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Early effects of acute γ-radiation on vascular arterial tone

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1 To determine the acute effects of irradiation on the functionality of vessel, rat aortic rings were mounted in an organ bath for isometric tension measurements and irradiated (^{60}Co, 1 Gy min^{-1}, 15 min).

2 Irradiation, which is without effect on non-contracted or endothelium-denuded vessels, led to an immediate and reversible increase in vascular tone on (−)-phenylephrine (1 μM)-precontracted aortic rings. The tension reached a plateau about 5 min after the beginning of irradiation.

3 The maximal radiation-induced contraction occurred on aortic rings relaxed by acetylcholine (ACh) (1 μM). In this condition, the addition of catalase (1000 u ml^{-1}), which reduces hydrogen peroxide, and DMSO (0.1% v/v), which scavenges hydroxyl radical, had no influence on tension level while superoxide dismutase (SOD) (100 u ml^{-1}), a superoxide anion scavenger, reduced the observed contraction. A similar result was obtained in the presence of indomethacin (10 μM), a cyclo-oxygenase blocker.

4 Pretreatment of rings with the nitric oxide synthase inhibitor, N_ω-nitro-L-arginine methyl ester (L-NAME) (10–100 μM) inhibited the radiation-induced contraction.

5 This effect was dose rate-dependent and even occurred for a very low dose rate (0.06 Gy min^{-1}).

6 The present results indicate that γ-radiation induces an instantaneous vascular tone increase that is endothelium and dose rate-dependent. This effect is (i) maximal when nitric oxide (NO) is produced, (ii) greatly reduced by SOD and (iii) inhibited by L-NAME, suggesting a major involvement of complexes between NO and superoxide anion.

Keywords: γ-Radiation; vascular tone; nitric oxide; endothelium; free radicals; arachidonic acid

Introduction

Endothelial cells play an active role in a variety of physiological functions including maintenance of the fluidity of the blood, modulation of the vascular tone, inflammatory and immunological processes (Lüscher & Vanhoutte, 1990). Endothelial dysfunctions could lead to hyperactivity of vascular smooth muscle, hence, to pathological elevated vascular tone and to activation, adhesion and aggregation of platelets (Rubanyi, 1991). Some of these consequences have been experimentally demonstrated in a variety of animal models as well as in human coronary artery disease. In particular, it has been suggested that radiation therapy could be a direct cause of coronary artery disease (Benoff & Scheitzer, 1995). Some studies have described post-irradiation changes of smooth muscle cells and/or endothelium in vessels (Hopewell *et al*., 1986; Fajardo *et al*., 1988), leading to obliteration and ischaemic effects. All these alterations are typically delayed lesions and usually appear several months to a few years after initial exposure. *In vitro*, relationships between endothelial cell damages produced by irradiation and their altered biochemical and functional properties have also been studied (Lam *et al*., 1985; Rubin *et al*., 1991).

It has been hypothesized that irradiation-induced alterations could result from the generation of reactive oxygen species during γ-radiation exposure which is considered as the most important indirect mechanism of radiation injury (Dubner *et al*., 1995). Free radicals can be generated by water radiolysis to act on Ca^{2+}, Na^+ and K^+ channels, Na^+/K^+ and Ca^{2+}-ATPases, and $Na^+/K^+/2C1^-$ cotransporter (Eliott & Koliwad, 1995). Nevertheless, despite these results, no data are available concerning the immediate effects of acute irradiation on the functionality of the endothelium as a regulator of vasculator tone.

In this study, we have examined the acute effects of γ-irradiation on arterial vascular tone. Our results demonstrated that irradiation induces an immediate and reversible constriction of rat precontracted aortic rings. This effect required endothelium integrity and may be due to the formation of complexes between free radicals and NO.

Methods

Preparation of aortic rings and perfusion conditions

Female Wistar rats, 4–6 month old, were anaesthetized with sodium pentobarbitone (60 mg kg^{-1}). A segment of descending thoracic aorta, 4–5 cm long, was quickly excised and placed in an oxygenated (CO_2 5%, O_2 95%) Krebs bicarbonate buffer of the following composition (mM): NaCl 118, KCl 5.6, $CaCl_2$ 2.4, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 20 and D-glucose 11, pH 7.4. The tissue was cleaned of adhering fat and connective tissue and cut into 1.5 mm long transverse segments. The experiments were performed in an organ bath (0.9 ml) filled with thermostated (37°C) Krebs bicarbonate solution. A peristaltic pump delivered the solution (1.5 ml min^{-1}) from a reservoir in which pharmacological agents were added. The overflow from the organ bath was run

