Pharmacology Biochemistry & Behavior, Vol. 14, pp. 831–834, 1981. Printed in the U.S.A.

Quipazine-Induced Behavior in Neonatal Rat Pups

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Received 24 January 1981

SPEAR, L. P. AND L. A. RISTINE. *Quipazine-induced behavior in neonatal rat pups.* PHARMAC. BIOCHEM. BEHAV. **14**(6) 831–834, 1981.—Three day old Sprague-Dawley rat pups were placed in an incubator at room temperature (22±1°C) or nest temperature (35±1.5°C) and observed using a behavioral-time sampling procedure following injection of saline or quipazine (1, 2.5, 5, 10 mg/kg). Quipazine administration induced a marked behavioral activation that was characterized by increases in forward locomotion, wall climbing, forelimb paddling and hindlimb treading, the magnitudes of which were not dependent upon ambient temperature. In a second experiment, quipazine was also observed to induce mouthing behavior that was dependent upon the length of the mother-pup separation interval. These results suggests that administration of a serotonergic agonist can have marked behavioral effects in the neonatal rat pup, which reinforces previous neurochemical observations that have indicated the presence of ample postsynaptic serotonin substrates in neonatal rats. Possible implications of these results are discussed.

Developmental psychopharmacology Quipazine Serotonin Neonates Behavioral activation Mouthing behavior

THE EARLY ontogenetic responses to drugs potentiating the catecholamine neurotransmitter systems have been examined in several studies. During the first postnatal week catecholamine agonists such as L-DOPA [5] and amphetamine [9] induce increases in locomotor movements, suggesting that there may be an early ontogentic development of the catecholamine systems. In contrast, however, the development of the serotonergic system has been postulated to occur later in ontogeny, during the second and third week of life. For example, serotonergic antagonists such as methysergide stimulate suckling in pups from 15 days of age, while serotonergic agonists such as quipazine inhibit suckling behavior in deprived 10- and 21-day old rat pups [7]. Similarly, serotonin depletion has been shown to increase stabilimeter cage activity beginning at 15 days postnatally [6].

Although these early studies suggest that serotonin does not become functional in affecting behavior until the second to third postnatal week, there is neurochemical evidence that serotonin substrates are present a good deal earlier. For example, the amount of serotonin binding as well as serotonin-sensitive adenylate cyclase is greater in the early neonatal period than in adulthood in a variety of brain areas [11,12], which may indicate the presence of hypersensitive serotonergic receptor-enzyme systems that serve some special function in the immature brain (see [12]). Yet, there is a dearth of studies examining psychopharmacological responsiveness to serotonergic manipulation in the neonatal period. In the suckling and stabilimeter cage studies discussed above [6,7], the youngest animals tested were 10 days of age. Jacobs [3] reported in a review article that serotonergic agonists did not induce adult-typical behaviors, such as resting tremor, rigidity, reciprocal forepaw treading, hindlimb abduction, Straub tail and lateral head weaving, in rats until 14–17 days postnatally. Yet, in the same article Jacobs [3] briefly mentioned that such agonists had "clear and dramatic behavioral effects" in neonatal rat pups, although the specific behaviors induced by the drugs were not specified.

In view of the neurochemical evidence for an early development of serotonergic substrates, it is important to examine the effects of serotonergic stimulation on behavior in the neonatal period. The present study examined the psychopharmacological effects of the serotonergic agonist, quipazine (e.g., [8]), in 3-day old rat pups.

EXPERIMENT 1

METHOD

Three day old Sprague-Dawley rat pups bred from established breeding pairs in our laboratory were used as experimental subjects. Litters were culled to 10 within 24 hours after birth. The animals were maintained on a 12/12 hr light-dark cycle with lights on at 0700 hrs. All testing occurred between 1100 and 1600 hrs. At the beginning of testing, the entire litter was removed from the home nest and placed in a holding incubator kept at 35°C. Pups were singly removed from the incubator immediately prior to injection and testing, and then replaced in the holding incubator with littermates after testing.

