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COMMUNICATIONS

Postnatal behavioural effects of maternal nicotine exposure in rats

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Abstract—The effects of nicotine on locomotor activity have been studied in neonate rats exposed to nicotine (1·5 mg kg^{-1} day^{-1}) throughout the gestational period. Both 14 day old male and female offspring demonstrated an increase in spontaneous locomotor activity when compared with saline-exposed controls. However, systemic administration of (+)-amphetamine was effective in attenuating the hyperactivity of these nicotine-exposed pups.

Exposure of pregnant and lactating rats to nicotine has been shown to adversely alter the survival rate, growth and development of the offspring (Becker et al 1968; Dalby 1978; Peters et al 1979; Peters & Tang 1982; Sershen et al 1982). These effects can be attributed to the actions of nicotine, with or without contributing factors associated with hypoxia and ischaemia (Martin & Becker 1971; Slotkin et al 1986).

In most studies examining the effect of maternal nicotine exposure on the offspring, nicotine was administered to pregnant rats either by repeated parental injections once or twice daily or in drinking water. These methods of nicotine administration may expose both the mother and foetuses to sudden spike concentrations of nicotine. It has been suggested that these spikes of nicotine can produce hypoxia in the foetus due to constriction of placental vasculature (Werler et al 1985; Benowitz 1986).

To circumvent this problem of nicotine administration and to administer nicotine at a dose that closely mimics the amount of nicotine intake as with cigarette smoking in humans, nicotine was administered to pregnant rats via subcutaneous implantation of osmotic mini-pumps which delivered nicotine at a dose of 1·5 mg kg^{-1} day^{-1} (Murrin et al 1987). Recent studies have shown that osmotic mini-pumps delivering that dose will produce plasma nicotine levels in rats similar to those found in humans smoking one pack of cigarettes per day (Hill et al 1983; Murrin et al 1987). Therefore, the animal model used in the present study will more closely resemble the human situation. The effects of prenatal exposure to nicotine on motor coordination and spontaneous locomotor activity of the 14 day old male and female pups were examined.

Materials and methods

Animals. Sprague-Dawley rats (Sasco, Inc. Omaha, NE), ca 230 g, were used. They were allowed free access to food (Purina Lab. Chow) and water and maintained on a normal 12 h light/12 h dark cycle at 21 ± 1°C. Male and female rats were paired for mating and sperm positive females were anaesthetized with Equithesin (0·25 mL/100 g wt). An incision was made in the skin posterior to the shoulder and an osmotic mini-pump Model 2ML4 (Alza corp. Palo Alto, CA) containing sterile physiological saline or nicotine (1·5 mg kg^{-1} day^{-1}) was implanted subcutaneously. The incision was closed with a wound clip and covered with a mixture of benzocaine and Betadine to alleviate discomfort and to prevent infection. The model 2ML4 osmotic mini-pump contains 2 mL of solution and has a pumping life of 28 days. Nicotine was administered as the free base, using nicotine tartrate (Sigma Chemical Co. St Louis, MO) dissolved in sterile physiological saline solution. The litter size, litter viability, birth weight and length of all pups in each litter were determined immediately after birth (postnatal day 1). The saline and nicotine-exposed pups were cross-fostered to drug free (surrogate) females who had delivered at the same time. This was done to isolate the effect of nicotine to the prenatal period. Litter size was maintained at 8 pups (4 males and 4 females) per mother. Fourteen day old male and female saline and nicotine-exposed pups were used in this study.

Assessment of motor coordination. For determining the degree of interlimb coordination and for assessing locomotor development, these tests which included position reflex, surface righting and negative geotaxic tests were used (Ryan & Pappas 1986). To pass the position reflex test, the pups had to assume a normal position within 10s when the hind leg was lowered over the edge of a horizontal table. To pass the surface righting test, the pup had to turn right-side up with all four limbs in a normal standing position within 15s when placed on its back in a supine position on a level surface. Pups were placed head downward on a textured surface with a 25° slope and were allowed a maximum of 60s to make a 180° turn to pass the negative geotaxic test. Control pups are able to pass all these tests by day 14.

Determination of spontaneous locomotor activity. Locomotor activity of pups was measured with a motor activity cage equipped with photocells and an automatic counter (Lehigh Valley Electronics, Allentown, NJ). Each pup was allowed to adapt to the cage for 10 min before the administration of saline (s.c.) or (+)-amphetamine (1 mg kg^{-1} s.c.). After drug injection, the locomotor activity of each pup was determined at 10 min intervals for 2 h. All behavioural studies were conducted between 0800 and 1600 h.

