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A DISBALANCE BETWEEN BETA-ADRENERGIC AND MUSCARINIC RESPONSES CAUSED BY HYDROGEN PEROXIDE IN RAT AIRWAYS IN VITRO

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Summary: The effect of hydrogen peroxide on adrenergic and muscarinic responses of rat airway smooth muscle was studied.The trachea muscle and the lung parenchymal strip were contracted with methacholine and relaxed with (-)-isoprenaline. Recording of three (-)-isoprenaline curves on the trachea muscle and the lung parenchymal strip was followed by treatment for 30 min with hydrogen peroxide (H_2O_2) (1mM) after which a new dose response curve for (-)-isoprenaline was constructed.Using the trachea muscle this treatment with H_2O_2 resulted in a decrease of 61% of the maximum contraction by methacholine compared with the control and a complete inhibition of the relaxation by (-)-isoprenaline. In the lung parenchymal strip preparation we found, after the same treatment no reduction of the contraction by methacholine and 61% reduction of the relaxation by (-)-isoprenaline, compared with the control.The results demonstrate that the adrenergic response in rat airways is more susceptible to hydrogen peroxide than the muscarinic response.

Szventivanyi (1) developed a general theory in which atopy and asthma were considered to be clinical expressions of beta-adrenoceptor dysfunction. Under normal psysiological conditions in airway smooth muscle, the bronchomotor tone of the muscle is the result of a balance between the sympathetic and parasympathetic activity.

To study the direct effect of drugs in vitro on airway smooth muscle, preparations of the large airways are used mostly. It is known that the fine peripheral bronchioles contain more smooth muscle tissue in relation to their diameter when compared with large airways (2).

Free radicals in general, as well as oxygen metabolites, may be important causes of lung injury. Recently, the effects of free radicals and oxygen radicals, like the hydroxyl radical, on receptor density and function in various tissues have been studied by several investigators (3-8). We found that the number of beta-adrenergic receptors in airway smooth muscle decreased if the membranes were challenged with oxidative stress (4,5). Others reported a decrease in tracheal relaxation in response to isoprenaline by oxygen radicals produced by macrophages (3). Hydrogen peroxide produces consistent contraction in canine lung strips and bovine tracheal strips, probably mediated by effects on prostaglandin metabolism (7), via free radical formation (6).

Here we studied the effect of hydrogen peroxide on adrenergic and muscarinic responses using two different parts of rat airway smooth muscle, the trachea muscle and the lung parenchymal strip, in order to investigate if oxygen radicals are important for the hypothesized disbalance between adrenergic and cholinergic responses, as this might play an important role in asthmatic conditions.

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Materials and methods

Male Wistar rats (240 - 300 g, TNO, Zeist, The Netherlands) were killed by a blow on the head and bled. The organs were rapidly excised and after preparation mounted in a water - jacketed organ bath with a temperature of 37 °C containing the appropriate buffer (see below) which was gassed with a mixture of 95% O_2 and 5% CO_2; pH = 7.40.
Tracheal strips were prepared according to Timmerman and Scheffer (9), with the modification that the cartilage rings were also cut at the site opposite the muscles. After preparation in a Krebs buffer of the following composition (mM): NaCl, 117.5 ; KCl, 5.6; $MgSO_4$, 1.18; $CaCl_2$, 2.5; NaH_2PO_4, 1.28; $NaHCO_3$, 25.0 and glucose, 5.5, the tracheal strips were immediately suspended in the Krebs buffer. Each tracheal strip contained six rings and to each tracheal strip a passive force of 0.5 g was applied. Recording was performed isotonically using a Hugo Sachs TL-2 Hebelaufnehmer.
After an equilibration period of one hour with six intermediate washings the muscle tone was increased with 3.10^{-7} M methacholine and a cumulative dose response curve of (-)-isoprenaline was recorded in the same bath using a Rikadenki B-261 recorder. Three dose response curves with (-)-isoprenaline were obtained first, the third one serving as the control curve. Between each curve a washing period of 30 min with five intermediate washings was applied. After the third curve and a washing period the tracheal strips were incubated for 30 min with 1mM hydrogen peroxide and washed again for 30 min. After this washing period another dose response curve of (-)-isoprenaline was recorded. The same procedure was used for studying the effect of hydrogen peroxide on a cumulative dose response curve of methacholine.
Parenchymal strips were prepared according to Vornanen (10) and suspended in the Krebs buffer. Recording was performed isotonically and a passive force of 0.5 g was applied. After an equilibration period for 60 min with six intermediate washings the muscle tone was increased with 3.10^{-5} M methacholine and a cumulative dose response curve of (-)-isoprenaline was recorded in the same bath. With this preparation three (-)-isoprenaline curves were obtained, the third curve being the control. Between each curve a washing period of 30 min with five intermediate washings was applied. After the third curve and a washing period the parenchymal strips were incubated for 30 min with 1mM hydrogen peroxide and washed again for 30 min. After that another dose response curve of (-)-isoprenaline was recorded. The same procedure was used for studying the effect of hydrogen peroxide on a cumulative dose response curve of methacholine.
Results were statistically evaluated by using the Student 's t-test.
Drugs used in this study were: (-)-isoprenaline hydrochloride and methacholine chloride, obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Hydrogen peroxide was obtained from Merck, Darmstadt, F.R.G. All other reagents used were of reagent grade.

