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Abstract: Total or partial body irradiation is a distinct risk of accidental, wartime, or terrorist events. Total body irradiation is also used as conditioning therapy before hematopoietic stem cell transplantation. This can result in injury to multiple tissues and may result in death due to multi-organ failure. The hypothalamic-pituitary-adrenal (HPA) axis could play a causative role in those injuries, in addition to being activated under conditions of stress. In a rat model of TBI, we have established that radiation nephropathy is a significant lethal complication, due to hypertension and uremia. The present study assessed HPA axis function in rats undergoing total body irradiation (TBI). Using a head-shielded model of TBI, we found an enhanced response to corticotropin-releasing hormone (CRH) *in vitro* in pituitaries from irradiated compared to non-irradiated rats at both eight and seventy days after 10 Gy single fraction TBI. At seventy, but not eight days, plasma ACTH and corticosterone levels were significantly increased in irradiated compared to non-irradiated rats. Plasma aldosterone was not affected by TBI at either time point, while plasma renin activity was decreased in irradiated rats at 8 days. Basal and stimulated adrenal steroid synthesis *in vitro* was not affected by TBI. In addition, plasma epinephrine was decreased at 70 days after TBI. Hypothalamic expression of CRH mRNA and hippocampal expression of glucocorticoid receptor mRNA were unchanged by irradiation. We conclude that the hypertension of radiation nephropathy is not aldosterone or catecholamine-dependent, but that there is an abscopal activation of the HPA axis after 10 Gy TBI. This activation was at least partially attributable to enhanced pituitary ACTH production.

Introduction

Illness after total body irradiation (TBI) is complex, and can present as multi-organ injury ([1]). The hypothalamic-pituitary-adrenal (HPA) axis could play a significant causative or reactive role in this multi-organ injury. There have been limited studies on the effects of TBI on the HPA axis. In humans undergoing TBI for hematopoietic stem cell transplantation, acute increases in plasma ACTH have been reported at four hours after TBI ([2]). Rats undergoing 1 to 10 Gy TBI showed increased plasma ACTH and corticosterone at one hour to three days after TBI ([3]–[5]). This suggests pituitary-adrenal activation soon after TBI. Changes in the HPA axis at later times have not been thoroughly studied.

In preliminary studies, we have found an increase in urinary aldosterone and corticosterone excretion between one and ten weeks after 10 Gy TBI in rats (unpublished). This could contribute to the

hypertension of radiation nephropathy, either through aldosterone itself or via mineralocorticoid effects of corticosterone. Additional complications of TBI, such as osteopenia or impaired hippocampal neurogenesis, could also be caused by an activated HPA axis. These studies were designed to test whether TBI activates the HPA axis in rats. The effects of TBI were assessed by measuring plasma hormones, evaluating *in vitro* hormone production, and quantifying the expression of pertinent genes.

Methods

Radiation model

WAG/Rij/MCW male rats aged 5 to 8 weeks were maintained on sterilized rat chow in a moderate-security barrier facility at the Biomedical Resource Center of the Medical College of Wisconsin (MCW) (Milwaukee, WI, USA). Rats received TBI with a single dose of 10 Gy with a posterior-anterior field at a dose rate of 1.95 Gy/min. This dose was chosen because it is a dose that has been used clinically and because we have ample data in this model at this dose. Irradiation was with 300 kVp orthovoltage x-rays, with a half value layer of 1.4 mm Cu. The heads were protected by a lead shield that reduced the radiation dose by over 98%; this prevents long term buccal and salivary gland injury. The brain and pituitary gland are shielded in this model. The use of a barrier facility eliminated the risk of infectious morbidity and mortality. One to 2 hr after TBI, rats were given fresh bone marrow cells from a syngeneic littermate as previously published (6). The bone marrow cells prevent hematologic death from the TBI, and the use of a syngeneic donor avoids graft versus host disease. Control rats were sham-irradiated and did not receive a bone marrow transplant. In this model, there is reduced food intake during the first week after TBI. The animals then recover, gain weight, and are apparently healthy until they become hypertensive and uremic at three months or more after TBI. A standard rodent diet (Harlan Teklad 8604, 25% protein and 0.3% sodium) and water were provided *ad libitum*. Rats were housed two per cage on a 12 hour light-dark cycle at an ambient temperature of 21 degrees C. Rats were handled daily on the three days before experimentation in order to minimize stress responses at the time of sacrifice. Rats were sacrificed by rapid

decapitation at 8 or 70 days after TBI, all between 8 and 9 a.m. The eight and seventy day time points are within the latent period where there is little evidence for radiation injury in this model. Thus, detectable abnormalities at these time points are highly relevant to the mechanisms of injury. Animal care was in accordance with NIH guidelines. The Animal Care and Use Committee at the MCW approved all procedures, which were done in accord with its ethical guidelines. .

Eight irradiated and eight non-irradiated rats were sacrificed on each study day. Trunk blood was obtained from all rats. Four pituitary glands from each group were placed in liquid nitrogen and four were placed in ice-cold buffer. Twelve adrenal glands from each group were placed in ice-cold buffer, and four were placed in liquid nitrogen. Brains were frozen in methylbutane on dry ice, and then kept frozen on dry ice. All frozen tissue was subsequently stored in a −70° freezer until further analysis.

Plasma hormone levels

Plasma ACTH, corticosterone, and aldosterone were measured by radioimmunoassay as described previously ([7](#)). Plasma renin activity was measured as angiotensin I generation in vitro as described previously ([8](#)). Plasma catecholamines were measured by HPLC and electrochemical detection as described previously ([9](#)). Rat plasma angiotensinogen (Agt) was measured by ELISA (IBL, Gunma, Japan). Standards ranged from 0.08 to 5.0 ng/ml. Plasma samples were diluted 1:3100 and are reported in µg/ml.

Pituitary ACTH secretion in vitro

Pituitary corticotroph function was assessed by measurement of basal and CRH-stimulated ACTH release from pituitary fragments *in vitro* as described previously ([10](#)).

