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Differences in Morphine-Induced Antinociception and Locomotor Activity in Mature Adult and Aged Mice

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HOSKINS, B., C. K. BURTON AND I. K. HO. *Differences in morphine-induced antinociception and locomotor activity in mature adult and aged mice.* PHARMACOL BIOCHEM BEHAV **25**(3) 599–605, 1986.—Mature adult (3–6 months old) and aged (24–27 months old) male ICR mice were injected with 10 to 100 mg/kg morphine, SC. The ED50 values for locomotor behavior representing 5 times control activity were 7.5 mg/kg for aged mice and 17.8 mg/kg for the mature adults. There were striking age- and dose-dependent differences in both intensities and durations of morphine-induced locomotor activity. The ED50 values for antinociception 1 hour after morphine administration were 70 mg/kg for the aged mice and 13 mg/kg for the mature adults. One hour after injecting 30 and 100 mg/kg morphine tagged with ^{3}H-morphine, 0.13 and 0.14 percent of the doses appeared in brains of aged and mature adult mice, respectively. Distribution of morphine among brain regions was the same for both age groups. The results suggest that the differences in response to morphine by the two age groups were due to age-related differences in affinities, numbers and/or functioning of opioid receptors and not to pharmacokinetic differences.

Aging in mice Morphine-analgesia, antinociception Morphine-induced locomotor activity Brain morphine distribution

EVIDENCE that the functioning of the central nervous system deteriorates during the advanced stages of the aging process is convincing. Equally convincing is the evidence that the central dopaminergic system is the one that seems to be most affected by advancing age [1, 4, 8, 11, 13, 15]. Major and Pleuvry [12] and Vander Wende and Spoerlein [17,18] have demonstrated that some effects of morphine, including antinociception, are antagonized by pretreatment with the dopamine precursor, L-dopa. Eidelberg and Erspamer [5] have also reported that dopamine is involved in morphine-induced antinociception and morphine-induced locomotor excitation. Specifically they found that the dopamine receptor blocking agent, haloperidol, potentiated morphine-induced antinociception and enhanced morphine tolerance in mice. They also showed that morphine-tolerant mice exhibited enhanced sensitivity to the locomotor excitatory actions of L-dopa. Therefore, since the central dopaminergic system has been implicated in both the antinociceptive effects of morphine [5, 12, 17, 18] as well as in morphine-induced running behavior of mice [2, 3, 5, 14, 16], we sought to determine the effects of aging, particularly in the advanced stages, upon these two responses to morphine in an outbred strain of mice. We further sought to determine if any age-related differences in these responses to morphine could be due to age-induced alterations in the distribution of morphine to and/or within the brain.

METHOD

Male ICR mice of two different ages, i.e., mature adult (3–6 months old) and aged (24–27 months old) were used in these experiments. Initially, there were 20 mice in each age group, however, over the 3 months required for this study, one aged mouse died (apparently of old age) and 3 others had to be euthanized because of their development of massive tumors. In order to perform all of these studies, each mouse had to be used more often than once, however no mouse was ever injected with morphine more often than once a week. Furthermore, each mouse served as its own control throughout the study, such that control values of all mice were determined each time they were tested. These control values did not change throughout the duration of the study.

Test for Morphine-Induced Antinociception

Prior to dosing with morphine, each mouse was carefully and non-traumatically restrained such that its tail could be immersed to a depth of approximately 1.5 inches into a constant temperature water bath maintained at 60°C. Simultaneously with the immersion, a digital stopwatch was started; the moment at which each mouse responded to the hot temperature by flicking its tail or removing its tail from the water was recorded. This procedure was repeated at specific time intervals (0.5, 1.0, 1.5, 2.0, 3.0 and 4.0 hours) after injections

