ELSEVIER

Pharmacology Biochemistry & Behavior, Vol. 55, No. 3, pp. 397–403, 1996

0091-3057/96 $15.00 + .00

PII S0091-3057(96)00109-8

Ritanserin Blocks DOI-Altered Embryonic Motility and Posthatch Learning in the Developing Chicken

GEORGE BOLLWEG AND SHELDON SPARBER[1]

Department of Pharmacology, University of Minnesota, 3-249 Millard Hall, Minneapolis, MN 55455

Received 6 November 1995; Accepted 27 February 1996

BOLLWEG, G. AND S. SPARBER. *Ritanserin blocks DOI-altered embryonic motility and posthatch learning in the developing chicken.* PHARMACOL BIOCHEM BEHAV **55**(3) 397–403, 1996.—Developing chicken embryos exposed to cocaine show altered motility, hatchability, and posthatch detour learning. Pretreating such subjects with the serotonin$_2$ (5-HT$_2$) antagonist ritanserin (RIT) can block the motility suppression and reduced hatchability, indicating 5-HT$_2$ receptor involvement in these cocaine effects. To study behavioral consequences of more selective 5-HT$_2$ receptor stimulation and its blockade during development and to compare such exposure with that of cocaine, we injected eggs with 15-day-old chicken embryos with the 5-HT$_2$ agonist dimethoxyiodophenylaminopropane (DOI, 1.0 mg/kg egg) and 1 h later, with RIT (0.3 and 0.9 mg/kg egg). Motility was recorded 2.5 or 24 h after DOI. This DOI dose suppressed motility 2.5 h but not 24 h after administration. Both RIT doses blocked DOI's motility suppression. No treatment affected hatchability. Subjects were tested on posthatch days 6–9 for detour learning acquisition. DOI "enhanced" learning (i.e., reduced latency), a cocaine-like effect observed in prior work, which was also blocked by both RIT doses. Thus, some consequences of DOI exposure late during embryonic development resemble cocaine's and are blocked by RIT, suggesting a therapeutic role for RIT-like drugs against cocaine's potential developmental toxicity.

DOI 5-HT$_2$ receptor Cocaine Motility Learning Developmental toxicity

IN spite of a decade of active recent investigation, the developmental consequences of human cocaine exposure appear less well established at present than was the case several years ago when the term "crack baby" was coined. Much clinical evidence is difficult to interpret because of poorly defined and nonrepresentative study populations; misclassification of cocaine users and nonusers; inadequate information regarding timing, quantity, and duration of cocaine exposure; confounding maternal variables; concurrent exposure to other abused substances; and limited outcome measures that exclude potential long-term effects. Because of these shortcomings, it seems reasonable to argue that the issue of cocaine's teratogenic potency remains open (16).

This uncertainty reinforces the importance of animal models for studying "mechanistic" aspects of cocaine's potential for altering development. One animal model that can complement mammalian studies is the developing chicken. In addition to rapid development, relative ease of manipulation, and fairly well-characterized developmental stages, its use avoids maternal confounding variables (e.g., maternal–neonate interaction, nutritional perturbations) inherent in mammalian developmental cocaine studies, because properly incubated eggs develop without maternal influence. Observation of direct developmental effects of the agent of interest is facilitated.

The developing chicken also appears to be a useful model for studying "critical periods" in development, a time of increased toxicant susceptibility more likely to result in longer term (or permanent) consequences. For example, injection of various agents including the neurotransmitters norepinephrine, epinephrine, dopamine, and serotonin (5-HT) into chicken embryos over embryonic days 4–14 (E4–E14) greatly increased lethality (up to 60%) after E8–E12 exposures, but not after earlier exposure (32). Earlier work also demonstrated critical periods for biochemical alterations subsequent to drug exposure during development in the chicken embryo (15): whole-brain tyrosine hydroxlase activity increased and whole brain catecholamine levels decreased in 3-day-old chicks following injection of eggs with reserpine prior to incubation, but not

[1]To whom requests for reprints should be addressed.

if it was injected into yolk sac on E7 or E14. Thus, critical periods in chick embryonic development have been observed for the effects of exogenous and endogenous chemicals, as well as environmental manipulations such as incubation temperature (1) manifest functionally or biochemically.

Cocaine's pharmacological activity is mediated in part by its ability to block synaptic reuptake of dopamine, norepinephrine, and most potently, 5-HT (23). We have reported that a range of cocaine HCl doses (11.25–67.5 mg/kg egg) injected 2–3 mm beneath the shell of eggs with embryos, suppresses embryonic motility 20 min after injection, and in late development reduces hatchability after 22.5 and 67.5 doses (26–28). We have also found that E17 pretreatment with the 5-HT_2 antagonist ritanserin (RIT, 0.4 mg/kg egg) can block effects of cocaine on motility and hatchability when cocaine is injected on E18 (10), supporting the idea that 5-HT_2 receptor stimulation is involved in these manifestations of cocaine-mediated toxicity. In addition, we reported motility suppression, hatch interference, and herniated umbilici in the developing chicken after administering dimethoxyiodophenylaminopropane, DOI (25), a selective 5-HT_2 agonist (7), over a range of doses and developmental days, as well as RIT's efficacy against these effects.

