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Serotonin Modulation of Pain Responsiveness in the Aged Rat

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AKUNNE, H. C. AND K. F. A. SOLIMAN. *Serotonin modulation of pain responsiveness in the aged rat.* PHARMACOL BIOCHEM BEHAV **48**(2) 411–416, 1994.—This study was designed to examine differences in basal nociceptive responsiveness between young (3 months) and old (25 months) male Fischer-344 rats and also to evaluate the effects of methysergide and fluoxetine on this behavioral paradigm. The results indicate that the aged animals were less sensitive than young animals to pain responsiveness in simple nociceptive tests such as the tail-flick, hot plate (55°C), and hind-paw pressure tests. In both groups of animals, this behavioral response followed a circadian rhythm, with peak of pain latency during the dark phase and trough occurring in the light phase. In all three analgesic tests, treatment with methysergide, which is a serotonin antagonist, resulted in hyperalgesia in both groups of animals within the first hour, followed by a return to basal response level after 2 h. Fluoxetine treatment resulted in a nonsignificant increase in nociceptive response at 30 min posttreatment which returned to the baseline by 1 h. Moreover, in both young and old animals morphine produced moderate analgesia in the hot plate and hind-paw tests, which was potentiated by simultaneous treatment with fluoxetine. This study shows that noxious response was reduced in the aged male Fischer-344 rats, and the data obtained provide evidence that the serotonergic system modulates pain sensitivity similarly in young and old animals.

Analgesia Hyperalgesia Fischer-344 male rats Fluoxetine Methysergide

SEROTONIN (5-HT) is a neurotransmitter that is well distributed in the CNS and has been implicated in a variety of physiological functions. One of these functions is its role in mediating pain sensitivity. The early demonstration by Tenen (22) that a pharmacologically induced alteration in the concentration of brain serotonin, using *para*-chlorophenylalanine (*p*-CPA) and 5-hydroxytryptophan, was associated with a change in the behavioral response to noxious stimuli. Anatomical mapping of the pain modulatory pathway (9,13,17) suggests the involvement of at least the descending serotonergic tract in enhancing the antinociceptive effect of morphine (16). In addition, the administration of 5,6-dihydroxytryptamine, a drug which causes a preferential destruction of the descending serotonin system, is effective in reducing morphine analgesia (22). Tryptophan and morphine interactions have recently been postulated in postoperative pain. It was suggested that the serotonergic system in the brain can antagonize the dissociative state produced by morphine, which helps patients tolerate pain (11). Nonetheless, results of clinical tests with tryptophan as analgesic agent has been inconsistent.

Both the opioid and the serotonergic systems have been reported to be altered with aging (1,20). Decline in synaptic terminal fields (5) and decreased neurotransmitter content or turnover (1,10,14) are some of the degenerative processes that accompany aging. Studies have not been directed towards the neurochemical correlates of pain responsiveness in younger versus older subjects. Therefore, a detailed study on how the serotonergic system might affect pain sensitivity during aging is important.

The objective of this investigation is to study differences in nociceptive response in young and old animals. We will also provide preliminary evidence for the importance of the serotonergic system as a nonopioid modulator in pain responsiveness. To assess the role of 5-HT in this regard, two serotonergic agents are employed: methysergide and fluoxetine. Because of the involvement of brain serotonin in the antinociceptive action of morphine (15), we also studied the

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interaction of morphine and fluoxetine in pain latency in young and aged animals.

MATERIALS AND METHODS

Animals

Male Fischer-344 rats maintained by Harlan Sprague–Dawley and obtained through the National Institute of Aging (NIA) were used in these studies. Upon arrival, animals were 3 months (young) and 25 months (old) and weighed between 200 and 500 g for the two ages, respectively. Animals were housed in stainless steel cages and maintained in a controlled environment of a 12-h light–dark cycle with temperature at 21 ± 1°C. Food and water were provided ad lib.

Chemicals

Fluoxetine hydrochloride was a complimentary gift from Eli-Lilly (Indianapolis; Illy 86-101-199). Morphine sulfate, USP, was purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ). Methysergide maleate was a complimentary gift from Sandoz Research. Free base solutions were prepared for all injections, and all injections were made by the IP route.

