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Enhancing effect of carazolol on chemiluminescence accompanying decomposition of hydrogen peroxide in the presence of copper ions

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The aim of this study was to investigate the effect of carazolol on the generation of the oxygen species with respect to chemiluminescence (CL) from the copper ion/H_2O_2 system. The reactions are carried out in buffered aqueous solution. The quantum yield of the CL from the copper ion/H_2O_2 system is increased by a factor of 2^{-10} in the presence of carazolol. The CL spectra from the carazolol/copper ion/H_2O_2 system are spread through the full visible region and four emission bands are shown at maxima 480–500, 580, 640 and 700 nm. The excitation spectrum obtained from the above mentioned system shows the main maxima at around 273, 310 and 340 nm ($\lambda_{obs} = 365$ nm). The fluorescence emission spectrum exhibits only one maximum at 365 nm ($\lambda_{exc} = 340$ nm) corresponding to carazolol. The light emission decreases in the presence of several biologically important compounds which are typical scavengers of oxygen radicals and singlet oxygen ($^1O_2$). The results demonstrate that carazolol enhances the production of oxygen radicals and $^1O_2$ under our experimental conditions. Generation of $^1O_2$ was additionally confirmed using ESR spin trapping and spectrophotometry.

1. Introduction

Carazolol is a beta-2-adrenoceptor antagonist which is clinically used in the treatment of hypertension, cardiac arrhythmias and angina pectoris [1–3]. Mejean et al. [4] recently showed that carazolol also is a potent selective beta 3-adrenoceptor agonist.

There is a growing awareness of the role of reactive oxygen species like superoxide free radical ($O_2^{\cdot-}$), hydroxyl radical ($HO^{\cdot}$), hydrogen peroxide (H_2O_2) and singlet oxygen (the lowest excited state of molecular oxygen, $^1O_2$) in etiology of several human diseases because of their great destructive effect [5, 6]. Imbalance between production of oxygen species and protection against their overabundance can lead to the interaction of these species at the nuclear level that may cause carcinogenesis and cellular injury. Among several sources generating toxic oxygen species in biological systems are the breakdown of H_2O_2 catalyzed by trace metal contaminants, and interaction between $O_2^{\cdot-}$ and H_2O_2 [7].

Since free radical-mediated damage is often suggested to be an important mechanism in the cause of heart failure [8], it seems interesting to check the behaviour of carazolol in a system generating the reactive oxygen species.

The aim of the present study was to investigate the effect of carazolol on the oxygen species generation with respect to chemiluminescence (CL) produced from the Cu(II)/H_2O_2/buffer system.

2. Investigations, results and discussion

The interaction between Cu(I) and H_2O_2 generates HO radicals via the following reaction:

$$Cu(I) + H_2O_2 \rightarrow Cu(II) + HO^{\cdot} + HO^- \qquad (1)$$

The simultaneous presence of the redox metal and molecular oxygen leads to $O_2^{\cdot-}$ formation:

$$Cu(I) + O_2 \rightarrow Cu(II) + O_2^{\cdot-} \qquad (2)$$

Further, an interaction between $O_2^{\cdot-}$ and H_2O_2, in the presence of redox metals in basic media, generates chemiluminescence by Haber-Weiss type of reaction [9]:

$$O_2^{\cdot-} + H_2O_2 \rightarrow HO^{\cdot} + HO^- + {}^1O_2 \qquad (3)$$

Several reducing agents (AH_2) such as catecholamines or ascorbate are capable of driving $HO^{\cdot}$ formation from reaction (1) transforming Cu(II) into Cu(I) as follows [10, 11]:

$$Cu(II) + AH_2 \rightarrow Cu(I) + AH^{\cdot} + H^+ \qquad (4)$$

Van Dyke et al. [10] reported that the yield of the $HO^{\cdot}$ formation in reactions (1) and (3) depends on the buffer effects. Accordingly, it was important to check the oxygen species generation in the presence of different buffers (Fig. 1). Of the buffers tested, carbonate was found to be the best choice for measuring oxygen species formation under our experimental conditions. As shown in Fig. 1 the addition of carazolol enhances the light emission in the tested buffers.

Direct evidence for the emitters of observed light emission can be obtained by measuring the fluorescence and CL spectra. Fig. 2 (part A) shows the fluorescence excitation spectra (curves 1, 2) and emission spectra (curves 3, 4) obtained from carazolol solution in carbonate buffer and from the carazolol/$CuSO_4$/H_2O_2/buffer reaction system. The excitation spectra exhibit a complicated structure in both cases. The fluorescence emission spectra exhibit only one band with the maximum at 365 nm (λ_{exc} = 280 to 340 nm).

The excitation and emission bands from the reaction mixture rapidly decrease with the reaction time. The rate determining factor of the decrease in the band intensity is the H_2O_2 concentration (Fig. 2 part B). On the contrary, the CL intensity increased with the H_2O_2 concentration. These results indicate that the observed fluorescence is due to $^1S^* \rightarrow S_0$ transition from excited molecules of carazolol.