Adrenocortical steroid synthesis in vitro

Adrenal steroidogenesis was assessed by measurement of basal and stimulated aldosterone release from dispersed zona glomerulosa cells (adrenal capsules), and basal and stimulated corticosterone

release from dispersed zona fasciculata/reticularis cells (adrenal subcapsules) as described previously (11).

Pituitary and adrenal gene expression

CRH receptor and pro-opiomelanocortin (POMC) mRNAs were analyzed by qPCR of frozen anterior pituitaries. Essential components of the adrenal steroidogenic pathway were evaluated by measurement of LDL receptor, StAR, P450scc, and late pathway enzyme mRNAs by qPCR of frozen adrenal glands (12). Total RNA for real-time PCR was isolated from adrenal glands (1 adrenal per group per experimental day) and anterior pituitaries (4 pooled pituitaries per group per experimental day) using the RNeasy Mini Protocol (Qiagen). The concentration of RNA was quantified using a Qubit Fluorometer (Invitrogen Corporation, Carlsbad, CA). All RNA samples were diluted to a final concentration of 10–20 ng/μL in the PCR assay. Real-time PCR was performed using the Taqman One-Step RT-PCR protocol and pre-made primers and probes (Applied Biosystems, Inc., Foster City, CA). Primer/probe sets are listed in Table 1. The final reaction volume of 20 uL consisted of 1X AmpliTaq Gold® DNA Polymerase mix, 1X RT enzyme mix containing MultiScribe™ Reverse Transcriptase and RNase Inhibitor, 1X primer/probe mix, and 50–100 ng of total RNA. Amplification and detection were performed with the ABI Prism 7900HT Sequence Detection System with the following thermal cycler conditions: 48 ºC for 30 min (RT), 95 ºC for 10 min, and 40 cycles at 95 ºC for 0.25 min and 60 ºC for 1 min. Each sample was assayed in triplicate. Gene expression was quantified using the number of cycles to reach a predetermined threshold value in the intensity of the PCR signal (the Ct value).

Table I. TaqMan gene expression assays used for real-time reverse-transcription PCR

Gene	Symbol	Assay ID#
Melanocortin 2 receptor (ACTH receptor)	Mc2r	Rn02082290_s1
Low-density lipoprotein receptor	Ldlr	Rn00598438_m1
Steroidogenic acute regulatory protein	Star	Rn00580695_m1
Cytochrome P450, subfamily 11A	Cyp11a	Rn00568733_m1
Cytochrome P450, subfamily 21A, polypeptide 1	Cyp21a1	Rn00588996_g1
Cytochrome P450, subfamily 11B, polypeptide 1	Cyp11b1	Rn02607234_g1
Cytochrome P450, subfamily 11B, polypeptide 2	Cyp11b2	Rn01767819_g1
Angiotensin II receptor, type 1a	Agtr1a	Rn01435427_m1
Tyrosine hydroxylase	Th	Rn00562500_m1
Dopamine beta-hydroxylase	Dbh	Rn00565819_m1
Phenylethanolamine-N-methyltransferase	Pnmt	Rn01495588_m1
Catechol-O-methyltransferase	Comt	Rn00561037_m1
Corticotropin releasing hormone receptor 1	Crhr1	Rn00578611_m1
Proopiomelanocortin	Pomc	Rn00595020_m1
Actin, beta	Actb	Rn00667869_m1
Ribosomal protein L19	Rpl19	Rn00821265_g1

Notes: Each gene expression assay consisted of a mix of sequence specific forward/reverse primers and a FAM-labeled probe. Primer/probe sets that cross an exon-exon boundary are designated "m1" at the end of the Assay ID#. Primer/probe sets designated "g1" cross an exon-exon boundary but may still detect genomic DNA, whereas an "s1" designation describes an assay target found within a single exon (i.e., detects genomic DNA).

Brain studies

In situ hybridization histochemistry (ISHH) was used to assess hypothalamic paraventricular (PVN) CRH mRNA and hippocampal glucocorticoid receptor mRNA expression as described previously ([13]–

[15](#)). All ISHH data were analyzed by digitizing the X-ray images, with optical densities measured using NIH Image software (courtesy W. Rasband; http://rsb.info.nih.gov/nih-image). For each section, background optical density was measured in white matter (corpus callosum) and subtracted from raw density measurements for CRH or GR mRNA signal to yield "corrected gray level." For CRH mRNA, data from 2–3 sections per rat were averaged; for GR mRNA, 2–4 sections were averaged.

Statistical Analyses

Body weight and plasma hormone data were analyzed by two-factor analysis of variance (days after ±TBI vs. ±TBI) followed by Duncan's multiple range test (Sigmastat 3.0). ACTH secretion from pituitary fragments was analyzed by three-factor analysis of variance (±CRH vs. ±TBI vs time after TBI) followed by Duncan's multiple range test. qPCR data were analyzed by t-test. The observed corticosterone and aldosterone levels were log-transformed to allow modeling of multiplicative effects (fold-changes in hormone production) and improve normality of the residuals. We tested whether the production of corticosterone or aldosterone differs between irradiated and non-irradiated rats, at 8 days and also at 70 days, using a paired t-test on the unstimulated observations. We also tested whether the stimulatory effect of ACTH and cAMP on corticosterone or aldosterone differs, between irradiated and non-irradiated rats, at 8 days and also at 70 days. For this analysis we fit a mixed model with fixed effects for the drug, radiation group, and their interaction, and a random intercept for each experimental run. This takes into account the repeated measurements for each experiment, and adjusts for different baseline rate of hormone production in each run. For each of the four outcomes (the log of ACTH vs corticosterone, ACTH vs. aldosterone, cAMP vs corticosterone and cAMP vs aldosterone) the hypothesis of interest is the presence of interaction of either ACTH or cAMP and treatment (radiation vs. no radiation). Adrenal cell statistical analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

