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The influence of antioxidants and cycloheximide on the level of nitric oxide in the livers of mice in vivo

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Abstract

When injected into mice prior to the NO generation increase induced with lipopolysaccharide (LPS) from Escherichia coli, exogenous antioxidants diethyldithiocarbamate (DETC) or phenazan (sodium 3.5-di-*tert*-butyl-4-oxiphenylpropionate) as well as the inhibitor of protein biosynthesis, cycloheximide (CHI) attenuated the NO production in mouse liver in vivo. These data demonstrated the key role of free radicals, which were likely, active oxygen species, in the synthesis of inducible NO-synthase (iNOS) responsible for the NO production in this organ. Similar effects of phenazan and CHI were observed in livers of mice treated with γ-irradiation or LPS + Fe^{2+}-citrate, which suggested that these treatments also induced INOS synthesis through initiating the action of active oxygen species. The rate of NO synthesis was estimated by accumulation of paramagnetic mononitrosyl iron complexes with DETC (MNIC-DETC) detected using the EPR method. The formation of MNIC-DETC complexes was found in the brain of mice pre-treated with LPS + Fe^{2+}-citrate which seemed to be due to iNOS synthesis stimulated by this treatment.

Keywords: Active oxygen species; Antioxidant; Inducible NO synthase; Nitric oxide

1. Introduction

It has been recently shown that dithiocarbamates, inhibitors of the nuclear transcription factor κB (NFκB) prevent synthesis of inducible NO synthase (iNOS) in renal mesangial cells treated with interleukin 1 or tumour necrosis factor α, as well as in murine alveolar and bone marrow-derived macrophage cells stimulated with bacterial lipopolysaccharide (LPS) and interferon-γ (INF-γ) [1–3]. These data demonstrated the key role of active oxygen species in this iNOS biosynthesis, inactivated with well-known antioxidants, dithiocarbamates.

In the present study similar investigations were undertaken on the animal organism. There, the effects were studied of diethyldithiocarbamate (DETC) and of another antioxidant, phenazan as well as the action of a protein biosynthesis inhibitor, cycloheximide (CHI) on NO synthesis induced by bacterial LPS or γ-irradiation via the L-arginine-dependent pathway in mouse liver in vivo [4–6]. γ-Irradiation is well known to produce active oxygen species in animal tissues. Therefore we proposed that these species can induce iNOS synthesis through the transduction pathway similar to that stimulated with bacterial LPS.

To detect NO in mouse liver we used a method based on utilizing a complex of Fe^{2+} with DETC as an exogenous ligand. Fe^{2+}-DETC complex acts as a specific trap for NO and forms with it paramagnetic mononitrosyl complexes (MNIC-DETC). This complex is characterized by an EPR signal at $g_{\perp} = 2.035$ and $g_{\parallel} = 2.02$ with a triplet hyperfine structure (HFS) at $g_{\perp}$ [7]. This approach makes it possible to demonstrate and quantify NO formation in animal organs in vivo following various pretreatments [4–6]. Thus in our experiments DETC was used as both an antioxidant and a ligand component of MNIC.

Abbreviations: DETC, Diethyldithiocarbamate; EPR, electron paramagnetic resonance; HFS, hyperfine structure; INF-γ, interferon-γ; L-NNA, N^G-nitro-L-arginine; LPS, lipopolysaccharide from *Escherichia coli*; MNIC-DETC, mononitrosyl iron complex with DETC; NO, nitric oxide; cNOS or iNOS, constitutive or inducible NO synthase, respectively; PDTC, pyrrolidine dithiocarbamate.

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2. Materials and methods

2.1. Materials

Lipopolysaccharide (LPS) from *Escherichia coli* (serotype 005:B5), cycloheximide (CHI), and N^G-nitro-L-arginine (L-NNA) were all from Sigma. $NaNO_2$, sodium diethyldithiocarbamate (DETC), sodium citrate and $FeSO_4 \cdot 7H_2O$ were obtained from Serva. Sodium dithionite was from Merck. A water-soluble antioxidant phenazan (sodium 3,5-di-tert.-butyl-4-oxiphenylpropionate) was a generous gift of Prof. V.V. Ershov and Dr. L.G. Plekhanova. Gaseous NO was obtained by the reaction of $FeSO_4$ and $NaNO_2$ in acid environment (0.1 M HCl) and then purified by fractional sublimation in a high-vacuum system ($P < 0.01$ mmHg).

