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Age-Dependent Changes in Serotonergic Modulation of Yawning in the Rat

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URBÁ-HOLMGREN, R., B. HOLMGREN, B. A. LEON AND A. UGARTE. *Age-dependent changes in serotonergic modulation of yawning in the rat.* PHARMACOL BIOCHEM BEHAV 43(2) 483–486, 1992.—Serotonin (5-HT) effects on physostigmine (PHY)-induced yawning were studied in LY Sprague-Dawley rats by injecting Lu 10 171 (citalopram), a specific 5-HT uptake blocker, and two antagonists—methiothepine and ritanserin—which differ slightly in the selectivity of their actions on different 5-HT receptor subtypes. Infant and young rats show significant increases in PHY-induced yawning when preinjected with citalopram (5–10 mg/kg). Two-month-old animals show this effect only with 10 mg/kg. With adult animals (3–5 months old), the effect is the opposite: Yawning decreases. The facilitatory effect in infant and young rats was counteracted by methiothepine but not by ritanserin, suggesting that it is mediated through 5-HT_{1A} or 5-HT_{1B} receptor subtypes. The inhibitory effect of citalopram in adult rats was unmodified by the two antagonists used, leaving open the possibility that it is mediated by 5-HT_3 receptors.

Yawning Ontogeny Serotonin Physostigmine Lu 10 171 (citalopram) Methiothepine Ritanserin

IN a previous article (23), it was reported that physostigmine (PHY)-induced yawning in infant (6–7 days old) and young (45 days old) rats was significantly increased when animals were pretreated with Lu 10 171, (citalopram), a selective serotonin (5-HT) uptake inhibitor (12). Because this effect was counteracted by metergoline, which blocks 5-HT receptors, and this drug per se did not show any action on yawning, it was suggested that 5-HT may exert a positive modulatory effect on this behavior. Similar results were obtained when infant rats were tested with fluoxetine and pirandamine, two other 5-HT uptake inhibitors (11).

A few years later, Okuyama et al (17) reported that both apomorphine (APO)- and PHY-induced yawning in adult rats were reduced by administration of the 5-HT precursor 5-hydroxytryptophan. Moreover, they found that APO- but not PHY-induced yawning was enhanced by pretreatment with *p*-chlorophenylalanine, a 5-HT synthesis inhibitor, or by the serotonin neurotoxin 5-7 dihydroxytryptamine. Because these drugs do not elicit yawning when given alone, their results led them to suggest that 5-HT exerts a negative modulatory influence on yawning.

Although different serotonergic drugs were used in the above-mentioned studies, perhaps the main difference between them was the age of rats, a methodological fact that might have led to the opposite findings. Therefore, it was decided to repeat the experiments with citalopram, exploring its effects on rats 15 days to 5 months old from a Sprague-Dawley inbred subline of rats characterized by its low incidence of spontaneous yawning (LY subline) (24). From this last point of view, these animals may be comparable to the Wistar rats used in the former experiments (23).

The discovery of several subtypes of central 5-HT receptors (3,5) and the subsequent development of selective serotonergic agonists and antagonists allows a new approach for research on the role of this neurotransmitter in yawning behavior. To extend previous findings, two of these compounds were used—methiothepine (5-HT_1/5-HT_2 antagonist) and ritanserin (5-HT_{1C}/5-HT_2 antagonist)—to counteract citalopram effects (3,5,7,13).

METHOD

Animals

Male Sprague-Dawley rats from the LY subline (24) were used. They were born in our animal house, weaned when 30 days old, and then housed in Plexiglas collective cages in groups of four. They were maintained under standard conditions with a 12 L : 12 D cycle (lights on at 0700 h); food (standard Purina rodent pellets) and drinking water were given ad lib.

