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Differences in Central and Peripheral Responses to Oxotremorine in Young and Aged Rats

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ESPÍNOLA, E. B., M. G. M. OLIVEIRA AND E. A. CARLINI. *Differences in central and peripheral responses to oxotremorine in young and aged rats.* PHARMACOL BIOCHEM BEHAV **62**(3) 419–423, 1999.—Few studies have investigated the pharmacological response of agents that act on the cholinergic system from the point of view of age. The present article investigated central responses (tremor) and peripheral responses (chromodacryorrhea) subsequent to the administration of oxotremorine to young (3–6 months of age) and aged rats (24–30 months of age). The aged rats presented greater duration and intensity of tremor in three doses utilized (0.25, 0.5, and 1.0 mg/kg) compared to young rats. These two groups of animals did differ in latency for the onset of the tremor. The aged rats presented more intense chromodacryorrhea than the young rats in all utilized doses. These data are indicative that both responses—central and peripheral—are affected by aging, possibly as a result of pharmacokinetic alterations and/or alterations in functionality of the cholinergic system in aged rats. © 1999 Elsevier Science Inc.

Oxotremorine Aging Cholinergic system Tremor Chromodacryorrhea

POPULATIONAL aging is today a universal phenomenon that occurs both in rich and Third World countries (44). Furthermore, the problems associated with aging of the population are increasingly felt by health organizations (29). The functional decline associated with aging reduces the quality of life and, in response to this, there is a large number of studies for the purpose of elucidating mechanisms of aging (31) where alterations have been reported—anatomical (9,21), histological (9,23,45), neurochemical (27), and behavioral (2,8,12,20). In these studies, the cholinergic system has been awarded a great deal of attention (42), owing to the evidence that it is involved in the mediation of cognitive processes (2,7,42). Anticholinergics, for example, may impair learning and memory (17). Other studies show that the lesion of cholinergic neurons in animals leads to a deficit of memory (3,10,14), and that there is a quantitative relationship between cognitive damage and cholinergic deficit observed in patients with Alzheimer's disease (26).

Several studies indicate that a cholinergic dysfunction occurs with the passing years (7,13,42). For example, choline uptake and acetylcholine release (1,22,23), choline-acetyltransferase, and acetylcholinesterase activities (6,23) diminish in some regions of the brain of aged animals and elderly humans. There is a loss of cholinergic neurons (4,22,30) and of muscarinic and nicotinic receptors in aged animals and elderly humans (1,23). Moreover, cholinergic cells transplanted to the brains of aged rats induce a significant improvement in the memory impairment of those rats (10).

There are conflicting reports in the literature, showing that the sensitivity to cholinergic drugs is augmented (25), diminished (11), or unaltered (18) in aged rats. Up to the present time, there have been no studies evaluating the cholinergic peripheral responses in vivo in aged rats that might contribute to the elucidation of the factors responsible for the modified responses of the cholinergic agonists in these animals. Besides, there is a lack of studies that simultaneously evaluate the effects of an agonist on the central and peripheral cholinergic systems of aged rats. The present study, therefore, investigated the central cholinergic system, in young and aged rats, through tremor resulting from stimulation of muscarinic receptors in striatum (39,46), and through chromodacryorrhea, a red secretion attributed to the stimulation of the peripheral muscarinic receptors of the Harder's gland (19,37).

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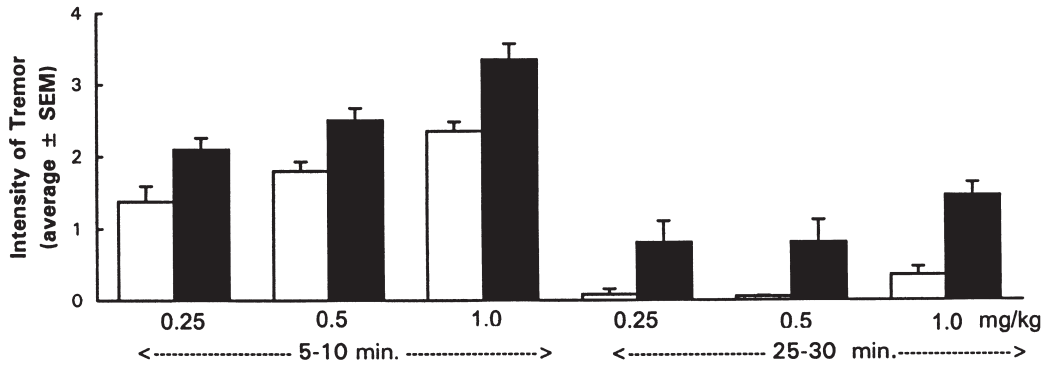

FIG. 1. Intensity of tremor measured in grades in young (□) and aged (■) rats induced by three doses of oxotremorine administered intraperitoneally. Tremor was measured 5–10 min and 25–30 min subsequent to treatment. Data are expressed in average ± SEM. Statistically significant group and dose effects (two-way ANOVA).

