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Neurotoxic Effects of Secalonic Acid D in Mice During Subchronic Postnatal Exposure

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MONTELLA, P. G. AND C. S. REDDY. *Neurotoxic effects of secalonic acid D in mice during subchronic postnatal exposure.* PHARMACOL BIOCHEM BEHAV **40**(2) 241–247, 1991.—To establish a dose-response of neurotoxic effects to daily oral doses of the mycotoxin secalonic acid D (SAD), as well as to correlate the neonatal behavioral responses to smaller doses of SAD with the attendant neurochemical effects in mice, 5 neonates of each sex were placed with each mother and 4 litters were treated orally with 0 to 5 mg/kg of SAD daily from postnatal day (PND) 3 through 35. Body weights, toxic signs, and mortality were used to arrive at a no observable effect level (NOEL). Performance in several behavioral tests and changes in regional norepinephrine and dopamine levels in the brains of neonates treated with SAD at NOEL (1.25 mg/kg/day) or below were evaluated at selected times during SAD exposure. Doses as low as 1.25 mg/kg/day reversibly reduced body weights in both sexes on PND 12 and 13 compared to controls, whereas doses of 2.5 mg/kg/day or greater were lethal (LD_{50} of 2.5 mg/kg/day). Toxic signs observable in neonates receiving 2.5 mg/kg/day or more of SAD included fine body tremors, uncoordinated movements, hindlimb weakness, circling, loss of righting reflex, paddling, and terminal coma. Ontogeny of cliff avoidance (PND 5, 7, and 9), hindlimb grip response (PND 14, 17, and 20), olfactory discrimination (PND 8 through 11) and swimming (PND 13 through 21) were significantly delayed by SAD exposure: some even at 0.625 mg/kg/day. Dopamine levels significantly increased on PND 13 and decreased on PND 20 only in the olfactory lobe of SAD-exposed neonates. Norepinephrine levels were unchanged in all the brain regions examined. These results suggest a NOEL of <0.625 mg/kg/day for SAD in mouse neonates and a role for regional alterations in dopamine levels in SAD-induced behavioral deficits.

Secalonic acid D Neurotoxic effects Cliff avoidance Hindlimb grip response Olfactory discrimination Swimming Dopamine Norepinephrine Olfactory lobe Neonatal mice

SECALONIC acid D is a teratogenic mycotoxin (22) known to exert behavioral effects in offspring of mice following both prenatal (26) and postnatal (4) exposures. A maternal dose of 25 mg/kg of SAD on day 11 of gestation delayed the ontogeny of several behavioral responses in association with a selective decrease in forebrain dopamine levels (26) in the resulting offspring. Bolon and St. Omer (4) demonstrated similar behavioral effects following oral dosing of lactating dams during the first 10 days of lactation. Neurochemical changes demonstrated in this study (3) were regionally nonspecific and involved both catecholamines and indolamines. Two problems associated with the above studies include the absence of direct exposure in the offspring (maternal dose of 15–25 mg/kg/day) and the inability to estimate the role of maternal effects in the pathogenesis of SAD-induced changes in the offspring. The direct oral dosing of the offspring employed in the present study clearly circumvents these two problems. Establishment of the exposure-to-effect relationship is critical in assessing the risk associated with a chemical and in designing guidelines to prevent such effects.

Although behavioral effects observed in the above studies following in utero (26) or lactational (4) exposure are similar, the neurochemical effects were clearly different involving only dopamine in a specific brain region (forebrain) in the former study and nonspecific, in terms of both the regions and the biogenic amines affected, in the latter study. This study was undertaken to establish a dose response and NOEL for SAD-induced neurotoxic effects, to assess if the earlier reported neurobehavioral effects could result from exposure at NOEL or below, and finally to resolve the issue of regional vs. generalized neurochemical effects (raised by the above studies) and to correlate them with the observed neurobehavioral alterations.