Pain Sensitivity Tests

The tail-flick test. The apparatus used for this test is Tail Flick Analgesi-Meter, model 33 (Innovators Instrumentation, IITC, Landing, NJ). The procedure is based on the method of D'Amour and Smith (6). It consisted of a radiant heat source, photocell, automatic timer, and power supply. The rat's tail was placed in a slit over the photocell. When the light switch was turned on (it also turned on the timer), a sudden typical flick of the tail allowed light to penetrate the photocell and stop the timer. This represented and measured the animal's reaction to the heat stimulus. The cutoff point was set at 30 s with low intensity setting.

The hot plate test. A modified hot plate method of Eddy and Leimbach (7) was used. In this procedure, a hot plate was utilized with a thermostat set to maintain temperature at 55°C. The reaction to heat included an escape attempt and/or licking of paw, any of which indicates reaction.

The hind-paw pressure test. An analgesiometer (Ugo Basile, Milan, Italy) was used in this pressure-based test, which was modified from the method of Randall and Sellito (18). In this test, a foot pedal was applied on a surface of 1 mm^2, and applied pressure was measured in grams. The right hind paw was placed on a marked spot under the movable weight unit, and pressure was increased gradually. The end point of stimulation was determined by the withdrawal of the paw or attempt to do so or vocalization, whichever comes first. The reaction time was therefore recorded in grams and the cutoff weight was set at 250 g.

Nociceptive Testing

Prior to recording any pain measurements, each animal was habituated to the testing environment and to the stimulus. Briefly, three days before the actual testing animals were exposed twice daily to the testing apparatus in the test room. Then, one day before the experiments were conducted animals were exposed to the functional testing apparatus for 20 and 15 s and 150 g for the tail-flick, hot plate, and hind-paw apparatus, respectively. On the test day animals were taken into the test room 2 h before the actual tests. Appropriate measures were also taken to avoid any learning or sensitization during testing design. Essentially, we used one group of animals only once and retested after an interval when necessary in each particular experimental protocol.

In all studies we adhered to the guidelines for "Investigations of Experimental Pain in Conscious Animals" as adopted in 1983 by Zimmerman (24).

Experimental Design

Experiment 1: Effect of age on pain sensitivity. Young and aged animals were divided randomly into six groups of five animals each. Pain measurements were taken at 4-h intervals for a 24-h period beginning at 0800. Each group was tested only once at the designated time period. The three pain tests described previously were used. A red diffused light of low intensity enabled measurements to be taken during the dark phase without light interfering with the testing.

Experiment 2: The effect of methysergide on pain sensitivity. Eight animals from each age group were subjected to different pain tests following the administration of methysergide (3 mg/kg, IP). Measurements were taken before drug administration and at 30 min and 1, 2, and 4 h following drug treatment.

Experiment 3: The effect of fluoxetine treatment on pain sensitivity. Young or aged animals, five per group, were treated at 1000 and 2200 daily for three consecutive days with 10 mg/kg of fluoxetine hydrochloride. The interaction effect of fluoxetin on morphine-induced analgesia was also studied in the two groups of animals. In this experiment, fluoxetine (10 mg/kg, IP) was administered 30 min prior to morphine injection (5 mg/kg, IP) and the analgesic measurements were performed before drug administration and at 15, 30, and 60 min after treatments.

Statistical Analysis

Two-way analysis of variance (ANOVA) with repeated measures was used to compare differences between means at different time periods with Neuman–Keuls post hoc test. All statistical analyses were based on a significance level of $p < 0.05$.

RESULTS

Effect of Age on Pain Sensitivity

Figures 1, 2, and 3 represent the results of various pain sensitivity measurements in young and old animals during a 24-h period. The data indicate a significant ($p < 0.01$) decrease in basal nociceptive response (increase in latency) in old animals across the 24-h period. The only insignificant response between ages occurred at 2000 in the tail-flick test and at 1600 with the hot plate and the hind-paw pressure tests (Figs. 2 and 3). The peak and trough of response with the tail-flick test were at 2400 and 2000, respectively, for the old animals and at 1600 and 2000 for the young animals (Fig. 1). The results of the hot plate and hind-paw tests (Figs. 2 and 3) were similar. The peak for pain latency occurred at 2000 for both young and aged animals, although the troughs were at 1600 for aged animals and 1200 for young animals.

