X-irradiation attenuates relaxant responses in the rabbit ear artery

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1 The relaxant actions of acetylcholine, substance P and calcitonin gene-related peptide, and the levels of neuropeptide Y and calcitonin gene-related peptide were assessed in the rabbit central ear artery 1, 4 and 6 weeks after a single dose of 45 Gy X-irradiation, a dose similar to that used clinically in intraoperative radiotherapy.

2 Relaxant responses induced by acetylcholine and substance P (both endothelium-dependent) and calcitonin gene-related peptide (endothelium-independent) were reduced, and endogenous neuropeptide Y and calcitonin gene-related peptide levels were unaffected after X-irradiation.

3 The mechanism(s) by which a single dose of 45 Gy X-irradiation may selectively damage relaxant, but not direct, contractile responses of the smooth muscle (as we have shown previously) of the rabbit central ear artery are discussed.

Keywords: Rabbit ear artery; intraoperative radiotherapy; vasodilatation

Introduction

There is now considerable evidence for dual control of vascular tone by perivascular nerves and endothelial cells (for review, see Lincoln & Burnstock, 1990). Neuropeptide Y (NPY)-like immunoreactivity has been localized in sympathetic perivascular nerves of the rabbit central ear artery (REA), and exogenous NPY is known to have neuro-modulatory effects on the sympathetic transmission in this preparation (Wong-Dusting & Rand, 1988; Saville *et al.*, 1990). The REA also has a sensory-motor innervation with perivascular nerves containing substance P- (SP) and calcitonin gene-related peptide (CGRP)-like immunoreactivity (Morris & Bevan, 1986; Maynard *et al.*, 1990). SP and CGRP induce vasodilatation on precontracted REA preparations in an endothelium-dependent (Bolton & Clapp, 1986) and endothelium-independent (Maynard *et al.*, 1990) manner, respectively.

Much has been reported on the effects of radiation on the structure but not on the functioning of the blood vessels (for reviews see Law, 1981; Fajardo & Berthrong, 1988; Reinhold *et al.*, 1990). Recent evidence has suggested that endothelial cell function (see Fajardo, 1989) and vascular smooth muscle (Hopewell *et al.*, 1989) may be affected by irradiation. In a previous publication, we showed that a single dose of 45 Gy X-irradiation, similar to that used in intraoperative radiotherapy (Abe *et al.*, 1980; Kinsella & Sindelar, 1989; Le Couteur *et al.*, 1989), significantly affected the sympathetic transmission but not the direct contractile action of noradrenaline (NA) on the REA (Stewart-Lee *et al.*, 1991).

Since our previous findings indicated that there is X-irradiation-induced perivascular nerve damage, in this study we have examined the effects of a single dose of 45 Gy X-irradiation on the content of NPY and CGRP in the perivascular nerves, and on the endothelium-dependent (acetylcholine (ACh) and SP) and endothelium-independent (CGRP) relaxant actions on precontracted REA preparations.

Methods

X-irradiation procedure

Details of the X-irradiation experimental procedure have been described previously (Stewart-Lee *et al.*, 1991). Briefly, both

ears of New Zealand White rabbits (4–6 months; 2–3.5 kg) were locally irradiated with a single dose of X-rays (45 Gy, 215 kV, 12.5 mA). There were 6 to 9 animals in each of the age-matched control and X-irradiated animal groups. The latter were examined at 1, 4 and 6 weeks after X-irradiation.

Peptide assays

NPY and CGRP assays were performed on mid-segments of the REA which were measured, weighed and the peptides extracted. NPY and CGRP levels were quantitated by an inhibition enzyme-linked immunosorbent assay as described earlier (Belai *et al.*, 1988).

Pharmacology

Proximal segments of the REA were mounted under isometric conditions and optimal resting conditions of 1.0 g for at least 1 h, and used for pharmacological investigations as previously described (Stewart-Lee *et al.*, 1991). Cumulative concentration-response curves were established for NA (0.1–100 μ M) and were unaffected after 45 Gy X-irradiation (Stewart-Lee *et al.*, 1991).

To study relaxant responses to ACh, SP and CGRP, REA segments were precontracted with NA (3–10 μ M) which produced approximately 70% of the maximal NA contraction (Stewart-Lee *et al.*, 1991). As ACh does not cause desensitization of receptors, it was added cumulatively (0.1–100 μ M) to the organ bath, whereas SP (0.1 μ M) and CGRP (0.01 μ M) were added as single, predetermined concentrations (Illes & van Falkenhausen, 1986; Maynard *et al.*, 1990) and washed out once a maximal relaxant response was established.

Drugs and peptides

NA and ACh were obtained from the Sigma Chemical Company, Poole, U.K. SP, CGRP, NPY and anti-CGRP were purchased from Cambridge Research Biochemicals, Cambridge, U.K., and anti-NPY was obtained from Immunodiagnosics Ltd., U.K.

Expression of results and statistical methods

Relaxant responses are all expressed as a percentage change of the control response (taken as 100%). pD_2 values for ACh were calculated from the mean of the $-\log EC_{50}$ [ACh], i.e. the concentration of ACh which produced 50% of the maximal relaxant response. Slope values (Table 2) were obtained by use of a Pharmacologic Calculation System based on the *Manual of Pharmacologic Calculations with Computer*

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Table 1 Tissue content of neuropeptide Y (NPY) and calcitonin gene-related peptide (CGRP) in the rabbit central ear artery from control segments and preparations 6 weeks after a single dose of 45 Gy X-irradiation

	NPY (pmol cm ⁻¹)	CGRP (pmol cm ⁻¹)
Control segments	0.65 ± 0.10 (6)	0.13 ± 0.03 (6)
Irradiated segments	0.59 ± 0.11 (6)	0.14 ± 0.01 (6)

Values are mean ± s.e.mean. (n) indicates the number of preparations.