METHOD

Drugs

Oxotremorine sesquifumarate (Sigma Chemical Co.) was dissolved in saline solution and injected intraperitoneally in a volume of 0.1 ml/100 g.

Animals

Young (3–6 months of age) and aged rats (24–30 months of age) were housed three in each home cage, in a room with a light–dark cycle of 12 h, and a temperature of 23 ± 2°C. Food and water were available ad lib.

Forty-three young rats and 41 aged rats were employed in this study. The animals were treated with 0.25 (13 young and 11 aged rats); 0.5 (10 and 10) or 1.0 mg/kg (20 and 20) of oxotremorine. Immediately after the administration of oxotremorine, each rat was individually placed in a wire cage (15 × 20 × 30 cm) and observed.

Tremor

Latency for the appearance of the first tremor was recorded. The intensity of the tremor was assessed using the Santos and Carlini scale (36): Grade 0—absence of tremor; Grade 1—one to five tremors of the head, each with a duration of less than 1 min; Grade 2—six or more tremors of the head with a duration of less than 1 min; Grade 3—one to five tremors of the head with a duration equal to or greater than 1 min; Grade 4—tremor(s) of the head lasting more than 1 min and one to five tremors of the body, each with a duration of less than 1 min; Grade 5—tremor(s) of the head of more than 1 min and six or more tremors of the body lasting less than 1 min each; Grade 6—tremors of the head and body lasting more than 1 min.

The time and intensity of the tremor were evaluated in the intervals of time 5–10, 25–30, and 55–60 min after the administration of the drug.

Chromodacryorrhea (Red Lacrimation)

The presence or absence of red tears was evaluated, in the same animals used for tremor, 20 min subsequent to administration of oxotremorine, in accordance with the Santos and Carlini scale (37): Grade 0—absence of lacrimation in both eyes; Grade 1—discrete presence of lacrimation in one eye; Grade 2—discrete presence of lacrimation in both eyes or in-

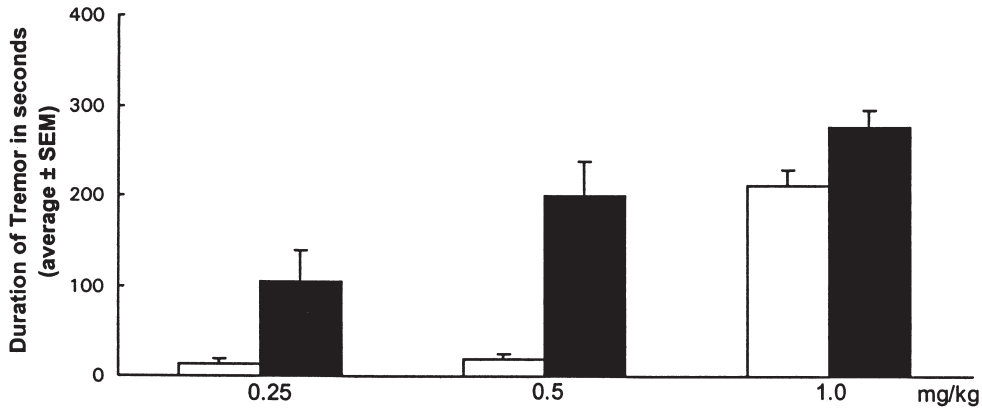

FIG. 2. Total sum of duration of tremor in seconds observed in the periods 5–10, 25–30, and 55–60 min subsequent to administration of three doses of oxotremorine in young (□) and aged (■) rats. Data are expressed in average ± SEM. Statistically significant group and dose effects. Two-way ANOVA.

