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Behavioral Effects of Mianserin on the Developmental Toxicity of Cocaine

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ABSTRACT : *To investigate the involvement of 5-HT_{2A}/5-HT_{2C} receptors in the developmental toxicity of cocaine in rats, mianserin (2.5 mg/kg), a 5-HT_{2A}/5-HT_{2C} receptor antagonist, and/or cocaine HCl (45 mg/kg) were administered intraperitoneally (i.p.), during postnatal days (PND) 7-13. Behavioral assessments for the rat pups were done after 100 days of age by using the progressive ratio schedule of reinforcement (FR 1-FR 128, doubled everyday) and cocaine challenge (5, 15 or 30 mg/kg i.p.) upon established FR 32 behavior. Cocaine injected just prior to the FR 32 session suppressed the established FR 32 responding in a dose-dependent manner. The low dose of cocaine did not affect the FR 32 responding, while the high dose of cocaine suppressed it in all experimental groups. However, by the middle dose of cocaine, rats previously received water-cocaine in their early life showed a marked resistance to cocaine-induced behavioral suppression, and this resistance was not observed in rats received both mianserin and cocaine in their early life. These results suggest that 5-HT_{2A}/5-HT_{2C} receptors may have an important role for the persistently altered behavioral sensitivity to cocaine caused by exposure to cocaine during development.*

Key Words : *Cocaine, Mianserin, 5-HT_{2A}/5-HT_{2C} receptor, Neurobehavioral toxicity, Development*

I. INTRODUCTION

Cocaine has been known to possess harmful effects on the outcome of pregnancy. Cocaine abuse during pregnancy increases the risk of premature delivery, abruptio placentae, meconium staining, intrauterine growth retardation and cerebral infarction (Acker *et al.*, 1983; Chasnoff *et al.*, 1985, 1989; Hadeed and Siegel, 1989; Little *et al.*, 1990; Pitts and Weinstein, 1990). Also included in these effects are structural anomalies which are possibly caused by severe vasoconstriction (Doering *et al.*, 1989; Hoyme *et al.*, 1990), and behavioral state changes during the neonatal period (Chasnoff *et al.*, 1989; Hume *et al.*, 1989).

It is now well established that these pharmacological actions of cocaine result from its indirect agonistic action at monoaminergic neuronal synapses, whereby cocaine inhibits the neuronal reuptake of the neurotransmitters via their own transporters (Azzaro and Rutledge, 1973; Koe, 1976; Ritz *et al.*, 1987; Terry *et al.*, 1994). A plethora of data has suggested that dopamine plays a primary role in the behavioral effects of cocaine (Goeders and Smith, 1983; Ritz *et al.*, 1987; Einhorn *et al.*, 1988; Witkin *et al.*, 1991b; Koob, 1992; McGregor and Roberts, 1993; Batsche *et al.*, 1994). By potentiation of mesolimbic dopamine transmission, cocaine appears to mediate the rewarding (Pettit *et al.*, 1984), locomotor stimulatory (Delfs *et al.*, 1990), and discriminative stimulus effects (Witkin *et al.*, 1991a; Callahan *et al.*, 1994).

However, dopamine alone does not account the overall behavioral profile of cocaine because not all of the behavioral effects of cocaine are consistently inhibited by dopamine antagonists (Colpaert *et al.*, 1976; Spyraki *et al.*, 1982; Loh and Roberts, 1990). Cocaine also binds to the 5-hydroxytryptamine (5-HT) and norepinephrine transporters (Koe, 1976). In addition, cocaine is a more efficacious and potent inhibitor of the impulse activity of 5-HT neu-

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rons than that of either dopamine or norepinephrine neurons (Pitts and Marwah, 1987; Cunningham *et al.*, 1992), while it has a relatively equal potency for inhibition of dopamine, 5-HT and norepinephrine reuptake as well as ligand binding to dopamine, 5-HT, and norepinephrine transporter sites (Ritz *et al.*, 1987).

Therefore, recent studies have begun to focus on the interaction of 5-HT system with the pharmacological and behavioral effects of cocaine. Cocaine influences on the 5-HT system not only by inhibition of the neuronal reuptake mechanism as stated above (Cunningham and Lakoski, 1988), but also by its action on the 5-HT receptors (Kilpatrick *et al.*, 1989; Fan *et al.*, 1994). Furthermore, the effects of cocaine are also influenced by the modulatory action of 5-HT through the interaction with dopamine (Chen and Reith, 1993).