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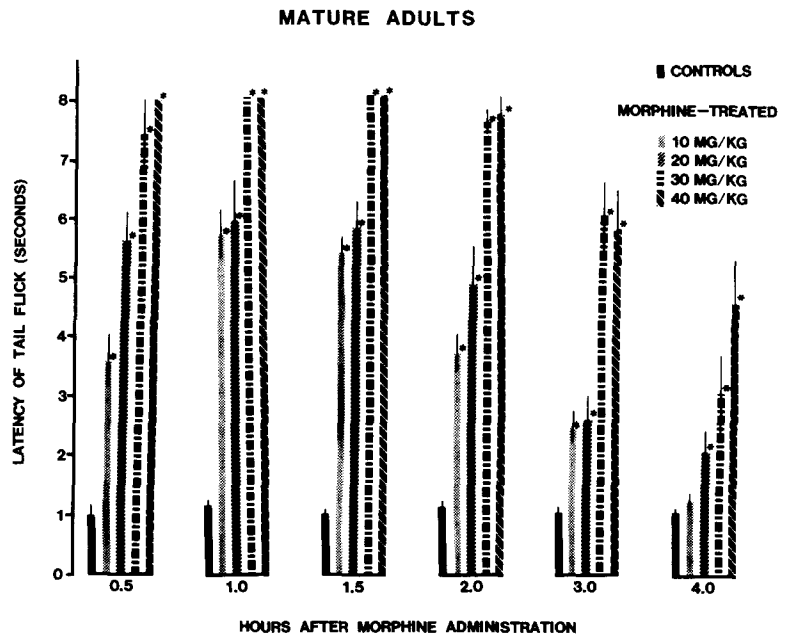

FIG. 1. Morphine-induced analgesia in mature adult (3–6 months old) mice. Data are provided as time (in seconds) tails remained immersed in a 60°C water bath without movement. Each represents the mean±S.E. of determinations on 8–10 mice. Asterisks denote significant difference from control values.

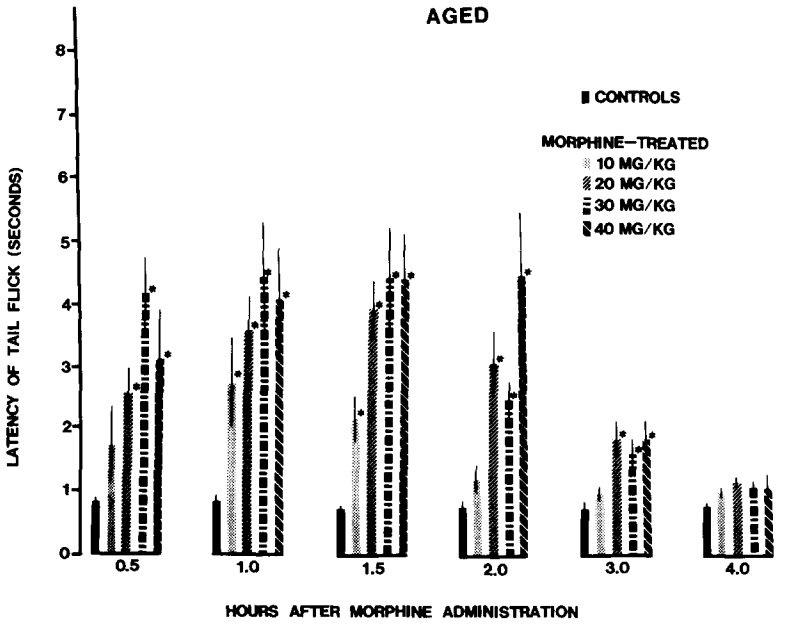

FIG. 2. Morphine-induced analgesia in aged (24–27 months old) mice. Data are provided as time (in seconds) tails remained immersed in a 60°C water bath without movement. Each bar represents the mean±S.E. of determinations on 8–12 mice. Asterisks denote significant differences from control values.

