Vitamin E and selenium regulate balance between β-adrenergic and muscarinic responses in rat lungs

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Received 5 April 1988

The effects of hydrogen peroxide on the β-adrenergic and muscarinic responses of the rat trachea muscle were studied in vitro, after feeding rats, for 6 weeks, either a diet deficient in vitamin E and selenium or a control diet. In the control situation after incubation with 1 mM hydrogen peroxide for 30 min, a reduction of the maximal response to methacholine of 39% occurred whereas no pD_2 shift could be demonstrated. Moreover, no response to isoprenaline after precontraction with 3×10^{-7} M methacholine was left. In the deficient situation, we found a reduction to 64% of the response to methacholine after incubation with 1 mM hydrogen peroxide. Again isoprenaline became inactive, i.e. no relaxation with isoprenaline was observed after precontraction with 3×10^{-7} M methacholine. We therefore conclude that vitamin E and selenium protect against oxidative stress in lung tissue and thus regulate the (patho-) physiological balance between adrenergic and muscarinic responses.

Vitamin E; Selenium; β-Adrenergic receptor; Muscarinic receptor; (Lung)

1. INTRODUCTION

Vitamin E (mainly α-tocopherol) is important in defence mechanisms of the cell against (lipid-peroxy) radicals [1]. The process of lipid peroxidation, as a result of oxidative stress, disturbs the membrane structure and this process can be antagonised by vitamin E. Selenium is essential for the activity of the selenium-dependent enzyme glutathione peroxidase, that converts hydrogen peroxide into water. Hydrogen peroxide, a generator of hydroxyl radicals, is capable of initiating the process of lipid peroxidation. It is known that vitamin E and selenium may cooperate in the protection against lipid peroxidation [2].

A diet deficient in both vitamin E and selenium decreases the cellular defence against radicals. Previously, we investigated the effect of lipid peroxidation on the density of β-adrenergic receptors and found a reduction in their density after treatment with cumene hydroperoxide or paraquat [3]. Here we determined the effect of hydrogen peroxide on the muscarinic and β-adrenergic responses in rat trachea muscle in vitro from rats fed a diet either with or without vitamin E and selenium.

Vitamin E and selenium deficiency exacerbates the effect of hydrogen peroxide. In both cases, deficient and control, the muscarinic response was found to be less susceptible to hydrogen peroxide than the β-adrenergic response.

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2. MATERIALS AND METHODS

2.1. *Organ preparation*

Male Wistar rats (20–40 g, TNO, Zeist, The Netherlands) were fed either a control diet or a diet deficient in vitamin E and selenium (Hopefarms, Woerden, The Netherlands) for 6 weeks. After 6 weeks, the rats weighing 220–240 g, were killed by a blow on the head. The trachea was rapidly excised and prepared according to Timmerman and Scheffer [4], with the modification that the cartilage rings were also cut at the site opposite the muscle. The trachea muscle was mounted in a water-jacketed

Published by Elsevier Science Publishers B.V. (Biomedical Division)

organ bath, at a temperature of 37°C, containing a buffer solution of the following composition (mM): 117.5 NaCl, 5.6 KCl, 1.18 $MgSO_4$, 2.5 $CaCl_2$, 1.28 NaH_2PO_4, 25.0 $NaHCO_3$, 5.5 glucose. The buffer solution was gassed with a mixture of 95% O_2 and 5% CO_2, pH 7.4. The tracheal strips contained 6 rings and a passive force of 0.5 g was applied to each strip. Contraction and relaxation were recorded isotonically.

After an equilibration period of 45 min with 6 intermediate washings, the effect of a cumulative dose of methacholine (contraction) was measured. The relaxation of the trachea muscle was recorded with a cumulative increase in the concentration of (−)-isoprenaline after contraction with 3×10^{-7} M methacholine. Between each curve a washing period of 30 min, with 5 intermediate washings, was applied. The third dose response curve served as the control since the fourth curve did not differ from the third one. After 3 dose response curves the trachea muscle was incubated with a single dose of hydrogen peroxide for 30 min. After this incubation period the muscle preparation was washed again following the same procedure as described above and a dose response curve of either methacholine or isoprenaline was recorded.