Table 1: ESR parameters for the formation of nitroxide radicals

Reaction mixture	g	Linewidth (G)	Splitting (G)
$CuSO_4$ + H_2O_2 + buffer + TEMP	2.0065	0.37 ± 0.02	16.2 ± 0.02
Carazolol + $CuSO_4$ + H_2O_2 + buffer +TEMP	2.0064	0.38 ± 0.02	16.0 ± 0.02
TEMPO + buffer	2.0068	0.39 ± 0.02	16.1 ± 0.02

Conditions: 0.25mol/l TEMP, 0.5 mmol/l carazolol, 0.5 mmol/l $CuSO_4$, 0.5 mmol/l H_2O_2, 0.1 mmol/l TEMPO, carbonate buffer, pH 9.3, temperature 294 K.

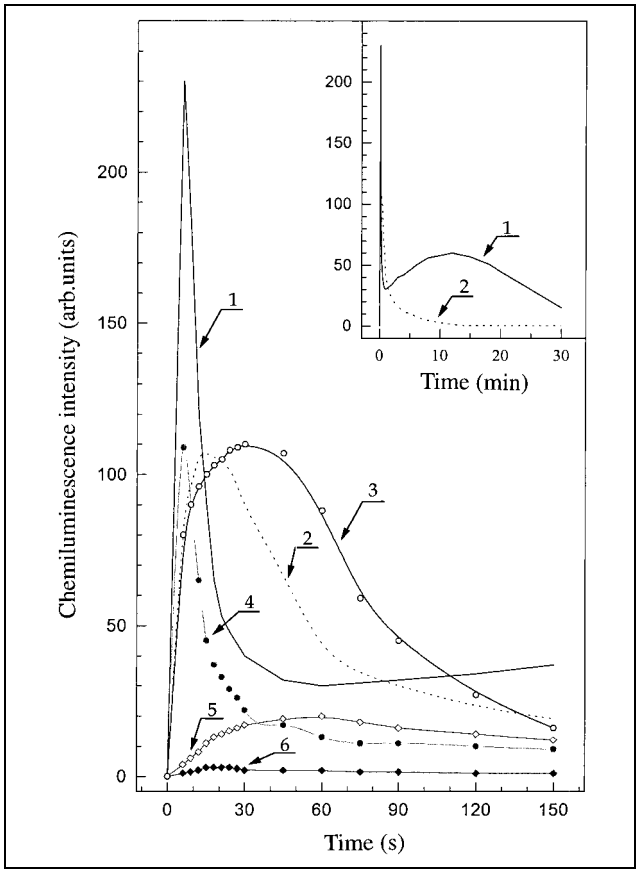

Fig. 1: Effects of buffers and metal ions on CL from the metal ion/H_2O_2/HO^- system (curves 2, 4, 6) and the above mentioned system in the presence of carazolol (curves 1, 3, 5). Curves 1 and 2: $CoCl_2$/EDTA, 0.1 mol/l carbonate buffer pH 9.3; curves 3 and 4: $CuSO_4$, 0.1 mol/l carbonate buffer pH 9.3; curves 5 and 6: $CuSO_4$, 2 mmol/l borate buffer, pH. 9.2. The inset shows curves 1 and 2 measured for t = 30 min.
Concentrations of substrates after mixing: 0.5 mmol/l carazolol, 0.5 mmol/l $CoCl_2$, 0.5 mmol/l EDTA, 0.5 mmol/l $CuSO_4$, 0.5 mmol/l H_2O_2. Temperature 310 K

