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Serotonergic Modulation of the Acoustic Startle Response in Rats During Preweaning Development[1]

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SHEETS, L. P., L. L. COOK AND L. W. REITER. *Serotonergic modulation of the acoustic startle response in rats during preweaning development.* PHARMACOL BIOCHEM BEHAV 33(2) 415–422, 1989.—The involvement of serotonin (5-HT) in modulating the acoustic startle response (ASR) is well established in adult rats, but 5-HT involvement during the preweaning period, when 5-HT neurons undergo extensive development, has not previously been described. Three 5-HT receptor subtypes are reported to modulate the ASR in adult rats: 5-HT_{1A} and 5-HT_2 receptor agonists facilitate the ASR, whereas 5-HT_{1B} agonists decrease the response. In the present study, the effects of 5-HT agonists and generalized 5-HT depletion on the ASR were studied in preweanling animals, using independent groups of Long-Evans rats tested on postnatal day (PND) 13, 17 and 21. 8-Hydroxy-2-(di-n-propylamino) tetralin (8OHDPAT, 62–1000 μg/kg), a 5-HT_{1A} receptor agonist, and 5-methoxy-N,N-dimethyl tryptamine (MeODMT, 2–4 mg/kg), a nonselective 5-HT agonist, had no effect on PND 13 and then increased the ASR on PND 17 and 21. The 5-HT_2 receptor antagonists cyproheptadine (5 mg/kg) and ketanserin (5 mg/kg) blocked the effect of MeODMT at both ages, providing some evidence that MeODMT increased the ASR through 5-HT_2 receptors. 1-(m-Chlorophenyl) piperazine (mCPP, 1–5 mg/kg), a 5-HT_{1B} agonist, had no effect on ASR amplitude on PND 13 or 17 and then produced a dose-related decrease in the response on PND 21. Generalized depletion of 5-HT by 80–90% in whole-brain and spinal cord, using p-chlorophenylalanine (PCPA, 300 mg/kg 24 hr prior to testing), did not alter ASR amplitude at any age. These results support the development of 5-HT facilitation and inhibition of the ASR during the third postnatal week. 5-HT involvement in facilitating the ASR (5-HT_{1A} and 5-HT_2) developed earlier than inhibition (5-HT_{1B}). The failure of PCPA to increase the ASR in preweanling rats tends to indicate that 5-HT tonic inhibition has not developed by PND 21. The results with PCPA are inconclusive, however, since there is some discrepancy regarding the effect of PCPA on the ASR in adult rats.

Acoustic startle response Startle Serotonin Development Rat Preweaning Behavior

SEROTONIN (5-HT) modulates the startle response in a complex manner in adult rats (8) but its involvement in modulating the startle response in immature animals has not previously been described. In the adult, 5-HT is involved in both facilitation and inhibition of the startle response. These opposing effects have been demonstrated with agonists and antagonists selective for certain 5-HT receptor subtypes and with localized administration of 5-HT agonists to brain and spinal cord. Because 5-HT neurons undergo extensive development during the preweaning period their role in modulating the startle response may develop during this period.

In adult rats, there is evidence supporting the involvement of 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 receptor subtypes in modulating the startle response. 5-HT_{1A} and 5-HT_2 receptor agonists both increase startle response amplitude, whereas 5-HT_{1B} agonists decrease the response. 8-Hydroxy-2-(di-n-propylamino) tetralin (8OHDPAT), a 5-HT_{1A} agonist, and 5-methoxy-N,N-dimethyl tryptamine (MeODMT), a nonselective 5-HT agonist (34), both increase the startle response (9, 11, 32). The efficacy of cyproheptadine, a 5-HT_2 antagonist, in blocking the effect of MeODMT on startle amplitude (9) suggests that 5-HT_2 receptors may be involved in mediating this effect. In contrast to the increased startle response that occurs with 5-HT_{1A} and 5-HT_2 receptor agonists, 1-(m-chlorophenyl) piperazine (mCPP), a 5-HT_{1B} receptor agonist (30), decreases startle amplitude (11).

Davis and co-workers (9) have proposed that 5-HT increases the amplitude of the startle response at spinal levels and attenuates the response at supraspinal levels. The involvement of 5-HT in

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facilitating the startle response at the spinal cord level is supported by the finding that the startle response is augmented by the intrathecal administration of 5-HT, MeODMT or 8OHDPAT to intact rats or systemic administration of MeODMT to decerebrate animals (9–11). Forebrain-mediated 5-HT inhibition of the startle response is supported by decreased startle amplitude following intraventricular 5-HT, MeODMT or mCPP (5, 8–11, 15, 17). The response is not altered with intraventricular 8OHDPAT or intrathecal mCPP (11), indicating further that these drugs modulate startle at spinal (facilitation) and supraspinal (inhibition) levels, respectively.

There is considerable evidence that 5-HT neurons in the forebrain tonically inhibit the startle response. Response amplitude increases with generalized depletion of 5-HT, accomplished either by placing animals on a tryptophan-deficient diet (35) or by treatment with PCPA (4,6). Electrolytic lesions in the dorsal and median raphe nuclei deplete 5-HT primarily in the forebrain and increase startle amplitude (13,16). The administration of 5,7-dihydroxytryptamine into the ventral tegmentum also depletes 5-HT in the forebrain and increases startle (8).

