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Radiation effects on cyclic AMP, cyclic GMP, and amino acid levels in the CSF of the primate

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Research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

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CSF samples were taken at predetermined times postirradiation. Significant increases in cyclic AMP and cyclic GMP levels were observed following irradiation. An analysis of brain areas obtained by biopsy of irradiated animals showed significant decreases in only the cerebellar cyclic AMP and cyclic GMP. No appreciable changes were found in the CSF amino acid composition.

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Radiation Effects on Cyclic AMP, Cyclic GMP, and Amino Acid Levels in the CSF of the Primate

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INTRODUCTION

Previous studies have indicated that exposure of animals to ionizing radiation affects the adenyl cyclase system in different tissues. Decreases in adenyl cyclase and phosphodiesterase activities have been observed in the liver of newborn rats exposed to low levels of radiation (*1*). Similar changes have also been found in adenyl cyclase and, to a lesser degree, phosphodiesterase and protein kinase activities in the posterior lobe of the pituitary gland following irradiation of rats with supralethal doses of γ radiation (*2*). On the other hand, no changes in cyclic AMP levels were observed in irradiated thymocytes with γ radiation, whereas heating at 43°C was found to cause a massive rise in its levels within the cell (*3*). Increases in cerebral (*4*) and cerebrospinal fluid (CSF) (*5*) cyclic AMP levels have resulted from *in vivo* administration of putative adrenergic biogenic amines. Daily fluctuations in the levels of CSF cyclic AMP in the Rhesus monkey have also been demonstrated (*6*). Both cyclic AMP and cyclic GMP are found in the CSF and, although it is not yet clear what their function is, there is evidence that changes in their levels in the CSF are associated with neurologic (*7–9*) and cerebrovascular (*10*) disorders. There is not much information available on the effects of radiation on cyclic nucleotide metabolism in the mammalian CSF; therefore, the purpose of this study was to determine if and to what extent cyclic AMP and cyclic GMP levels in CSF are affected by exposure of animals to ionizing radiation.

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METHODS

Silastic Pudenz catheters were chronically implanted in the fourth ventricle of four male young adult cynomolgus monkeys (2.5–3.5 kg) according to a published method (*11*). The catheters were connected to compressible polyethylene Ommaya reservoirs placed subcutaneously over the occiput. This method permitted sterile aspiration of cerebrospinal fluid in the awake, unrestrained animal by means of a needle and syringe. Thorough mixing of the CSF in the reservoir and that in the fourth ventricle and posterior fossa subarachnoid space was accomplished by digital compression and passive filling of the reservoir four or five times. This was shown by radiocistenography following the injection of 3 mCi of ^{99}Tc into the reservoir. Cistenograms taken even after more than 20 pumpings revealed isotope primarily in the fourth ventricle and posterior fossa. Computerized tomography showed the animals to be neuroradiographically healthy with absence of hydrocephalus.

Irradiation Procedures

A standard primate chair modified to hold a lead body shield and a collimator was used to restrain the animal during irradiation. The collimator was a 7.6 × 10 × 20-cm lead block containing an aperture corresponding to the sagittal silhouette of the cranial cavity of the monkey. Its purpose was to make certain that only the brain area of the animal was exposed to the impinging radiation.

The animal's head was further restrained during irradiation by a dental acrylic form molded in the shape of the monkey's left hemicranium. Prior to irradiation duplicate baseline CSF samples were taken 24 hr apart after repeated reservoir pumping to ensure good mixing with the CSF in the ventricle. The heads of the animals were exposed unilaterally to 300 or 900 rad of 6.5-MeV average energy electromagnetic radiation (bremsstrahlung) from the AFRRI linear accelerator. The average dose rate was 70 rad/min. Bremsstrahlung was produced by impinging the transmitted electron beam on a 12.65 g/cm^2 tantalum target. After irradiation the animals were returned to their cages and CSF samples were taken 1, 24, 48, and 72 hr postirradiation. Sample collection time was at 1300 hr.

In a second experiment a group of monkeys was exposed to 900 rad bremsstrahlung, head only. The animals were anesthetized 24 hr postirradiation with an initial loading dose of 65 mg ketamine hydrochloride and maintained and immobilized with ketamine hydrochloride (7 mg/kg/hr iv), 60% nitrous oxide, and pancuronium bromide (0.04 mg/kg, iv). Subsequent hemicraniectomy permitted removal of frontal and occipital cerebrocortical and cerebellar biopsies. Specimens were plunged into liquid nitrogen immediately and subsequently assayed or cyclic AMP and cyclic GMP content. Controls were treated identically except that they were not irradiated.

