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Suckling Behavior of the Infant Rat: Modulaton by a Developing Neurotransmitter System

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NOCK, B., C. L. WILLIAMS AND W. G. HALL. *Suckling behavior in the infant rat: modulation by a developing neurotransmitter system.* PHARMAC. BIOCHEM. BEHAV. 8(3) 277–280, 1978. – Drugs which alter serotonin receptor activity modified the suckling behavior of 20-day-old rat pups. Suckling could be reinstated in nondeprived pups, which normally do not suckle, by blockade of serotonin receptors with methysergide. Stimulation of serotonin receptors with quipazine inhibited suckling in deprived pups, and this effect was prevented by methysergide pretreatment. This evidence suggests that suckling in weaning age pups is controlled by a serotonergic inhibitory mechanism.

Suckling behavior Development Serotonin Methysergide Quipazine

SUCKLING is the rat pup's principal behavior during an important phase of postnatal neural development, and as such may provide an opportunity to relate the development of a complex behavioral system to neurophysiological, neuroanatomical, and neurochemical maturation. The experiments reported here are based on earlier findings which demonstrated the emergence of an inhibitory control of suckling between the 10th and 15th day of age [13,14]. Prior to this age, rat pups will rapidly attach to the nipples of their anesthetized mother, whether deprived (of food, water, and suckling) or nondeprived. After 15 days of age only deprived pups reliably attach to their mother's nipples and suckle. Because this transition in suckling occurs during the period when monoaminergic neurotransmitter systems are maturing [3, 20, 21, 22], and since these systems have been implicated in the expression of adult ingestive behavior [2, 4, 5, 27, 28, 29], we have begun to investigate their involvement in the development of suckling behavior. As an initial step in this analysis, we report here several interesting effects of acute manipulations of serotonergic neurotransmission on suckling behavior in the 20-day-old rat pup.

EXPERIMENT 1

If the emergent inhibitory control of suckling is dependent on serotonergic (5–HT) activation, acute blockade of 5–HT neurotransmission could conceivably cause 20-day-old pups to suckle in a fashion similar to that displayed by younger animals (i.e., suckle, regardless of deprivation state). We tested this hypothesis by observing the suckling behavior of 20-day-old rat pups after treatment with the 5–HT receptor antagonist methysergide [1, 7, 12].

Method

Animals. Eight litters, the progeny of primiparous Charles River Breeder CD strain rats were used. After mating, the pregnant females were housed in large cages on wood shavings bedding with chow and water always available. Females were checked for births each afternoon and pups discovered then were considered born on that day (day of birth = 0-days old). The colony room was maintained at a temperature of 22–24°C on a 14:10 light-dark cycle. Pups were 20 days old at testing.

Procedure. The testing paradigm utilized the pups' anesthetized mothers in order to isolate the specific capabilities and characteristics of the pup which are normally obscured by the mother's active participation in suckling [6, 8, 13, 14]. In this situation the anesthesia also blocks milk letdown, so that during suckling tests, pups received no milk [19].

For each of the eight litters, two pups were deprived by removing them for their home cages 18–20 hr prior to testing and placing them together in a small cage with no food or water available. Nondeprived pups remained with their mother until she was removed for anesthetization (with 2 ml/kg Chloropent, IP, Fort Dodge Laboratories). At the time of testing, one deprived pup and one nondeprived pup received a single injection of methysergide maleate (Sandoz Pharmaceuticals, 20 mg/kg salt, 10 ml/kg, IP), and one deprived pup and one nondeprived littermate control

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received saline (10 ml/kg). Methysergide in this dose range is behaviorally effective in adult rodents [18,22]. Higher doses produced ataxia.

Behavioral tests began about 1400 hr and lasted for one hr. The anesthetized mother was placed on her back in a clear plastic tub, with both nipple lines exposed. Pups were then injected and immediately placed at their mother's side. The four littermates (one pup in each experimental group) were checked for nipple attachment (on or off) at five-min intervals (12 checks). This time-sampling technique provided a good estimate of time attached in the 60 min test, since episodes of suckling at this age usually last for many minutes after the initial attachment. In addition, the general behavior of the pups was observed throughout the test.

Results

Methysergide produced a clear and significant stimulation of suckling in nondeprived pups (drug by deprivation interaction, $F(1,7) = 10.8$, $p<0.05$). Nondeprived pups treated with methysergide spent a mean of 37.5 (± 5.6) min attached to the nipple compared to 17.5 (± 8.0) min for vehicle controls (difference significant by Scheffé's Test). Nipple attachment differences between deprived and nondeprived pups obtained in these tests replicated results obtained using shorter tests [13,14]. Saline injected, deprived pups attached for a mean of 58.5 (± 0.2) min compared to only 17.5 (± 8.0) min for nondeprived pups, $F(1,7) = 27.2$, $p<0.05$.