The Effect of Methysergide on Pain Sensitivity

At 4 h following methysergide treatment young animals showed a significant increase in latency for noxious stimuli in the tail-flick test and a significant decrease in latency in the hot plate and the hind-paw tests (Fig. 4). The responses of

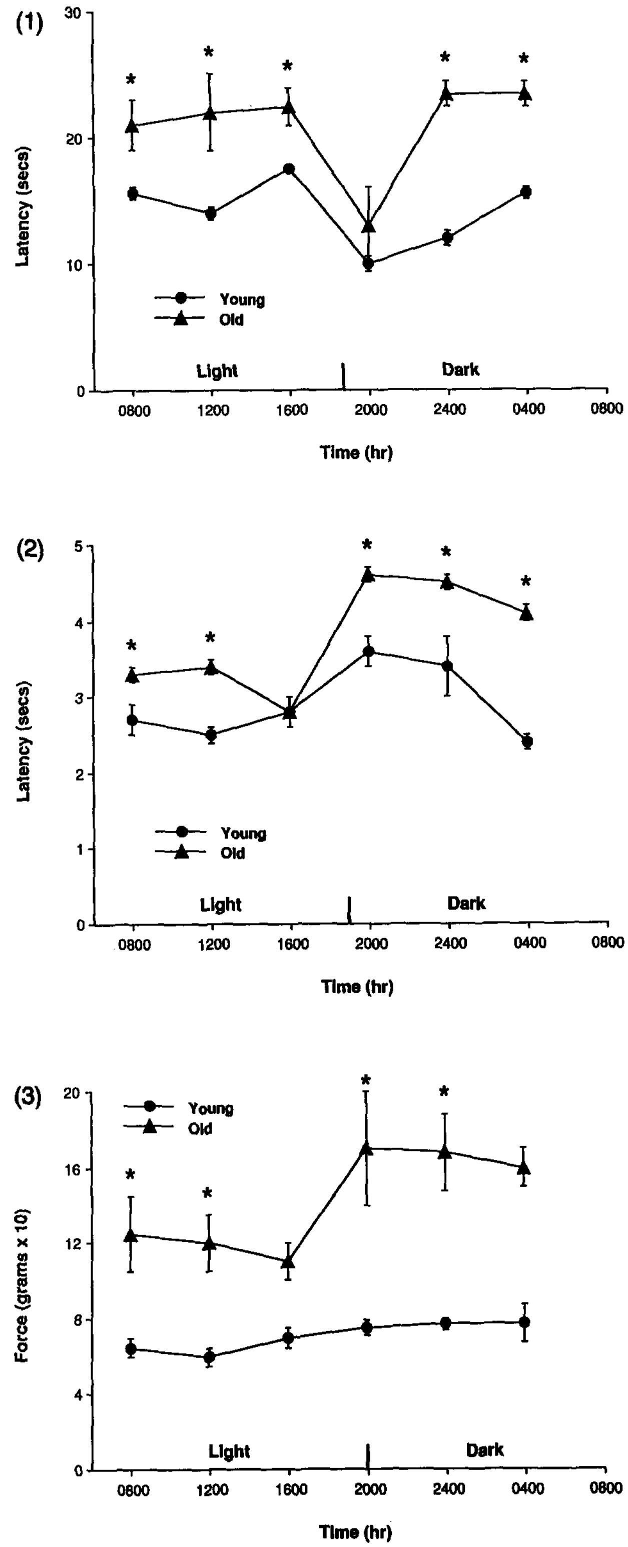

FIG. 1-3. Pain sensitivity measurements in young and aged rats using the tail-flick test (Fig. 1), hot plate (Fig. 2), and hind-paw pressure test (Fig. 3). There were six groups of young or aged animals; each group corresponded to testing at 4-h intervals (24-h period). Each group consisted of five animals and each point represents the mean ± SEM. * $p < 0.05$, representing significance from pain measurements obtained from young animals.

