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Implication of nitric oxide synthase in radiation-induced decrease in hippocampal noradrenaline release in rats

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Introduction

The hippocampus is important in critical functions such as learning, memory, and motor performance, and these functions are impaired after exposure to ionizing radiation [1]. Noradrenergic systems are important in mediating arousal, food intake and to some extent motor functions; histochemistry and immunohistochemical techniques have shown noradrenergic pathways in the hippocampus [2]. Several factors can contribute to acute nervous system damage *in vivo*: systemic blood pressure is reduced following exposure to 25–100 Gy γ radiation [3, 4], and cerebral blood flow decreases in a variety of brain regions, including the hippocampus [5]; the ischaemia produced by decreased blood flow is likely to affect neuronal activity [6]; ionizing radiation generates free radicals, and resulting oxygen radicals have been implicated in cell damage following ischaemia; brain ischaemia induces the release of an excessive amount of glutamate in the hippocampus, and glutamate acts on nitric oxide (NO) synthase to form NO through N-methyl-D-aspartate receptors, causing toxic effects [7]. The purpose of this study was to examine the effect of ionizing radiation on hippocampal noradrenaline (NA) release *in vitro*, stimulated by KCl 0.5, 24, 48, and 72 h after irradiation/sham-irradiation and to determine the role of NO synthase in the radiation-induced decrease in NA release.

Methods

Male Sprague-Dawley rats, weighing 200–300 g, were used in these experiments. Rats were killed by decapitation, and brains were removed. The hippocampus was dissected using the method of Glowinski & Iversen [8]. NA was measured by h.p.l.c. coupled with electrochemical detection. Release of hippocampal NA *in vitro* was stimulated by KCl and the irradiation procedures were carried out as described previously [9]. Rats were exposed bilaterally to varying doses of γ rays using a ^{60}Co source at a rate of 10 Gy min⁻¹. Statistical analysis was performed using Student's *t* test. Multiple comparisons with a sham-irradiated control were done by analysis of variance and Dunnett's test. Data were identified as significant if $P < 0.05$.

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Radiation dose/ sham-irradiation	NA release (pmol mg ⁻¹ of protein)			
	0.5 h	24 h	48 h	72 h
Sham	15.8 ± 0.3	15.2 ± 0.4	15.5 ± 0.2	15.3 ± 0.4
5 Gy	16.5 ± 0.8	14.5 ± 0.9	12.0 ± 0.5*	12.0 ± 0.2*
10 Gy	16.5 ± 0.6	12.4 ± 0.8	8.4 ± 0.65*	8.0 ± 0.5*
30 Gy	16.7 ± 0.5	12.0 ± 0.4*	8.0 ± 0.85*	8.0 ± 0.6*

Effect of exposure to γ rays (10 Gy min⁻¹) on NA release

Values are means \pm s.e.m. of three separate experiments. * Significantly different from sham-irradiated values; $P < 0.05$.

L-NA concentration (mg/kg)	NA release (pmol mg ⁻¹ of protein)			
	Sham irradiation	Saline + irradiation	L-NA + sham- irradiation	L-NA + irradiation
0	16.0 ± 0.3	8.4 ± 0.6*	16.9 ± 0.9	8.6 ± 0.4*
3	15.8 ± 0.6	8.0 ± 0.4*	17.5 ± 0.6	12.0 ± 0.4**
5	15.4 ± 0.4	8.2 ± 0.3*	21.5 ± 0.4*	16.5 ± 0.8**
10	15.5 ± 0.65	8.0 ± 0.3*	24.0 ± 0.6*	19.5 ± 0.6**

Effect of pretreatment with L-NA on NA (pmol mg⁻¹ of protein) release 48 h after exposure to 10 Gy of γ rays

Values are means \pm s.e.m. of three separate experiments. * Significantly different from sham-irradiation values; $P < 0.05$. ** Significantly different from irradiated values; $P < 0.05$.

Results

There was no significant difference in NA release between irradiated and sham-irradiated rats when the hippocampal NA concentration was determined 0.5 h after radiation exposure (5–30 Gy at 10 Gy min⁻¹). However, there were significant decreases in hippocampal NA release 48 and 72 h after exposure to 5 and 10 Gy and 24, 48, and 72 h after exposure to 30 Gy of γ rays (Table 1). Based on the above data, a post-irradiation time period of 48 h and a γ -radiation dose of 10 Gy at 10 Gy min⁻¹ were chosen for further studies with an NO synthase inhibitor, N^G-nitro-L-arginine (L-NA). Pretreating rats with 1 mg kg⁻¹ of L-NA administered i.p. 1 h before irradiation or sham-irradiation had no effect on the radiation-induced decrease in NA (data not shown). However, 3 mg kg⁻¹ of L-NA prevented the radiation-induced decrease in NA release, and 5 and 10 mg kg⁻¹ L-NA not only prevented the decrease in NA release in irradiated rats but also enhanced NA release in sham-irradiated rats (Table 2). Similar pretreatment with 1–10 mg kg⁻¹ of L-arginine before irradiation or sham-irradiation had no effect on either the radiation-induced decrease in NA release or the basal hippocampal NA release (data not shown).

Discussion

This study demonstrates that radiation had no effect on hippocampal NA release 0.5 h after exposure but decreased NA release 24, 48 and 72 h after exposure, depending on the radiation dose. At the present time we have no data to explain the differences in NA release at post-irradiation time intervals. However, it has been suggested that the blood-brain barrier could be disrupted by ionizing radiation [10, 11]. This would allow radiation-released neurotransmitters such as prostaglandins (PGs), histamines, and 5-hydroxytryptamine, as well as other circulating factors, (abnormal) access to neurons that modulate hippocampal NA release [12]. It has been shown that PGs of the E series inhibit the release of NA from sympathetic nerves both in the periphery and the central nervous system; conversely, inhibition of PG synthesis leads to an increase in NA release [13].

Immunochemical localization of NO synthase has been demonstrated in most areas of the rat brain, including the hippocampus. NO synthase forms NO from L-arginine [7]. Pretreatment with L-NA (a selective inhibitor of brain NO synthase) reversed the radiation-induced decrease in NA release, suggesting that NO synthase is involved in this phenomenon. Pretreatment with L-NA also enhanced NA release in sham-irradiated rats, suggesting that NO is involved in the regulation of NA under normal conditions. However, inhibition of PG synthesis by L-NA (thereby increasing NA levels) should not be excluded at this time. Although no results are currently available on the measurement of glutamate release in the hippocampus following exposure to radiation, our results support the hypothesis that toxic overstimulation of glutamate receptors by radiation or excitotoxicity contributes to overproduction of NO that can be toxic to neurons.

In conclusion, these results suggest that ionizing radiation induces a decrease in hippocampal NA release 24, 48 and 72 h after exposure, and NO synthase is implicated in this radiation-induced decrease in NA release.

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