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Embryonic cocaine exposure and corticosterone: Serotonin$_2$ receptor mediation

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Abstract

Cocaine activates the mature hypothalamic–pituitary–adrenal (HPA) axis, increasing corticosterone concentrations in animals and humans and serotonin$_2$ receptors (5-HT$_2$) are involved in this effect. Although prenatal cocaine exposure is associated with altered responsiveness of the HPA axis to "stress" and serotonergic compounds postnatally, it is unknown whether cocaine directly activates the embryonic HPA axis or if 5-HT$_2$ receptors are involved. Domestic chicken eggs with viable embryos were exposed to either the 5-HT$_2$ receptor agonist dimethoxyiodophenylaminopropane (DOI: 0.4, 0.8, or 1.2 mg/kg egg) or saline on embryonic day 18 (E18). In a second study, the 5-HT$_2$ antagonist ritanserin (0.3 mg/kg egg, a dose found effective against other effects of DOI or cocaine) or vehicle was administered on E17, prior to treatment on E18 with either saline or cocaine (5 injections of 12 mg/kg egg, equivalent to a total dose of 3.5 mg/egg). Radioimmunoassay was used to measure serum corticosterone from blood samples taken approximately 1–2 h after drug injections. DOI significantly raised corticosterone in a dose-related fashion. Cocaine-induced corticosterone elevations were blocked by pretreatment with ritanserin, whereas ritanserin by itself did not affect corticosterone concentrations. These data indicate that 5-HT$_2$ receptors are involved in cocaine's effect on the HPA axis during late chicken embryogenesis.

Keywords: Development; HPA axis; DOI; Prenatal stress; Chicken; Ritanserin

1. Introduction

Cocaine is known to affect the hypothalamic–pituitary–adrenal (HPA) axis, leading to increased corticosterone in rats (Mello and Mendelson, 1997) and cortisol in sheep (Owiny et al., 1991), rhesus monkeys, and humans (Mello and Mendelson, 1997). This suggests that cocaine can, like many other drugs of abuse, act as a generalized stressor in postnatal subjects. Serotonin (5-HT) is involved in regulating HPA axis activity in general (Fuller, 1992), and is implicated in altered HPA axis activity following cocaine use. 5-HT agonists, for example, increased corticosterone, while drugs that reduced 5-HT activity (e.g., PCPA, a 5-HT depleter, or 5,7-DHT, a 5-HT neurotoxin) attenuated or blocked cocaine-induced corticosterone increases (Levy et al., 1991). Additionally, chronic cocaine exposure has been shown to alter corticosterone responses to serotonergic agents. For example, paradoxically, chronic cocaine potentiated the corticosterone response to the selective 5-HT$_2$ agonist dimethoxyiodophenylaminopropane (DOI) (Levy et al., 1992), and reduced the corticosterone response to the nonselective 5-HT releaser *p*-chloroamphetamine (Van de Kar et al., 1992).

Of the 3.8 million Americans that used cocaine in 1998, 1.4 million were women of childbearing age (Substance Abuse and Mental Health Services Administration, 1999). Thus, there is a compelling rationale to examine the impact of cocaine exposure on the developing HPA axis, both with respect to acute in utero effects, as well as long-term consequences. Prenatal cocaine exposure has been reported to have long-term effects on postnatal HPA axis reactivity (Choi et al., 1998; Magnano et al., 1992; Molina et al., 1994), as well as HPA axis responsiveness to serotonergic compounds postnatally in mammals (Battaglia et al., 1995, 1998, 2000; Cabrera et al., 1993, 1994). However, it is not known if the effects of prenatal cocaine are a consequence of altered maternal HPA axis reactivity, or that of the fetus,

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and whether either (or both) of these effects are mediated in utero by activating specific 5-HT receptors in the embryo or fetus. Using the chick model, we can examine the more direct effects of cocaine on the fetal HPA axis, as well as the involvement of 5-HT receptors. In ovo drug administration eliminates indirect effects on the fetus caused by the mother's physiological responses to cocaine, such as HPA axis activation or vasoconstriction. Furthermore, similar findings across species (e.g., chickens, rats, nonhuman primates, etc.) strengthens the potential for findings of clinical relevance to human populations.

