PARTIAL SPECTRAL ANALYSIS OF THE HYDROPEROXIDE-INDUCED CHEMILUMINESCENCE OF THE PERFUSED LUNG

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1. Introduction

Evaluation of the oxidative stress in the intact organ can be approached with different experimental techniques such as glutathione release [1–3], hydrocarbons expiration [4–6] and chemiluminescence. Low level chemiluminescence of the intact organ permits an assessment of the oxidative stress that does not involve an invasion or destruction of the tissue and establishes a great advantage over the thiobarbituric acid assay (malonaldehyde) [7] as indicated [8].

The detection of chemiluminescence from the liver in situ was reported in 1961 [9] and therefrom an extraordinary development has been made in chemiluminescence applied to organs, organelles and model systems.

A low level chemiluminescence from the in vivo and in vitro liver was reported [10,11], rekindling great interest in the subject. Light emission from tissue homogenates [12,13], mitochondria [14,15], and microsomal fractions [16,17], as well as model systems [18,19] helps to build up a possible mechanism for the chemiluminescence of the intact organ that per se presents a great difficulty.

We have reported on the hydroperoxide-induced chemiluminescence of the perfused lung [20] emphasizing the relationship between chemiluminescence and pulmonary edema, as well as the effect of paraquat on light emission. Here, we summarize the dual effect on lung chemiluminescence induced by either H_2O_2 or *t*-butyl hydroperoxide and offer a first approach to the nature of light emission by means of a partial spectral analysis of the lung chemiluminescence.

2. Materials and methods

2.1. *Chemicals*

t-Butyl hydroperoxide (*t*-BOOH) was obtained from Aldrich Chemical Co. (Milwaukee, WI) and H_2O_2 from J. T. Baker, Chem. Co. (Phillipsburg, NJ).

2.2. *Lung perfusion*

The lungs of Sprague-Dawley male rats (Charles River Breeding Labs., Inc., Wilmington, MA) (300–350 g body wt) were isolated by the technique in [21]. Lungs were perfused at a rate of 10 ml/min with a Krebs-Ringer phosphate buffer (pH 7.3–7.4) that was kept at 37°C. The perfusate was not recirculated. Ventilation and perfusion pressures were monitored continuously with pressure transducers (Statham Instr. 123 FD) and recorded with an oscillograph (MFE Corp., Salem, NH). *t*-Butyl hydroperoxide or hydrogen peroxide was infused with a syringe pump, model 341, (Sage Instr., Orion Labs., Cambridge, MA) into a cannula situated in the inflow of the pulmonary artery. The rate of infusion was ~0.3 ml/min.

2.3. *Photon counting*

Chemiluminescence was measured in a photon counter as in [10,11] using an EMI 9658 photomultiplier responsive in the range 300–900 nm connected to a photon counter system [11]. The perfused lung was placed in a special lighttight box as close as possible to the end of the light guide. Kodak wratten filters (Eastman Kodak Co., Rochester, NY) with selective spectral transmittance in the visible or red regions were placed in front of the phototube in

order to obtain a partial spectral distribution of the light observed. The estimated spectra of the hydroperoxide-induced chemiluminescence of the lung were calculated by superimposing the relative chemiluminescence yields (ratio of counts/s with a filter to counts/s without a filter), corrected for the photomultiplier efficiency, and read by a densitometer.

3. Results

3.1. t-*Butyl hydroperoxide- and/or* H_2O_2*-induced chemiluminescence of the lung*

In these experimental conditions, and with a red-sensitive EMI phototube, a background level of about 350 counts/s was observed; the darkcurrent was ~50 counts/s. A total chemiluminescence yield of 745 ± 60 counts/s was observed in the perfused lung upon infusion with H_2O_2 and of 1600 ± 200 counts/s upon infusion with *t*-butyl hydroperoxide.