Analytical Procedures

Cyclic AMP and cyclic GMP radioimmunoassay kits were purchased from Schwarz/Mann, Orangeburg, NY. The CSF samples (1 ml) were thoroughly mixed at 4°C with equal volumes of ice-cold 6% trichloroacetic acid and centrifuged at

TABLE I

Effects of Radiation on CSF Cyclic AMP Levels (pmole/ml CSF)

Monkey No.	Radiation dose (rad)	Preirradiation 24 hr ($n = 3$)	Postirradiation ($n = 3$) 1 hr	24 hr	48 hr	72 hr
1	300	29.4 ± 0.31[a]	39.5 ± 0.25*	36.5 ± 0.22*	42.0 ± 0.19*	34.8 ± 0.10*
2	300	36.9 ± 0.39	46.7 ± 0.19*	47.9 ± 0.16*	44.4 ± 0.15*	50.1 ± 0.16*
3	900	39.5 ± 0.20	60.0 ± 0.14*	55.6 ± 0.20*	55.1 ± 0.20*	85.0 + 0.23*
4	900	37.0 ± 0.14	55.9 ± 0.21*	59.0 ± 0.13*	57.0 ± 0.22*	61.4 ± 0.21*

[a] ±SEM

* Statistically significant compared to controls ($P < 0.05$).

12,000g for 5 min in a refrigerated centrifuge. The supernatant fluids were removed and extracted four times with 7.5 ml water-saturated ethyl ether to remove the acid. The aqueous solutions were then lyophilized, dissolved in 1 ml of 0.05 M acetate buffer, pH 6.2, and adjusted to this pH. Analyses for cyclic AMP and cyclic GMP were performed by radioimmunoassay (*12*). Amino acid analyses were performed using a Technicon amino acid analyzer. Student's t test was used for statistical analyses.

RESULTS

Irradiation of the animals caused significant increases in the levels of both cyclic AMP and cyclic GMP which were observed as early as 1 hr postirradiation and were maintained for the duration of the experiment (Tables I and II). These increases were found to be more pronounced in animals that received 900 rad than in the ones that received 300 rad, especially in the levels of cyclic GMP.

Radiation-induced changes in brain cyclic nucleotide levels are shown in Table III. Significant decreases in the levels of both cyclic AMP and cyclic GMP were found in the cerebellum of the irradiated animals. No appreciable changes in cyclic nucleotide levels were observed in any of the cerebrocortical regions examined.

Amino acid analyses before and 24 hr after irradiation were performed on the CSF of one monkey which had been exposed to 900 rad (monkey No. 3, Table I).

TABLE II

Effects of Radiation on CSF Cyclic GMP Levels (pmole/ml CSF)

Monkey No.	Radiation dose (rad)	Preirradiation 24 hr ($n = 3$)	Postirradiation ($n = 3$) 1 hr	24 hr	48 hr	72 hr
1	300	3.9 ± 0.04	5.9 ± 0.10*	4.7 ± 0.06*	4.7 ± 0.06*	6.4 ± 0.11*
2	300	5.0 ± 0.11	7.6 ± 0.16*	7.0 ± 0.07*	8.1 ± 0.05*	9.8 ± 0.11*
3	900	5.9 ± 0.07	19.7 ± 0.08*	21.4 ± 0.16*	30.7 ± 0.14*	40.1 ± 0.30*
4	900	6.9 ± 0.13	31.3 ± 0.21*	24.9 ± 0.11*	27.0 ± 0.12*	N[b]

[a] ±SEM

[b] N: assay was not carried out

* Statistically significant compared to controls ($P < 0.05$)

TABLE III

Effect of Radiation on Cerebellar and Cerebrocortical Cyclic AMP and Cyclic GMP Content in Anesthetized Monkeys (pmole/mg protein)

	Cerebellum (n = 4)		Cortex (n = 3)			
			Frontal		Occipital	
	cAMP	cGMP	cAMP	cGMP	cAMP	cGMP
Controls	5.95 ± 0.26[a]	4.81 ± 0.19	5.06 ± 0.29	0.38 ± 0.06	4.09 ± 0.17	0.46 ± 0.09
900 rad	3.89 ± 0.31*	1.42 ± 0.16*	4.87 ± 0.18	0.39 ± 0.11	4.20 ± 0.26	0.43 ± 0.12

[a] ±SEM.

