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Development of Serotonin-Mediated Behavioral Inhibition in the Hyperthyroid Mouse

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NAGY, Z. M. AND M. J. FORSTER. *Development of serotonin-mediated behavioral inhibition in the hyperthyroid mouse*. PHARMAC. BIOCHEM. BEHAV. **16**(2) 203–206, 1982.—Experimental hyperthyroidism was induced in neonatal mice by thyroxine injections at 1–3 days of age. Control and thyroxine-treated mice were subsequently tested daily for locomotor activity following injections of methysergide, a serotonin antagonist, or saline from 10 through 15 days of age. Although thyroxine-treated mice were more active than controls, the ontogeny of methysergide-induced disinhibition of locomotor activity was similar for both thyroxine-treated and control mice. The results suggest that the early maturation of serotonin-mediated inhibition of behavioral arousal is not affected by thyroxine-accelerated development. Results are discussed in terms of the specificity of the pharmacological agent, methysergide.

Neonatal mice Thyroxine Locomotor activity Methysergide Serotonergic development Arousal

EXPERIMENTAL procedures which result in excess thyroid hormone in the neonatal rat or mouse accelerate CNS development as evidenced by a variety of maturational indices. Anatomical [14, 23, 24, 28], physiological [23,27], and biochemical [7, 22, 25] aspects of CNS maturation are accelerated by experimental hyperthyroidism, as are sensory, motoric, and reflexive capacities [2, 18, 26]. In addition, experimental hyperthyroidism appears to accelerate development of CNS processes necessary for learning and memory [18,25].

It has been suggested that CNS development in altricial rodents proceeds in a caudal to rostral sequence, with brainstem excitatory areas maturing somewhat earlier than those rostral areas mediating behavioral inhibition. Pharmacological studies have indicated that brainstem excitatory systems are catecholaminergic and are modulated by later maturing cholinergic or serotonergic systems [1, 6, 15]. A variety of biochemical evidence suggests that development of catecholamine and acetylcholine systems is affected by early thyroxine treatment [7, 22, 25]. Recent studies have indicated that the effects of hyperthyroidism can also be detected pharmacologically. The disinhibitory effect of the anticholinergic, scopolamine, can be detected at an earlier age in thyroxine-treated mice than in controls [16,17].

The present study was conducted to investigate the possibility that development of serotonin-mediated capacities for behavioral inhibition is also affected by neonatal thyroxine treatment. The postsynaptic serotonergic blocking agent, methysergide, was employed using dosage and time course parameters determined previously [20]. If development of serotonin-mediated inhibitory capacities is accelerated by neonatal thyroxine treatment, then thyroxine treated mice would be expected to show the disinhibitory effects of methysergide at an earlier age than controls.

METHOD

Animals

The animals were 96 Swiss-Webster mice (*Mus musculus*) born in the Bowling Green State University psychology department mouse colony. Litters were reared in 30.4×18×12.8 cm polyethylene cages with wire grid tops and wood chips on the floor. Nesting material was provided and ad lib food and water were available to the mothers. Pups remained with the mothers at all times except during test sessions and daily recording of body weights. The colony room was maintained on a 12 hr light-dark cycle beginning at 0800 hrs, and temperature was maintained at 24±1°C. Pregnant females were checked for births twice daily at 1200 and 2000 hours.

Apparatus

Activity was monitored in 19.4×6.4×9 cm clear Plexiglas cages with grid floors constructed of 1 mm dia. stainless steel rods spaced 4 mm center-to-center and extending parallel to the length of the cage. Photocells were spaced 3.4 cm from each end of the cage, each opposite an infrared light source. The activity cages were located in sound attenuated chambers, each equipped with a 6-W incandescent ceiling light for illumination and a ventilation fan which provided a 74-dB ambient noise level within the chamber.

Activity counts were recorded by printout counters located outside the sound-attenuated chamber each time the

animal traversed the full 12.6 cm distance between the photocells within each 15 min interval.

Procedure

Litters were selected which had been born on the previous day and contained 10–13 pups. Four males and 4 females with near equal weights were selected for treatment, while the remaining pups were sacrificed in order to maintain constant litter size. Two pups of each gender were randomly assigned to receive 1 μg/g body weight IP injections of thyroxine (L-thyroxine, crystalline, Sigma Chemical Company) in 0.9% saline suspension on days 1, 2, and 3 postpartum. The remaining 4 pups in each litter received equivalent volumes of 0.9% saline at the same ages.