Statistical analysis. For postnatal data, the pups of each dam were considered to represent a single determination. Chi square or Student's *t*-test was used to analyse characteristic of pups when appropriate. In locomotor studies, data were analysed by SAS followed by least significant difference test. For each sex, the data was analysed as a two way analysis of variance with (+)-amphetamine-nicotine interaction. Analysis of variance was performed with time as repeated measures. Wilks criterion F statistics for time (+)-amphetamine-nicotine interaction effect was $F(11, 11) = 11{\cdot}56$ for males and $F(11, 11) = 7{\cdot}10$ for females. Both values were significant at the 0·01 levels.

Results and discussion

We monitored the gain in body weight, food and water consumption of all pregnant control and nicotine-treated females throughout the gestational period. In agreement with earlier studies, nicotine administered via osmotic mini-pumps at a dose of 1·5 mg kg^{-1} day^{-1} had no effect on daily gain in body

weight, or daily food and water consumption of the animals (Murrin et al 1987). Although nicotine did not affect the birth weight and body length of pups (data not shown), the total number of pups born to nicotine-treated females ($n = 10{\cdot}3 \pm 0{\cdot}4$) was significantly reduced ($P < 0{\cdot}05$ Student's t-test) when compared with saline-treated controls ($n = 12{\cdot}7 \pm 0{\cdot}6$) in the 30 litters studied.

Both the saline and nicotine-exposed pups passed the criteria for the determination of surface righting, position reflex and negative geotaxic tests (data not shown). This suggests that prenatal exposure to nicotine may not alter the development of motor coordination in these offspring. However, both the nicotine-exposed male and female pups were found to be spontaneously hyperactive when compared with saline-treated controls. Interestingly, (+)-amphetamine was found to be effective in reducing this hyperactivity in nicotine-exposed pups, but stimulated the locomotor activity in saline-exposed pups (Figs 1, 2). Thus, delivering nicotine to rats via the osmotic mini-pumps creates an animal model which resembles attention deficit disorder (ADD) in human. ADD is characterized by involuntary hyperactivity, restlessness, short attention span and a variety of cognitive and perceptual problems in children and can be treated with (+)-amphetamine (Brown et al 1980; Wender 1984).

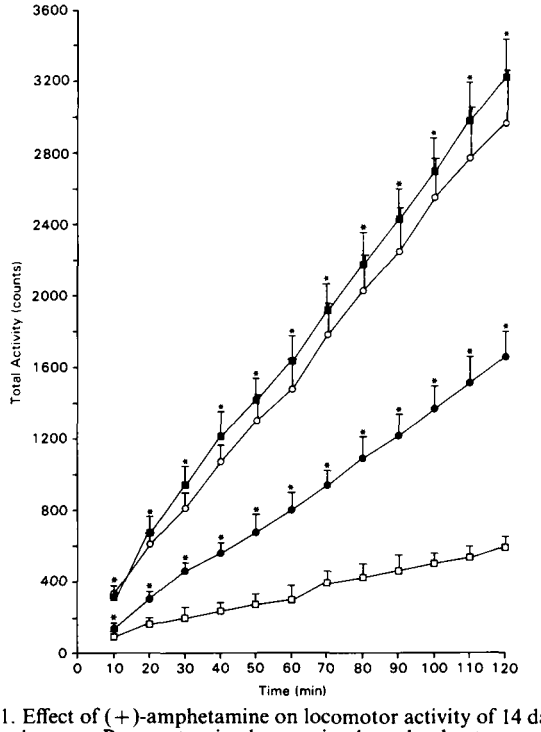

FIG. 1. Effect of (+)-amphetamine on locomotor activity of 14 day old male pups. Pregnant animals were implanted subcutaneo isly with osmotic mini-pumps containing either physiological saline or nicotine ($1{\cdot}5$ mg kg^{-1} day^{-1}) for the gestational period. At birth, all pups were cross-fostered to drug free surrogate mother. Results are mean $\pm$ s.e.m. of 7 pups. □——□ pups were exposed prenatally to saline followed by saline injection, ■——■ pups were exposed prenatally to saline followed by (+)-amphetamine (1 mg kg^{-1} s.c.) injection, ○——○ pups were exposed prenatally to nicotine followed by saline injection, ●——● pups were exposed prenatally to nicotine followed by (+)-amphetamine (1 mg kg^{-1} s.c.) injection. * Significantly different from corresponding control group ($P < 0{\cdot}01$, Least significant difference test).