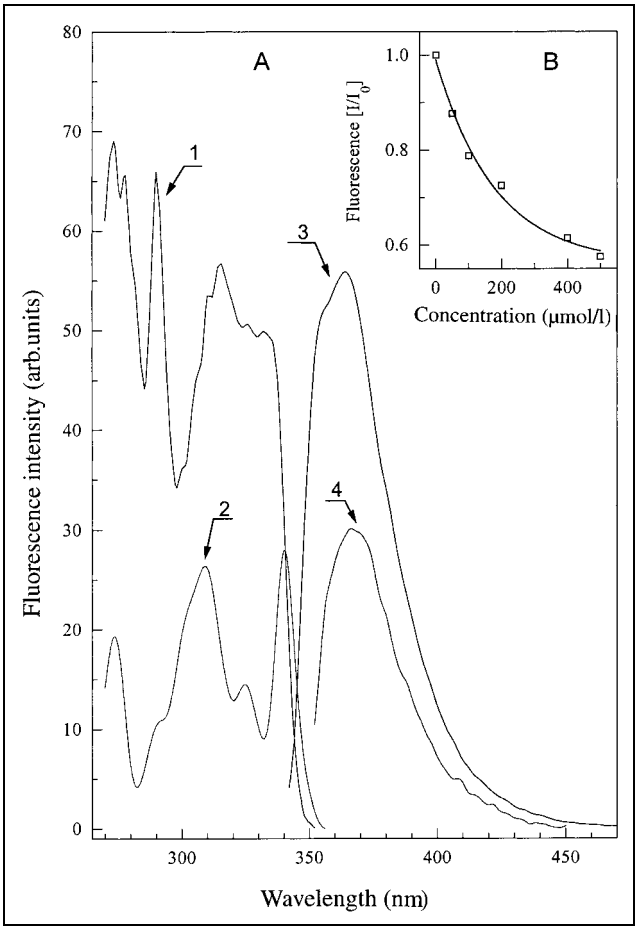

Fig. 2: Part A. Fluorescence excitation (curve 1) and emission (curve 3) spectra of carazolol in the buffer; fluorescence excitation (curve 2) and emission (curve 4) spectra of carazolol in the $CuSO_4$/H_2O_2/buffer system. Conditions: 0.5 mmol/l carazolol, 0.5 mmol/l $CuSO_4$, 0.5 mmol/l H_2O_2, carbonate buffer, pH 9.3; temperature 293 K. Curves 1 and 2 λ_{obs} = 365 nm; curves 3 and 4 λ_{exc} = 340 nm.
Part B. Influence of the H_2O_2 concentration on the fluorescence emission intensity from the carazolol/$CuSO_4$/buffer system. Under the same conditions as in part A

The decrease in the fluorescence emission band intensity was also observed from the drug dissolved in the buffer in the presence of molecular oxygen. A similar decrease of the fluorescence band intensity to that for oxygen-saturated solution was obtained in the air-saturated solution. This could be explained by the presence of larger concentrations of the oxygen in the air-saturated solution (about 0.28 mmol/l) than of the carazolol concentration (0.1 mmol/l). The oxygen quenching of the fluorescene emission spectrum suggests the direct interaction of carazolol with O_2 e.g. the transfer of an electron from the electronically excited states of the drug to O_2 results in O_2^- formation.

The CL spectra from reaction mixtures in the presence and absence of carazolol are shown in Fig. 3. Both spectra are spread through the full visible region and have maxima at around 460, 500, 580, 640 and 700 nm.

As shown in Figs. 2 and 3, the fluorescence emission band does not cover the CL spectrum, thus the CL could be interpreted as neither emission from the excited carazolol nor from excited products of its degradation. This suggestion is confirmed by measurements of the optical absorption spectra of carazolol in the buffer and in the carazolol/$CuSO_4$/H_2O_2/buffer system. The solution of carazolol in the buffer shows absorption with maxima at 220, 235, 285, 320, and 332 nm. In the presence of H_2O_2 and Cu ions the absorbance rapidly decreased with an increase in oxidation time without formation of new bands, as checked in ultraviolet and visible regions (data not shown).

These results suggest that there are two potential emitters of the observed CL: singlet oxygen and excited CO_2. The position of the bands at 580, 640, 700 nm corresponds to the simultaneous transition in O_2-dimoles with vibrational quantum numbers (1,0) for 580 nm, (0,0) for 640 nm and (0,1) for 700 nm of the $2O_2[^1\Delta_g] \rightarrow 2O_2[^3\Sigma_g^-]$ transition. The emission at about 500 nm is the (0,0) transition in the $2O_2[^1\Delta_g, ^1\Sigma_g^+] \rightarrow 2O_2[^3\Sigma_g^-]$ system [12, 13]. The band at 460 nm corresponds to the emission from excited singlet and/or triplet groups of CO_2 formed in the carbonate buffer [14].

When the Fenton type reaction is used as the oxygen species source, the CL should be decreased by addition of O_2^-, $HO^\cdot$ scavengers and $^1O_2$-quenchers [5, 6]. Fig. 4 shows the inhibition of CL by several biologically important antioxidants and $^1O_2$-quenchers. Most of the examined compounds act synergistically, i.e. both as oxygen free radicals-inhibitors and as $^1O_2$-quenchers. For this reason it is difficult to distinguish the kind of oxygen species being removed by the particular scavenger. However, the main function of trolox, captopril, glutathione, cysteine, thiourea, cimetidine, tryptophan, or ascorbic acid is due to their high reactivity with $HO^\cdot$ radicals, while