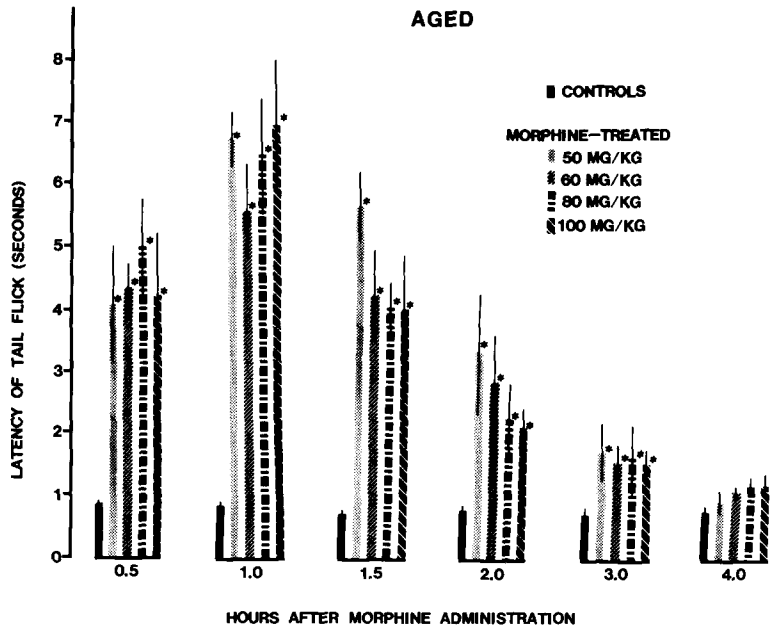

FIG. 3. Morphine-induced analgesia in aged (24–27 months old) mice. Data are provided as time (in seconds) tails remained immersed in a 60°C water bath without movement. Each bar represents the mean±S.E. of determinations on 8–12 mice. Asterisks denote significant differences from control values.

of morphine. The maximum time allowed for tails to remain immersed in the hot water was 8 seconds. Doses of morphine injected SC for these studies were: for mature adults, 10, 20, 30, and 40 mg/kg body weight; for the aged mice, 10, 20, 30, 40, 50, 60, 80, and 100 mg/kg body weight.

Test for Morphine-Induced Locomotor Activity

At the beginning of each testing session, each mouse was placed in an opaque plastic shoe-box (9×20×7 inches) type rat cage which rested on an electronic activity sensor (Stoelting Co., Model 31410). These were housed in a wooden cabinet with a glass front to minimize any influences of external noise.

Mirrors were mounted above the cages such that constant visualization of each mouse and its entire cage was possible. The control unit with digital display of activity units was positioned outside the cabinet such that separate calibration of each sensor could be performed without disturbing the mice or the sensors. Calibration of each activity monitor was performed such that only locomotor activity of each mouse was measured, i.e., sniffing, rearing, scratching, etc., were not registered in the digital display of activity on each sensor. The mice were allowed to adapt to these conditions for at least one hour, then each mouse was removed from and placed back in the cage three times such that its activity was observed and recorded for three separate 10-minute periods. The average of these three 10-minute activity values (steps) was taken as the control activity of each mouse. Each mouse was then injected, SC, with morphine and activity was again recorded at 10-minute intervals until the activity returned to the control levels. The doses of morphine used were: 10, 20, 30, 40, 50, 60, and 80 mg/kg body weight.

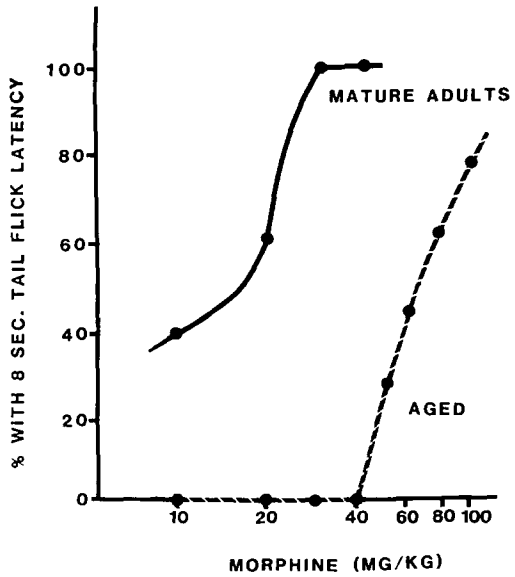

FIG. 4. Dose-response curves for morphine-induced analgesia using 8 seconds of tail flick latency as the maximal analgesic response one hour after morphine administration. Each circle represents the % responding of 8 to 12 mice tested.