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Behavioral Observations

Rats were allowed to habituate to the experimental room conditions for 1 h. In the case of infant rats, they were separated from their mother immediately before the experiment began. Observations were performed with each rat placed in a transparent glass cylinder (diameter 19 cm, height 10 cm), the floor of which was covered with a clean filter paper and the top with a Plexiglas plate, leaving a 1-cm wide segment open for ventilation. The observations were done between 0900–1000 h to minimize circadian variations (1,2). Yawning was monitored by two observers sitting on opposite sides of the table on which animals were placed. A yawn was scored when a rat opened its mouth wide and gradually, retained the open position during a couple of seconds, and closed the mouth rapidly. The movement was usually (but not always) accompanied by extension of the neck and one or both forelimbs. The results are expressed as frequencies (number of yawns/time).

Drugs

The following compounds were used: citalopram HBr (H. Lundbeck & Co. A/S, Copenhagen-Valby, Denmark); physostigmine sulfate (Sigma Chemical Co., St. Louis, MO); methiothepine maleate (Hoffmann-La Roche, Basle, Switzerland); and ritanserin (Janssen Pharmaceutical Ltd., Beerse, Belgium).

Drugs were dissolved in distilled water and further diluted with saline to reach the standard injection volume of 2 ml/kg body weight. Drug doses refer to the weight of the base. Methiothepine (IP) and ritanserin (SC) were administered 60 min before and citalopram (IP) 30 min before PHY, which was injected (IP) at the standard dose of 0.10 mg/kg. Controls received saline. Animals were used only once.

Statistics

Results are expressed as means ± SE. A Kruskal–Wallis analysis of variance (ANOVA) was first carried out and, if significant, a Mann–Whitney *U*-test was used to compare controls with drug-treated groups (20). Significance was accepted at $p < 0.05$.

RESULTS

Effect of Citalopram on Spontaneous Yawning

To test the action of citalopram per se, 15-day- and 2-month-old rats were injected with 5, 10, and 20 mg/kg and observed for 1 h. Results are shown in Table 1. No significant changes in yawning frequency were observed with infant or 2-month-old animals.

TABLE 1

EFFECT OF CITALOPRAM ON SPONTANEOUS YAWNING

	15 Days		2 Months	
	n	MYF SE	*n*	MYF SE
Control	24	1.9 ± 0.5	18	2.9 ± 0.9
Citalopram (mg/kg)				
5	8	2.3 ± 1.0	8	3.6 ± 1.4
10	8	4.6 ± 1.4	8	2.6 ± 0.6
20	8	2.8 ± 0.7	8	2.1 ± 0.6

Animals were injected with the drug and observed for the following hour. *n*, number of Sprague-Dawley LY rats; MYF, mean yawning frequency; SE, standard error. Kruskal–Wallis test, NS for both ages.

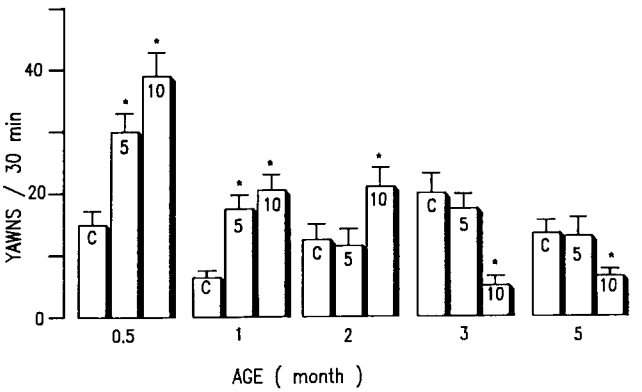

FIG. 1. Age-dependent effects of citalopram on PHY-induced yawning. Control rats were injected with saline (−30 min) and PHY, at the standard dose of 0.10 mg/kg. Citalopram (5 or 10 mg/kg) was injected 30 min before PHY administration time, at which yawn monitoring began, rats being observed for 30 min. $n = 14$–16 control rats were used for each age group, 8–10 citalopram-pretreated rats as experimental subjects. Statistics: Kruskal–Wallis test, $p < 0.01$ for both sets of doses; Mann–Whitney *U*-test, $*p < 0.02$ or less when each pretreated group was compared with its control.