METHOD

Toxicity Study

Timed-pregnant CD1 mice (Charles River Laboratories, Wilmington, MA) were housed individually in plastic cages in rooms maintained under standard conditions (temperature, 22 ± 1°C; humidity, 40 ± 10%; light/dark cycle, 14 h/10 h). Pups born on day 19 (PND 0) were pooled, sexed, and randomly reformed into litters of 5 males and 5 females. Groups of two litters, all members of which received the same treatment, were given 0, 0.625, 1.25, 2.5, or 5.0 mg/kg of SAD daily from PND 3 until PND 35 or death. This was replicated once more immediately following completion of the above replication, thus giving a total of four litters per each dose group. Sodium bicarbonate (5%

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TABLE 1

PHYSIOLOGICAL BASIS AND SCORING OF BEHAVIORAL TEST PERFORMANCE

Test (PND) *	Function	Criterion	Scoring
Negative Geotaxis (5–7, 20–22)	Integration of vestibular function with motor responses for space orientation	Change from a head-down position on an incline to a head-up position with 60 s.	Time (s) to response
Olfactory Discrimination (8–12)	Nest-seeking behavior mediated by smell	Recognition of soiled "Home-Cage" bedding within 120 s.	Time (s) to response
Cliff Avoidance (5, 7, 9)	Interpretation of tactile cues (visual after eyes open)	Turn around when forelimbs and face were placed over a cliff — 15 s.	Time to response 0—no response 1—10 to 15 s. 2—5 to 10 s. 3—less than 5 s.
Swimming Performance (7, 9, 11, 13, 15, 17, 19, 21)	Stress adaptation requiring coordination of neuromuscular responses by higher brain centers	Swimming with nose and ears above water when dropped into an aquarium for 10 s.	0—nose below surface 1—nose at surface 2—nose and top of head above surface, ears below surface 3—same as 2, with water at mid-ear 4—nose, top of head and ear above surface
Hindlimb Grip (14, 17, 20, 22)	Motor coordination and muscle strength	Grasping a horizontal wire with hindlimbs when suspended on the wire with the forelimb grip — in 15 s.	Response (+ or −) in 15 s.

*Numbers in parentheses represent postnatal days on which each test was performed.

v/v in distilled water) was used as solvent. All the pups were weighed daily and observed for any toxic signs and mortality several times daily. It was observed that the weight gain of pups derived from timed-bred mice was considerably lower than was observed in earlier studies with SAD (4,26). A comparative study using mature (>8 weeks old) nulliparous female CD1 mice bred on site to CD1 males was conducted to evaluate if site of breeding also influences mortality.

Behavioral Study

Following the establishment of a dose of 1.25 mg/kg/day of SAD as the apparent NOEL (in the above study), doses of 0, 0.625 or 1.25 mg/kg/day were chosen for behavioral testing. Due to earlier-mentioned differences in weight gains between pups derived from timed-bred mice and those from females bred on site, behavioral ontogeny was also compared between the two groups using 4 litters of pups derived from each source in each treatment group. Each day, containers of toxin solutions and the vehicle were color coded so that the investigator dosing the animal and making behavioral measurements was blind to the treatment received by each group of animals. Two pups of each sex per litter were identified exclusively to study the ontogeny of behavior. The remainder of the pups in each litter were used to study the development of physical landmarks.

Development of physical and maturational landmarks. Physical and maturational landmarks studied included incisor eruption (PND 7 to criterion), eye opening (PND 12 to criterion), testes descent in males (PND 21 to criterion) and vaginal opening in females (PND 30 to criterion). Pups were observed daily during the indicated postnatal periods for the completion of the event studied.

Behavioral test battery. Test battery used in this study is similar to the one used in the Collaborative Behavioral Teratology Study conducted by NCTR (5) with some modifications as used in the two earlier studies with SAD (4,26). The behavioral tests used in this study, their physiological basis, duration of testing, criterion and scoring are described in Table 1. Ontogeny of negative geotaxis was studied at two levels of incline, i.e., 25° (PND 5, 6, 7) and 45° (PND 20, 21, 22). Neonates failing the cliff avoidance test were allowed to fall into a soft bedding.

