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BRIEF COMMUNICATION

The GABA-A Agonist Muscimol Facilitates Muscular Twitches and Locomotor Movements in the Neonatal Mouse

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TIRELLI, E. *The GABA-A agonist muscimol facilitates muscular twitches and locomotor movements in the neonatal mouse.* PHARMACOL BIOCHEM BEHAV 33(2) 497–500, 1989.—The effects of nonsedative doses of muscimol, a postsynaptic GABA-A agonist, on neurobehavioral activities in 5- and 11-day-old newborn mice were assessed using an observational point sampling procedure. Muscimol activated the emission of muscular twitches after injections of 0.025 or 0.050 mg/kg in 5-day-old pups, and 0.075 mg/kg in 11-day-old pups. At 0.075 mg/kg, the GABA agonist also produced an increase of locomotor movement levels in the younger age group. Given that muscimol at low dosages typically produces an increase of locomotion in mature mice, it is suggested that the GABAergic activity involved in locomotor behaviors is functional very early in life. Furthermore, since twitching behavior exhibited while lying presumably indicates paradoxical sleep early in life, it is speculated that muscimol may have activated this form of sleep in our newborn mice.

Muscimol GABA-A receptors Neonatal mouse Muscular twitches Locomotion Ontogenetic ethopharmacology

IN mature rodents, systemic administration of muscimol, a specific GABA agonist acting on the bicuculline-sensitive GABA-A receptors, typically induces an inhibition of spontaneous locomotor activity at relatively high doses (higher than 1–1.5 mg/kg), but causes activation of this behavior with dosages ranged from 0.025 to 1 mg/kg (3, 7, 17, 29). Increasing evidence for an involvement of the striato-nigral and striato-pallidal GABA systems in the mechanisms of expression of locomotor behaviors suggests that a systemic GABAergic compound could exert its behavioral effects by modifying the activity of these systems (16). However, the precise mechanisms by which low doses of muscimol induce effects opposite to those of higher doses are unknown.

As far as the sedative consequences of a net GABA activation are concerned, it has been reported that immature rodents aged less than 2 weeks may respond behaviorally to muscimol, at doses much smaller than those used in adults. Thus, in neonatal rats aged between 3 and 12 days, muscimol at 0.2 or 0.4 mg/kg can induce an attenuation of body rolling movements when pups are placed on a warm plate and of turning behavior while they are held in air by the tail (5,6). Furthermore, systematically given GABA (20 mg/kg) has also been reported to reduce general motility monitored by an electronic activometer in neonatal 8–9- and 12–13-day-old rats, as is the case in adult rodents intraperitoneally treated with 100 mg/kg GABA (19,22). Of the relatively few studies that have examined the early ontogeny of GABAergic function, only one has reported that muscimol may produce activating effects in neonatal rodents that resemble those observed in adulthood. Spear and her colleagues (24) have shown that muscimol at 0.125 mg/kg was able to reduce time spent in lying still behavior (behavioral inactivity) in neonatal 3–4-day-old rat pups. However, this behavioral energization was not specifically observed in locomotor behaviors, indicating a broad activating effect of the GABA agonist on general activity.

It is of interest that selective motoric inhibition as well as activation have been described in rat pups aged 3 to 12 days subcutaneously injected with benzodiazepines, which act on targets structurally linked to the GABA-A receptor complex (4, 13–15, 23). The motoric inhibition can be induced by chlordiazepoxide and diazepam (at 1 to 10 and 0.5 to 4.5 mg/kg, respectively) on turning behavior when 9–12-day-old rats are held in air by the tail and on head-raising in 4–16-day-old rats (5, 6, 15). The motoric activation induced by these benzodiazepines, as well as by flurazepam, clonazepam and lorazepam in a large range of doses, involved locomotor behaviors (such as swimming and wall progression) and/or an increase in the emission of muscular twitches while the pup lies still. The latter is one of the most incident behaviors in the ethogram of the neonatal rodent [brief and intermittent jerks of varying amplitude involving the head, limb(s), tail or the whole body when lying still], and is probably an early expression of 'REM' sleep (1, 8, 11, 23, 27).