Irradiated rats gained less weight than non-irradiated rats, as previously reported for this model ([16](#)) ([Figure 1](#)). Two of 86 irradiated

rats died before the 70 day time point. Plasma ACTH, corticosterone, and aldosterone concentrations did not differ between irradiated and non-irradiated rats at 8 days after TBI (Figure 2). At 70 days after TBI, plasma ACTH and corticosterone concentrations were significantly higher in the irradiated compared to the non-irradiated rats. Plasma renin activity was lower at the 8-day time point in irradiated compared to non-irradiated rats; at 70 days after TBI, plasma renin activity was not different between irradiated and non-irradiated rats. Plasma angiotensinogen (Agt) concentrations (N=12–13 per group) were unaffected by TBI. Plasma Agt at 8 days after TBI was 4.0± 0.4 µg/ml compared to 4.5±0.5 µg/ml in unirradiated rats. Plasma Agt at 70 days after TBI was 5.2±0.8 µg/ml compared to 4.9±0.3 µg/ml in unirradiated rats. Plasma aldosterone did not differ at the 70-day time point. There was no effect of irradiation on norepinephrine levels at either time point, whereas epinephrine levels were significantly lower in irradiated rats at 70 days after TBI (Figure 3).
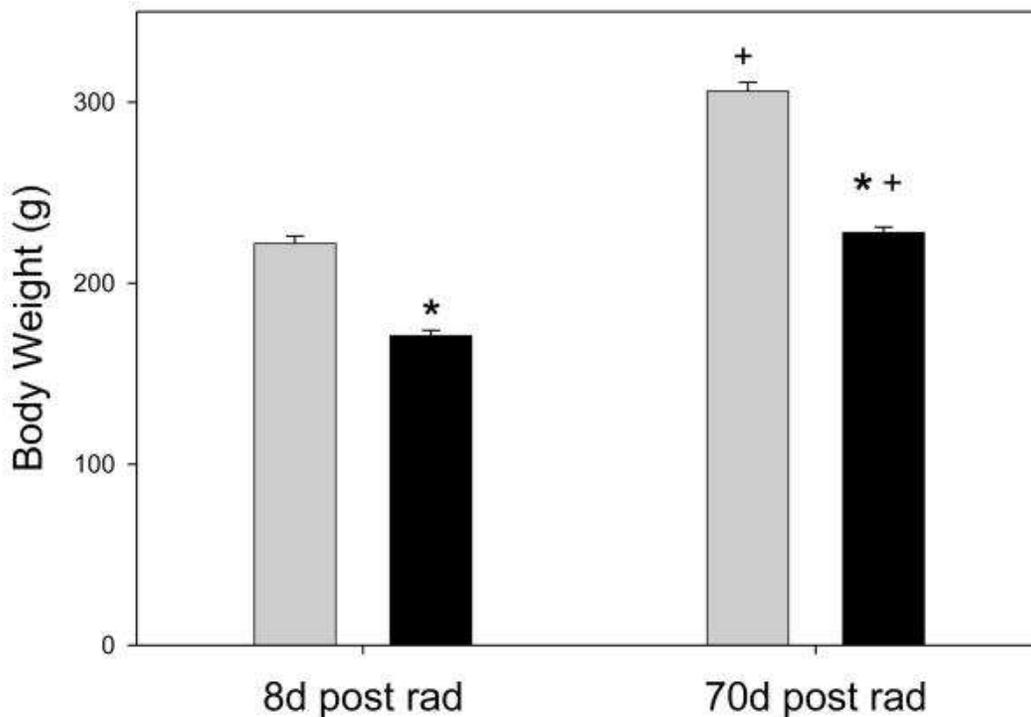

Figure 1 Body weights in the eight and seventy day unirradiated and irradiated rats. + indicates a significant difference from the corresponding value at the different time point, and * indicates a significant difference from the control value at the same time point, both p<0.05. N=32 (4 experimental days) for the eight day time point, and n=48 (six experimental days) for the 70 day time point.
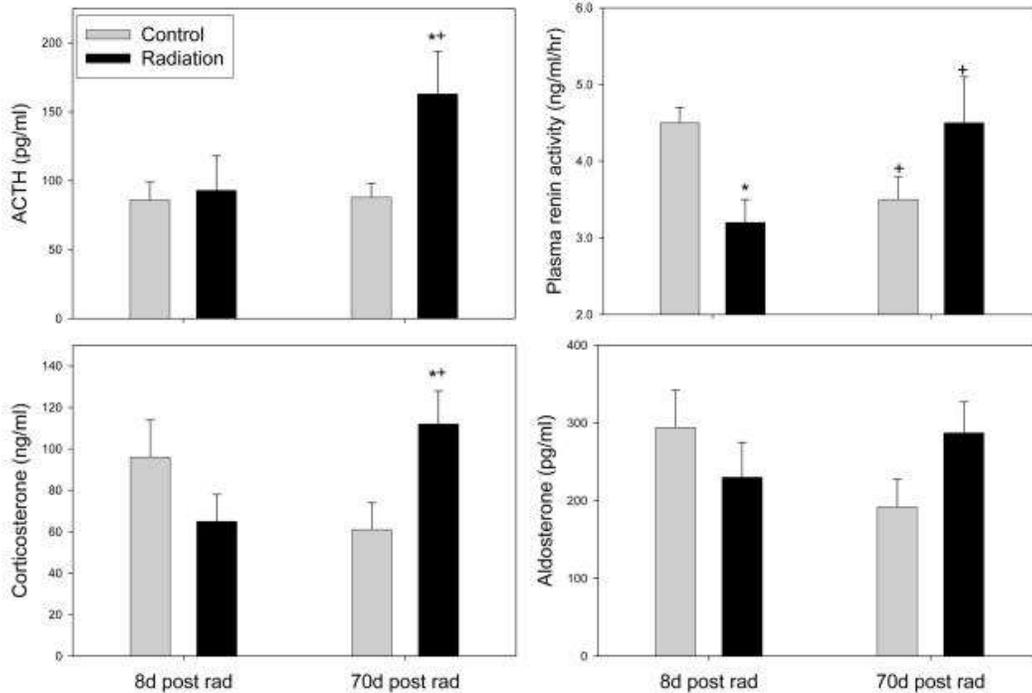
Figure 2 Plasma hormone levels at eight and seventy day rats after TBI. + indicates a significant difference from the corresponding value at the different time point, and * indicates a significant difference from the control value at the same time point, both $p<0.05$. N=24 (six experimental days) for the eight day time point, and n=20 (five experimental days) for the seventy day time point.
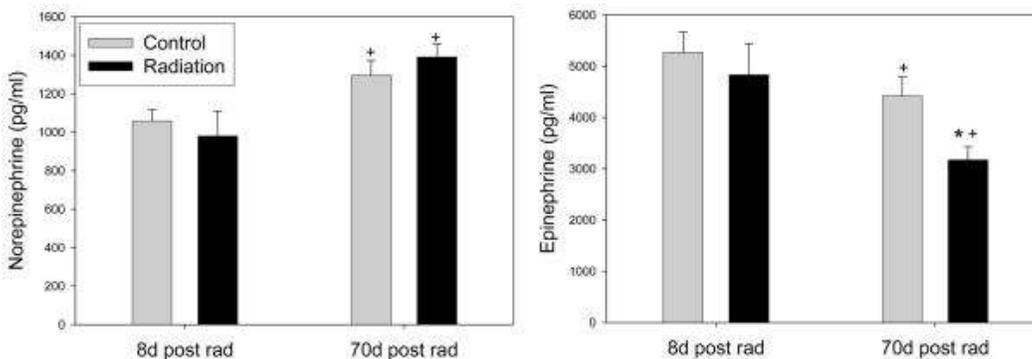