Fig.1 shows the chemiluminescence of the perfused lung upon the successive infusion of both hydroperoxides. The light emission signal began immediately after the starting of the infusion of H_2O_2, reached its maximum in ~1.5 min, and kept constant at its maximum level as long as the infusion of H_2O_2 was maintained. Light emission decayed slowly to the background upon withdrawal of the H_2O_2. A successive infusion of the tertiary hydroperoxide, *t*-butyl hydroperoxide, produced a different kinetic pattern, increasing the total chemiluminescence yield, but with a slower rate than that of the H_2O_2-enhanced chemiluminescence; a tendency to saturation was observed only after 3 min infusion with *t*-butyl hydroperoxide.

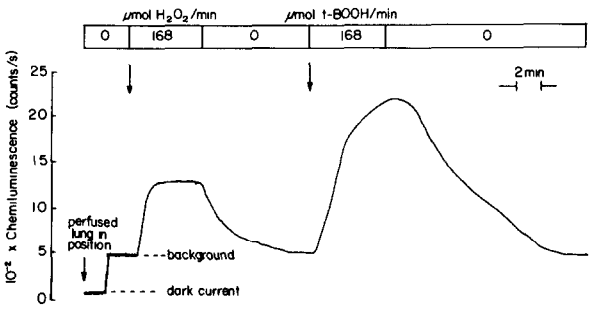

Fig.1. Lung chemiluminescence successively induced by H_2O_2 and *t*-butyl hydroperoxide (*t*-BOOH). The infusion rate of the peroxides was kept constant as indicated in the upper ordinate. Start or withdrawal of the peroxide infusion is indicated by 0 in the ordinate. The rate of lung perfusion was maintained at 10.5 ml/min. Other assay conditions as in section 2.

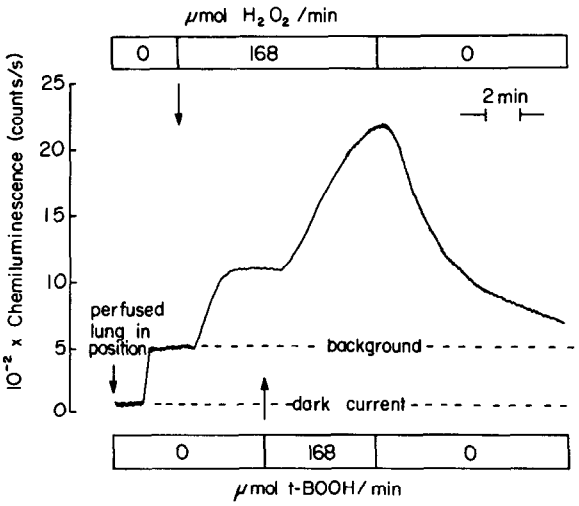

Fig.2. Lung chemiluminescence simultaneously induced by H_2O_2 and *t*-butyl hydroperoxide. Assay conditions as described in fig.1. When the constant level of chemiluminescence of the lung induced by H_2O_2 was achieved, *t*-butyl hydroperoxide infusion was started.

In each experiment, the lungs showed a higher rate of chemiluminescence and a lower chemiluminescence yield with H_2O_2.

The effect of the simultaneous addition of both peroxides on the perfused lung chemiluminescence is shown in fig.2. The infusion of *t*-butyl hydroperoxide was started when the constant level of light emission induced by the H_2O_2 infusion was achieved. Photoemission rose over the H_2O_2 constant level, showing once more a different kinetic pattern of chemiluminescence. When the infusion of both peroxides was halted, the signal decayed slowly and approached the background in ~10–15 min.

3.2. *Partial spectral distribution of lung chemiluminescence*

By means of an interference Wedge filter, in a model system formed by cytochrome *c* and hydroperoxide, we have obtained maximal emission of chemiluminescence at 662 nm and 590 nm with *t*-butyl hydroperoxide and hydrogen peroxide, respectively (E. C. unpublished). The use of an interference filter in organ chemiluminescence is restricted by the low chemiluminescence yield of the system; therefore it was replaced by gelatin filters (Kodak wratten filters) that give a wide, though not exact, approach

to the wavelength of the light emission observed.