Eighty 3-day old pups were given subcutaneous injections of 1.0, 2.5, 5.0 or 10.0 mg/kg/5 cc quipazine maleate (Miles Laboratories) or a 0.9% saline control solution and individually placed in a test chamber (11.4 mm×10.0 mm diameter)

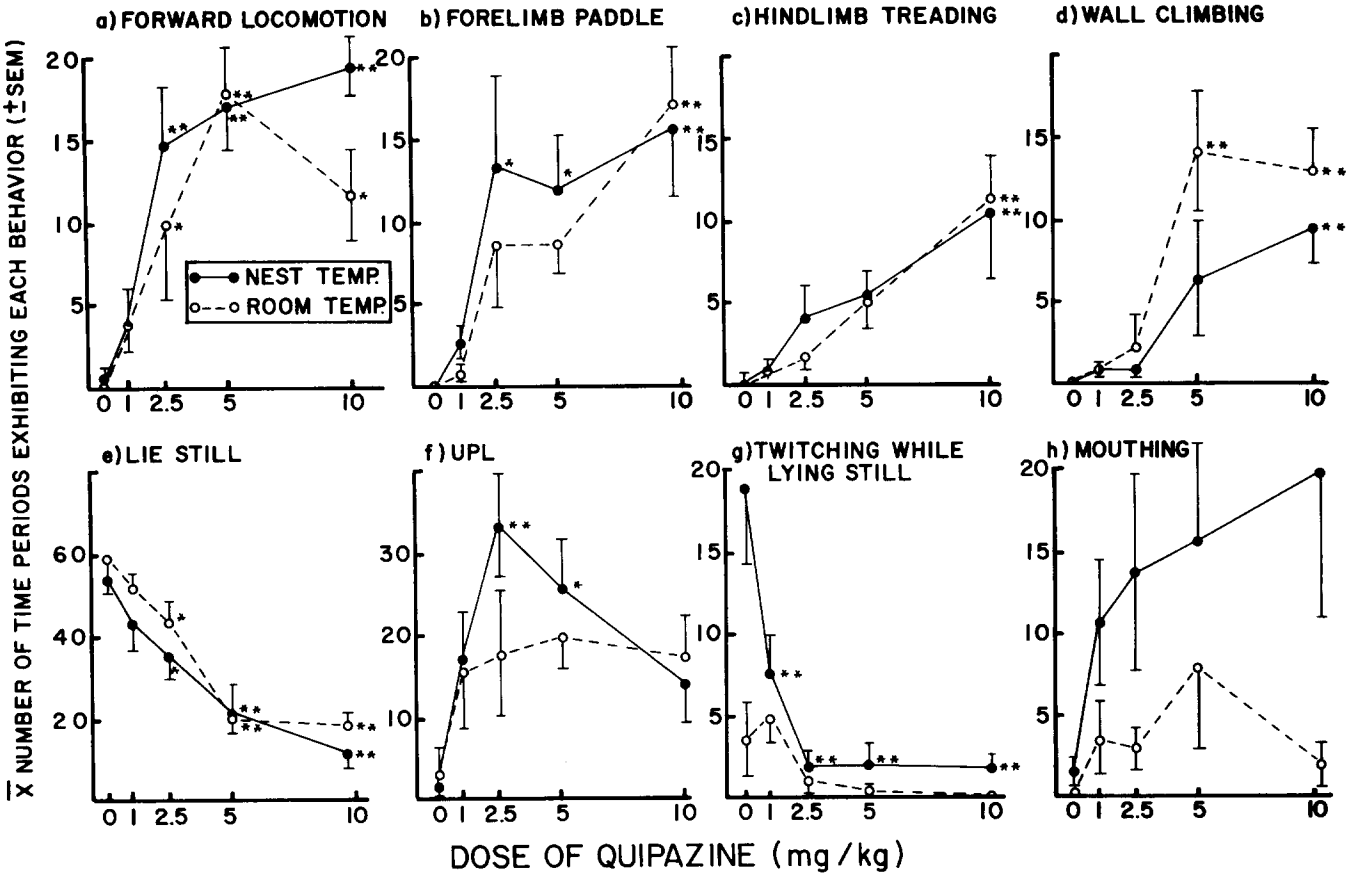

FIG. 1. Effects of 0 (saline), 1, 2.5, 5 and 10 mg/kg quipazine and room (23±1°C) or nest (35±1.5°C) ambient temperatures on behaviors measured by the behavioral time-sampling technique in 3-day old rat pups. *Significantly different at a $p \leq 0.05$ from saline control pups from the same ambient temperature condition (Tukey-A tests). **Significantly different at a $p \leq 0.01$ from saline control pups from the same ambient temperature condition (Tukey-A tests).