The effectiveness of methysergide in stimulating suckling is particularly impressive when it is recognized that there is an absorption delay before the drug becomes effective; in the second half of the test, the suckling of the nondeprived, methysergide-treated pups actually equaled that of the deprived pups ($\bar{X}$ = 27.5 ± 2.3 and 29.5 ± 0.6 min, respectively).

Figure 1 depicts this time course effect of methysergide on nipple attachment. The difference between the drug and vehicle groups progressively increased during the test.

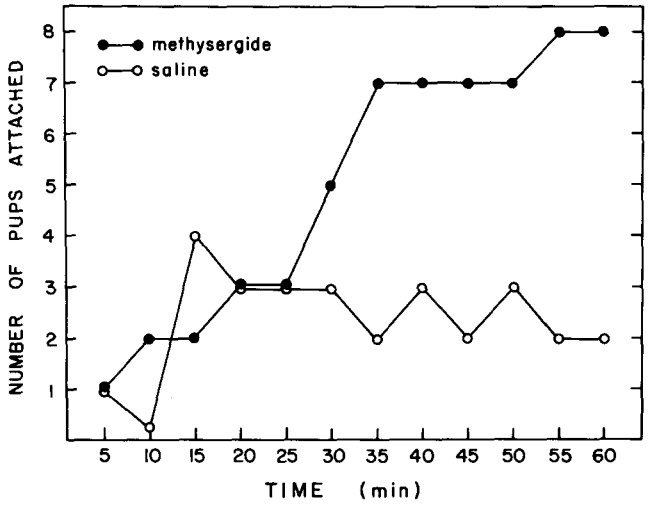

FIG. 1. Stimulation of suckling behavior in nondeprived, 20-day-old rat pups by methysergide (20 mg/kg). Data are expressed as total number of pups in each group (n = 8) attached at 5 min intervals of a 60 min test.

Since all deprived pups suckled for almost the entire 60 min, there was no significant difference in mean time attached for methysergide-treated, deprived pups (59.4 ± 0.6 min) and saline-treated, deprived pups (58.5 ± 0.2 min). However, in a follow-up study using a similar testing paradigm, we have noted that the drug-treated pups tend to suckle from a single nipple while deprived control animals shift from nipple to nipple during the test (vehicle 16.8 ± 2.3 shifts, methysergide 3.0 ± 0.7 shifts, $t = 5.7$, $p<0.05$, $n = 6$). This lack of nipple shifting in the methysergide-treated pups is similar to that seen in young pups (less than 15 days) which do not shift from one nipple to another once they become attached [13,14]. This lack of nipple shifting, along with methysergide induced suckling, suggests a regression to an earlier behavioral stage.

EXPERIMENT 2

In an attempt to further assess serotonergic involvement in the mediation of suckling behavior, we treated 20-day-old deprived pups with the 5–HT receptor agonist quipazine [15, 16, 26], either alone or in combination with a methysergide pretreatment. Since we found in Experiment 1 that blockade of 5–HT receptors with methysergide increased suckling, stimulation of 5–HT receptors with quipazine should decrease suckling in deprived animals. Specificity to the 5–HT system would be further indicated if methysergide pretreatment blocked any quipazine-induced effects.

Method

Animals. Eight, 20-day-old litters bred and maintained as in Experiment 1 were used.

Procedure. Three pups from each litter were deprived for 18–20 hr prior to testing. Pups in all groups received two injections (10 ml/kg each, IP), one 45 min prior to testing (pretreatment) and one 30 min prior to testing (treatment). One deprived pup from each litter received a saline pretreatment followed by quipazine (Miles Labs., Inc., 10 mg/kg), a second pup received a methysergide maleate (20 mg/kg salt) pretreatment followed by quipazine (10 mg/kg); and a third pup received two saline injections. Quipazine in this dose range is behaviorally and physiologically effective in adult rats [17,27]. Higher doses produced behavioral stereotypies. The testing and scoring procedures were identical to those used in the first experiment.

Results

As seen in Fig. 2, quipazine inhibited nipple attachment in deprived, 20-day-old pups, dramatically reducing mean time suckling (drug effect, $F(2,14) = 1006$, $p<0.05$; the quipazine group was significantly different than the other two groups by Scheffé's Test). The quipazine-treated animals were alert and they actively investigated and occasionally licked the nipples of their anesthetized mother but they rarely attached. Pretreatment with methysergide blocked the inhibitory effects of quipazine on suckling behavior (Fig. 2). The pups in this group did not differ from the deprived, saline-treated control animals; pups in both groups rapidly attached to their mothers' nipples and suckled for most of the 60 min test.