5-HT neurons undergo extensive development during the preweaning period (27). From postnatal day (PND) 12 to 21, the formation of 5-HT axon terminals is very active in forebrain areas, including the cerebral cortex and hippocampus (25). 5-HT innervation of the spinal cord also undergoes considerable development postnatally. The pattern and density of 5-HT immunoreactivity reach maturity around PND 14 in cervical cord and by PND 21 in thoracic and lumbar cord (3). The appearance between PND 14 and 17 of the "5-HT syndrome" in response to 5-HTP, MeODMT and p-chloroamphetamine (20) provides evidence for the functional development of 5-HT neurons that enhance the activity of spinal motor neurons at this time.

Our interest in the involvement of 5-HT in modulating the ASR during preweaning development began with the observation that ASR amplitude increases nonmonotonically over the preweaning period; decreasing from PND 15 to 18, in contrast to the increase over the remainder of the preweaning period (29). We proposed that this decline in response amplitude may reflect the development of neurons which tonically inhibit the ASR, in a manner similar to that of 5-HT inhibition of locomotor activity beginning about the same age (26). The development of 5-HT terminals in forebrain areas that are associated with 5-HT inhibition of the ASR at this time prompted us to speculate that inhibitory 5-HT neurons could be involved.

The purpose of this study was to examine the role of 5-HT in modulating the ASR during the preweaning period. One objective was to determine whether the ontogeny of 5-HT involvement in modulating the startle response could be dissociated for the three classes of receptors. A second objective was to determine whether 5-HT exerts a tonic inhibitory effect on the startle response during the preweaning period. Two experimental approaches were used in this study. The first approach involved examining the effect of 5-HT_{1A}, 5-HT_{1B} and 5-HT_2 agonists on the response, testing each at three ages that encompass the preweaning period. The second approach involved examining the effect of 5-HT depletion on the response at these ages.

METHOD

Animals

Pregnant Long-Evans hooded rats (Charles River) were received on day 15 of gestation. Animals were housed individually in suspended plastic cages with food (Purina lab chow) and water available ad lib. The animal facility was maintained on a 12:12-hr photoperiod L:D (0600:1800 hr), with controlled temperature (22 ± 2°C) and relative humidity (50 ± 10%). On postnatal day 1 (day of birth = PND 0) pups from all litters were randomized and redistributed with each dam assigned 4 males and 4 females. One paw of each pup was tattooed for unique identification within the litter [modified from Avery and Spyker (2)].

Treatment

A within-litter treatment design was employed with independent groups tested on PND 13, 17 and 21. For each compound, a male and female in each litter received one of the treatments and all pups in the litter were tested concurrently. Doses, calculated as the salt, were administered in 5 μl saline/g body weight.

8OHDPAT (Research Biochemicals Inc., Wayland, MA) was given SC, while MeODMT (Sigma, St. Louis, MO) and mCPP HCl (Aldrich, Milwaukee, WI) were administered IP immediately before animals were tested. Cyproheptadine HCl (Sigma, St. Louis, MO) was administered IP at 5 mg/kg, 15 minutes prior to MeODMT and ketanserin tartrate (Research Biochemicals Inc., Wayland, MA) was injected SC at 5 mg/kg, 30 minutes before MeODMT. Pups were returned to their home cage between injections.

PCPA methyl ester HCl (Calbiochem, San Diego, CA) was administered IP in saline 24 hours prior to behavioral testing. Two males and two females in each litter received PCPA (300 mg/kg) and an equal number of littermates received saline. Tissues were collected from one male and one female per treatment in each litter immediately after animals were tested. Animals were killed by decapitation, the brain rapidly excised and the tissue caudal to the cerebellum was discarded. The full length of the spinal cord was collected by cutting away the dorsal portion of the spinal column. Tissues were weighed, placed in vials and kept briefly on dry ice before storage at −80°C.

Biogenic Amine Analysis

The effect of PCPA on biogenic amine levels in brain and spinal cord was determined by high performance liquid chromatography (HPLC) with electrochemical detection. Tissues were homogenized in 0.2 N perchloric acid containing 2.0 mM sodium metabisulfite and 3,4-dihydroxybenzylamine (DHB) as an internal standard. Homogenates were centrifuged ($20{,}000 \times g$) for 15 minutes at 4°C and concentrations of 5-HT, norepinephrine and dopamine in the supernatant were measured. Quantitation was based on sample peak height ratios (amines/DHB) relative to a series of chemical standards. 5-HT determinations were made by direct injection of sample supernatant (23). Norepinephrine and dopamine measurements were preceded by an alumina adsorption procedure, using minor modifications of published methods (14). The HPLC system was a Waters Associates (Milford, MA) model 6000A pump, model 710A automatic sample injector, and a C-18 reverse phase column (3.9 mm × 30 cm μ-Bondapak). A model LC-4 amperometric detector with glassy carbon electrode (Bioanalytical Systems, West Lafayette, IN) was used, with the detector potential set at +0.70 V vs. Ag/AgCl reference electrode. The mobile phase was 30 mM Na_2HPO_4, 0.27 mM EDTA, 4.0 mM heptane sulfonic acid, 5% acetonitrile, and 0.5% triethylamine, pH 3.1.

