Effect of Antihistamines, Disodium Cromoglycate (DSCG) or Methysergide on Post-irradiation Cerebral Blood Flow and Mean Systemic Arterial Blood Pressure in Primates after 25 Gy, Whole-body, Gamma Irradiation

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Exposure to ionizing radiation causes hypotension, cerebral ischemia and release of histamine (HA) and serotonin (5-HT). To investigate the relationship among these responses, rhesus monkeys (Macaca mulatta) received physiological saline (i.v.), disodium cromoglycate (DSCG), antihistamines (AH, mepyramine and cimetidine), or methysergide (METH), then were given 25 Gy whole-body irradiation. Monkeys receiving DSCG, AH or METH had higher post-irradiation mean arterial blood pressure (MBP) than saline-treated controls. Compared to levels in controls, post-irradiation hippocampal blood flow (rCBF) levels were higher in monkeys receiving DSCG, AH or METH. Treatment with the 5-HT2 receptor antagonist methysergide was the most effective in maintaining both rCBF and MBP after irradiation. Results support the hypothesis that the irradiation-induced cerebral ischemia and, to some extent, the hypotension is mediated by serotonin through 5-HT2 receptor sites.

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INTRODUCTION

Studies have shown elevated levels of circulating blood histamine (HA) in humans undergoing radiation therapy and increased levels of HA in plasma of nonhuman primates following irradiation. HA is implicated in irradiation-induced hypotension and post-irradiation reduced cerebral blood flow. However, HA is not the only biological mediator involved in irradiation injury and may not be the mediator directly responsible for the irradiation-induced decrease in cerebral blood flow.

The irradiation-induced reduction in cerebral blood flow may employ intermediate mediators such as free radicals produced by exposure to ionizing irradiation. Free radical interactions are implicated in many pathological conditions including irradiation injury, ischemia, microvascular injury and cell membrane damage. Disodium cromoglycate (DSCG), a known mast-cell stabilizer and an efficient free-electron scavenger, was employed successfully to diminish the irradiation-induced decrease in regional cerebral blood flow (rCBF) without altering significantly the irradiation-induced release of HA. However, DSCG and the antihistamines (AH) mepyramine and cimetidine, given in combination, significantly altered post-irradiation hypotension and the reduction in rCBF. Therefore, a hypothesis that attempts to explain the irradiation-induced hypotension and reduced rCBF solely through the free electron-histaminergic mechanisms may be incorrect.

Serotonin (5-hydroxytryptamine, 5-HT) is released after irradiation and causes contraction of vascular smooth muscle by direct and indirect actions. Intracarotid infusion of neurotensin into the isolated, perfused head of rats triggered 5-HT and HA release within one minute and elicited vasoconstriction. The vasoconstrictor response was greatly attenuated by the 5-HT receptor antagonist methysergide (METH) but unaffected by the antihistaminic drugs mepyramine and cimetidine. This suggests that the vasoconstriction was mediated by 5-HT rather than by HA.

In an attempt to elucidate mechanisms underlying the irradiation-induced decrease in rCBF in primates, the effects of AH, DSCG or METH on post-irradiation hippocampal blood flow (rCBF) and mean systemic arterial blood pressure (MBP) were compared after a bilateral whole body irradiation of animals corresponding to a midline tissue dose of 25 Gy of 60Co gamma rays. The hippocampus was selected as the region of interest because this area of the brain is particularly vulnerable to oxygen deprivation associated with ischemia and shows alteration of neuronal activity following irradiation.

MATERIALS AND METHODS

In this study we used 22 male rhesus monkeys (Macaca mulatta) weighing between 3.0 kg and 3.9 kg (3.25 ± 0.06 SEM). The animals were divided randomly into four groups: (1) six given physiological saline (i.v.) for 60 minutes before and after irradiation, (2) six given mepyramine (0.5 mg/min, i.v.) and cimetidine (0.25 mg/min, i.v.) in saline for 60 minutes before and after irradiation, (3) six given DSCG (0.5 mg/min, i.v.) in saline for 60 minutes before and after irradiation, (4) six given METH (0.5 mg/min, i.v.) in saline for 60 minutes before and after irradiation.
irradiation, (3) four given DSCG (100 mg/kg, i.v.) five minutes before irradiation, and (4) six given methysergide orally (4 mg/day) for two days before irradiation and by infusion (0.476 µg/kg/min, i.v.) in saline for 60 minutes before and 60 minutes after irradiation. Food was withheld from all animals for 18 hours before the experiment, but water was available ad libitum. Research was conducted according to the principles enumerated in the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources, National Research Council (U.S.A.). Monkeys were initially anesthetized in their cages with ketamine hydrochloride (20 mg/kg, i.m.) supplemented with 0.015 mg/kg atropine sulfate, and were then moved to the laboratory for the remainder of the experiment.

A systemic venous catheter was used to administer physiological saline and the principal anesthetic, α-Chloralose (100 mg). Supplemental infusions were provided as needed, based on heart rate, blood pressure, blood pH, and peripheral reflexes. A femoral arterial catheter was used to withdraw blood for blood gas determinations and to measure systemic arterial blood pressure.