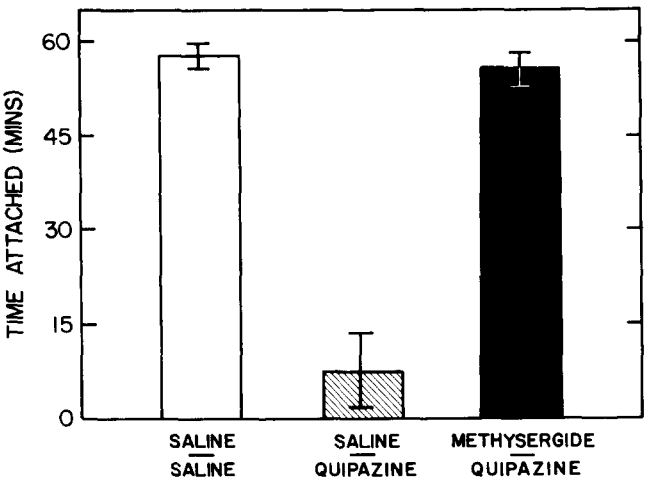

FIG. 2. Inhibition of suckling behavior in deprived, 20-day-old rat pups by quipazine (10 mg/kg) and reestablishment of suckling with a methysergide (20 mg/kg) pretreatment. Data were collected using a 5 min interval time-sampling technique (see text) and are expressed as mean (± SEM) min attached per group (n = 8), during a 60 min test.

DISCUSSION

The results of these experiments indicate that 5–HT receptor blockade with methysergide stimulates suckling in nondeprived pups, and that 5–HT receptor stimulation with quipazine has the opposite behavioral effect, inhibiting the suckling of deprived pups. Although the specificity of both quipazine and methysergide has been questioned [9, 10, 11, 18], a common serotonergic receptor site for the behavioral effects of these drugs is supported by the data which shows that methysergide pretreatment antagonizes the behavioral effects of quipazine. In addition, we have recently found that the 5–HT receptor blocker metergoline [30] also stimulates suckling in older rat pups, whereas drugs that affect catecholaminergic receptor activity (pimozide, phenoxybenzamine, and apomorphine) have little or no effect on suckling behavior. Together, these results suggests a serotonergic inhibitory mechanism controlling the suckling of weaning age rat pups.

It is also known that 5–HT mechanisms are involved in temperature regulation [23], and we have found that methysergide at the dose used in these experiments is hypothermic. However, it is unlikely that methysergide-treated pups suckle because they are cold, since we have found that cooling nondeprived pups to 32°C in a refrigerator does not stimulate suckling behavior. Furthermore, quipazine (10 mg/kg) which inhibits suckling, also appears to be slightly hypothermic. Thus, the changes in suckling behavior reported here do not appear to be related to drug-induced changes in body temperature. In addition, because reinstatement of suckling behavior was induced by acute pharmacological manipulations, it can not be attributed to chronic metabolic changes.

Central 5–HT mechanisms have been implicated in the control of food intake in adult rats [2, 4, 5, 27, 28, 29], yet, it is unlikely that the reinstatement of suckling observed in this study is simply the result of making the nondeprived pups hungry. Two kinds of evidence support this statement. First, we have preliminary data which indicates that methysergide reliably stimulates suckling in 30-day-old pups (deprived and nondeprived). At this age, even 24 hr food deprivation fails to reliably stimulate suckling behavior. Thus, simply making a pup hungry is not a sufficient cause for it to suckle. Second, given the choice between eating rat chow or attaching to their mother's dry nipples, deprived 20-day-old pups eat food pellets and suckle while nondeprived methysergide-treated pups only suckle (Williams and Hall, unpublished observations). Therefore, food deprivation and methysergide treatment appear to have different effects on the appetitive behavior of 20-day-old rat pups.

These data, in fact, suggest hypotheses about feeding effects obtained with serotonergic manipulations in adult animals [10]. Adult effects may be related to specific disinhibition or inhibition of only one component of the adult feeding system, rather than to general satiety or hunger processes. That is, a treatment in the weaning age pup which may alter perception of oral stimulation and reinstate suckling in preference to feeding, may in the adult lead to an increased attention to food and feeding in the absence of other appropriate oral manipulanda. In this way, the presumed reestablishment of suckling in the present study may also reflect the types of processes which underlie neurological regression and the reappearance of neonatal reflexes (e.g., suckling, rooting) in humans suffering from neurological trauma or senility [25].

In neonatal pups, suckling has the appearance of a reflex behavior. However, in the brief preweaning period, this initially simple behavior sequentially develops internal controls. The pharmacological stimulation and inhibition of suckling in 20-day-old rats reported here, suggest that, in part, the development of these controls may be related to the emergence of an inhibitory serotonergic system. This study also suggests that suckling may provide a behavioral system for investigating the emergence of neurotransmitter function and points to the usefulness of suckling as a model system for the study of the development of complex motivated behaviors.

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