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Is There a "Serotonergic Syndrome" in Neonatal Rat Pups?

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RISTINE, L. A. AND L. P. SPEAR. *Is there a "serotonergic syndrome" in neonatal rat pups?* PHARMACOL BIOCHEM BEHAV 22(2)265–269, 1985.—Neonatal rat pups given the serotonergic agonist quipazine exhibited alterations in the frequency of several behaviors including an increase in mouthing, forward locomotion, forelimb paddling, unusual position of limbs (UPL), and a decrease in twitching and lying still. The serotonergic antagonist metergoline potently blocked quipazine-induced mouthing and UPL, and partially attenuated the increase in forward locomoting and decrease in lying still induced by quipazine. Pretreatment with the α-noradrenergic antagonist phentolamine partially attenuated the quipazine-induced increase in mouthing, with both phentolamine and the dopaminergic antagonist haloperidol exhibiting some tendency to suppress the forward locomotion induced by quipazine. Thus, serotonin appears to be most important for the expression of quipazine-induced behaviors in the neonate, although there appears to be some catecholaminergic involvement as well. While quipazine may induce in neonates some components of the adult "serotonergic syndrome" [8], there are some clear age differences in the response patterns. Serotonergically-influenced behaviors seen only early in ontogeny may subserve adaptive functions for the young organism.

Ontogeny Quipazine Metergoline Phentolamine Haloperidol Serotonin Catecholamines "Serotonergic syndrome" Mouthing Ingestion

PSYCHOPHARMACOLOGIC manipulations of the serotonergic system in adult rats have been reported to produce alterations in pain sensitivity (e.g., [17]), feeding behaviors (e.g., [7]), sleep (e.g., [10]), and behavioral arousal (e.g., [14]). One widely recognized behavioral model of pharmacological stimulation of the serotonin (5HT) system has been advanced by Jacobs [8]. When adult animals are administered either 5-HT precursors or 5-HT agonists, they are often hyperactive [6] and exhibit a behavioral syndrome characterized by reciprocal forepaw treading, tremor, rigidity, hindlimb abduction, lateral head weaving, and Straub tail. This syndrome has been suggested to be specifically related to central 5-HT receptor activation (e.g., [8]), although there is some controversy as to whether there may be catecholaminergic involvement in the expression of some of these behaviors as well (e.g., [3,5] vs. [8,20].

During ontogeny, adult-typical behavioral alterations in response to serotonergic manipulations do not become evident until around the second to third postnatal week. For instance, Jacobs has reported that 5-HT agonists do not induce the "serotonergic syndrome" in rat pups until 14–17 days postnatally [8]. Similarly, serotonin depletion does not induce increases in stabilimeter cage activity until pups are 15 days of age and older [15]. Although pharmacological manipulations of the serotonergic system do not elicit adult-typical behaviors in rat pups during the first few postnatal weeks, it is clear that such manipulations are effective in altering behavior early in life. For example, we have observed that serotonergic antagonists potently attenuate both suckling behavior [22] and intra-oral milk-induced mouthing behavior [1] in 3–4 day old rat pups. In neonatal rat pups of this age, the serotonergic agonist, quipazine, also has marked behavioral consequences [21].

In neonates, quipazine produces deprivation-dependent mouthing behavior and a number of behaviors, including forward locomotion, forelimb paddling, hindlimb treading, wall climbing and an unusual position of the limbs (UPL), that are not dependent on the period of separation from the dam prior to testing [21]. From this study alone, one cannot classify these behaviors as being characteristic of a neonatal "serotonergic syndrome" given that some or all of the observed effects may be the result of quipazine's interaction with other neurotransmitter systems. In adult animals at least, it has been suggested that quipazine also has partial agonist effects on the catecholaminergic sytems [4]. In order to assess the differential contribution of the serotonergic and catecholaminergic systems to quipazine-induced behaviors in neonatal rat pups, in this experiment 3–4 day old rat pups were pretreated with antagonists of the serotonergic, noradrenergic, and dopaminergic systems prior to quipazine administration. Quipazine-induced behaviors that are primarily a result of serotonergic stimulation should be attenuated by serotonergic antagonist pretreatment, but should remain relatively intact after pretreatment with antagonists of other neurotransmitter systems.

METHOD

One hundred and twelve Sprague-Dawley rat pups at 3–4 days postnatally were used as subjects in this experiment.