In addition to its role in cocaine's actions, 5-HT and its receptors are involved in the expression of withdrawal from other abused substances. Prior work in this laboratory showed that the 5-HT_2 antagonists mianserin (21) and ketanserin and pirenpirone (22) blocked several signs of naloxone-induced withdrawal after induction of acute or chronic morphine dependence in rats. Others have reported that mianserin attenuated the decline in time spent in the open arm of an elevated plus-maze, a sign of withdrawal, after 4 days of ethanol exposure in young rats (13), again demonstrating serotonergic involvement. Morphine withdrawal is demonstrable at E12–E14 in the chicken embryo (5), and opiate withdrawal in this species can also be induced by E19 naloxone administration following E3 injection of the long-acting opiate *N*-desmethyl-*l*-α-acetylmethadol, NLAAM (12). Opiate withdrawal and acute cocaine actions appear similar in terms of excessive autonomic activation and CNS arousal, and both may be developmental hazards. Thus, drugs that block opiate withdrawal may also block some acute and potential functional teratogenic actions of opiate withdrawal or cocaine.

To better characterize the involvement of excessive 5-HT_2 receptor stimulation in drug-associated developmental toxicity in the chicken embryo, the present study investigated acute and longer term behavioral effects of a single DOI dose. The purpose of the present work was to determine whether E15 DOI (1.0 mg/kg egg) could mimic the short-term motility depressant effect of E15 cocaine (26), and if so, whether treatment 1 h later with RIT (0.3 or 0.9 mg/kg egg) could block this effect. To gain initial information on the duration of the DOI motility suppressive effect, motility was also recorded 24 h after DOI administration in a group of eggs separate from those studied on E15. Hatchability was also observed. To determine whether these treatments might result in posthatch behavioral effects, chicks were also tested 6–9 days posthatch for acquisition of a detour learning response.

METHOD

Subjects and Their Treatment

Fertilized eggs with embryos (White Leghorn × White Leghorn) were obtained from Midwest Hatchery & Poultry, Dassel, MN. Upon arrival in the lab, incubation was continued in a rotating forced air incubator (Hatchette model, Humidaire Co., New Madison, OH) maintained at 37.5°C and 58% relative humidity. Eggs were candled for viability and nonviable eggs were discarded. Sufficient eggs were procured to allow treatment of two separate sets of 28 eggs each, one for motility recordings 2.5 h after DOI or saline, and one for recording motility 24 h after DOI or saline. Subjects from both sets were used in the hatchability and detour learning parts of the experiment. Except for brief transfer and handling periods (e.g., injections), embryonic subjects were maintained in incubators throughout the prehatching period.

The day before drug administration holes were drilled in eggshells for injections and electrode placement. Shell surfaces where holes were to be drilled were disinfected with a drop of 2% tincture of iodine, then immediately wiped with a gauze pad moistened with 70% ethanol to remove the iodine. A 1.2 mm diameter dental burr and a small variable speed drill (Dremel Moto-Tool Model 260, Dremel Mfg. Co., Racine, WI) was used to drill holes, using care to avoid puncturing the membranes below the shell. Immediately after drilling, each hole was covered with an approximately 1 cm square piece of transparent plastic tape (3M, St. Paul, MN). Average egg weight after drilling was ~57 g. Eggs were numbered and randomly assigned to four treatment groups for drug administration and motility recording as follows, n = 7/group: 1) saline (0.85%)-tartrate 0.05 M; 2) DOI 1.0 mg/kg egg-tartrate; 3) DOI 1.0 mg/kg egg-RIT 0.3 mg/kg egg; 4) DOI 1.0 mg/kg egg-RIT 0.9 mg/kg egg. No saline-RIT groups were used because prior work indicated a lack of effect of E14 RIT 0.1–2.7 mg/kg egg on detour learning (4).

Drugs

Drug solutions were prepared the day of the motility experiment and kept chilled on ice until administration. Saline (0.85%) and DOI (RBI Inc., Natick, MA) were administered in 20 μl injection volumes with Hamilton 50 μl syringes (Reno, NV) fitted with a small stop approximately 2.5 mm from the needle tip to ensure standard injection depth. RIT (RBI Inc., Natick, MA) solution or its vehicle, 0.05 M (+)-tartartic acid (Calbiochem, Los Angeles, CA) were injected in 40 μl volumes. These vehicle injections have not affected any variable studied in prior work. The DOI dose of 1.0 mg/kg egg was chosen on the basis of preliminary E15 work showing that it suppressed motility without evidence of lethality.

Motility Recordings

Motility was measured on a recording apparatus within an incubator. During recordings eggs were placed on a triangular configuration of phonograph cartridges to minimize transmission of ambient room vibration to eggs (11). To conduct electric potential produced by embryonic movement, two 28 gauge platinum wire electrodes were inserted approximately 2–3 mm into holes drilled earlier (9,17). Electrodes were held and positioned with micromanipulators (Model M3301, WPI, Inc., Sarasota, FL). Recordings were scheduled such that half were made before 1200 h on E15 and half after 1200 h on E15 to control for possible effects of age differences at recording time. A similar schedule was observed for E16 recordings.