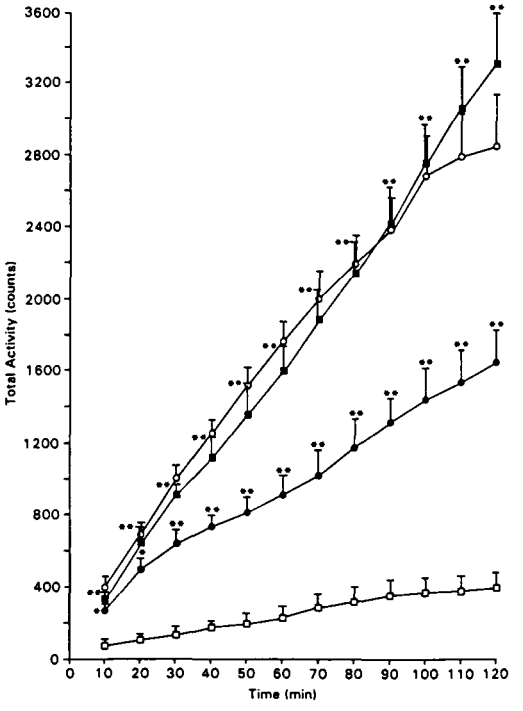

FIG. 2. Effect of (+)-amphetamine on locomotor activity of 14 day old female pups. Pregnant animals were implanted subcutaneously with osmotic mini-pumps containing either physiological saline or nicotine ($1{\cdot}5$ mg kg^{-1} day^{-1}) for the gestational period. At birth, all pups were cross-fostered to drug free surrogate mother. Results are mean $\pm$ s.e.m. of 7 pups. □——□ pups were exposed prenatally to saline followed by saline injection, ■——■ pups were exposed prenatally to saline followed by (+)-amphetamine (1 mg kg^{-1} s.c.) injection, ○——○ pups were exposed prenatally to nicotine followed by saline injection, ●——● pups were exposed prenatally to nicotine followed by (+)-amphetamine (1 mg kg^{-1} s.c.) injection. * Significantly different from corresponding control group ($*P < 0{\cdot}05$, $**P < 0{\cdot}01$, Least significant difference test).

Several human studies indicate a strong correlation between exposure to nicotine in utero and the increased incidence of ADD (Denson et al 1975; Dunn & McBurney 1977; Brown et al 1985). Although the specific etiology of ADD remains unclear, nicotine has been implicated in this disorder by altering the normal development of nigrostriatal dopaminergic system. This is supported by observations that depletion of brain dopamine in development animals produced a hyperactivity pattern similar to that seen in ADD children (Shaywitz et al 1976; Concannon & Schechter 1981). Furthermore, the cerebrospinal fluid of children with ADD has significantly less homovanillic acid, a major metabolite of dopamine as compared with the level of normal children (Shaywitz et al 1977).

The exact mechanism whereby prenatal exposure to nicotine caused hyperactivity in these pups remain uncertain. Acute and chronic administration of nicotine have been shown to alter the activity of dopaminergic neurons in the striatum (Westfall 1974; Andersson et al 1981; Lichtensteigen et al 1982; Fung & Lau 1987). Furthermore, nicotine has been shown to exert a greater effect in releasing dopamine from the nucleus accumbens than the striatum in adult rats (Imperato et al 1986). If nicotine modifies the normal development of striatal dopaminergic neurons, a deficit in dopaminergic neuronal activity may reduce the modulatory effect of dopamine on excitatory noradrenergic activity, resulting in hyperactivity (Shaywitz et al 1976). The administration of (+)-amphetamine, which is thought to act by

stimulating the formation and release of newly synthesized dopamine from nerve terminals in the striatum and nucleus accumbens (Chiueh & Moore 1975; Jackson et al 1975; Fung & Uretsky 1982) may thus reduce the hyperactivity of the nicotine-exposed pups. The biochemical effects of nicotine on the pups appear to be complex and warrant further investigation.

In conclusion, this study shows that prenatal exposure to nicotine induced hyperactivity in 14 day old rat pups. Administration of (+)-amphetamine was effective in reducing this hyperactivity. Nicotine may induce this hyperactivity by altering dopaminergic function in the central nervous system.

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