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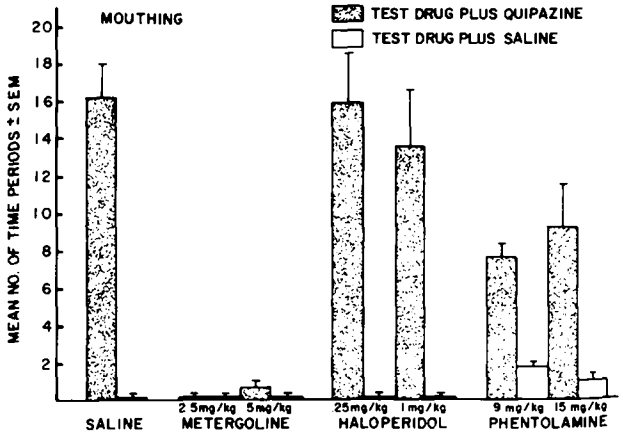

FIG. 1. Effects of serotonergic, dopaminergic and α-adrenergic antagonists on mouthing induced by quipazine in 3–4 day old rat pups. Clear bars represent pretreatment with the antagonist or saline followed by saline treatment. Stippled bars represent pretreatment with the antagonist or saline followed by treatment with 5 mg/kg quipazine.

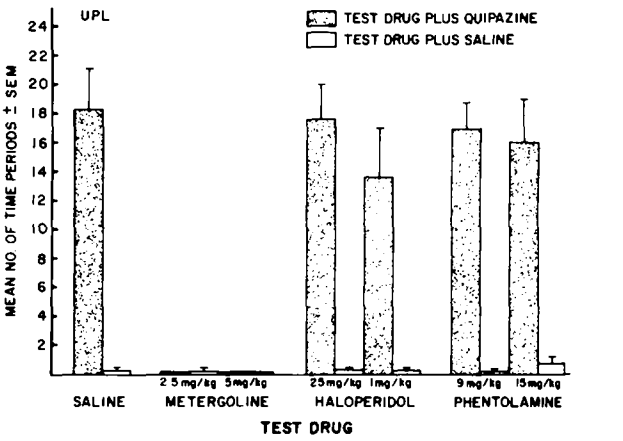

FIG. 2. Effects of serotonergic, dopaminergic and α-adrenergic antagonists on UPL induced by quipazine in 3–4 day old rat pups. Clear bars represent pretreatment with the antagonist or saline followed by saline treatment. Stippled bars represent pretreatment with the antagonist or saline followed by treatment with 5 mg/kg quipazine.

All subjects were derived from established breeding pairs in our colony. Pups were housed with their parents and littermates in standard maternity cages in a vivarium maintained on a 12/12 hour light/dark cycle (lights on at 0700 hr). Litters were culled to ten pups within 24 hours after birth, with the date of parturition being designated as postnatal day 0. Litters containing fewer than seven pups were not used.

Behavioral testing was conducted between 1100 and 1600 hr. All subjects were deprived from the dam for 2–4 hr prior to testing. During the deprivation period, pups were housed with littermates in an incubator maintained at an ambient temperature of 34±1°C. Prior to testing, the pups were subcutaneously injected with saline, 9 or 15 mg/kg/5 cc of the α-noradrenergic antagonist, phentolamine, 0.25 or 1 mg/kg/5 cc of the dopaminergic antagonist, haloperidol, or 2.5 or 5 mg/kg/5 cc of the serotonergic antagonist, metergoline. The animals were then returned to the incubator for 10 min (following saline or phentolamine) or 20 min (following saline, haloperidol or metergoline) prior to being subcutaneously injected with either 5 mg/kg/5 cc quipazine or saline. Ten min following the second injection, each animal was placed in a cylindrical test chamber (10 cm diameter × 11.4 cm high) within a temperature and humidity controlled incubator maintained at 34±1°C. The behavior of each animal in the testing chamber was then videotaped for 10 minutes using a GBC model CTC-2200 video camera with a 8 mm F1.6 lens. The camera was located outside the incubator with the lens positioned approximately 3 cm from the front edge of the testing chamber and tilted at a 45° angle such that the entire testing chamber was visible at all times. A black background and overhead light from a 25 watt bulb in a reflective shield were placed outside the incubator to minimize reflections cast onto the plastic sides of the incubator by the normal fluorescent room lighting. All video data were stored for later behavioral scoring by being recorded on video cassettes via an RCA model VET650 video cassette recorder. Internal body temperatures of all subjects were taken prior to the first injection and immediately following the videotaping session.