The 5-HT system has also been reported to be involved in the developmental toxicity of cocaine. It has been reported that 5-HT may play a trophic role in the prenatal development of the CNS (Lauder and Krebs, 1978; Lauder *et al.*, 1981; Hellendall *et al.*, 1993). Extracellular 5-HT affects the growth of fetal 5-HT neurons in culture by an autoregulatory mechanism (Whitaker-Azmitia and Azmitia, 1986). Cocaine binding sites have been reported to be present in the fetal rat brain (Meyer *et al.*, 1993). Cocaine also elicited high-affinity [^{3}H]5-HT release response from cortical synaptosomes prepared from rat fetal tissues (Kramer *et al.*, 1994).

These overall studies suggest that 5-HT system may exert some effects on the behavioral alterations induced by exposure to cocaine during developmental period. However, experimental evidences for the role of 5-HT in the developmental toxicity of cocaine are not fully understood and show inconsistencies that have yet to be resolved. We have previously reported that ritanserin, a 5-HT_{2A}/5-HT_{2C} (formerly designated as 5-HT_{1C}; Hoyer *et al.*, 1994) antagonist, protects against cocaine-induced decrease in the mobility and the hatchability of developing chicken fetus (Sparber *et al.*, 1992). Meert *et al.* (1991) also reported that ritanserin reduced the self-administration of cocaine in rats. These reports indicate that 5-HT_{2A}/5-HT_{2C} antagonists may play a role in protecting against the developmental toxicity of cocaine.

Therefore, this experiment was designed with the following two specific aims: 1) to determine whether exposure to cocaine during early postnatal period results in changed behavioral sensitivity to cocaine later in life, 2) to examine the role of 5-HT_{2A}/5-HT_{2C} receptors on the developmental toxicity of cocaine by using mianserin as a probe for the 5-HT_{2A}/5-HT_{2C} receptor.

II. Materials and Methods

1. Animals

Nulliparous female and proven breeder male Sprague-Dawley rats (200-250 g) were used for breeding. Genetic heterogeneity was ensured by selecting no more than two rats of each sex from the same litter. Mating and delivery were done in a well controlled manner. From gestational day (GD) 20 until delivery, all nesting cages were examined at 0800 hr and 2000 hr to see the presence of the pups. The litters contained up to 14 pups at birth and were culled to a maximum of 10 within one day. Twelve to twenty four hr (average 18 hr) after birth, pups were removed from their biological mother, divided by sex, counted and individually weighed. Pups were fostered to a lactating dam which had given birth 36 to 48 hr earlier. Each foster litter had 6 males and 6 females which came from 3 biological mothers (2 males and 2 females from a biological mother).

2. Drug treatment during early postnatal period and handling of offspring

During postnatal days (PND) 7-13, pups were intraperitoneally administered mianserin (2.5 mg/kg)-saline (1 ml/kg), water (1 ml/kg)-cocaine HCl (45 mg/kg) or mianserin (2.5 mg/kg)-cocaine HCl (45 mg/kg) daily. The interval between successive administration of two drugs was 30 min. Water (1 ml/kg)-saline (1 ml/kg) were also administered to another group for control because mianserin or cocaine was dissolved in water or saline respectively. Each group consisted of 6 males and 6 females in-

itially. They were weighed and toe-clipped for identification. Weights were recorded daily during PND 7-20 and weekly thereafter until behavioral tests began. The final numbers of rats from each experimental group when subjected to behavioral tests were 12 (water-saline), 12 (water-cocaine), 11 (mianserin-saline), and 10 (mianserin-cocaine).

