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Effect of Perinatal Amphetamine Administration on Behavior and Amine Concentration in Brains of Rat Pups

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Abstract: Daily intraperitoneal administration of d-amphetamine sulphate at a dose of 8 mg kg^{-1} body weight to rats during 6-15 days of gestation has resulted in significant effect on sensory and motor reflexes of their pups. These pups have shown increased righting and rotating reflexes and cliff avoidance activity. Significant increase in brain noradernaline, 5-hydroxytryptamine and decrease in monoamine oxidase activity was observed in these pups and their mothers compared to their control counterparts.

Key words: Amphetamine, perinatal, rats, pups, behavior

INTRODUCTION

Amphetamines are indirect-acting sympathomimetic amines with potent stimulant action on central nervous system and cardiovascular system (Hoffman and Lefkowistz, 2005). They have been used for fatigue reduction, mood elevation and appetite suppression, often with no acceptable medical use, since they may produce psychological dependence and chemical tolerance (Stowe *et al*., 1976; Pedro *et al*., 2003; Diniz *et al*., 2003). The drug is metabolized and excreted more slowly in adult female rats compared to male (Groppetti and Costa, 1969). This persistence of unmetabolized amphetamine in numerous female tissues following its systemic administration has been associated with protracted behavioral and physiological actions of the drug in female rats compared to male (Meyer, 1977; Meyer *et al*., 1977; Beatty and Holzer, 1978). Newborns that have been exposed to drugs of abuse in utero may have characteristic physical and mental development problems throughout their lives (Kwong and Ryan, 1997). Therefore, this study was carried out to investigate the effects of administration of amphetamine during perinatal period in rats on the behavior of the offspring.

MATERIALS AND METHODS

Animals: Thirty mature Wister rats weighing 160-180 g, at the time of mating were used for this study. They were kept at controlled temperature of 24±1°C, a relative humidity of 40-65% and a 12 h light/dark cycle. They were fed on a commercial lab Chow (Arasco, Saudi Arabia) and tap water *ad libitum*. In each cage 1 male rat was housed with 2 females overnight. The day on which a vaginal plug or sperms were found was considered as 0 day of pregnancy. The pregnant dams were divided into 2 groups. Group 1 animals were injected intrapertinaly (I.P.) with saline during 6-15 days of gestation. Group 2 animals were treated similar to group 1 but with d-amphetamine sulfate (Sigma, UK) at a dose of 8 mg kg^{-1} body weight. This dose has stimulated the release of noradrenaline in the brain (Altar *et al*., 1984). On the day of birth, the pups were culled to only 6 per dam and left with their mother. Pups from control (group 1) and ampheteamine treated mothers (group 3) were designated as 2 and 4 group, respectively. Pups from each litter were subjected to various behavioral tests as follows:

Righting reflex: The time taken by a pup placed on its back to correct itself was recorded.

Rotating reflex: The time taken for a pup placed downwards on an 30° in claimed surface to rotate its body through 180° and face its head upwards was recorded.

Cliff avoidenace: The time taken for a pup placed on the edge of a table to back away and turn from the cliff was recorded.

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Assay of brain amins: Pups were decapitated on 7 day postnatal Brains were quickly removed, frozen in liquid nitrogen and then stored at -30°C until the assay of amines. The brain amines were estimated by methods previously validated and described (Homeida and Cooke, 1982 a, b). Each brain was divided by sagittal section into portions of 260-280 mg. Brain concentrations of amines were measured spectrophotoflurometrically. Noradrenaline (NA) in one section using iodinc oxidation (Miller *et al*., 1970) and 5-hydroxytryptamine (5-HT) and 5-hydroxyindole acetic acid (5-HIAA) in the other using O-phthaldehyde oxidation (Maickel *et al*., 1968). Fluorescence was measured on a Perkin-Elmer Model 203 fluorescence spectrophotometer using quartz microcells (type 17, Chandos Intercontinental, UK). Excitation and emission wavelengths were 385 and 485 nm, respectively, for NA 360 and 470 nm for 5-HT and 5-HIAA. All standard curves were linear over the range 0.05-3.0 μg. Recoveries of exogenous amines added to assay tubes, before the first shaking step, were 78-88% for NA, 97-101% for 5-HT and 70-72% for 5-HIAA. Monoamine Oxidase (MAO) activity was estimated by measuring the fluorescence of 4-hydroxyquinolone, produced by the oxidative deamination of kynuramine (Krajil, 1965). Deproteinisation was achieved with 0.6 M perchloric acid as recommended by Century and Rupp (1968). Fluorescence was measured on a Perkin-Elmer Model 203 fluorescence spectrophotometer using quartz microcells. Excitation and emission wavelengths were 315 and 380 nm, respectively. All standard curves were linear over the range 0.015-0.035 μmol and the recovery of 4-hydroxyquinolone added to brain homogenates was 97.1±0.4.2 (SD)%. Results were assessed using Student's t-test.

RESULTS

Perinatal treatement of pregnant rats with amphetamine had a significant effect on all sensory and motor reflexes of pups. During the first week postnatal, the pups from the amphetamine-treated mothers (group 4) showed significant ($p<0.001$) increase in their righting, reflexes (Fig. 1), rotating reflaxes (Fig. 2) and cliff avoidance (Fig. 3) compared to group 3 (controls).

Significant ($p<0.01$) increase in the concentration of AN, 5-HT but not 5-HIAA in brain of pups (group 4) was observed. In these pups a significant ($p<0.01$) decrease of MAO activity in the brain was seen (Table 1).