Neurochemical measurement in brain areas. Based on the findings of earlier studies (3, 4, 26), as well as the analysis of behavioral effects of SAD following postnatal oral exposure in this study, sampling of various brain areas for catecholamine analysis was performed on PND 9, 13, 17, 20, and 22. Since the behavioral ontogeny was similar between pups derived from timed-pregnant mice and those derived from mice bred on site, four litters (each with 5 males and 5 females) derived from only the mice bred on site were used at each dose level (0, 0.625, and 1.25 mg/kg/day) of SAD. One pup of each sex per litter was sacrificed at each time point by immersion in liquid nitrogen.

Following extraction of the brain from the skull at 0°C, regions of brain areas comprising olfactory lobe (including olfactory tubercle and nucleus accumbens), thalamus and hypothalamus (with cortex intact), and cerebellum were dissected out. Care was taken to prevent the tissues from thawing, by performing the dissection at 0°C, by periodic immersion of the dissecting instruments in liquid nitrogen and by limiting the time needed to dissect each brain to less than 3 minutes. Tissue samples were stored frozen at −70°C for no more than 48 h prior to homogenization in 0.5 ml/20 mg of tissue of 0.1 N perchloric acid (PCA) containing 1 mM EDTA and 50 ng/ml of the internal standard, dihydroxybenzylamine (DHBA). A portion of each tissue was used for protein determination by the bicinchonic acid

method of Smith et al. (25). The method for catecholamine analysis utilized high performance liquid chromatography (HPLC) with electrochemical detection (15). The PCA homogenate was centrifuged at 15,000 rpm for 20 min and the supernatant (0.4 ml) was stored frozen at −70°C prior to analysis. The supernatant was diluted with 2.0 ml of Tris buffer (pH 8.6) and was passed through a column containing 20 mg of heat-activated acid-washed alumina (Bioanalytical Systems, West Lafayette, IN). The column was washed with 2.0 ml of double-distilled water prior to elution of alumina-bound catecholamines with 250 μl of 0.1 N PCA. Duplicate (20–50 μl) samples of the eluate were injected onto a 5 μm reverse phase (C_{18}) column (Perkin-Elmer, Norwalk, NJ) and catecholamines were separated by eluting with 1 ml/min of a 10% aqueous methanol mobile phase (pH 4.8) containing 0.1 M sodium acetate, 0.03 M citric acid, and 0.0003 M octyl sodium sulfate, using a Perkin-Elmer Series 10 LC. The detection of norepinephrine (NE), DHBA, and dopamine (DA) were accomplished using an LC-4B amperometric detector (Bioanalytical Systems) containing a TL-5 flow cell with glassy carbon and Ag/Cl reference electrodes. The detector potential was set at 0.62 V and the range set at 5 nA. Working standard containing 34 ng NE, 50 ng DHBA, and 38 ng DA/ml were similarly processed along with brain samples to evaluate recovery, which varied between 75 and 80%. The peak height ratios of NE or DA/DHBA were used to calculate NE or DA concentration in brain samples as follows:

$$(\text{NE or DA})_{\text{unk}} = \frac{(\text{NE or DA/DHBA})_{\text{unk}} \times (\text{NE or DA})_{\text{kno}}}{(\text{NE or DA/DHBA})_{\text{kno}}}$$

where, $(\text{NE or DA/DHBA})_{\text{unk or kno}}$ represent peak height ratios of NE or DA and DHBA in samples or standards, respectively. $(\text{NE or DA})_{\text{unk or kno}}$ represent concentration of NE or DA in samples or standards, respectively. These concentrations were subsequently expressed as ng of NE or DA per mg protein in each tissue.

