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Ionizing Radiation Alters Beta-Endorphin-Like Immunoreactivity in Brain but not Blood[1]

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MICKLEY, G. A., K. E. STEVENS, G. H. MOORE, W. DEERE, G. A. WHITE, G. L. GIBBS AND G. P. MUELLER. *Ionizing radiation alters beta-endorphin-like immunoreactivity in brain but not blood.* PHARMACOL BIOCHEM BEHAV **19**(6) 979–983, 1983.—Previous behavioral and pharmacological studies have implicated endorphins in radiation-induced locomotor hyperactivity of the C57BL/6J mouse. However, the endogenous opiate(s) responsible for this behavioral change have not been identified. The present study measured beta-endorphin-like immunoreactivity (beta-END-LI) in brain, blood, and combined brain and pituitary samples from irradiated and sham-irradiated C57BL/6J mice. After radiation exposure, levels of beta-END-LI decreased significantly in the brain. A similar, but not statistically significant, decline was measured in combined brain and pituitary samples. Concentrations of blood beta-END-LI were not changed by irradiation. These radiogenic changes in beta-END-LI are in some ways similar to those observed after other stresses. However, radiation-induced locomotor hyperactivity may be mediated more by alterations of beta-END-LI in the brain than in the periphery. Other endogenous opiate systems may also contribute to this behavioral change in the C57BL/6J mouse.

Radiation exposure Beta-endorphin-like immunoreactivity Locomotor hyperactivity Endorphins
Mice Brain Blood Pituitary Opiates

A VARIETY of recent studies have suggested that exposure to ionizing radiation, like exposure to other stressors [1, 3, 4, 5, 7] may stimulate the release of endorphins and that these endogenous opiates might play some part in radiogenic behavioral change. For example, cross tolerance has been demonstrated between the effects of radiation and morphine in that morphine-tolerant rats suffer less of a behavioral incapacitation after irradiation than do non-tolerant subjects [23]. Additionally, another species (the C57BL/6J mouse) exhibits a naloxone-reversible locomotor hyperactivity almost immediately after irradiation [22]. This radiogenic locomotor response is similar to the "running fits" observed in mice after either a peripheral injection of morphine [38] or an intraventricular injection of enkephalin [16]. Further, chronic preexposure to the stresses of restraint or footshock (known to increase blood beta-END-LI) [27] has been shown to significantly reduce the subsequent radiogenic activation of the C57BL/6J mouse [24]. This finding is analagous to others which have revealed a cross tolerance between various stress-induced analgesias [5] and it lends support to the hypothesis that endogenous opiates mediate radiogenic locomotor hyperactivity in the C57BL/6J mouse.

Although behavioral and pharmacological evidence suggests a role for endorphins in radiation-induced behavioral change, the endogenous opiate(s) which produce these effects and the locus of their action have yet to be identified. The endogenous opiate beta-endorphin is known to be released in response to several stressful stimuli [26,27] and exhibits potent morphine-like effects under experimental conditions [14,37]. To examine the possibility that beta-endorphin, or closely related peptides, may mediate radiation-induced behavioral changes we measured beta-

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END-LI in samples of brain, combined brain and pituitary, and blood of the C57BL/6J mouse following ^{60}Co irradiation.

METHOD

Male C57BL/6J mice (20–25 g) were obtained from Jackson Laboratories, Bar Harbor, ME and group-housed under a 12 hour "on" 12 hour "off" light/dark cycle (lights on at 7:00 a.m.). Animals had free access to standard laboratory rat chow and tap water. At the beginning of the experiment subjects (N=120) were removed from their home cages and placed in individual polyethylene holders (7.7×2.9×3.0 cm). Sixty mice were then exposed to 1500 rads ^{60}Co. The remaining half of the subjects underwent a sham irradiation procedure in which they were confined in holders for a time equal to that of the irradiated subjects. All irradiations occurred at approximately the same time of day during the animals' light cycle, in an attempt to control for circadian variations.