made of Plexiglas with a fine wire mesh floor. The entire test chamber was housed within a clear Plexiglas incubator maintained at either room temperature (22±1°C) or nest temperature (35±1.5°C). One pup from each litter was placed into each of the 10 (5 drug dose×2 ambient temperature) treatment conditions. Beginning 5 min post-injection, behavioral time-sampling data were collected every 20 sec for a test duration of 20 min (see [10] for details of the behavioral time-sampling procedure). At each of the 60 sampling periods, the pup was observed for 5 sec and the emitted behavior was recorded. Categories included: lie still, groom, mouth, twitch, forward locomotion, tread with hindlegs, paddle with forelimbs, wall climb, and other (used for description of all noncategorized behaviors). Internal body temperatures were taken immediately pre- and post-test using a rectal probe (YSI #511). To assess differences in total number of instances of each behavior during the 60 sampling periods, a 2 (ambient temperature)×5 (drug dose) analysis of variance was conducted for each of the behaviors and post-hoc Tukey-A tests were applied.

RESULTS

At both ambient temperatures, quipazine induced a marked dose-dependent increase in many behaviors, including forward locomotion, $F(4,70)=16.179$, $p \leq 0.001$, forelimb padding, $F(4,70)=10.100$, $p \leq 0.001$, hindlimb treading, $F(4,70)=11.209$, $p \leq 0.001$, and wall climbing, $F(4,70)=13.446$, $p \leq 0.001$, as well as a dose-dependent decrease in the amount of time spent lying still, $F(4,70)=29.380$, $p \leq 0.001$ (see Fig. 1a–e). Quipazine also induced, at both ambient temperatures, a characteristic posture and tremor of the forelimbs and hindlimbs frequently evident when the animals were stationary—the paws were held up in the air with the "elbows" on the floor of the apparatus (abbreviated "UPL" for "usual position of limbs," see Fig. 1f). There were no significant main effects of ambient temperature, nor any interaction between drug dose and ambient temperature, for any of these behaviors.

Twitching behavior emitted while lying still is presumably indicative of REM sleep early in life [4]. For this measure there were significant effects of Dose, $F(4,70)=10.026$, $p \leq 0.001$, Ambient Temperature, $F(1,70)=12.269$, $p \leq 0.001$ and Dose×Ambient Temperature, $F(4,70)=4.566$, $p \leq 0.05$. Quipazine decreased twitching behavior of pups tested at nest, but not room temperature, while saline control pups twitched more at nest temperature than at room temperature (all p's ≤ 0.01) (see Fig. 1g).

The only significant effect on mouthing behavior was an effect of Ambient Temperature, $F(1,70)=10.267$, $p \leq 0.001$,

with animals at nest temperature mouthing more than those at room termperature. Although Fig. 1h suggests that quipazine might have induced selective increases in mouthing behavior, this was not significant in the overall analysis, due to marked variability in the amount of mouthing behavior within treatment conditions. Examination of the mouthing data suggested that variability in the amount of mouthing might be related to the length of the deprivation period. The pups were away from the mother throughout the entire testing period (which took approximately 4 hrs for a complete litter). This possibility was tested in terms of a correlation between time of testing within the test session and amount of mouthing for animals tested at nest temperature. This correlation, $r(32)=.51$, was significant ($p\leq0.01$), suggesting that pups tested after long periods of deprivation exhibit more mouthing than those tested after relatively short periods of separation from the mother and nest.

A 2 (ambient temperature)×5 (drug dose)×2 (pre-test vs post-test) analysis on the internal body temperatures indicated that Dose had no main effect and did not enter into any significant interaction; thus, quipazine had no effect on body temperature. As expected, post-test body temperatures of animals tested at room temperature were lower than those of animals tested at nest temperature (all Tukey-A tests with p's≤0.01; Temperature, $F(1,89)=133.99$, $p\leq0.001$; Trials, $F(1,79)=213.69$, $p\leq0.001$; Interaction, $F(1,79)=100.35$, $p\leq0.001$, effects).