Approximately 2 hours before irradiation, the animals were intubated with a cuffed endotracheal tube and ventilated using a forced volume respirator to maintain stable blood pH and oxygen tension. After insertion of the endotracheal tube each animal was placed on a circulating water blanket to maintain body temperature between 36°C and 38°C. A rectal probe monitored body temperature.

Using a technique previously described2,4,7, platinum-iridium wire electrodes were placed in the left and right hippocampi (CA1 region) to measure rCBF by hydrogen clearance. Measurements were taken for 30 minutes before irradiation and for 60 minutes after. This technique is essentially an amperometric method that has been successfully employed in similar studies2,4,7,8).

After 30 minutes of pre-irradiation measurements the animals were disconnected from the respirator and recording apparatus and irradiated in a separate room, using a bilateral, whole-body exposure to gamma ray photons from a 60Co source located at the Armed Forces Radiobiology Research Institute. All doses quoted are midline abdomen tissue doses. Prior to animal irradiations the midline abdomen tissue (MLAT) dose rate was measured by placing a 0.5 cc tissue equivalent ionization chamber (calibration factor traceable to the National Institute of Standards and Technology) at the center of a 23.6 cm diameter, cylindrical acrylic phantom, 28 cm in length. Exposure time was adjusted so that each animal received a total 25 Gy MLAT at a nominal dose rate of 47 Gy per minute. The tissue-to-air ratio (TAR), defined as the ratio of the dose rate in free air to the dose rate measured in the phantom, was 0.95. The techniques used for these measurements were in accordance with the AAPM protocol for the determination of absorbed dose from high-energy photon and electron beams26). [The Gray (Gy), the Système Internationale (SI) unit for absorbed dose, corresponds to an energy absorption of 1 J/kg or 100 rad.]

The animals were reconnected to the respirator and recording apparatus at 4 minutes post-irradiation and measurements were continued for a minimum of 60 minutes. At 30 and 10 minutes before irradiation, and at 2, 4, and 6 minutes after irradiation, blood samples were taken via the arterial catheter to monitor stability of blood pH and oxygen tension, and
respiration was adjusted to maintain pre-irradiation levels. MBP was determined via the arterial catheter during the experiment. After the experiment, the animals were humanely euthanized with an i.v. injection of saturated MgSO₄ while still under anesthesia. Brains were removed and dissected for visual verification of electrode placement.

Blood pressure and blood flow data were grouped into 10 minute periods, measured in relation to midtime of radiation. Data from each period were averaged and plotted at the middle of the period. The Shapiro-Wilk Test was used to assess normality of values of the various sample groups. The Wilcoxon Rank Sum Test was used for the statistical analysis of the blood pressure and blood flow data. A 95% level of confidence was employed to determine significance. Because all animals were treated identically before irradiation, and because the data for control and test animals showed no significant difference among monkeys at 30 minutes and 10 minutes before irradiation, pre-irradiation data were combined for each monkey.

| 5-HT    | = | 5-Hydroxytryptamine, Serotonin |
|---------|---|---------------------------------|
| AAPM    | = | American Association of Physicians in Medicine |
| AH      | = | Antihistamines |
| CBF     | = | Cerebral Blood Flow |
| DSCG    | = | Disodium Cromoglycate |
| Gy      | = | Gray, SI unit for absorbed dose |
| HA      | = | Histamine |
| MBP     | = | Mean Arterial Blood Pressure |
| METH    | = | Methysergide |
| MLAT    | = | Midline Abdomen Tissue |
| rCBF    | = | Regional Cerebral Blood Flow |
| SEM     | = | Standard Error of the Mean |
| SI      | = | Système Internationale |
| TAR     | = | Tissue-to-Air Ratio |

## RESULTS

The Shapiro-Wilk test, which assesses the composite hypothesis of normality, indicated that data from many samples were sufficiently inconsistent (p<0.05) with a normal distribution. This finding encouraged us to use alternate methods, such as distribution-free techniques or non-parametric statistical procedures. Therefore, the Wilcoxon Rank Sum Test was used for the final statistical analysis.

The mean systemic arterial blood pressure (MBP) of the four groups of irradiated animals decreased from the pre-irradiation mean of 106 ± 3.4 mm Hg within 10 minutes after irradiation (Figure 1 and Table 2). The irradiated group given saline showed a drop only to the 10 minutes post-irradiation level that was approximately 35% of the pre-irradiation value. The irradiated group treated with DSCG showed blood pressure levels that dropped to approximately the same
Figure 1. Percent of preirradiation mean arterial blood pressure (MBP) after exposure to 25 Gy, whole-body, gamma irradiation (± SEM), compared to a preirradiation mean of 106.0±3.4 mm Hg. Six animals were given physiological saline for 60 min before and after irradiation. One group (n=4) received the saline infusion for 60 min before and after irradiation, and disodium cromoglycate (DSCG) by i.v. infusion (100 mg/kg) 5 min before irradiation. The third group (n=6) received the antihistamines (AH) mepyramine (0.5 mg/min) and cimetidine (0.25 mg/min) in the saline infusion for 60 min before and after irradiation. The fourth group (n=6) received methysergide (METH) orally (4 mg/day) for two days before irradiation and by infusion (0.476 μg/kg/min, i.v.) in saline for 60 min before and after irradiation.