2.2. Sample preparation

Experiments were carried out on white mongrel male mice (20–22 g) in February–April. All agents were injected into mice in 0.2 ml of physiological saline intraperitoneally (i.p.) (CHI, DETC, L-NNA, $NaNO_2$ or phenazan) or subcutaneously in the thigh (Fe^{2+}-citrate complex) at the following doses: DETC, 500 mg/kg; $FeSO_4 \cdot 7H_2O$, 37.5 mg/kg; citrate-Na, 187.5 mg/kg; CHI, 3 mg/kg; $NaNO_2$, 50 mg/kg; phenazan, 100 mg/kg; L-NNA, 250 mg/kg (administration terms are shown in the text below). After decapitation of the animals, the liver and the brain were isolated, frozen in liquid nitrogen, and analyzed with an EPR-V (Russia) or an ESR-'Radiopan' (Poland) radiospectrometer. In some experiments the tissues isolated from mice were treated with gaseous NO (300 mmHg). To this aim, isolated tissue was placed into a closed vessel. After evacuation, the vessel was filled with NO gas at 300 mmHg for 30 min. The NO was pumped out and the tissue was frozen in liquid nitrogen for EPR analysis. One group of mice was also subjected to γ-irradiation from a ^{60}Co-source at a sublethal dose (700 Röntgen) for 10 min. Special experiments have shown that of 30 mice exposed to such irradiation, 20 survived a month (~ 70%). A solution of ^{57}Fe to be injected subcutaneously into mouse upper leg was prepared as follows. A powder of $^{57}Fe_2O_3$ was heated with $H_2SO_4 + H_2O$ (8:3) in a glass test tube on a water bath until decoloration (~ 10 h). The concentration of $^{57}Fe^{3+}$ was adjusted to 1 mg/ml (pH 3–4), and the solution was stored in a refrigerator. Before injection into the animals, sodium citrate (3.75 mg in 0.2 ml) was added, increasing the pH to 6. Then sodium dithionite was added until decoloration to reduce $^{57}Fe^{3+}$ to $^{57}Fe^{2+}$, and this solution was used for the injection.

2.3. EPR spectrometry

EPR spectra were recorded at 77 K, the microwave frequency of 9.330 GHz, the microwave power of 5 mW, and the modulation amplitude of 0.5 mT. Quantification of MNIC-DETC complexes was accomplished using the comparision of the amplitudes of EPR signals with those of a standard MNIC-DETC complex of known concentration prepared in dimethylsulfoxide solution as described elsewhere [7].

3. Results

3.1. Effects of antioxidants and CHI on NO formation induced with LPS in mouse liver in vivo

LPS from *E. coli* induced NO generation in mouse liver after 3.5–4 h of its administration to mice. The method used in this investigation permitted estimation of the rate of MNIC-DETC complex formation over 30 min, i.e., the rate of NO formation which could be trapped over this time by the Fe^{2+}-DETC complexes. It is noteworthy that our method will only quantify the relative concentrations of NO within the tissues because not all of the NO which has been formed by the tissue is evidently included into MNIC-DETC complex.

The rate of MNIC-DETC complex formation increased from 1 nmol/g of wet liver tissue/30 min in control mice to 18 nmol/g of wet liver/30 min in mice pretreated with LPS (Table 1). This rate was constant between 3.5 and 9.5 h after the LPS administration but then it returned to the control level at 15 h (not shown). The values of these rates

Table 1
Quantification of MNIC-DETC complexes formed in the liver of mice treated with DETC, Fe^{2+}-citrate complex (Fe), phenazan or CHI at the doses indicated in Section 2