Test Apparatus

Testing was conducted in eight sound-attenuated chambers ($30.5 \times 57 \times 35$ cm), each containing a wire mesh plastic-framed cage ($9 \times 5 \times 5.7$ cm) mounted on a load cell/force transducer assembly (Gould Statham Model UC3 with 5-lb adaptor Model

UL4-5) designed to measure vertical force. Two speakers were suspended from the ceiling of each chamber. A Motorola piezoelectric tweeter (5.0 × 12.5 cm) presented the eliciting stimulus (13 kHz, 120 dB, 40 msec tone with a 2.5 msec rise/decay). A second speaker (Creative Acoustics, 16 ohm) delivered broad-spectrum background noise at 45 to 75 dB. The output from this speaker, measured at 80 dB with a Spectrascope Model SD330 Real Time Analyzer, had approximately equal power from 200 Hz to 15 kHz (± 10 dB); output dropped 40 dB from 15 kHz to 20 kHz. All sound measurements were made with a Bruel and Kjaer Model 2209 Sound Level Meter (A-scale) with a Model 4165 half-inch free field microphone.

Test Procedure

Animals received a total of 30 trials following a 10-minute adaptation period at ambient noise levels (approximately 42 dB). The adaptation period was reduced to 3 minutes with tests involving MeODMT because of this compound's short duration of action. Each trial began with 20 seconds of background noise prior to the presentation of the eliciting stimulus; the background noise continued throughout stimulus presentation and data collection. Three noise levels were used in a session (45, 60 and 75 dB) to examine effects on sensitization to background noise. The order in which noise levels were presented was computer-generated in a semi-random but balanced fashion (i.e., the three background noise levels were presented in random order within each of ten sets). Data collection began with the presentation of the eliciting stimulus and continued for 64 msec. The analog signal for each response was digitized at 1 kHz and converted to grams using a previously determined calibration curve for each load cell.

Response amplitude and latency to onset measurements were taken from each animal's average response curve, calculated across all trials at a given background noise level. Baseline was defined as the average force (g) exerted on the platform during the first 10 msec following the eliciting stimulus, a time period which precedes response onset (19). The latency to onset was defined as the time (msec) following the onset of the eliciting stimulus when the amplitude exceeded the baseline by more than four standard deviations. If this criterion was not reached within 40 msec, then no latency to onset was recorded. Response amplitude (g) was defined as the maximum value of the average curve, minus the baseline. Sensitization was estimated as the difference in amplitude occurring for trials at 75 vs. 45 dB.

The replacement of some test equipment during the period over which these experiments were conducted resulted in a decrease in our measure of latency to onset. This change primarily reflects a decrease in baseline standard deviation, and the fact that latency was defined on the basis of this measure (see above). Thus, the latency measures in the later experiments (mCPP) are shorter than in the earlier experiments (8OHDPAT and MeODMT).

Statistics

All data were analyzed using the univariate general linear model procedure (GLM) on SAS (1985). Greenhouse-Geisser adjusted degrees of freedom were used for the estimate of the p value. Separate analyses were performed for each response measure. Data were analyzed using sex, age of testing and treatment as between-animal factors and background noise level as the within-animal factor (repeated measure). When interactions were significant, simple main effects were examined. Post hoc comparisons of means were made using Tukey's (a) statistic. For all statistical tests, the critical value at $p<0.05$ was accepted as significantly different.

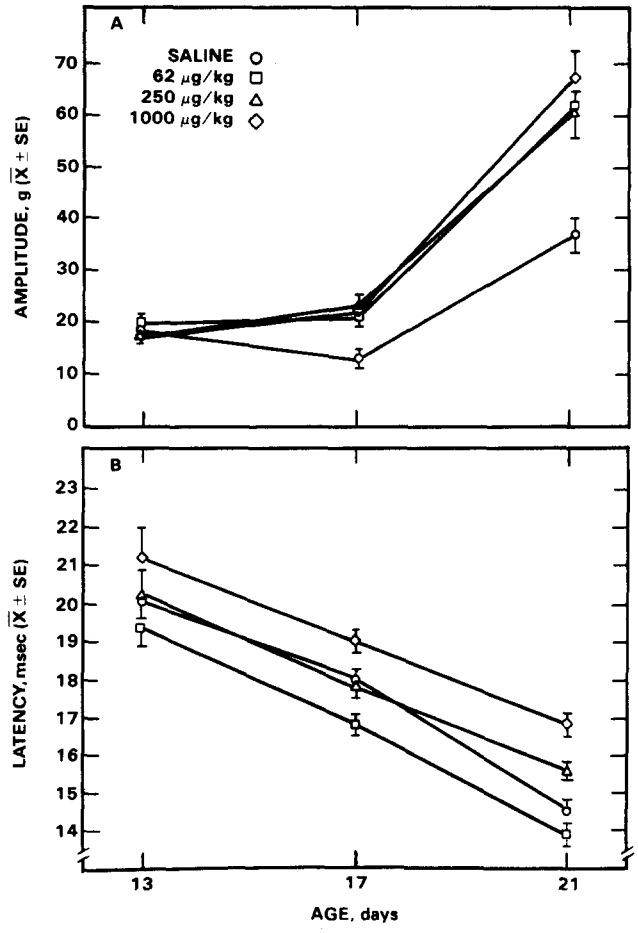

FIG. 1. Startle response amplitude and latency to onset immediately following 8OHDPAT (0, 62, 250 or 1000 μg/kg), administered SC in saline on PND 13, 17 and 21 (n = 12/group). a) Response amplitude was not affected by treatment on PND 13 and was increased at all doses on PND 17 and 21. b) Latency was increased by the high dose at all 3 ages (*$p<0.05$, Tukey's).