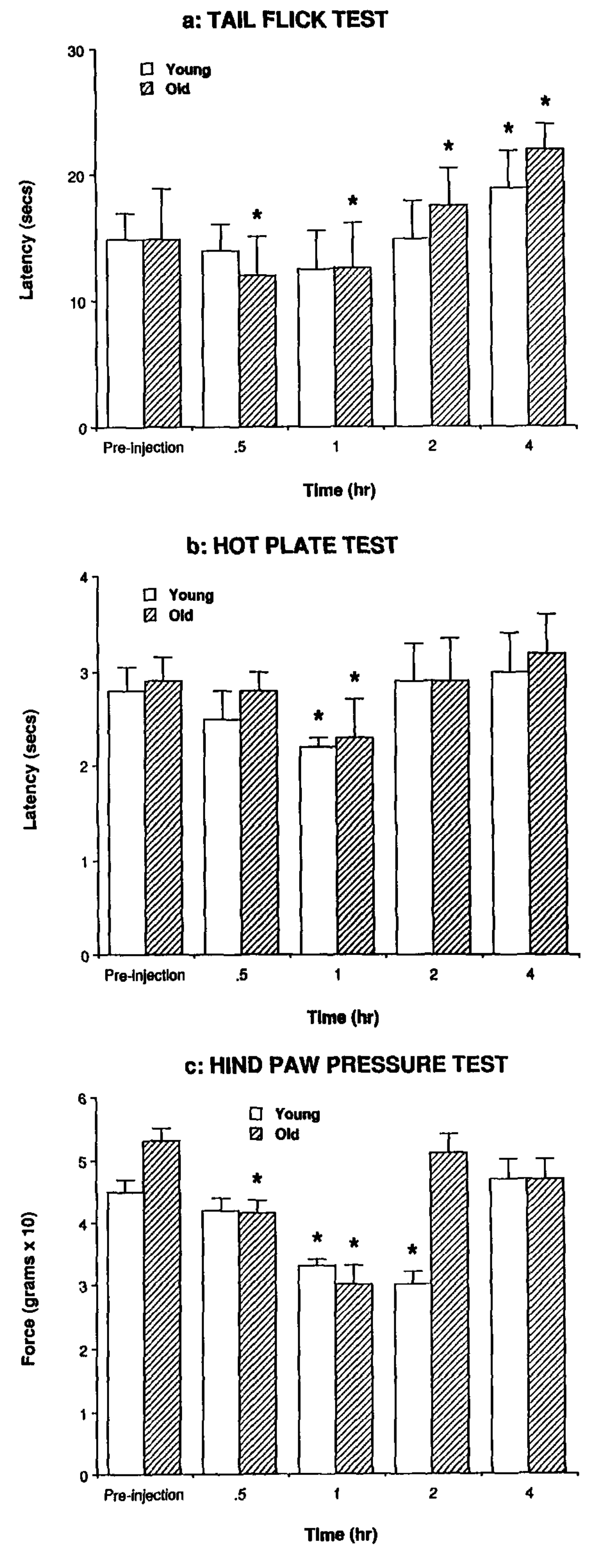

FIG. 4. The effect of methysergide on pain sensitivity. Young and aged animals were treated with 3 mg/kg (IP) of methysergide. They were tested for pain responsiveness to noxious stimuli before and at different intervals following drug administration using the tail-flick (a), hot plate (b), and hind-paw pressure (c) tests. Each group consisted of eight animals and each value represents the mean ± SEM. * $p < 0.05$, representing significance from control (preinjection).

aged animals were different. Methysergide administration resulted in significant decrease in pain latency at 0.5 h and 1 h after treatment with a rebound to increased latency at 2 h and 4 h posttreatment in the aged animals. This rebound phenomenon was not significant in the hot plate and the hind-paw tests (Fig. 4, b and c).

The Effects of Fluoxetine and Morphine Treatments on Pain Sensitivity

A three-day fluoxetine treatment resulted in a significant increase ($p < 0.05$) in pain sensitivity (decreased latency) with the hind-paw (Fig. 5a) and hot plate (Fig. 5b) tests for both age groups of animals. Morphine treatment (5 mg/kg, IP) resulted in moderate but significant increase in latency that was potentiated by simultaneous treatment with fluoxetine in both the young and old animals at 15 and 30 min postinjection.