3. Apparatus for behavioral assessments

Standard operant chambers (Skinner Box, BRS/LVE, Beltsville, MD, USA) enclosed in custom-made sound-attenuating outer cubicles were used. The boxes were 31 cm wide × 25 cm long × 18 cm high with grid floors of stainless steel bars (0.5 cm diameter, spaced 2 cm apart). Each box contained two 7.5 cm wide stainless steel strips with the lower edge 15 cm above the grid floor, one on the rear and another on the side wall. Contacts with the strip, indicative of exploratory rearing behavior, were monitored through a high resistance drinkomotor circuit. Each box was equipped with a retractable lever (Model 143-22; BRS/LVE, Beltsville, MD, USA), a dispenser for delivery of 45 mg food pellets (Formula 21, Bioserv, Frenchtown, NJ, USA), and a speaker for introduction of white masking noise. A lever pressing was recorded through a microswitch by pressing the extended lever with at least 20 g force for the fixed ratio behavioral analysis. The boxes were controlled and data collected by TRS-80 Color Computers (Tandy Corp., Fort Worth, TX, USA) connected to the boxes by custom-built interfaces. Cumulative recorders (Ralph Gerbrands, Arlington, MA, USA) were used to make backup continuous recordings of behavioral responses. Rats from each treatment group were evenly distributed among the experimental chambers to control for any slight variations in the strip and/or lever sensitivity of each behavioral box.

4. Progressive ratio behavior analysis

When the offspring reached PND 100, they began to receive behavioral tests. For this procedure, the daily food intake of the rats was restricted until body weights were gradually stabilized to about 80% of their free-feeding levels. Then, rats were initially allowed to eat twenty 45 mg food pellets (Formula 21, Bioserve, Frenchtown, NJ, USA) prior to testing to avoid confounding experiments due to neophobia. The subjects were then tested on a progressive fixed ratio (FR) schedule. For this session, a lever was extended for 15 min, during which food pellets as a reinforcement were delivered contingent upon completion of a fixed number of extended lever presses. At the first session, the rats earned a food pellet for each lever press (FR 1) and were maintained on the FR 1 schedule for the following 4 days to allow stabilization of the lever press responding. Thereafter, the number of presses required for reinforcement (fixed ratio) was doubled daily until the animal should make 128 lever presses to obtain a single food pellet (FR 128).

5. Cocaine challenge on established FR 32 behavior

To determine if hitherto unexpressed neurobehavioral toxicity would be unmasked by acute cocaine challenge (Hughes and Sparber, 1978), and/or whether exposure to cocaine in early postnatal days could lead to differential sensitivity to cocaine exposure at adulthood, cocaine challenge was done upon established behavior. Completing the progressive FR task, the rats' behavior was restored to and maintained at FR 32 schedule of reinforcement. They were habituated to intraperitoneal injection with saline (1 ml/kg) during 4 daily sessions. Then, challenging cocaine by intraperitoneal administration was performed just prior to the beginning of 15 min session of FR 32 schedule once per day in increasing doses (5, 15 and 30 mg/kg on day 1, 2 and 3 respectively).

6. Statistics

Differences in body weights of the rats among treatment groups were analyzed by a one-way analysis of variance (ANOVA) followed by Scheff's F-test procedure. Lever pressings during the progressive FR schedule were expressed as a percent of FR 1 value, and comparisons were made using the Kruskal-Wallis test. A one-way ANOVA was also used to analyze the data for the cocaine chal-

lenge experiments. Data from behavioral assessments were analyzed by collapsing across sex if no overall sex differences were found. Preplanned comparisons of treatment groups with the control group were made using two-tailed Dunnetts t-statistic.

III. RESULTS

1. Weight changes

There were no statistically different weight changes in rats received mianserin-saline or water-cocaine during PND 7-13 compared with the control rats when the weight changes were separately analyzed by sex.

The mean weights of rats of all experimental groups persistently increased without reduction until the beginning of behavioral tests while the mianserin-cocaine treated rats showed reduced weight gains during a few days of their early life when compared with the water-saline group (male: PND 12-14, female: PND 11-18) (Table 1, not all data shown). However, this depressed rate of weight gains was soon recovered to the similar level thereafter as those of other groups.

2. Effects of neonatal cocaine and/or mianserin on the progressive ratio behavior at maturity

The number of lever presses of the rats increased until the fixed ratio went up to 8, maintained at the similar level from 8 to 32, and decreased at the higher fixed ratios (Fig. 1). However, the level of responding at FR 128 was not below that of the FR 1 value. Except for the mianserin-cocaine group, the highest value of responding was observed during the FR 8 session. At each session, behavioral differences between experimental groups were statistically insignificant (Kruskal-Wallis test).