2.2. *Determination of vitamin E and glutathione peroxidase activity*

The lung membranes were isolated as described previously by Kramer et al. [3]. Vitamin E was extracted from the lung membranes according to Driskill et al. [5] and assayed by HPLC, as described by Rammell et al. [6] using fluorometric detection. The selenium-dependent and total (selenium-dependent and selenium-independent) glutathione peroxidase activity was measured from the cytosol of the lungs, according to Wendel [7] using either H_2O_2 (selenium-dependent) or cumene hydroperoxide (total) as substrates.

2.3. *Statistics*

Maximal responses (compared to respective controls) and pD_2 values are given ± SD. pD_2 values for either methacholine or isoprenaline did not change significantly after H_2O_2 incubations. Dose response curves as presented in the figures are the mean of at least six experiments; levels of significance were tested with Student's *t*-test.

2.4. *Chemicals*

Drugs used were: methacholine hydrochloride, (−)-isoprenaline hydrochloride and cumene hydroperoxide (Sigma); hydrogen peroxide (Merck); glutathione reductase (Boehringer). All other reagents used were of reagent grade.

3. RESULTS

As documented in table 1, after a diet deficient in vitamin E and selenium for 6 weeks, the lung membranes were deficient in vitamin E. In the deficient lung cytosol fraction, selenium-dependent glutathione peroxidase activity was not detectable. The Se-independent, glutathione peroxidase activity in the cytosol was also reduced in the deficient lungs compared to the control lungs (2.86 ± 0.6 vs 7.00 ± 3.8 nmol NADPH/min × mg protein, respectively).

Fig.1 presents the methacholine (contraction) dose response curves of the trachea muscle in the control situation. We found a pD_2 value of 5.37 ± 0.06 for the control curve. After incubation with 1 µM or 1 mM hydrogen peroxide for 30 min no change in the pD_2 value could be found (5.29 ± 0.05 after 1 µM H_2O_2 and 5.40 ± 0.21 after 1 mM H_2O_2), whereas the maximal contraction was reduced to $17 \pm 10\%$ (not significant) and $61 \pm 10\%$ ($p < 0.01$ compared to control), respectively, compared to the control methacholine dose response curve.

Concentration-dependent relaxation by isoprenaline, after contraction with 3×10^{-7} M methacholine is shown in fig.2 for the control rats. The pD_2 value for isoprenaline remained the same before or after H_2O_2 pretreatment (7.45 ± 0.17 for the control and 7.61 ± 0.13 after 1 µM H_2O_2), whereas the maximal response was reduced to $86 \pm 10\%$ compared to the control curve ($p < 0.01$) after 1 µM H_2O_2. After incubation with 1 mM hydrogen peroxide no relaxation with isoprenaline could be found.

In the deficient situation, the measurement of the methacholine dose response curves (fig.3) resulted in pD_2 values of 5.20 ± 0.09 (control curve) and 5.31 ± 0.05 (incubation with 1 µM

Table 1

Vitamine E in lung membranes and glutathione peroxidase activity in lung cytosol from rats fed a diet deficient in vitamin E and selenium or a control diet

	Vitamine E (nmol vitamin E/mg protein)	Glutathione peroxidase activity (nmol NADPH/min × mg protein)	
		Se-dependent	Se-independent
Control	1.31 ± 0.08 (6)	13.7 ± 3.7 (7)	7.00 ± 3.8 (7)
Deficient	ND (6)	ND (7)	2.86 ± 0.6 (7)

Each result represents the mean of a number of experiments (indicated in parentheses) ± SD. ND, not detectable