DISCUSSION

Several lines of evidence indicate that the brain serotonin system is involved in antinociceptive action of morphine (17,23), although the specific mechanism has not been clearly defined. Furthermore, studies have not been directed towards investigating the possible implication of the serotonergic system in pain sensitivity during aging. The present study describes differences in basal responsiveness to noxious stimulus and alterations in pain responsiveness following manipulations of the serotonergic system in two age groups of rats. In all three analgesic tests, response latency to noxious stimulation was greater in old animals (Figs. 1, 2, and 3). The peak

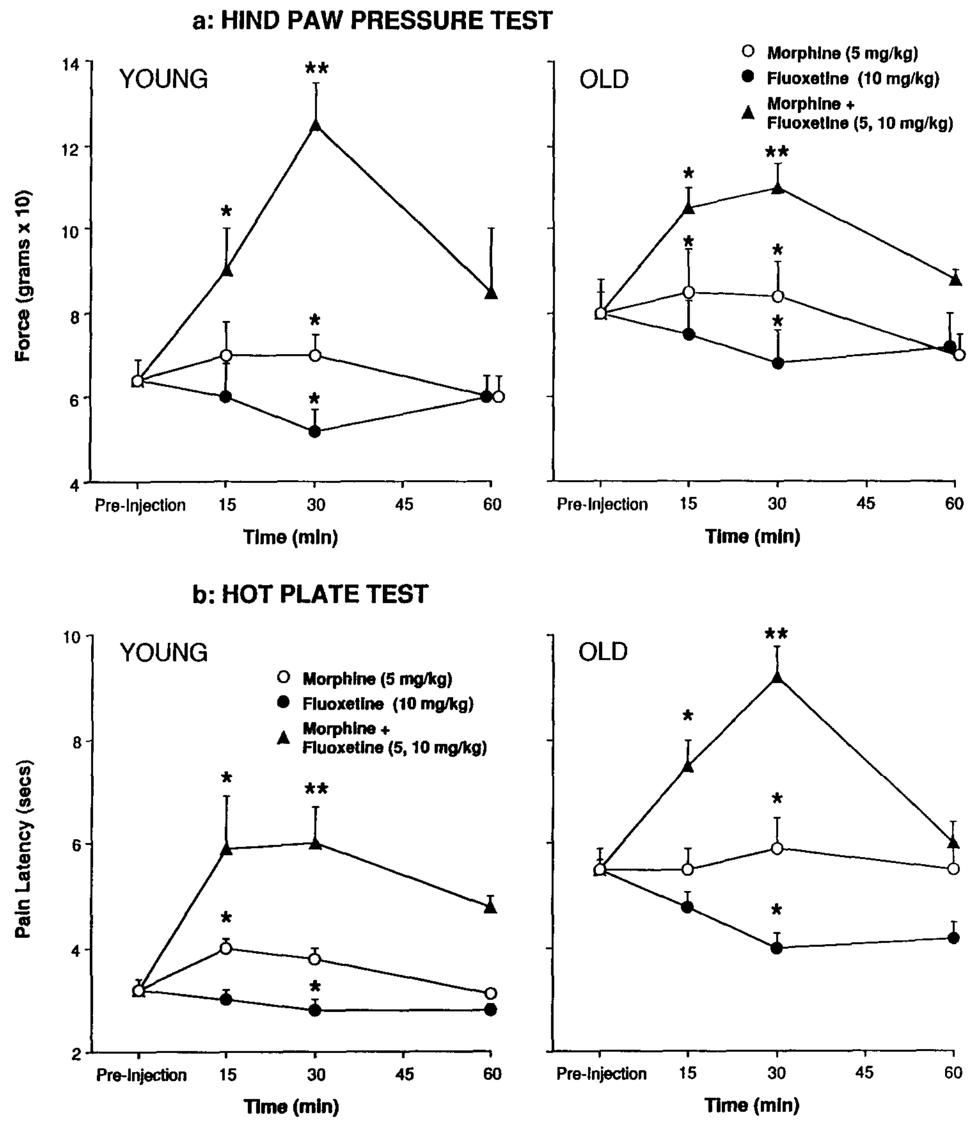

FIG. 5. The effect of three-day fluoxetine treatment on pain sensitivity in young and aged animals. Fluoxetine was administered twice daily for three days in one group of young or old animals. The other groups received morphine or fluoxetine followed by morphine. Nociceptive response was measured using the (a) hind-paw and (b) hot plate tests. Each value represents the mean ± SEM of five animals. *$p < 0.05$, **$p < 0.01$, representing significance from control (preinjection).

of this response occurred during the dark phase and the trough was towards the late stages of the light phase. Similar rhythms in pain sensitivity have been reported in rodents (12). The rhythm reported in man is reversed, with peak of latency during the light phase (19). These data are indicative of an increase in pain latency occuring during the active phase for man and animal.

Manipulation of the serotonergic system with methysergide and fluoxetine affected responsiveness to noxious stimuli similarly in both age groups of animals. Methysergide treatment resulted in increased response to noxious stimuli, while fluoxetine treatment resulted in increased response to noxious stimuli. Fluoxetine potentiated morphine antinociceptive action in both young and aged animals. It apppears that the two systems interact in modulating noxious response (16). Specifically, morphine appears to directly or indirectly activate the descending serotonergic system, resulting in depolarization of primary afferent terminals that carry pain impulses and presynaptically inhibiting pain impulses. There are some interesting findings when the serotonergic system is isolated. The administration of fluoxetine (a serotonin uptake inhibitor) resulted in an increase in latency to noxious stimuli, which might suggest that this response is due to an apparent increase in synaptic content of serotonin. In an unpublished work we showed that brain contents of serotonin and metabolites were decreased in aged animals when compared to young animals. But the behavioral response to noxious stimulation in the present work showed that both groups of animals exhibited similar response patterns. Therefore the significance of the changes in brain content of serotonin in the aged animals is not clear.