Figure 3 Plasma catecholamine levels at eight and seventy day rats after TBI. + indicates a significant difference from the corresponding value at the different time point, and * indicates a significant difference from the control value at the same time point, both $p<0.05$. N=22 (six experimental days) for the eight day time point, and n=20 (five experimental days) for the seventy day time point.

Pituitary glands from irradiated rats evaluated *in vitro* had significantly increased basal and CRH-stimulated ACTH secretion

compared to non-irradiated rats at both 8 and 70 days after TBI (p < 0.001; Figure 4). The effect of time after TBI was not significant (p = 0.102). None of the interaction terms in the 3-factor analysis of variance were significant (p = 0.464–0.828) indicating that the TBI-induced increase basal and CRH-stimulated ACTH secretion represented a parallel shift. Unstimulated and cAMP-stimulated (Figure 5) and ACTH-stimulated (not shown) corticosterone and aldosterone release from adrenal cells *in vitro* were not altered by TBI at either time point.
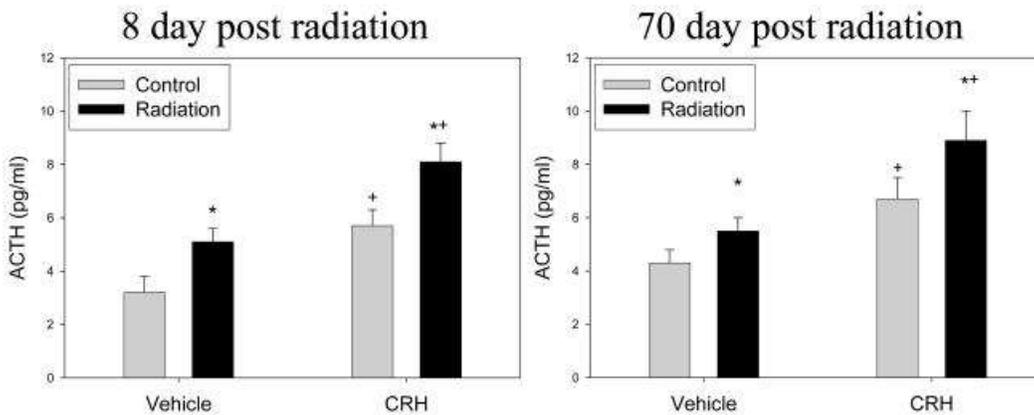

Figure 4 Basal and CRH-stimulated ACTH release by pituitary fragments in vitro. + indicates a significant difference from the corresponding unstimulated value, and * indicates a significant difference of the irradiated values compared to the nonirradiated ones, both p<0.05. N=5 experimental days for the eight and seventy day time points.
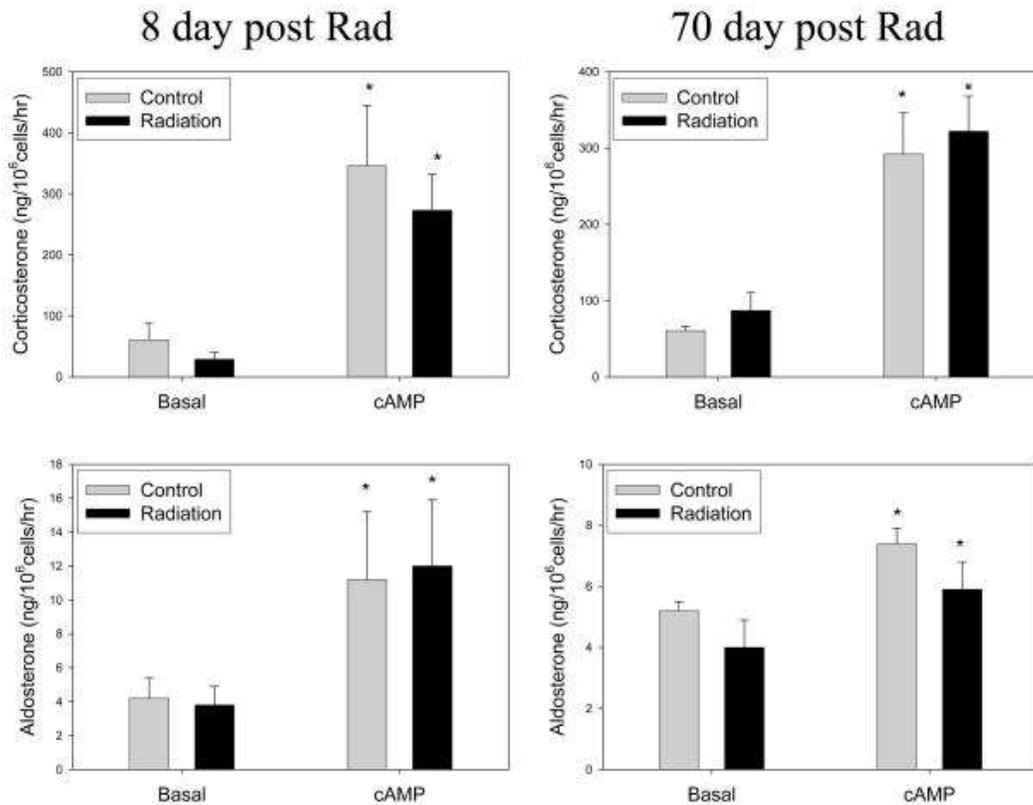
Figure 5 Basal and cAMP-stimulated adrenal steroid synthesis by adrenal fragments in vitro. * indicates a significant difference from the corresponding unstimulated value, $p<0.05$. N=5 experimental days for the 8- and 70-day time points.