Table 2. Percent of Preirradiation Mean Arterial Blood Pressure (MBP) After Exposure to 25 Gy, Whole-Body, Gamma Irradiation*

| Time (min) Post-irradiation | Saline      | DSCG        | AH          | METH        |
|----------------------------|-------------|-------------|-------------|-------------|
| 10                         | 35.20±4.31  | 35.50±4.72  | 59.60±9.86  | 53.43±6.15* |
| 20                         | 40.67±5.34  | 47.50±8.74  | 66.80±8.73  | 67.43±7.55* |
| 30                         | 50.17±5.03  | 51.00±7.30  | 68.80±10.69 | 71.00±4.70* |
| 40                         | 50.00±5.33  | 47.00±8.49  | 60.60±6.93  | 66.57±4.17* |
| 50                         | 42.00±4.41  | 43.75±9.72  | 56.00±5.96  | 56.14±3.92* |
| 60                         | 38.17±4.95  | 41.75±8.62  | 57.40±4.57  | 52.14±3.33* |

* Significantly different from saline treated (p<0.05)
* Mean ± SEM
levels (36%) as those treated with saline only. The group treated with AH showed blood pressure levels that dropped to approximately 60% of the pre-irradiation mean while the group treated with METH showed a decline to 53.43% of the pre-irradiation mean. However, the METH treated group was the only group to show a significant difference between its post-irradiation MBP values and those of the saline treated group. A significant difference (p ≤ 0.05) was seen between the MBP values of the METH treated group of monkeys and the saline treated group at any time after irradiation. In each of the four groups, the respiration of each subject was maintained at pre-irradiation levels, and the blood gas data revealed a general stability of blood pH and oxygen tension throughout the experiment (data not shown).

Within 10 min post-irradiation, blood flow values for the saline treated animals showed a rapid, significant decline to approximately 53% of the pre-irradiation levels of 75.1 ± 5.9 ml per

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**Figure. 2.** Percent of preirradiation mean hippocampal blood flow (rCBF) after exposure to 25 Gy, whole-body, gamma irradiation (±SEM), compared to a preirradiation mean of 75.1 ± 5.9 ml/100 g of tissue/min. Six animals were given physiological saline for 60 min before and after irradiation. One group (n = 4) received the saline infusion for 60 min before and after irradiation, and disodium cromoglycate (DSCG) by i.v. infusion (100 mg/kg) 5 min before irradiation. The third group (n = 6) received the antihistamines (AH) mepyramine (0.5 mg/min) and cimetidine (0.25 mg/min) in the saline infusion for 60 min before and after irradiation. The fourth group (n = 6) received methysergide (METH) orally (4 mg/day) for two days before irradiation and by infusion (0.476 mg/kg/min, i.v.) in saline for 60 min before and after irradiation.
100 g of tissue per min in the hippocampus (Figure 2, Table 3). At all measurements after irradiation, rCBF was not significantly different between the saline and DSCG groups. The remaining two groups of irradiated animals (one given AH and the other given METH) did not show initial decreases in rCBF and were significantly different (p<0.05) at all post-irradiation measurements from the group given saline. In fact, after irradiation, rCBF levels for both the AH and the METH treated groups showed increases within two minutes to levels that were higher than the pre-irradiation levels. The rCBF in the AH treated group decreased to a level less than the pre-irradiation level by the 20-min post-irradiation observation, but remained significantly different (p<_0.05) from the levels in the saline treated group for the remainder of the experiment. However, the rCBF in the METH treated group did not decrease to a level less than the pre-irradiation level until after the 40-min post-irradiation observation and was significantly different (p<_0.01) from the levels in the saline treated group during this time.

**DISCUSSION**

The initial precipitous decline in post-irradiation MBP and rCBF reported here has been well documented in other studies of the rhesus monkey\(^2,4,7,8,29\). The immediate post-irradiation decrease in rCBF is usually associated with the decline in the MBP since it is thought that a critical MBP of 50% to 60% of normal is necessary in primates for adequate autoregulation of cerebral circulation\(^29-31\).

The inability of DSCG to alter significantly the post-irradiation hypotension when given alone corroborates a previous report\(^4\) in which there was not a significant difference between the post-irradiation MBP of DSCG-treated monkeys and the post-irradiation MBP of saline-treated monkeys. However, in contrast to the previous report, this study does not show a statistically significant difference in post-irradiation rCBF between the DSCG-treated group of monkeys and the saline-treated group of animals. The only treatment difference between the previous report and this report was the level of irradiation. Monkeys in the previous experiment were exposed to

| Time (min) | Post-irradiation | Treatment |
|------------|-------------------|-----------|
| 10         | 52.83±9.28        | Saline    |
| 20         | 56.67±7.89        | DSCG      |
| 30         | 64.83±8.44        | AH        |
| 40         | 66.00±6.44        | METH      |
| 50         | 65.67±7.98        |           |

\* Significantly different from saline treated (p≤0.05)

\** Significantly different from saline treated (p≤0.01)

\a Mean ± SEM
100 Gy, whole body, \( \gamma \) irradiation while the animals in this experiment were exposed to 25 Gy, whole body, \( \gamma \) irradiation. This comparison may then suggest a dose dependent variation resulting in a significant difference in post-irradiation rCBF at a high dose but not at a relatively lower dose.