Results

Figure 1 shows the changes in dose response curves of the contraction caused by methacholine after pretreatment of the trachea muscle of the rat with hydrogen peroxide (1mM). This resulted in a decrease of 61% of the maximal contraction. In Figure 1 it is also shown that treatment of the lung parenchymal strip of the rat with the same concentration of hydrogen peroxide does not cause a decrease of the maximal contraction caused by methacholine. The data of Table 1 indicate that the pD_2 - values of methacholine for the contraction of trachea muscle and lung parenchymal strip, do not change significantly upon pretreatment with hydrogen peroxide.

Figure 2 shows that pretreatment of tracheal strips with hydrogen peroxide, washing and subsequent contraction with 3.10^{-7} M methacholine, resulted in a complete disappearance of the relaxation by (-)-isoprenaline.

After pretreatment with hydrogen peroxide of the lung parenchymal strip and subsequent contraction with 3.10^{-5} M methacholine we found a decrease of 61% of the maximal attainable effect of (-)-isoprenaline.

The pD_2 - values calculated from the (-)-isoprenaline dose response curves for the trachea muscle and the lung parenchymal strip (relaxation), appeared not to be significantly different before and after hydrogen peroxide (Table 2).

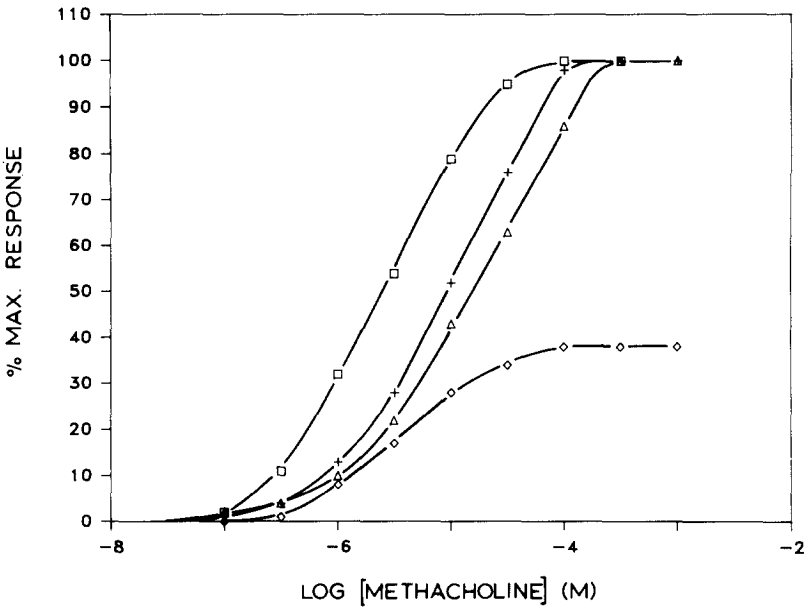

Figure 1. Dose response curves of methacholine chloride on trachea muscle and lung parenchymal strip of the rat, without (□-□ trachea, + - + lung) or with (◊ - ◊ trachea, Δ - Δ lung) pretreatment of 1mM hydrogen peroxide for 30 min. Curves are the mean of at least six independent experiments.

We also used lower concentrations of hydrogen peroxide (10^{-4}M, 10^{-5}M and 10^{-6}M), but did not find an effect neither on the response to methacholine nor to isoprenaline.

The results of Figure 3 illustrate that hydrogen peroxide caused an instantaneous contraction of the lung parenchymal strip, whereas no direct effect on the tracheal preparation was observed.