In situ hybridization studies of the brain showed no effect of TBI with head shield on the expression of CRH mRNA in the paraventricular nucleus, or glucocorticoid receptor mRNA in the hippocampus (Table 2). Further, there were no significant differences between irradiated and non-irradiated rats for pituitary POMC or CRH receptor-1 mRNAs assessed by qPCR, at either 8 or 70 days after TBI (Table 3). Adrenal mRNAs for steroidogenic factors and enzymes showed no consistent differences at either time point (Table 3). Modest but significant increases in mRNAs for catecholamine synthetic enzymes were found at 8 days in irradiated compared to non-irradiated rats, but not at 70 days.

Table II. *In situ* hybridization histochemistry results for hypothalamic PVN CRH mRNA and hippocampus GR mRNA (corrected gray area)

	8 days post TBI			70 days post TBI		
	PVN CRH	CA1 GR	DG GR	PVN CRH	CA1 GR	DG GR
Control	23.1 ± 3.4	5.2 ± 0.6	6.4 ± 0.7	42.5 ± 3.2	20.7 ± 1.5	24.3 ± 1.5
TBI	23.7 ± 2.4	6.8 ± 1.1	7.1 ± 1.3	45.5 ± 3.4	20.3 ± 1.6	22.8 ± 0.5

Abbreviations: CA1 and DG GR, hippocampal CA1 and dentate gyrus glucocorticoid receptor mRNA ($N = 4$ to 5 per mean + standard deviation).

Table III. qPCR results for adrenal and pituitary (cycles to threshold)

Tissue	Gene symbol	8 days post RAD			70 days post RAD		
		No RAD	RAD	P	No RAD	RAD	P
Adrenal Cortex	Star	20.74 ± 0.04	20.80 ± 0.19	0.746	22.04 ± 0.25	21.85 ± 0.12	0.514
	Ldlr	32.82 ± 0.22	32.76 ± 0.24	0.864	35.09 ± 0.27	35.64 ± 0.38	0.268
	Cyp11a1	22.41 ± 0.37	21.91 ± 0.16	0.252	23.60 ± 0.11	23.71 ± 0.12	0.512
	Cyp21a1	21.43 ± 0.08	21.50 ± 0.12	0.666	23.59 ± 0.18	23.72 ± 0.06	0.520
	Mc2r	27.43 ± 0.12	27.55 ± 0.07	0.422	24.00 ± 0.11	24.23 ± 0.09	0.164
	Cyp11b1	25.68 ± 0.18	25.71 ± 0.11	0.899	25.91 ± 0.09	25.26 ± 0.11*	0.002
	Cyp11b2	28.68 ± 0.14	28.07 ± 0.28	0.270	28.87 ± 0.49	28.40 ± 0.14	0.380
	Agtr1a	30.02 ± 0.14	29.70 ± 0.07	0.065	29.58 ± 0.26	29.71 ± 0.13	0.657
Medulla	Th	23.31 ± 0.07	22.75 ± 0.11*	0.003	23.87 ± 0.12	24.04 ± 0.10	0.307
	Dbh	27.78 ± 0.06	27.30 ± 0.12*	0.007	26.53 ± 0.13	26.60 ± 0.12	0.707
	Pnmt	25.95 ± 0.06	25.04 ± 0.18*	0.001	24.23 ± 0.19	24.01 ± 0.16	0.388
	Comt	31.55 ± 0.12	31.21 ± 0.08*	0.047	31.90 ± 0.09	31.87 ± 0.10	0.824
	Actb	20.27 ± 0.20	20.11 ± 0.14	0.516	22.12 ± 0.36	21.76 ± 0.06	0.352
	Rpl19	27.72 ± 0.13	27.13 ± 0.06*	0.004	21.99 ± 0.06	21.80 ± 0.13	0.279
Anterior Pituitary	Pomc	19.02 ± 0.12	19.37 ± 0.28	0.288	22.09 ± 0.13	23.00 ± 0.54	0.138
	Crhr1	31.02 ± 0.09	30.87 ± 0.09	0.247	33.73 ± 0.15	33.81 ± 0.16	0.719
	Rpl19	28.38 ± 0.01	28.41 ± 0.07	0.741	29.54 ± 0.57	29.35 ± 0.13	0.756

Abbreviations: CT, cycles to threshold.
Notes: The full name of each gene listed above can be found in Table I. $N = 5$ per experimental group at each age studied. Remember that a decrease in Ct represents an increase in mRNA concentration.
*Significantly different from no rad.