3. Effects of neonatal cocaine and/or mianserin on cocaine challenge upon the established FR 32 responding at maturity

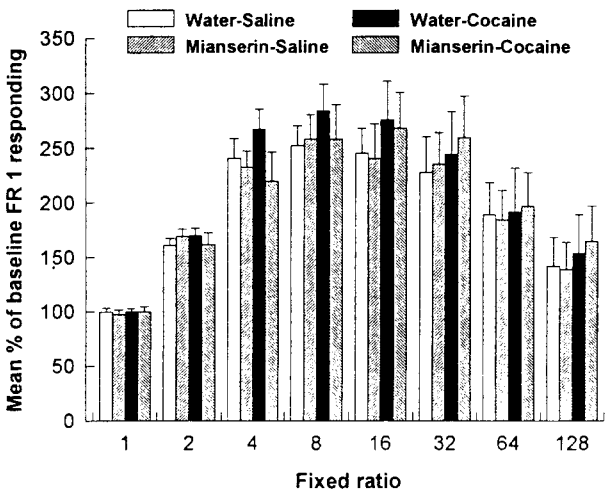

Fig. 1. Effect of exposure to cocaine and/or mianserin during PND 7-13 on the progressive ratio behavior of mature rats. The lever extended for 15 min and the number of lever presses required for reinforcement (fixed ratio) was doubled after each daily session up to 128. Detailed methods are described in the materials and methods. Kruskal-Wallis test shows that the differences among the 4 experimental groups are not statistically significant at each FR session.

Table 1. Body weights(g) of rats received cocaine and/or mianserin during postnatal days 7-13

Group	Water-Saline		Mianserin-Saline		Water-Cocaine		Mianserin-Cocaine	
PND	male	female	male	female	male	female	male	female
7	16.4±0.5	15.5±0.6	16.7±0.8	16.2±0.6	16.9±0.6	16.3±0.5	16.9±0.9	15.7±1.2
9	21.7±1.0	20.6±0.9	21.6±1.0	20.5±0.7	20.9±0.9	20.6±0.8	20.3±1.2	18.6±1.2
11	27.0±0.9	26.0±1.1	26.7±1.1	25.4±0.7	24.6±1.1	24.3±1.0	23.2±1.2	21.0±1.5*
13	33.1±1.1	31.8±1.2	31.8±1.5	30.6±1.2	29.0±1.4	28.6±1.1	26.8±1.5*	23.6±2.5*
18	45.7±1.4	43.6±1.3	45.4±1.8	43.8±1.6	40.9±1.0	41.1±1.5	39.6±1.8	34.5±1.8**
21	57.2±1.7	54.0±1.7	57.7±2.1	54.3±2.7	52.7±1.0	51.6±1.6	51.6±2.5	45.1±3.4
27	100.0±2.3	89.6±1.6	103.9±2.6	89.0±3.5	95.1±1.6	86.4±1.3	94.7±3.3	77.6±4.0
48	284.2±5.0	204.5±3.2	298.8±6.5	199.7±5.6	277.8±13.5	201.8±3.3	276.8±6.0	189.0±6.7
84	474.8±6.6	292.7±4.8	486.3±8.0	283.0±8.9	456.2±17.3	289.5±6.1	463.5±12.9	274.8±9.6

Each value indicates mean±S.E., PND; postnatal day
*P<0.05, **P<0.01 by Scheffe's F-test, compared to the water-saline group of each sex

Intraperitoneal injection of saline did not change the baseline responding of the established FR 32 behavior. During the first 1 min session, immediately after saline injection of the 4 experimental groups, the mean values of responding ranged between 65.1±9.3 and 78.3±15.1/min, and during the last 1 minute session, the respond rates were from 50.8±6.9 to 82.0±12.0/min (Fig. 2A). The baseline responding was maintained fairly throughout the 15 min session. An analysis of the 4 groups using ANOVA also shows that the responding of each group is not different from each other.

Challenge of cocaine showed a dose-dependent behavioral suppression. Water-saline group of 15 or 30 mg/kg of cocaine challenge demonstrated a marked behavioral suppression (Fig. 2C and 2D), although the behavioral suppression at lower dose (5 mg/kg) of cocaine was not apparent (Fig. 2B). The behavioral suppression was more marked at 30 mg/kg of cocaine than at 15 mg/kg of cocaine (0.2±0.2/min and 21.3±11.2/min respectively). All rats of water-saline group at 30 mg/kg of cocaine challenge did not even show any lever pressing response at all during some 1 min periods (7, 9, 10 and 13 mins). The behavioral pattern induced by a challenge of cocaine (15 or 30 mg/kg) was similar in both rat groups received mianserin-saline or mianserin-cocaine during PND 7-13.