Statistics. Each neonate was considered an experimental unit. Body weights of pups on each PND were compared using a one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test. In addition, the slopes of body weight gains between the three lower dose groups (0, 0.625 and 1.25 mg/kg SAD) were also analyzed for significance. Lethality data in each replicate was used to calculate LD_{50} by Weil's (30) method. Postnatal age of attainment of criterion in maturational indices, time to response in the negative geotaxis and olfactory discrimination tests, and performance scores in the cliff avoidance and swimming tests were analyzed for treatment effects at each testing point by the ANOVA followed by LSD test. Binomial response in the hindlimb grip test was analyzed by Chi-square test. Data for each catecholamine in each region were compared for differences between treatments by the ANOVA followed by LSD test. Data for both sexes were pooled (as was the case for all parameters except for incisor eruption, body weights, and of course, the vaginal opening and testicular descent) when neither sex differences nor dose × sex interaction were significant in ANOVA or when similar effects were found among sexes between the treatment groups for data analyzed by the Chi-square test. Other factors (litter, etc.) were similarly treated and discussed when pertinent. All p values of <0.05 were considered significant.

RESULTS

Toxic Signs and Lethality

Irrespective of the site of breeding, daily oral exposure to 2.5 mg/kg of SAD or more produced significant reduction in body weights (Fig. 1; data from pups derived from timed-pregnant mice not shown), and a dose-dependent increase in the severity of toxic signs and mortality. Doses of 1.25 mg/kg/day or less of SAD failed to produce either mortality or toxic signs. At a dose of 1.25 mg/kg/day, although transiently lower body weights were seen between PND 12 and 17, such an effect was reversible at later time points. The slopes of body weight gains throughout this period, however, were parallel with controls (Fig. 1). A comparison between pups born to timed-bred females and those bred on site, indicated significantly lower body weights in the former group compared to the latter at all ages beginning PND 12 (data not shown). As expected, males among all groups were heavier than females.

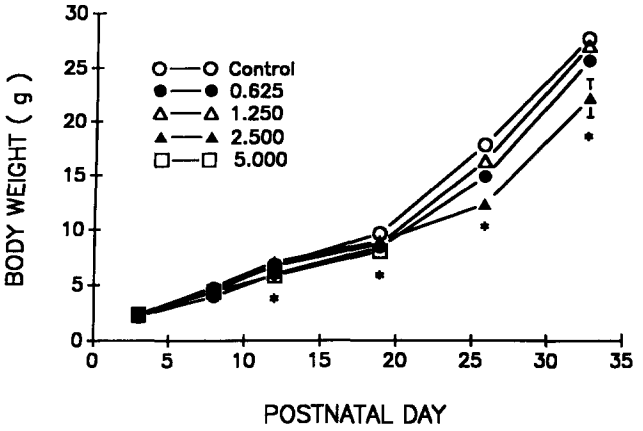

FIG. 1. Body weights (g; mean ± SE) of male CD1 mice (pups derived from females bred on site) following postnatal oral exposure to secalonic acid D. Asterisk indicates significant difference in body weight of mice receiving 5.0 mg/kg/day (PND 12 and 19) and 2.5 mg/kg/day (PND 26 and 33) compared to controls. N ranged from 20/group on PND 3 to 9 in 2.5 mg/kg group on PND 33 and to 6 in 5.0 mg/kg group on PND 19.

Toxic signs began to appear on PND 14 in pups receiving 5 mg/kg/day and slightly thereafter in 2.5 mg/kg group. Early signs included excitement and circling inside the cage followed by fine body tremors, uncoordinated movements, leading to hindlimb weakness characterized by dragging of the hindlegs, loss of righting reflex, paddling and coma prior to death. All the animals in the 5 mg/kg group succumbed to the toxic effects of SAD prior to PND 26. The LD_{50} at PND 21 in both sexes in each replicate was 2.5 mg/kg/day. Irrespective of the site of breeding, 50% of the mice of both sexes receiving 2.5 mg/kg died by PND 21. Only one additional animal (5% of the animals in the group) died between PND 21 and 35 although all mice given this dose became increasingly moribund.

Necropsy of animals dying from toxin exposure revealed congested liver, pale kidneys and gross hemorrhages on the surface of the brain. Stomachs were filled with milk. Histological examination failed to reveal any significant lesions in heart, kidney, liver, lungs, spleen and brain.