A recent report showed that serotonin is a direct-acting agonist resulting in the hyperalgesia in the mechanical paw withdrawal nociceptive threshold (21). It has been reported that systemic administration of *p*-CPA resulted in selective decreases in both basal and analgesic pain thresholds. This effect has been attributed to the inhibition of tryptophan hydroxylase and subsequent depletion of brain serotonin (4). Therefore, the increase in pain latency and the reduced brain contents of serotonin in old animals appear to contradict these reports (4,21). In our parallel unpublished study, brain levels of beta endorphin were increased in older animals, and this might have offset any changes in pain threshold that have been independently observed in aged animals. It is still not clear what the effects of interactions of other neurotransmitters are on pain sensitivity.

Neuronal degeneration that accompanies aging has been well described (5,10,20). Changes in neurotransmitter (10) and hormonal (4) levels may be responsible for the decreased ability of old animals to respond to noxious stimulus. Nonetheless, it appears that the serotonin receptor systems in these old animals are functional, since methysergide affected both age groups similarly. The effect of methysergide treatment appears to be biphasic, with decreased latency to noxious stimuli in the first phase and increased pain latency during the second phase (possibly a rebound effect). The biphasic effects may also be a reflection of an earlier report that serotonin may inhibit or excite dorsal horn neurons (3). We selected methysergide for these tests because, unlike the other antagonists, it is highly specific without histamine or acetylcholine interference (8).

It has been suggested that a major component of morphine antinociception is mediated through the action of morphine on endogenous opioid receptors that stimulate 5-HT release in the spinal cord, thereby inhibiting nociceptive afferent transmission (2). This may be an explanation for the previous observations of morphine and serotonergic effects on pain sensitivity. In this study fluoxetine potentiated morphine antinociceptive action in both age groups of animals, again providing evidence that the functioning capacity of this biological loop is still intact at an older age.

In summary, we have shown that aged male Fischer-344 rats respond less to noxious stimulus than young animals in three simple nociceptive tests. The data also show that there are no differences between young and old animals in their repsonse to pain when the contents of serotonin were altered by methysergide and fluoxetine.

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