Diphenyleneiodonium Inhibits the Cell Redox Metabolism and Induces Oxidative Stress*

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Diphenyleneiodonium (DPI) and the structurally related compound diphenyliodonium (DIP) are widely used as inhibitors of flavoenzymes, particularly NADPH oxidase. Here we report further evidence that DPI and DIP are not specific flavin binders. A 3-h incubation of N11 glial cells with DPI significantly inhibited in a dose-dependent way both the pentose phosphate pathway and the tricarboxylic acid cycle. In parallel, we observed a dose-dependent increase of reactive oxygen species generation and lipoperoxidation and increased leakage of lactate dehydrogenase activity in the extracellular medium. The glutathione/glutathione disulfide ratio decreased, whereas the efflux of glutathione out of the cells increased. This suggests that DPI causes an augmented oxidative stress and exerts a cytotoxic effect in N11 cells. Indeed, the cells were protected from these events when loaded with glutathione. Similar results were observed using DIP instead of DPI and also in other cell types. We suggest that the DPI-elicited inhibition of the pentose phosphate pathway and tricarboxylic acid cycle may be mediated by the blockade of several NAD(P)-dependent enzymes, such as glucose 6-phosphate dehydrogenase, glyceraldehyde 3-phosphate dehydrogenase, and lactate dehydrogenase. In light of these results, we think that some effects of DPI or DIP in in vitro and in vivo experimental models should be interpreted with caution.

Diphenyleneiodonium (DPI) and the structurally related compound diphenyliodonium (DIP) are widely used as uncompetitive inhibitors of flavoenzymes. Firstly identified as a hypoglycemic agent able to block gluconeogenesis and respiration in rat liver (1), DPI has been subsequently shown to inhibit the activity of NADH:ubiquinone oxidoreductase (2, 3), NADPH oxidase (4–6), nitric-oxide synthase (7), xanthine oxidase (6), and NADPH cytochrome P450 oxidoreductase (8). DPI and other iodonium derivatives have been shown to react via a radical mechanism, whereby an electron is abstracted from inhibitors or DIP in in vitro and in vivo experimental models should be interpreted with caution.

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1 The abbreviations used are: DPI, diphenyleneiodonium; DIP, diphenyliodonium; PPP, pentose phosphate pathway; G6PD, glucose 6-phosphate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; SOD, reactive oxygen species; MDA, malondialdehyde; LDH, lactate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; PBS, phosphate-buffered saline; DHEA, dehydroepiandrosterone.

In most experimental works of the last years, DPI or DIP have been used as inhibitors of NADPH oxidase. NADPH oxidases are a group of plasma membrane-associated enzymes found in a variety of cells of mesodermal origin. The most thoroughly studied is the leukocyte isoform, which catalyzes the production of superoxide (O2·−) by the one-electron reduction of oxygen, using NADPH as the reducing agent (11). The O2·− generated by NADPH oxidase serves as the starting material for the production of a vast assortment of reactive oxidants used by phagocytes to kill invading microorganisms or tumor cells (11). A low activity NADPH oxidase is present in a variety of nonphagocytic cells, wherein this enzyme is a source of second messengers. It has been postulated, for instance, that the O2·− generated by the aorta functions as a blood pressure regulator by consuming nitric oxide, a well known hypotensive agent with which it reacts at a diffusion-limited rate, and NADPH oxidases have been suggested to serve as components of oxygen sensors in various tissues (11). The large spectrum of physiopathological situations potentially involving the activation of NADPH oxidase accounts for the wide use of its inhibitors, in both in vitro and in vivo experiments. Although the effects of DPI and DIP are often accepted as evidence of NADPH oxidase inhibition, these compounds are nonspecific flavin binders. Moreover, experimental evidence suggests that the action of DPI and other iodonium-containing compounds is not restricted to flavoenzymes only. Indeed, DPI caused reversible blockade of K+ and Ca2+ currents in isolated type I carotid body cells (12) and in pulmonary smooth muscle cells (13), indicating that DPI is a nonselective ion channel blocker. Both DPI and DIP have been found to be antagonists of the N-methyl-D-aspartate receptors, through inhibition of opening processes of the ion channel (14); by this way, DPI protects neurons against glutamate toxicity (15). The above mentioned effects do not apparently involve the inhibition of flavoenzymes, such as NADPH oxidase or nitric-oxide synthase.