Physical and Maturational Landmarks

The results of these evaluations are presented in Table 2. In control pups of both sexes incisor eruption occurred between PND 12 and 13, and eye opening occurred between PND 14 and 16. Testes descent and vaginal opening occurred between PND 22 and 23, and PND 29 and 31 in male and female offspring, respectively. All responses were independent of the breeding site

TABLE 2

POSTNATAL DAY OF APPEARANCE OF PHYSICAL LANDMARKS OF MATURATION IN NEONATAL MICE EXPOSED TO SECALONIC ACID D*

Landmark	n	Control	SAD mg/kg 0.625	1.25
Incisor Eruption	48	13.37 ± 1.15	12.13 ± 0.34	13.81 ± 0.98
Eye Opening	48	15.25 ± 0.78	15.56 ± 0.35	15.44 ± 0.73
Testes Descent	24	22.25 ± 0.46	22.13 ± 0.35	22.00 ± 0.00
Vaginal Opening	24	30.125 ± 0.84	30.63 ± 0.74	30.38 ± 0.74

*Data are pooled from pups derived from timed pregnant females and females bred on site.

of the dams. Treatment with SAD, at either dose, failed to significantly affect any of the parameters in this category (Table 2).

Behavioral Tests

Due to earlier described differences in body weights of pups derived from timed-pregnant females compared to those of pups derived from females bred on site, all the behavioral parameters between these two groups of pups were compared within each sex. The ontogeny of the behaviors tested, surprisingly, was unaffected by the site of breeding of the dams. As a result, comparisons of behavioral performance were conducted on pooled data for each sex. When no differences were found between sexes, the data for both sexes also were pooled for analysis.

Cliff avoidance. Data in Fig. 2 indicated that in the control group a majority of the pups turned away from the cliff in less than 5 seconds (score 3) as early as on PND 5, all the pups achieving this score by PND 9. Pups exposed to the lowest dose (0.625 mg/kg/day) of SAD exhibited significantly increased latency (lower score) on both PND 7 and 9, whereas the high (1.25 mg/kg/day) dose exposure prolonged the latency to response as early as PND 5 which returned to normal by PND 9. Such treatment-related effects were evident in both males and females (data not shown).

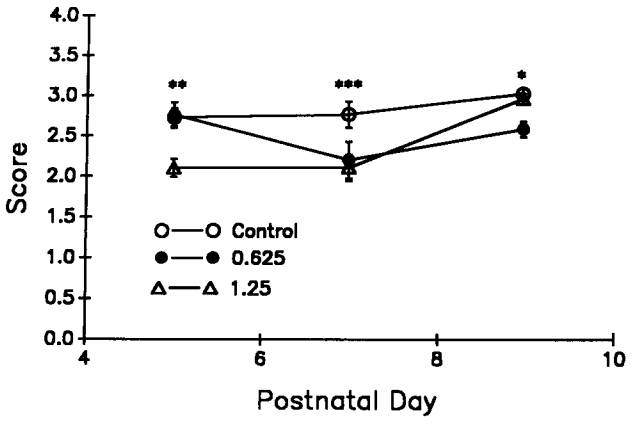

FIG. 2. Effect of postnatal secalonic acid D exposure on cliff avoidance response (mean score ± SE) in mouse neonates (pooled data). Asterisks indicate significant ($p \leq 0.05$) difference between mice receiving 0.625 mg/kg/day (*), 1.25 mg/kg/day (**), or both groups (***) and controls on the postnatal day indicated. N = 32 per dose at each time point.

