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Electrical Field Stimulation Causes Oxidation of Exogenous Histamine in Krebs–Henseleit Buffer: A Potential Source of Error in Studies of Isolated Airways

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Electric field stimulation (EFS) relaxes human histamine-precontracted airways in vitro. This relaxation is only partly neurally mediated. Nonneural relaxation has been also shown in blood vessels and is due to the generation of oxygen radicals by EFS. In isolated airways the origin of the nonneural component of the relaxation is not clear. Because exogenous catecholamines are oxidized during EFS of carbogenated Krebs–Henseleit (K-H) buffer, we questioned whether this is also the case for exogenous histamine. Human airways precontracted with histamine or methacholine were exposed to either EFS-stimulated carbogenated K-H buffer that also contained histamine or methacholine or unstimulated buffer. Airways exposed to EFS-stimulated buffer that contained histamine relaxed, whereas airways exposed to buffer containing methacholine or exposed to unstimulated buffer did not. It appeared that the histamine concentrations in the organ baths decreased during 30 min of EFS. This decrease was significantly reduced in the presence of ascorbic acid. We conclude that EFS causes oxidation of histamine in carbogenated K-H buffer, and this may at least partly explain the nonneural component of EFS-induced relaxations of precontracted human isolated airways. Therefore, histamine should not be used to induce precontraction in EFS experiments.

Keywords: Electric field stimulation; Nonadrenergic noncholinergic response; Human airways; Histamine

Introduction

Electric field stimulation (EFS) of human isolated airways, precontracted with histamine, causes a neurally mediated cholinergic contraction followed by an inhibitory nonadrenergic noncholinergic (i-NANC) response which is largely insensitive to the nervous conductance blocker tetrodotoxin (TTX) (Taylor et al., 1985; De Jongste et al., 1987a). The mechanism of this TTX-insensitive component of EFS-induced relaxations is not clear. Nonneural relaxation in response to EFS has been also shown in isolated blood vessels and appears to be due to the generation of oxygen radicals during stimulation of the buffer solution bathing the isolated tissue (Lamb and Webb, 1984; Greenberg et al., 1986). It can be hypothesized, therefore, that the TTX-insensitive component of the i-NANC response is due to the generation of oxygen radicals relaxing the airway preparation. Isolated human airways precontracted by methacholine, however, were not relaxed by EFS-stimulated Krebs–Henseleit (K-H) buffer (Jongejan et al., 1989). Furthermore, only a small TTX-insensitive component of i-NANC response was seen when EFS was performed on human airways at resting tone (De Jongste et al., 1987b; Belvisi et al., 1992).

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Since it has been shown that catecholamines can be oxidized by EFS (Wyse, 1977; Lamb and Webb, 1984), we questioned whether this may also occur with histamine. Therefore, we studied the effect of replacement of the organ bath fluid of histamine- or methacholine-precontracted airways by EFS-stimulated K-H buffer with histamine or methacholine. In addition, we measured the histamine concentration in the organ baths during EFS.

Methods

Human lung tissue was obtained from four patients (mean age: 62, range: 49–78 years) who underwent a thoracotomy for bronchial carcinoma. Transverse strips of central bronchi (3rd to 5th generation) were dissected and studied at 37°C in 10 mL siliconized double-jacketed organ baths containing carbogenated (95% O_2, 5% CO_2) K-H buffer (composition in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 5.55). Contractile responses were measured with isotonic transducers (type 3810/60, Penny & Giles Instrumentation Ltd., Christchurch, UK) against a load of 500 mg. Preparations were equilibrated with histamine (4×10^{-6} M, $n = 4$) or methacholine (10^{-5} M, $n = 4$) to obtain a submaximal stable contraction. In separate organ baths without bronchi, carbogenated K-H buffer of 37°C containing histamine or methacholine was exposed to EFS (50V, 1-30 Hz, 0.3 ms, rectangular pulses of alternating polarity). EFS was applied via two platinum plate electrodes (40 × 5 mm, distance 15 mm) by a custom-made high-power tissue stimulator. Organ baths containing K-H buffer with histamine or methacholine not exposed to EFS served as a control. After a stable contraction level was obtained, transfer experiments were done where the buffer in the organ baths with an airway preparation was replaced by either the EFS-stimulated buffer or the unstimulated buffer. After the experiments, the airways were fully relaxed by adding L-isoproterenol (10^{-4} M) and EDTA (4×10^{-3} M) to the organ baths. Because we found that only the transfer of prestimulated buffer that contained histamine relaxed precontracted bronchi, we performed additional experiments to evaluate the effects of EFS on histamine-containing buffer.