Another influence on the statistical significance of the difference between rCBF in the saline treated group and the DSCG group was the number of animals in the latter group. Since DSCG had been successfully employed to alter post-irradiation rCBF in a previous experiment\(^4\) only four animals were assigned to the group to be treated with DSCG. The inconsistency of data indicated by the Shapiro-Wilk test\(^27\), when applied to these four animals, probably contributed to the lack of statistical significance between the saline treated group and the DSCG treated group in Table 3. Therefore, even though the difference looks biologically significant, it is not statistically significant.

The regional brain concentration of biological mediators such as catecholamines, histamine, serotonin and the eicosanoids may be a third factor playing an important role in rCBF response to irradiation. For example, the two regions selected for study in the previous report\(^4\) vary dramatically in mast cell and histamine content and responded differently to irradiation and to the administration of DSCG. The hypothalamus contains numerous mast cells and the highest histamine concentrations in the brain\(^32-34\) while the postcentral gyrus is an area with few mast cells and little histamine\(^32,33\).

The addition of the H\(_1\) and H\(_2\) receptor blockers, mepyramine and cimetidine, in combination with DSCG, resulted in an alteration of the post-irradiation MBP not achieved with the administration of DSCG alone\(^2\). Animals given the mast cell stabilizer DSCG and the antihistamines before and after irradiation did not exhibit an abrupt decline in blood pressure but displayed a gradual decrease in MBP below pre-irradiation levels. Also, the treated, irradiated monkeys displayed rCBF values that were not significantly different from the non-irradiated controls. Since post-irradiation rCBF and MBP were not measured in primates given the antihistamines without DSCG, before and after irradiation, it was difficult to determine if it was the DSCG or the antihistamines that was responsible for the response.

Monkeys given the antihistamines mepyramine and cimetidine before and after irradiation in this experiment showed higher MBP and rCBF than those given either saline or DSCG. The post-irradiation mean rCBF of the antihistamine treated group was significantly different (\( p \leq 0.05 \)) from that seen in the saline treated group. Although the post-irradiation MBP of the antihistamine treated monkeys was not statistically different from that of the saline treated monkeys, the difference may have been biologically significant because of the effect hypotension may have on cerebral blood flow. Besides the potential for HA to alter rCBF through its hypotensive actions, HA is also a direct acting cerebral vasodilator when applied in \textit{in vitro} or topically\(^34\). Furthermore, infusing HA into humans resulted in decreased MBP and altered rCBF\(^35,36\). However, in another study, similar treatment altered neither MBP nor rCBF\(^37\). Therefore, the net effect of small concentrations of HA or rCBF is unclear. In any case, the antihistamines were more effective than DSCG in blocking the hypotensive and reduced rCBF responses to irradiation.

The greater effectiveness of the antihistamines may be due to cimetidine, a specific
histamine H₂ antagonist, which blocked the immediate bradycardia and hypotension caused by intravenous injection of serotonin³⁸). Furthermore, the hypotensive effect of 5-HT infusion is likely due to an action in the cell body regions of the raphe nucleus³⁹). This suggests that a post-irradiation release of serotonin, and not histamine, may be directly responsible for the irradiation-induced decrease in rCBF since serotonin is normally released after irradiation⁵,⁶,⁴⁰) and causes contraction of vascular smooth muscle by direct and indirect actions¹⁷–¹⁹). Indeed, Rioux et al²⁰), has shown with the isolated, neurotensin-perfused head of the rat that it is not the release of histamine that may be responsible for a decreased rCBF, but the release of serotonin. This agrees with an earlier report by Edvinsson and Owman⁴¹) that serotonin is the most efficient vasoconstrictor agent for human pial arteries with its effect inhibited by the serotonin antagonist, methysergide.

In this experiment MBP followed a triphasic pattern consisting of an initial reflex hypotension, a secondary pressor response and a delayed hypotension in all four groups of monkeys (Figure 1, Table 2). However, the post-irradiation hypotension was altered significantly (p<0.05) with the administration of methysergide (METH). A possible explanation for the action of METH is that it is a selective 5-HT₂ antagonist⁴²) and 5-HT is implicated in coronary artery constriction and reduced coronary blood flow¹⁹). This could contribute to irradiation-induced cardiovascular dysfunction⁴³) resulting in a decreased cardiac output and post-irradiation hypotension. However, Freed et al²⁹) concluded that it is brain serotonin rather than serotonin in heart or other peripheral tissues which causes the hypotension.

The serotonin antagonist, methysergide, was the most effective agent used in this study to block the irradiation-induced decrease in rCBF (Figure 2, Table 3). Hippocampal blood flow in the METH treated monkeys actually increased above the pre-irradiation mean for 40 min post-irradiation and was significantly different (p≤0.01) from the rCBF in the saline treated group for that time. For the last 20 min of the measurements, the rCBF of the METH treated animals remained significantly different (p≤0.05) from the rCBF of the saline treated group, although falling slightly below the pre-irradiation level. These results are consistent with a cerebrovascular vasoconstrictor role for serotonin and suggest serotonin involvement in the irradiation-induced decrease in rCBF.

