Effects of Fetal Exposure to Gamma Rays on Aggressive Behavior in Adult Male Mice

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Aggressive behavior (AB) in first generation (F1) hybrid male C57BL/6×C3H mice irradiated on the 14th day of gestation was studied at 100-135 days of age. Gravid female mice were irradiated with 1.0 or 2.0 Gy of gamma rays to the whole body. The AB of pairs of mice were recorded with a capacitance-induction motility monitor and on videotape. Recordings were continued for 90 min, starting at 2:00 PM. Vigorous wrestling, boxing and biting were regarded as AB. Data recorded at 15-min intervals were stored on micro-computer discs. The body weight for the irradiated group was significantly lower than that for the control group. The number of instances of AB was significantly higher in the irradiated group. The AB of the 2.0 Gy group was significantly more intensive than that of the control group. No difference in the duration of AB was found for the 2 irradiated and the control groups. Results demonstrate that male mice irradiated prenatally show increased aggressiveness.

INTRODUCTION

Exposure to ionizing radiation prior to parturition is known not only to cause a variety of morphological changes but to produce behavioral changes in animal offspring. D'Amato and Hicks1) reported that rats irradiated with 2.0 Gy on the 17th day of gestation had disordered locomotor rhythm. Moreover, rats irradiated with X-rays on day 17 of gestation showed a greater conditioned avoidance response than did the controls2-3). In the open field test, rats that had been irradiated with 1.25 Gy on gestational day 15 showed greater activity than the controls at 5 months of age4). Most of the evidence presented, however, has been for the behavior of animals that were maintained alone. Little is known about the social behavior of prenatally irradiated animals.

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The study reported here was done to investigate social behavior in particular aggressive behavior (AB) in mice exposed prenatally to ionizing radiation. The 14th day of gestation was chosen for irradiation because Sasaki (personal communication) has shown that male mice irradiated with 3.0 Gy of gamma rays on that day showed marked aggressive behavior, sometimes even muricide.

MATERIALS AND METHODS

Animals

First generation (F1) C57BL/6 × C3H hybrid male mice were used. All were bred and reared in the laboratory. C57BL/6 females, 10–14 weeks old, were caged nightly with C3H males of the same age. The presence of sperm in the vagina the next morning was taken as presumptive evidence of pregnancy, the day on which this was determined being designated as 0 day of gestation. All offspring were weaned at 4 weeks of age and separated according to sex. The mice were maintained under Specific Pathogen Free (SPF) conditions up to 2 months of age, thereafter, under clean maintenance conditions in the laboratory where the behavioral testing was done. Each mouse was housed individually in a rearing cage for about 1 month before testing. The ambient regulated temperature was 22±2°C. A 12-hour day-night cycle was maintained with light on at 8:0 AM. Food and water were given ad libitum. The mice between 100 and 135 days of age were tested. The experiment was done in accordance with the established guidelines in “Animal Experiments” compiled by Yamanashi Medical College.

Irradiation

On the morning of the 14th day of gestation, pregnant mice were exposed to single whole-body gamma irradiation from a cesium-137 source irradiator. During irradiation the unanesthetized mouse was restrained in a plexiglass box. The dose rate to the midline of the mouse was 0.25 Gy per minute. The dose given was 1.0 or 2.0 Gy. The offspring were fostered by normal mothers. The control mother was handled in the same way and placed on the platform in the plexiglass box but given no radiation.

The Aggressive Behavior (AB)

AB was recorded with a capacitance-induction motility monitor (Natumex KN-70H). This device operates on the principle of a tuned oscillator circuit which is off-tuned when any new mass is introduced into its electromagnetic field. Movement of an animal across this field therefore leads to a change in tuning that is recorded as a “count”. The apparatus is housed in a box measuring 40×30×15 cm. Immediately below the top of the box is a mounted system of two high frequency oscillator coils. The box also contains the rest of the oscillator circuit, an amplifier, a pulse former and a counter5–7. A pair of mice was removed from the home cage and placed in a plastic cage (20×17×13 cm) that rested on the stage of the apparatus. The sensitivity of the apparatus was adjusted for 4 scores that were indicated on the monitor when a plastic cylinder (1.5 cm dia) filled with 20 ml of physiological saline solution was moved at the
rate of 10 cm/sec on the stage. The AB and motility scores of these animals were recorded simultaneously on videotape. Two observers, "blinded to" treatment condition, analyzed the videotape records. The sum of the vigorous wrestling, boxing and biting that took place was regarded as AB. The duration of the AB was measured from a slow motion replay. The beginning and end of AB were confirmed at 0.1 second units. All recordings were made for 90 min, starting at 2:00 PM. During the experimental period, the mice were deprived of water. Pairs were formed randomly using mice from different litters in the same group. A mouse was never paired with the same mouse more than once.