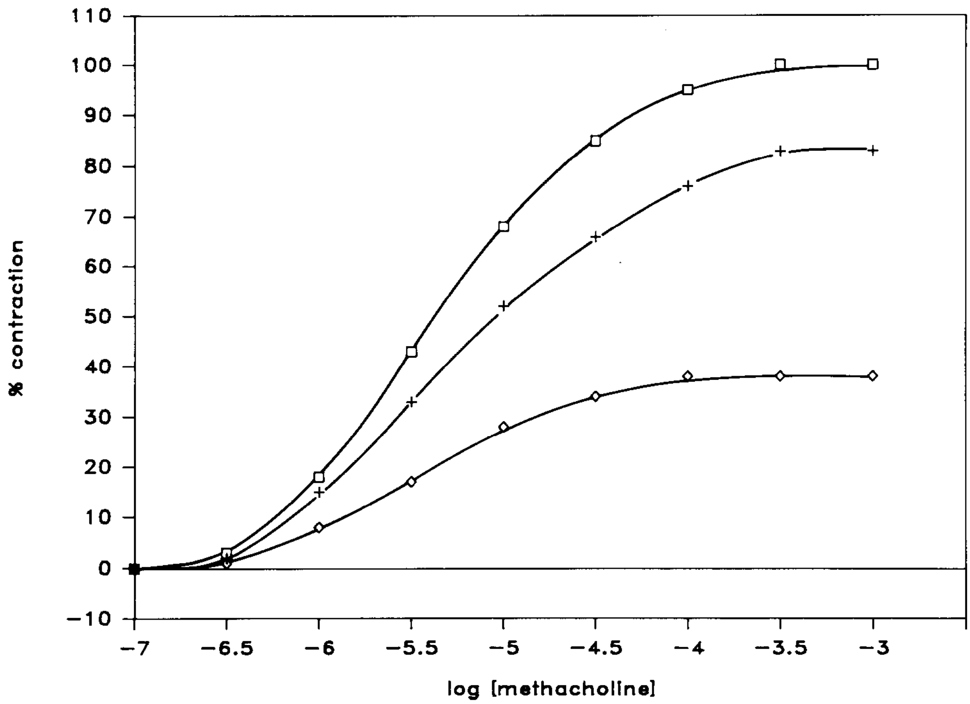

Fig.1. Dose response curves of methacholine in the case of rat trachea muscle after a diet without (□), with 1 μM (+) or 1 mM (◇) hydrogen peroxide pretreatment for 30 min.

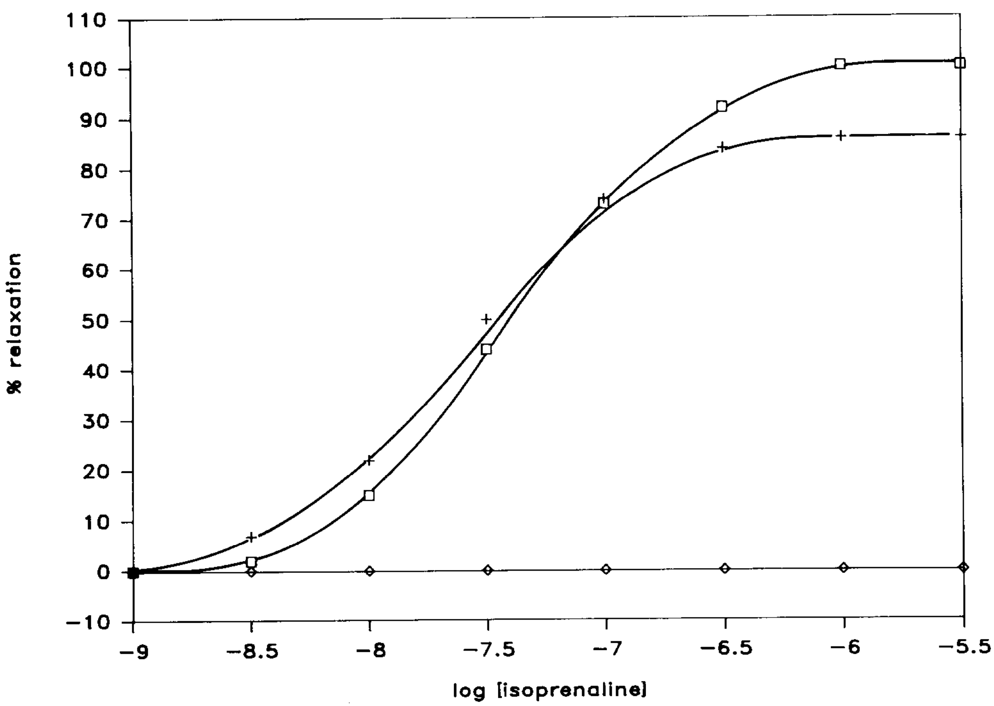

Fig.2. Isoprenaline dose response curves (precontraction with 3×10^{-7} M methacholine) after a control diet for 6 weeks. (□) Control curve; (+) and (◇) following incubation with 1 μM and 1 mM hydrogen peroxide for 30 min, respectively.