Discussion

There was a significant increase in plasma ACTH and corticosterone levels at 70 days after TBI in this model of radiation nephropathy. This is the latest time after TBI that HPA axis stimulation has been reported, to our knowledge. This effect was not present 8 days after TBI. There was no effect on hypothalamic CRH mRNA or hippocampal GR mRNA expression. There was significant enhancement of the *in vitro* pituitary response to CRH in irradiated rats at both time points. There was no effect of TBI on basal and stimulated steroidogenesis in adrenal cells *in vitro*. Plasma catecholamine levels were not increased by TBI, and epinephrine was decreased at the 70 day time point.

Over sixty years ago, increased adrenal weight was reported in rats at one week after 650r TBI ([17]). Later studies tested adrenal

function in rhesus monkeys after successive weekly or bimonthly doses of TBI, and found some evidence for an enhanced adrenal response to ACTH ([18](#)). Plasma corticosterone was increased after 500 r TBI to rats, at one hour and at two days after irradiation, with similar findings in mice after 1500 r TBI ([19](#), [20](#)). On the other hand, inhaled plutonium was reported to cause hypocortisolism in beagles ([21](#)). At the level of the pituitary gland, mice exposed to planned atomic detonations developed ACTH-secreting tumors ([22](#)), while cranial irradiation of rats at 20 to 24 Gy caused generalized pituitary trophic hormone deficiencies, including that of ACTH ([23](#)). More recently, a lesser sensitivity to dexamethasone suppression of the HPA axis was reported at six weeks after 10 Gy whole brain irradiation ([24](#)).

Our finding of a late increase in activity of the HPA axis runs counter to the clinical evidence for central hypoadrenalism after brain or whole body radiation exposures. This may be the case because this model does not involve appreciable brain irradiation. The stimulatory effect of whole body irradiation is thus evident. An increase of plasma corticosterone was also found after body-only irradiation of burros with 275 cGy and higher doses of fast neutrons ([25](#)).). Use of an intermediate dose of TBI could show a dose-response effect of HPA activation, but we did not do those studies.

Mechanism of HPA activation

The mechanism of the increase in plasma ACTH at 70 days post-TBI is unknown. The *in vitro* pituitary cell studies suggest an enhanced responsiveness to CRH, although a similar enhancement was found at 8 days with no increase in plasma ACTH. A direct effect of brain irradiation is unlikely, because the heads were shielded. Systemic effects of irradiation and/or the bone marrow transplant procedure could be relevant. These might include cytokines such as interleukin-1 or interleukin-6 ([2](#), [5](#)). But the cytokine response to TBI and the marrow transplant is likely to be dissipated by 70 days. Metabolic acidosis can elicit ACTH secretion ([26](#)) and could exist at the 70 day time point, because the rats have renal injury and a BUN of ~ 40 mg/dl. Acidosis probably does not explain the enhanced pituitary ACTH response at the eight day time point, when there is no reduction of kidney function. A role for energy stores in the HPA axis has been

proposed ([27](#)); in that model, the lesser weight and body fat at the both the 8 and 70 day time point might lead to a feedback-mediated increase in ACTH secretion.

The hypothalamic response to TBI was invoked over 50 years ago as the mechanism whereby TBI caused activation of the HPA axis ([28](#)). The present studies do not show evidence for enhanced hypothalamic CRH mRNA expression or a change in GR mRNA expression in the hippocampus as the cause for the activated HPA axis at 70 days after TBI. A lack of change in CRH or GR mRNA does not exclude changes in translation or post-translational processing, or a change in neuronal activity not reflecting in changes in mRNA expression. It is possible that TBI without head shielding could have a different effect from that of the present studies. Any direct effect of pituitary irradiation would have to take into account the findings of the present study, which suggest an indirect, abscopal stimulatory effect of TBI on pituitary release of ACTH. The present head-shielded model is particularly relevant to partial body exposures, as are likely in war, accident, or dirty bomb events.

Our studies do not replicate those that show an early increase in plasma ACTH and/or corticosterone after TBI. This may be because most previous studies did not control for the stress of handling the animals. By the daily handling of the rats for the three days before their rapid decapitation, we minimized the confounding acute stress-induced surge of ACTH that could occur. In fact, despite being only 8 days after TBI, BMT, and consequent weight loss associated with those treatments, plasma ACTH and corticosterone levels were normal, which shows the general health of these rats.

It is possible that pituitary corticotroph hyperplasia could underlie the persistent elevation in ACTH at 70 days after TBI. Mice exposed to atomic detonations can form ACTH-secreting tumors by two years after radiation exposure ([22](#)). But in the present studies, pituitary tumors were not observed during the dissection. Histological studies could clarify this issue.

Consequences of HPA activation

Pituitary-adrenal activation could have beneficial or deleterious effects. TBI as low as 2 Gy may cause bone thinning in mice ([29](#)), and we have preliminary data in our rats that show a decline in trabecular number after 10 Gy TBI (Bateman, personal communication). Increased corticosterone concentrations could certainly reduce bone mass. Increased corticosterone levels could also influence radiation nephropathy. Two of the three reported studies of attempted treatment of radiation nephropathy with corticosteroids have reported a poorer outcome ([30](#)–[32](#)). Thus, increased plasma corticosterone at 70 days after TBI could worsen the observed renal injury. Finally, the doubling of plasma corticosterone in our study may have led to an attenuation of hippocampal neurogenesis, an effect that would be additive to that of radiation itself when using TBI without head shielding.

The increase in ACTH and corticosterone at 70 days occurred at a time when rats in this model show renal injury. Proteinuria is evident by 40 days after 10 Gy TBI, azotemia soon after that, and the systolic blood pressure is significantly elevated by 70 days ([33](#)). It is possible that the increase in plasma corticosterone plays a role in the increase in blood pressure. This could depend on the activity of renal11-beta hydroxysteroid dehydrogenase type 2 and enhanced corticosterone-induced activation of the mineralocorticoid receptor. We have no information on the activity of this enzyme in this model. Its gene expression was not reported as changed in a gene array study of irradiated mouse kidney ([34](#)). At this time point in our study, plasma aldosterone was higher than it was in the unirradiated rats, but just short of statistical significance. It appears unlikely that the hypertension in this model of radiation nephropathy depends on plasma aldosterone.