Morphine Distribution in Brain

Tritiated morphine [N-methyl-^{3}H-morphine (New England Nuclear, Boston, MA)], 72.7 Ci/mmol, was added to morphine injection solutions such that the specific activities

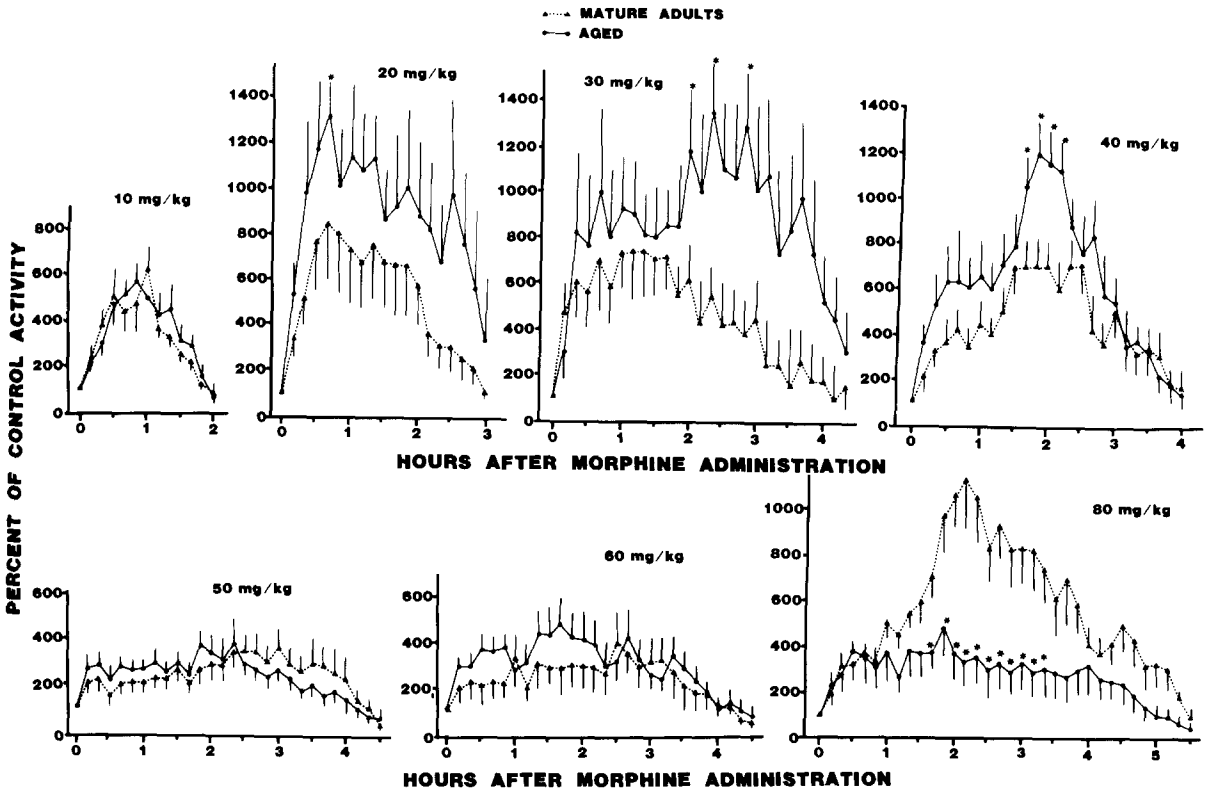

FIG. 5. Morphine-induced locomotor activity in mature adult (3–6 months old) and aged (24–27 months old) mice. Each dot represents the mean activity (as % of control activity) of 8 to 10 mice. Asterisks indicate significant difference from mature adult mice.

of the final solutions were: for the 30 mg/kg body weight dose, 6.5×10^6 cpm/mg morphine; for the 100 mg/kg body weight dose, 2.2×10^6 cpm/mg morphine. One hour following injections of each dose, SC, into 8 mice of each age group, the mice were killed by decapitation and their brains were dissected on ice into the following regions according to the procedure described by Glowinski and Iversen [7]: cortex, midbrain, cerebellum, medulla, pons and striatum. The periaqueductal gray area was obtained using the diagram provided by Khachaturian *et al.* [10] as a guide. Each brain region was carefully weighed, digested and prepared for liquid scintillation spectrometric determination of radioactivity due to the tritiated morphine.