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to waste. Aortic rings were placed horizontally between a stationary stainless steel hook and an isometric force transducer (Biologic, Claix, France) which was connected to a microcomputer. An initial passive tension of 15 mN was applied to the aortic rings. After an equilibration period of 60 min, this passive tension was readjusted to 15 mN for 15 min before the start of the experiment. For each experiment, the functionality of aortic rings was evaluated by two pharmacological tests: (−)-phenylephrine (PE) (1 μM) which induces contraction of smooth muscle cells, and acetylcholine (ACh) (1 μM) which exerts a vasodilator endothelium-dependent effect. The experiments were performed only on aortic rings where integrity of both endothelial and smooth muscle cells has been confirmed. In some rings, the endothelium was removed by gentle rubbing of the intimal surface with a section of polyethylene catheter and absence of functional endothelium was then confirmed by the lack of a response to ACh.

Irradiation experiments

A sealed ^{60}Co source was used for γ-irradiation (1 Gy min^{-1} for 15 min). Irradiation was performed under different conditions: (1) aortic rings contracted by PE (1 μM), with or without endothelium; (2) non contracted aortic rings; (3) PE-precontracted aortic rings, dilated by ACh (1 μM); (4) PE-precontracted aortic rings, dilated by ACh + indomethacin (10 μM); (5) PE-precontracted aortic rings, dilated by ACh + antioxidants (DMSO 0.1% v/v or SOD 100 u ml^{-1} or catalase 1000 u ml^{-1}); (6) PE-precontracted aortic rings, dilated by ACh after L-NAME (10 μM or 100 μM) preincubation.

To study the effect of γ dose rate on induced contraction, experiments were performed on PE-precontracted aortic rings, dilated by ACh with three irradiation dose rates (1 Gy min^{-1}, 0.25 Gy min^{-1}, 0.06 Gy min^{-1}) for 15 min.

Drugs

PE, ACh, catalase, SOD, DMSO, L-NAME were purchased from Sigma (St-Quentin Fallavier, France). Indomethacin was obtained by Merck (Sharp & Dohme-Chibret, Paris, France).

Expression of results and data analysis

Results are expressed as the mean ± s.e.mean of aortic ring tension, in mN. For comparisons, values were normalized to the mean PE-induced contraction. A paired *t* test was used to study the effect of irradiation. The effect of drugs was analysed by one way ANOVA and Dunnett's method after a Kolmogorov-Smirnov normality test. A *P* value less than 0.05 was considered to be statistically significant.

Results

Effect of irradiation on precontracted and vasodilated aortic rings

γ-Irradiation (15 Gy) induced a significant increase in vascular tone of PE-contracted aortic rings (8.95 ± 0.69 mN to 11.95 ± 0.85 mN ($n=6$) (Figure 1a). This effect was rapid (70% of maximal effect is reached in 70 s) and immediately reversible. Under similar conditions, when the endothelium was removed, no response to irradiation was observed ($n=4$) (Figure 1b). When irradiation was carried out on non-precontracted aortic rings, there was no detectable radiation effect on vascular tone ($n=4$) (Figure 1c). However, irradiation induced a marked increase in vascular tone on PE-precontracted aortic rings relaxed by ACh (1 μM) (1.66 ± 0.51 mN to 8.10 ± 0.71 mN) ($n=7$) (Figure 1d).

Effect of indomethacin on irradiation-induced contraction

Indomethacin, a cyclo-oxygenase blocker which was added at the beginning of the experiment, did not modify the PE and ACh effects. Irradiation was carried out on ACh-dilated aortic rings in the presence of indomethacin (10 μM) ($n=4$) (Figure 2a). Under these conditions, the radiation-induced contraction was significantly decreased as compared to the