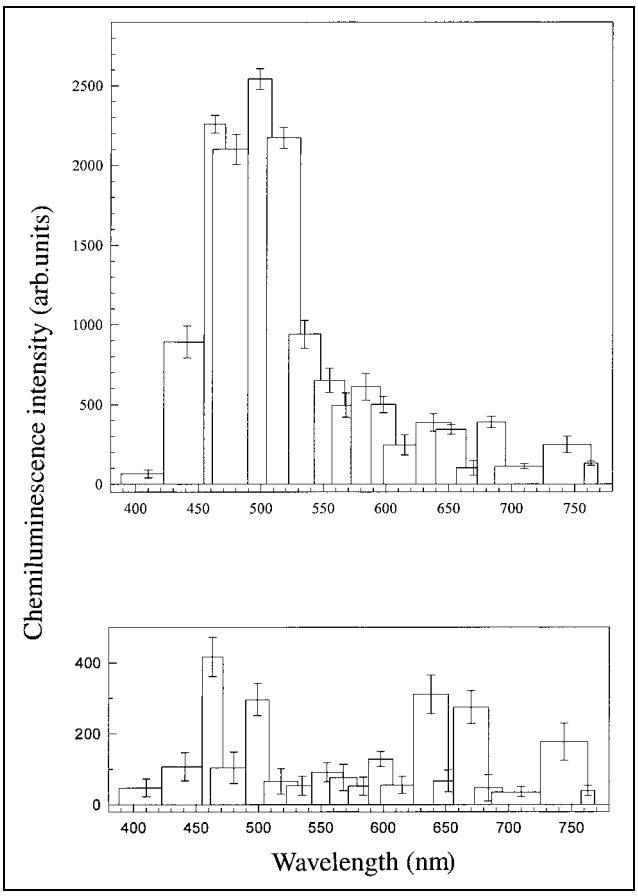

Fig. 3: Chemiluminescence spectra from the 0.5 mmol/l carazolol 0.5 mmol/l $CuSO_4$ 0.6 mmol/l H_2O_2 system in carbonate buffer, pH 9.3 (light rectangles) and from the above system without carazolol (dark rectangles) measured 150 s after the beginning of the reaction. The height of the rectangle corresponds to the mean value of 10 measurements of the relative intensity. The size of the bars approximates the standard deviation. Temperature 294 K

NBT, tiron, carnosine, lactic acid dehydrogenase strongly decrease the $O_2^{\cdot-}$ concentration, myoglobin decomposes H_2O_2 in a similar way as does catalase and scavengers $O^{\cdot}$ and $O_2^{\cdot-}$. β-Carotene, DPBF, DABCO, sodium azide, methionine, histidine and 5,5-dimethyl-1,3-cyclohexanedione are the most active of all established scavengers for $^1O_2$ [5, 6, 15]. The results obtained show that the tested compounds revealed very strong quenching effects on the light emission independent on the time of introduction to the reaction system. These results give evidence that oxygen species such as $O_2^{\cdot-}$ and $HO^{\cdot}$ play an important role as precursors of $^1O_2$ being an emitter of the light emission.

Direct evidence for the carazolol enhanced production of activated oxygen species and finally $^1O_2$ in the reaction system can be obtained by the spin-trapping technique using 2,2,6,6-tetramethylpiperidine (TEMP) as a trap. The reaction of $^1O_2$ with TEMP results in the formation of stable nitroxide radicals with a typical triplet spectrum. The product of this reaction is expected to be 2,2,6,6-tetramethylpiperidine-*N*-oxide (TEMPO) [16]. The mixing of TEMP with the $Cu(II)/H_2O_2/HO^-$ system results in the appearance of an ESR signal. The addition of carazolol to the above mentioned system led to a 20-fold increase in the signal amplitude. The obtained ESR spectra could be analysed in terms of the parameters listed in the Table. Within the limits of experimental uncertainty, the obtained parameters for both investigated systems are almost the same and similar to that observed for TEMPO [16]. Moreover it has been demonstrated that TEMP is the specific $^1O_2$ spin trap since neither $O_2^{\cdot-}$ nor $HO^{\cdot}$ and H_2O_2 generated the nitroxide radical [17].

In addition, in order to check the generation of $^1O_2$ in the tested reaction mixtures the spectrophotometric method was used. Fig. 5 presents the bleaching of RNO in the $RNO/A/carazolol/Cu(II)/H_2O_2/HO^-$ and $RNO/A/carazolol/Cu(II)/EDTA/H_2O_2/HO^-$ systems. In the absence of imidazole the loss of RNO was not detectable which means that neither H_2O_2 nor $^1O_2$ generated in the process of H_2O_2 decomposition causes the bleaching of RNO. It can be seen from Fig. 5. that the rate of RNO bleaching in the system containing a chelating agent is slower than in its absence. A similar effect of EDTA was observed in the case of the chemiluminescence kinetics (data not shown).