The pentose phosphate pathway (PPP) is a metabolic route operating in all tissues. Its first, oxidative phase converts glucose 6-phosphate into ribulose 5-phosphate and CO2, leading to the synthesis of NADPH, a redox cofactor for many antioxidant enzymes. The metabolic flux through the PPP is a sensitive index of the cell exposure to oxidant molecules since glucose 6-phosphate dehydrogenase (G6PD), which catalyzes the first step of the pathway, is activated by any oxidative stress, via a decrease of the NADPH/NADP+ ratio (16, 17). As the G6PD reaction is the rate-limiting step, the measurement of glucose flux through the PPP allows us to monitor the real levels of G6PD activity in whole cells (18). Starting from the observation in our laboratory that DPI inhibited the PPP in N11 glial cells, this work has been aimed to investigate the mechanism by
which DPI exerts such a metabolic effect and to investigate whether this effect is associated with the occurrence of an oxidative stress.

**EXPERIMENTAL PROCEDURES**

**Cells and Reagents**—The N11 mouse glial cell line was a gift from Dr. Marco Righi (CNR Institute of Neuroscience, Section of Cellular and Molecular Pharmacology, Milan, Italy). Cells were cultured up to the confluence in 100- or 25-cm² dishes with Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and then incubated for 1–6 h in the absence or presence of DPI and other reagents, before or during the assays, as described in the following paragraphs. The protein content of cell monolayers or suspensions was assessed with the BCA kit from Pierce. When not differently indicated, reagents were from Sigma.

**Measurement of Glucose Flux through PPP and Tricarboxylic Acid Cycle**—Cells were washed with fresh medium, detached with trypsin/EDTA (0.05%/0.02% v/v), washed with PBS, and resuspended at 2 × 10⁵ cells in 1 ml of Hepes buffer (in mM: 145 NaCl, 5 KCl, 1 MgSO₄, 10 Hepes sodium salt, 10 glucose, 1 CaCl₂, pH 7.4, at 37 °C) containing 2 μCi of [1-¹⁴C]glucose or [3-¹⁴C]glucose (58 and 55 mCi/mM, respectively, from Amersham/GE Healthcare). Cell suspensions were incubated in the absence or presence of DPI, menadione, and/or H₂O₂ at the concentrations and for the time periods indicated under “Results,” using a closed experimental system to trap the ¹⁴CO₂ developed from [¹⁴C]glu- trations and for the time periods indicated under “Results,” using a Magic Lite Analyzer (Ciba Corning Diagnostic Corp.) for 30 min and afterwards. For the production of ¹⁴CO₂, cells were resuspended in a quartz cuvette at 0.15 mg of cell proteins/ml in Krebs-Ringer phosphate buffer, supplemented with 5 mM glucose, 1 mM CaCl₂, and 10 μM luminol (total volume: 1 ml). The basal luminescence was recorded (Magic Lite Analyzer; Ciba Corning Diagnostic Corp.) for 30 min and expressed as cps/mg of cell proteins.

**Measurement of GSH and GSSG**—After having incubated the cells under the experimental conditions described under “Results,” intracellular and extracellular GSH and GSSG were measured spectrophotometrically as described (22) and expressed as pmol/mg of cell proteins.

**Measurement of Glycolytic and Oxidative Phosphogluconate Dependent Intermediate**—Cells were incubated with glucose 6-phosphate. However, the product of G6PD activity, 6-phosphogluconate, is largely oxidized further in the 6-phosphogluconate dehydrogenase (6PGD) reaction so that more than 1 (nearly 2) mole of NADP⁺ is reduced per each mole of glucose 6-phosphate oxidized. The assay results can be corrected for the 6PGD-mediated oxidation as follows. A first measurement is performed by adding to the assay system a saturating amount of both 6-phosphogluconate and glucose 6-phosphate; the rate of NADP⁺ reduction is the rate of both G6PD and 6PGD activities. A second assay is performed with 6-phosphogluconate only as a substrate; this procedure allows us to measure 6PGD activity alone. G6PD activity is obtained by subtracting the rate of the second assay from the rate of the first one (27). Each reaction kinetics was linear throughout the 20 min of observation. Enzymatic activity was expressed as pmol of NADP⁺ reduced/min/mg of cell proteins.