However, the behavioral pattern in the water-cocaine group by the middle dose (15 mg/kg) of cocaine challenge was not similar to those of the other groups, i.e., rats of the water-cocaine group made an increased lever touch responses during

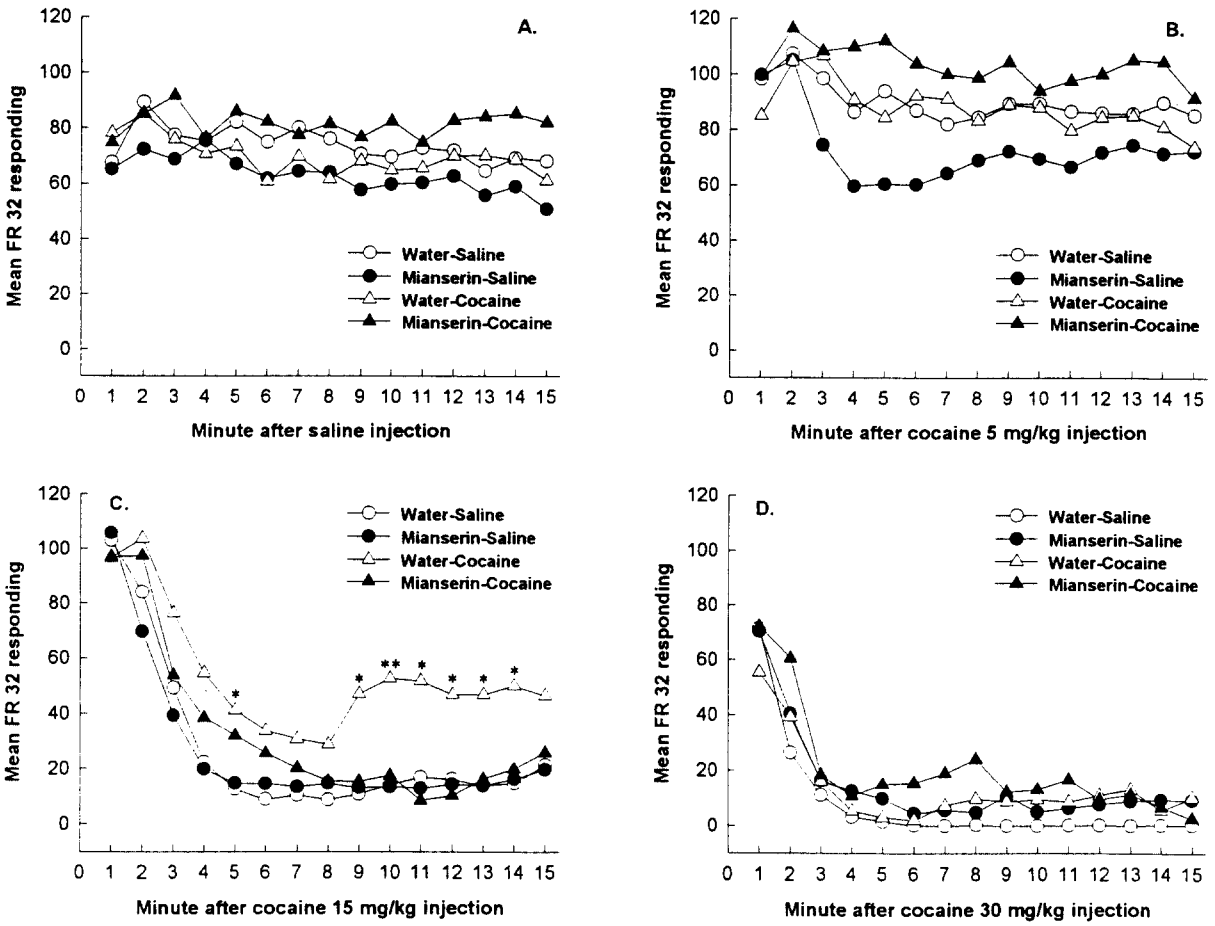

Fig. 2. Effect of exposure to cocaine and/or mianserin during PND 7-13 on cocaine challenge upon established FR 32 responding of mature rats. Saline (1 ml/kg: A.) or cocaine (5 mg/kg, 15 mg/kg, 30 mg/kg: B, C, and D respectively) was injected intraperitoneally just prior to the beginning of the 15 min session. Values indicate the mean number of lever presses of rats for 1 min period. *P<0.05, **P<0.01 by two-tailed Dunnett's test compared to the water-saline group.

the second half of the 15 min period comparing with other experimental groups (Fig. 2C). An analysis using ANOVA followed by two-tailed Dunnett's t-test revealed significant differences during this period when the water-saline group was used as control. Namely, the water-cocaine group exhibited a resistance to the suppressive effect of cocaine challenge during the second half of the FR 32 session. In contrast, the mianserin-cocaine group showed the similar pattern to that of the water-saline group.