Carbogenated K-H buffer with histamine (4×10^{-6} M) was either stimulated with continuous EFS (50 V, 2 Hz, 0.3 ms) for 30 min or exposed to EFS of stepwise (every 5 min) increasing frequency (1, 2, 5, 10, 20, and 30 Hz). In order to assess a possible role of oxygen radicals, we added ascorbic acid (5×10^{-4} M), a non-specific oxygen radical scavenger, to the organ baths prior to EFS. Samples of the organ bath fluid were taken at 5-min intervals, and histamine concentration was measured by an automated fluorometric assay (Technicon Inc., Tarrytown, NY; Siraganian, 1974).

The following drugs were used: methacholine hydrobromide (Janssen Pharmaceutica, Beerse, Belgium), histamine dihydrochloride (Janssen), L-isoproterenol (Janssen), EDTA (Sigma Chemical Co., St. Louis, MO), and L-ascorbic acid (Sigma). The results of the transfer experiments with prestimulated buffer containing histamine or methacholine and unstimulated buffer containing histamine or methacholine were compared by Student's t test for paired data (two-tailed, $\alpha = 0.05$). Analysis of variance (ANOVA) was applied to test the difference between histamine concentration in organ baths with and without ascorbic acid. All data are expressed as mean ± SEM.

Results

All airway preparations contracted to histamine or methacholine and reached a stable contraction plateau that was maintained during at least 1 hr. Figure 1 shows a typical tracing of a transfer experiment. Transfer of prestimulated buffer with histamine relaxed the histamine-precontracted airway preparations to 19.6% ± 1.4% of their initial contraction. The addition of isoproterenol and EDTA resulted in further relaxation, indicating that the preparations were not fully relaxed after the transfer of prestimulated buffer but retained some basal tone. In contrast, prestimulated buffer with methacholine or unstimulated buffer with either methacholine or histamine did not relax precontracted airways ($n = 4$, t test: $p < 0.05$ compared to prestimulated buffer with histamine). The histamine concentration in the EFS-stimulated organ baths decreased both in time and with increasing EFS frequency (Figures 2 and 3). This decrease was largely and significantly prevented in the presence of ascorbic acid (ANOVA: $p < 0.005$, Figure 3). In the control experiments, where no EFS was applied, the histamine concentration was stable (Figure 2).

Discussion

Our experiments show that transfer of histamine-containing, electrically stimulated carbogenated K-H buffer relaxes histamine-precontracted airways, whereas transfer of unstimulated histamine-containing buffer and stimulated buffer with methacholine did not. The histamine concentration in the stimulated organ baths decreased both in time and with increasing EFS frequency. This decrease in histamine concentration was partly prevented by ascorbic acid indicating that EFS causes oxidation of histamine via generation of activated oxygen molecules. Because stimulated buffer with methacholine did not relax precontracted air-

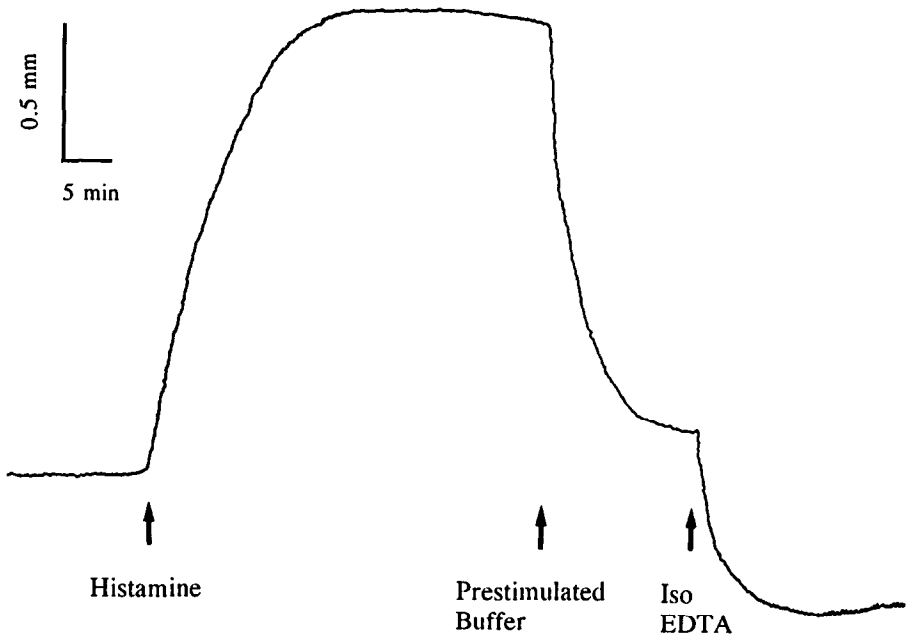


Figure 1. Recording of a typical response of histamine-precontracted human bronchus to prestimulated K-H buffer containing histamine. At the points indicated by the arrows histamine (4×10^{-6} M), prestimulated K-H buffer, isoprenaline (10^{-4} M), and EDTA (4×10^{-3} M) were added to the organ bath.