Serotonin has a complex action in the cerebrovasculature, causing contraction or relaxation depending on the species involved, the cellular structure first encountered and the type of 5-HT receptors present¹⁷,¹⁸,⁴²,⁴⁴). Serotonin receptors may be subdivided into at least six subtypes called 5-HT₁A, 5-HT₁B, 5-HT₁C, 5-HT₁D, 5-HT₂ and 5-HT₃⁴²). Vascular constriction is mediated primarily through the 5-HT₂ receptors with some 5-HT₁ involvement⁴⁵). 5-HT₁ receptors are involved primarily with arteriolar dilatation. Other proposed functional correlates of 5-HT₁ receptor subtypes are dorsal raphe cell and CA1 hippocampal cell inhibition, thermoregulation, and hypotensive effects⁴²).

Emesis is a common symptom of the early prodromal phase of radiation sickness in humans⁴⁶) and the ferret has proven an excellent small animal model for the study of irradiation-induced emesis⁴⁷,⁴⁸). The area postrema has been implicated in irradiation-induced emesis⁴⁹) and specific 5-HT₃ binding sites have been shown in the brains of several species, including humans and ferrets⁵⁰). The use of 5-HT₃ receptor antagonists such as ondansetron, granisetron, zacop-
ride and Y-25130 in controlling irradiation-induced emesis further strengthened the relationship between 5-HT and some of the pathophysiological results of irradiation.

In contrast to the subtypes of the 5-HT1 and 5HT3 sites, 5HT2 sites are quite homogeneous. The highest concentration of 5-HT2 binding sites in the brain are in the caudate and layer IV of the cerebral cortex where the application of 5-HT caused a slow depolarization of cortical neurons, resulting in decreased conductance. Proposed functional correlates of 5-HT2 receptor subtypes include contraction of vascular smooth muscle, contraction of bronchial smooth muscle, head twitches seizures and edema. Infusion of 5-HT produced brain edema, increased blood-brain barrier permeability and decreased rCBF, all of which can be prevented with a 5-HT2 receptor antagonist.

Serotonin also may be associated with impairment of CBF autoregulation as shown with renal autoregulation in the low pressure range. In fact, no correlation was seen between MBP and CBF as demonstrated by red cell velocity after intracarotid injection of 5-HT even though correlation existed between MBP and CBF following arterial blood withdrawal. However, following exposure to moderate irradiation (4 Gy) with 137Cs, ferrets showed no significant change in MBP but a general reduction of rCBF ranging from 7 to 33%. Certainly, the involvement of 5-HT in autoregulation of CBF following irradiation is still a matter of discussion requiring further investigation.

Many of the sequelae of 5-HT administration are similar to the results of irradiation, notably hypotension, emesis, brain edema, altered blood-brain barrier permeability and a marked reduction in rCBF as well as total CBF. As noted before, some of these can be prevented with the proper selective 5-HT receptor antagonist. Irradiation-induced emesis can also be altered with the proper 5-HT3 receptor antagonist. This study reports the amelioration of irradiation-induced hypotension and diminished rCBF in the hippocampus with the 5-HT2 receptor antagonist, methysergide and the greater effectiveness of methysergide compared to the antihistamines mepyramine and cimetidine, and the mast cell stabilizer, disodium cromoglycate, in this action. We believe that these results, although not conclusive, support the hypothesis that irradiation-induced diminished hippocampal blood flow and, to some extent, post-irradiation hypotension is mediated by 5-HT through 5-HT2 receptor sites.

The phenomenon of CNS radiation abnormalities and damage that are not associated with the tumor under treatment but occur with the radiotherapy has been well documented but remains poorly understood. Necrosis in the tissue surrounding the tumor may be secondary to radiation-induced edema and ischemia and the cerebrovasculature of the normal parenchyma may be more sensitive to the effects of cranial irradiation than that found in the tumor. There is now increased interest in the possible use of agents which might limit irradiation-induced changes in cerebral blood flow and the extent of tissue injury. We suggest that the use of a 5-HT2 receptor antagonist will not only diminish the irradiation-induced alteration in cerebral blood flow, thereby ameliorating the associated pathologic changes in tissue surrounding the tumor, but also may improve the blood flow in tumors under radiotherapy and alter the response of the radioresistant hypoxic cells that have been shown to occur in nearly all animal tumors and may exist in some human cancers.