**Measurement of GAPDH and LDH Activity**—Cells were washed with fresh medium, detached with trypsin/EDTA, washed with PBS, resuspended at 0.1 × 10⁶ cells/ml in 0.1 mM Tris, 0.5 mM EDTA, pH 8.0, and sonicated on ice with two 10-s bursts. This cell lysate was supplemented with 10 mM MgCl₂ and 0.25 mM NADP⁺, and each measurement was performed on 1 ml of this reaction mix. The reaction was started at 37 °C by adding 6-phosphogluconate (0.6 mM) with or without glucose 6-phosphate (0.6 mM) and measured in a Lambda 3 spectrophotometer (PerkinElmer Life Sciences) as the increase of absorbance/min at 340 nm (27). The most commonly used assays for G6PD activity measure the rate of reduction of NADP⁺ to NADPH when a cell lysate is incubated with glucose 6-phosphate. However, the product of G6PD activity, 6-phosphogluconate, is largely oxidized further in the 6-phosphogluconate dehydrogenase (6PGD) reaction so that more than 1 (nearly 2) mole of NADP⁺ is reduced per each mole of glucose 6-phosphate oxidized. The assay results can be corrected for the 6PGD-mediated oxidation as follows. A first measurement is performed by adding to the assay system a saturating amount of both 6-phosphogluconate and glucose 6-phosphate; the rate of NADP⁺ reduction is the rate of both G6PD and 6PGD activities. A second assay is performed with 6-phosphogluconate only as a substrate; this procedure allows us to measure 6PGD activity alone. G6PD activity is obtained by subtracting the rate of the second assay from the rate of the first one (27). Each reaction kinetics was linear throughout the 20 min of observation. Enzymatic activity was expressed as pmol of NADP⁺ reduced/min/mg of cell proteins.

**RESULTS**

A 3-h incubation of N11 cells with DPI caused a dose-depend- ent inhibition of both the PPP and the tricarboxylic acid cycle (Fig. 1). The DPI-induced inhibition was also time-dependent. After an incubation with 100 μM DPI for 1, 3, and 6 h, the PPP was decreased, respectively, to 40.1 ± 3.86%, 20.42 ± 1.52%, and 10.06 ± 0.75% of control (n = 6, p < 0.001 at each point). The tricarboxylic acid cycle was 54.6 ± 2.62%, 22.96 ± 2.12%, and 4.38 ± 0.71% of control after the same incubation times (n = 6, p < 0.001 at each point). The oxidizing agents menadione and H₂O₂ doubled the rate of the PPP (Fig. 2). Also, these increases were significantly inhibited by the coinubication with...
FIG. 2. Effect of DPI on PPP activity in N11 cells, during a 3-h incubation in the absence (ctrl) or presence of 100 μM menadione (men), 10 μM H2O2, 1 μM DPI, and 100 μM DHEA. Data are presented as means ± S.E. (n = 3) versus control (ctrl) (*, p < 0.001); versus menadione (men) (○) (p < 0.001); and versus H2O2 (△) (p < 0.001). prot, protein.

FIG. 3. Effect of DPI on the intracellular (in) and extracellular (out) level of GSH and GSSG in N11 cell cultures, after a 3-h incubation in the absence (ctrl) or presence of 1, 10, and 100 μM DPI. Data are presented as means ± S.E. (n = 3) versus control (ctrl): *, p < 0.01; **, p < 0.001. prot, protein.

FIG. 4. Effect of DPI on the generation of luminol-dependent chemiluminescence and on the production of H2O2 in N11 cells. At the end of a 3-h incubation in the absence (ctrl) or presence of 1, 10, or 100 μM DPI, cells were detached, and the luminol-dependent chemiluminescence (ROS) and the level of H2O2 were measured as described under "Experimental Procedures." To verify the contribution of the superoxide anion to the chemiluminescence, aliquots of cells were incubated with superoxide dismutase (SOD) as described under "Experimental Procedures." Data are presented as means ± S.E. (n = 4) versus control (ctrl): *, p < 0.02; **, p < 0.001. prot, protein.

1 μM DPI. The G6PD inhibitor dehydroepiandrosterone (DHEA) markedly inhibited the PPP, both in resting conditions and after activation with H2O2 (Fig. 2). The tricarboxylic acid cycle was not significantly modified by menadione, H2O2, and DHEA (data not shown). The impairment of the PPP by DPI could result in a lowered content of GSH. Indeed, after a 3-h incubation, DPI dose-dependently induced in N11 cells a decrease of GSH, an increase of GSSG, and an increased efflux of both molecules (Fig. 3).