Electrical signals detected by the electrodes were amplified 1000-fold with a custom-built preamplifier and sent through a custom-built low-pass filter (low end cutoff frequency = 0.1 Hz; high end cutoff frequency = 12 Hz), then to an analog-

to-digital converter (MacADIOS 8AIN, GW Instruments, Somerville, MA, gain = 1). The digital signal was processed and initially analyzed with a commercial wave analysis application (Superscope, GW Instruments, Somerville, MA) run on a Macintosh IIci computer (Apple Computer, Cupertino, CA). Voltages detected across the electrodes were processed by the hardware and software and saved as minimum (min), maximum (max), range, and standard deviation (SD; all measures in volts) data in spreadsheet format for subsequent statistical analysis.

Injections and recordings occurred in the following sequence: subjects were removed from the incubator, tape over injection holes was removed, and injections with DOI or saline were made. Eggs were then replaced in the incubator. One hour later, subjects were removed from the incubator and injected with RIT or tartrate and again replaced. Two and a half hours after DOI or saline (1.5 h after RIT or tartrate) subjects from the first set of 28 eggs were placed on the recording stand and electrodes were inserted. After a 5-min acclimation period, motility was recorded (20 15-s "waves" or recording periods, 80 Hz sampling rate, 5 min total recording time). Tape was replaced over the holes after every injection and recording step. After recordings were complete, these subjects were returned to the incubator until motility was recorded in the second set of 28 eggs, 24 h after DOI administration. After these recordings all subjects were returned to the rotating incubator until E18 when they were placed in an adjacent hatcher for hatchability assessment.

Motility Statistics

Motility data were analyzed with a commercial statistics application (Statview, Abacus Concepts, Berkeley, CA) by one-factor (treatment) ANOVA for minimum, maximum, range, and standard deviation (voltage). Planned comparisons (saline-tartrate vs. each of DOI 1.0-tartrate, DOI 1.0-RIT 0.3, DOI 1.0-RIT 0.9) were made by analysis of variance (ANOVA) followed by Dunnett's test.

Hatchability and Body Weight; Posthatch Banding and Housing

Eggs were placed in the hatcher on E18 and checked for hatchlings on E19–21. All eggs hatched over E20–21. Because 100% of treated subjects hatched, no statistical analysis of hatchability was done. After hatching, all chicks were weighed, numbered with small leg bands for identification, then placed in a heated, five-level community brooder with ad lib food and water. Body weight was analyzed with one-factor ANOVA followed by Dunnett's test.

Detour Learning

Because there were too many hatchlings to test on one day, and for logistical reasons, 48 of the 56 total hatchlings were randomly selected and separated into two equivalent sets (n = 24/set) for alternate day testing the day before detour learning sessions began . The night before posthatch day 6 (set 1) or 7 (set 2), chicks were deprived of food in preparation for detour learning assessment. Detour learning has been used as a test of maturation (24) and for the detection of postnatal consequences of prenatal exposure to drugs (e.g., reserpine (30), cocaine (28), and ethanol (18)) and toxicants such as methylmercury (8).

The detour learning apparatus used in this experiment is a fluorescently illuminated metal enclosure with a hinged lid,

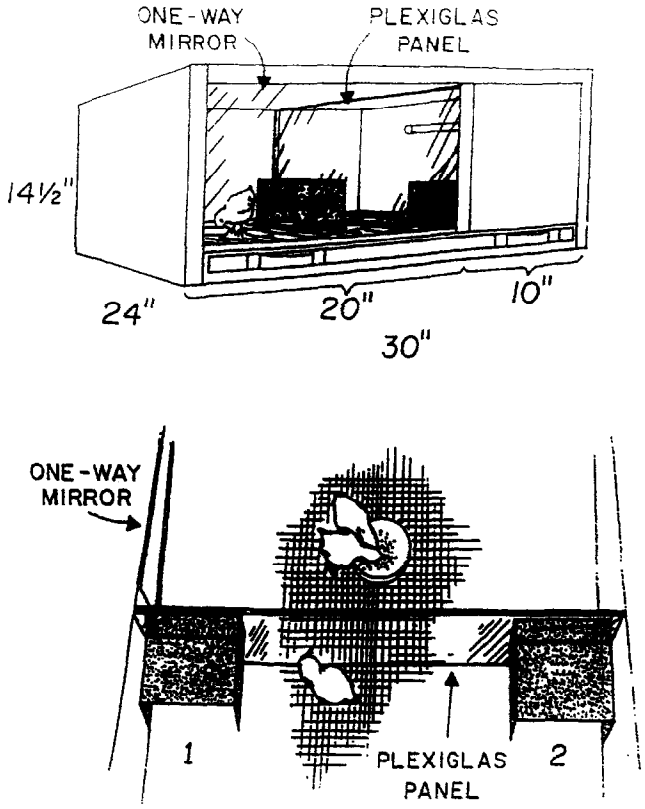

FIG. 1. Detour learning was assessed in a two-tunnel apparatus depicted in this figure. Chicks were deprived of food overnight, then allowed access to starter food and social reinforcement for 30 s, after which one was placed behind the Plexiglas partition. This chick was allowed 3 min (180 s) to face away from the reinforcing complex and detour through one tunnel, the other being blocked throughout the experiment. If the chick did not respond (i.e., emerge from the tunnel within 180 s), it was gently guided through the tunnel with a wooden probe, allowed access to communal feeding for 30 s, and its latency scored as 180 s. The next chick in the group was then placed on the isolation side of the partition, initiating its trial.

