Superoxide Anion Generation during Airway Anaphylaxis

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Abstract. Extracellular release of superoxide anion (O$_2^-$) during in vitro anaphylaxis in guinea pig trachealis was found to exhibit both a time- and a concentration-dependent generation of O$_2^-$. Trachealis O$_2^-$ release was unaffected by treatment with diphenhydramine HCl (10$^{-5}$ M, 10$^{-6}$ M). While indomethacin (10$^{-5}$ M) augmented anaphylaxis tension it did not enhance O$_2^-$ release. However, the semiselective SRS-A antagonist FPL 55712 (10$^{-5}$ M) resulted in essentially complete inhibition of O$_2^-$ generation. These data indicate that O$_2^-$ generation occurring during acute airway anaphylaxis is associated with the activation of SRS-A products.

Introduction

Leukotrienes (LT) are highly potent bronchoconstrictors in man and guinea pig and appear to be important putative mediators of immunologic anaphylaxis [1, 2]. Recent work from this laboratory indicated that LTD$_4$-induced histamine hyperreactivity in guinea pig trachealis in vitro was inhibited by superoxide dismutase (SOD), implying a role for toxic oxygen radicals in this interaction [3, 4]. Since exogenous LTD$_4$ was found to concurrently cause the extracellular release of superoxide anion (O$_2^-$) we sought to determine whether anaphylaxis (ANA) was also associated with O$_2$- generation in guinea pig trachealis.

Materials and Methods

Male Hartley guinea pigs weighing 400-500 g were sacrificed with pentobarbital sodium (500 mg/kg). The trachealis was rapidly excised, cut into rings, and isometric tension responses obtained after equilibration at a resting tension of 2.0 g for 60 min at 37°C in Krebs-Henseleit (KH) buffer gassed with 95% O$_2$-5% CO$_2$; full details of isometric tension methods and KH composition have been recently detailed [5]. Passive in vitro sensitization (Schult-Dale reaction) was accomplished as follows: each trachealis ring was placed in a 6.0-ml plastic tube containing a final total volume of 1.5 ml KH buffer. 0.02 ml of normal saline reconstituted rabbit anti-chicken egg albumin antiserum (ICN, Cleveland, Ohio) was added for 20 min at 37°C, then excess antibody was eluted with multiple fresh KH washes. ANA was initiated with 0.1 ml antigen (14.7% N$_2$), 0.1-1,000 µg/ml final bath concentration. O$_2^-$ trachealis release was assayed at 37°C by a modification of the superoxide dismutase-inhibitable reduction of ferricytochrome-C at 550 nm [6, 7]. The reaction mixture consisted of one tracheal ring (mean wet weight 13 mg), 120 nM ferricytochrome-C, antiserum and ovalbumin with the anaphylactic effluent assayed at time points specified in the text. All samples were assayed in duplicate in the presence or absence of bovine, erythrocytic SOD (300 U/ml), in room air. For inhibition studies, FPL 55712 (10$^{-5}$ M) (gift of Hoffmann-La Roche, Nutley, N.J.) or diphenhydramine HCl (10$^{-5}$ M, 10$^{-6}$ M) was incubated 30 min prior to inducing ANA with ovalbumin; the effect of indomethacin (10$^{-5}$ M) (Sigma Chemical Co., St. Louis, Mo.) was tested similarly. After the appropriate time periods, the reaction was terminated by immersion of the reaction tubes in an ice bath, the trachea removed and rapidly frozen at -4°C for subsequent creatine phosphokinase (CPK) assay. The remaining ferricytochrome-KH buffer was centrifuged at 1,200 g for 5 min at 4°C and the supernant then assayed for cytochrome-C production in a Beckman DV spectrophotometer at 550 nm. The OD values were converted to nmol O$_2^-$ using a molar extinction coefficient of 2.1 x 10$^4$ cm$^{-1}$/M. O$_2^-$ production was calculated as the difference between the amount of ferricytochrome-C reduction obtained between parallel samples in the presence or absence of SOD. Appropriate blanks and controls in the presence of ferricytochrome-C but without the addition of the stimulating agent were examined in parallel with the experimental samples. Neither antigen ovalbumin, antibody, SOD nor trachealis alone affected this O$_2^-$ assay. The remaining trachealis was analyzed colorimetrically for muscle CPK activity by creatine formation in a CPK-catalyzed ADP/phosphocreatine reaction using Sigma kit No. 520 (Sigma). Final trachealis superoxide generation is expressed as nM O$_2^-$/CPK unit x 10$^3$ over specified reaction times. Differences between observations were analyzed by Student's t test for grouped data with p < 0.05 taken as significant; results are expressed as mean ± SEM.

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$^2$ Note that the term 'trachealis' is used as short form for 'smooth tracheal muscle'.

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Table 1. Effect of diphenhydramine and FPL 55712 on O2 generation

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>nM O2/CPK × 10^-2 (30 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control ANA</td>
<td>5</td>
<td>4.85 ± 0.29</td>
</tr>
<tr>
<td>Diphenhydramine HCl, M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-3</td>
<td>4</td>
<td>4.15 ± 0.59</td>
</tr>
<tr>
<td>10^-6</td>
<td>4</td>
<td>4.45 ± 0.10</td>
</tr>
<tr>
<td>FPL 55712, M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^-5</td>
<td>4</td>
<td>0.35 ± 0.08</td>
</tr>
</tbody>
</table>

1. Over 30 min reaction time.
2. Not significant (p > 0.7), compared to control ANA.
3. Significant (p < 0.001), compared to control ANA.