RESULTS

The data for males and females were combined and collapsed across trials at the three background noise levels for each treatment, since there were no treatment × sex or treatment × background interactions. None of the treatments affected sensitization to background noise, defined as the difference in response amplitude between trials with 75-dB background noise versus ones with 45-dB background; therefore, these data are not shown.

8OHDPAT

8OHDPAT did not affect the ASR on PND 13 and then increased startle amplitude on PND 17 and 21 at all doses (62 to 1000 μg/kg) (Fig. 1A). In fact, over this broad (16-fold) dose range all doses were equally effective, producing about a 65% increase in the response. The age × treatment interaction, $F(6,158) = 3.73$, $p<0.0017$, reflects this age-related difference. Response amplitude was not affected by treatment on PND 13, $F(3,40) = 0.92$, $p<0.4378$, and was increased by treatment on both PND 17, $F(3,46) = 11.21$, $p<0.0001$, and PND 21, $F(3,48) = 6.34$, $p<0.0010$. For startle latency, there was an overall treat-

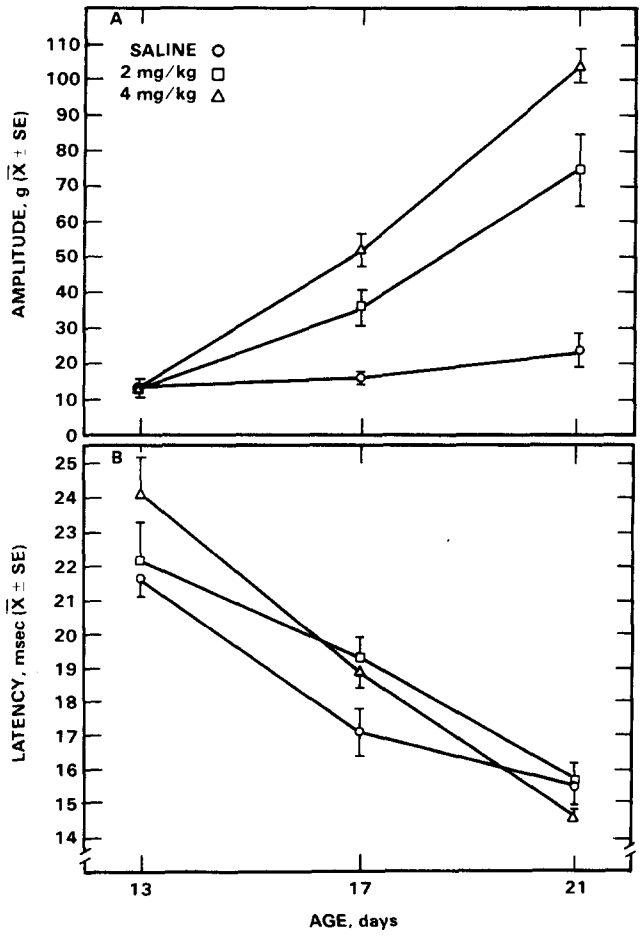

FIG. 2. Startle response amplitude and latency to onset immediately following MeODMT (0, 2 or 4 mg/kg), administered IP in saline on PND 13, 17 and 21 (n=8/group). a) Response amplitude was not affected by treatment on PND 13 and was increased at both doses on PND 17 and 21 (*$p<0.05$, Tukey's). b) Latency was not affected by treatment.