Unexplained findings

There are aspects of the TBI model that are not explained by the increase in ACTH and corticosterone at 70 days. Rats undergoing TBI at doses of 6 Gy and above have a significantly lower weight gain and body fat than their un-irradiated littermates ([16](#)). One might

expect greater weight gain and increased body fat in a state of hypercortisolism and *ad lib* food intake. As described above, activation of the HPA axis could result from a negative energy balance caused by other factors after TBI.

The modest but significant increase in adrenal medullary genes for catecholamine synthesis at 8 days is unexplained. Hasan *et al.* showed some evidence for adrenal medullary cell degranulation and an increase in blood epinephrine and norepinephrine at 14 days after 600 r to male rats ([35]). Older data suggest that the adrenal medulla is radioresistant ([36]). The gene expression data notwithstanding, we did not find increased plasma catecholamines 8 days after TBI, and there was actually a decrease in plasma epinephrine at day 70 in these studies. Therefore, the hypertension in our model of radiation nephropathy does not seem to involve increased plasma catecholamines.

We have previously found that plasma renin was unchanged for up to 9 weeks after 17 Gy TBI given in six fractions ([33]). That irradiation schedule is very similar in its biological effect to the 10 Gy single fraction TBI used in the present studies. It is not clear why we found significantly lower PRA at 8 days in the present studies. It was not due to differences in plasma angiotensinogen concentrations. Extracellular volume expansion is unlikely, since the rats usually have a lower oral intake at this time. Hypertension is not observed in this model until nine weeks or more after TBI. Thus, hemodynamic causes for renin suppression appear unlikely. Plasma catecholamines were unchanged, so their role in causing this low PRA is unlikely. In previous studies, we have found neither serum nor intrarenal increases in Ang II ([33]), which reduces the likelihood that an increase in Ang II would cause the lower PRA. Injury to the juxtaglomerular apparatus was reported after 11 Gy renal irradiation to rats ([37]), so that a direct injury to renin-producing cells in the kidney is possible. The origin of the higher PRA at 70 days after irradiation is also unexplained. At this time, the animals begin to have an increase in blood pressure, and the increased PRA could play a role in causing that hypertension. There is a parallel trend for a higher plasma aldosterone at the 70-day time point ([Figure 3]), but this was not statistically significant. We have tested the role of spironolactone in this model and found it to have no effect to mitigate renal injury or to significantly lower the blood

pressure (38). Therefore, neither an increased plasma renin activity nor an increased plasma aldosterone explains the pathophysiology of this model of radiation nephropathy.

Conclusions

We have found that there is a pituitary-dependent activation of the HPA axis at 70 days after 10 Gy total body irradiation in rats. This appears to be an indirect, abscopal effect of whole body irradiation. It is possible that the ACTH-mediated increase in plasma corticosterone at this time may contribute to impaired neurogenesis and osteopenia, and may complicate other organ injuries after TBI. On the other hand, there is no evidence of HPA axis activation *in vivo* at 8 days after 10 Gy TBI, which belies the notion of a generalized stress response to irradiation at this early time point. Finally, changes in renin, aldosterone, and catecholamines do not appear to explain the development of hypertension that is a well-established feature of this model.

Acknowledgments

Abbreviations

TBI total body irradiation
Gy gray
HPA axis hypothalamic pituitary adrenal axis
CRH corticotrophin releasing hormone
ACTH adrenocorticotrophic hormone
Agt angiotensinogen
StAR steroidogenic acute regulatory protein
qPCR quantitative polymerase chain reaction
LDL low density lipoprotein

Footnotes

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References

1. Meineke V, Fliedner TM. Radiation-induced multi-organ involvement and failure: Challenges for radiation accident medical management and future research. BJR Suppl. 2005;27:196–200.

2. Girinsky TA, Pallardy M, Comoy E, Benassi T, Roger R, Ganem G, et al. Peripheral blood corticotropin-releasing factor, adrenocorticotropic hormone and cytokine (interleukin beta, interleukin 6, tumor necrosis factor alpha) levels after high- and low-dose total-body irradiation in humans. Radiat Res. 1994 Sep;139(3):360–3.

3. Van Cauwenberghe H, Fischer P, Vliers M, Bacq ZM. Irradiation of the rat and blood levels of 17-hydroxycorticosteroids. Arch Int Physiol Biochim. 1957 Feb;65(1):143–7.

4. Lebaron-Jacobs L, Wysocki J, Griffiths NM. Differential qualitative and temporal changes in the response of the hypothalamus-pituitary-adrenal axis in rats after localized or total-body irradiation. Radiat Res. 2004 Jun;161(6):712–22.

5. Kandasamy SB, Thiagarajan AB, Harris AH. Possible involvement of prostaglandins in increases in rat plasma adrenocorticotropic hormone and corticosterone levels induced by radiation and interleukin-1 alpha alone or combined. Fundam Appl Toxicol. 1995 May;25(2):196–200.

6. Moulder JE, Fish BL. Late toxicity of total body irradiation with bone marrow transplantation in a rat model. Int J Radiat Oncol Biol Phys. 1989 Jun;16(6):1501–9.

7. Raff H, Lee JJ, Widmaier EP, Oaks MK, Engeland WC. Basal and adrenocorticotropin-stimulated corticosterone in the neonatal rat exposed to hypoxia from birth: Modulation by chemical sympathectomy. Endocrinology. 2004 Jan;145(1):79–86.

8. Rieder MJ, Roman RJ, Greene AS. Reversal of microvascular rarefaction and reduced renal mass hypertension. Hypertension. 1997 Jul;30(1 Pt 1):120–7.

9. Bruder ED, Henderson LM, Raff H. Adrenal lipid profiles of chemically sympathectomized normoxic and hypoxic neonatal rats. Horm Metab Res. 2006 Dec;38(12):807–11.

10. Buckingham JC, Hodges JR. The use of corticotrophin production by adenohypophysial tissue in vitro for the detection and estimation of potential corticotrophin releasing factors. J Endocrinol. 1977 Feb;72(2):187–93.