Five minutes after irradiation, forty mice were removed from their containers and quickly decapitated. Brains (tissue rostral to the cerebellum but excluding the most anterior portions of the olfactory bulbs) from 10 mice were quickly dissected and individually frozen in liquid nitrogen. The same procedure was followed for 10 additional mice but in these samples, pituitary glands were included along with brain tissue. Trunk blood was collected from 20 other animals. Due to the small volume of blood which could be obtained from each mouse, subjects were paired and blood was pooled to make up a total of 10 samples for the beta-END-LI assay. Blood was collected into tubes containing 0.5 ml of 10% EDTA plus bacitracin (30 mg/ml) and kept on ice. The tubes were centrifuged and resulting plasma was stored at −70°C until assayed.

Forty other mice were decapitated 5 minutes after sham-irradiation and brain, (N=10), brain and pituitary (N=10) and blood samples (N=20, yielding 10 samples, 2 mice/sample) were collected as described above.

After irradiation (N=20) or sham exposure (N=20) the remaining mice were removed from their holders and placed back in a cage for 1 hour. After this period these animals were decapitated and brain samples (similar to those described previously) were taken from half the irradiated and sham-irradiated subjects. Brain and pituitary samples were collected from the remaining mice. Blood was not taken at this later time period.

Two irradiation facilities were used to deliver the standard midline whole-body dose of 1500 rads ^{60}Co. This dose of ^{60}Co radiation exceeds the LD95(30) for these mice (Personal communication, Dr. G. D. Ledney, Armed Forces Radiobiology Research Institute, Bethesda, MD) but has been shown to produce an acute locomotor hyperactivity [22]. Mice from which we collected brain and combined brain and pituitary samples were irradiated through the use of a Theratron 80 irradiator. Subjects were placed 80 cm away from the cobalt source and covered with a 0.5 mm lead bolus. The dose rate averaged 119 rads/minute. Dosimetry was accomplished in air with ionization chambers (Spokas Model 1000) which were equipped with a ^{60}Co build-up cap. Doses and backscatter factors were computed from depth/dose tables [8]. Mice from which blood was collected received the same 1500 rad ^{60}Co dose as did the other animals, however, this irradiation was accomplished through the use of the ^{60}Co facility of the Armed Forces Radiobiology Research Institute, Bethesda, MD. Here mice were 106 cm away from the cobalt source and the dose rate averaged 292 rads/minute. Dosimetry was accomplished through the use of a 0.5 cc Exradin Ionization Chamber. Calibration of all ionization chambers was traceable to the National Bureau of Standards.

Brain and combined brain and pituitary beta-END-LI was measured with a double antibody radioimmunoassay using commercially-available reagents supplied by Immuno Nuclear Corporation, Stillwater, MN. The antibody was raised in rabbits against synthetic human beta-endorphin conjugated by carbodiimide to keyhole limpet hemocyanin. The antibody detects beta-endorphin and cross reacts, on an equal molar basis with beta-lipotropin and several modified forms of beta-endorphin (i.e., Des-Tyr^1 beta-endorphin, 2-Me-Ala^2 beta-endorphin). Peptides related to the N-terminal amino acid sequence of beta-endorphin (i.e., Leu- and Met-enkephalin, alpha-endorphin), however, do not cross react in the assay. The radioimmunoassay of brain and pituitary tissue followed acid extraction with 5 Normal acetic acid and utilized phenylmethylsulfonyl fluoride and iodoacetamide (both 30 mg/ml of ethanol) as protease inhibitors [20]. Clarification by preparative centrifugation was followed by concentration using lyophilization.

Plasma levels of beta-END-LI were measured by a radioimmunoassay as previously described [26, 27, 31, 32]. The antibody used (antiserum C-55) detects beta-endorphin (beta-END), purified human beta-lipotropin (gift of A. Parlow, University of California Medical Center, Torrance, CA and D. Orth, Department of Medicine, Vanderbilt University Medical School, Nashville, TN) and several modified forms of beta-END (i.e., N-acetyl beta-END 1–31, 1–27, and 1–26; beta-END 1–27 and 1–26) on an equal molar basis. Peptides related to the N-terminal amino acid sequence of beta-END (i.e., Leu-and Met-enkephalin, alpha-endorphin), however, do not cross-react in the assay.