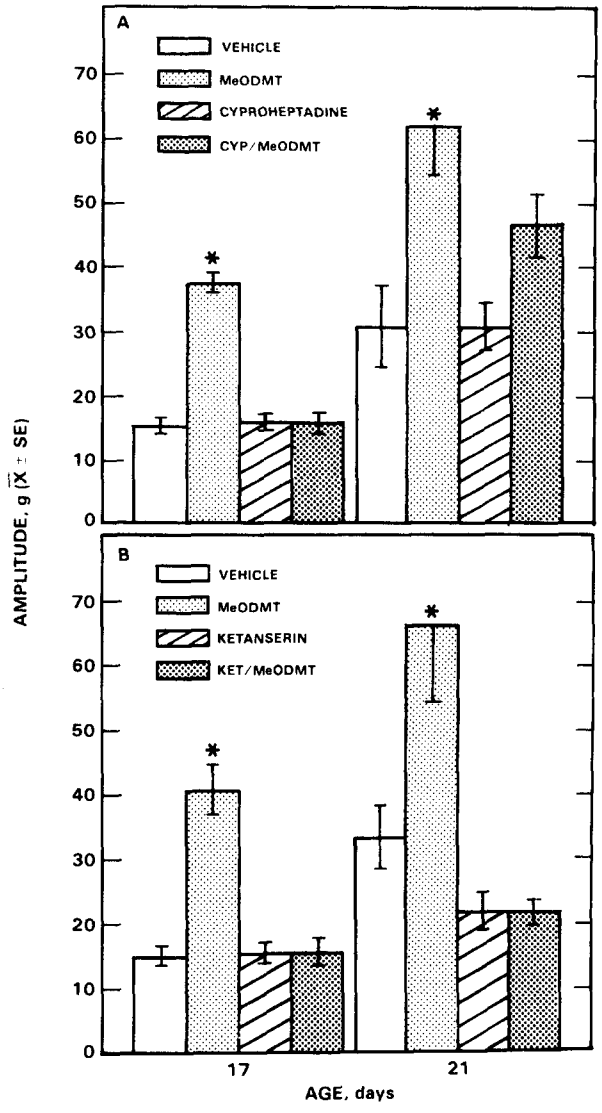

FIG. 3. The effect of cyproheptadine and ketanserin on ASR amplitude in 17- and 21-day-old rats treated with MeODMT (4 mg/kg) or saline immediately prior to testing. a) Cyproheptadine (5 mg/kg) was given IP in saline 15 minutes prior to MeODMT (n=12/group). b) Ketanserin (5 mg/kg) was administered SC in saline, 30 minutes before MeODMT (n=10/group). (*Different from control; $p<0.05$, Tukey's.)

ment effect, $F(3,158)=16.54$, $p<0.0001$, but no age × treatment interaction, $F(6,158)=0.75$, $p<0.6074$. The high dose increased latency at all three ages (Fig. 1B).

MeODMT

MeODMT produced a dose-related increase in amplitude beginning on PND 17 (Fig. 2A). The high dose (4 mg/kg) increased the response by 228% and 346% on PND 17 and 21, respectively. The age × treatment interaction, $F(4,59)=19.31$, $p<0.0001$, supports an age-related difference. There was no treatment effect on PND 13, $F(2,17)=0.05$, $p<0.9482$, whereas there was a treatment effect on PND 17, $F(2,12)=17.60$, $p<0.0003$, and PND 21, $F(2,12)=48.29$, $p<0.0001$. Startle latency was not affected by treatment, $F(2,59)=2.22$, $p<0.1175$, and there was no age × treatment interaction, $F(4,59)=2.45$, $p<0.0557$ (Fig. 2B).

Both cyproheptadine and ketanserin blocked the increase in ASR amplitude that occurred following MeODMT in 17- and 21-day-old animals. Cyproheptadine blocked augmentation of the response by MeODMT at both ages, at a dose that did not alter the response when given alone (Fig. 3A). There was an overall treatment effect, $F(3,76)=20.26$, $p<0.0001$, and no age × treatment interaction, $F(3,76)=1.87$, $p<0.1412$. The response of animals treated with MeODMT alone exceeded that of all other groups. The response of the groups that received cyproheptadine, either alone or prior to MeODMT, was not different from control. Ketanserin also blocked the effect of MeODMT on response amplitude, at a dose that did not alter the response when given alone (Fig. 3B). Again, there was an overall treatment effect, $F(3,60)=20.93$, $p<0.0001$, with no age × treatment interaction, $F(3,60)=1.67$, $p<0.1827$. The response of the group treated with MeODMT alone exceeded that of all other groups; the response of the groups treated with ketanserin was not different from control.

mCPP

mCPP affected startle amplitude only on PND 21 but increased

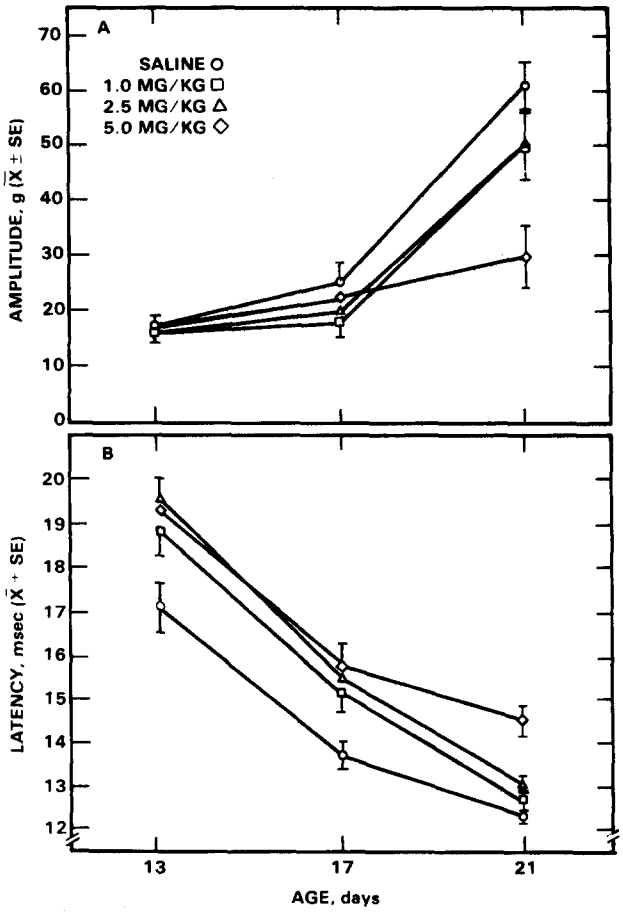

FIG. 4. Startle response amplitude and latency to onset immediately following mCPP (0, 1.0, 2.5 or 5.0 mg/kg), administered IP in saline on PND 13, 17 and 21 (n = 10/group). a) Startle ampltiude was not affected by treatment on either PND 13 or 17 and was decreased at the 5.0 mg/kg dose level on PND 21. b) Latency was increased at all ages following 2.5 and 5.0 mg/kg (*$p<0.05$, Tukey's). The latency to onset measured in this experiment is shorter than other experiments due to a change in test equipment (see the Method section).

