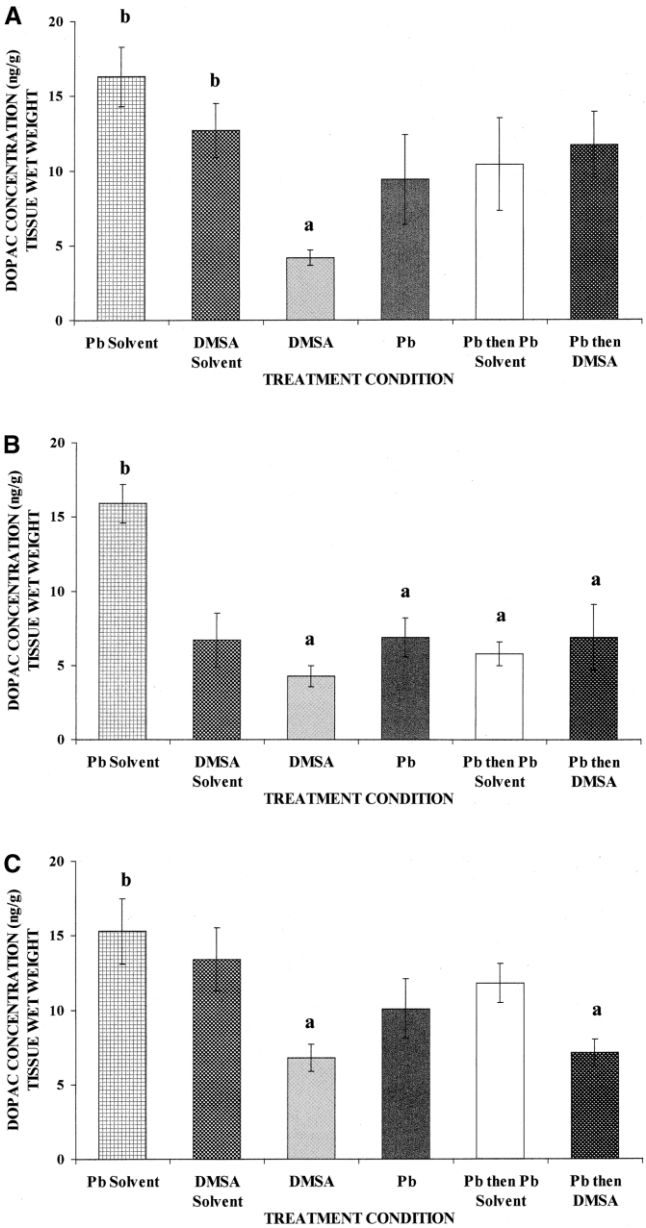

Fig. 3. (A) Mean (±S.E.M.) concentration of DOPAC in the medulla grouped by treatment condition. (B) Mean (±S.E.M.) concentration of DOPAC in the optic tectum grouped by treatment condition. (C) Mean (±S.E.M.) concentration of DOPAC in the cerebellum grouped by treatment condition. Bars labeled as "b" indicate a statistically significant difference when compared to the bars labeled as "a."

3.3. 3,4-Dihydroxyphenylacetic acid

Significant effects were found for the DOPAC in the medulla [$F(5,44) = 2.561$, $P < .05$], optic tectum [$F(5,44) = 5.932$, $P < .001$], and cerebellum [$F(5,44) = 4.633$, $P < .01$]. Post hoc Tukey's HSD comparisons revealed that there were no differences in DOPAC levels between the two solvent control groups in the medulla ($P > .05$) and cerebellum ($P > .05$). In the optic tectum, DOPAC levels were greater in the Pb solvent control group (2% nitric acid) compared to DOPAC levels in the DMSA solvent control group (0.1 N NaOH; $P < .01$).

In the medulla, DOPAC levels were greater in the Pb solvent control group compared to the condition in which trout were exposed to dietary DMSA ($P < .05$). In the optic tectum, DOPAC levels were greater in the Pb solvent control group compared to the condition in which trout were exposed to dietary Pb followed by dietary DMSA ($P < .01$), the condition in which trout were exposed to dietary Pb followed by no Pb in the diet ($P < .01$), the condition in which trout were exposed to dietary DMSA ($P < .001$), and the condition in which trout were exposed to dietary Pb ($P < .01$). In the cerebellum, DOPAC levels were greater in the Pb solvent control group compared to the condition in which trout were exposed to dietary DMSA ($P < .01$) and the condition in which trout were exposed to dietary Pb followed by dietary DMSA ($P < .01$). These data are illustrated in Fig. 3A–C.