The brain and brain and pituitary data were analyzed by two separate 2×2 (irradiation/sham × sampling times) analyses of variance while the data derived from the blood assay were examined via a *t*-test [43].

RESULTS

As compared to the sham-irradiated mice, exposure to ionizing radiation produced a statistically significant reduction in the concentration of brain beta-END-LI, $F(1,36)=9.72$, $p=0.004$ (see Table 1). An "a priori" test [43] revealed that this radiogenic reduction in beta-END-LI was most prominent by 5 minutes after irradiation ($p=0.002$). After 60 minutes there was no statistically significant difference ($p>0.05$, a priori test) between the brain beta-END-LI levels in irradiated and sham-exposed mice. Beta-END-LI brain concentrations increased over time in irradiated mice but decreased in sham-irradiated subjects. However, within the analysis of variance design, these changes did not achieve statistical significance, $F(1,36)=0.04$, $p=0.84$. Neither was there a significant interaction between sampling time and irradiation/sham treatments, $F(1,36)=2.43$, $p=0.13$.

Samples of brain, which also included the pituitary, revealed a trend towards a radiogenic decline in beta-END-LI relative to sham irradiated controls (see Table 1). This decrease just failed to achieve statistical significance, $F(1,36)=3.90$, $p=0.06$. The subjects sacrificed 60 minutes after irradiation/sham had combined brain and pituitary concentrations which were significantly higher than those sacrificed 5 minutes, post irradiation/sham, $F(1,36)=14.64$, $p<0.001$. This enhancement was not observed solely in irradiated mice and may reflect a reaction to the stress of

TABLE 1

MEAN CONCENTRATIONS OF BETA-ENDORPHIN-LIKE-IMMUNOREACTIVITY IN THE C57BL/6J MOUSE AT TWO DIFFERENT TIMES AFTER RADIATION EXPOSURE OR SHAM IRRADIATION

Sample	Treatment	Sample Time (minutes post radiation or sham)	N	Mean beta-END-LI concentration (standard error of the mean)	p
Brain	Radiation	5	10	0.74 ng/g (0.09)	0.002*
Brain	Sham	5	10	1.57 ng/g (0.25)	
Brain	Radiation	60	10	0.98 ng/g (0.12)	NS*
Brain	Sham	60	10	1.25 ng/g 0.23)	
Brain and Pituitary	Radiation	5	10	21.74 ng/g (1.96)	NS†
Brain and Pituitary	Sham	5	10	24.42 ng/g (1.66)	
Brain and Pituitary	Radiation	60	10	27.05 ng/g (1.25)	NS†
Brain and Pituitary	Sham	60	10	29.95 ng/g (0.85)	
Blood	Radiation	5	10‡	10.95 ng/ml (5.26)	NS§
Blood	Sham	5	10‡	11.65 ng/ml (5.16)	

*Two-way ANOVA, a priori comparison. NS=Not significant ($p>0.05$).
†Two-way ANOVA.
‡Two mice per pooled sample.
§Student's t-test.

confinement which occurred during both irradiation and sham irradiation exposure. A significant interaction between radiation treatment and sampling time was not present in the data derived from the brain and pituitary specimens, $F(1,36)=0.01$, $p=0.94$.

Blood beta-END-LI levels (taken at 5-minutes post irradiation/sham) were not altered by radiation exposure. The variability of these samples was large (see Table 1). A "t-test" revealed no significant difference ($p>0.05$) between the endorphin levels in irradiated and sham irradiated mouse blood.