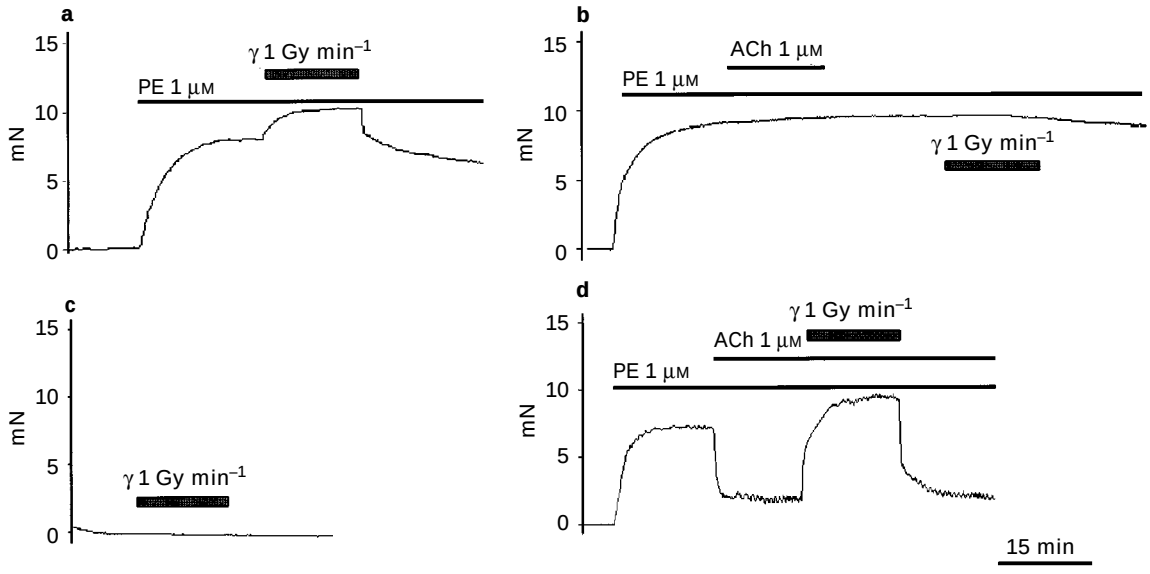

Figure 1 Effect of γ-irradiation on vascular tone of aortic rings. The traces show the typical effect of γ-irradiation (1 Gy min^{-1}, 15 min) on (−)-phenylephrine (PE; 1 μM)-contracted aortic rings: (a) with endothelium ($n=6$), (b) without endothelium ($n=4$), (d) relaxed by acetylcholine (ACh; 1 μM) ($n=7$) and on non-contracted aortic rings ($n=4$) (c).

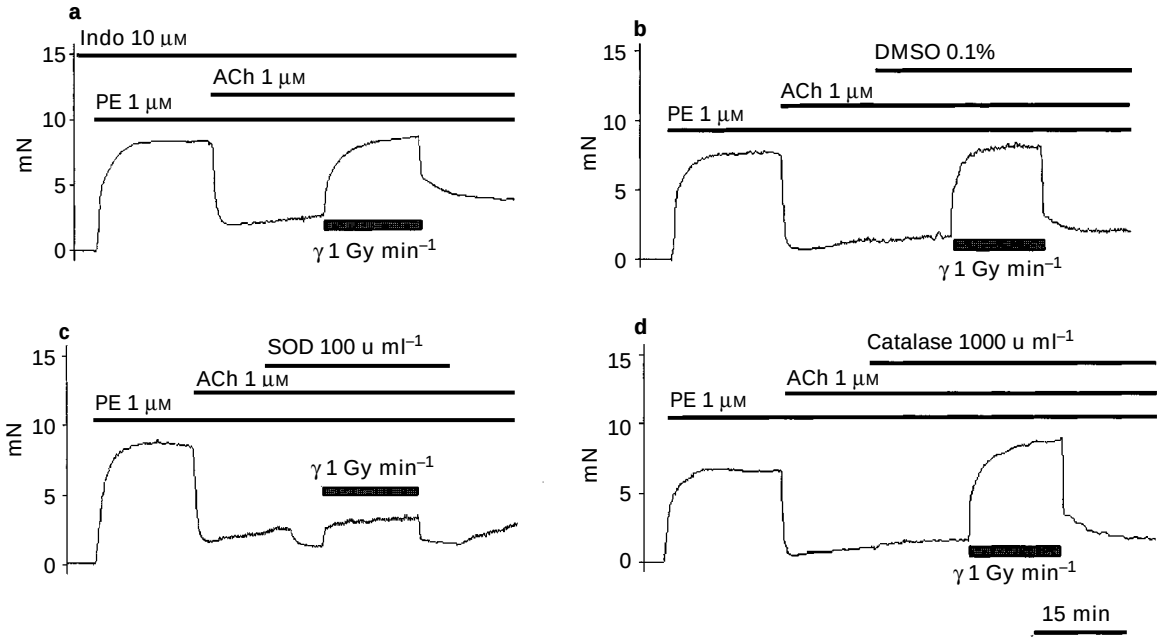

Figure 2 Action of indomethacin and antioxidants on the radiation-induced contraction. The traces show the typical effect of γ-irradiation (1 Gy min^{-1}, 15 min) on (−)-phenylephrine (PE; 1 μM)-precontracted aortic rings, dilated by acetylcholine (ACh; 1 μM): (a) effect of indomethacin (Indo, 10 μM), (b) effect of dimethyl sulphoxide (DMSO) (0.1% v/v), (c) effect of superoxide dismutase (SOD) (100 u ml^{-1}) and (d) effect of catalase (1000 u ml^{-1}); $n=3$ or 4 for each treatment.

radiation-induced contraction in the absence of indomethacin (Table 1).