All animals were tested for locomotor activity in 4-hr daily sessions from 8 through 15 days postpartum. Prior to testing at 10 days of age, 12 males and 12 females from each of the neonatal treatment groups were assigned to methysergide or saline groups such that activity was nearly equal within each neonatal treatment group for the first two activity sessions. Animals were injected immediately prior to testing at 10–15 days of age with methysergide (1 mg/kg, IP, Methysergide Maleate, Sandoz Pharmaceuticals) or equivalent volumes of 0.9% saline. All activity sessions took place during the light period of the light-dark cycle, and at approximately the same time each day.

RESULTS

An analysis of variance was conducted upon body weights recorded from 10 to 15 days of age, with hormone, drug, sex, and age as factors. Body weight reliably increased with age, $F(5,440)=63.28$, $p<0.0005$, while no significant effect was obtained for drug or sex, F's <1.0. A significant main effect of hormone, $F(1,88)=7.69$, $p<0.01$, reflected the smaller mass of thyroxine-treated animals as compared to controls.

Analysis of variance conducted on the pre-injection activity sessions on days 8 and 9 yielded no pretreatment activity differences for mice assigned to methysergide or saline groups, F<1.0. However, the greater activity of thyroxine-treated animals relative to controls on day 9 was largely responsible for a significant hormone × interval × age interaction, $F(7,616)=3.57$, $p<0.001$.

Figure 1 depicts activity as a function of hormone, drug, age, and 30-min intervals (data are graphed in 30 min intervals for clarity; all analyses conducted over 15 min intervals) for repeated sessions over days 10–15 postpartum (note different ordinate scales for upper and lower panels). From 10 through 15 days of age, activity increased for all groups, $F(5,440)=64.71$, $p<0.0005$, although the main affect for hormone, $F(1,88)=12.29$, $p<0.001$ and a hormone × age interaction, $F(5,440)=3.59$, $p<0.005$, reflected the greater increase in activity with age by thyroxine-treated animals relative to controls.

Methysergide potentiated activity of both thyroxine and saline groups on days 11 through 15, resulting in a main effect of drug, $F(1,88)=7.56$, $p<0.01$ and a drug × intervals interaction, $F(7,616)=11.85$, $p<0.0005$. Neither the drug × hormone nor the drug × hormone × age interaction was significant, F's<1.0. It is evident in the figure that the pattern of activity following methysergide varied with age in a similar fashion for thyroxine and saline groups. Methysergide groups were less active than their respective control

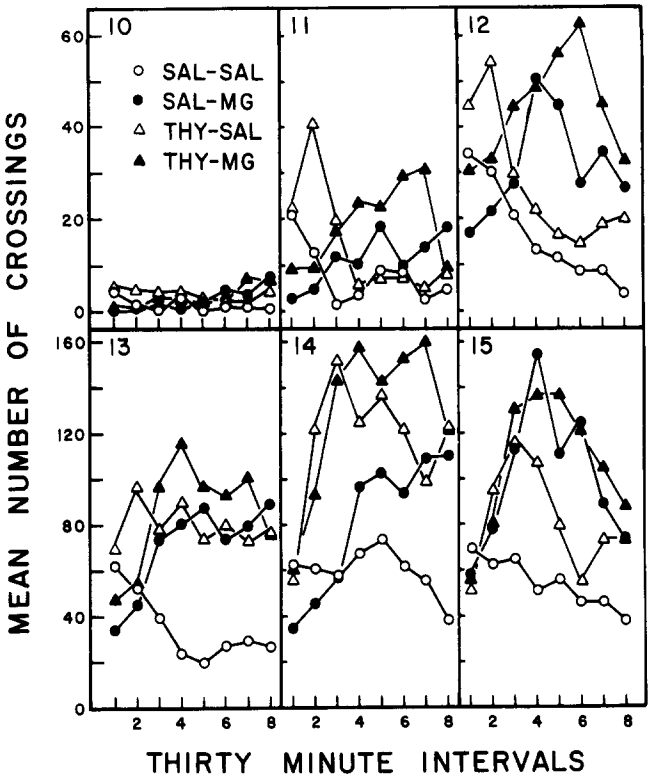

FIG. 1. Mean activity scores as a function of hormone, drug, age, and 30 min intervals (note different ordinate scales for upper and lower panels).

groups during the first hour after injection from 11 through 13 days of age, though this effect occurred only in the saline groups at 14 days of age. Methysergide groups were more active than their controls by the 3rd hour following injection at 11 days, and by the 2nd hour from 13 through 15 days of age. While the differences between methysergide and control groups were greater for saline-treated than for thyroxine-treated animals on days 13 through 15, the pattern of activity of both methysergide groups was similar at these ages.