Treatment	MNIC-DETC complex formation over 30 min of (nmol/g of wet liver tissue)
DETC (0.5 h)–decap.	1 ± 1
DETC, Fe (0.5 h)–decap.	3 ± 1
LPS (4.5 h) + DETC (0.5 h)–decap.	18 ± 4
LPS (4.5 h) + DETC,Fe (0.5 h)–decap.	460 ± 20
LPS,phenazan (4.5 h) + DETC (0.5 h)–decap.	1 ± 1
LPS (6 h) + DETC (2 h)–decap.	1 ± 1
LPS (4.5 h) + DETC (3.5 h) + DETC (0.5 h)–decap.	0.8 ± 0.3
LPS (6.5 h) + DETC (2.5 h) + DETC (0.5 h)–decap.	13 ± 2
LPS (4.5 h) + CHI (2 h) + DETC (0.5 h)–decap.	6 ± 2
LPS (9 h) + DETC (0.5 h)–decap.	20 ± 5
LPS (9 h) + DETC,Fe (0.5 h)–decap.	430 ± 20
LPS (9 h) + CHI (2 h) + DETC (0.5 h)–decap.	20 ± 5
LPS (9 h) + CHI (2 h) + DETC,Fe (0.5 h)–decap.	200 ± 20

Data shown are expressed as mean ± S.E. of 5–20 individual observations from different mice (* $P < 0.01$ versus corresponding different animals). Time between the reagent administrations and decapitation (decap.) of animals is given in brackets.

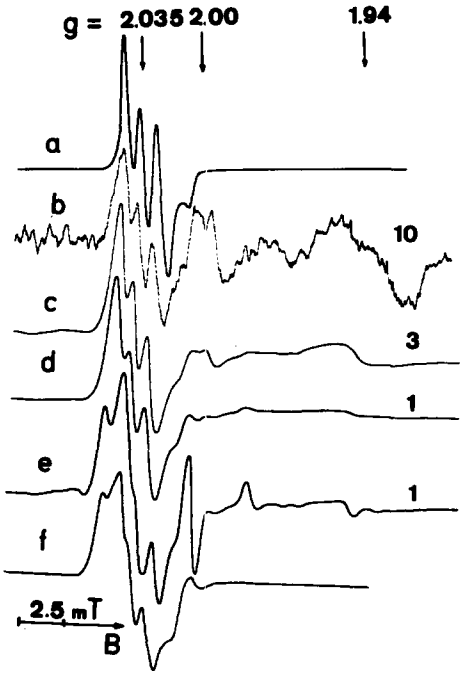

Fig. 1. Typical EPR spectra of mouse liver preparations from a control animal injected with DETC (b), an animal injected with LPS (4 h) + DETC (c), an animal injected with LPS (4 h) + DETC + $^{56}Fe^{2+}$-citrate (d) or $^{57}Fe^{2+}$-citrate (e) complexes. (a,f) EPR spectra of MNIC-DETC complexes in dimethylsulfoxide solution, including ^{56}Fe (a) or ^{57}Fe (f). The MNIC-DETC complex gives an EPR signal at $g_{\perp} = 2.035$, $g_{\parallel} = 2.02$. The EPR signal at $g = 1.94$ is due to reduced iron-sulfur proteins. Records were made at 77 K, $p = 5$ mW, $f = 9.330$ GHz, modulation amplitude = 0.5 mT. Relative gain settings of the spectrometer are shown at the right side of spectra.

were estimated by the intensities of EPR signals from MNIC-DETC complexes, which are presented in Fig. 1.

DETC administration to the animals did not practically affect the NO formation in mouse liver if DETC was injected into mice during the period of elevated LPS-induced NO synthesis. This was demonstrated by the experiments when DETC was injected into mice 4 h after LPS treatment and 2.5 h before decapitation (Table 1, line 8). However, DETC completely inhibited the effect of LPS treatment of mice on the NO formation when it was injected into mice 1 h after LPS administration, i.e., prior to the LPS-induction of iNOS (Table 1, line 7). It is worth noting that administration of DETC to the animals for 2 h prior to decapitation within the period of elevated NO-synthesis (without the additional DETC injection) did not lead to the accumulation of MNIC-DETC in mouse liver (Table 1, line 6). Evidently these complexes were decomposed by this time and the additional DETC injection for 30 min was required to synthesize new MNIC-DETC (Table 1, line 8). The inhibitory effect of DETC on MNIC-DETC formation observed in our experiments seemed to be due to antioxidant properties of DETC. A similar effect was obtained for another antioxidant phenazan when it was injected into mice simultaneously with LPS (Table 1, line 5).