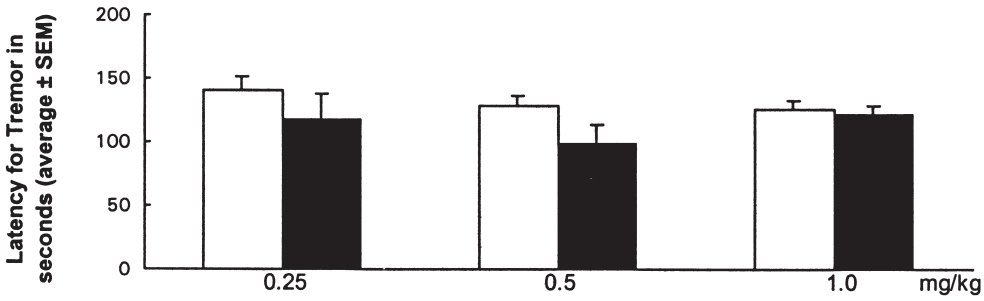

FIG. 3. Latency in seconds for the appearance of first tremor in young (□) and aged (■) rats subsequent to acute treatment with three doses of oxotremorine. Data are expressed in average ± SEM. Statistically significant group effect. Two-way ANOVA.

tense in one eye; Grade 3—discrete presence of lacrimation in one eye and intense in the other; Grade 4—intense lacrimation in both eyes.

Statistics

Results of latency, intensity, and time of tremor as well as results of chromodacryorrhea were analyzed using the two-way ANOVA to carry out comparisons between young and aged groups.

RESULTS

Tremor

Figure 1 shows that for all of the three doses of oxotremorine tested, the aged rats showed significantly greater intensity of tremor in both periods of observation. This effect was dose dependent. There was a group effect, $F(1, 77) = 25.29$, $p < 0.0001$, for a period of 5–10 min; $F(1, 78) = 32.62$, $p < 0.0001$, for a period of 25–30 min, a dose effect, $F(2, 77) = 19.97$, $p < 0.008$ for a period of 25–30 min, but no interaction group × dose was observed.

As can be seen in Fig. 2, the aged rats experienced also greater duration of tremor as compared to the young rats [group effect: $F(1, 66) = 36.05$, $p < 0.0001$]. It can be observed a dose–response curve [dose effect: $F(2, 66) = 39.41$, $p < 0.0001$], and the interaction group × dose was statistically significant, $F(2, 66) = 3.33$, $p < 0.042$. The Duncan post hoc test showed that the interaction was due to the greater increase of the tremor duration of the aged animals at dose 0.25 to 0.50 compared to the young animals. A dose–response curve was not observed for the latency for the beginning of tremor [dose effect: $F(2, 78) = 0.876$, $p < 0.42$]. The aged rats had a diminished latency [group effect: $F(1, 78) = 4.14$, $p < 0.04$], but no interaction group × dose was seen (Fig. 3).

Chromodacryorrhea

As seen in Fig. 4, aged rats had greater intensity of chromodacryorrhea than young rats when treated with oxotremorine, in all the doses studied [group effect: $F(1, 78) = 7.39$, $p < 0.008$]. A dose–response curve was also observed [dose effect: $F(2, 78) = 42.74$, $p < 0.0001$], but no interaction group × dose was seen.

DISCUSSION

Up to the present there have been no studies evaluating the in vivo peripheral cholinergic responses in aged rats; in addition, there are also a paucity of data simultaneously analyzing the central and peripheral cholinergic responses to direct muscarinic agonists.

The present study utilized parameters that measure the cholinergic central and peripheral activities subsequent to acute administration of oxotremorine, a direct muscarinic receptor agonist, comparing young and aged rats. Tremor is a

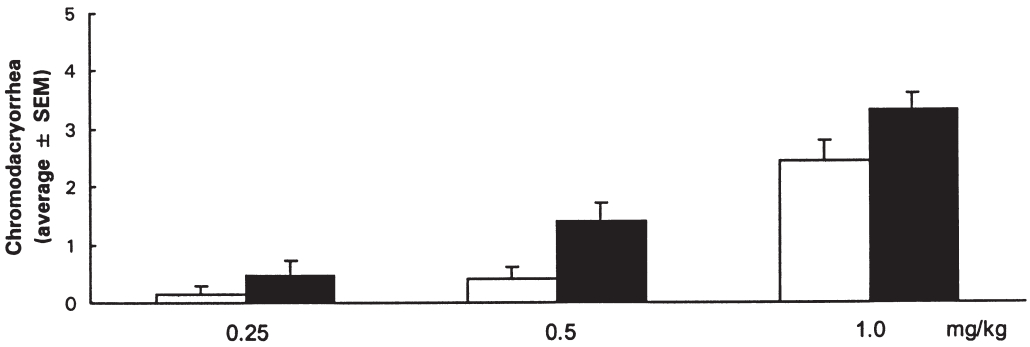

FIG. 4. Intensity of chromodacryorrhea, measured in grades, in young (□) and aged (■) rats subsequent to acute treatment with three doses of oxotremorine. Data are expressed in average ± SEM. Statistically Significant group and dose effects. Two-way ANOVA.