Videotapes were scored for behaviors by a trained observer who had no knowledge as to the drug condition of any subject. A time-sampling scoring method was used in which the behaviors of each subject were sampled at 20 sec intervals for a test duration of 10 min, yielding a total of 31 sampling periods. In each sampling period, the pup was observed for 5 sec and the following behaviors were noted if present: mouthe, forward locomotion, forelimb paddle, hindlimb tread, wall climb, lie still, UPL (unusual position of limbs, see [21]), twitch, probe, head weave, groom, and roll or curl. An "other" category on the data sheet was used for description of non-categorized behaviors emitted during the sampling period.

RESULTS

Quipazine-Induced Behaviors

In order to assess the effects of quipazine administration on behavior of the neonatal rat pup, saline control animals (pups receiving both pretreatment and treatment injections of saline) were compared with quipazine-treated animals (pups pretreated with saline followed by 5 mg/kg quipazine). Quipazine significantly increased mouthing, $F(1,14)=88.67$, $p<0.001$, UPL, $F(1,14)=38.20$, $p<0.001$, and forward locomotion, $F(1,14)=9.95$, $p<0.01$, as seen in Figs. 1–3. Quipazine also significantly increased forelimb paddling, $F(1,14)=6.92$, $p<0.025$, (saline/saline: 0.2 ± 0.2; saline/quipazine: 16.4 ± 1.7). The amount of time spent lying still was significantly decreased by quipazine, $F(1,14)=36.00$, $p<0.001$, (see Fig. 4). Twitching behavior was also decreased by quipazine, $F(1,14)=7.33$, $p<0.025$, (saline/saline: 8.9 ± 3.0; saline/quipazine: 0.7 ± 0.4). None of the other sampled behaviors were found to be significantly affected by quipazine administration in these one-way analyses of variance (ANOVAs).

Effects of Antagonists

To evaluate the efficacy of the antagonists for attenuating or otherwise altering the incidence of quipazine-induced be-

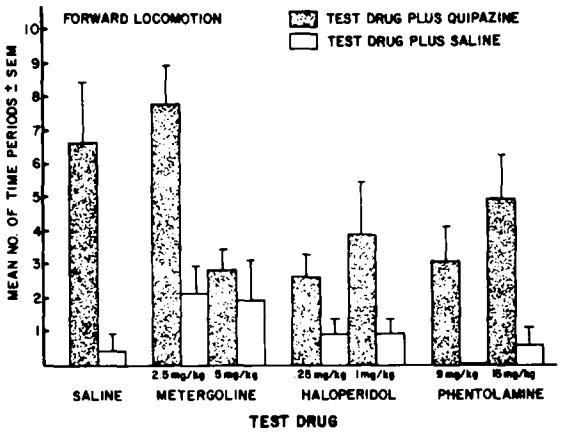

FIG. 3. Effects of serotonergic, dopaminergic and α-adrenergic antagonists on forward locomotion induced by quipazine in 3–4 day old rat pups. Clear bars represent pretreatment with the antagonist or saline followed by saline treatment. Stippled bars represent pretreatment with the antagonist or saline followed by treatment with 5 mg/kg quipazine.

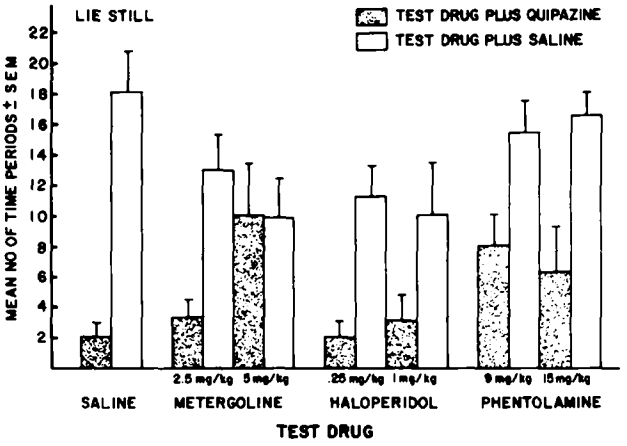

FIG. 4. Effects of serotonergic, dopaminergic and α-adrenergic antagonists on the quipazine-induced decrease in amount of time spent lying still in 3–4 day old rat pups. Clear bars represent pretreatment with the antagonist or saline followed by saline treatment. Stippled bars represent pretreatment with the antagonist or saline followed by treatment with 5 mg/kg quipazine.

haviors, for each of the three antagonists, a 3 Pretreatment (dose of antagonist or saline) × 2 Treatment (quipazine or saline) ANOVA was performed on data for each behavior.