The attenuated formation of MNIC-DETC complexes in livers of mice following administration of these antioxidants might have been caused by a decrease in the amount of NO traps (Fe^{2+}-DETC complexes) available in the animal tissue. To verify this hypothesis we investigated the effect of administration of DETC or phenazan to mice (1 h after LPS or simultaneously with LPS, respectively) on MNIC-DETC complex formation induced by NO exogenous donor $NaNO_2$ (50 mg/kg) in the liver. The NO formation from $NaNO_2$ in animal organisms has been demonstrated earlier by formation of nitrosyl hemoglobin or MNIC-DETC complexes [5,8]. No influence of DETC or phenazan on the MNIC-DETC complex formation was observed in these experiments (data not shown).

Similar results were obtained in experiments with CHI administration to mice. The CHI treatment of mice 2.5 h after LPS administration resulted in the attenuation of NO formation in mouse liver (Table 1, line 9). This was not the case when CHI was administrated after the induction of iNOS with LPS (Table 1, line 12). Experiments using $NaNO_2$ as an exogenous donor of NO showed that CHI administration to animals did not affect the amount of NO traps, Fe^{2+}-DETC complexes (data not shown).

3.2. *Effects of phenazan and CHI on the hepatic NO formation induced with γ-irradiation in mice*

γ-Irradiation of mice at a dose of 700 Röntgen potentiated the MNIC-DETC complex formation in the liver (Table 2, lines 2 and 7). This effect was observed in 2–15 h, whereafter the rates of MNIC-DETC formation decreased (not shown). CHI or phenazan treatments before or immediately after γ-irradiation resulted in the attenuation

Table 2
Quantification of MNIC-DETC complexes in livers of CHI- or phenazan-treated mice under γ-irradiation (γ-)

Treatment	MNIC-DETC complexes formation over 30 min (nmol/g of wet liver tissue)
DETC (0.5 h)–decap	2.3 ± 0.3
γ-(4.5 h) + DETC (0.5 h)–decap.	9.0 ± 1.0
Phenazan, γ-(4.5 h) + DETC (0.5 h)–decap.	3.3 ± 1.3
CHI (6 h) + γ-(4.5 h) + DETC (0.5 h)–decap.	1.4 ± 0.3
γ-,CHI (4.5 h) + DETC (0.5 h)–decap.	2.1 ± 0.7
γ-(6 h) + CHI (2 h) + DETC (0.5 h)–decap.	5.5 ± 1.3
γ-(7.5 h) + DETC (0.5 h)–decap.	7.3 ± 1.6
γ-(7.5 h) + CHI (2 h) + DETC (0.5 h)–decap.	8.2 ± 2.3
γ-,CHI (7.5 h) + DETC (0.5 h)–decap.	2.1 ± 0.5

Data shown as the mean ± S.E. of 5–15 observations from different mice (* $P < 0.01$ versus corresponding different animals). Time between γ-irradiation with reagent administration and decapitation (decap.) is given in brackets.

of MNIC-DETC formation (Table 2, lines 3–6 and 9). The increase in the interval between γ-irradiation and the following CHI administration to mice led to diminishing the CHI effect. For example, if CHI was administrated to mice 5.5 h after γ-irradiation for 2 h prior to decapitation of animals, no influence of this agent on the MNIC-DETC complex formation was observed (Table 2, line 8).

Pretreatment of mice with LPS changed the effect of γ-irradiation on the NO formation in mouse liver. γ-Irradiation potentiated the MNIC-DETC complex formation if the LPS pretreatment of animals stimulated the formation of MNIC-DETC complex at rates below 3 nmol of MNIC-DETC/g of wet liver/30 min. On the contrary, γ-irradiation diminished the MNIC-DETC complex formation if the LPS pretreatment stimulated this formation with the rates above 20 nmol of MNIC-DETC/g of wet liver/30 min.