Using systemic administration of muscimol, the most representative GABA-A agonist, the present study was aimed at specifying the behavioral role of GABA receptors in the neonatal mice. Considering that muscimol at small doses is able to induce an increase of locomotion in mature mice and that benzodiazepines can activate locomotor behaviors as well as twitching in newborn rats, it can be expected that low doses of the GABA agonist may bring about such effects in neonatal mice. In order to maximize a reliable assessment of selective excitatory effects of the GABA-A agonist, a period of observation of the behavioral repertoire much longer than those from the above-mentioned ontogenetic studies, was used (75 instead of 1 to 10 minutes).

METHOD

Animals and Breeding

The subjects were 128 Carworth Farms-1 newborn mice, aged 5 or 11 days, obtained from 16 litters born at our laboratory colony. Animals were kept under colony conditions of 23 ± 1°C, 12:12 light-dark cycle beginning at 0800 hours, uncontrolled humidity and free access to full-protein pellet food with tap water. Four virgin females were placed with a sire and pregnancy was confirmed by the presence of sperm in a vaginal smear. Pregnant females were checked for births daily at 1100 and 1700 hours, with the day following birth being considered as the first day of postnatal life. At birth, the dams were housed separately with their litters, which were culled on day 2 to eight pups in a balanced representation of males and females. Pups were used only once.

Procedure

Muscimol hydrobromide (SYNTHELABO) was dissolved in 0.09% NaCl saline vehicle so that the volume to be injected was always to 0.01 ml/g body weight. Pilot studies have indicated that muscimol at doses higher than 0.3 mg/kg produces in mice aged 11 days a prolonged behavioral inertia and a total loss of righting reaction. Nonsedative doses of the GABA agonist were chosen on the basis of these results. Two pups from each litter, one per sex, were randomly assigned to one of the four drug treatment conditions in a factorial design, where Muscimol (four levels) was crossed with Sex (two levels). In this experimental design the total number of litters is equal to the number of subjects in each cell (n = 8). Pups received in the nape of the neck a subcutaneous injection of saline solution or 0.025, 0.050, 0.075 mg/kg muscimol. They were immediately thereafter put singly into the experimental chambers which consisted of 32 × 11 × 11 cm clear Plexiglas boxes with a paper flooring. A red 40-W bulb suspended 18–20 cm above each field maintained the chamber temperature at 34 ± 1°C. Neither blind procedure nor simultaneous scoring by two observers were used, since pilot studies had shown that these procedures were not necessary. Behavioral evaluations began 30 min after muscimol injection. They were performed using a point sampling technique for a duration of 75 min. With this technique the behavioral score is expressed as the total amount of all instantaneous samples on which the behavior pattern occurs (number of occurrences). Pups were checked for various behavioral categories (see Table 1) at the initiation of the test and every 1 min, for a total of 76 sample points.

All testing was done in the light cycle (between 0900 and 1700), in a diffusely illuminated room maintained at 23 ± 1°C.

Statistical Analysis

Sex (2 levels) and dose of Muscimol (4 levels) were taken as between-subjects factors in an analysis of variance where the litter was considered as random factor in a randomized blocks design. No comparisons were conducted across ages and animals given each dose treatment were compared with subjects of the same age given the vehicle. Treatment effects were further evaluated for meaningful individual comparisons by Dunnett's multiple comparison test. Differences were considered significant when the probability of falsely rejecting the null hypothesis (alpha level) was less than 0.05.