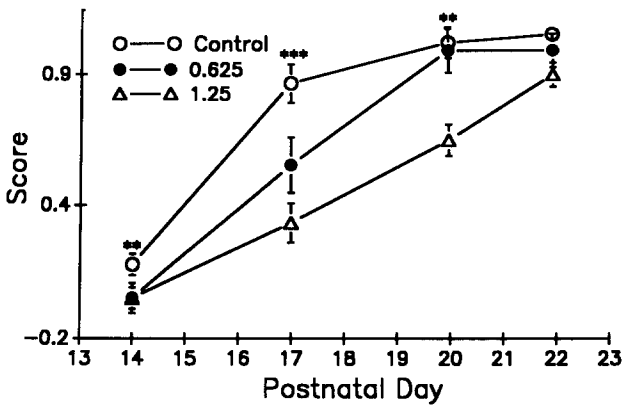

FIG. 3. Hindlimb grip response (mean score ± SE) in neonatal mice (pooled) exposed postnatally to oral doses of secalonic acid D. Asterisks indicate significant ($p < 0.05$) difference between mice receiving 0.625 mg/kg/day (*), 1.25 mg/kg/day (**), or both groups (***) and controls on the postnatal day indicated. N = 32 per dose at each time point.

Hindlimb grip. Ontogeny of this response in the control pups occurred between PND 14 and 22 with 12.5, 81, 97 and 100 percent of pups responding positively on PND 14, 17, 20 and 22, respectively (Fig. 3). A clear dose-dependent delay in the ontogeny of this response was noted in SAD-treated pups. Significantly fewer pups responded positively on PND 14 and 17 (0 and 50%) in the lower dose group, whereas in pups receiving 1.25 mg/kg/day of SAD response rates of only 0, 28, 59 and 84% were seen on PND 14, 17, 20 and 22 respectively (Fig. 3). Analysis of the data by sex showed similar results (not shown).

Negative geotaxis. Analysis of the pooled data for control dams indicated that, following an initial increase, the mean response time decreased gradually to their shortest by PND 7 (25° incline, data not shown). Subsequent testing between PND 20 and 22 at an incline of 45° suggested that control mice retained their fast response throughout this period. No significant effects were produced by daily SAD exposure at either dose in males or females.

Olfactory discrimination. As shown in Fig. 4, the latency to reach home bedding for the control pups decreased gradually over the five-day testing period. Significantly longer response times were seen in SAD-treated mice on PND 8 and 9 (0.625 mg/kg/day) and PND 11 (both doses). Both males and females showed delayed response following SAD exposure on one or more days of testing.

Swimming performance. Figure 5 illustrates swimming performance scores for the pooled data. All groups started with a mean score close to zero on PND 7. Both control and low dose (0.625 mg/kg/day of SAD) groups of mice showed similar ontogeny to each other with a gradual increase in score to 4 by PND 19 and 17, respectively. The pups in the high dose (1.25 mg/kg/day of SAD) group were significantly outperformed by control pups beginning PND 13 and were unable to attain a score of 4 during the testing period. This trend was apparent even when the data were separately analyzed by sex.

Neurochemistry

Only samples from pups derived from females bred on-site were analyzed for neurochemistry. Since no significant sex-dose interaction was evident in any of the parameters examined, the

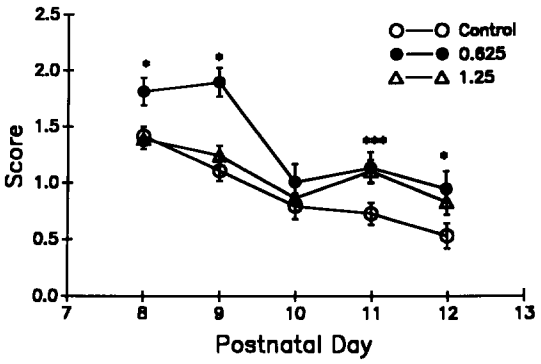

FIG. 4. Delay in the ontogeny of olfactory discrimination (mean response time in min ± SE) following secalonic acid D exposure orally in neonatal (pooled). Asterisks indicate significant ($p \leq 0.05$) difference between mice receiving 0.625 mg/kg/day (*), 1.25 mg/kg/day (**), or both groups (***) and controls on postnatal day indicated. N = 32 per dose at each time point.

results of the analysis of pooled data are presented.