The incubation of N11 cells with DPI for 3 h elicited an increased, dose-dependent production of ROS, as determined by luminol-amplified chemiluminescence experiments and by the detection of H2O2 with the fluorescent probe A12222 (Fig. 4). The G6PD inhibitor DHEA was able to induce in N11 cells a production of ROS superimposable to that obtained after incubation with DPI (Fig. 4). The increase of chemiluminescence induced by DPI was mainly dependent on superoxide production as it was markedly inhibited by the presence of the superoxide scavenger superoxide dismutase (Fig. 4). On the other hand, DPI did not evoke any increase of luminescence or fluorescence when added to the cell suspension immediately before the measurement (data not shown). A 3-h incubation with DPI also caused a dose-dependent increase of intracellular MDA and an increased leakage of LDH activity in the extracellular medium (Fig. 5). Incubating N11 cells with 1 mM GSH for 4 h, intracellular GSH doubled, increasing from 10.65 ± 0.64 (n = 6) to 23.54 ± 0.8 pmol/mg of cell proteins (p < 0.001). A 3-h incubation of GSH-loaded cells with 100 μM DPI did not significantly increase the MDA level and LDH release (Fig. 5) and did not significantly decrease the high intracellular GSH level (22.03 ± 0.9 pmol/mg of cell proteins, n = 6). The protective role of GSH loading against the lipoperoxidative and cytotoxic effects of an oxidative stress in N11 cells was confirmed by using H2O2 as a positive control in the place of DPI (Fig. 5).

After a 1-h incubation with the lysate of N11 cells, DPI significantly inhibited in a dose-dependent way the activity of G6PD, LDH and GAPDH; 6PGD activity was decreased only by the highest DPI concentration (Fig. 6). A similar effect was observed when whole cells were incubated for 3 h in the absence or presence of DPI and then lysed and checked for the above mentioned enzyme activities (data not shown). When we measured the activity of the purified enzymes (G6PD, LDH, and GAPDH) in a cell-free system, the presence of DPI in the reaction mixture caused a dose-dependent inhibition of each enzyme as well (Fig. 7).

Since LDH catalyzes a reversible reaction and can use both NAD(H) and NADP(H) as redox cofactor, we investigated whether the DPI-induced inhibition could be influenced by the type of cofactor and by its redox state. At the lowest concentration, DPI appeared to inhibit more efficiently the LDH reaction in the presence of NADPH than NADPH, but at higher concentrations, DPI exerted a similar inhibition of the enzyme activity in each reaction mixture (Fig. 8).

**DISCUSSION**

In most experimental works of the past years, DPI and DIP have been used as inhibitors of NADPH oxidase. The Ki for inhibition of NADPH oxidase by DPI or DIP has been reported to vary, depending on the biological system investigated and the incubation time. The half-maximal inhibition of the enzyme has been obtained at DPI concentrations ranging from 0.1 μM in phorbol ester-stimulated human monocytes (28) to 5.6 μM in human neutrophils (9). The Ki for DIP does not differ signifi-
Diphenyleneiodonium Induces Oxidative Stress

FIG. 5. Effect of DPI on the production of MDA and on the extracellular release of LDH in N11 cells. Cells were incubated for 1 h in the absence or presence of 1 mM GSH and then incubated for 3 h further in the absence (ctrl) or presence of 1, 10, or 100 μM DPI or 10 μM H2O2. After this time period, cells were checked for their MDA content, whereas LDH activity was measured on 100 μl of the supernatant, as described under “Experimental Procedures.” Data are presented as means ± S.E. (n = 3) versus control: *, p < 0.05; **, p < 0.02; ***, p < 0.005; versus the corresponding experimental condition without GSH loading: ◇, p < 0.05; ◇◇, p < 0.005. prot, protein.

FIG. 6. Effect of DPI on the activity of purified G6PD, 6PGD, LDH, and GAPDH in N11 cells. The cell lysates were incubated for 1 h in the absence (ctrl) or presence of 1, 10, or 100 μM DPI and, after this time period, were checked for the above mentioned enzyme activities, as described under “Experimental Procedures.” Data are presented as means ± S.E. (n = 3) versus control: *, p < 0.02; **, p < 0.001. prot, protein.