TABLE 1

BEHAVIORAL CATEGORIES

1. Lying still quietly
2. Muscular twitches while lying still (twitching)
3. Head-raising (sniffing in air)
4. Locomotion, turning and rolling on back (locomotor movements)
5. Immobility
6. Grooming
7. Wall-climbing position with front paws treading
8. Wall-climbing position without treading
9. Oral activities (licking, mouthing)

RESULTS

Muscimol had significant effects only on locomotor movements and on muscular twitches while lying still quietly. There were neither effects of Sex, nor interactions between dose of Muscimol and Sex, for any of the behaviors under study. The effects of muscimol on lying still, twitching, locomotor movements and head-raising, the most incident behaviors of neonatal mice when placed in our experimental conditions, are depicted in Fig. 1.

The GABA agonist at 0.075 mg/kg enhanced the mean number of occurrences for locomotor movements in 5-day-old pups, $F(3,21) = 9.22$, $p<0.001$, while the two lower doses (0.025 and 0.050 mg/kg) facilitated the emission of muscular twitches when pups are in lying position, $F(3,21) = 15.63$, $p<0.0005$.

Among the 11-day-old pups, muscimol did not substantially modify the levels of locomotor movements at any dose, but did significantly increase twitching at 0.075 mg/kg, $F(3,21) = 3.40$, $p<0.050$. The muscimol-induced increase of muscular twitches seems to be selective at both ages, since the time spent lying still, during which twitches were emitted, did not increase at all.

At both ages head-raising tended to be slightly reduced, but without reaching statistical significance.

DISCUSSION

The GABA-A agonist muscimol can induce a reliable motoric activation when injected at relatively small dosages in neonatal rodents. This activation had two distinct aspects: at 0.075 mg/kg, muscimol produced a clearcut locomotor excitation, and at lower dosages (0.025 and 0.050 mg/kg), a multiplication of muscular twitches while lying still in 5-day-old pups. The emission of muscular twitches can also be facilitated by the GABA-A agonist at 0.075 mg/kg in the 11-day-old pups.

The muscimol-induced locomotor activation obtained in our neonatal mice is reminiscent of that observed in adult mice where low muscimol doses (0.025 to 1 mg/kg) have been reported to increase levels of locomotion (3, 7, 17). Furthermore, as reported in the Method section, pilot studies have indicated that doses higher than 0.3–0.4 mg/kg muscimol produce marked behavioral depression in neonatal mice, as is the case in adults treated with

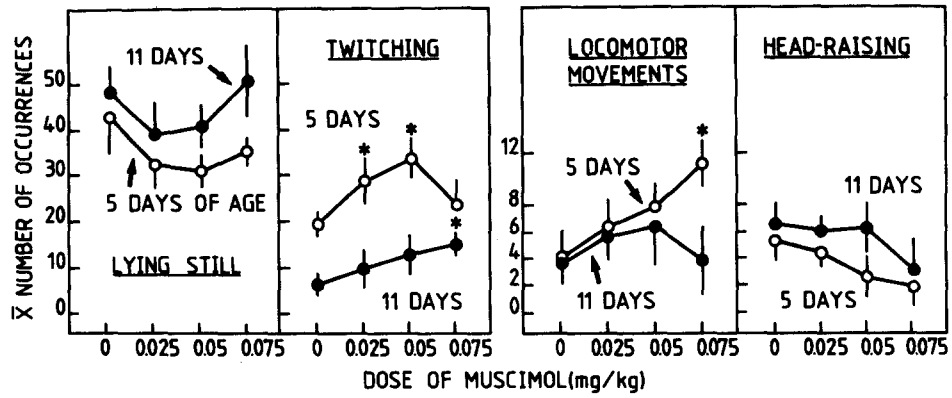

FIG. 1. Effect of muscimol 0.025, 0.050 and 0.075 mg/kg and saline vehicle subcutaneously injected on the mean number of occurrences of locomotor movements, head-raising, lying still and twitching while lying still quietly in neonatal mice aged 5 or 11 days. The direct GABA-A agonist was given 30 min prior to the beginning of a 75-min observation period. Means (± S.E.M.) result from cell means pooled across sex (n = 16). *$p<0.050$ as compared to the saline-treated pups group (Dunnett's test after analysis of variance).