Levels of NE in all brain regions tended to be stable (1.65 to 5.4 ng/mg protein) with only minor increments with age. No significant alterations in NE levels were produced, in any brain region examined, by SAD exposure at any postnatal age through PND 22 (data not shown). In the control mice, DA levels were low (0.35 to 1.58 ng/mg protein) in the cerebellum and moderate (2.45 to 5.4 ng/mg protein) in the thalamus/hypothalamus regions. The levels of DA in these regions remained stable between PND 9 through 22 (not shown). The highest levels of DA and the most rapid changes in its levels in control mice occurred in the olfactory lobe (Fig. 6). A rapid increase in DA with a peak on PND 20 followed by some decline by PND 22 occurred in this region. Significant changes in DA levels following SAD exposure also occurred in this region with a significant dose-dependent increase (250% of controls) on PND 13 and a significant decrease (to 50% of controls) following high dose SAD exposure on PND 20 (Fig. 6).

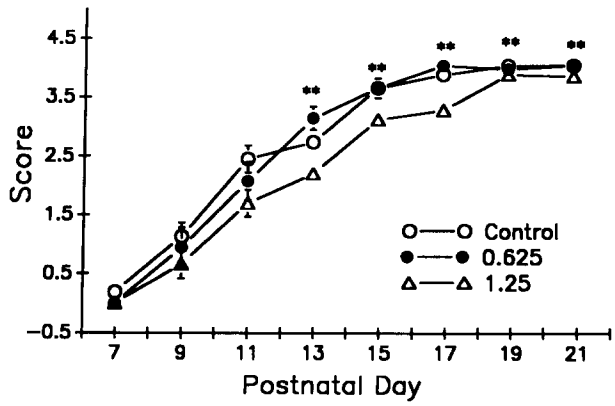

FIG. 5. Ontogeny of swimming performance (mean score ± SE) following postnatal exposure to oral doses of secalonic acid D in CD1 mouse offspring (pooled). Asterisks indicate significant difference ($p \leq 0.05$) difference between mice receiving 0.625 mg/kg/day (*), 1.25 mg/kg/day (**), or both groups (***) and controls on the postnatal day indicated. N = 32 per group at each time point.

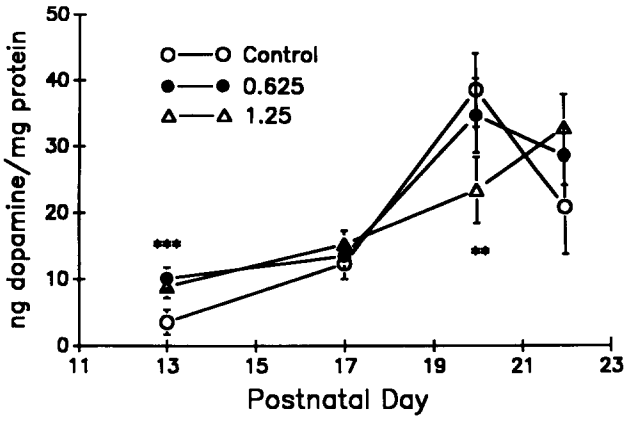

FIG. 6. Secalonic acid D-induced changes in dopamine levels (mean ± SE) in the olfactory lobe region of neonatal mice (N = 5 to 10 at each time point per dose). Asterisks indicate significant ($p \leq 0.05$) difference between mice receiving 0.625 mg/kg/day (*), 1.25 mg/kg/day (**), or both groups (***) and controls on the postnatal day indicated.

DISCUSSION

The toxic signs observed in this study following higher doses of SAD are similar to those reported earlier in adult mice (21). The lack of sex differences in mortality and in the dose response to other toxic effects evaluated in this study is in contrast to earlier reported sex differences in acute toxicity (21) and may suggest a lack of establishment of hormone-based sexual differences in immature mice. Initial toxicity studies also suggested a dose of 1.25 mg/kg/day of SAD to be close to a "no observable effect level" (NOEL) and its suitability as the highest dose in the behavioral testing.