The results of this experiment indicate that the serotonergic agonist quipazine induces a marked behavioral activation in 3-day old rat pups tested at both room and nest temperature. The results also tentatively suggest that the amount of mouthing after quipazine may be positively related to length of deprivation as well as ambient temperature, a hypothesis examined more systematically in Experiment 2.

EXPERIMENT 2

METHOD

Eighty 3- to 4-day old Sprague Dawley rat pups were deprived from their mother in a temperature and humidity controlled incubator maintained at 35°C for 0, 2, 4 or 16 hrs prior to testing. At the time of test, pups were given subcutaneous injections of saline or 2.5 mg/kg/5 cc quipazine and placed in a test chamber (see Experiment 1) housed in an incubator maintained at 35° (+1.5°) C. One pup from each litter was placed into each of the 8 (4 deprivation×2 drug dose) treatment conditions. Beginning 5 min after injection, for a test duration of 20 min, behavioral time sampling data were collected every 20 sec using the procedures outlined in Experiment 1.

RESULTS

As in Experiment 1, quipazine increased wall climbing, $F(1,72)=20.948$, $p\leq0.001$, forward locomotion, $F(1,72)=53.424$, $p\leq0.001$, forelimb paddling, $F(1,72)=12.751$, $p\leq0.001$, hindlimb treading, $F(1,72)=6.085$, $p\leq0.005$, and UPL, $F(1,72)=201.17$, $p\leq0.001$, and decreased the amount of lying still, $F(1,72)=42.060$, $p\leq0.001$. There was no main effect of deprivation on these behavioral measures. Amount of twitching while lying still was decreased by quipazine, $F(1,72)=2.994$, $p\leq0.05$, and higher levels of deprivation, $F(3,72)=8.771$, $p\leq0.001$, although there was no significant interaction between quipazine and deprivation.

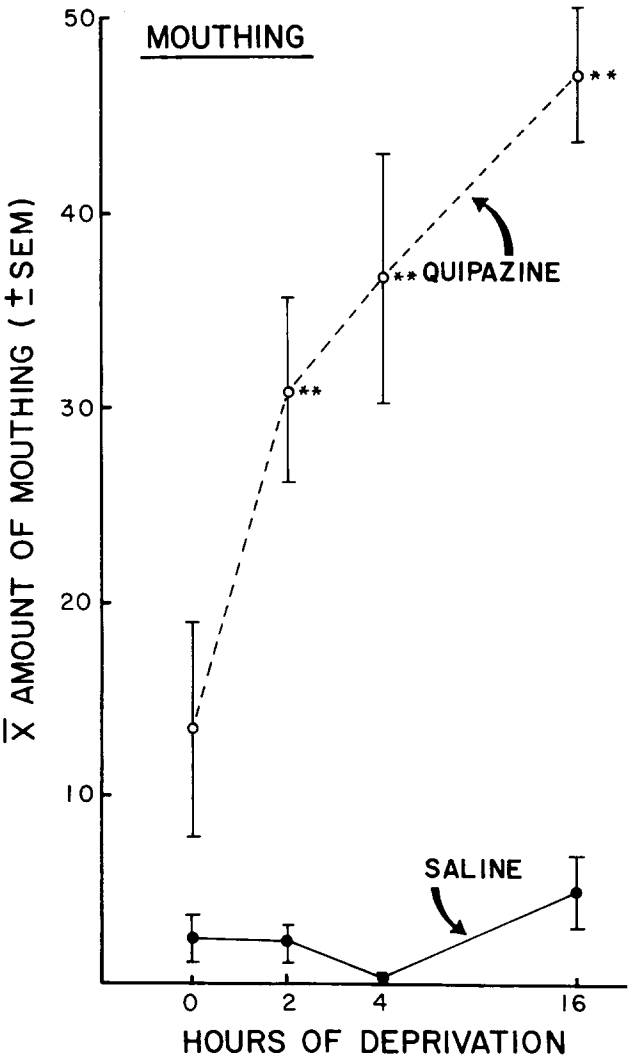

FIG. 2. Effects of varying the mother-pup separation interval on mouthing behavior of 3–4 day old rat pups given saline or 2.5 mg/kg quipazine. **Significantly different at a $p\leq0.01$ from the saline control group under the same deprivation condition (Tukey-A tests).