Because it was possible that interactions of drug, hormone, and age were obscured by the relatively higher activity of all animals at 13, 14, and 15 days of age, separate analyses were conducted on activity data at 10–12 and 13–15 days of age. The analysis for 10–12 days yielded significant drug × age $F(2,176)=3.40$, $p<0.005$ and drug × age × intervals, $F(14,1232)=3.17$, $p<0.0005$ interactions, due to the fact that methysergide did not substantially potentiate activity until 12 days of age. The analysis for 13–15 days of age resulted in a significant drug × intervals interaction, $F(7,616)=6.42$, $p<0.0005$, but drug × age and drug × age × intervals interactions were not significant, F's <1.0, due to the similar effect of methysergide at 13, 14, and 15 days of age. Neither analysis yielded significant drug × hormone or drug × hormone × age interactions, all F's <1.0.

DISCUSSION

The elevated activity and reduced body weight of

thyroxine-treated animals in the present study are in general accord with previous findings for thyroxine-treated animals [16, 17, 18]. Also in accord with previous findings [20], methysergide was found to potentiate locomotor activity of control animals by 13 days of age during the 3rd and 4th hr following injection. Although activity of thyroxine-treated animals was considerably higher than controls from 11 through 15 days of age, the time course of locomotor response to methysergide was similar for thyroxine-treated animals and controls. Moreover, the age at which the disinhibitory behavioral effect of methysergide was first evident was not altered by thyroxine treatments. Thus, the present study failed to provide evidence for altered ontogeny of serotonin-mediated capacities for behavioral inhibition in thyroxine-treated animals.

The present findings were somewhat surprising in light of the widespread effects of neonatal hyperthyroidism on the development of catecholamine [22] and acetylcholine [7,25] systems, and the fact that serotonin systems are affected by acute hyperthyroidism [11] and neonatal hypothyroidism [21]. The failure to obtain effects in the present study is clearly not due to an overall lack of effect of the thyroxine treatments employed, insofar as studies conducted in our laboratory indicate that effects on cholinergic and catecholaminergic systems can be detected following the same thyroxine-treatment regimen used in the present study. In addition to its general acceleration of physical and behavioral development [17], the present thyroxine-treatment regimen produces chronic hyperactivity [16,17] associated with altered catecholamine function [22] and alters the development of those cholinergic systems involved in the modulation of locomotor activity [16]. Notwithstanding these effects, however, it is possible that longer treatments or higher doses of thyroxine are necessary to alter development of those serotonin systems mediating behavioral inhibition. While it is also possible that effects of neonatal hyperthyroidism on serotonin-mediated behavioral inhibition could be detected with higher or lower doses of methysergide than that used in the present study (1 mg/kg), a previous dose-response study [20] indicated that 1 mg/kg methysergide afforded maximal sensitivity to altered ontogenesis of serotonergic behavioral inhibitory capacities. The earliest manifestations of serotonin-mediated inhibition were detected using this dosage, while this dose was also sufficient to detect delayed maturation of serotonin-mediated inhibition in undernourished mice. Thus, it does not seem likely that higher or lower doses of methysergide would have produced effects of great significance in the present study.

It may be premature, however, to rule out the possibility that the ontogeny of serotonin systems was altered by the present neonatal thyroxine treatment. First, the present study examined the effect of only one pharmacological agent, methysergide. Evidence suggests that methysergide does not antagonize the action of serotonin in some brain areas receiving serotonergic input. Methysergide blocks the excitatory synaptic action of serotonin in the reticular formation [9,10], and the inhibitory action of serotonin in the dorsal hippocampus [10], an area postulated to be involved in the control of locomotor activity [12,13]. However, methysergide does not block the inhibitory action of serotonin in the ventral lateral geniculate, the optic tectum, or the amygdala, areas with a heavy serotonin input [9]. The anatomical specificity of methysergide is consistent with recent evidence suggesting that methysergide may affect distinct populations of serotonin receptors [19]. Thus, the behavioral response to methysergide probably reflects only selected aspects of serotonin function. Therefore, it is possible that the ontogeny of the behavioral response to methysergide was unaffected by neonatal hyperthyroidism despite undetected changes in other aspects of serotonin function. Second, the present study examined only one dependent measure, locomotor activity. It is possible that examination of more specific behaviors, in the context of a less selective serotonin antagonist, might yield some effect of thyroxine treatment on serotonergic development.

Because catecholamine systems apparently mediate behavioral arousal [8], enhanced synthesis and utilization of brain catecholamines [21,22] and increased receptor sensitivity [3, 4, 5] may mediate the increased behavioral arousal following neonatal hyperthyroidism. It has been suggested that the apparent accelerated maturation of the forebrain cholinergic inhibitory system following hyperthyroidism represents a necessary compensatory response to this enhanced arousal [16]. If such is the case, the present study provides evidence that serotonin systems which are affected by methysergide, although they are believed to mediate behavioral inhibition, do not participate in such compensation.

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