Effect of three antioxidants on irradiation-induced contraction

In these experiments, the influence of three antioxidants on irradiation effect was tested on PE-precontracted aortic rings relaxed by ACh. The drugs were added before irradiation and maintained during experimentation. DMSO (0.1% v/v), a powerful scavenger of hydroxyl radicals, induced no modification of the radiation-induced contraction ($n=3$) (Figure 2b, Table 1). Similar results were obtained in the presence of diethyltriamine pentaacetic acid (DETAPAC) (200 μM), a Fe^{3+} metal ion chelator that limits the Fenton reaction (data not shown). In contrast, SOD (100 u ml^{-1}), a superoxide anion scavenger, reduced the radiation-increased tension and potentiated ACh endothelium-dependent relaxation ($n=3$) (Figure 2c, Table 1). Catalase (1000 u ml^{-1}), which reduces hydrogen peroxide, slightly modified the basal arterial tone but had no effect on radiation-induced contraction ($n=4$) (Figure 2d, Table 1).

Effect of L-NAME preincubation on irradiation-induced contraction

Firstly, we observed that L-NAME (10 μM) preincubation (45 min) increased significantly PE-contraction ($n=4$) (Figure 3a). Secondly, L-NAME incubation led to the reduction of both ACh-induced vasodilatation and radiation-induced vasoconstriction (Figure 3a, Table 1). Furthermore, at this concentration, a correlation was observed between the inhibition level of ACh-induced relaxation and the increase in vascular tone during irradiation (Figure 3b).

Table 1 Effect of indomethacin, antioxidants and L-NAME on irradiation-induced contraction of aortic rings

	ΔRI (mN)	*Reduction of γ-induced contraction towards PE+ACh alone* (%)
PE + ACh	8.66 ± 0.55	
+ indomethacin	6.02 ± 0.32*	30
+ DMSO	8.55 ± 0.91	1
+ SOD	2.70 ± 0.21*	69
+ catalase	7.99 ± 1.13	8
+ L-NAME (10 μM)	2.27 ± 0.55*	74
+ L-NAME (100 μM)	0.29 ± 0.05*	97

The values represent the mean ± s.e.mean ($n=3$ to 7) of aortic ring tension increase during irradiation (ΔRI), expressed in milliNewton (mN). The initial tension was normalized to the average PE-contraction level. *Significant effect of drugs on radiation-induced contraction compared to PE + ACh alone (ANOVA followed by Dunnett's test, except for L-NAME (100 μM) the effect of which was significantly different from PE + ACh alone by using Fischer's test). With all these treatments, there was a significant effect of irradiation (paired t test). A P value lower than 0.05 was considered to be significant.

The use of L-NAME (100 μM) led to complete inhibition of the ACh effect ($n=4$). Under these conditions, a residual contraction was observed during irradiation (Figure 3c, Table 1).

Effect of dose rate on irradiation-induced contraction

When different dose rates were used for γ-radiation on the same PE-precontracted ACh-dilated ring, a correlation was observed between the dose rate and the corresponding contraction ($n=4$) (Figure 4a). Furthermore the radiation-induced contraction did not vary with repeated irradiations (Figure 4a).