IV. DISCUSSION

The present study shows that early exposure to cocaine brings about an altered behavioral sensitivity to cocaine itself at adulthood. Challenging moderate dose of cocaine at adulthood revealed this otherwise unexpressed effects of developmental toxicity of cocaine. Cocaine has a suppressive effect on motor activity of animals (Ruth *et al.*, 1988; George, 1989, 1990; Sparber *et al.*, 1992) which may be related to the 5-HT system (Sparber *et al.*, 1992). In the present study, rats exposed to cocaine during their early postnatal life exhibited a resistance to the cocaine-induced behavioral suppression. However, these rats did not show behavioral alterations in the progressive fixed ratio schedule of reinforcement. This measurement of behavior has been reported to be sensitive to motivational variables, such as degree of food deprivation (Hodos, 1961) and strength of the reinforcer (Hodos and Kalman, 1963). Moreover, this technique has been proved sensitive to brain stimulation (Hodos, 1965) or brain lesion (Hothersall, 1970), and to minor changes of brain function from prenatal exposure to phenobarbital (Middaugh *et al.*, 1975a, b) or to amphetamine (Zemp and Middaugh, 1975). Therefore, early exposure to cocaine may not affect motivational level caused by food deprivation at maturity. This result indicates that early exposure to cocaine may have subtle changes of brain function which could not be revealed by a progressive ratio technique.

In the field of behavioral teratology, completely normally appearing animals at maturity often shows abnormal responding when challenged with pharmacological agent (Eccles and Annau, 1982; Annau and Cuomo, 1988). On the basis of these reports, we decided challenging cocaine upon established FR 32 behavior in rats which have completed progressive fixed ratio test. Then we found significant resistance to cocaine-induced behavioral suppression in the rats which have been exposed to cocaine previously in their early life.

In the present study, the weight changes revealed some differences between rats received mianserin and/or cocaine for a few days in their early life. However, the weight changes were soon recovered to normal. This observation indicates that the neurobehavioral alteration can be masked by a compensatory mechanism such as a neuronal plasticity at young age. Accordingly, any individual who has been exposed to cocaine in his early life but are grossly normal may have a possibility of expressing the neurobehavioral toxicity in the future when the compensatory mechanism works no longer.

The rats received mianserin 30 min prior to cocaine administration did not express the resistance as shown by the water-cocaine group. The behavioral pattern presented by the mianserin-cocaine group was by no means statistically different from the water-saline group. This indicates that mianserin was able to block the neurobehavioral toxic effects of cocaine on the developing rat brain. As mianserin is a potent 5-HT_{2A}/5-HT_{2C} antagonist, its ability to abolish the resistance to the cocaine challenge-induced behavioral suppression is highly responsible for its antagonism of the cocaine's action mediated through a 5-HT_{2A}/5-HT_{2C} receptor activity. When taken together, these results support that cocaine has a neurobehavioral effects on the developing rat brain which can persist until adulthood or throughout life, and that the 5-HT_{2A}/5-HT_{2C} receptor activity is involved in mediating the behavioral effects of cocaine. In addition, the results suggest that mianserin protects brain function from the behavioral toxic effects of cocaine. Similarly another 5-HT_{2A}/5-HT_{2C} receptor antagonist, ketanserin, has been reported to alleviate the cocaine-induced decrease of motility and hatchability of developing chicken fetus (Meert *et al.*, 1991) and the self-administrative behavior of rats (Sparber *et al.*, 1992).

In conclusion, 5-HT_{2A}/5-HT_{2C} receptors may have an important role for the persistently altered behavioral sensitivity to cocaine caused by exposure to cocaine during development, and antagonism to the 5-HT_{2A}/5-HT_{2C} receptor activity may prevent cocaine-induced neurobehavioral toxicity at least in part.

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