The heavy water effect was also applied to detect the involvement of $O_2^{\cdot-}$ in the $carazolol/Cu(II)/H_2O_2$/buffer system [18]. We observed an 1.8-fold increase in the quantum yield of the CL which is much lower than that expected from lifetime (τ) of $O_2(^1\Delta_g)$ in used mixture of solvents: $D_2O + H_2O$ (9 : 1), (τ ranges from 3.1 μs in H_2O to approximately 22 μs in this mixture). There are two

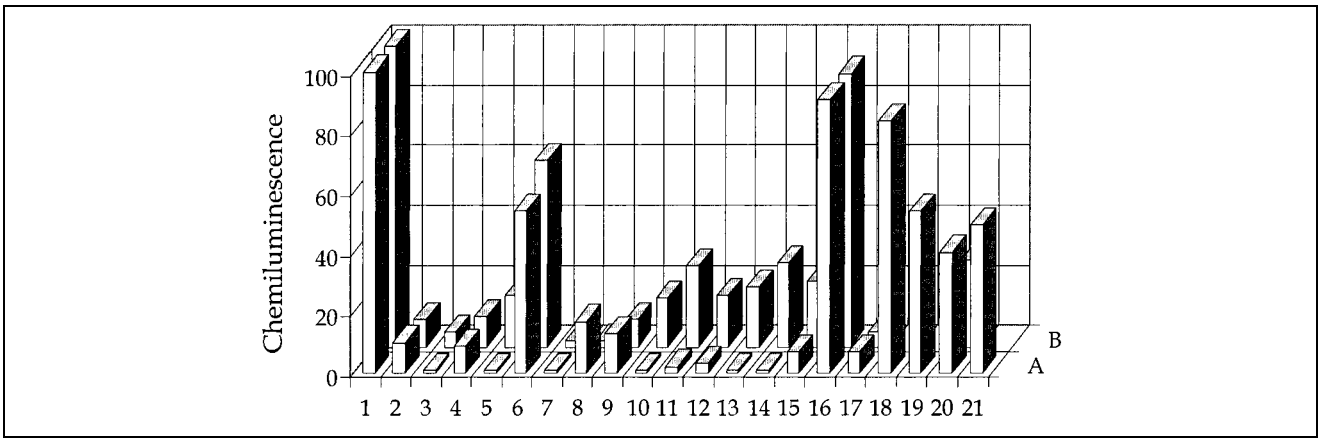

Fig. 4: The effect of $O_2^{\cdot-}$, $HO^{\cdot}$-scavengers and $^1O_2$-quenchers on the CL intensity of the $carazolo/CuSO_4/H_2O_2/HO^-$ system in the carbonate buffer, pH 9.3. Part A. Inhibitors were added to the reaction system at the start of the reaction; Part B. Inhibitors were added 2 min after mixing of the reagents. Remaining conditions as in Fig. 1. Concentration of inhibitors were as follows: 1: without inhibitor, 2: 1 mmol/l carnosine, 3: 1 mmol/l trolox, 4: 0.1 mmol/l myoglobin, 5: 5 mmol/l tiron, 6: 1 mmol/l NBT, 7: 5 mmol/l glutathione, 8: 5 mmol/l captopril, 9: 2 mmol/l cysteine, 10: 5 mmol/l thiourea, 11: 5 mmol/l cimetidine, 12: 0.5 g/l lactic acid dehydrogenase, 13: 5 mmol/l methionine, 14: 5 mmol/l histidine, 15: 0.5 mmol/l tryptophan, 16: 5 mmol/l sodium azide, 17: 5 mmol/l ascorbic acid, 18: 1 mmol/l DABCO, 19: 1 mmol/l DPBF, 20: 1 mmol/l 5,5-dimethyl-1,3-cyclohexanedione, 21: 1 μmol/l β-carotene