separated into two compartments (social and isolation sides) by a clear Plexiglas wall (Fig. 1). The two sides are connected by two tunnels in the wall, with the same one open throughout the experiment, the other being blocked by a Plexiglas barrier. The Plexiglas allows isolated subjects to observe those on the social side, while a one-way mirror on one enclosure wall allows the experimenter to observe subjects' behavior. To return to the social side, isolated subjects must turn away from the Plexiglas wall and detour through the open tunnel. Under the experimental conditions the opportunity for access to food and broodmates are appropriate stimuli for reinforcing the detour response, resulting in shorter response latencies as learning occurs.

On experimental days, one chick per group (four chicks total) from the food deprived set was randomly selected from the community brooder and placed on the social side of the detour apparatus, which contained a Petri plate with a small amount of moistened chick food. These subjects were allowed access to the food and social reinforcement for 30 s, after which one was selected and placed in the center of the isolation side facing the other three broodmates engaged in eating. The

TABLE 1

RITANSERIN (RIT) INJECTED 1 H AFTER DOI OR SALINE BLOCKS SUPPRESSION OF EMBRYONIC MOTILITY 2.5 H AFTER DOI OR SALINE INJECTIONS IN EGGS WITH E15 CHICKEN EMBYOS

	Motility Measure (Mean ± SD, volts)			
Treatment	Minimum	Maximum	Range	Std. dev.
Saline-tartrate	−1.499 ± 0.174	1.878 ± 0.226	3.376 ± 0.389	0.677 ± 0.102
DOI 1.0-tartrate	−0.953 ± 0.523*	1.037 ± 0.647*	1.99 ± 1.168*	0.396 ± 0.28
DOI 1.0-RIT 0.3	−1.333 ± 0.398	1.534 ± 0.666	2.867 ± 1.062	0.569 ± 0.25
DOI 1.0-RIT 0.9	−1.452 ± 0.416	1.738 ± 0.758	3.189 ± 1.168	0.661 ± 0.301

Values represent mean volts ± SD for groups of seven embryos each.
*$p < 0.025$ vs. saline-tartrate, one-tailed Dunnett's test.

subject was allowed 180 s to face away from the reinforcing complex and detour through the open tunnel. If no detour response was made during this time, latency was recorded as 180 s and the subject was gently guided through the tunnel with a wooden probe, terminating the trial, and beginning another 30 s period of access to food and broodmates. After 30 s the sequence was repeated with a subject from the next group, until each of the four chicks had received four trials. Subject testing sequence for each group of four chicks was rotated to avoid possible order effects. They were then returned to another brooder level furnished with water and ad lib food, and another group of four food-deprived chicks was selected. The procedure was repeated until all 24 chicks in the set had completed four trials. After four days of alternate day testing, all tested chicks ($n = 48$) received eight trials.

Detour Learning Statistics

Response latency (seconds) was measured with a stopwatch. Latency data were analyzed by repeated measures ANOVA over trials 1–8 and by one factor (treatment) ANOVA at each session. Planned comparisons were made with Dunnett's test.

RESULTS

Motility

Planned comparisons between treated and control groups were made for the 2.5 h results and are shown in Table 1. Minimum, maximum, and range of voltage were significantly suppressed 2.5 h after saline or DOI in the DOI 1.0-tartrate group compared to saline-tartrate controls, but the numeric reduction vs. controls for the mean standard deviation of voltage in the DOI 1.0-tartrate group was not statistically significant. With control values defined as 100%, the pattern shown by all four motility measures is that DOI-tartrate is suppressed 36–45% compared to saline-tartrate controls, DOI 1.0-RIT 0.3 values are 11–16% less than saline-tartrate controls, and DOI 1.0-RIT 0.9 values are 2–7% less than saline-tartrate controls. Representative analog motility recordings are shown in Fig. 2, which depicts 15 s of spontaneous embryonic motility during the same (15th of 20) 15-s recording period for four consecutive subjects, one from each group. DOI suppression of motility (smaller deflections from zero) and attenuation of DOI's action by RIT posttreatment (reversion towards control responding) are visually apparent.

Motility data recorded 24 h after DOI administration were also analyzed by one-factor (treatment) ANOVA for minimum, maximum, range, and standard deviation (volts). Comparisons between treated and control groups were made and results are shown in Table 2. The motility of DOI 1.0 mg/kg egg-treated subjects was not suppressed compared to controls at this time, nor was it affected by either RIT dose.

Hatchability and Body Weight

All chicks hatched in all groups, precluding the need for statistical analysis. One chick in the DOI 1.0-tartrate group had a herniated umbilicus. Body weight (Table 3) showed no effect of treatment, $F(3, 52) = 1.10$, $p = 0.36$.