DISCUSSION

Previous studies have suggested that endogenous opiates may mediate radiation-induced locomotor hyperactivity of the C57BL/6J mouse [22,24] and there has been a preliminary report of enhanced blood levels of beta-END-LI in irradiated rats [2]. However, the present experiments found that exposure to ionizing radiation produced an immediate decline in the levels of brain beta-END-LI in the C57BL/6J mouse. To a lesser extent, this reduction is also observed in combined brain and pituitary samples. Levels of blood beta-END-LI, however, were not altered by irradiation in this strain of mouse.

The concentrations of whole-brain beta-END-LI reported here are lower than previously published values for the rat [28, 30, 35]. C57BL/6J mice also apparently have less pituitary beta-END-LI than do rats [19,21]. In addition significant differences have been reported between the levels of pituitary beta-END-LI of various mouse strains [9]. Cross-study comparisons should take into account this apparent genetic variability between the beta END-LI concentrations of difference species and mouse strains.

Beta endorphin is a potent analgesic [37] and is thought to mediate the antinociceptive effects observed after various stress treatment [5]. Therefore the reductions of brain Beta-END-LI concentrations reported here might predict a radiogenic hyperalgesia or, alternatively, the lack of change in blood beta-END-LI may suggest that pain perception would be unaltered after irradiation. In either case, given our neurochemical data, one would not expect an analgesic reaction to accompany radiation exposure. Although analgesia was not measured in the present experiments, previous studies of pain perception are consistent with our data. They reveal that radiation exposure may either reduce [18] or not discernibly alter [10] morphine-induced analgesia. Apparently irradiation does not produce analgesia itself [23].

Like the radiation-exposure treatment described here, other stressors (i.e., footshock and restraint) have also been shown to reduce concentrations of brain endorphins [12, 34, 36]. However, this similarity should not be interpreted as confirmation of a common endorphin substrate which mediates a variety of stress-induced behavioral changes. The present findings could be produced by several mechanisms (e.g., increased release and breakdown of endorphin, radiation-induced cleavage of the assayed molecules, etc.)

which may or may not be common with other stress reactions. Additionally, increases in blood endorphin (perhaps of pituitary origin) have been reported after footshock and restraint stesses in rats [12, 27, 34] whereas, in this study, no such change occurred after radiation exposure in mice. Whether this difference is due to the different species used or type of stresses employed remains to be determined.

Although some evidence exists for a relationship between the stress-induced changes in brain, pituitary and blood endorphins [34], alterations of brain beta-END-LI may well be independent of changes in pituitary and blood endorphins. A variety of studies have suggested the existence of two separate endogenous opiate systems [19, 25, 29, 30, 39, 40, 41]: (a) an endocrine system involving the pituitary and (b) a neuronal system wherein beta-END-LI is synthesized and released by brain neurons. The present data are consistent with the hypothesis that the neural beta-END-LI system may be activated by ^{60}Co radiation (perhaps causing release and breakdown) while the pituitary system may be less affected by this treatment. As others have suggested [15], perhaps endorphins are acting centrally to produce behavioral changes even during times when endogenous opiate levels are undetectable or unaltered in the blood stream.

Administration of morphine produces a locomotor hyperactivity similar to that seen in irradiated mice [22,38]. Therefore, since blood and brain concentrations of beta-END-LI were not found to increase after irradiation, an alternative explanation of the present data is that other endogenous opiates might provide a more likely substrate of radiation-induced behavioral activation. A recent study [33] has revealed that beta-endorphin, administered intracerebroventricularly (ICV) does not produce locomotor hyperactivity in the C57BL/6J mouse but rather evokes a depression of movement. However, in this same experiment, D-Ala^2, Leu^5) enkephalinamide (ICV) stimulated locomotor activity in the mouse. Enkephalins are located in different areas of the brain [6], have different break-down rates [13] and seem to have functions which differ dramatically from those of beta-endorphin [11,42]. Therefore additional interpretations of the present data should include the possibility that beta-END-LI may play an indirect or non-primary role in the production of radiogenic locomotor activation in the C57BL/6J mouse.

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