The chick embryo HPA axis is functional during the second half of embryogenesis. From E14 forward, the chick embryo is capable of mounting a corticosterone response to both ACTH and a physical stress (Wise and Frye, 1973), and respond to pituitary transplants with an increase in corticosterone (Woods et al., 1971). In a preliminary study, we found that cocaine treatment during late development, on embryonic day 18 (E18), increased concentrations of serum corticosterone and reduced binding sites of ^{3}H-corticosterone in the hippocampal region approximately 1–2 h after the fifth and final injection of a "binge" model of cocaine exposure (Bordone et al., 1998). These results indicated that the older chicken embryo is capable of mounting a corticosterone response to cocaine. However, the involvement of 5-HT_2 receptors in the corticosterone response to cocaine in this phenomenon in the chick or its milieu has yet to be explored. We have focused on the 5-HT_2 receptor because these receptors are involved in mediating some potentially deleterious effects of embryonic cocaine exposure in the developing chick, such as vasoconstriction, umbilici herniation (Zhang et al., 1998), altered exploratory behavior in a novel environment (Schrott et al., 1998), and suppressed immune responsiveness (Schrott and Sparber, 1999). Additionally, there is strong evidence for 5-HT_2 receptor involvement in cocaine's effects on the HPA axis in the adult rat (Levy et al., 1991; Borowsky and Kuhn, 1991). As such, we determined if the selective 5-HT_2 receptor agonist DOI could mimic the neuroendocrine effects of cocaine and increase serum corticosterone in the embryo on E18 in Experiment 1. In Experiment 2, we determined if E17 pretreatment with the 5-HT_2 receptor antagonist ritanserin could block or attenuate the effect of E18 cocaine on serum corticosterone.

2. Method

2.1. Subjects

Fertilized chicken eggs from a Rhode Island Red × White Leghorn cross were acquired from the St. Paul Poultry Nutrition Research Center at the University of Minnesota. Since the eggs for Experiments 1 and 2 were derived from flocks of different ages and breeding histories, it was deemed important to include contemporaneous controls in each study. Eggs were set (E0) in a rotating forced air incubator/hatcher (Humidaire, New Madison, OH) with the temperature at 37–38°C and the relative humidity at 58–60%, as suggested by the manufacturer. The eggs were candled on E12–E14, and nonfertilized eggs and eggs containing embryos that failed to develop, as evidenced by poor extraembryonic vascularization, were removed prior to randomized treatment assignment. Injection sites were pencil-marked about 2.0 cm below the air cell at an area free of, but adjacent to, observable membrane-bound blood vessels. The external injection site was sterilized with a drop of 2% iodine (dissolved in 70% ethanol) followed by a swipe with a gauze pad moistened with 70% ethanol to remove the iodine. A small drill with a 1.2 mm diameter dental burr was used to make a hole at the injection site, taking care not to puncture the underlying membrane. A small piece of plastic Scotch tape (3M, St. Paul, MN) was used to cover the hole until the time of injection and was replaced following injections.

2.2. Treatment: Experiment 1

Eggs containing viable embryos were randomly assigned to receive either (±) DOI (RBI, Natick, MA) or saline. DOI (0.4, 0.8, or 1.2 mg DOI/kg egg weight) was dissolved in avian saline (0.85% NaCl) and administered as a single bolus injection at 1500 h on E18. All drug solutions were prepared fresh and filtered with a 0.2 μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI) just prior to injection. Injection volume was 21 μl for all injections, which were made 2–3 mm beneath the eggshell via the predrilled holes.

2.3. Treatment: Experiment 2

Eggs containing viable embryos were randomly assigned to receive the following two treatments: (1) pretreatment at 1500 h on E17 with ritanserin (RBI, 0.3 mg/kg egg weight) or its vehicle, 0.1 M tartaric acid (TA); (2) E18 treatment with avian saline or cocaine HCl (five injections of 12 mg/kg egg, equivalent to a total dose of 3.5 mg/egg). Thus, there were four treatment groups: TA + saline controls; TA + cocaine; ritanserin + saline; and ritanserin + cocaine. Cocaine was provided by the National Institute on Drug Abuse and was dissolved in distilled water. All treatments on E18 were made as a series of five injections at 90 min intervals beginning at 0900 h, a "binge" model we have used previously (Schrott et al., 1998; Zhang et al., 1998). Drug and vehicle solutions were prepared and injected as in Experiment 1. Using ^{3}H-cocaine, we have previously demonstrated that cocaine is present in the embryo heart and brain within 20 min of injection beneath the eggshell and remains there for at least 2.5 h (Sparber et al., 1993).