The *t*-butyl hydroperoxide-induced chemiluminescence of the perfused lung indicated a chief composition of red light-emitting species with little contribution of green light-emitting species. Fig.3 shows the light emission of the perfused lung when the Wratten filters were placed in front of the photomultiplier; maximal light emission was observed with red filters (no. 29,70) with spectral transmittance beyond 600 and 650 nm (fig.3b,c), and almost negligible light emission was detected beyond 730 nm with a dark red filter (no. 88A) (fig.3d); a photoemissive component with a wavelength between 400 and 600 nm (green filter no. 64) was also small compared with the red species contribution. Fig.3B sums up the spectral distribution of the *t*-butyl hydroperoxide-induced chemiluminescence of the lung and shows that the major contribution is done by species emitting between 650 and 720 nm, with a small band between 600 and 650 nm.

On the other hand, the H_2O_2-enhanced lung chemiluminescence showed some differences from the spectral distribution of the *t*-butyl hydroperoxide-induced chemiluminescence that are shown in fig.4. Fig.4A indicates the direct light emission signal obtained with the superposition of different filters. At variance with the *t*-butyl hydroperoxide-induced chemiluminescence, components beyond 650 nm (red filter 70) are of smaller importance (fig.4e), whereas maximal light emission is obtained with filters with spectral transmittance beyond 520 and 600 nm (filters 16,29) (fig.4b and c, respectively). Furthermore, the H_2O_2-induced chemiluminescence of the lung, more than the *t*-butyl-enhanced chemiluminescence, is associated with components emitting between 400 and 600 nm (filter 64; fig.4d). Fig.4B shows that the lung chemiluminescence obtained upon infusion of H_2O_2 is increasingly associated with components that have an emission wavelength from 520 toward 650 nm.