FIG. 7. Effect of DPI on the activity of purified G6PD, LDH, and GAPDH. The reaction mixtures (see “Experimental Procedures”) were checked for the above mentioned enzyme activities, in the absence (ctrl) or presence of 1, 10, or 100 μM DPI, added immediately before performing the measurement. Data are presented as means ± S.E. (n = 3) versus respective control: *, p < 0.005; **, p < 0.001.

FIG. 8. Effect of DPI on the activity of purified LDH. Four reaction mixtures were prepared, containing different substrates: NADH + pyruvate (NADH+pyr), NADPH + pyruvate (NADPH+pyr), NAD+ + lactate (NAD+lact), NADP+ + lactate (NADP+lact) (see “Experimental Procedures”). In each of these reaction mixtures, the enzyme activity was checked in the absence (ctrl) or presence of 1, 10, or 100 μM DPI, added immediately before performing the measurement. Data are presented as means ± S.E. (n = 3) versus respective control (ctrl): *, p < 0.05; **, p < 0.001.

DPI significantly inhibited both basal and oxidative stress-stimulated PPP, ruling out that DPI inhibits the PPP activity by blocking NADPH oxidase and suggesting that such inhibition is exerted at a different level, more likely at the G6PD step. Indeed, DHEA, a potent uncompetitive inhibitor of G6PD (34) or with H2O2; in both conditions, the ROS synthesis was significantly from that of DPI (30). To achieve a complete inhibition of NADPH oxidase, a concentration range of 1–100 μM DPI and, after this time, phenomenon has usually been thought to be the consequence of the reduced production of O2− by NADPH oxidase. To check whether this mechanism was operating in N11 cells, we measured the PPP activity in cells stimulated with menadione (which exerts an oxidative stress by generating ROS through its redox cycling and by forming a conjugate with glutathione) (35, 36), significantly inhibited the PPP and abolished its activation by H2O2, confirming that also in N11 cells, G6PD is the rate-limiting step in the PPP and that the PPP measurement is a useful tool to monitor the actual G6PD activity in whole cells (18).

GSH is the most important antioxidant agent in the cells,
Diphenyleneiodonium Induces Oxidative Stress

where it is present at mM concentrations. In the presence of oxidant agents, GSH is oxidized in the place of lipids, proteins, and nucleic acids; its regeneration is obtained thanks to glutathione reductase, which uses the NADPH produced in the PPP as a reducing cofactor. A decrease of the PPP potential may impair the ability of the cell to oppose oxidative stress. As oxidant molecules are also produced relentlessly in physiological conditions, as a consequence of cell metabolic activity, the inhibition of the PPP by DPI may account for the dose-dependent decrease of GSH, increase of GSSG, and efflux of both molecules induced by DPI in N11 cells. Interestingly, it has recently been reported that DPI decreases intracellular GSH in T24 bladder carcinoma cells, and this effect has been suggested to account for the ability of DPI to sensitize T24 cells to Fas-mediated cell death (37); the decrease of GSH content was attributed to an augmented efflux of glutathione as the GSH/GSSG ratio did not change. Differently, we have observed that DPI, besides promoting the efflux of GSH, also elicits an increased oxidation of GSH into GSSG, leading to a change of GSH/GSSG ratio. This difference may be attributable to the different cell types investigated; T24 cells could be more efficient than N11 cells in extruding GSH before it is oxidized. Anyway, it is interesting that the effect of DPI on the intracellular level of GSH in the two cell types appears to be very similar.