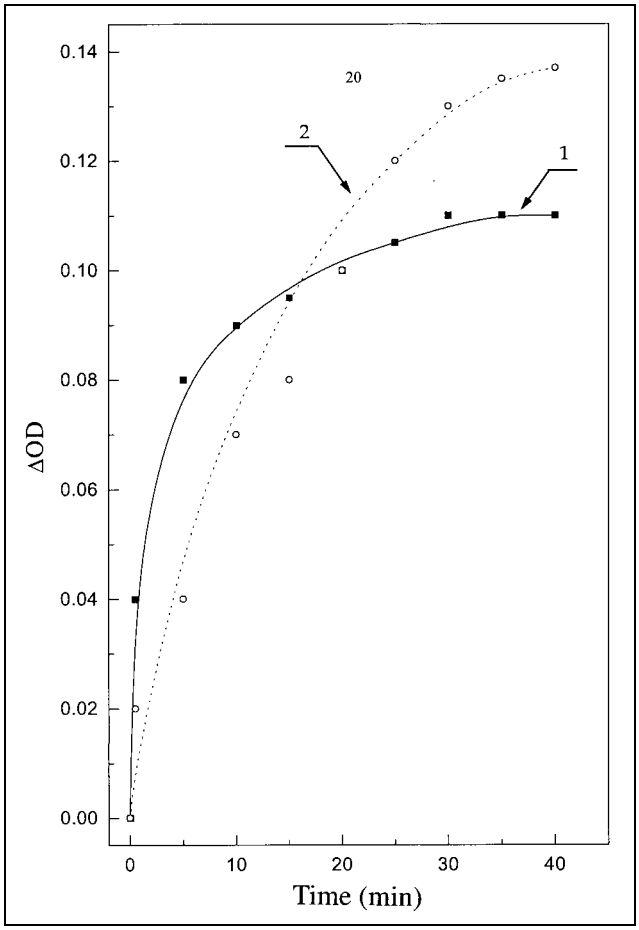

Fig. 5: Time course of the bleaching of RNO at 440 nm in the RNO + A + carazolol + Cu(II) + H_2O_2 (curve 1) and RNO + A + carazolol + Cu(II)-EDTA + H_2O_2 (curve 1) systems. Reaction conditions: 80 μmol/l RNO, 60 mmol/l A, 0.5 mmol/l carazolol, $CuSO_4$, Cu(II)-EDTA and H_2O_2. Carbonate buffer, pH 10.8, 293 K (0.2 cm cell)

main reasons of the observed small effect: (1) a large effect of D_2O will only be observed when $^1O_2$ undergoes relaxation by radiationless process and reacts with acceptor molecules; (2) the rate constant of H_2O_2 decomposition is less in D_2O than in H_2O, and amounts of $^1O_2$ bubbles escaping from the solution may also be different in the two solvents.

The results presented here show that the radical generating system of the Fenton type reaction is sensitive to the presence of carazolol. The ability of the drug to stimulate the production of reactive oxygen species by the $CuSO_4$/H_2O_2/buffer is very strong.

The question arises what elementary reactions generating emitters observed CL occur with the participation of carazolol. In order to answer this question we studied the oxidation of the drug in the presence of Cu(II) ions in the absence of H_2O_2 as well as the effect of EDTA as a chelating agent on the Cu(II)-catalyzed oxidation of carazolol. Solutions of 0.5 mmol/l carazolol in carbonate buffer (pH 10.8) saturated with air show a weak CL lasting a few minutes in the presence of Cu(II) ions. The observed emission may result from a sequence of the following reactions [19]:

$$\text{carazolol} \xrightarrow{\text{Cu(II), O}_2} \text{oxidized carazolol} + O_2^- \quad (5)$$

$$\text{Cu(II)} + O_2^- \rightarrow \text{Cu(I)} + O_2 \quad (6)$$

$$2O_2^- \xrightarrow{2H^+} H_2O_2 + {}^1O_2 \quad (7)$$

The Cu(I) ion in the presence of molecular oxygen undergoes oxidation generating O_2^- according to reaction (2). Reaction (7) is known as a source of $^1O_2$, whereas the interaction of O_2^- with H_2O_2 also gives $^1O_2$ according to reaction (3).

When Cu(II) ions were chelated with EDTA prior to the addition of carazolol a small increase in the CL was observed in comparison to that observed in the presence of the copper ion alone. The Cu(II)-EDTA complex may induce oxidation of carazolol generating O_2^-, that is responsible for the reduction of Cu(II)-EDTA to Cu(I)-EDTA as follows:

$$\text{carazolol} \xrightarrow{\text{Cu(II)-EDTA, O}_2} \text{oxidized carazolol} + O_2^- \quad (8)$$

$$\text{Cu(II)} - \text{EDTA} + O_2^- \rightarrow \text{Cu(I)} - \text{EDTA} + O_2 \quad (9)$$

The stimulation of the CL by Cu(II)-EDTA may be attributed to increased generation of $HO^\cdot$ e.g. by reaction (1). The interaction of $O_2^{\cdot-}$ with $HO^\cdot$ leads to the $^1O_2$ generation at a very high rate constant $k = 1.5 \cdot 10^{10}\ mol^{-1} \cdot s^{-1} \cdot l$ [20]:

$$O_2^- + HO^\cdot \rightarrow {}^1O_2 + HO^- \quad (10)$$

In experiments conducted in DMSO using KO_2 as a generator of O_2^- we have also observed an enhancing effect of carazolol on CL (Fig. 6.). Superoxide radicals can initiate the carazolol oxidation as follows:

$$\text{carazolol} + O_2^- \xrightarrow{2H^+} \text{oxidized carazolol} + H_2O_2 \quad (11)$$