Data Analysis

The number of instances of AB that occurred during the six 15-min blocks was counted, after which AB/sec scores and the duration (sec) of AB during the 6 blocks were calculated. All data were stored on micro-computer discs (NEC 9801Vm). Because the data were not normally distributed, Mann-Whitney U tests were used for the analysis.

RESULTS

The numbers of mothers, offspring (male), and recordings, as well as the body weights at 3 and 5 months of age of the 2 irradiated and the control groups are shown in Table 1. The irradiated groups had significantly lower body weights than the control group at 3 and 5 months of age. As one mouse in the 2.0 Gy group was killed by its partner during the testing, the number of recordings for the 2.0 Gy group is smaller than the numbers for the other groups.

Table 1. Numbers of pregnant mothers, offspring (male) and recordings, and the means and standard deviations (S.D.) for body weight at 3 and 5 months of age

|                     | Control | 1.0 Gy | 2.0 Gy |
|---------------------|---------|--------|--------|
|                     | mean    | S.D.   | mean   | S.D.   | mean   | S.D.   |
| Number of mothers   | 3       | 3      |        |        | 3      | 3      |
| Number of offspring (male)| 13 (4, 4, 5) | 10 (3, 3, 4) | 9 (2, 3, 4) |
| Number of animals used | 9       | 10     | 7      |
| Number of recordings | 9       | 9      | 8      |
| Body weight (g)     |         |        |        |
| 3 months of age     | 36.8 ± 2.2| 31.0 ± 1.2*| 29.1 ± 1.0* |
| 5 months of age     | 41.3 ± 1.8| 38.0 ± 2.1*| 32.3 ± 3.8* |

Significant difference from the control value (t-test): *p<0.05. Numbers in parentheses are the number of mice in each litter.
The AB

The control group showed an initial slight decrease in the number of instances of AB per block, and subsequent lower numbers during the observation period. The irradiated group had a time-related decrease in number, the number in the early part of the observation period being significantly higher in the irradiated than in the control group (Fig. 1, upper panel). The AB scores of the 1.0 Gy and control groups decreased slightly, but not significantly, throughout the 90-min observation period. Scores for the 2.0 Gy group remained stable until the 5th block, then decreased. For these 5 blocks, the 2.0 Gy group registered significantly more scores than the control group. No significant difference was found between the 1.0 Gy and control groups (Fig. 1).
The duration of AB in each 15-min block for the 2 irradiated and the control groups was roughly uniform throughout the 90-min observation period. There was no significant difference in duration among the 3 groups (Table 2).

**Table 2.** Means and standard deviations (S.D) of the duration (sec) of AB in six 15-min blocks

| Time (min) | Control mean ± S.D. | 1.0 Gy mean ± S.D. | 2.0 Gy mean ± S.D. |
|-----------|---------------------|-------------------|-------------------|
| 15        | 13.6 ± 6.8          | 13.8 ± 5.1        | 15.0 ± 9.5        |
| 30        | 14.9 ± 8.8          | 12.4 ± 4.9        | 15.9 ± 7.1        |
| 45        | 11.0 ± 5.3          | 10.9 ± 7.0        | 12.2 ± 5.2        |
| 60        | 13.5 ± 12.3         | 12.3 ± 3.9        | 11.4 ± 8.3        |
| 75        | 11.8 ± 6.8          | 13.2 ± 5.1        | 9.8 ± 3.6         |
| 90        | 9.3 ± 6.4           | 12.6 ± 10.3       | 15.0 ± 6.2        |

Numbers of recordings are given in Table 1.

**DISCUSSION**

Although recordings made with devices such as the motility monitor used in this experiment clearly can not distinguish between different kinds of AB, our results demonstrate that adult male mice that had irradiated with gamma rays during the fetal period showed increased aggressiveness. There is little information in the literature with which to compare our findings. Upton et al. reported that adult male mice exposed to 3.0 Gy of X-rays on day 14.5 of gestation showed hyper-irritability. No quantitative functional changes however have been reported.

The data reported here indicate that the number of instances of AB was significantly higher in the irradiated groups than in the control group and that the AB of the 2.0 Gy group was significantly more intensive than that of the control group. These results are considered to indicate that the 2.0 Gy group was hyperactive. Several reports have shown that adult rats irradiated with X-rays during the late stage of their fetal development exhibited hyperactivity, but it is impossible to make a direct comparison because of the differences in the animals and behavioral tests used. The number of squares traversed in an open field test given adult rats that had been irradiated with 1.0 Gy on the 15th day of gestation was greater than that for non-irradiated rats. Norton has reported that in the modified open field test the number of times that adult rats dipped their head into the holes placed in the floor of the field was greater for rats exposed to 1.25 Gy on gestational day 15 than for non-irradiated rats.