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REFERENCES

1. Lasser, E. C. and Stenstrom, K. W. (1954) Elevation of circulating blood histamine in patients undergoing deep roentgen therapy. Am. J. Roentgenology 72: 985–988.
2. Cockerham, L. G., Pautler, E. L., Carraway, R. E., Cochrance, D. E. and Hampton, J. D. (1988) Effect of disodium cromoglycate (DSCG) and antihistamines on postirradiation cerebral blood flow and plasma levels of histamine and neurotensin. Fundam. Appl. Toxicol. 10(2): 233–242.
3. Doyle, T. F. and Strike, T. A. (1977) Radiation-released histamine in the rhesus monkey as modified by mast-cell depletion and antihistamine. Experimentia 33: 1047–1048.
4. Cockerham, L. G., Doyle, T. F., Pautler, E. L. and Hampton, J. D. (1986) Disodium cromoglycate, a mast cell stabilizer, alters postradiation regional cerebral blood flow in primates. J. Toxicol. Environ. Health 18: 91–101.
5. Walden, T. L., Jr., and Farzaneh, N. K. (1990) Biochemistry of Ionizing Radiation. Raven Press, New York.
6. Walden, T. L., Jr., and Farzaneh, N. K. (1991) Biochemical responses of normal tissues to ionizing radiation. In “Radiation Injury to the Nervous System”. Eds. P. H. Gutin, S. A. Leibel, and G. E. Sheline, pp. 17–36, Raven Press, New York.
7. Cockerham, L. G., Arroyo, C. M. and Hampton, J. D. (1988) Effects of 4-hydroxypyrazolo (3, 4-d) pyrimidine (Allopurinol) on postirradiation cerebral blood flow: Implications of free radical involvement. Free Radic. Biol. Med. 4(5): 279–284.
8. Cockerham, L. G., Prell, G. D., Cerveny, T. J., O’Brien, M. M. and Hampton, J. D. (1991) Effects of aminoguanidine on pre- and postirradiation regional cerebral blood flow and systemic blood pressure in the primate. Agents and Actions 32: 237–244.
9. Del Maestro, R. F. (1980) An approach to free radicals in medicine and biology. Acta Physiol. Scand. Suppl. 492: 153–168.
10. Del Maestro, R. F., Thaw, H. H., Bjork, J., Planker, M. and Arfors, K.-E. (1980) Free radicals as mediators of tissue injury. Acta Physiol. Scand. Suppl. 492: 43–57.
11. Kennedy, A. R., Troll, W. and Little, J. B. (1984) Role of free radicals in the initiation and promotion of radiation transformation in vitro. Carcinogenesis 5(10): 1213–1218.
12. Hammond, B., Kontos, H. A. and Hess, H. L. (1985) Oxygen radicals in the adult respiratory distress syndrome, in myocardial ischemia and reperfusion injury, and in cerebral vascular damage. Can. J. Physiol. Pharmacol. 63: 173–187.
13. Konat, G. W. and Wiggins, R. C. (1985) Effect of reactive oxygen species on myelin membrane proteins. J. Neurochem. 45: 1113–1118.
14. Kontos, H. A. (1985) Oxygen radicals in cerebral vascular injury. Circ. Res. 57(4): 508–516.
15. McCord, J. M. (1985) Oxygen-derived free radicals in postischemic tissue injury. New England J. Med.
16. Carmichael, A. J., Arroyo, C. M. and Cockerham, L. G. (1988) Reaction of disodium cromoglycate (DSCG) with hydrated electrons. Free Radic. Biol. Med. 4(4): 215–218.
17. Vanhoutte, P. M. (1986) Serotonin, adrenergic nerves, endothelial cells and vascular smooth muscle. Prog. Appl. Microcirc. 10: 1–11.
18. Reneman, R. S. and Bollinger, A. (1986) Vascular and microvascular effects of serotonin—Some conclusive remarks. Prog. Appl. Microcirc. 10: 83–86.
19. Hollenberg, N. K. (1988) Serotonin and vascular responses. Ann. Rev. Pharmacol. Toxicol. 28: 41–59.
20. Rioux, F., Kerouac, R. and St-Pierre, S. (1985) Release of mast cell mediators, vasoconstriction and edema in the isolated, perfused head of the rat following intracarotid infusion of neutrotensin. Neuropeptides 6: 1–12.
21. Suzuki, R., Yamaguchi, T., Li, C-L. and Klatzo, I. (1983) The effects of 5-minutes ischemia in mongolian gerbils: II. Changes of spontaneous neuronal activity in cerebral cortex and CA1 sector of hippocampus. Acta Neuropathol. 60: 217–222.
22. Kirino, T. and Sano, K. (1984) Selective vulnerability in the gerbil hippocampus following ischemia. Acta Neuropathol. 62: 201–208.
23. Tolliver, J. M. and Pellmar, T. C. (1987) Ionizing radiation alters neuronal excitability in hippocampal slices of the guinea pig. Radiat. Res. 112: 555–563.
24. Hollinden, G. E. and Pellmar, T. C. (1989) Attenuation of synaptic transmission in hippocampal slices following whole animal exposure to ionizing radiation. Soc. Neurosci. Abstr. 15: 134.
25. Pellmar, T. C., Schauer, D. A. and Zeman, G. H. (1990) Time- and dose-dependent changes in neuronal activity produced by X radiation in brain slices. Radiat. Res. 122: 209–214.
26. AAPM Task Group 21 (1983) A protocol for the determination of absorbed dose from high-energy photon and electron beams. Med. Phys. 10: 741–771.
27. Shapiro, S. S. and Wilk, M. B. (1965) An analysis of variance test for normality. Biometrika 52: 591–611.
28. Remington, R. D. and Schork, M. A. (1970) “Statistics with Applications to The Biological and Health Sciences”, pp. 313–315, Prentice-Hall, Inc., Englewood Cliffs, New Jersey.
29. Champman, P. H. and Young, R. J. (1968) Effect of cobalt-60 gamma irradiation on blood pressure and cerebral blood flow in the Macaca mulatta. Radiat. Res. 35: 78–85.
30. Doyle, T. F., Curran, C. R. and Turnes, J. E. (1974) The prevention of radiation-induced, early transient incapacitation of monkeys by an antihistamine. Proc. Soc. Exper. Biol. Med. 145: 1018–1024.
31. Farrar, J. K., Gamache, F. W., Jr., Ferguson, G. G., Barker, J., Varkey, G. P. and Drake, C. G. (1981) Effects of profound hypotension on cerebral blood flow during surgery for intracranial aneurysms. J. Neurosurg. 55: 857–864.
32. Taylor, K. M., Gfeller, E. and Snyder, S. H. (1972) Regional localization of histamine and histidine in the brain of the rhesus monkey. Brain Res. 41: 171–179.
33. Edvinsson, L., Cervos-Navarro, J., Larsson, L. -I., Owman, C. H. and Ronnberg, A. -L. (1977) Regional distribution of mast cells containing histamine, dopamine, or 5-hydroxytryptamine in the mammalian brain. Neurology 27: 878–883.
34. Gross, P. M. (1982) Cerebral histamine: Indications for neuronal and vascular regulation. J. Cereb. Blood Flow Metabol. 2: 3–23.
35. Shenkin, H. A. (1951) Effects of various drugs upon cerebral circulation and metabolism of man. J. Appl. Physiol. 3: 465–471.
36. Alman, R. W., Rosenberg, M. and Fazekas, J. F. (1952) Effects of histamine on cerebral hemodynamics and metabolism. A. M. A. Arch. Neurol. Psychiat. 67: 354–356.
37. Krabbe, A. A. and Olesen, J. (1982) Effect of histamine on regional cerebral blood flow in man. Cephalgia 2: 15–18.
38. Wiggins, R. C., Glatfelter, A., Campbell, A. M., Kunkel, R. C. and Ulevitch, R. J. (1985) Acute
hypotension due to platelet serotonin-induced chemoreflexes after intravenous injection of dextran sulfate in the rabbit. Circ. Res. 57: 262-277.