4. Discussion

There is now an extensive body of evidence that supports the efficacy of using chelation therapy to reduce the levels of metal contaminants and regain normal physiological function in children, adults, and animal models (Gong and Evans, 1997; Graziano et al., 1992; Khalil-Manesh et al., 1992; Pappas et al., 1995; Smith and Flegal, 1992; Smith et al., 1998). In addition, it is now being observed that such treatment may also be of value in reversing some of the behavioral deficits induced by toxic metal exposure (Laughlin et al., 1999b; Stewart et al., 1996). To date, however, the biological mechanisms of these drugs had been examined in only a few studies. Several authors have hypothesized that compounds such as DMSA chelate toxic metals such as Pb or Hg (Egorova, 1972; Rivera et al., 1989) and, therefore, remove them from chemical interactions with or within tissues and cells. Others have suggested that DMSA metabolites (e.g., mixed disulfides or glucuronide conjugates) may play a major role in ameliorating heavy metal toxicity. For example, one major metabolite of DMSA has been reported to be a 1:2 DMSA/cysteine-mixed disulfide (Maiorino et al., 1989). This 1:2 DMSA/cysteine-mixed disulfide has been synthesized and administered to Pb-poisoned rats to determine its efficacy. The 1:2 metabolite increased urinary Pb excretion when compared to saline or cysteine-treated animals. Pb excretion was comparable to that observed in DMSA-treated rats. Because DMSA and its mixed disulfides were detected in the urine of both 1:2 metabolite- and DMSA-treated animals, the authors could not conclude that the 1:2 metabolite was the sole active compound (Maiorino et al., 1993). Evidence suggesting that Pb and free DMSA are not excreted together (Asiedu et al., 1995) supports the possibility that other binding species are involved. Different isoforms of DMSA (e.g., dimethyl or diisopropyl DMSA) may have a more pronounced effect on metal redistribution, brain neurotransmitter levels, or neurotransmitter enzyme activity (Tripathi et al., 1997). This is important because, to date, it is still unclear what the active form of DMSA is and/or whether a DMSA/ligand complex is necessary to activate an important metabolite. Upon entry to the blood, DMSA binds primarily to albumin and is, in this form, transported to other tissues (Maiorino et al., 1990).

Interestingly, it has been reported that DMSA alone can alter behavior (Laughlin et al., 1999a) suggesting that the drug's reversal of Pb-induced behavioral changes observed by others (Stewart et al., 1996) may have less to do with Pb–DMSA interactions than DMSA–neuron interactions. Since there have been, to our knowledge, no published data to either confirm or reject this hypothesis, this study was designed to evaluate the effects of DMSA alone on brain neurochemistry, as well as its ameliorative effects when preceded by short-term dietary Pb exposure.

Because of other behavioral tests (e.g., agonistic displays, feeding, learning, startle responses) conducted on Pb-exposed fish in our laboratory, the three brain regions of primary interest were the cerebellum, the medulla, and the optic tectum. DA, HVA, and DOPAC levels varied across the exposure conditions and the brain regions examined. The mechanisms underlying Pb-induced behavioral deficits, DMSA-induced alterations of behavior and regional neurochemical changes currently are not well understood.

Contrary to our hypothesis, short-term Pb exposure had no effect on DA levels in the medulla, optic tectum, and cerebellum. This finding does not support the notion that Pb-induced DA level alterations are involved in Pb-induced neurobehavioral toxicity. This finding is consistent with researchers that have reported no change in DA levels in Pb-exposed animals (Golter and Michaelson, 1975; Silbergeld and Goldberg, 1975; Sobotka et al., 1975) and is inconsistent with others that have reported a decrease in DA levels (Dubas and Hrdina, 1978; Sauerhoff and Michaelson, 1973). Future research is aimed at testing the hypothesis that extended exposure to Pb and/or exposure to higher sublethal doses would result in a significant decrease in DA levels in the medulla, optic tectum, and cerebellum. Although DMSA had no effect on DA levels in the medulla and optic tectum, it significantly increased DA levels in the cerebellum. Similarly, the removal of Pb had no effect on DA levels in the medulla and optic tectum but increased DA levels in the cerebellum. The finding that the replacement in the diet with DMSA resulted in significant increases in DA

levels in the medulla, optic tectum, and the cerebellum is not consistent with our hypothesis that Pb followed by DMSA exposure would result in near-control levels of DA.