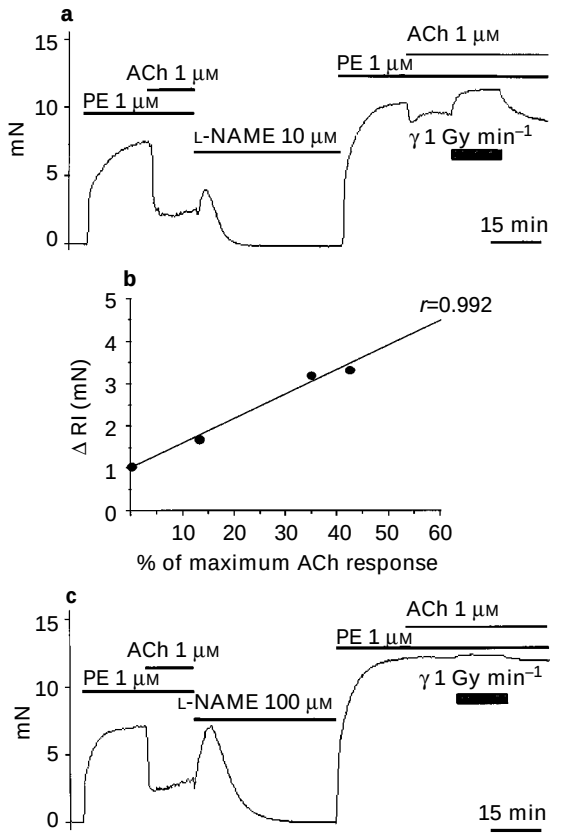

Figure 3 Effect of L-NAME on the radiation-induced contraction. The traces show the typical effect ($n=4$) of γ-irradiation (1 Gy min^{-1}, 15 min) on (−)-phenylephrine (PE; 1 μM)-precontracted aortic rings, relaxed by acetylcholine (ACh; 1 μM), after preincubation (45 min) with L-NAME (10 μM) (a) or L-NAME (100 μM) (c). To show the modifications induced by L-NAME pretreatment, pharmacological tests with PE and ACh are presented (left). The trace in (b) shows the increase in vascular tone (ΔRI), in mN, during irradiation (normalized to the mean PE-precontraction), for four aortic rings (each point represents a preparation), relaxed by ACh (1 μM) after L-NAME (10 μM) preincubation.

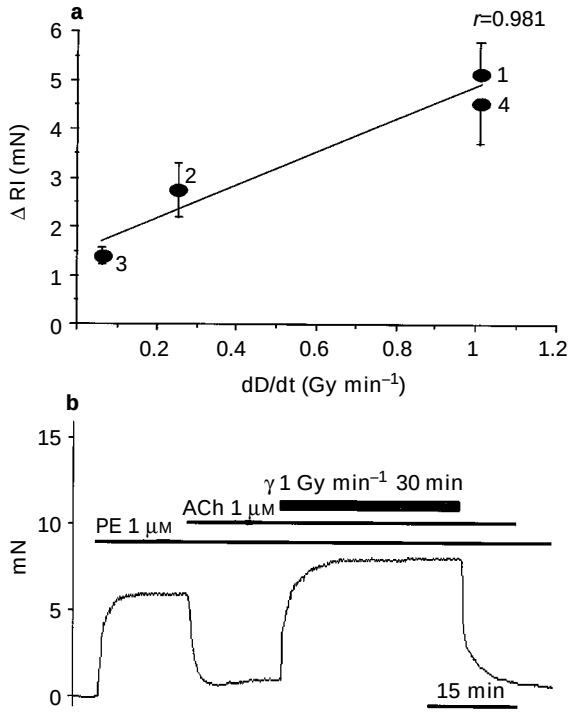

Figure 4 Effect of γ dose-rate on vascular tone increase. The trace (a) shows the increase in vascular tone (ΔRI), in mN, of ACh-relaxed aortic rings (normalized to the mean PE-precontraction) submitted to successive irradiations at different dose-rates (dD/dt). At the end of the experiment the initial dose rate was readministered to observe eventual irradiation-induced irreversible alterations. The values are expressed as mean and vertical lines show s.e.mean ($n=4$). The trace in (b) shows the typical effect of γ-irradiation (1 Gy min^{-1}) on (−)-phenylephrine (PE; 1 μM)-precontracted aortic rings relaxed by acetylcholine (ACh; 1 μM) for 30 min ($n=4$). In (a): the number beside each symbol is the rank of successive irradiations.

Discussion

The present study describes the acute effect of ionizing γ-radiation on the vascular tone of excised rat aortic rings. On PE-contracted as well as PE-precontracted then ACh-dilated aortic rings, irradiation induced a significant, immediate and reversible increase in vascular tone. This effect was endothelium-dependent since it did not occur on aortic rings without endothelium. Moreover, this effect was correlated with the irradiation dose rate used, even at a very low one. Furthermore, for a given dose rate, the time and consequently the dose did not influence the range of radiation-induced contraction, demonstrating that ionizing radiation induced no cumulative and irreversible alterations in the conditions of the experiment.