The inhibition of the PPP and the decrease of intracellular GSH are likely to favor the onset of a more oxidizing intracellular environment. If so, we could expect that cells incubated with DPI exhibit signs of increased oxidative stress and of cytotoxicity. Indeed, when N11 cells were incubated with DPI, a significant production of ROS was detected. The marked decrease of ROS generation observed with the chemiluminescence experiments in the presence of superoxide dismutase suggests that DPI induces the formation of the superoxide anion, which subsequently dismutates to hydrogen peroxide. Indeed, H$_2$O$_2$ production was augmented by the incubation with DPI. When DPI was added to the cells immediately before the measurement, no increase of ROS generation was observed; this suggests that the induction of ROS generation by DPI needs the time necessary for the PPP flux and GSH level to decrease significantly, thus leading to a progressive inability of the cell to counteract the ROS coming from cell metabolism. The G6PD inhibitor DHEA induced a ROS generation similar to that observed with DPI under the same experimental conditions, confirming that the inhibition of G6PD is sufficient to elicit an oxidative stress. DPI also increased the level of intracellular MDA, a lipid peroxidation marker, and the leakage of LDH activity in the extracellular medium, a sensitive index of the loss of cell membrane integrity. The preventive role played by GSH against DPI-elicted lipoperoxidation and cytotoxic effect was demonstrated by loading N11 cells with GSH. In GSH-loaded cells, the incubation with the highest concentration of DPI did not significantly increase the MDA level and LDH release; the cell loading with GSH also exerted a protective effect against the direct oxidative stress exerted by H$_2$O$_2$. The absence of DPI effects was in agreement with the inability of this compound to decrease significantly the intracellular GSH in GSH-loaded cells. This suggests that the effect of DPI on the intracellular level of GSH is not direct, but rather mediated by the blockade of the PPP, and that the inhibition of the PPP is harmful for the cells by depleting GSH. When the cells were provided with exogenous GSH, the inhibition of the PPP did not exert toxic effects.

To clarify the mechanism by which DPI inhibits the metabolic activity in N11 cells, we measured the activity of some NADP- and NAD-dependent oxidoreductases in the lysate of N11 cells incubated with different concentrations of DPI; G6PD, LDH, and GAPDH were significantly inhibited by DPI. The same inhibitory effect was observed on the activity of purified G6PD, LDH, and GAPDH. These results suggest that DPI inhibits the PPP by blocking the activity of its regulatory enzyme, G6PD. Furthermore, NAD-dependent enzymes such as LDH and GAPDH are also inhibited. The subsequent blockade of glycolysis could, at least partly, account for the inhibition of glucose flux through the tricarboxylic acid cycle, in addition to the already described impairment of the cell respiratory activity (2, 3). The inhibition of LDH was observed in the presence of both NAD(H) and NADP(H).

The above mentioned consequences of DPI incubation could be reproduced in N11 cells with DIP as well (data not shown), confirming that these two compounds share a common metabolic effect. Furthermore, we could reproduce the DPI-elicted inhibition of the PPP, production of MDA and ROS, and release of LDH in other cell types, i.e. murine alveolar macrophages MH-S, human monocyte-like U937 cells, and human erythrocytes (data not shown), thus confirming that these DPI effects are not restricted to N11 cells.

Taken as a whole, our data suggest that DPI inhibits the cell metabolic activity not only by blocking the mitochondrial NADH dehydrogenase and other flavoenzymes but also by impairing the activity of NAD(P)-dependent enzymes. The concentration of DPI necessary to achieve the half-maximal inhibition of G6PD, LDH, and GAPDH appears to be around 10 $\mu$M, a concentration 2–10-fold higher than the $K_i$ reported for NADPH oxidase but within the concentration range usually utilized in previous reports. A consequence of this effect is the inhibition of one of the main antioxidant pathways of the cells, i.e. the PPP, leading to the onset of an oxidative stress. This interference with redox metabolism poses serious caveats to the interpretation of results from experiments performed with DPI and its derivative DIP, in particular those aimed to investigate the role of NADPH oxidase in any in vivo and in vitro experimental system. Indeed, some effects of DPI are attributed to the reduced production of ROS via NADPH oxidase. When this enzyme is activated by several stimuli, such as bacterial endotoxin or phorbol esters, DPI has been found to be effective in inhibiting the NADPH oxidase-mediated ROS generation (11). Our results provide a new mechanism by which DPI could inhibit the respiratory burst, i.e. by decreasing the availability of NADPH as a consequence of the PPP inhibition. On the other hand, by this mechanism, DPI is capable of decreasing intracellular GSH and increasing lipoperoxidation. This is not the only work suggesting that DPI elicits an oxidative stress. Recently, it has been observed that DPI can cause apoptosis in rat heart cultured cells via induction of mitochondrial superoxide production (38) and induces in rat pulmonary aortic endothelial cells the transcription of heme oxygenase-1, a gene very sensitive not only to hypoxia but also to oxidative stress (39). Furthermore, in porcine aortic endothelial cells DPI potentiated the generation of ROS, both basally and after stimulation with vascular endothelial growth factor (29). Our results could then provide an explanation to such observations, reporting a “paradoxical” induction of oxidative stress by DPI. It is likely that, in conditions wherein NADPH oxidase is not maximally activated, the prevailing effect of DPI is to stimulate, rather than to inhibit, ROS generation. For this reason, we think that other inhibitors than DPI and DIP should be used in experimental systems aimed to block the activity of NADPH oxidase and other flavoenzymes.