Discussion

The experiments executed show that the larger airway (the trachea muscle) is more sensitive to muscarinic and adrenergic agonists than the fine peripheral airway (the lung parenchymal strip) (Figures 1-2). The pD_2 values calculated from the effect of (-)-isoprenaline dose response curves

Table 1. Effect of hydrogen peroxide on muscarinic response by methacholine chloride.

	Control		Pretreated	
	pD_2	% max effect	pD_2	% max effect
Trachea	5.54 ± 0.20 (10)	100	5.40 ± 0.21 (6)	39
Lung	5.03 ± 0.13 (8)	100	4.71 ± 0.11 (7)	100

pD_2 values (mean ± SE, number of experiments between brackets) maximal and response of rat trachea muscle and lung parenchymal strip to methacholine chloride (relative to the control), with or without pretreatment with 1mM hydrogen peroxide.

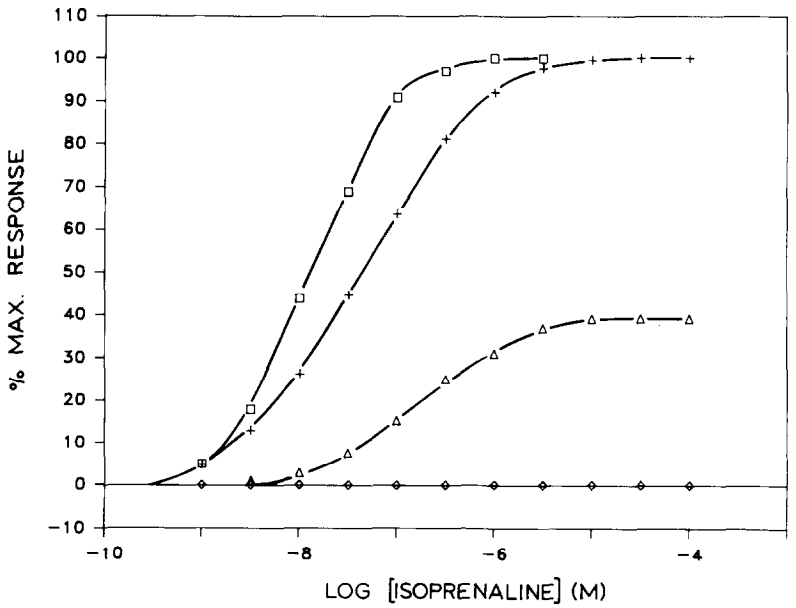

Figure 2. Dose response curves of (-)-isoprenaline hydrochloride on trachea muscle and lung parenchymal strip of the rat, without (□ - □ trachea, + - + lung) or with (◊ - ◊ trachea, Δ - Δ lung) pretreatment of 1mM hydrogen peroxide for 30 min. Curves are the mean of at least six independent experiments.

for the trachea muscle and the lung parenchymal strip were in contrast with the results reported earlier (10).Vornanen found a pD_2 value of 6.39 ± 0.13 (trachea muscle) and 6.70 ± 0.09 (lung parenchymal strip), whereas we found a pD_2 value of 7.85 ± 0.18 (trachea muscle) and 7.35 ± 0.26 (lung parenchymal strip) (Table 2).This discrepancy may be explained by the differences in concentration of the spasmogenic applied for achieving a contraction of the trachea muscle and the lung parenchymal strip.We used 3.10^{-7} M methacholine (10% of maximum contraction) for the trachea muscle and 3.10^{-5} M methacholine (50% of maximum contraction) for the lung parenchymal strip. However, Vornanen (10) tested the relaxing potency of isoprenaline after maximum contraction by carbacholine was achieved. As reported by Buckner and Saini (11) and

Table 2. Effect of hydrogen peroxide on adrenergic response by (-)-isoprenaline hydrochloride.

	Control		Pretreated	
	pD_2	% max effect	pD_2	% max effect
Trachea	7.85 ± 0.18 (14)	100	no response (10)	0
Lung	7.35 ± 0.26 (11)	100	6.91 ± 0.32 (7)	39

pD_2 values (mean ± SE, number of experiments between brackets) and maximal response of rat trachea muscle and lung parenchymal strip to (-)-isoprenaline hydrochloride (relative to the control), with or without pretreatment with 1mM hydrogen peroxide.

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least partly caused by the effect of free radicals on receptor function, thereby leading to a disbalance between adrenergic and cholinergic activity (1), is strengthened by this study, as we show that the adrenergic response is more susceptible to radicals than the cholinergic response. Moreover a possible role of oxygen radicals in the pathology of several lung diseases is emphasized (16).

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