39. Freed, C. R., Echizen, H. and Bhaskaran, D. (1985) Brain serotonin and blood pressure regulation: Studies using in vivo electrochemistry and direct tissue assay. Life Sciences 37: 1783-1793.

40. Franzen, F., Gross, H., and Thielicke, G. (1963) Biogene amine in urin und blut von ratten nach subletaler ganzzkörperbestrahlung. Strahlentherapie 120: 598-610.

41. Edvinsson, L. and Owman, Ch. (1976) Amine receptors in brain vessels. In “The Cerebral Vessel Wall”. Eds. J. Cervós-Navarro, et al, pp. 197-206, Raven Press, New York.

42. Peroutka, S. J. (1988) 5-Hydroxytryptamine receptor subtypes. Ann. Rev. Neurosci. 11: 45-60.

43. Hawkins, R. N. and Forcino, C. D. (1988) Effects of radiation on cardiovascular function. Comments Toxicology 2: 243-252.

44. Parsons, A. A. (1991) 5-HT receptors in human and animal cerebrovasculature. Trends Pharmacol. Sci. 12: 310-315.

45. Saxena, P. R., Bom, A. H. and Verdonw, P. D. (1989) Characterization of 5-hydroxytryptamine receptors in the cranial vasculature. Cephalalgia 9(Suppl 9): 15-22.

46. Baum, S. J., Anno, G. H., Young, R. W. and Withers, H. R. (1985) Symptomatology of Acute Radiation Effects in Humans after Exposure to Doses of 75 to 4500 Rads (cGy) Free-In-Air. DNA-TR-85-50, Defense Nuclear Agency, Washington, DC.

47. Tuor, U. I., Kondysar, M. H. and Harding, R. K. (1988) Emesis, radiation exposure, and local cerebral blood flow in the ferret. Radiat Res. 114: 537-549.

48. King, G. L. (1988) Characterization of radiation-induced emesis in the ferret. Radiat. Res. 114: 599-612.

49. Harding, R. K, Hugenholtz, H., Keaney, M. and Kucharzyk, J. (1985) Discrete lesions of the area postrema abolish radiation-induced emesis in the dog. Neurosci. Lett., 53: 95-100.

50. Frazer, A., Maayani, S. and Wolfe, B. B. (1990) Subtypes of receptors for serotonin. Ann. Rev. Pharmacol. Toxicol. 30: 307-348.

51. Priestman, T., Challoner, T., Butcher, M. and Priestman, S. (1988) Control of radiation induced emesis with GR38032F (GR). Proc. Am. Soc. Clin. Oncol. 7: 281 (Abstr. 1089).

52. Bermudez, J., Boyle, E. A., Miner, W. D., and Sanger, G. J. (1988) The anti-emetic potential of the 5-hydroxytryptamine, receptor antagonist BRL 43694. Br. J. Cancer 58: 644-650.