The number of instances of AB in the 1.0 Gy group was higher than for the control group, but changes in the AB scores were not as clear as those for the 2.0 Gy group. The number of instances of AB appears to be a more sensitive indicator of radiation-induced effects than the AB scores, under the experimental protocol we used.
Body weights of the irradiated groups at 3 and 5 months of age were significantly below the weights of the control group. The studies of Rugh et al.\textsuperscript{10} on the monkey, of Sikov et al.\textsuperscript{11} and Norton\textsuperscript{4} on the rat, and of Minamisawa and Sasaki\textsuperscript{12} on the mouse gave similar results. Jensh et al.\textsuperscript{13} stated that changes in body weight are a more sensitive indicator of the effects of prenatal exposure to X-radiation than is postnatal behavior. Our results show that both body weight and AB were equally sensitive to prenatal irradiation.

Various studies on behavioral changes produced by low level prenatal radiation exposure have been reported. Rats irradiated at 0.25 or 0.5 Gy on day 10 or 20 of gestation performed at a significantly higher levels in the Lashley III maze\textsuperscript{9}. Jensh et al.\textsuperscript{2} reported that in a conditioned avoidance test, rats that had received 0.6 Gy on the 9th day of gestation had significantly higher shock rates than their control counterparts. Furthermore, activity in a continuous corridor at 21 days of age was decreased in rats that had received 0.25 Gy of X-rays on gestational day 15\textsuperscript{4}. Our data do not show the effects on AB of prenatal exposure to low levels of ionizing radiation. The analysis of different kinds of AB (wrestling, boxing and biting) does, however, make clear whether the AB of adult male mice exposed to low level prenatal irradiation is changed.

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REFERENCES

1. D'AMATO, C. J. and HICKS, S. P. (1980) Development of the motor system: effects of radiation on developing corticospinal neurons and locomotor function. Exptl. Neurol. 70: 1–23.
2. JENSH, R. P. BRENT, R. L. and VOGEL, W. H. (1987) Studies of the effect of 0.4-Gy and 0.6-Gy prenatal X-irradiation on postnatal adult behavior in the Wistar rat. Teratology 35: 53–61.
3. TAMAKI, Y. and INOYUE, M. (1979) Avoidance of and anticipatory responses to shock in prenatally X-irradiated rats. Physiol. Behav. 22: 701–705.
4. NORTON, S. (1986) Behavioral changes in preweaning and adult rats exposed prenatally to low ionizing radiation. Toxicol. Appl. Pharmacol. 83: 240–249.
5. SVENSSON, T. H. and THIEME, G. (1969) An investigation of a new instrument to measure motor activity of small animals. Psychopharmacologia 14: 157–163.
6. HOFECKER, G. KMENT, A. NIEDERMULLER, H. and SAID, H. (1974) Assessment of activity patterns of one- and two-year-old rats by electronic recording. Exptl. Gerontol. 9: 109–114.
7. MARTIN, J. R. FUCHS, A. BENDER, R. and HARTING, J. (1986) Altered light/dark activity difference with aging in two rat strains. J. Gerontol. 41: 2–7.
8. UPTON, A. C. CONKLIN, J. W. and POPP, R. A. (1966) Influence of age at irradiation on susceptibility to radiation-induced life-shortening in RF mice. In “Radiation and Ageing”, Ed. P. J. Lindop and G. A. Sacher, pp. 337–344. Taylor and Francis, London.
9. WERBOFF, J. HAVLENA, J. and SI Kov, M. R. (1962) Effects of prenatal X-irradiation on activity, emotionality, and maze-learning ability in the rat. Radiat. Res. 16: 441–452.
10. RUGH, R. DUHAMEL, L. SKAREDOFF, L. and SOMOGYI, C. (1966) Gross sequelae of fetal X-irradiation of the monkey (Macaca mulatta). Atompraxis 12: 468-473.
11. SIKOV, M. R. RESTA, C. F. and LOFSTROM, J. E. (1969) The effects of prenatal X-irradiation of the rat on postnatal growth and mortality. Radiat. Res. 40: 133-148.
12. MINAMISAWA, T. and SASAKI, S. (1983) The electrocorticograms of the adult and aged mouse X-irradiated during the perinatal period. Electroenceph. Clinical Neurophysiol. 55: 73-81.
13. JENSH, R. P. BRENT, R. L. and VOGEL, W. H. (1986) Studies concerning the effects of low level prenatal X-irradiation on postnatal growth and adult behaviour in the Wistar rat. Int. J. Radiat. Biol. 50: 1069-1081.