11. Raff H, Jankowski BM, Engeland WC, Oaks MK. Hypoxia in vivo inhibits aldosterone synthesis and aldosterone synthase mRNA in rats. J Appl Physiol. 1996 Aug;81(2):604–10.

12. Bruder ED, Taylor JK, Kamer KJ, Raff H. Development of the ACTH and corticosterone response to acute hypoxia in the neonatal rat. Am J Physiol Regul Integr Comp Physiol. 2008 Oct;295(4):R1195–203.

13. Herman JP, Adams D, Prewitt C. Regulatory changes in neuroendocrine stress-integrative circuitry produced by a variable stress paradigm. Neuroendocrinology. 1995 Feb;61(2):180–90.

14. Raff H, Jacobson L, Cullinan WE. Augmented hypothalamic corticotrophin-releasing hormone mRNA and corticosterone responses to stress in adult rats exposed to perinatal hypoxia. J Neuroendocrinol. 2007 Nov;19(11):907–12.

15. Ziegler DR, Cullinan WE, Herman JP. Organization and regulation of paraventricular nucleus glutamate signaling systems: N-methyl-D-aspartate receptors. J Comp Neurol. 2005 Mar 28;484(1):43–56.

16. Moulder JE, Fish BL, Holcenberg JS, Sun GX. Hepatic function and drug pharmacokinetics after total body irradiation plus bone marrow transplant. Int J Radiat Oncol Biol Phys. 1990 Dec;19(6):1389–96.

17. Patt HM, Swift MN. Adrenal response to total body X-radiation. Am J Physiol. 1947 Sep;150(3):480–7.

18. Wolf RC, Bowman RE. Adrenal function in the rhesus monkey after total-body X-irradiation. Radiat Res. 1964 Oct;23:232–8.

19. Groza P, Vladescu C. Plasma corticosterone secretion in rats after irradiation. Rev Roum Morphol Embryol Physiol. 1975;12:53–55.

20. Flemming K, Hellwig J. Radiation effects and adrenal cortex. Acta Radiol. 1971;310(suppl):124–136.

21. Weller RE, Buschbom RL, Dagle GE, Park JF, Ragan HA, Watson CR. Hypoadrenocorticism in beagles exposed to aerosols of plutonium-238 dioxide by inhalation. Radiat Res. 1996 Dec;146(6):688–93.

22. Furth J, Gadsen EL, Upton AC. ACTH secreting transplantable pituitary tumors. Proc Soc Exp Biol Med. 1953 Oct;84(1):253–4.

23. Robinson IC, Fairhall KM, Hendry JH, Shalet SM. Differential radiosensitivity of hypothalamo-pituitary function in the young adult rat. J Endocrinol. 2001 Jun;169(3):519–26.

24. Velickovic N, Djordjevic A, Matic G, Horvat A. Radiation-induced hyposuppression of the hypothalamic-pituitary-adrenal axis is associated with alterations of hippocampal corticosteroid receptor expression. Radiat Res. 2008 Apr;169(4):397–407.

25. Haley TJ, Komesu N, Brown DG. Plasma corticosterone levels after fast neutron exposure in burros. Radiat Res. 1966 Apr;27(4):566–9.

26. Welbourne TC. Acidosis activation of the pituitary-adrenal-renal glutaminase I axis. Endocrinology. 1976 Oct;99(4):1071–9.

27. Dallman MF, Warne JP, Foster MT, Pecoraro NC. Glucocorticoids and insulin both modulate caloric intake through actions on the brain. J Physiol. 2007 Sep 1;583(Pt 2):431–6.

28. Bacq ZM, Martinovitch P, Fischer P, Pavlovitch M, Sladitch DS, Radivojevitch DV. Nervous control of the reaction of anterior hypophysis to x-irradiation as studied in grafted and newborn rats. Radiat Res. 1957 Oct;7(4):373–85.

29. Willey JS, Lloyd SA, Robbins ME, Bourland JD, Smith-Sielicki H, Bowman LC, et al. Early increase in osteoclast number in mice after whole-body irradiation with 2 gy X rays. Radiat Res. 2008 Sep;170(3):388–92.

30. Berdjis CC. Cortisone and radiation. III. histopathology of the effect of cortisone on the irradiated rat kidney. Arch Pathol. 1960 Apr;69:431–9.

31. Caldwell WL. The effect of prednisolone on fatal postirradiation nephritis in rabbits. Radiology. 1971 Feb;98(2):431–3.

32. Geraci JP, Sun MC, Mariano MS. Amelioration of radiation nephropathy in rats by postirradiation treatment with dexamethasone and/or captopril. Radiat Res. 1995 Jul;143(1):58–68.

33. Cohen EP, Fish BL, Moulder JE. The renin-angiotensin system in experimental radiation nephropathy. J Lab Clin Med. 2002 Apr;139(4):251–7.

34. Kruse JJ, te Poele JA, Velds A, Kerkhoven RM, Boersma LJ, Russell NS, et al. Identification of differentially expressed genes in mouse kidney after irradiation using microarray analysis. Radiat Res. 2004 Jan;161(1):28–38.

35. Hasan SS, Sarkar FH, Sharma TR, Prasad GC, Pant GC, Udupa KN. Response of adrenal gland to whole body 60Co irradiation. Indian J Exp Biol. 1977 Jul;15(7):513–6.

36. Rubin P, Casarett GW. The endocrine glands. In: Rubin P, Casarett GW, editors. Clinical Radiation Pathology. Philadelphia: WB Saunders; 1968.

37. Fisher ER, Hellstrom HR. Pathogenesis of hypertension and pathologic changes in experimental renal irradiation. Lab Invest. 1968 Nov;19(5):530–8.

38. Cohen EP, Fish BL, Moulder JE. Mitigation of radiation injuries via suppression of the renin-angiotensin system: Emphasis on radiation nephropathy. Curr Drug Targets. 2010 Jun 28;

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