startle latency at all ages. For ASR amplitude, there was an age × treatment interaction, $F(6,96)=4.02$, $p<0.0012$, with no treatment effect on either PND 13, $F(3,32)=0.19$, $p<0.9004$, or PND 17, $F(3,32)=1.13$, $p<0.3509$, and a decrease in startle amplitude on PND 21, $F(3,32)=5.57$, $p<0.0034$ (Fig. 4A). The high dose decreased the response by 50% on PND 21. For latency, there was an overall treatment effect, $F(3,96)=15.85$, $p<0.0001$, and no age × treatment interaction, $F(6,96)=1.37$, $p<0.2364$, reflecting increased latency at the intermediate and high doses at all three ages (Fig. 4B). Latency was increased about 2 msec in the high dose group.

PCPA

Tissue monoamine determinations are shown in Table 1. 5-HT levels increased with age in brain and spinal cord, a total of 27% and 19%, respectively. The effect of PCPA on tissue monoamine levels did not vary with age or sex. 5-HT levels were reduced 80% in whole-brain and 88% in the spinal cord, whereas norepinephrine and dopamine levels were reduced 8 and 9%, respectively.

PCPA did not alter the ASR. Although response amplitude was modestly elevated on PND 17 (26%), the effect was not statistically significant. There was no overall treatment effect, $F(1,107)=1.67$, $p<0.1992$, or age × treatment interaction, $F(2,107)=1.01$, $p<0.3684$, on startle amplitude (Fig. 5). Startle latency was also not affected by treatment, $F(1,107)=3.21$, $p<0.0758$ (data not shown).

DISCUSSION

These results support the proposal that putative 5-HT receptor involvement in facilitating and inhibiting the ASR develops between PND 13 and 21. 8OHDPAT and MeODMT, 5-HT_{1A} and nonselective 5-HT receptor agonists, respectively, had no effect on ASR amplitude on PND 13, and then both treatments increased the response on PND 17 and 21. The effect of MeODMT on response amplitude was blocked with cyproheptadine and ketanserin, indicating that 5-HT_2 receptors were involved. mCPP, a 5-HT_{1B} receptor agonist, did not affect ASR amplitude on either PND 13 or 17 and then decreased the response on PND 21. In contrast to the other treatments, 5-HT depletion with PCPA did not alter startle amplitude during the preweaning period. The absence

TABLE 1

EFFECT OF PCPA ON TISSUE MONOAMINES DURING PREWEANING DEVELOPMENT

	Tissue Weight*		Serotonin†		Norepinephrine†		Dopamine†	
	Brain	Sp. Cord	Brain	Sp. Cord	Brain	Sp. Cord	Brain	Sp. Cord
				PND 13				
Saline	1097 ± 18	110 ± 3	338 ± 13	473 ± 39	281 ± 7	565 ± 19	368 ± 6	N.D.‡
PCPA	1055 ± 13	108 ± 2	88 ± 4	55 ± 12	267 ± 5	528 ± 25	355 ± 5	N.D.
				PND 17				
Saline	1258 ± 20	144 ± 2	351 ± 17	526 ± 35	291 ± 12	557 ± 19	408 ± 14	N.D.
PCPA	1201 ± 10	142 ± 3	72 ± 5	55 ± 5	286 ± 6	535 ± 21	333 ± 37	N.D.
				PND 21				
Saline	1299 ± 22	175 ± 4	431 ± 9	562 ± 18	382 ± 13	577 ± 29	529 ± 11	N.D.
PCPA	1298 ± 17	166 ± 4	80 ± 3	76 ± 9	331 ± 6	501 ± 19	459 ± 8	N.D.

*mg wet weight (mean ± SE) (N = 10/group).
†ng/g wet weight.
‡N.D. = not detectable.

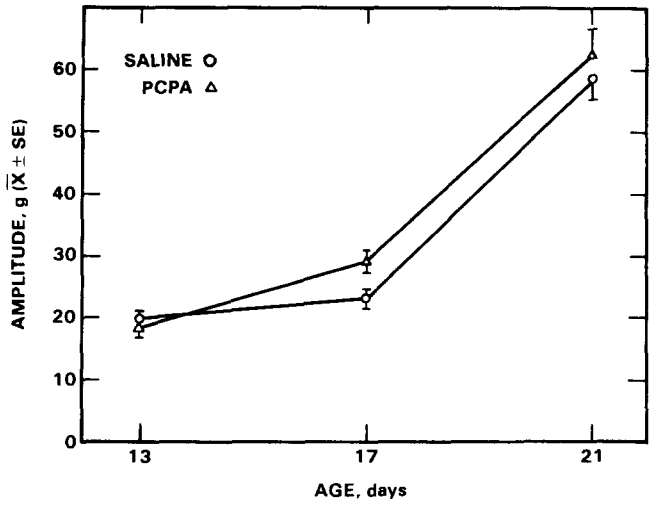

FIG. 5. Startle response amplitude following PCPA (300 mg/kg) or saline, administered IP 24 hours before testing on PND 13, 17 and 21 (n = 20/group). The response was not affected by PCPA at any age.

of an increase in startle amplitude with PCPA could indicate either that 5-HT neurons involved in tonic inhibition of the startle response develop after PND 21 or that generalized 5-HT depletion with PCPA is not an effective means for demonstrating this effect.