* Statistically significant compared to controls ($P < 0.05$).

The results are shown in Table IV. No appreciable changes, except perhaps for alanine and arginine, the levels of both of which decreased, were found to occur in CSF amino acid composition as a result of irradiation of the head of the animal.

DISCUSSION

It has been previously reported that in neurologic disorders (*7–10*) and also in the induction of fever (*13*), the levels of cyclic AMP in the CSF increase. Increases in CSF cyclic AMP levels have also been observed following administration of biogenic amines or probenecid, thus showing the central nervous system origin of CSF cyclic AMP (*5*). Our results indicate that irradiation of the head of monkeys caused pronounced increases in the levels of both cyclic AMP and cyclic GMP which were maintained for the duration of the experiment (72 hr postirradiation).

TABLE IV

Effects of Radiation on CSF Amino Acid Composition (nmole/ml CSF)

Amino acid	Preirradiation	Postirradiation
Taurine	25.0	26.1
Aspartic acid	16.0	16.6
Threonine Serine	360.0	351.7
Glutamic acid	37.2	32.9
Glycine	41.7	38.4
Alanine	71.7	47.8
Valine	66.7	66.0
Cystine (1/2)	55.6	55.1
Methionine	8.9	8.9
Isoleucine	22.2	22.2
Leucine	—	—
Tyrosine	28.1	24.6
Phenylalanine	20.0	16.5
Lysine	76.1	83.3
Histidine	212.8	221.7
Arginine	55.6	33.3

To avoid possible variations in CSF cyclic nucleotide levels because of diurnal variations (*6*), special care was taken to collect the samples of CSF at exactly the same time of the day (1300 hr).

As shown in Table III, cyclic AMP and cyclic GMP levels in the cerebellum were significantly decreased by irradiation of the head of the animal, whereas cerebrocortical cyclic nucleotide levels were not appreciably affected. Since brain biopsy, as described under Methods, is a very involved surgical procedure, it was not practically possible to use more than one radiation dose and time postirradiation for brain cyclic nucleotide analysis. A 900-rad dose and 24-hr postirradiation time were selected because, under these conditions, the levels of both CSF cyclic nucleotides postirradiation were significantly higher than in the controls. Because of the stress that would be involved during a cerebral biopsy from an unanesthetized animal, we could not compare cyclic nucleotide levels in brain from unanesthetized and anesthetized monkeys. However, unpublished work in our laboratory has shown no significant change in CSF cyclic AMP and cyclic GMP levels as a function of a light anesthesia regimen as the one described above. Thus these data suggest that the radiation-induced drop in cerebellar cyclic nucleotide levels might also be seen in the awake animal. Although the data do not prove that CSF cyclic nucleotides rise and cerebellar cyclic nucleotides fall in the same animal, the magnitude of the observed changes leads us to speculate that both events would occur in the same animal. The cause of the radiation-induced rise in CSF cyclic nucleotides is not known. However, the fact that the reservoir communicates primarily with fourth ventricular fluid which is closely associated with the cerebellum and that the cerebrocortical tissues failed to lose cyclic nucleotides following irradiation suggests that the CSF cyclic nucleotide increases observed in this study may result from cyclic nucleotide transport from cerebellum to CSF. In favor of this interpretation is the finding that cyclic AMP in the CSF is of central nervous system origin and that introduction of biogenic amines directly into the cisterna magna enhanced its secretion from nervous tissue (*5*). In this respect it is of interest to note that exposure of animals to high doses of radiation resulted in activity changes of brain enzymes responsible for neurotransmitter metabolism, especially of monoamine oxidase, which catalyzes the oxidative deamination of biogenic amines and whose activity was found to be markedly affected by irradiation of the animal (*14*). However, other periventricular brain areas not assayed in these experiments might affect posterior fossa CSF cyclic nucleotide levels following irradiation. Further delineation of transport mechanisms of cyclic nucleotides into and out of CSF, as well as work on their synthesis and degradation in the various brain areas and in CSF, is certainly required.

Although a systematic amino acid analysis of the various CSF samples collected was not performed in this study, the fact that no appreciable changes in the CSF amino acid composition were found in one of the monkeys that received the higher radiation dose makes us believe that, under our experimental conditions, irradiation of the animal did not cause any significant changes in the CSF amino acid content.

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