Contrary to our hypothesis, short-term dietary Pb exposure had no effect on DOPAC levels in the medulla and cerebellum, but resulted in a significant decrease in optic tectum DOPAC levels. A significant decrease in DOPAC levels was observed for all brain regions exposed to dietary DMSA. Contrary to our hypothesis, the replacement of Pb with DMSA did not result in near-control levels of DOPAC. Similarly, the removal of Pb did not result in a significant alteration of DOPAC levels versus Pb alone.

The data for the DA metabolite, HVA, were less consistent across brain regions for the various dietary exposure conditions. Contrary to our hypothesis, short-term dietary Pb exposure had no effect on HVA levels. Pb removal resulted in elevated HVA levels in the medulla, optic tectum, and the cerebellum. The replacement of Pb with DMSA resulted in elevated HVA levels in the medulla, cerebellum, but not the optic tectum. Exposure to dietary DMSA resulted in elevated HVA levels in the medulla, optic tectum, and cerebellum. This finding for fish is similar to those found using dietary exposures in mammals (Silbergeld and Chisholm, 1976) and waterborne exposures in other fish species (Spieler et al., 1995). Elevated HVA levels have been interpreted as an index of increased DA turnover (Silbergeld and Chisholm, 1976).

A potential model of Pb and DMSA interference with neurotransmitter metabolism is through the interaction with enzyme cofactors. Pb may indirectly inhibit DA synthesis by inhibiting the synthesis of the cofactor, biopterin. Because biopterin may be at subsaturating concentrations within a catecholamine-containing neuron, it may play an important role in regulating synthesis (Purdy et al., 1981). Tyrosine hydroxylase, a mixed-function oxidase that uses oxygen and L-tyrosine as substrates and biopterin as a cofactor, is found in cells that synthesize catecholamines (Shiman et al., 1971). This enzyme hydroxylates either tyrosine directly or phenylalanine to form L-tyrosine. Tyrosine is transformed to L-dopa, which is, in turn, decarboxylated to form DA. Pb interference with the biopterin cofactor is a possible explanation for the trend of lower DA levels observed in all three brain regions. It does not explain the higher DA levels in fish exposed to dietary DMSA.

Three other hypotheses exist as to the function of DMSA in altering DA and DA metabolite levels. First, just as Pb can alter the biochemical properties of central DA synapses (Wince et al., 1980), DMSA may also interact with receptor sites on neural cell surfaces. Second, it may be possible that because DMSA is a succinic acid with two internal sulfhydral groups, that it mimics succinic acid in the Krebs cycle of cellular respiration. If so, then DMSA could directly affect cellular metabolism, including that of a neuron, which would then change neurotransmitter synthesis, release, and/or metabolism. Third, Pb is a calcium (Ca^{2+}) antagonist and, therefore, interferes with Ca^{2+}-mediated intracellular signaling (Silbergeld and Hruska, 1980). DMSA, however, elicits concentration-dependent increases in renal intracellular Ca^{2+} levels. Apparently, Pb blocks this effect (Pokorski et al., 1999). It is also possible that the differential alterations in brain neurochemistry observed in both dietary Pb- and DMSA-exposed fish are due to opposing effects on neuronal Ca^{2+} balance. One difficulty with this hypothesis is that fish treated with dietary DMSA alone responded similarly to those treated with DMSA after dietary Pb treatment. To our knowledge, there is no evidence to support any of these possibilities.

In conclusion, contrary to our hypothesis, short-term Pb exposure alone had no effect on DA and DA metabolite levels in the medulla, optic tectum, and cerebellum. This finding does not support the notion that Pb-induced DA and/or DA metabolite level alterations are involved in Pb-induced neurobehavioral toxicity. Future research is aimed at testing the hypothesis that extended exposure to Pb and/or exposure to higher sublethal doses would result in significant alterations of DA and DA metabolite levels in the medulla, optic tectum, and cerebellum. Contrary to our hypothesis, Pb exposure followed by DMSA exposure did not restore DA or DA metabolites to near-control levels. Hence, DMSA-induced alterations of DA, HVA, and DOPAC may not be involved in behavioral improvement following DMSA exposure. However, it appears that DMSA alone is sufficient to alter levels of DA, HVA, and DOPAC in the medulla, cerebellum, and optic tectum. Removing Pb from the diet often results in similar changes in DA, HVA, and DOPAC level as replacing Pb with DMSA. Last, the animal model of short-term dietary exposure to Pb and DMSA, both alone and sequentially, to mimic dietary exposure to Pb and the oral delivery of DMSA, that our laboratory has developed, may be useful in future studies aimed at characterizing the neurobiological mechanisms by which Pb and/or DMSA alters neurotransmitter dynamics and behavior.

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