Detour Learning

Repeated measures ANOVA for all groups over detour learning trials 1–8 showed no treatment effect, $F(3, 44) = 1.88$, $p = 0.15$. However, significant effects of repeated measure, $F(7, 308) = 32.29$, $p < 0.0001$, and a treatment by repeated measures interaction, $F(21, 308) = 1.99$, $p = 0.007$, were observed. One-factor ANOVA at each of trials 1–7 showed no overall treatment effect, but on trial 8 this effect emerged, $F(3, 44) = 3.86$, $p = 0.02$. Trial 8 latency for the DOI 1.0-tartrate group (40.8 s) was significantly lower than that for the saline-tartrate group (119.3 s), while that for both RIT-treated groups (DOI 1.0-RIT 0.3, 86.3 s; DOI 1.0-RIT 0.9, 128.0 s) did not differ from saline-tartrate controls. The detour learning latency data are depicted in Fig. 3.

DISCUSSION

We studied acute and longer term behavioral effects of a 5-HT_2 agonist, DOI, and a 5-HT_2 antagonist, RIT, on the developing chicken. The key results of the study were 1) DOI 1.0 mg/kg egg suppressed embryonic motility 2.5 h after injection 2–3 mm beneath the shell, indicating that it is rapidly distributed from the injection site, interacts with functional 5-HT_2 receptors, and produces a biological effect; 2) this DOI dose did not appear overtly toxic, because hatchability, body weight, and subjective appearance of DOI-treated hatchlings differed little from control subjects (though one herniated umbilicus was observed in the DOI-tartrate group); 3) RIT (0.3 and 0.9 mg/kg egg) administered 1 h after DOI blocked DOI's motility suppressive effect in a dose-related manner; 4) DOI altered detour learning at posthatch days 6–9; and 5) both RIT doses blocked DOI's effects on detour learning. Thus, suppression of embryonic motility by DOI and its reversal by RIT on E15 demonstrate the presence of and a role for 5-HT_2 receptors in this effect. DOI exposure resulted in posthatch detour learning changes 1–2 weeks after its adminis-

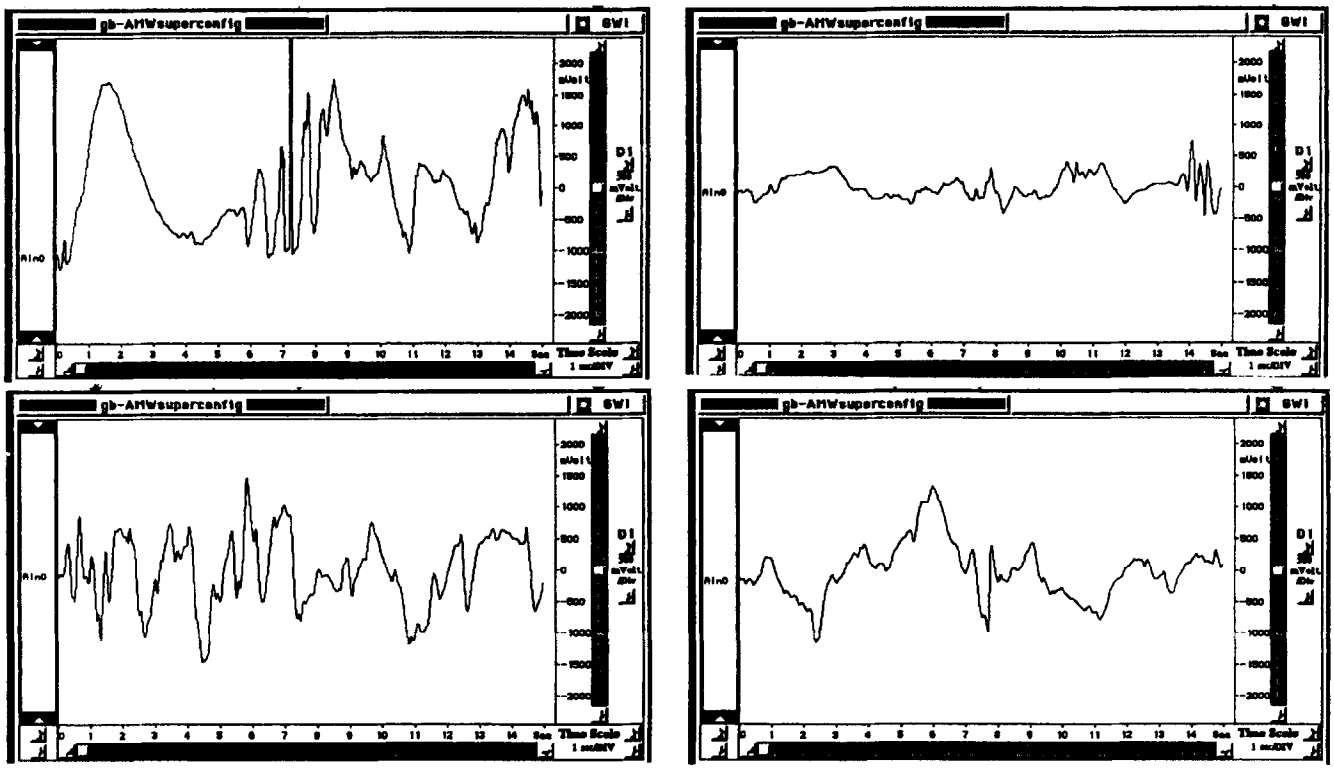

FIG. 2. Representative analog records showing 15 s of spontaneous embryonic motility in each of the four experimental groups approximately 2.5 h after saline or DOI, with time (s) on the x-axis and voltage (mVolt) on the y-axis. Upper left, saline-tartarte, egg 24; upper right, DOI 1.0-tartrate, egg 21; lower left, DOI 1.0-RIT 0.3, egg 22; lower right, DOI 1.0-RIT 0.9, egg 23; all recordings made 8/11/94. The records show the 15th of 20 traces for four consecutively recorded embryos. Suppressed motility in the DOI 1.0-tartrate group relative to saline-tartrate controls was attenuated in both RIT-treated groups (DOI 1.0-RIT 0.3, DOI 1.0-RIT 0.9).