The large effect of irradiation on ACh-dilated rings as well as the inhibition of radiation-induced contraction after L-NAME (100 μM) preincubation suggest an involvement of nitric oxide (NO) in these phenomena. This hypothesis is supported by the significant correlation existing between the reduction of radiation-induced contraction and the degree of the inhibition by L-NAME (10 μM) of the ACh-induced vasodilatation. The observed variability of L-NAME maximum effect between rings is probably due to variations in uptake by endothelial cells (Rees *et al.*, 1990). This suggests that radiation acts on NO production or availability. This hypothesis is in accordance with the results obtained on PE-precontracted aortic rings which exhibit, as opposed to non-precontracted vessels, an enhanced basal production of NO and a response to irradiation. The basal production of NO was confirmed by the observation of the PE constricting effect that was significantly increased after inhibition of NO synthesis by L-NAME preincubation (8.54 ± 0.34 mN to 12.79 ± 0.72 mN, Student's t test, $P<0.05$), consistent with previous findings (Palmer *et al.*, 1988).

Reactive oxygen species, particularly hydroxyl radical and superoxide anion, are known to be produced during irradiation by water radiolysis (Stark, 1991). Suspecting a contribution of free radicals in the mechanism of radiation-induced contraction of aortic rings, we tested the effect of three antioxidants (DMSO, catalase, SOD). DMSO and catalase had no influence on the increased tension during irradiation, leading us to conclude that the hydroxyl radical and hydrogen peroxide do not play a key role in the γ-radiation effect. The very low contraction observed after catalase adjunction was possibly due to the basal presence of H_2O_2 in the medium that induces a vasodilator action, as previously demonstrated (Rubanyi, 1988). The results indicate that superoxide anion is probably involved in the radiation-induced contraction, since the presence of SOD during irradiation significantly reduced the observed effect. A direct action of superoxide anion was excluded, since no response to irradiation was

observed without contraction and endothelium integrity. This observation led us to hypothesize that superoxide anion is involved in radiation-induced contraction probably by scavenging NO. This hypothesis is in agreement with the observation that SOD markedly stabilized NO (Gryglewski *et al*., 1986). This property of SOD as well as SOD-mediated stimulation of the release of NO (Langenstroer & Pieper, 1992) could partly explain the small increase of ACh-induced vasodilatation during SOD adjunction before irradiation. Finally, as suggested before, it is possible that during irradiation when NO is produced by endothelial cells, it reacts with $O_2^{\bullet -}$ to give the unstable peroxynitrite $ONOO^-$ (Huie & Padmaja, 1993). Nevertheless, an opposing hypothesis could be formulated since it has been shown that peroxynitrite acts as a vasodilator agent (Liu *et al*., 1994). However, peroxynitrite is less potent (50 fold) than NO for induction of vasodilatation (Tarpey *et al*., 1995) and a peroxynitrite-related vasoconstrictor response has been observed (Villa *et al*., 1994; Voelkel *et al*., 1995). So the formation of peroxynitrite during irradiation would result in an increase in vascular tone by consuming NO or/and by a direct constrictor effect.

Although L-NAME-preincubated aortic rings inhibited the increase tension due to radiation, the adjunction of indomethacin was found to reduce the γ-irradiation effect. Thus, it is possible that prostanoids are produced under γ-irradiation and act on the formation, release or activity of NO as previously described (Katusic *et al*., 1993; Tesfamariam, 1994). So, the constrictor effect of irradiation would result from two associated mechanisms: a minor action on the metabolism of arachidonic acid and a major action on NO. However, a residual effect occurred with L-NAME (100 μM) preincubation without (Figure 3c) or with indomethacin (data not shown), suggesting a role for a third element, such as an endothelium-derived contracting factor.

In this study, we have shown that acute γ-radiation has an immediate and reversible constrictor effect on arterial tone of precontracted and dilated aortic rings even at a very low dose rate. This effect requires endothelium integrity and is inhibited by L-NAME and prevented by SOD administration. The peroxynitrite, a complex formed between NO and $O_2^{\bullet -}$ leading to inactivation of NO, seems to be implicated as well as and to a lesser extent, prostanoid formation. Although there has been some scepticism in the past about the causal role of radiation in coronary artery disease, multiple studies now suggest that it is an important late effect, particularly of irradiation in childhood (Stewart *et al*., 1995). Further experiments are needed to demonstrate to what extent these phenomena, which may occur during radiation therapy, have an influence on long-term deleterious effects of radiation and what happens in pathological conditions where superoxide anions are produced to react with nitric oxide.

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