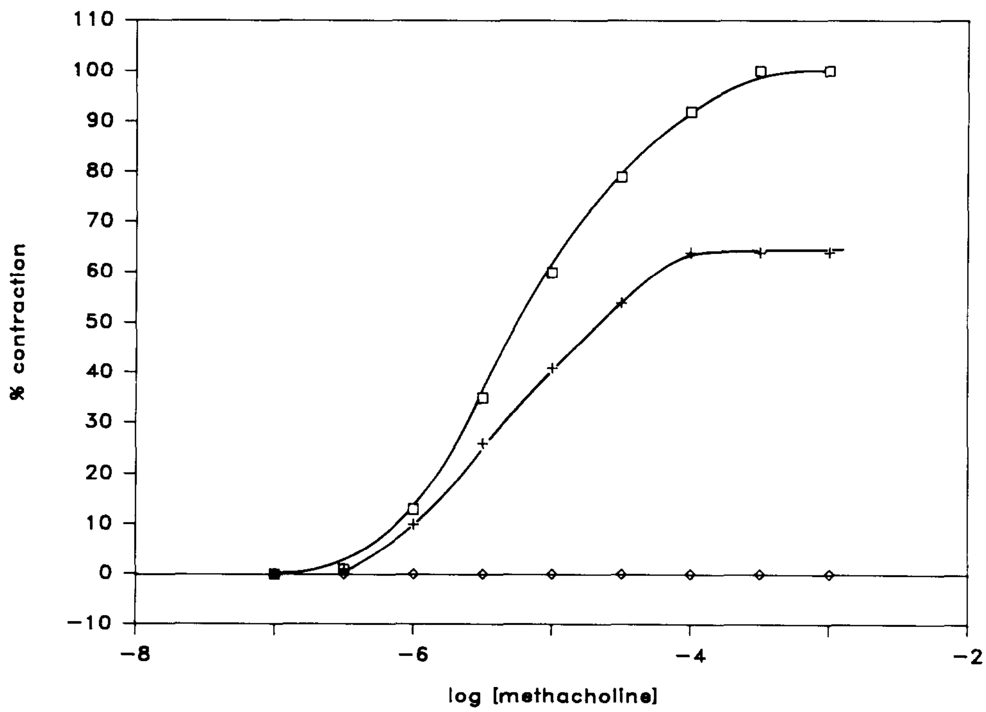

Fig.3. Dose response curves of methacholine, after a diet deficient in vitamin E and selenium, from rat trachea muscle. (□,+,◇) Control, 1 μM and 1 mM hydrogen peroxide pretreatment for 30 min.