Programs (Tallarida & Murray, 1981). Results are expressed as the mean ± standard error of the mean (s.e.mean), and statistical significance was calculated by use of Student's unpaired *t* test. A probability level of *P* ≤ 0.05 was considered significant.

Results

Peptide assays

Immunoassays were performed on control and 6 weeks post-irradiated REA preparations. There was no significant change in the amount of either NPY or CGRP in the REA 6 weeks after 45 Gy X-irradiation in comparison with control preparations (Table 1).

Pharmacology

In precontracted control preparations, ACh produced concentration-dependent relaxant responses. The maximum relaxant effect of ACh measured in control preparations (80.9 ± 3.7% relaxation of NA-induced precontraction) was significantly reduced 1, 4 and 6 weeks after 45 Gy irradiation (Table 2). The diminution of the relaxant response increased with time after irradiation, being greatest after 6 weeks. The pD₂ value measured in segments from control animals, however, was not significantly different up to 6 weeks after 45 Gy X-irradiation (Table 2).

Both SP (0.1 μM) and CGRP (0.01 μM) produced relaxant responses in precontracted REA segments from control animals (27.4 ± 4.4% and 57.6 ± 5.4% relaxation of NA-induced precontraction, respectively) which were significantly reduced after 45 Gy X-irradiation (Figure 1a,b).

Table 2 Statistical analysis of the effect of acetylcholine (ACh) on isolated transverse ring preparations of the rabbit ear artery as influenced by X-irradiation treatment: age-matched control preparations are compared with those 1, 4 and 6 weeks after a single dose of 45 Gy X-radiation

Time after irradiation (weeks)	Maximum relaxation to acetylcholine	pD ₂ value	Slope
Control (7)	100.0 ± 4.6	5.9 ± 0.2	57.1 ± 4.6
1 (6)	50.5 ± 10.3***	6.2 ± 0.1 ^{NS}	34.6 ± 7.0*
4 (6)	42.6 ± 5.1***	6.0 ± 0.2 ^{NS}	31.2 ± 5.0**
6 (6)	36.9 ± 7.0***	5.8 ± 0.1 ^{NS}	21.9 ± 3.3***

All values are given as mean ± s.e.mean and *n* (in parentheses) is the number of observations. Asterisks denote significant differences (**P* ≤ 0.05; ***P* ≤ 0.01; ****P* ≤ 0.001); NS denotes no significant differences between control and irradiated preparations. pD₂ values were calculated as the -log molar concentration of ACh producing 50% of the maximal response. Maximal relaxation to ACh is expressed as a percentage of the control response (taken as 100%).

Figure 1 Graphs illustrating the percentage change of relaxations mediated by substance P (0.1 μM) (a) and calcitonin gene-related peptide (CGRP, 0.01 μM) (b) on precontracted preparations (noradrenaline, 3–10 μM) of the rabbit isolated central ear artery. Preparations were taken from age-matched control animals and animals 1, 4 and 6 weeks after a single dose of 45 Gy X-irradiation. Symbols represent means and vertical bars denote s.e.mean (***P* ≤ 0.01; ****P* ≤ 0.001).

Discussion

A single dose of 45 Gy X-irradiation reduced the levels of NA (Stewart-Lee *et al.*, 1991), but not NPY (this study) in the REA. This finding suggests that X-irradiation may have a differential effect on these two vasoactive substances, which are thought to be colocalised in the same sympathetic nerve terminals in the REA (Saville *et al.*, 1990). Alternatively, 45 Gy X-irradiation may damage sympathetic fibres that do not contain NPY. NA is stored in both small and large dense-cored vesicles (Miserez *et al.*, 1988), whereas NPY, which was unaffected 6 weeks after 45 Gy X-irradiation, is stored exclusively in large dense-cored vesicles (Wharton & Gulbenkian, 1987). Therefore, it is possible that the small dense-cored, NA-containing vesicles are selectively radiation-sensitive. This possibility is consistent with the observation that endogenous levels of CGRP were unaffected, since this polypeptide is also stored in large dense-cored vesicles (Wharton & Gulbenkian, 1987) in sensory-motor nerves of the REA (Maynard *et al.*, 1990).

The finding that after 45 Gy X-irradiation, the endothelium-dependent relaxant responses to ACh (Owen & Bevan, 1985; Maynard *et al.*, 1990) and SP (Bolton & Clapp, 1986) in the REA were progressively attenuated with time may be a reflection of the structural changes to the endothelium caused by this treatment (Kristek *et al.*, 1990). However, since the relaxant responses to CGRP, which acts independently of the endothelium in the REA (Maynard *et al.*, 1990), were also reduced to a similar degree, X-irradiation may have induced a general impairment of the relaxant capacity of the REA smooth muscle. It should be noted that there was no effect on

NA-induced contractions after 45 Gy X-irradiation (Stewart-Lee *et al.*, 1991). This suggests that the vasodilator mechanism(s) may be more radiation-sensitive than the vasoconstrictor mechanism(s) of the REA smooth muscle. It is possible that the selective damage may be at the level of the transduction mechanisms associated with vasodilatation, but not involved in vasoconstriction, e.g. calcium sequestration (Kuriyama, 1981).

Our previous studies have shown that perivascular nerves, endothelial cells and smooth muscle cells (Kristek *et al.*, 1990), and sympathetic transmission (Stewart-Lee *et al.*, 1991) are all adversely affected by a single dose of 45 Gy X-irradiation. The present paper reveals that even though levels of endogenous CGRP in perivascular nerves are unaffected after 45 Gy X-irradiation, the relaxant action of this polypeptide is impaired.

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