relatively high doses (more than 1–1.5 mg/kg) of the GABA agonist (17,20). Therefore, it may reasonably be concluded that the pattern of behavioral responsiveness to muscimol typically seen in adult mice is present in newborns even though sensitivity to muscimol is higher in developing mice. Muscimol appeared to be more efficient in the younger pups aged 5 days than in the 11-day-old ones, confirming the already reported continuous decline in sensitivity to muscimol as the animals mature until adulthood (5, 6, 26). Such a differential effect between early ages may be due to differences in capacity of the mice to metabolize and/or excrete muscimol, or to factors affecting the penetration of the drug into the brain, such as maturing blood-brain barrier. This latter factor may be of particular significance because muscimol is metabolized in the periphery with only a small percentage of the parent compound reaching the site of action (14). Therefore, it might be that a muscimol dose higher than 0.075 mg/kg can activate locomotor movements in 11-day-old mouse pups, by compensating the lower sensitivity to the GABA-A agonist at this age. However, in absence of precise data dealing with the early ontogeny of the blood-brain barrier permeability to muscimol, no robust interpretations about the potential role of a maturing blood-brain barrier in the behavioral effects of systemic muscimol in newborn mice can be offered.

Our results are in substantial agreement with those published by Spear and associates (24) even if they did not consider twitching in their report. In fact, these authors described a robust decrease of mean time spent in lying still after 0.125 mg/kg muscimol in 3–4-day-old pups. Even though this dose of muscimol did not conversely produce a significant increase in any of the motoric behaviors under study (forward locomotion, regularly alternating limb placing movements, rolling on back or side), such a trend was evident, which collectively accounts for the decrease in lying. Since behavioral effects of muscimol may last more than 60 min in rodents, the duration of the observational period in that study (10 min) might have been insufficient to adequately reflect a potential increase of locomotor behaviors.

A number of studies suggest a dopaminergic and serotonergic engagement in the expression of twitching in the newborn rat, injections of apomorphine (a dopamine agonist) and quipazine (a serotonin agonist) having been shown to specifically inhibit its exhibition (20,25). Furthermore, chlorpromazine and L.S.D., two enhancers of the serotonergic activity, simultaneously suspend electroencephalic signs of paradoxical sleep and muscular twitches in neonatal rats and kittens (2,22), while diazepam, which acts on the GABA/benzodiazepine receptor complex, promotes the occurrence of electrocorticographic sleep spindles (23). In this latter experience it is not clear, however, whether the motoric activation which accompanies sleep spindles related to twitches. Our results, along with those showing a stimulating action of benzodiazepines on twitching (see introduction), provide further information on the potential mechanisms subserving this age-specific behavior and suggest that it may have not only monoaminergic but also GABAergic bases. Taken together these findings are consistent with the view that monoaminergic and GABAergic receptors participate in the regulation of 'REM' sleep in adult altricial mammals (9, 12, 18, 28). Therefore, it raises the possibility that the facilitatory action of muscimol on muscular twitches may reflect a concomitant promotion of paradoxical sleep in our neonatal mice. Clearly, it is only through simultaneous measurement of electroencephalic activity and behavioral twitching, as done for example by Corner and Mirmiran with chlorpromazine (2), that is would be possible to determine if the enhancing action of GABA agonists on this behavior depends on a specific increase of paradoxical sleep.

In conclusion, the results of the present report indicate that the administration of the GABA-A agonist muscimol to neonatal mice may result in an energization of two distinct forms of motoric activity, probably reflecting different functions: muscular twitching, possibly related to paradoxical sleep, and at a stronger GABAergic mobilization, locomotor activity. Such effects confirm studies describing neonatal neurochemical and physiological activities in rodent GABA systems (10,26), and strongly suggest an early functional maturation of the GABAergic output systems involved in motoric behaviors of the newborn rodent.

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