3.3. The effect of CHI on the increase in LPS-induced NO formation with Fe^{2+}-citrate in mouse liver

Administration of the Fe^{2+}-citrate complex to mice 30 min prior to decapitation in the period of elevated LPS-induced NO synthesis sharply increased the rates of MNIC-DETC formation (Table 1, lines 4 and 11). This effect might be solely due to an increase in the content of NO traps, the Fe^{2+}-DETC complexes. Results of experiments with injections of $^{57}Fe^{2+}$-citrate complexes into mice were in line with this proposition. If ^{57}Fe were to be included in the MNIC-DETC complex, its EPR signal would be characterized with an HFS originating from interaction of the unpaired electron with the ^{57}Fe nucleus (its spin, $I = 1/2$) [7]. Such HFS was recorded in the EPR signal from the MNIC-DETC complex formed in the liver of mice treated with LPS + $^{57}Fe^{2+}$-citrate (Fig. 1). This result demonstrated that exogenous iron can form Fe^{2+}-DETC complexes as endogenous iron, thereby increasing the content of these NO traps in the mouse liver. Nevertheless, when CHI was administrated to mice 7 h after the LPS treatment, the effect of Fe^{2+}-citrate complex administration on the MNIC-DETC formation diminished two-fold (Table 1, line 13). These findings indicate that the increase in the rate of MNIC-DETC complex formation induced by the Fe^{2+}-citrate complex might be partially caused by the elevation of NO generation due to iNOS induction.

Administration of Fe^{2+}-citrate to non-LPS-pretreated mice increased the MNIC-DETC complex formation in mouse liver as well but its rate was much lower than that in the animals pretreated with LPS (Table 1, line 2).

3.4. MNIC-DETC complex formation in mouse brain stimulated with LPS and Fe^{2+}-citrate

LPS also induced the formation of NO in mouse brain within 3.5–4 h of its administration to the animals. The

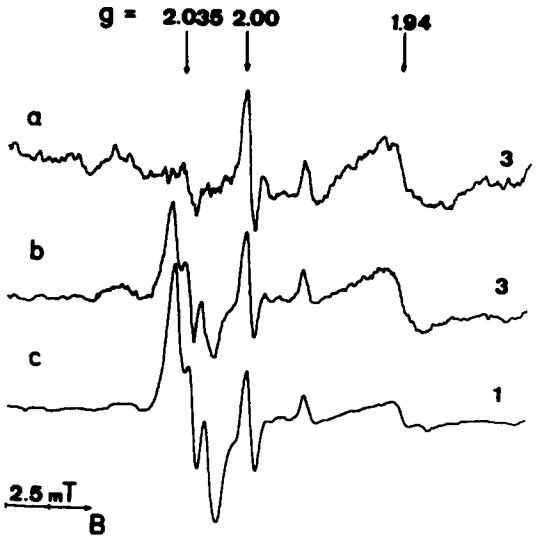

Fig. 2. Typical EPR spectra of a mouse brain preparations from an animal injected with DETC (a), an animal injected with DETC + Fe^{2+}-citrate complex (b), an animal injected with LPS (4 h) + DETC + Fe^{2+}-citrate complex (c). The MNIC-DETC complex gives an EPR signal at $g_{\perp} = 2.035$, $g_{\parallel} = 2.02$ and triplet HFS. The EPR signal at $g = 1.94$ is due to reduced iron-sulfur proteins. The EPR signal at $g = 2$ is the third component of HFS in the EPR signal due to Cu^{2+}-DETC complex. Recording conditions are the same as indicated in Fig. 1.

rate of MNIC-DETC complex formation was 10 ± 2 nmol of MNIC-DETC/g of wet brain tissue/30 min ($n = 5$). However, this formation was observed only if Fe^{2+}-citrate complex was injected into animals simultaneously with DETC. The Fe^{2+}-citrate + DETC administration to non-LPS-pretreated mice stimulated the MNIC-DETC complex formation at the rate of 2 ± 1 nmol of MNIC-DETC/g of wet brain tissue/30 min ($n = 5$). Without the Fe^{2+}-citrate administration, the MNIC-DETC complex formation was observed neither in the brains of animals pretreated with LPS nor in the brains of untreated mice. The formation of DETC complexes with endogenous copper (Cu^{2+}) was detected only in these preparations. Typical EPR spectra from the brains of mice treated with LPS, DETC, and Fe^{2+}-citrate are shown in Fig. 2.