tration, a change also blocked by RIT. Taken together, the results show that excessive 5-HT_2 receptor stimulation during development in this species has both acute and longer lasting functional consequences that can be blocked by a 5-HT_2 antagonist. In addition, toxicant exposure during development less than that required for increased lethality can be demonstrated to result in relatively long-lasting effects.

DOI-mediated motility suppression and altered (enhanced) detour learning in the developing chicken resemble cocaine's effects in this species. We observed cocaine-mediated enhancement of the detour response in chicks tested on posthatch days 6–9 following E19 cocaine (45 and 90 mg/kg egg) administration (28), similar to the effect of DOI in the present results. We have also found that RIT can block the suppression of embryonic motility and hatchability induced by certain cocaine doses (10). Consistent with findings that RIT decreased drinking of a cocaine solution in rats that had developed a preference for it when given the choice between it and water (19), and that DOI-induced head shaking increases after chronic cocaine administration (3), the present results reinforce the therapeutic potential of 5-HT_2 antagonists in treatment of cocaine dependence and/or withdrawal from it or other drugs.

That the detour learning response in the DOI treated group is "enhanced" (latency is reduced) relative to controls may be an inappropriate anthropomorphic characterization. We

TABLE 2

DOI (1.0 mg/kg EGG) INJECTED INTO EGGS WITH E15 CHICKEN EMBRYOS DOES NOT SUPPRESS EMBRYONIC MOTILITY 24 H AFTER ADMINISTRATION

	Motility Measure (Mean ± SD, Volts)			
Treatment	Minimum	Maximum	Range	Std. dev.
Saline-tartrate	−1.292 ± 0.272	1.430 ± 0.545	2.722 ± 0.805	0.525 ± 0.198
DOI 1.0-tartrate	−1.468 ± 0.261	1.825 ± 0.437	3.294 ± 0.676	0.653 ± 0.152
DOI 1.0-RIT 0.3	−1.480 ± 0.341	1.766 ± 0.570	3.246 ± 0.904	0.640 ± 0.207
DOI 1.0-RIT 0.9	−1.323 ± 0.401	1.658 ± 0.597	2.981 ± 0.997	0.573 ± 0.236

Values represent mean volts ± SD for groups of seven embryos each.

TABLE 3

CHICK BODY WEIGHTS AFTER INJECTION OF DOI OR DOI PLUS RIT ON E15

Treatment	Body weight (g)
Saline-tartrate	44.4 ± 3.0
DOI 1.0-tartrate	44.4 ± 2.7
DOI 1.0-RIT 0.3	44.9 ± 3.5
DOI 1.0-RIT 0.9	46.3 ± 3.2

Values represent the mean hatch weight (g) ± SD of 14 hatchlings per group.

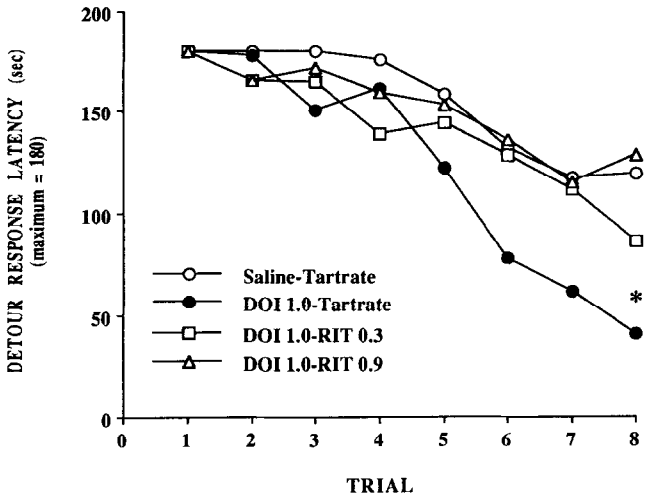

FIG. 3. Detour response latency (s) of 6–9-day-old chicks (n = 12/group) hatched from eggs injected on embryonic day 15 (E15) with saline vehicle or DOI 1.0 mg/kg egg followed by tartrate vehicle or RIT 0.3 or 0.9 mg/kg egg. DOI exposure significantly decreased response latency on trial 8 and both RIT doses blocked DOI's effect. Overall $F(3, 44) = 3.51$, $^*p < 0.025$ vs. saline-tartrate, one-tailed Dunnett's test.