53. Hunter, A. E., Prentice, H. G., Pothecary, K., Coumar, A., Collis, C., Upward, J., Murdoch, R., Gandhi, L., Hamon, M., Butler, M. and Wells, J. (1991) Granisetron, a selective 5-HT3 receptor antagonist, for the prevention of radiation induced emesis during total body irradiation. Bone Marrow Transplant 7(6): 439-441.

54. King, G. L. and Landauer, M. R. (1990) Effects of zacopride and BMY25801 (batanopride) on radiation-induced emesis and locomotor behavior in the ferret. J. Pharmocol. Exp. Ther. 253(3): 1026-1033.

55. Fukuda, T., Setoguchi, M., Inaba, K., Shoji, H. and Tahara, T. (1991) The antiemetic profile of Y-25130, a new selective 5-HT3 receptor antagonist. Eur. J. Pharmacol. 196(3): 299-305.

56. Sharma, H. S., Dey, P. K and Olsson, Y. (1989) Brain edema, blood-brain barrier permeability and cerebral blood flow changes following intracarotid infusion of serotonin: modification with cyproheptadine and indomethacin. In “Pharmacology of Cerebral Ischemia 1988”. Ed. J. Krieglstein, pp. 317-323, CRC Press, Inc., Boca Raton, Florida.

57. Sharma, H. S., Olsson, Y. and Dey, P. K. (1990) Changes in blood-brain barrier and cerebral blood flow following elevation of circulating serotonin level in anesthetized rats. Brain Res. 517: 215-223.

58. Endlich, K., Kuhn, R. and Steinhausen, M. (1993) Visualization of serotonin effects on renal vessels of rats. Kidney Int. 43(2): 314-323.

59. Bing, R. J., Chang, B. L., Santillan, G. and Sato, M. (1983) The effect of 5-hydroxytryptamine and arterial blood withdrawal on cerebral microcirculation in the cat, arterial permeability in the rabbit.
Adv. Exp. Med. Biol. 161: 327–345.

60. Gunter-Smith, P. J. (1987) Effect of ionizing radiation on gastrointestinal physiology. In “Military Radiobiology”. Eds. J. J. Conklin and R. I. Walker, pp. 135–151, Academic Press, Inc., New York.

61. Hawkins, R. N. and Cockerham, L. G. (1987) Postirradiation cardiovascular dysfunction. In “Military Radiobiology”. Eds. J. J. Conklin and R. I. Walker, pp. 153–163, Academic Press, Inc., New York.

62. Young, R. W. (1987) Acute radiation syndrome. In “Military Radiobiology”. Eds. J. J. Conklin and R. I. Walker, pp. 165–190, Academic Press, Inc., New York.

63. Russell, L. B., Fike, J. R., Cann, C. E. and Susskind, C. (1984) Dual energy CT scanning for analysis of brain damage due to X-irradiation. Ann. Biomed. Eng. 12: 15–28.

64. Ludwig, R., Calvo, W., Kober, B. and Brandeis, W. E. (1987) Effects of local irradiation and i.v. methotrexate on brain morphology in rabbits: early changes. J. Cancer Res. Clin. Oncol. 113: 235–240.

65. Lo, E. H., Frankel, K. A., Steinberg, G. K., DeLaPaz, R. L. and Fabrikant, J. I. (1992) High-dose single-fraction brain irradiation: MRI, cerebral blood flow, electrophysiological, and histological studies. Int. J. Radiat. Oncol. Biol. Phys. 22: 47–55.

66. Cockerham, L. G., Mickley, G. A., Walden, T. L., Jr. and Stuart, B. O. (1994) Ionizing radiation. In “Principles and Methods of Toxicology”, 3rd Ed. Ed. A. W. Hayes, pp. 447–496, Raven Press, New York.

67. Delattre, J. Y., Shapiro, W. R., Posner, J. B. (1989) Acute effects of low-dose cranial irradiation on regional capillary permeability in experimental brain tumors. J. Neurol. Sci. 90: 147–153.

68. Horsman, M. R., Chaplin, D. J. and Overgaard, J. (1991) The use of blood flow modifiers to improve the treatment response of solid tumors. Radiother. Oncol. 20: Suppl 1: 47–52.

69. Gobble, G. T., Seilhan, T. M. and Fike, J. R. (1992) Cerebrovascular response after interstitial irradiation. Radiat. Res. 130: 236–240.

70. Chaplin, D. J., Durand, R. E. and Olive, P. L. (1986) Acute hypoxia in tumors: implications for modifiers of radiation effects. Int. J. Radiat. Oncol. Biol. Phys. 12: 1279–1282.

71. Cockerham, L. G., Forcino, C. D., Pellmar, T. C. and Smart, S. W. (1987) Effect of methysergide on postirradiation hypotension and cerebral ischemia. Proceedings of the Cerebral Hypoxia and Stroke Symposium, Budapest, Hungary, August 22–24, 1987.

72. Cockerham, L. G., and Forcino, C. D. (1988) Post-irradiation alterations in cerebral blood flow. In “Terrestrial Space Radiation and It’s Biological Effects”. Eds. P. McCormack, C. E. Swenberg and H. Büecker, pp. 495–507, Plenum Press, New York.