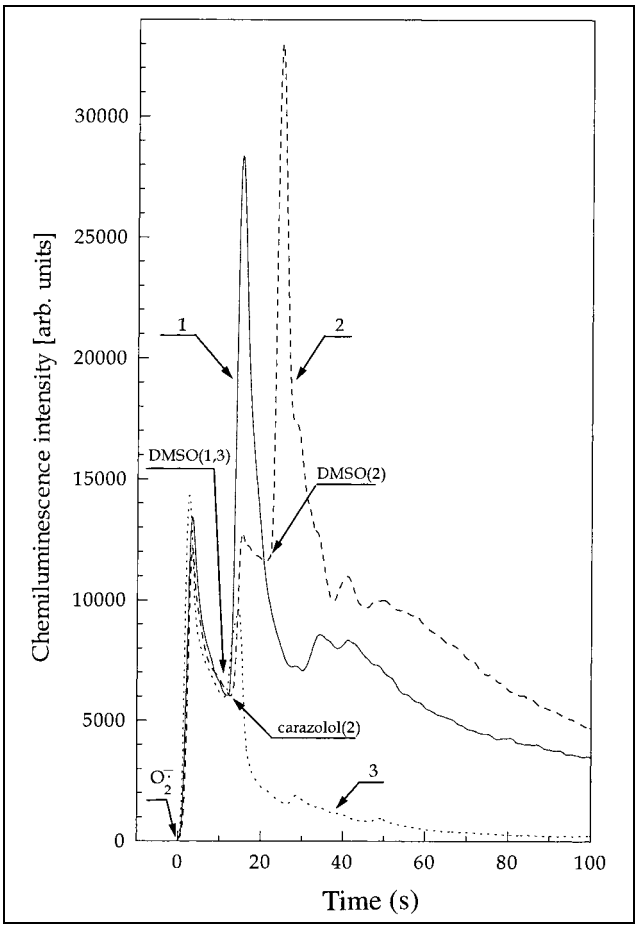

Fig. 6: Kinetics of CL resulted from the system generating $O_2^{\cdot-}$ in DMSO. Curve(1): 1 mmol/l O_2^- in DMSO; curve (2): as curve (1) but in presence of 5 mmol/l carazolol; curve (3): as curve(1) but in the presence of 0.5 mmol/l $CuSO_4$. Arrows indicate moment of the reagent addition, numbers in a bracket indicate responsible curve. Temperature 310 K

This explains the relatively large increase in CL observed in the presence of carazolol (curve 2). The CL from the O_2^- generating system (curve 1) was strongly decreased by Cu(II) ions (curve 3) according to reaction (6). The latter reaction is known to precede at a rate of $4.81 \cdot 10^9$ $mol^{-1} \cdot s^{-1} \cdot 1$ [20]. The presence of carazolol in the system generating O_2^- decreased the quenching effect of Cu(II) ions.

If Cu(II) ion was replaced by Cu(II)-EDTA the decrease in the CL was much weaker, and the presence of carazolol in the reaction mixture caused an increase of the light emission (data not shown). A relatively small quenching effect exerted by Cu(II)-EDTA results probably from much lower rate of O_2^- reaction with the complex ($k < 10^5\, mol^{-1} \cdot s^{-1} \cdot l$) [20].

The results presented here give evidence that carazolol behaves multifunctionally when exposed to the Cu(II)-H_2O_2-HO^- system. It is important that such interactions result in the formation of more toxic oxygen species such as $^1O_2$ and $HO^{\cdot}$ in comparison to H_2O_2 or O_2^-. Since increased generation of these active oxygen species can contribute to the oxidative stress, mutagenicity and toxicity in biological systems, precautions should be considered during carazolol treatment.

3. Experimental

Carazolol 4-(2-hydroxy-3-isopropylaminopropoxy)-carbazole was kindly provided by Klinge Pharma GmbH (Münich, Germany); cysteine and glutathione were obtained from Reanal (Budapest, Hungary); 2,2,6,6-tetramethylpiperidine-N-oxide (TEMPO) and 2,2,6,6-tetramethylpiperidine (TEMP) were purchased from Sigma (St. Louis, MO). 1,4-Diazobicyclo-[2,2,2]-octane (DABCO), dimethylsulfoxide (DMSO) were obtained from Aldrich (Milwaukee, WI); 1,3-diphenylisobenzofurane (DPBF) was recrystallized twice from benzene, KO_2 and 18-crown-6 ether were obtained from Fluka (Buchs, Switzerland). Compounds used as scavengers: carnosine, trolox, myoglobin, tiron, nitroblue, tetrazolium, captopril, glutathione, cysteine, cimetidine, thiourea, lactic acid dehydrogenase, methionine, histidine, alanine, tryptophan, sodium azide and ascorbic acid, were purchased from Merck (Darmstadt, Germany). Other reagents were purchased from POCH (Gliwice, Poland). Heavy water (D_2O, 99.8%) was obtained from IBJ wierk (Poland).

Superoxide anion radicals were generated according to the following procedure: a stock solution of KO_2 was prepared by dissolving 60 mg of 18-crown-6 ether in 10 ml of dry DMSO and then 7 mg of KO_2 were added quickly to avoid contact with air humidity. The mixture was stirred with a magnetic stirrer for one hour to give a pale yellow solution of 10 mM O_2^-. The O_2^- solution is stable at room temperature for at least one hour.