The selective affinity of 8OHDPAT for 5-HT_{1A} receptors (34) and the effectiveness of 5-HT_2 antagonists in blocking facilitation of the response with MeODMT in adult (9) and preweanling rats, provide evidence that 8OHDPAT and MeODMT increase the startle response through 5-HT_{1A} and 5-HT_2 receptors, respectively. Since neither drug affected startle amplitude on PND 13 and both increased the ASR on PND 17, it is suggested that 5-HT_{1A} and 5-HT_2 receptors involved in increasing the ASR both develop within the period from PND 13 to 17. Further testing with a selective 5-HT_2 agonist is required to test this proposal. The ontogenic profile for controls in the MeODMT experiment was uncharacteristic in that the startle response increased very little from PND 17 to 21. An explanation for this is not available; however, the low control response on PND 21 cannot account for the treatment effect. The startle response of animals that received MeODMT on PND 21 exceeded that of all other control groups. Furthermore, MeODMT increased the ASR significantly in both antagonist experiments, where the control response was normal in amplitude. Finally, MeODMT also consistently increased the response on PND 17.

The effects of MeODMT and 8OHDPAT on the ASR of 17- and 21-day-old rats were similar to those seen in adults. Both treatments increase the ASR in adult (9,32) and preweanling rats. The sensitivity of preweanling rats to MeODMT, and the magnitude of response facilitation that occurred with MeODMT, resembled that of adults. At 2 mg/kg, MeODMT approximately doubles the ASR in both adult (9) and preweanling rats. A direct comparison of sensitivity to 8OHDPAT cannot be made with these data since different routes of administration were used in adult (IP) and preweanling (SC) animals. 8OHDPAT increased startle latency in both adult (32) and preweanling rats, but only at doses that were 16-fold greater than required to increase startle amplitude. In contrast to the age-related effect of 8OHDPAT on startle amplitude, latency was affected equally at all ages. Increased startle latency occurred at a dosage (1 mg/kg) that produced postural changes (hindlimb abduction) and severe hypothermia (3°C). At the intermediate dose level, startle latency was not affected and postural changes were not evident, however, pups were hypothermic (1.7°C) (temperature data not shown).

Evidence in adult rats that 8OHDPAT and MeODMT increase the startle response at spinal, and not supraspinal, levels suggests that the effect of 8OHDPAT and MeODMT on ASR amplitude that develops from PND 13 to 17 reflects 5-HT development in the spinal cord. Intrathecal administration of 8OHDPAT or MeODMT increases the startle response in adult rats, whereas intraventricular administration decreases (MeODMT) or has no effect (8OHDPAT) on startle amplitude (9,11). Caudal raphe nuclei innervate spinal motor neurons (31) and there is evidence that these nuclei facilitate the startle response through a mechanism that involves 5-HT. Electrical stimulation of caudal raphe nuclei facilitates startle; this effect is blocked with 5-HT depletion and is restored with 5-HT repletion (1). Furthermore, iontophoretic application of 5-HT facilitates the depolarization of spinal motor neurons in response to the excitatory amino acid glutamate (36). Thus, the increased startle response with 8OHDPAT and MeODMT that develops between PND 13 and 17 may reflect the innervation of spinal motor neurons by caudal raphe nuclei. The appearance of the "5-HT syndrome" at this time, between PND 14 and 17 (20) provides evidence that 5-HT receptors that facilitate the activity of spinal motor neurons develop at this age. This syndrome consists of characteristic behavioral signs of motor activation that accompany elevated levels of 5-HT or relatively high doses of certain 5-HT agonists (18) which are mediated by the brainstem and spinal cord (21).

One objective of this study was to determine whether the processes underlying 5-HT_{1A} and 5-HT_2 receptor-mediated facilitation of the ASR could be dissociated by differences in ontogenic profiles. Instead, it appears that they developed concurrently from PND 13 to 17. This assumes that the effect of MeODMT is mediated through 5-HT_2 receptors; an assumption based on the efficacy of ketanserin and cyproheptadine for blocking this effect. Again, this must be tested using a selective 5-HT_2 agonist.

The failure of mCPP to affect startle amplitude on PND 17 suggests that 5-HT_{1B} involvement in startle inhibition develops later than facilitation through 5-HT_{1A} and 5-HT_2 receptors. In adult rats, forebrain involvement in mediating the effect of mCPP on ASR amplitude is indicated by the much more potent effect of mCPP on ASR amplitude following intraventricular versus intrathecal administration (11). The relatively late development of this inhibitory effect is consistent with the ontogeny of locomotor activity, in which inhibition develops around 15 days of age (26). The efficacy and potency of mCPP in decreasing startle amplitude in 21-day-old rats is similar to that seen in adults; 5 mg/kg decreasing the response about 65% in adults (11) and 50% in 21-day-old animals. Like 8OHDPAT, mCPP increased startle latency at all ages. In this instance, rectal temperature was reduced 1.7°C in 21-day-old animals treated with the high dose, a decrease in temperature that occurred with 8OHDPAT, at a dose that did not affect latency. Response latencies for controls in this experiment were shorter than for other control groups, reflecting a change in test equipment (see the Method section). Startle response latency to onset is currently 11 msec in adult rats (data not shown), compared with about 12 msec with the previous equipment (7). The current measure in adult rats equals that reported by others (19) and is closer to the 8 msec ASR latency of EMG activity in the hindlimb of adult rats (12,33).