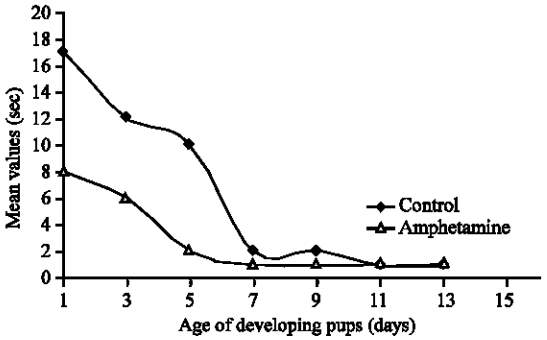

Fig. 1: Effect of perinatal amphetamine administration on the mean (sec) righting reflex of rat pups

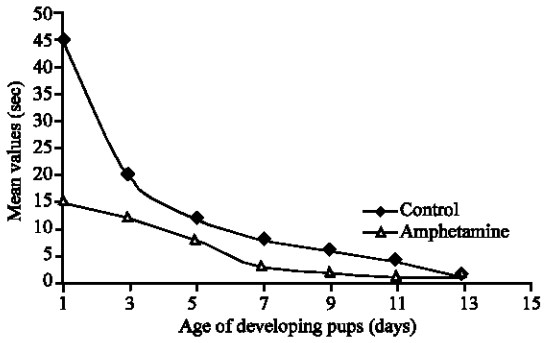

Fig. 2: Effect of perinatal amphetamine administration on the mean (sec) cliff avcoidance activity of rat pups

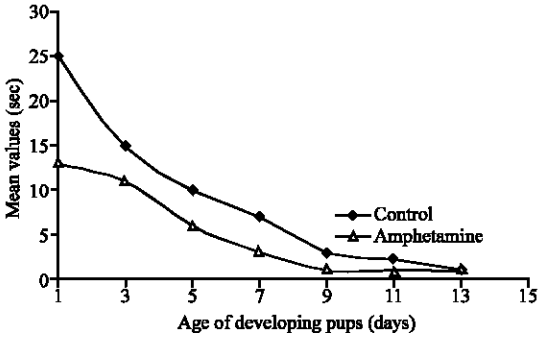

Fig. 3: Effect of perinatal amphetamine administration on the mean (sec) rotating reflex of rat pups

Table 1: Mean brain concentration of Noradrenaline, 5-hydroxytryptamine, 5-hydroxyindole acetic acid and 5-hydroxyquinolone in amphetamine-treated mothers and their pups

	Mothers		Pups	
Brain concentration	Control	Treated	Control	Treated
NA (μg g^{-1})	0.551	0.941*	0.451	0.821*
5-HT(μg g^{-1})	0.511	1.031*	0.412	0.872*
5-HIAA (μg g^{-1})	0.271	0.031*	0.264	0.021*
MAO (μmol)	1.712	0.644*	1.310	0.511*

NA = Noradrenaline; 5-HT = 5-hydroxytryptamine; 5-HIAA = 5-hydroxyindole acetic acid; MAO = Monoamine Oxidase; Significantly different from controls ($p<0.001$)

DISCUSSION

Pups from amphetamine-treated mother were found to be very active and agile from the day they were born. Rigting and rotating reflexes and cliff avoidance activity were significantly increased compared to their control counterparts. Induced locomotor effects of amphetamine reported to occur in rats and mice (Fairchild and Alles, 1967; Ross, 1979; Kalix and Braenden, 1985). Pups may have been exposed to amphetamine in utero. Most drugs are capable of crossing the placenta at rates controlled by their molecular size, ionization state, lipophilicity and degree of plasma protein or placental tissue binding (Chan *et al*., 2003). Placental transfer of most drugs of abuse takes place primarily by passive diffusion due to their small molecular size and high lipophilicity because of this, placental blood flow may be the most critical limiting factor regarding drug transport to the fetus (Chan *et al*., 2003). Ultimately, fetal exposure is a product of maternal consumption, metabolism and elimination, placental transfer and metabolism and fetal metabolism (Chan *et al*., 2003). The continual observed hyperactivity of pups maybe related to the fact that neonates are deficient in their ability to metabolize amphetamine in their circulation (Homeida *et al*., 1993).

Increased concentration of NA and 5-HT were observed in the brain of amphetamine treated mothers and their pups. Amphetamine-induce alterations in behavior and physiology have been ascribed to the ability of this drug to block the reuptake and enhance the release of catecholamine neurotransmitters in presynaptic nerve terminals of both the central and peripheral nervous system (Costa and Garattini, 1970). Amphetamine has been reported to increase brain noradrenaline (Kalix, 1983) and 5-hydroxytryptamine in a dose-dependent manner (Azzaro and Rutledge, 1973) in rats. The metabolite of amines, 5-HIAA concentration was reduced in brain. This was expected since amphetamine caused brain MAO inhibition and resulted in defective in metabolic pathway of catecholamines metabolism thus, further contributing to elevated concentration of amines (Paulo *et al*., 2003). Furthermore, alterations in brain enzymes are among the factors responsible for disturbances in behavioural activites of affected animals (Kellog *et al*., 1998; Ajarem and Brain, 1993). These findings indicate the desirable need to diagnose and begin treatment of individuals exposed to drugs of abuse in utero (Lopez *et al*., 2007).

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