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Neurochemical Consequences Following Administration of CNS Stimulants to the Neonatal Rat[1]

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WAGNER, G. C., C. R. SCHUSTER AND L. S. SEIDEN. *Neurochemical consequences following administration of CNS stimulants to the neonatal rat.* PHARMAC. BIOCHEM. BEHAV. 14(1) 117–119, 1981.—The possible induction of long-lasting catecholamine depletions in discrete brain regions by psychomotor stimulant drugs was examined in neonatal rats. Three agents, methylamphetamine, *d*-amphetamine, and methylphenidate were administered to groups of rat pups from days 10 to 40 of life. Pups were killed 2 weeks beyond the last drug administration. Catecholamine levels of various brain regions from groups of rats receiving 12.5, 25 or 50 mg/kg/day of each drug were compared with levels of vehicle treated pups. Both methylamphetamine and *d*-amphetamine at the higher doses induced long-lasting dopamine depletions in the caudate of rat pups. Methylphenidate had no long-term effect on dopamine while norepinephrine levels were not altered by any treatment.

Neurochemical consequences CNS stimulants Neonatal rat Catecholamine depletion

REPEATED administration of high doses of methylamphetamine (MA) to rhesus monkeys, rats or guinea pigs causes long-lasting depletions of regional brain catecholamine (CA) levels [14, 19, 20]. A similar long-lasting depletion of central serotonin levels was observed following the repeated administration of high doses of MA to rats [11]. It was also demonstrated that, in rats, these depletions are dose-dependent, last at least 6 months after the last injection, and are accompanied by a decrease in the number of high-affinity DA uptake sites and neuronal degeneration [2, 10, 19]. Furthermore, these disruptions of the monoamine system are induced by MA and *d*-amphetamine (AMPH) [5, 7, 17, 18], but are not found after the repeated administration of high doses of methylphenidate (MP) [18].

To date, little work has been done on possible long-lasting neurochemical changes consequent to the repeated administration of these psychomotor stimulants to neonates. Long-lasting changes of CA levels have been reported in the offspring of rats following the administration of MA to pregnant mothers [6, 8, 9, 16] but additional reports in which the drug is administered directly to the neonate are not available. Since MA, AMPH, and MP have been used in the clinical treatment of minimal brain dysfunction [12], this experiment was conducted to examine for possible long-lasting central CA depletions following the repeated administration of psychomotor stimulants to neonatal rats.

METHOD

Fourteen day, sperm-positive Sprague-Dawley rats were obtained from Holtzman Co. (Madison, WI) and were housed individually with ad lib access to food and water. The colony room was maintained at 21+1°C with an automatic, 12 hour light-dark cycle.

Rat pups were born in approximately 1 week and on their third day of life were pooled and redistributed to form litters of 10 without regard to sex. Pups were weaned on their 21st day of life.

Each litter of rats served as a group and was randomly assigned to one of the following drug treatments: (1) Twice daily, subcutaneous (SC) injections of methylamphetamine hydrochloride dissolved in physiological saline to a concentration of 25 mg/ml. The doses were 12.5, 25 or 50 mg/kg/day. (2) Twice daily, SC injections of methylphenidate hydrochloride dissolved in physiological saline in a concentration of 25 mg/ml. The doses were 12.5, 25 or 50 mg/kg/day. (3) Twice daily, SC injections of *d*-amphetamine sulfate dissolved in physiological saline to a concentration of 25 mg/ml. The doses were 12.5, 25 or 50 mg/kg/day. (4) Twice daily, SC injections of equivalent volumes of the vehicle, physiological saline.

Each drug was administered to at least two litters. The total daily dose was given in two equally divided, daily in-

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TABLE 1

DOPAMINE (DA) AND NOREPINEPHRINE (NE) LEVELS IN μg/g OF TISSUE FROM VARIOUS BRAIN REGIONS OF RAT PUPS TREATED WITH 12.5, 25 OR 50 mg/kg/day OF METHYLPHENIDATE HYDROCHLORIDE (MP), *d*-AMPHETAMINE SULFATE (AMPH), METHYLAMPHETAMINE HYDROCHLORIDE (MA) OR THE VEHICLE, SALINE (C)