Effect of Citalopram on PHY-Induced Yawning in Rats of Different Ages

When PHY was injected in citalopram-pretreated rats (5 or 10 mg/kg), different effects were observed. All animals, whatever their age, showed noticeable increases in oral activity, gaping, and salivation. In addition, some 15-day-old rats exhibited short and occasional tremor episodes (four of eight animals injected with 10 mg/kg). In regard to yawning, Fig. 1 shows the results obtained: The lower dose of citalopram (5 mg/kg) led to important increases in yawning frequency in the 15-day- and 1-month-old rats, but older animals yawned at the same level as controls. On the other hand, with a higher dose (10 mg/kg) yawning also increased in frequency, even in the 2-month-old rats, but decreased significantly in the 3- and 5-month-old animals.

Effect of Methiothepine and Ritanserin on Citalopram Actions on PHY-Induced Yawning

Figure 2 shows the results obtained when these two antagonists were used to counteract citalopram effects. In general, methiothepine (0.25 mg/kg) + citalopram (10 mg/kg) + PHY (0.10 mg/kg)-treated animals showed less oral activity, salivation, and gaping than saline + citalopram + PHY-treated ones. Among infant rats, tremor persisted after methiothepine. When ritanserin (0.25 and 0.50 mg/kg) was used, the trembling episodes disappeared. Methiothepine (0.25 mg/kg) antagonized completely the citalopram facilitation of PHY-induced yawning in 15-day-old rats, but was quite ineffective in modifying the decrease in PHY-induced yawning produced by citalopram in adult animals. On the other hand,

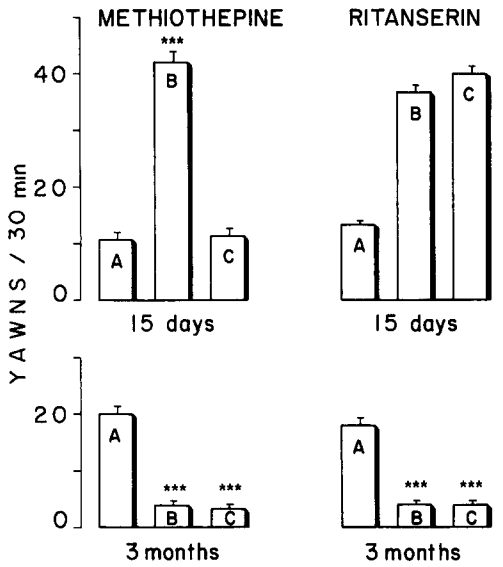

FIG. 2. Methiothepine and ritanserin effects on citalopram actions on PHY-induced yawning. Methiothepine (0.25 mg/kg) and ritanserin (0.25 mg/kg) were injected 60 min before PHY; citalopram (10 mg/kg) was injected 30 min before yawning induction with PHY. Ordinate: number of yawns observed during 30 min after PHY administration at the standard dose of 0.10 mg/kg. (A), saline-saline-PHY; (B), saline-citalopram-PHY; (C), antagonist-citalopram-PHY. n = 8-10 male rats in each group. Statistics: For the four experiments, $p < 0.01$ (Kruskal-Wallis test); ***$p < 0.001$ when compared with control (Mann-Whitney U-test).

ritanserin (0.25 mg/kg) did not antagonize citalopram effects in either infant or adult rats. Ritanserin was also tried at a higher dose (0.5 mg/kg): The same results were obtained.