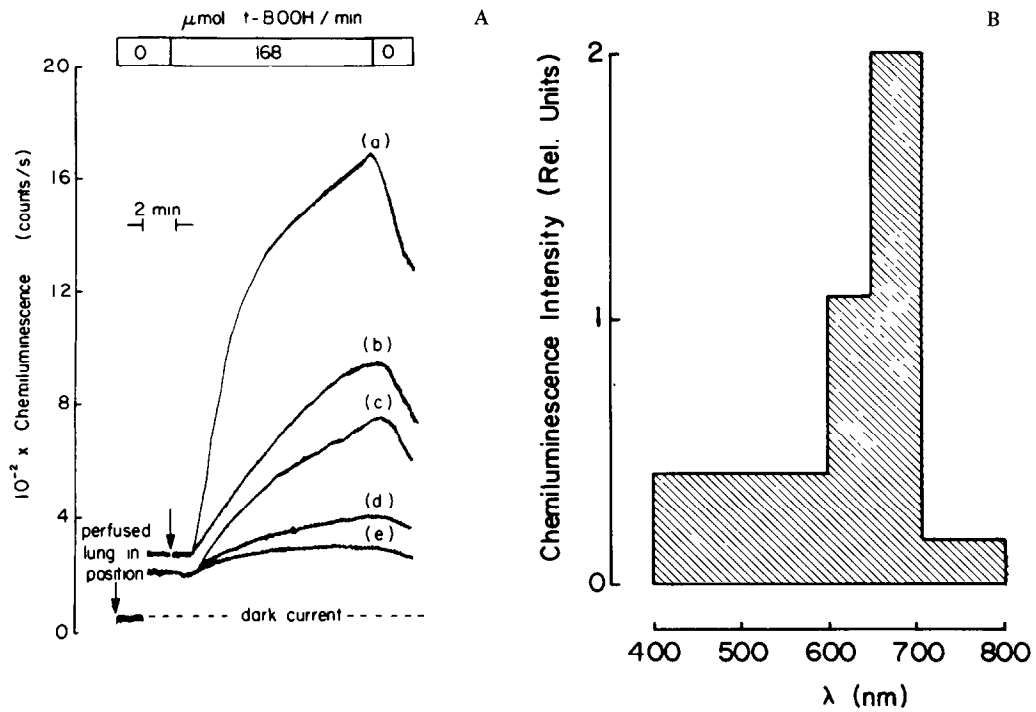

Fig.3. Partial spectral analysis of *t*-butyl hydroperoxide-induced chemiluminescence of the lung. (A) Assay conditions as in fig.1. Kodak wratten filters were placed in front of the EMI phototube: (a) control; (b) filter 29; (c) filter 70; (d) filter 64; (e) filter 88A. Explanations in the text. (B) Diagram illustrating the relative spectral distribution of *t*-butyl hydroperoxide-induced light emission of the lung obtained from the data of (A).

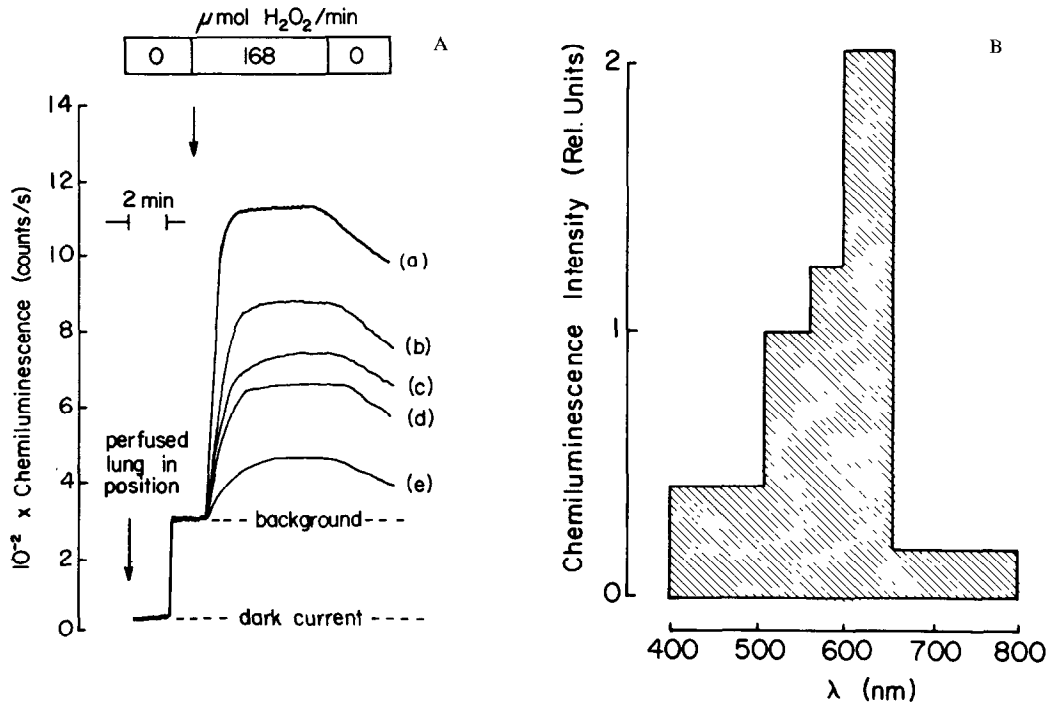

Fig.4. Partial spectral analysis of H_2O_2-induced chemiluminescence of the lung. (A) Assay conditions as in fig.3 and further explanations in the text. Trace: (a) control; (b) filter 16; (c) filter 29, (d) filter 64; (e) filter 70. (B) Diagram illustrating the relative spectral distribution of the H_2O_2-induced photoemission of the lung obtained from the data of (A).