	C	MP			AMPH			MA		
	—	12.5	25	50	12.5	25	50	12.5	25	50
CAU	5.16	4.76	4.79	5.43	4.89	4.54	3.27*	4.22	3.58*	3.56*
DA	(0.28)	(0.20)	(0.21)	(0.29)	(0.40)	(0.22)	(0.84)	(0.36)	(0.27)	(0.28)
HT	1.20	1.43	1.24	1.29	1.46	1.30	1.17	1.23	1.41	1.20
NE	(0.13)	(0.22)	(0.11)	(0.07)	(0.24)	(0.18)	(0.12)	(0.12)	(0.16)	(0.17)
MID	0.50	0.57	0.52	0.56	0.56	0.56	0.57	0.50	0.65	0.54
NE	(0.04)	(0.03)	(0.03)	(0.02)	(0.02)	(0.04)	(0.04)	(0.11)	(0.07)	(0.15)
PONS	0.40	0.37	0.38	0.43	0.40	0.40	0.35	0.35	0.47	0.49
NE	(0.02)	(0.04)	(0.02)	(0.07)	(0.03)	(0.03)	(0.02)	(0.03)	(0.08)	(0.04)
TELE	0.58	0.64	0.64	0.65	0.61	0.63	0.61	0.51	0.62	0.37
NE	(0.03)	(0.04)	(0.03)	(0.03)	(0.02)	(0.07)	(0.05)	(0.07)	(0.20)	(0.04)

Lower values are standard errors.

*=$p<0.05$, *t* test. CAU—caudate nucleus; HT—hypothalamus; MID=midbrain; PONS=pons-medulla; TELE=telencephalon.

jections approximately 12 hours apart. The pups were injected between 10 and 40 days of age. Two weeks after the last injection, pups from each treatment group were killed. Their brains were removed and dissected (as described in detail elsewhere [11]) to yield portions of caudate, midbrain, hypothalamus, pons-medulla and telencephalon. Brain parts were stored in liquid nitrogen until assayed. DA levels were determined by high performance liquid chromatography with electrochemical detection and NE levels were determined by alumina adsorption with spectroflurometric analysis [3,15].

RESULTS

Table 1 presents the CA levels for the various brain regions of rat pups treated on days 10 to 40 of life with one of three doses of a psychomotor stimulant or with the vehicle solution. A one-way analysis of variance revealed a significant difference in caudate DA levels of rats treated with MA or with AMPH, $F(3,23)=7.00$; $F(3,24)=3.34$, respectively. The pups treated with 25 or 50 mg/kg/day of MA had caudate DA levels significantly lower than vehicle treated pups (to 69% control). The pups treated with 50 mg/kg/day of AMPH also had significantly lower caudate DA levels (to 63% of control). No dose of MP produced any significant decrease in DA levels. One-way analysis of variance revealed no significant differences in norepinephrine levels in any brain region after any treatment.

DISCUSSION

This study extends the previously reported findings of long-lasting DA depletions induced by administration of MA and AMPH [2, 5, 7, 18, 19, 20] to the neonate. The lack of norepinephrine depletion in the neonatal rat induced by either of these two compounds concurs with previous studies in which no long-lasting alteration of norepinephrine levels were observed after treating adult rats or guinea pigs with MA [18, 19, 20]. MP did not induce long-lasting CA depletions in any brain region after any dose tested. This is in agreement with the lack of CA level alteration after treatment of adult rats or rhesus monkeys with MP [18].

In adult rats, the caudate DA depletions induced by the administration of 50 mg/kg/day for 30 days of either MA or AMPH were to 40% [19] and 56% [18] of control values, respectively. In this study, neonatal rats were also treated with 50 mg/kg/day of either MA or AMPH. However, the induced depletions were only to 69% and 63% of control levels. This observation of a decreased sensitivity to the effects of systematically administered amphetamines in neonates is in agreement with other reports comparing the lethal effects of the amphetamines in neonates and adults [1]. In addition, parachloroamphetamine was shown to have a decreased effect on serotonergic levels subsequent to its administration to neonatal rats as compared with adults [4].

Finally, these psychomotor stimulants have been shown to release CAs from the central nervous system nerve terminals. The amphetamines release CAs from an alpha-methyltyrosine sensitive pool while MP releases CAs from a reserpine sensitive pool [13]. All three agents are efficacious in the treatment of minimal brain dysfunction yet only AMPH and MA induce long-lasting DA depletions. However, the doses which produced the DA depletion far exceed the therapeutic dose range used in the treatment of minimal brain dysfunction. This implies that the common mechanism of release of CAs as opposed to the induction of long-lasting DA depletions is involved in their therapeutic attenuation of this syndrome.

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