Data were analyzed using Student's *t*-test to analyze the ^{3}H-morphine distribution data and analysis of variance in order to study the effects of age, treatment (control versus morphine and different doses of morphine), and any possible interaction of these factors. Since significant treatment differences were found in the presence of significant interactions with age, the control and morphine differences for each age were compared using the least significant multiple range test with $\alpha=0.05$. In order to obtain ED50 values and slopes of dose-response curves for the antinociception and excitatory locomotor responses, probit analyses of dose-response relationships were performed using the method of Finney [6]. Differences were considered significant at $p<0.05$.

RESULTS

Morphine-Induced Antinociception

There was a dose-dependent antinociceptic action of morphine in mature adult mice up to a dose of 40 mg/kg at which all mice failed to respond to the hot water by withdrawing or otherwise moving their tails (Fig. 1). The significant antinociception was not evident 4 hours after the 10 mg/kg dose but was still evident 4 hours after the higher doses of morphine. On the other hand, aged mice consistently displayed less antinociceptive response to morphine at these doses (Fig. 2). Also, the responses of the aged mice to doses above 30 mg/kg were not significantly different from each other although all were significant compared to controls. Figure 3 shows that doses up to 100 mg/kg were unable to elicit the maximal (8 sec tail flick latency) antinociceptive response in the aged mice. Four hours after morphine administration to the aged mice, all antinociceptive activity had disappeared.

By comparisons of the data in Fig. 1 with those in Figs. 2 and 3, it becomes apparent that not only was the duration of antinociception greater in the mature adult mice, but also the degrees of antinociception produced by a given dose of morphine were greater in the mature adults than in the aged mice. For example, whereas the dose of 10 mg/kg produced a latency of tail flick of 3.6 ± 0.4 seconds in mature adults, this

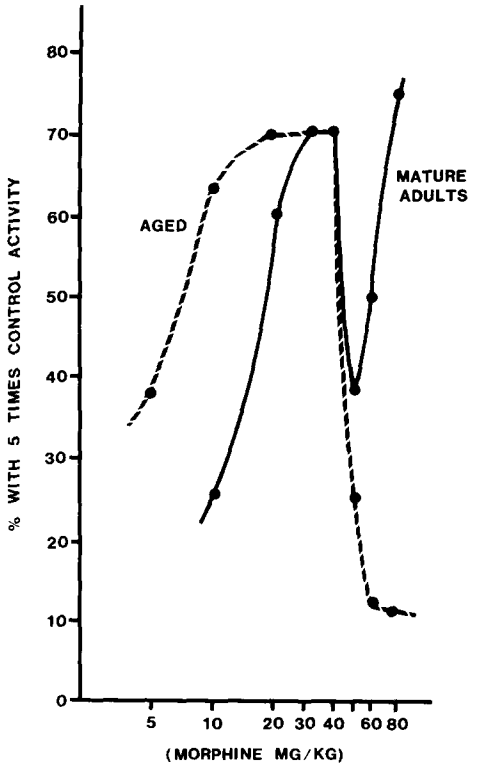

FIG. 6. Dose-response curves for morphine-induced locomotor activity using 5 times control activity as the response tested. Each circle represents the % responding of 8 to 10 mice tested.