Mouthing. For mouthing behavior, there were significant Pretreatment × Treatment interactions for both metergoline, $F(2,42)=78.29$, $p<0.001$, and phentolamine, $F(2,42)=10.29$, $p<0.001$. As can be seen in Fig. 1, metergoline potently blocked quipazine-induced mouthing behavior. Phentolamine also significantly attenuated the mouthing induced by quipazine, although this effect was much less pronounced than that seen with metergoline (see Fig. 1). The dopaminergic antagonist, haloperidol, did not alter quipazine-induced mouthing.

UPL. As can be seen in Fig. 2, metergoline administration potently attenuated quipazine-induced UPL (interaction: $F(2,42)=38.40$, $p<0.001$). Administration of the catecholaminergic antagonists did not affect the incidence of this quipazine-induced behavior.

Forward locomotion. There was a significant Pretreatment × Treatment interaction of metergoline on forward locomotion, $F(2,42)=3.62$, $p<0.05$. The higher dose of metergoline significantly attenuated the quipazine-induced increase in forward locomotion. There were no other significant effects of the antagonists on this quipazine-induced behavior although, as can be seen in Fig. 3, there appeared to be some trend toward suppression of quipazine-induced locomotion by the catecholaminergic antagonists.

Lie still. There was a significant Pretreatment × Treatment interaction for metergoline with lying still, $F(2,42)=6.62$, $p<0.01$. The higher dose of metergoline significantly attenuated the quipazine-induced decrease in lying still as can be seen in Fig. 4. The catecholamine antagonists had no effect on the incidence of this quipazine-influenced behavior, although there appeared to be a trend for phentolamine to attenuate the quipazine effect (see Fig. 4).

Forelimb paddle. There was a significant main effect of metergoline on forelimb paddling, $F(2,42)=8.76$, $p<0.001$. The lower dose of metergoline was observed to increase forelimb paddling (saline: 2.1±0.81; 2.5 mg/kg metergoline: 7.1±1.06; 5 mg/kg metergoline: 3.4±0.85). However, there were no significant interactions of either metergoline, phentolamine or halperidol with quipazine for forelimb paddling, suggesting that none of the antagonists significantly influenced the magnitude of the quipazine-induced increase in this behavior.

Twitch. There were no significant interactions in the ANOVAs for whole body twitching, a behavior that is presumably indicative of REM sleep early in life [11]. Thus, none of the antagonists significantly altered the quipazine-induced decrease in twitching behavior.

Probe. While probing behavior was not one of the behaviors found to be significantly altered by quipazine administration (see preceding summary of saline/saline vs. saline/quipazine one-way ANOVA's), there was a main effect of metergoline on this behavior, $F(2,42)=26.78$, $p<0.001$. Both doses of metergoline increased probing behavior (saline: 0.88±0.40; 2.5 mg/kg metergoline: 19.0±2.25; 5.0 mg/kg metergoline: 13.4±2.53). There were no significant interactions of either metergoline, haloperidol or phentolamine with quipazine for probing.

Body temperature. A 2 Trials (Pre-test vs. Post-test) × 3 Pretreatment (Dose of Antagonist or Saline) × 2 Treatment (Quipazine or Saline) ANOVA was performed on body temperature data from pups under each of the three antagonist conditions. There were no significant main or interactive effects of quipazine, metergoline or phentolamine on body temperature. Haloperidol administration at the 0.25 mg/kg dose produced slight hyperthermia, $F(2,42)=3.50$, $p<0.05$ (controls: 30.3±0.34°C; 0.25 mg/kg haloperidol: 31.6±0.37°C; 1 mg/kg haloperidol: 30.2±28°C).

DISCUSSION

Animals treated with the serotonergic agonist, quipazine, exhibited an increase in mouthing, forward locomoting, UPL and forelimb paddling and a decrease in lying still and twitching. Quipazine-induced mouthing and UPL were potently attenuated by administration of both doses of the serotoner-

gic antagonist, metergoline. The higher dose of metergoline also partially attenuated the increase in forward locomoting and the decrease in lying still seen following quipazine. Thus, it appears that 5-HT is important for the expression of these quipazine-induced behaviors in the neonate, although there may be partial catecholaminergic involvement as well. Quipazine-induced mouthing was partially attenuated by administration of phentolamine. In addition, both catecholamine antagonists tended to suppress the incidence of quipazine-induced forward locomotion and phentolamine tended to attenuate the decrease in lying still seen following quipazine administration, although these tendencies did not achieve statistical significance. Thus, in neonates, as in adults [4], quipazine appears to function primarily as a serotonergic agonist although it may have partial catecholaminergic agonist effects as well.