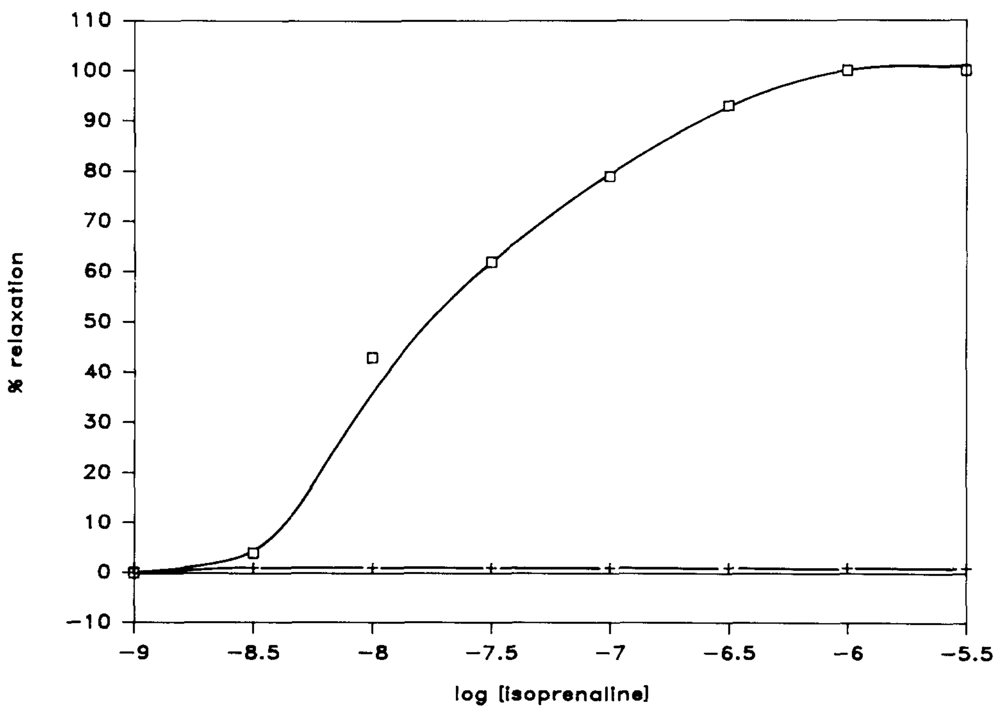

Fig.4. Isoprenaline dose response curves, after precontraction with 3×10^{-7} M methacholine, without (□) or with (+) 1 μM hydrogen peroxide treatment, for 30 min, of rat trachea muscle after a diet deficient in vitamin E and selenium.

H_2O_2 for 30 min). The maximal response was reduced to $64 \pm 10\%$ after 1 μM H_2O_2 ($p<0.01$ compared to control). The methacholine response was completely abolished after 1 mM hydrogen peroxide. For the isoprenaline dose response curves, after contraction with 3×10^{-7} M methacholine, we found, in the deficient situation, a pD_2 value of 7.55 ± 0.41 whereas after either 1 μM H_2O_2 or 1 mM H_2O_2 no response to isoprenaline was evident.

4. DISCUSSION

A diminished responsiveness towards endogenous β-agonists of the β-receptor-adenylate-cyclase-system has been proposed as a major defect in asthma [8]. Oxygen radicals, involved in inflammatory reactions, are generated during the catabolism of arachidonic acid metabolites and are also released from macrophages [9]. As we previously showed the density of the β-receptor diminishes after pretreatment with cumene hydroperoxide or paraquat, both generators of oxygen radicals [3].

Here we investigated the effect of 1 μM and 1 mM hydrogen peroxide on the β-adrenergic and cholinergic responses of rat trachea muscle after a control diet for 6 weeks. Neither the isoprenaline response nor the methacholine response was significantly affected by 1 μM hydrogen peroxide. However, after treatment with 1 mM hydrogen peroxide no β-adrenergic receptor response was measurable, whereas the maximal cholinergic response was decreased by 61% (comparison $p<0.01$). Apparently, the β-adrenergic response is more susceptible to hydrogen peroxide than the cholinergic response in the rat airways in vitro.

After a diet deficient in vitamin E and selenium, which diminishes the cellular defence mechanisms against oxygen radicals, methacholine and isoprenaline did not give a response after pretreatment with 1 mM hydrogen peroxide. Excessive free radical formation, under deficient conditions, could account for the aggravated damage.

Pretreatment with 1 mM hydrogen peroxide in the control situation, gave identical effects as pretreatment with 1 μM hydrogen peroxide in the deficient situation (no response for isoprenaline and a decrease of the maximal response for methacholine of 36%), again indicating a difference in sensitivity ($p<0.01$) towards oxidative stress between the adrenergic and the cholinergic component. An explanation for this difference might be sought in the difference of receptor structure and/or the stimulus transfer process. Wright and Drummond [10] reported that the enzyme adenylate cyclase was very susceptible to hydrogen peroxide. This may explain the relatively high sensitivity of the β-receptor response (which is coupled to the adenylate cyclase) for oxidative stress. However since the pD_2 values of both methacholine and isoprenaline did not change significantly (Student's t-test) after incubation with hydrogen peroxide, this indicates that not a change in receptor density or receptor-transducer coupling causes the effects. Rather the sensitivity of the relaxing or contracting processes is altered and affected differently.

Noticeable is the decrease in selenium-independent glutathione peroxidase activity found in the deficient situation. Probably the increase in oxidative stress as a result of the vitamin E and selenium deficiency in vivo diminishes the overall activity of the enzyme glutathione peroxidase compared to the control situation.

Oxygen radicals play a role in several forms of lung pathology. Vitamin E and selenium protect against oxidative stress in lung tissue and thus may regulate the (patho-) physiological balance between adrenergic and cholinergic responses.

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