have previously (29) and more recently (10) argued that behavior in control subjects reflects optimal responding from an evolutionary viewpoint and that significant differences from this behavior, increased or decreased, should be assumed to be adverse until shown to be otherwise. Chicks that have reduced latency to acquire the detour response may habituate too rapidly in novel environments. Such behavior may reflect altered sensory function, modified integrative processing, increased locomotor activity, some combination of these, and/or other effects. In nonlaboratory settings such changes could increase vulnerability to predators and threaten survival. The same issue can be addressed from an alternate perspective. For example, among rats lesioned with 3.0, 6.0, or 7.5 mg trimethyltin (TMT)/kg body weight, those given 7.5 mg TMT appeared to acquire a delayed reinforcement autoshaping response more readily than those given lower doses (6). However, a latent inhibition variation of the same test demonstrated that the "enhancement" was confounded by hyperreactivity to the environment and a failure to suppress behavior toward irrelevant stimuli. Later work (20) showed that rats given 7.5 mg TMT had significantly lighter hippocampi than those given 3.0 or 6.0 mg TMT/kg, gross anatomic confirmation of a larger lesion. Thus, the observation of "enhanced" performance after drug or toxin exposure requires cautious interpretation.

That altered developmental stimulation of 5-HT_2 receptors can lead to persistent as well as acute biological effects demonstrates a role of serotonin and its receptors in what has been referred to as "developmental pharmacology" (31): pharmacological manipulation of receptors in an immature animal can result in permanent changes in maturity (in the present case, substantially before adulthood). This may be due in part to developmental dynamics, for example, receptors may reach a prenatal peak number that later decreases (2) or may be expressed in areas in which they are later absent, and/or because ligands that later serve as neurotransmitters also function as developmental signals or morphogens (14). There are novel implications associated with this view; i.e., development is dependent on and can be directed by both the responding cell or tissue (by the extent of receptor expression) as well as by the signal inducing the change; and receptor function is not a fixed process, because it depends upon developmental stage (31).

The present results demonstrate that short-term motility suppression is not sufficient to induce detour learning deficits: motility was almost certainly suppressed in the DOI-RIT group after DOI and prior to injection of RIT 1 h later, yet this group learned the detour response like saline-tartrate controls. However, if part of the "mechanism" whereby DOI alters detour learning is related to motility suppression, it may be possible to define its temporal parameters with the present data. Motility was almost certainly suppressed equally in DOI-RIT and DOI-tartrate subjects for the hour after DOI, before RIT injections, assuming rapid uptake and distribution of both agents. Because motility of treated and control subjects did not differ at 24 h, this biologic insult must have occurred more than 1 h but less than 24 h after DOI administration. On the other hand, though both motility suppression and detour learning deficits result from exposure to the 5-HT_2 agonist DOI, they may be unrelated. The biologic effect(s) responsible for altered detour learning may have been induced much later than E15, long after motility had reverted to normal.

In summary, E15 injections of the 5-HT_2 agonist DOI suppressed short-term embryonic motility, an effect blocked by administration of the 5-HT_2 antagonist RIT. DOI also altered learning of a simple behavioral response 1–2 weeks after hatching, which was also prevented by RIT. The similarity of the present DOI effects with those of cocaine observed in prior work, as well as protection from such effects with the 5-HT_2 antagonist RIT, support the idea of 5-HT_2 receptor involvement in the potential developmental toxicity of cocaine. A possible therapeutic role for RIT is also suggested by these data.

ACKNOWLEDGEMENTS

This work was supported in part by USPHS Grants T32 DA 07097, DA 08131, and DA 04979.