The dministration of L-NNA, the NOS inhibitor, to mice 30 min before the addition of Fe^{2+}-citrate complex + DETC completely inhibited the MNIC-DETC complex formation in mouse brain (not shown). This result indicated that the formation of NO in mouse brain was induced via the L-arginine-dependent pathway; CHI administration did not influence the NO formation in mouse brain.

MNIC-DETC complexes were formed in mouse brain when $NaNO_2$ (50 mg/kg) was administrated to mice as an exogenous source of NO. The rate of this formation was 2.5 ± 0.5 nmol of MNIC-DETC/g of wet brain tissue/30 min ($n = 5$). The administration of Fe^{2+}-citrate complex to these animals increased this rate 4-fold. The treatment of brain samples with gaseous NO increased the content of

MNIC-DETC to 30 ± 5 nmol of MNIC-DETC/g of wet brain tissue ($n = 3$) and to 50 ± 10 nmol of MNIC-DETC/g of wet brain tissue ($n = 3$) isolated from the animals not treated with Fe^{2+}-citrate or treated with Fe^{2+}-citrate respectively.

4. Discussion

The induction of iNOS biosynthesis with LPS in animal liver in vivo has been demonstrated earlier by the increase of NO formation [4,5], the accumulation of NO_2^- and NO_3^-, the final products of NO oxidation [9,10], and by the expression of iNOS mRNA [11] in this organ. The data obtained in the present study allow us to propose that the regulation of iNOS biosynthesis in mouse liver may include the formation of active oxygen species initiating this protein synthesis. Our experiments are in line with those concerning the influence of antioxidants, dithiocarbamate derivatives, on the NO production by macrophage cells activated with LPS or LPS + INF-γ [1,2] or by renal mesangial cells treated with interleukin 1 or tumor necrosis factor [3]. Inhibition of this process with pirrolidine dithiocarbamate (PDTC) or DETC in cultured cells was suggested to be caused by the active oxygen species-scavenging effect of these antioxidants. They prevented the activation of NF-κB involved in the signal transduction pathway which links stimulation of macrophage or mesangial cells by bacterial endotoxin or cytokines with transcription of the gene encoding iNOS. It is noteworthy that PDTC interfered with induction of NOS rather than its catalytic activity: this antioxidant affected the NO production in macrophage cells being added only prior to the NO generation increase induced by LPS + INF-γ [1]. Similar results were obtained for CHI treatment of cells [2]. In our experiments DETC and CHI affected the NO production in livers of mice in a similar way. They attenuated the NO formation in mouse liver being added to the animals prior to the LPS-induced elevation of NO synthesis. However, neither DETC or CHI affected the NO formation in this organ when administered to mice during the increased NO generation. These results allow us to suggest that DETC as well as CHI operated only as an inhibitor of iNOS synthesis, but left the enzyme activity in mouse liver in vivo unaffected .

The antioxidant activity of DETC is primarily due to its capacity to bind ions of transition metals that catalyze formation of an active oxygen species [12]. In cells and tissues, however, DETC can act as a pro-oxidant as well. This is provided by its ability to chelate copper from the active center of copper-containing superoxide dismutase (SOD) [13]. According to the data reported in [13], the administration of DETC (500 mg/kg) to mice for 3 h decreased the SOD activity by 40% in the whole liver. Inactivation of the enzyme enhanced the amount of superoxide anion which easily oxidize NO molecules. Therefore, the decrease in hepatic MNIC-DETC observed in the liver on the DETC administration 1 h after LPS injection (Table 1, line 7) could be due to the decrease in NO readily transforming into peroxinitrites in the reaction with O_2^- [14] rather than to the blockade of iNOS biosynthesis.

This hypothesis is in disagreement with the results of experiments on DETC administration during the potentiation of NO generation by iNOS. Additional (for 2 h) administration of DETC did not practically decrease the MNIC-DETC level in murine liver (Table 1, lines 3 and 8). It seems that the decreased SOD activity resulting from the administration of DETC dose (500 mg/kg) was not sufficient to induce a considerable decrease in NO at the expense of NO reaction with O_2. Therefore in our experiments DETC acted primarily as an antioxidant.