O2/CPK × 10^-2 at 30 min and whose EC50 was 2.5 x 10^-8 M [4]. SOD (300 U/ml) had no effect on ANA tension development.

The extracellular release of O2 over the time course of ANA reveals trace amounts generated in the first few minutes of ANA, becoming maximal at 30 min (Fig. 2). In these time studies an ovalbumin concentration of 100 µg/ml was employed.

These observations imply an effect by SRS-A components, rather than histamine, upon O2 release. To evaluate this further, the specific inhibitor diphenhydramine (10^-3 M, 10^-6 M) and the semisynthetic inhibitor FPL 55712 (10^-5 M) were tested for their action on O2 release. The experimental conditions were antigen ovalbumin of 100 µg/ml and measured at 30 min of ANA. At these cited concentrations, ANA tension was inhibited by approximately 80% including the initial rapid phase by diphenhydramine and late tonic phase by FPL 55712 respectively. As shown in Table 1, diphenhydramine (10^-5 M) did not inhibit O2 generation (p > 0.7). In contrast, FPL 55712 reduced the maximal O2 release by 93%, from 4.85 to 0.35 nM O2/CPK × 10^-2 at 30 min of ANA (p < 0.001).

Indomethacin (10^-5 M) increased ANA isometric tension by 26%, measured at 45 min of ANA; ANA = 1,000 ± 55 mg vs. ANA + indomethacin = 1,260 ± 56 mg, n = 9, p < 0.01. However, indomethacin (10^-5 M) had no effect upon O2 release in the tracheal preparation: ANA = 4.65 ± 0.33 vs. ANA with indomethacin = 4.48 ± 0.32 nM O2/CPK unit × 10^-2 (n = 12, p > 0.7), assayed at 30 min.

Results

A typical anaphylactic contracture consists of an initial rapid phase (5 min) and a prolonged tonic phase of 90 min, with maximal tension occurring at approximately 45 min; maximal ANA tension was 1,200 ± 130 mg. As depicted in figure 1, an ovalbumin concentration-O2 response was observed from 0.1 to 1,000 µg/ml and with an EC50 of 4.5 µg/ml. The maximal O2 extracellular release was 4.65 nM/CPK × 10^-2 after 30 min of ANA. This is one-third of the maximum release observed with synthetic LTD4 (10^-6 M, maximal concentration) of 15.1 ± 1.1 nM O2/CPK × 10^-2 at 30 min and whose EC50 was 2.5 x 10^-8 M [4]. SOD (300 U/ml) had no effect on ANA tension development.

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Discussion

In vitro ANA in guinea pig trachealis was found to be associated with extracellular generation of $O_2^-$. Since the reaction assay mixture contains SOD we can infer the majority of released free radical oxidants was $O_2^-$ with a minimal component of $H_2O_2$. As we have observed with synthetic LTD$_4$, $O_2^-$ release occurred only in the late tonic phase of ANA indicating the association of SRS-A derivatives with toxic $O_2^-$ product release. There appears to be little role for histamine in this process of free radical generation [4]. This finding was substantiated by inhibition of $O_2^-$ formation with FPL 55712, a semiselective SRS-A antagonist, and not by diphenhydramine. The cellular origins of $O_2^-$ released during ANA in this trachealis preparation are not defined in this present study; the preparation does contain neutrophils, mast cells and smooth muscle elements, among others. While LT$_B$ has been reported to amplify the neutrophilic (PMN) release of oxygen metabolites, there are currently no reports of $O_2^-$ release in trachealis preparations as we have observed. For example, Gay et al. [6] have reported enhancement of chemotactic-factor stimulated PMN-oxidative metabolism by LT$_B$. PMNs incubated with $10^{-9}$ M LT$_B$ and FMLP (N-formyl-methionyl-leucyl-phenylalanine) released 17.7 nM $O_2^-$/1.5 x 10$^6$ PMN. Curnutte et al. [8] observed cis-un-saturated fatty acids (82 yAM arachidonate) stimulation of $O_2^-$ release at a rate of 105 nM $O_2^-$/min/10$^7$ human PMN. Rat peritoneal mast cells histamine release by PMN-activated neutrophils is reported to be dependent upon oxidative metabolites [9]. Finally, immunologic stimulation of rat peritoneal mast cells and human lung mast cells were shown to generate $O_2^-$, the release of which paralleled the release of histamine [10]. Hence, PMN and/or mast cells appear the most likely source of $O_2^-$ in our studies of trachealis anaphylaxis. However, differences in cell populations, methods and potency factors preclude quantitative comparisons of $O_2^-$ release among these preparations.

As previously reported, indomethacin enhancement of antigen-induced trachealis isometric tension by cyclooxygenase inhibition was demonstrated in our preparation [11]. However, since no increased release of $O_2^-$ occurred, it appears there is no role for $O_2^-$ in the enhancement of anaphylactic contractures by indomethacin.

We have previously reported that exogenous LTD$_4$ activates oxygen-derived free radicals which interact to induce an acquired hyperreactivity to agonist histamine in trachealis smooth muscle [4]. The present studies of ANA indicate that immunogenic activation of SRS-A products, e.g. LT, also generate $O_2^-$ in this guinea pig trachealis preparation which may also influence airways smooth muscle reactivity.

References


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