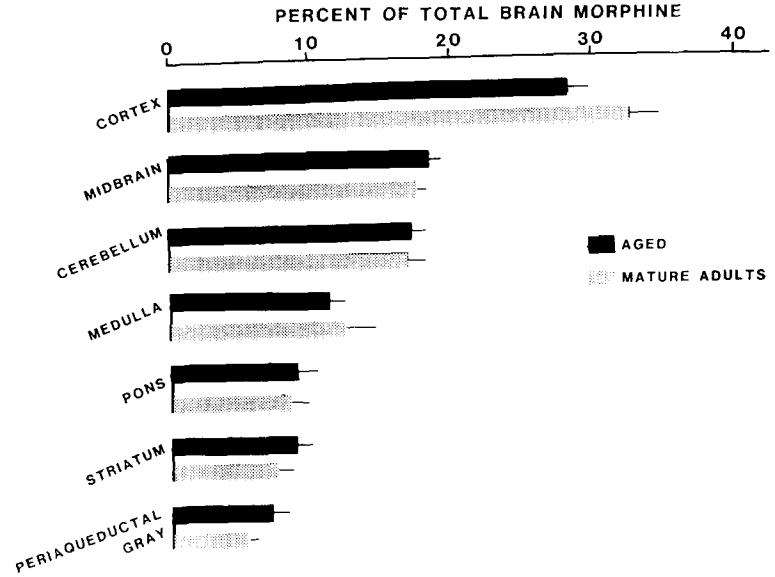

FIG. 7. Regional distribution of ^{3}H-morphine in brains of aged (24–27 months old) and mature adult (3–6 months old) mice. Each bar represents the mean±S.E. of values from 16 brains (data are combined for the 30 and 100 mg/kg doses since distributions, as percent of total in brain, were the same for both doses.

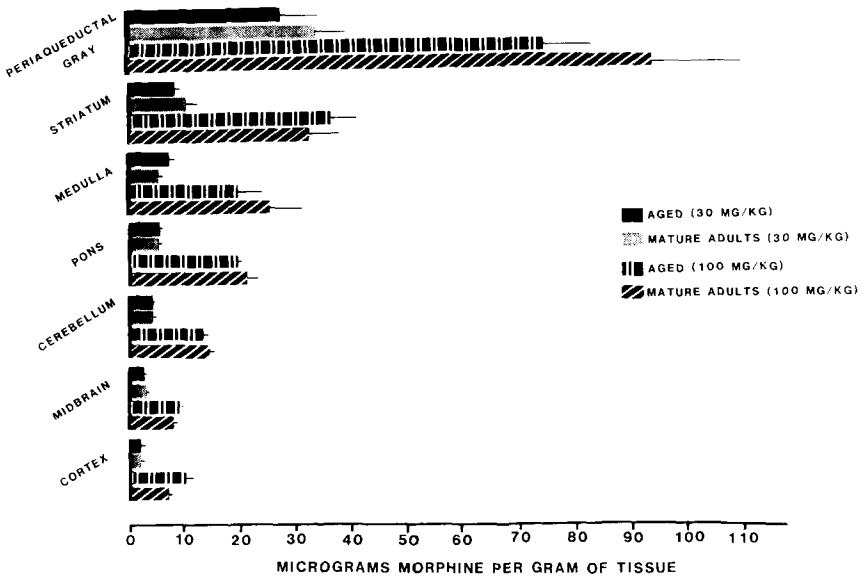

FIG. 8. Regional distribution of ^{3}H-morphine in brains of aged (24–27 months old) and mature adult (3–6 months old) mice. Each bar represents the mean ± S.E. of values from 8 brains.

dose produced a latency of only 1.7±0.5 seconds in the aged mice.

A diagram of the quantal dose-response curves for the two age groups (in terms of the percent of mice with latencies greater than 8 sec one hour after administration of morphine) are shown in Fig. 4. A dose of 30 mg/kg produced maximal antinociception (i.e., a tail flick latency of 8 sec) in all of the mature adult mice, whereas this same dose failed to give this degree of antinociception to any of the aged mice. Whereas this maximal antinociceptive response occurred in 4 of 10 mature adult mice 1 hour after a dose of 10 mg/kg, no aged mice responded maximally until 1 hour after a dose of 50 mg/kg when 3 of 11 aged mice failed to respond within the 8-second interval. Probit analyses revealed that the ED50 values for maximal antinociception were statistically different for the two age groups, being 12.95 (7.31–17.7) mg/kg for the mature adult mice, and 70.05 (60.07–86.69) mg/kg for the aged mice. Slopes of the dose-response curves were 4.15 and 5.98 for the mature adult and aged mice, respectively.