Serotonergic agonists (including quipazine, [12]) given to adult rats have been reported to elicit a number of behaviors such as head weaving, reciprocal forepaw treading, tremor of the head and forelimbs, hindlimb abduction and Straub tail (e.g., [8,9]) as well as hyperactivity [6]. The "serotonergic syndrome" elicited by administration of the serotonergic agonist, quipazine, to neonates is clearly different in several respects from that seen in the adult. Head weaving and Straub tail are not elicited by quipazine in neonates. Moreover, one of the prominent behavioral features of quipazine treatment in neonates is mouthing, a behavior that is not seen following 5-HT agonist administration to adult rats, although it has been reported to be a component of the "serotonergic syndrome" in adult pigeons and rabbits (see [8] for a discussion). Other behaviors elicited by quipazine in neonates may show some similarities to the "serotonergic syndrome" in adults. Forelimb paddling induced by quipazine in neonates may be similar to reciprocal forepaw treading seen as a part of the adult "serotonergic syndrome." Also, the quipazine-induced increase in forward locomoting and decrease in lying still seen in neonates is reminiscent of the hyperactivity induced by serotonergic agonists in adult rats. In both neonates and adults, one of the primary effects of serotonergic stimulation is an alteration in the position of the limbs characterized by hindlimb abduction in adults and what we call "UPL" in neonates. UPL is a characteristic posture of the fore- and hindlimbs involving upward flexion of the limbs with the paws being held in the air. In the forelimbs, paw elevation is often associated with reciprocal tremor of the paws (see [21] for a discussion). Thus, whereas tremor of the head and forelimbs is characteristic of serotonergic stimulation in adults, this tremor is restricted to the distal portion of the forelimbs in neonates. UPL in the hindlimbs is characterized by elevation of the paws with an exaggerated lateral angle of the limbs away from the body, a position that appears somewhat analogous to the hindlimb abduction seen after 5-HT agonist administration in adulthood.

Mouthing induced by quipazine and elicited by the intraoral infusion of milk to deprived neonates are both markedly attenuated by 5-HT antagonists ([1]; present study). Thus, there appears to be a strong serotonergic component to mouthing in neonates. Although the potential functional significance of serotonergically-mediated mouthing for the neonate is not yet ascertained, there is some evidence that such mouthing may be a critical component of normal ingestive behavior early in life. Neonatal rat pups given serotonergic antagonists search and probe for nipples but do not mouthe and lick the nipples (see [22]), behaviors that serve as normal precursors to attachment (e.g., [16]). Consequently, serotonergic blockade results in a failure of attachment [22]. Thus, serotonin may facilitate the mouthing component of suckling attachment in neonates. This facilitatory role, however, appears to be characteristic of only the early postnatal period. While serotonergic antagonists decrease suckling behavior of rat pups during the first week or so of life [19,22], they conversely increase suckling in older preweanling and weanling animals [19,28]. A similar ontogenetic shift is seen neurochemically with 24 hours of food and/or maternal deprivation significantly increasing 5HIAA levels in rat pups primarily during the first 7–10 days of life, and not thereafter [23].

Functional maturation of neurotransmitter systems may not be an all-or-none process. Nascent portions of neurotransmitter systems, in addition to serving as ontogenetic precursors of the adult nervous system, may subserve special functions early in development to mediate behaviors critical for the young organism. For instance, portions of the serotonergic system, particularly in caudal brain regions, appear to be functional in even neonatal rat pups [13, 18, 26, 27] and may serve to facilitate mouthing behavior critical for suckling attachment early in life. Later in ontogeny, with the maturation of other components of the serotonergic system, some of the early functions of these precocious portions of the serotonergic system may be suppressed as adult-typical responses emerge. When examining the functional ontogeny of neurotransmitter systems, it may be important to assess not only the emergence of adult-typical behaviors in response to pharmacological challenges, but also the influence of these manipulations on behaviors of particular significance to the developing organism.

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