4. Discussion

The different kinetic pattern of chemiluminescence of the lung obtained with the successive infusion of H_2O_2 and *t*-butyl hydroperoxide, along with the *t*-butyl hydroperoxide-enhancement of chemiluminescence over the already saturated light emission caused by H_2O_2, suggest the participation of different enzymatic activities for the metabolism of each peroxide. Hydroperoxides, either added or formed through a biologically iron-catalysed reaction, provide a chemiluminescence model reaction related to lipid peroxidation comprising certain enzymic activities such as glutathione peroxidase. Tertiary hydroperoxides, such as *t*-butyl hydroperoxide, are commonly used when measuring the glutathione peroxidase participation in oxidative metabolism in order to avoid any possible involvement of catalase [22]. Apparently, the different light emission signals reported here might be due to activities of catalase and glutathione peroxidase for H_2O_2 and *t*-butyl hydroperoxide. We want to hint at this phenomenon, but do realize the necessity of obtaining further evidence such as might be provided by the effects of lungs of rats fed a selenium-deficient diet on the hydroperoxide-induced chemiluminescence.

Most of the evidence on the nature of organ light emission will rest on data obtained from organelles and model systems, where a mechanism is easier to elucidate; this report, however, deals with the fact of a recognizable wavelength band, which permits certain approaches to the nature of the photoemission observed. Basically, singlet molecular oxygen is involved with chemiluminescence directly or indirectly. Its generation along with its participation in the light observed is stated in scheme 1. The two bands observed in the *t*-butyl hydroperoxide-stimulated chemiluminescence of the lung cover the 634 and 703 nm peaks of the singlet oxygen dimol emission when decaying to the ground state [23,24]; on the other hand, the participation of different light-emitting species, such as excited carbonyl groups, through a dioxetane mechanism [25], seems more feasible in the H_2O_2-induced light emission of the

A GENERATION OF $^1O_2$

Termination reactions of peroxy radicals

1) Primary and secondary peroxy radicals

$$ROO^{\cdot} + ROO^{\cdot} \longrightarrow {}^1O_2 + ROH + RO$$

2) Tertiary peroxy radicals

$$tBOO^{\cdot} + tBOO^{\cdot} \longrightarrow {}^1O_2 + tBOOBt$$

B EMISSION OF LIGHT

3) Monomol emission (1260, 1430 nm)

$$^1O_2 \longrightarrow {}^3O_2 + h\nu$$

4) Dimol emission (634,703 nm)

$$2\,{}^1O_2 \longrightarrow 2\,{}^3O_2 + h\nu$$

5) Dioxetane mechanism (350-480nm)

a) $^1O_2$ + >C=C< → >C(-O-O-)C< → >C=O + >C=O*

b) >C=O* → >C=O + $h\nu$

Scheme 1

lung, since it shows a stronger involvement of green light-emitting species.

It is worth noting that, because of the heterogeneity of the system studied, the wavelengths of the singlet oxygen dimol emission could be shifted; systems which are known to produce singlet oxygen, such as the $HClO/NH_3/H_2O_2$ system, were reported to have their spectral distribution of chemiluminescence shifted toward shorter wavelengths in the presence of certain amino acids [26]; moreover, the myeloperoxidase system and phagocytosing polymorphonuclear leukocytes show a maximal chemiluminescence emission at ~580 nm [27] in spite of the strong evidence supporting their generation of singlet oxygen [28].

At present, the utilization, in the perfused lung, of either quenchers of singlet oxygen, such as β-carotene [29] or enhancers of the singlet oxygen dimol emission, such as 1,4-diazabicyclo-[2,2,2]-octane [30] is very limited.