Morphine-Induced Locomotor Behavior

There were no statistically significant differences in baseline locomotor activities of the two age groups and as shown in Fig. 5, 10 mg/kg morphine affected both age groups alike, in terms of locomotor activity. The 20 mg/kg dose had a greater effect on the aged mice, with peak activity and significantly higher activity in aged mice occurring 40 minutes after morphine administration. Whereas peak activity was produced in the mature adults within 2 hours of administration of 30 mg/kg morphine, peak activity in the aged group occurred after 2 hours and the response at that time was significantly higher in the aged group. Peak locomotor activity occurred at approximately the same time after the dose of 40 mg/kg in both age groups, i.e., between 1.5 and 2.5 hours after the injections, and during this time, the activity was significantly higher in the older age group. Further increases in morphine dose to 50 and 60 mg/kg failed to have much effect on the locomotor activity of the two age groups, while a further increase in dose to 80 mg/kg greatly increased activity in the mature adults. Furthermore, this dose of morphine produced the greatest increase in activity of the mature adults. The aged mice, on the other hand, were little affected by this high dose of morphine.

Diagrams of quantal dose-response relationships in terms of producing locomotor activity which was 5 times that of control activity at anytime after morphine administration are shown in Fig. 6. Whereas 3 of 8 aged mice displayed a 5-fold increase in activity after a low dose of 5 mg/kg morphine, only 2 of 8 mature adult mice displayed such activity after 10 mg/kg morphine. In both age groups, the maximum number of such responders was 7 out of 10 mice. The ED50 values for the excitatory effect of 5 to 40 mg/kg, as determined by probit analysis, were statistically different, being 7.5 (4.10–8.60) mg/kg and 17.81 (9.02–21.62) mg/kg for the aged and mature adult mice, respectively. Slopes of the dose-response curves up to 50 mg/kg were 2.0 and 3.2 for the aged and mature adults, respectively. The figure also shows the loss of excitatory response at doses above 40 mg/kg in the aged animals as well as the decreased locomotor activity in the mature adult mice followed by another excitatory response at 80 mg/kg.

Brain Distribution of Morphine

One hour after SC administration of 30 and 100 mg/kg morphine tagged with ^{3}H-morphine, 0.13 and 0.14 percent of the doses appeared in brains of aged and mature mice, respectively. The regional distributions of morphine were the same in both age groups (Fig. 7) with the amounts corresponding with the sizes of the regions. When the distribution was calculated on a per weight basis, however, the reverse was true (Fig. 8), i.e., the highest concentration of morphine was found in the smallest region, the periaqueductal gray area and the lowest concentrations were found in the larger

areas (cortex, midbrain and cerebellum). These distributions were the same for both the 30 and 100 mg/kg doses of morphine.

DISCUSSION

This study has shown that at least two responses to morphine are altered by aging in ICR Mice. First, both the duration and intensity of morphine-induced antinociception were significantly decreased in the advanced stage of aging. This is in agreement with reports by Webster *et al.* [19] on C57BL/6J mice and Kavaliers *et al.* [9] on CF-1 mice. Second, morphine administration in doses of 20 to 40 mg/kg resulted in greater increases in locomotor activity in aged mice than in the younger group. On the other hand, while a dose of 80 mg/kg hardly affected the activity of aged mice, it greatly increased locomotor activity of the younger animals. These age-related differences were apparently not due to differences in levels of morphine attained in the brain, since such levels were the same in both age groups, nor were they due to differences in regional distribution of morphine within the brains since distributions were the same. Thus, they can only be explained in terms of differences in effectiveness of morphine in the central nervous system, i.e., in affinity and/or numbers and/or responsiveness of opioid receptors, or in responsiveness of the central dopaminergic or other neurotransmitter system(s) to morphine.

The differences we observed in the morphine-induced locomotor behaviors of the two age groups at morphine doses above 40 mg/kg are striking and may suggest dose-dependent interactions of morphine with multiple receptors, some of which are missing in aged animals or have become subsensitive to morphine. Alternatively, the excitatory and inhibitory effects of morphine on locomotor activity might be due to activation of a single opioid receptor subtype which is differentially and age-dependently related to activity in a variety of the complex neuronal systems of the CNS.

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