The kinetics of CL and the effect of scavengers on the light emission were measured using the integrated CL technique. Chemiluminescence intensity was measured using an M12FQC51 photomultiplier with S-20 cathode (Oberkochen, Germany) sensitive in the range 200–800 nm operating jointly with a Zeiss K-200 recorder (Oberkochen, Germany). Reagents were introduced to a thermostated glass cuvette placed in a light-tight camera through pipes from semi-automatic syringes. The cuvette was exhausted and washed using B-169 vacuum system Büchi (Flawill, Switzerland). CL spectra were estimated using a set of calibrates cut-off filters (GOST9411-66) and an EMI 9302 B photomultiplier [21].

The spectrophotometric determination of $^1O_2$ was performed according to the method described by Kraljiæ and Mohsni [22]. The method is based on the bleaching of *p*-nitrosodimethylaniline (RNO) by the intermediate product of the reaction of $^1O_2$ with imidazole (A) i.e. a transannular peroxide (AO_2):

$$^1O_2 + A \rightarrow AO_2$$

$$AO_2 + RNO \rightarrow RNO + \text{products}$$

The bleaching of RNO was followed by monitoring the decrease in the optical density (OD) at 440 nm. For spectrophotometric studies a Zeiss M40 spectrophotometer (Oberkochen, Germany) was used.

Electron spin resonance (ESR) measurements were made using a standard X-band spectrometer operating at 9.3 GHz with a 100 kHz modulation of the steady magnetic field. The spectra were recorded at a microwave power level of 2 mW and a modulation amplitude of 0.1 G. The samples were put into a quartz flat cell with the optical path length of 0.25 mm.

Fluorescence spectra were measured using a Hitachi MPF-3 spectrofluorimeter (Ratingen, Germany).

This work was presented at the VIII[th] International Symposium on Luminescence Spectroscopy in Biomedical and Environmental Analysis Detection Techniques and Applications in Chromatography and Capillary Electrophoresis, Las Palmas (Canary Islands), Spain, 26–29 May 1998.

References

1 Bartsch, W.; Dietmann, K.; Leinert, H.: Sponer G.: Arzneim.-Forsch. **27**, 1022 (1977)
2 Janiak, St.: Drugs of Today **17**, 55 (1981) and references were cited therein
3 Costin, B.; O'Donnell, S. R.; Wanstall, J. C.: J. Pharm. Pharmacol. **35**, 590 (1983)
4 Mejean, A.; Guillaume, J. L.; Strosberg, A. D.: Europ. J. Pharmacol. **291**, 359 (1995)
5 Halliwell, B.; Gutteridge, J. M. C.: Free Radicals in Biology and Medicine, 2nd edn, Clarendon Press, Oxford 1989
6 Kruk, I.: Environmental Toxicology and Chemistry of Oxygen Species; in: Hutzinger, O. (ed.): The Handbook of Environmental Chemistry. Vol. 2. Reactions and Processes. Part I. Springer-Verlag, Berlin, Heidelberg, New York 1998
7 Singh, A.: Can J. Physiol. Pharmacol. **60**, 1330 (1982)
8 Westlin, W.; Mullane, K.: Circulation **77** (suppl.I) I-30-I-39 (1988)
9 Haber, F.; Weiss, J.: Proc. R. Soc. London Ser. A**147**, 332 (1934)
10 Van Dyke, B. R.; Clopton, D. A.; Saltman, P.: Inorg. Chim. Acta **242**, 57 (1996)
11 Miller, J. W.; Selhub, J.; Joseph J. A.: Free Rad. Biol. Med. **21**, 241 (1996)
12 Khan, A. U.; Kasha, M.: J. Am. Chem. Soc. **92**, 3293 (1996)
13 Khan, A. U.: Photochem. Photobiol. **28**, 615 (1987)
14 Stauff, J.; Sander, U.; Jaeschke, W.: Chemiluminescence of perhydroxyl and carbonate radicals. in: Cormier, M. J. (ed): Chemiluminescence Biol. Rep. Int. Confer. Plenum New York, N.Y. 1973
15 Bartosz, G.: Druga twarz tlenu, Wydawnictwa Naukowe PWN, Warszawa (1995)
16 Lion, Y.; Delmelle, M., Van de Vorst, A.: Nature **263**, 442 (1976)
17 Rigo, A.; Agrese, E.; Stevanato, R.; Orsega, E. F.; Viglino, P.: Inorg. Chem. Acta **24**, 171 (1977)
18 Kearns, D. K.: Solvent and solvent isotope effects on the lifetime of singlet oxygen, in Wassermann, H. W.; Murray, R. W. (eds): Singlet Oxygen, Academic Press, New York 1979
19 Stohs, S. J.; Baghi, D.: Free Radic. Biol. Med. **18**, 321 (1995)
20 Bielski, H. B. J.; Cabelli, D. E.; Arudi, R. L.; Ross, A. B.: J. Phys. Chem. Ref. Data **14**, 1041
21 Kruk, I.; Lichszteld, K.; Michalska, T.: Z. Naturforsch **44C**, 39 (1989)
22 Kraljic, I.; El Mohsni, S.: Photochem. Photobiol. **28**, 577 (1978)

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