REFERENCES

1. Ahmad, G.; Zamenhof, S. Serotonin as a growth factor for chick embryo brain. Life Sci. 22:963–970; 1978.
2. Bar-Peled, O.; Gross-Isseroff, R.; Ben-Hur, H.; Hoskins, I.; Groner, Y.; Biegon, A. Fetal human brain exhibits a prenatal peak in the density of serotonin 5-HT_{1A} receptors. Neurosci. Lett. 127:173–176; 1991.
3. Baumann, M. H.; Brockington, A. M.; Rothman, R. B. Withdrawal from chronic cocaine enhances behavioral sensitivity to the 5-$HT_{2/1C}$ agonist DOI. Biol. Psychiatry 34:576–577; 1993.
4. Bollweg, G.; Sparber, S. B. Ritanserin (RIT) injected into eggs with chicken embryos on E14 does not affect detour learning 1–2 weeks after hatching. NIDA Res. Monogr. 153:170; 1994.
5. Bronson, M. E.; Sparber, S. B. Evidence of single dose opioid dependence in 12- to 14-day old chicken embryos. Pharmacol. Biochem. Behav. 34:705–709; 1989.
6. Cohen, C. A.; Messing, R. B.; Sparber, S. B. Selective learning impairment of delayed reinforcement autoshaping behavior caused by low doses of trimethyltin. Psychopharmacology (Berlin) 93:301–307; 1987.
7. Glennon, R. A.; Seggel, M. R.; Soine, W. H.; Herric-Davis, K., Lyon, R. A.; Titeler, M. [^{125}I]-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane. An iodinated radioligand that specifically labels the agonist high-affinity state of 5-HT_2 serotonin receptors. J. Med. Chem. 31:5–7; 1988.
8. Hughes, J. A.; Rosenthal, E.; Sparber, S. B. Time dependent effects produqced in chicks after prenatal injection of methylmercury. Pharmacol. Biochem. Behav. 4:507–513; 1976.
9. Jackson, H.; Rubel, E. W. Ontogeny of behavioral responsiveness to sound in the chick embryo as indicated by electrical recordings of motility. J. Comp. Physiol. Psychiatry 92:682–696; 1978.
10. Kim, D. G.; Sparber, S. B. Ritanserin blocks cocaine's motillity depressant and lethal effects upon chicken embryos. (in preparation).
11. Kovach, J. K.; Callies, D.; Hartzell, R. Procedures for the study of behavior in avian embryos. Dev. Psychobiol. 3(3):169–178; 1970.
12. Kuwahara, M. D.; Sparber, S. B. Prenatal withdrawal from opiates interferes with hatching of otherwise viable chick fetuses. Science 212:945–947; 1981.
13. Lal, H.; Prather, P. L.; Rezazadeh, S. M. Potential role of 5-HT_2/5-HT_{1C} receptors in the mianserin-induced prevention of anxiogeneic behaviors occurring during ethanol withdrawal. Alcohol Clin. Exp. Res. 17(2):411–417; 1993.
14. Lauder, J. M. Neurotransmitters as morphogens. Prog. Brain Res. 73:365–387; 1988
15. Lydiard, R. B; Sparber, S. B. Evidence for a critical period for postnatal elevation of brain tyrosine hydroxylase activity resulting from reserpine administration during embryonic development. J. Pharmacol. Exp. Ther. 189(2):70–79; 1974.
16. Mayes, L. C.; Granger R. H.; Bornstein, M. H.; Zuckerman, B. The problem of prenatal cocaine exposure: A rush to judgment. JAMA 267(3):406–408; 1992.
17. McCafferty, R. E.; Pressman, S. H.; Knisely, W. H. Recording techniques and results for amnionic and embryonic movement in situ during hen egg incubation. Biorheology 2:171–181; 1965.
18. Means, L. W.; McDaniel, K.; Pennington, S. N. Embryonic ethanol exposure impairs detour learning in chicks. Alcohol 6:327–330; 1989.
19. Meert, T. F.; Janssen, P. A. J. Ritanserin, a new therapeutic approach for drug abuse. Part 2: Effects on cocaine. Drug Dev. Res. 25:39–53; 1992.
20. Messing, R. B.; Bollweg, G; Chen, Q.; Sparber, S. B. Dose specific effects of trimethyltin poisoning on learning and hippocampal corticosterone binding. Neurotoxicology 9(3):491–502; 1988.
21. Neal, B. S.; Sparber, S. B. Mianserin attenuates naloxone-precipitated withdrawal signs in rats acutely or chronically dependent upon morphine. J. Pharmacol. Exp. Ther. 236:157–164; 1986.
22. Neal, B. S.; Sparber, S. B. Ketanserin and pirenpirone attenuate acute morphine withdrawal in rats. Eur. J. Pharmacol. 132:299–304; 1986.
23. Ritz, M. C.; Lamb, R. J.; Goldberg, S. R.; Kuhar, M. J. Cocaine receptors on dopamine transporters are related to self-administration of cocaine. Science 237:1219–1223; 1987.
24. Scholes, N. W. Detour learning and development in the domestic chick. J. Comp. Physiol. Psychol. 60:114–116; 1965.
25. Sparber, S. B.; Rizzo, A.; Berra, B. Excessive stimulation of serotonin$_2$ ($5HT_2$) receptors during late development of chicken embryos causes decreased embryonic motility, interferes with hatching and induces herniated umbilici. Pharmacol. Biochem. Behav. 53:603–611; 1996.
26. Sparber, S. B.; Wasserman, A.; Bollweg, G. Ritanserin (RIT) blocks the vasoconstriction caused by injection of cocaine (COC) into chicken eggs with 15 day-old embryos. NIDA Res. Monogr. 153:169; 1994.
27. Sparber, S. B.; Kim, D. G.; Kostarczyk, E. Cocaine's behavioral and lethal effects upon the developing chicken fetus are blocked by treatment with the 5-HT_{1C}/5-HT_2 antagonist ritanserin. Soc. Neurosci. Abstr. 229.7; 1992.
28. Sparber, G. R.; Lauerman, M.; Kim, D. G.; Sparber, S. B. Injection of cocaine into eggs on day 19 of development suppresses chicken embryonic motility and alters detour learning and distress vocalization 1–2 weeks postnatally. FASEB J. 5:A1588; 1991.
29. Sparber, S. B. Postnatal behavioral effects of in utero exposure to drugs which modify catecholamines and/or serotonin. In: Vernadakis, A.; Weiner, N., eds. Drugs and the developing brain. New York: Plenum Press; 1974:81–102.
30. Sparber, S. B.; Shideman, F. E. Detour learning in the chick: Effect of reserpine administered during embryonic development. Dev. Psychobiol. 2(2):56–59; 1969.
31. Whitaker-Azmitia, P. M. IV. Role of serotonin and other neurotransmitter receptors in brain development: Basis for developmental pharmacology. Pharmacol. Rev. 43(4):553–561; 1991.
32. Zamenhof, S. Selective sensitive period in chick embryo: Toxic effect of exogenous neurotransmitters. Cytobios 58:171–177; 1989.