Mouthing was the only variable other than twitching while lying still that was significantly affected by length of deprivation. There was significant Dose, $F(1,72)=22.859$, $p\leq0.001$, Deprivation $F(3,72)=40.140$, $p\leq0.001$, and Dose× Deprivation, $F(3,72)=6.385$, $p\leq0.001$, effects on mouthing behavior. While the amount of mouthing of saline-treated animals did not vary with length of deprivation, quipazine-induced mouthing markedly increased over the deprivation intervals (see Fig. 2).

Analysis of internal body temperatures revealed main effects of Dose, $F(1,72)=5.512$, $p\leq0.025$, and Trials, $F(1,72)=7.435$, $p\leq0.001$. Following the test, quipazine-treated animals had significantly lower body temperatures (32.2°C±0.21 SEM) than either their body temperatures pretest (33.1°C±0.28 SEM) or the post-test body temperatures of saline-treated animals (33.2°C±0.20 SEM) (both p's≤0.05).

GENERAL DISCUSSION

The results of the present study indicate that the serotonergic agonist, quipazine, induces marked behavioral activation characterized by increases in forward locomotion, wall climbing, forelimb paddling and hindlimb treading in infant rat pups. The magnitude of this activation is not dependent upon ambient temperature or length of deprivation. Quipazine also induces mouthing behavior that is dependent upon the length of the mother-pup separation interval. These results suggest that the postsynaptic substrates for serotonin are behaviorally functional early in postnatal life, reinforcing previous neurochemical observations that have indicated the presence of ample postsynaptic serotonin substrates in neonatal rats [11,12].

Quipazine not only induced increases in locomotor-type movements such as forward locomotion, forelimb treading, hindlimb paddling and wall climbing behavior. It also induced an unusual position of the limbs characterized by the "elbows" of both the fore- and hindlimbs being held on the floor with the paws elevated off of the floor surface. Whether this behavior is a precursor of the hindlimb abduction that is characteristic of serotonergic stimulation in adult animals [3] is a matter of speculation.

Another behavior induced by quipazine was mouthing behavior, the only quipazine-induced behavior found to be correlated with the length of the mother-pup separation interval. The significance of a deprivation-dependent induction of mouthing by quipazine is interesting though puzzling. Although deprivation has been shown to increase mouthing in rat pups given oral infusions of milk [2], studies examing the psychopharmacological characteristics of this response have not been published.

One could argue that quipazine may be inducing these marked behavior effects early in life through interaction with other neurotransmitter systems. Indeed, there is some evidence in adult animals that quipazine also has effects on the β-adrenergic system [1]. However, if the behavioral effects of quipazine in neonates were largely due to interactions with other neurotransmitter systems, one would expect that the effects of quipazine would be mimicked by psychopharmacological agents that affect these other neurotransmitter systems. From the limited data available, this does not appear to be the case. For example, while the catecholamine agonists, L-DOPA, apomorphine and clonidine have been reported to induce forward crawling as well as head-raising in neonatal rats [5], other behaviors induced by quipazine in the present experiments were not observed.

Clearly, more work is needed to clarify the effects of serotonergic manipulation early in postnatal life. Most psychopharmacological work with serotonin in development has focused on the development of typical adult behavior patterns after pharmacological manipulation of serotonin beginning at 10 days postnatally or later. However, the serotonergic agonist, quipazine, was shown in the present experiment to induce an impressive array of behaviors in neonatal animals. Moreover, neurotransmitter systems may have special functions during development, prior to the time that they control adult behaviors, to promote age-specific behaviors that although critical to the neonate, are quite different from those of the adult. These behaviors may not be noted in psychopharmacological investigations focusing on the development of adult-typical responses to the test drugs. For example, it is interesting to speculate that perhaps the deprivation-dependent increase in quipazine-induced mouthing seen in the present study may reflect the presence of an early maturing serotonergic system that is involved in mediating suckling behavior of neonates. Yet, whether early developing portions of the serotonergic systems are functional in modulating suckling or other critical behaviors characteristic of infancy is a matter for future consideration and more detailed investigation. The results of the present study form a basis for further work directed towards understanding the potential roles of serotonin in modulating unique age-specific behaviors of the neonatal period.

ACKNOWLEDGEMENTS

This research was supported in part by Grants 0144-03-240-78 O from the Research Foundation of the State of New York and RO1MH33215 from the National Institute of Mental Health.

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