ways, oxygen radicals generated by EFS do not have a direct relaxing effect. These findings suggests that the EFS-induced nonneural component of the i-NANC relaxation in histamine-precontracted human airways (De Jongste et al., 1987a; Taylor et al., 1985) is mainly due to oxidation of histamine.

The degree of histamine breakdown will probably depend on the method of EFS. We used plate electrodes with a relatively large surface (200 mm^2), and it is to be expected that the use of smaller electrodes will cause smaller artifacts due to histamine oxidation. Likewise, the power of the pulse generator will be important. Our tissue stimulator produces rectangular pulses of 0.3 ms, and maintains an actual 50-V potential difference over the 15-mm distance between the electrodes (De Jongste et al., 1987b). Most commercially available tissue stimulators are less powerful, and will deliver a much lower voltage in an organ bath, despite their specifications. Notwithstanding, the effect of EFS on histamine remains a potential source of error that should be avoided, and that may have biased a number of published studies. Instead of histamine,

Figure 2. Histamine concentration in organ baths either exposed to continuous EFS (50 V, 2 Hz, 0.3 ms) for 30 min (closed triangles) or not exposed to EFS (open circles). Horizontal axis displays time (min), vertical axis represents the histamine concentration (ng/mL) in the organ baths. Values are mean ± SEM; $n = 3$.

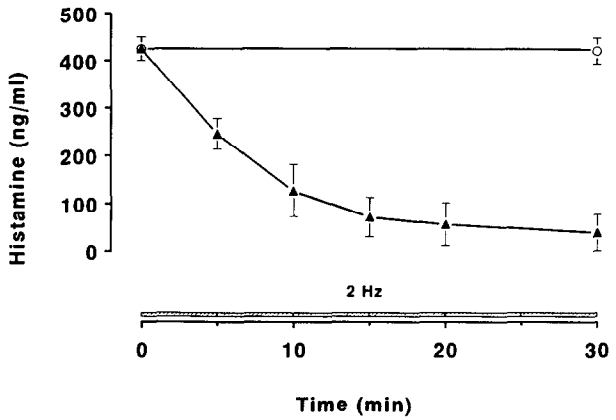
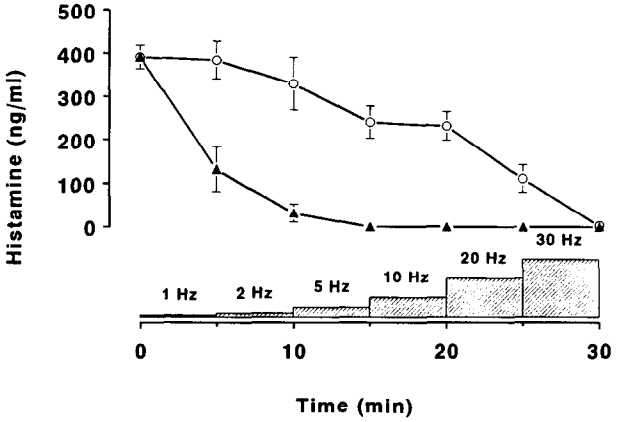


Figure 3. Histamine concentration in organ baths exposed to EFS of stepwise increasing frequency (1–30 Hz) in the absence (closed triangles) and presence of ascorbic acid (5×10^{-4} M, open circles). Horizontal axis displays time (min), vertical axis represents the histamine concentration (ng/mL) in the organ baths. Values are mean ± SEM; $n = 3$.



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methacholine could be used, although this would preclude the use of cholinergic blocking agents. Serotonin should probably also be avoided since this drug may be oxidized during EFS as well (Greenberg et al., 1986).

We conclude that exogenous histamine added to organ baths containing carbogenated K-H buffer is oxidized during EFS. This phenomenon may largely explain the nonneural component of EFS-induced relaxation of histamine-precontracted human airways. Therefore, histamine should not be used to induce tone in isolated airway preparations prior to EFS.

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