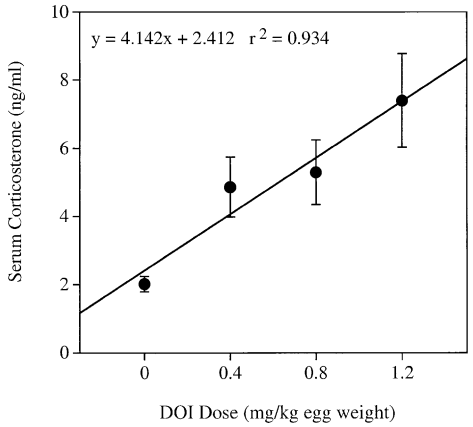

Fig. 1. Treatment with DOI on E18 increased serum corticosterone concentrations in a dose-related manner. Data points represent the mean ± S.E.M. of each treatment group. There were six subjects per treatment group.

2.4. Determination of serum corticosterone concentrations

Blood samples were obtained from the chick embryos via cardiac puncture approximately 1 h after the single injection in Experiment 1 and 1–2 h after the final injection in Experiment 2. No embryos died between the time of drug administration and blood sampling. Blood samples were obtained within 2 min of embryo externalization. Sera were isolated from the blood and stored at −70°C until analyzed for corticosterone via radioimmunoassay. The sera were diluted 1:50 and heated to denature corticosterone-binding globulin. The samples were then incubated with an antibody directed against corticosterone (ICN Biomedical, Costa Mesa, CA) and ^{3}H-corticosterone (New England Nuclear, Boston, MA). Charcoal was added and samples were centrifuged to separate bound ^{3}H-corticosterone. Bound ^{3}H-corticosterone was counted in a liquid scintillation counter to an error of 3%. All samples and standards were run in duplicate. Standards were used to generate a curve from which sample values were interpolated. These values were subsequently converted to nanograms per milliliter (ng/ml) for statistical analyses. Assay sensitivity was between 0.5 and 1 ng/ml, assay range from 1 to 1000 ng/ml, and the intra- and interassay percentage coefficients of variation were approximately 6% and 12%, respectively.

2.5. Statistical analyses

One-factor analysis of variance (ANOVA) followed by planned comparisons with Fisher's PLSD were used to examine embryonic treatment effects on corticosterone concentrations. Dose–response relationships in Experiment 1 were determined via regression analysis of treatment group means.

3. Results

3.1. Experiment 1: Effect of E18 DOI on E18 serum corticosterone

There was an overall treatment effect for serum corticosterone [$F(3,20) = 5.43$, $P < .007$). All doses of DOI (0.4, 0.8, and 1.2 mg DOI/kg egg weight) significantly increased corticosterone approximately 2.4 to 3.7-fold compared to controls ($P < .05$; Fisher's PLSD). Regression analysis revealed a significant dose–response relationship. As illustrated in Fig. 1, serum corticosterone concentrations increased linearly ($P < .04$) as the dose of DOI increased.

3.2. Experiment 2: Effect of E17 ritanserin–E18 cocaine on E18 serum corticosterone

A significant overall treatment effect was found for serum corticosterone concentrations [$F(3,24) = 6.38$, $P < .003$; Fig. 2]. Subsequent analyses revealed that E18 cocaine injections elevated corticosterone concentrations approximately 1.5-fold above TA + saline controls ($P < .05$). Ritanserin treatment on E17, followed by saline on E18, did not affect corticosterone concentrations. However, treatment with ritanserin on E17 blocked the cocaine-induced corticosterone increase on E18. Corticosterone concentrations of ritanserin + cocaine-treated subjects were significantly lower than TA + cocaine-treated subjects ($P < .05$), and were not different from TA + saline controls.

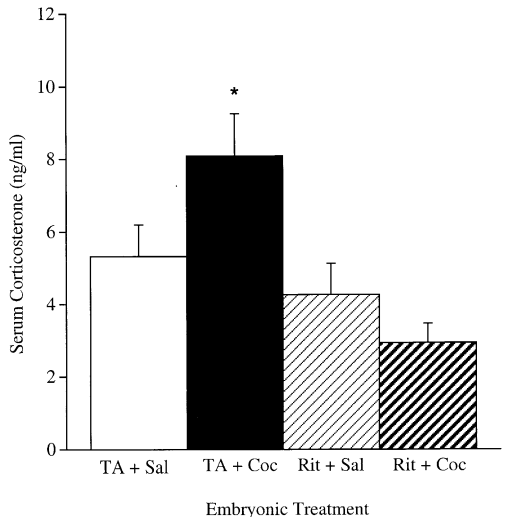

Fig. 2. E18 injected cocaine (Coc) increased serum corticosterone concentrations (ng/ml) above TA + saline controls (TA + Sal). E17 ritanserin (Rit) treatment blocked this effect, while having no effect on corticosterone when injected prior to E18 saline. Data are expressed as mean ± S.E.M. * $P < .05$ vs. all other groups (Fisher's PLSD). There were six to eight subjects per treatment group.