parameter used to assess activity of the central cholinergic system (19,34,36). Tremor induced by oxotremorine was significantly more intense (Fig. 1) in aged rats compared to young rats. Our data are in accordance with those obtained by other authors (24,25,40), who observed an augmented response in aged rats subsequent to administration of direct cholinergic receptor agonists. Even with indirect agonists, it was observed that the effects were larger in aged rats as referred to in a study carried out by Pintor et al. (28), utilizing the cholinesterase inhibitor diisopropylfluorophosphate.

The present results also show that peripheral cholinergic responses in aged rats are augmented, as measured through chromodacryorrhea. Therefore, our data indicate that both the peripheral and the central cholinergic systems are affected by aging in same direction.

Greater intensity of tremor in aged rats observed in the present study persisted for a longer time, and was observed even 30 min following the administration of oxotremorine (Figs. 1 and 2). The onset of tremor (latency) was more rapid in aged rats than young ones, suggesting that age, in this case, did alter the absorption and distribution of this drug. There are data showing the metabolization as slower (33,35,38,48) and a more prolonged rate of elimination in aged animals and humans for a number of drugs (33,38,47), although other publications disagree with this fact (15,41,43). Thus, more intense tremor in aged rats injected with oxotremorine could be due to higher plasmatic concentration of the drug, leading to greater arrival of the drug to its sites of action, augmenting the response (35,38). However, to the best of our knowledge, no pharmacokinetic studies with oxotremorine in aged rats have been performed to date; on the other hand, in another study it was observed that arecoline induced significantly larger tremors in aged rats compared to young rats, and that there were no pharmacokinetic differences between both groups of animals (40).

Another explanation for the increase of tremor and chromodacryorrhea in aged rats is the possible changes of central and peripheral muscarinic receptors, respectively. The study carried out by Pedigo et al. (25) supports this hypothesis. They found that oxotremorine hypothermic effects were markedly enhanced, and those of escopolamine were reduced, whereas muscarinic receptor density declined, in aged rats. These authors attributed such results to possible changes in subtypes of brain muscarinic receptors with advanced age. Furthermore, Pedigo (24) reported the same increased hypothermic response following intracerebroventricular injection of oxotremorine, a finding that speaks against a pharmacokinetic mechanism to explain the results. However, there are contradictory results when cholinomimetic-induced hypothermia is used to measure cholinergic responses in young and aged animals. Ferguson et al. (11) reported that 15–18-month-old rats failed to respond to carbachol, whereas a clear hypothermic response occurred in 3–5-month-old animals; Martin et al. (18) reported that oxotremorine produced the same maximal hypothermical response in senescent (34–40-month-old) and adult (8–9-month-old) rats. Apart from the different ages of the animals, in our study (24–30-month-old), and in those of Ferguson et al. (15–18-month-old) and Martin et al. (34–40-month-old), it is difficult to reconcile these results.

On the other hand, other reports describe a decline in the central cholinergic system with aging (22,23); in fact, there are reports describing a diminution in density of cholinergic receptors with aging (22,25). There is, thus, a contradiction between neurochemical data reported in the literature and the increased responses to oxotremorine in aged rats, making it difficult to equate these findings. Another possible explanation would be a change in conformation of the cholinergic receptors as a result of aging (5,16). The existence of two or more states of the receptor with different affinities for oxotremorine was postulated (5,16,25,32). It could, therefore be that, in aged rats, the number of muscarinic receptors with high affinity for oxotremorine is augmented, consequently resulting in a greater effect of the agonist.

Finally, subsequent works, like the study of the pharmacokinetic profile of oxotremorine in young and aged rats, will be necessary to definitively establish whether our results are due to pharmacokinetic alterations or whether they are the result of changes of cholinergic receptors.

It must be emphasized that studies such as this contribute towards clarifying the mechanisms and consequences of cholinergic disturbances related to aging, which may be important in understanding age-related diseases.

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