Behavioral tests used in screening batteries, such as in our study, frequently test overlapping functions so that toxicity usually appears as multiple effects (29). SAD-induced multiple behavioral changes observed in this study are in agreement with the earlier studies (4,26). Some differences in response to cliff avoidance, hindlimb grip test, and olfactory discrimination test between current study and that of St. Omer and Reddy (26) probably reflect differences in the timing of exposure (gestational in the later). Behavioral alterations were caused by doses as low as 0.625 mg/kg/day of SAD suggesting the no effect level to be below this dose: less than 50% of that suggested by toxicity study alone.

Although a clear dose response of SAD effects was seen for all parameters tested (except negative geotaxis) on certain PND's, several instances were noted where low dose significantly altered the behavioral response with no effect being seen at the high dose. Panksepp (19) noted the frequent reporting of such effects in the literature and suggested that low-dose effects rather than dose-response relationship may be physiologically more meaningful. The basis for such responses in behavioral studies is unclear at the present time, but may involve an interplay between factors such as the narrow dose-window needed for a response, selective effects on certain neural systems at low doses vs. more generalized effects at higher doses, disruption of normal phasic responses of certain neural systems, and the triggering of compensatory mechanisms.

Swimming behavior development is known to be affected by the prevailing levels of hormones such as thyroxine and cortisol (24). Cortisol is also known to delay maturation of brain responsiveness to sensory stimuli (23) possibly mediated by reduced

brain cell number (1) following postnatal exposure. Recent evidence that SAD is capable of elevating plasma corticosterone in adult male and pregnant female mice (7) may suggest the likelihood of such an effect in neonates. Confirmation of this SAD effect in neonates will help us with a definitive assessment of the extent of glucocorticoid involvement in SAD-induced behavioral deficits. Other factors influencing development of swimming behavior include abnormal otolith development (8) and dopaminergic dysfunction (14,28). Otolith development appears unaffected by SAD since such an effect would have altered the ontogeny of negative geotaxis, a response not affected by SAD in this study.

The levels of catecholamines in various brain regions in our study are in general agreement with those reported for rodents (2,9) and specifically for mice (12). Reported developmental pattern of stable to minor increases in the levels of NE with age in various brain regions (13,18) and steep increases in DA levels with age in the olfactory lobe and surrounding areas (18) also support the validity of catecholamine patterns and levels observed in our study. The dramatic decrease in the DA level in control olfactory lobe area on PND 22 compared to those on PND 20 appears to be a normal recurrent short-term fluctuation of DA in certain brain regions (13).

Effects of SAD on olfactory lobe DA levels without effects on NE levels in this region and lack of effects of SAD on either DA or NE in any other brain region examined indicates the specific nature of SAD effects. Dose-dependent increase in DA levels in the olfactory lobe (on PND 13), observed in our study, was not reported in earlier studies with SAD (3,26) which either pooled this brain region with others (26) or failed to study this region altogether (3). Such increases in biogenic amines, however, have been reported to result from exposure to other developmental toxicants (10, 13, 17). The subsequent reduction of DA levels in the mouse olfactory lobe by SAD on PND 20 as demonstrated in our study, however, is in agreement with both earlier studies with SAD (3,26), as well as with a number of other studies dealing with a variety of behavioral teratogens. Dopamine appears to be a key factor in the organization of motor behavior and in eliciting a number of other behavior patterns including aggression (20) and modulation of threshold in olfactory sensation in insects (16). Although the importance of specific alterations (especially biphasic as seen in our study) in olfactory DA levels in relation to the overall behavioral response in animals is difficult to ascertain, effects such as reduced motor activity following bulbectomy or lateral olfactory tract lesions (6,27), as well as both increase and decrease in DA levels in different brain regions by a single agent (13) during development, suggest the relevance of such changes to deficits in behaviors with a significant motor component, as those used in our study. The uncoupling of oxidative phosphorylation in liver mitochondria by SAD (11), if applicable to skeletal muscle and/or nervous tissue, may be an additional contributing factor in weaker or delayed behavioral responses in SAD-exposed mice.

Finally, although SAD-induced changes, observed in this study, seemed to reflect delays in the ontogeny and were reversed while SAD-exposure continued, the presence of other and potentially long-lasting effects that continue to exist following cessation of SAD-exposure cannot be excluded.

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