4. Discussion

The results presented herein confirm our prior findings (Bordone et al., 1997), and those of others (Wise and Frye, 1973; Woods et al., 1971), that the HPA axis is functional in the chicken embryo on E18 and that it responds to cocaine (Bordone et al., 1998), which is consistent with results in older fetal sheep (Owiny et al., 1991). We have also extended our observations with the E18 chick embryo by showing that excessive, direct stimulation of 5-HT_2 receptors by DOI can lead to dose-dependent increases in serum corticosterone. Whereas 5-HT neurons are present in the chick embryo hypothalamus at this age (Wallace, 1985), additional experiments are necessary to determine if the increased corticosterone results from direct 5-HT_2 receptor stimulation of neuroendocrine tissues (e.g., hypothalamus, pituitary, adrenals) or if it is an indirect consequence of 5-HT_2-mediated mechanisms at other sites (e.g., stressful cardiovascular effects, excessive contraction of extraembryonic membranous smooth muscle analogous to the placenta in mammals, etc.). Additionally, the results yield the first evidence of 5-HT_2 receptor involvement in cocaine's ability to activate the embryonic HPA axis, directly or indirectly, as pretreatment with ritanserin blocked the cocaine-induced elevated corticosterone. This supports the idea that, as in mature animals, 5-HT_2 receptor blockade can prevent cocaine-induced corticosterone increases (Borowsky and Kuhn, 1991; Levy et al., 1991). It is interesting to note that basal corticosterone concentrations differed in the two experiments, most likely as a consequence of the change in the egg-laying flock. However, the magnitude of the cocaine effect in Experiment 2 (1.5-fold increase above control) is consistent with that found in our preliminary study (Bordone et al., 1998). The magnitude of the DOI effect on corticosterone in Experiment 1 (2.4 to 3.7-fold above control) suggests that, for the doses examined, DOI caused a more robust effect on corticosterone than cocaine. This result is similar to our previous reports of 5-HT_2 receptor-mediated vasoconstriction and herniated umbilici induction, where the magnitude of the DOI effect after similar dose(s) was greater than that of cocaine (Sparber et al., 1996; Zhang et al., 1998).

Cocaine's ability to block reuptake of multiple neurotransmitters (e.g., dopamine, norepinephrine, and 5-HT), makes it difficult to develop selective treatment strategies that lack significant side effects for both cocaine addiction and cocaine-induced pathology. It is likely that brain areas and transmitter receptors involved in cocaine's reinforcing properties, are not identical to those involved in cocaine-induced pathology (Walsh and Cunningham, 1997). In fact, some aspects of cocaine-induced pathology may be peripheral in nature (e.g., cardiac and hemodynamic changes). Although the 5-HT_2 antagonists have not shown great promise in blocking psychological factors associated with cocaine (Ehrman et al., 1996; Johnson et al., 1997; Peltier et al., 1994), they have been more successful in blocking the physiological consequences of developmental drug exposure. For example, we have shown ritanserin to be efficacious in attenuating or blocking the behavioral, neural-immune, and vascular consequences of embryonic cocaine exposure in the embryonic and young chick (Schrott et al., 1998; Schrott and Sparber, 1999; Zhang et al., 1998). The present study adds another effect of embryonic cocaine developmental toxicity that ritanserin pretreatment can ameliorate. Thus, 5-HT_2 receptor antagonism may be useful for preventing adverse consequences of prenatal cocaine exposure, especially in light of it own relative safety. In the present study, a dose of ritanserin (0.3 mg/kg) that was efficacious in blocking cocaine's effects had no effect on serum corticosterone concentrations on its own. These data support other recent studies showing that ritanserin doses efficacious in blocking excessive 5-HT_2 activity during late chick embryogenesis do not alter a number of behavioral, neuroendocrine, neuroimmune, and cardiovascular measures (Bollweg and Sparber, 1996, 1998; Bollweg et al., 1998; Schrott et al., 1999; Zhang et al., 1998).

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