The occurrence of effects of 8OHDPAT, MeODMT and mCPP on ASR latency does not correlate with dose- and age-related effects on amplitude. MeODMT increased ASR amplitude on PND 17 and 21 and did not change latency at any age. 8OHDPAT and mCPP produced opposite age-related effects on ASR amplitude, while both increased latency at all ages. In contrast to mCPP, 8OHDPAT has been shown in adult (32) and preweanling rats to affect ASR latency only at doses that are 16-fold higher than ones

that increase amplitude. The presence of effects only at relatively high doses of 8OHDPAT suggests that 5-HT_{1A} receptors are not involved. Possible confounding effects that were evident at the high dose of 8OHDPAT were postural changes, primarily hind-limb abduction, and severe hypothermia (3°C). Such changes in posture could increase latency by impeding motor output. The second factor, hypothermia, has not been shown to increase startle latency, but factors such as slowing conduction velocity by reducing the temperature of nerve fibers could be involved. An evaluation of the effects of mCPP on ASR latency in adult rats has not been reported. Neither postural nor body temperature changes could appear to account for the increase in latency that occurred with mCPP. Postural changes were not observed at any dose and hypothermia was mild (1.7°C at the high dose) or absent (at the intermediate dose) at doses that increased latency. Thus, latency was affected by mCPP at doses required to change ASR amplitude and which produced no clinical signs. Although this may suggest that 5-HT_{1B} receptors mediated the effect of mCPP on latency, further evaluation is needed. The earlier occurrence of the effect on latency (by PND 13) compared with amplitude (after PND 17), indicates there is some difference in the mechanism involved.

The final portion of this study consisted of testing the effect of PCPA on the startle response to examine the involvement of 5-HT in tonic inhibition of startle through the preweaning period. In order to support our hypothesis that the development of inhibitory 5-HT neurons around PND 15 is responsible for producing the decline in startle amplitude that occurs from PND 16–18 (29), 5-HT depletion should increase the startle response on PND 17 and 21, but not on PND 13. PCPA depleted 5-HT in brain and spinal cord at all ages to an extent that is commensurate with that reported in whole-brain of adult rats (24). However, PCPA had no effect on the startle response at any age through the preweaning period. Therefore, these results do not support the involvement of 5-HT in tonic inhibition of startle during the preweaning period.

These results are not conclusive, however, since it is not clear that PCPA increases the ASR in adult rats. Reports on the effects of PCPA on the ASR in adult rats vary from no change to a modest increase in amplitude. One difference among studies with discrepant results is the dosing regimen that is used. Although this varies, the methods that are employed should produce comparable levels of 5-HT depletion (24). Therefore, it is likely that the discrepancy cannot be attributed to the extent of 5-HT depletion. The dose of PCPA that was used here does not alter ASR amplitude in adult rats, whether animals are tested 24 hours (6) or 72 hours following treatment (28). The two studies which report an increased startle response in adult rats used multiple doses of PCPA; either four successive daily 100 mg/kg doses (4) or two successive daily 300 mg/kg doses (6). This may indicate that a more rigorous treatment regimen is necessary to increase the response. Repeated treatment with PCPA was avoided in the present study since it has not been shown to be more effective in depleting 5-HT and since brain development during the preweaning period is sensitive to phenylketonuria, a condition that develops with repeated administration of PCPA (22).

The impact of PCPA on the startle response is not commensurate with the extent to which it depletes 5-HT (8). This indicates that the effect of PCPA on startle amplitude in adult rats does not reflect the magnitude of 5-HT involvement in tonic inhibition of the ASR. The relatively small and inconsistent effect of PCPA may relate to the dual role of 5-HT in modulating the ASR, acting both to increase and attenuate response amplitude, and the generalized depletion of 5-HT in the spinal cord and brain that occurs with PCPA. PCPA would be expected to attenuate both effects, each acting to oppose the other. The overall impact of PCPA could vary then, if the relative contribution of 5-HT facilitation and inhibition of the ASR varied with experimental conditions.

In summary, these findings support the development of 5-HT involvement in modulating the ASR during the preweaning period. 5-HT_{1A} (8OHDPAT) and 5-HT_2 (MeODMT) receptor agonists began increasing the amplitude of the ASR from PND 13 to 17, whereas a 5-HT_{1B} agonist (mCPP) began decreasing the response later, from PND 17 to 21. mCPP was the only treatment that affected latency at doses required to change response amplitude and in the absence of clinical signs. The increased latency that occurred with mCPP developed earlier than its effect on amplitude, being present at the earliest age tested (PND 13). Unlike these other treatments, generalized depletion of 5-HT with PCPA did not affect the ASR at any age through the preweaning period. The failure of PCPA to increase the ASR in preweanling animals would suggest that the tonic inhibitory effect that 5-HT exerts on the ASR in adults does not develop by the end of the preweaning period. However, the results with PCPA are inconclusive since there is some discrepancy regarding the effect of PCPA on the adult ASR.

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