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References

[1] Oshino, N. and Chance, B. (1977) Biochem. J. 162, 509–525.
[2] Sies, H., Koch, E., Martino, E. and Boveris, A. (1979) FEBS Lett. 103, 287–290.
[3] Bartoli, G. M. and Sies, H. (1978) FEBS Lett. 86, 89–91.
[4] Sagai, M. and Tappel, A. L. (1979) Toxicology in press.
[5] Riely, C. A. and Cohen, G. (1974) Science 193, 208–210.
[6] Kappus, H., Koster, U., Koster-Albretch, D., Kieczka, H. and Remmer, H. (1978) in: Functions of glutathione in liver and kidney (Sies, H. and Wendel, A. eds) pp. 176–182, Spring-Verlag, Berlin, New York.
[7] Comporti, M., Benedetti, A. and Chieli, E. (1973) Lipids 8, 498–502.
[8] Noguchi, T. and Nakano, M. (1974) Biochim. Biophys. Acta 368, 446–455.
[9] Tarusov, B. N., Polidova, A. I. and Zhuravlev, A. I. (1961) Radiobiologiya 1, 150–151.
[10] Boveris, A., Chance, B., Filipkowski, M., Nakase, Y. and Paul, K. G. (1978) in: Frontiers of Biological Energetics, vol. 2, Electron to Tissues (Scarpa, A. et al. eds) pp. 975–984, Academic Press, New York.
[11] Boveris, A., Cadenas, E., Reiter, R., Filipkowski, M. and Chance, B. (1980) Proc. Natl. Acad. Sci. USA 77, in press.
[12] Di Luzio, N. R. and Stege, T. E. (1977) Life Sci. 21, 1457–1464.
[13] Di Luzio, N. R. and Hartman, A. D. (1967) Fed. Proc. FASEB 26, 1436–1442.
[14] Stauff, J. and Ostrowski, J. (1967) Z. Naturforschg. 22b, 734–740.
[15] Suslova, T. B., Olenev, V. I. and Vladimirov, Yu. A. (1969) Biophysics (Moscow) 14, 540–548.
[16] Howes, R. M. and Steele, R. H. (1971) Res. Commun. Chem. Pathol. Pharmacol. 2, 619–626.
[17] Nakano, M., Noguchi, T., Sugioka, K., Fukuyama, H., Sato, M., Shimizu, Y. Tsuji, Y. and Inaba, H. (1975) J. Biol. Chem. 250, 2404–2406.
[18] Sugioka, K. and Nakano, M. (1976) Biochim. Biophys. Acta 423, 203–216.
[19] Cadenas, E., Boveris, A. and Chance, B. (1980) Biochem. J. 186, in press.
[20] Cadenas, E., Arad, I. D., Fisher, A. B., Boveris, A. and Chance, B. (1980) Biochem. J. in press.
[21] Basset, D. J. P. and Fisher, A. B. (1978) J. Appl. Physiol. 45, 943–949.
[22] Sies, H., Gertsenecker, C., Menzel, H. and Flohe, L. (1972) FEBS Lett 27, 171–175.
[23] Khan, A. V. and Kasha, M. (1963) J. Chem. Phys. 39, 2105–2106.
[24] Seliger, H. H. (1974) J. Chem. Phys. 40, 3133–3134.
[25] Foote, C. S. (1976) in: Free Radicals in Biology (Pryor, W. A. ed) vol. 2, pp. 85–133, Academic Press, New York.
[26] Andersen, B. R., Lint, T. F. and Bendzel, A. M. (1978) Biochim. Biophys. Acta 542, 527–536.

[27] Andersen, B. R., Bendzel, A. M. and Lint, T. F. (1976) Infec. Immunol. 17, 62–66.
[28] Rosen, H. and Klebanoff, S. J. (1976) Fed. Proc. FASEB 35, 1931.
[29] Foote, C. S. and Denny, R. W. (1968) J. Am. Chem. Soc. 90, 6233–6235.
[30] Deneke, C. F. and Krinsky, N. I. (1977) Photochem. Photobiol. 25, 299–304.