DISCUSSION

The age-related effects of citalopram on PHY-induced yawning here described agree with previous reports on the pharmacological manipulation of serotonergic systems modulating yawning. Former results with infant and young rats (23) were confirmed: These animals show a significant increase in PHY-induced yawning when preinjected with citalopram. On the other hand, and confirming Okuyama et al.'s (17) results, an increase in serotonergic tone in adult rats reduces yawning. Hence, it seems necessary to consider these results in the context of what is now known about the development of 5-HT neurones in the rat brain after birth.

It is well known that rats are born neurologically and behaviorally immature and that neuronal and glial growth in the CNS are mainly postnatal events. Loizou (14), studying the postnatal ontogeny of monoamine-containing neurones in the CNS of the rat with histochemical and biochemical techniques, found that at the end of the first postnatal week 5-HT terminals in the lower brain stem and the spinal cord have almost the same densities as in the adult. Mesencephalic, diencephalic, and telencephalic 5-HT terminal proliferation occurred later, between the second and third weeks. Loizou also reported that adult 5-HT concentration is reached at the end of the fourth week in all brain areas with the exception of the telencephalon, in which at this period it has only reached a level of 68%. The main features of these results have been confirmed by other authors (6,9,10,18,19). These data raise the possibility that serotonergic pharmacological manipulation might have different electrophysiological (19) and behavioral consequences depending upon the age of animals, as Frambes et al. (9) demonstrated studying feeding behavior in infant rats, and could be related to differential development of various 5-HT receptor subtypes. To explain the apparent discrepancies between Okuyama et al.'s (17) and Urbá-Holmgren et al.'s (23) results, it is suggested that a gradual decline in serotonergic facilitatory effects on yawning takes place due to a particular 5-HT receptor subtype disappearing with age or being gradually replaced or counteracted by another 5-HT receptor subtype population with opposite effects. The second alternative seems more attractive and is supported by histochemical, biochemical, and electrophysiological evidence (6,9,14,18,19). Nevertheless, Borton and Docherty (4) recently reported that 5-HT_{1B}-mediated responses in rat vas deferens are lost with maturation and aging. Therefore, the first alternative cannot be excluded without further research.

5-HT receptors have recently been classified into three major groups: 5-HT_1, 5-HT_2, and 5-HT_3 (5). The 5-HT_1 receptor subgroup is further subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, and 5-HT_{1D} subtypes. This classification has led to the development of new and relatively more selective 5-HT agonists and antagonists, such as methiothepine, which antagonizes both 5-HT_2 and all 5-HT_1 receptor subtypes, and ritanserin (5-HT_{1C}/5-HT_2 antagonist). Because methiothepine, but not ritanserin, counteracts citalopram positive modulatory effects on PHY-induced yawning, when infant rats are used as experimental subjects it might be suggested that this action is due to 5-HT_{1A} or 5-HT_{1B} receptor activation rather than through 5-HT_{1C} or 5-HT_2 receptor subtypes. In regard to yawning in adult animals, because both above-mentioned antagonists are ineffective the suggestion might be that the inhibitory effect of citalopram could be due to 5-HT_3 receptors and that 5-HT_1 and 5-HT_2 may be ruled out. Definitive and less speculative conclusions must await the development of (and experimental trials with) more specific antagonists of each of the different serotonergic receptor subtypes than methiothepine and ritanserin.

Interactions between serotonergic and cholinergic systems in the control of different functions and behaviors have been reported (15,16,21,25,26), some of them possibly exerted directly through presynaptic receptors localized on cholinergic terminals in the CNS. Serotonergic facilitatory or inhibitory effects on yawning behavior might be exerted directly through different types of serotonergic receptors localized on central cholinergic neurones, which play an important role in yawning behavior (22). But, the possibility should be explored that these serotonergic influences could be exerted indirectly by modulating, or interacting with, the activities of other monoaminergic neurotransmitters (dopamine or norepinephrine) or neuropeptides (corticotropin, oxytocin, prolactin), the effects of which on yawning behavior are also well known and have been reviewed recently (8). A deeper discussion of these possibilities is clearly beyond the purposes of this experimental report.

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