The inhibitory effect of DETC and of another antioxidant phenazan on the LPS-induced NO formation in mouse liver supports the proposition about the key role of active oxygen species in iNOS biosynthesis in this organ in vivo, which seem to act through the activation of NFκB. Obviously, this conclusion about the integrating role of active oxygen species in this NFκB-inducing signal also holds true for the effect of γ-irradiation on the NO formation in mouse livers in vivo. According to our data phenazan and CHI inhibited this process. The evidence that the Fe^{2+}-citrate complex, another prooxidant agent, is capable of inducing iNOS biosynthesis is rather obscure. Our data demonstrate that exogenous iron may elevate the content of NO traps, thereby increasing the efficiency of NO trapping by these complexes. However, the inhibitory effect of CHI administration on these animals indicates that Fe^{2+}-citrate may partially induce the increase of NO generation through iNOS induction.

It has recently been shown that another prooxidant agent pyrogallol stimulates the synthesis of iNOS mRNA in cultured human epithelial cells [15]. The iNOS mRNA level was seen to increase in a time- and concentration-dependent fashion, with a maximum at 1–2 h.

Obviously, LPS can also stimulate iNOS synthesis in the brain of mice. The main evidence for this was that the MNIC-DETC complex formation was potentiated by LPS after a long lag period, 4 h after the LPS treatment. This conclusion is in line with the data obtained by other authors [16,17], who observed an increase in iNOS mRNA level in the brains of animals treated with various agents.

The administration of the Fe^{2+}-citrate complex was obligatory for the MNIC-DETC complex formation in the brain of adult mice. This administration could sharply increase the amount of NO traps (Fe^{2+}-DETC complexes) available in mouse brain, thereby enhancing the probability of NO trapping by these complexes. Concurrently exogenous iron could potentiate NO synthesis in the brain tissue by a mechanism which was similar to that in mouse liver. This was especially the case when the animals were treated with LPS followed by Fe^{2+}-citrate administration. The rate

of MNIC-DETC complex formation in mouse brain reached 10 nmol/g of wet brain tissue/30 min. It was equal to the rate of MNIC-DETC complex formation in mouse brain induced by injections of $NaNO_2$ (50 mg/kg) + DETC + Fe^{2+}-citrate into animals. Without the administration of Fe^{2+}-citrate complex, $NaNO_2$ induced the MNIC-DETC complex formation at a 4-fold lower rate. Therefore, if administration of the Fe^{2+}-citrate complex to mice had only increased the efficiency of NO-trapping in LPS-pretreated animals, the MNIC-DETC complex formation would have been observed in the brains of mice without the Fe^{2+}-citrate administration at a 4-fold lower rate. Since this was not the case, we suggest that the administration of Fe^{2+}-citrate complex to mice pretreated with LPS induces both an increase in the amount of NO traps in the brain and a rise in the production of NO from endogenous sources.

The results of our experiments with CHI administration to mice made it impossible to suggest that the increase in NO formation in mouse brain induced by Fe^{2+}-citrate injection was due to iNOS synthesis in this tissue. The absence of CHI effects on NO formation in mouse brain may be explained by the inability of CHI to penetrate into brain tissue in vivo.

The appearance of MNIC-DETC complexes in the brain (10 nmol/g) might have been caused by blood transfer of a small part of these complexes from the liver or other tissues to the brain. However, in the blood from mice pre-treated with LPS and Fe^{2+}-citrate complex, the rate of MNIC-DETC complex formation has been recently shown not to exceed 0.5 nmol/ml of blood/30 min [5]. The absence of correlation between the effects of CHI on the content of MNIC-DETC complexes in the liver and the brain is another argument against the forementioned proposition.

An important role of exogenous Fe^{2+} ions in the NO-trapping in rat brain has been previously emphasized [18,19]. Those authors observed a rapid increase in NO formation in this tissue induced by cerebrale ischemia. However,according to the data reported in Ref. [20], a requirement for injection of exogenous iron to observe the formation of the MNIC-DETC complexes in the brain of kainate-injected rats was not necessary. Therefore, this problem remains obscure.

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