REFERENCES

1. Holland, P. C., Clark, M. G., Bloxham, D. P., and Lardy, H. A. (1973) J. Biol. Chem. 248, 6050–6056
2. Gatley, S. J., and Sherratt, S. A. (1976) Biochem. J. 158, 307–315
Diphenyleneiodonium Induces Oxidative Stress

3. Ragan, C. I., and Bloxham, D. P. (1977) Biochem. J. 163, 605–615
4. Cross, A. R., and Jones, O. T. (1986) Biochem. J. 237, 111–116
5. Cross, A. R. (1987) Biochem. Pharmacol. 36, 489–493
6. Cross, A. R., and Jones, O. T. (1986) Biochem. J. 237, 111–116
7. Cross, A. R. (1987) Biochem. Pharmacol. 36, 489–493
8. Doussiere, J., and Vignais, P. V. (1992) Eur. J. Biochem. 208, 61–71
9. Stuehr, D. J., Fasehun, O. A., Kwon, N. S., Gross, S. S., Gonzalez, J. A., Levi, R., and Nathan, C. F. (1991) J. Exp. Med. 176, 1033–1041
10. Mohanty, J. G., Jaffe, J. S., Schulman, E. S., and Raible, D. G. (1997) J. Immunol. Methods 202, 133–141
11. Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) Free Radic. Biol. Med. 11, 81–128
12. Gerard-Monnier, D., Erdelmeier, I., Regnard, K., Moze-Henry, N., Yadan, J. C., and Chaudiere, L. (1998) Chem. Res. Toxicol. 11, 1176–1183
13. Beutler, E. (1975) Red Cell Metabolism: A Manual of Biochemical Methods, Grune & Stratton, New York and London
14. Shuto, M., Ogita, K., and Yoneda, Y. (1997) Neurosci. Lett. 31, 73–82
15. Nakamura, Y., Tsuji, K., Shuto, M., Ogita, K., Yoneda, Y., Shimamoto, K., Shibata, T., and Kataklo, K. (1997) Neuroscience 66, 459–466
16. Luzzatto, L. (1987) Biochem. Biophys. Acta 146, 18–25
17. Eggleston, L. V., and Krebs, H. A. (1974) Biochem. J. 138, 425–435
18. Spolarics, Z. (1998) J. Leukocyte Biol. 63, 534–541
19. Pescarmona, G. P., Bosia, A., Arrese, P., Sartori, M. L., and Ghigo, D. (1982) Int. J. Biochem. 14, 245–245
20. Riganti, C., Aldieri, E., Bergandi, L., Fenoglio, I., Costamagna, C., Fubini, B., Bosia, A., and Ghigo, D. (2002) Free Radic. Biol. Med. 32, 938–949
21. Rieger, D., Loskutoff, N. M., and Betteridge, K. J. (1992) Reprod. Fertil. Dev. 4, 547–557
22. Vandeputte, C., Guizzoni, I., Genestie-Denis, I., Vannier, B., and Lorenzen, G. (1994) Cell Biol. Toxicol. 10, 415–421
23. Schwarzer, E., Turrini, F., Ulliers, D., Giribaldi, G., Ginsburg, H., and Arese, P. (1995) J. Exp. Med. 176, 1033–1041
24. Mohanty, J. G., Jaffe, J. S., Schulman, E. S., and Raible, D. G. (1997) J. Immunol. Methods 202, 133–141
25. Esterbauer, H., Schaur, R. J., and Zollner, H. (1991) Free Radic. Biol. Med. 11, 81–128
26. Gerard-Monnier, D., Erdelmeier, I., Regnard, K., Moze-Henry, N., Yadan, J. C., and Chaudiere, L. (1998) Chem. Res. Toxicol. 11, 1176–1183
27. Beutler, E. (1975) Red Cell Metabolism: A Manual of Biochemical Methods, Grune & Stratton, New York and London
28. Shuto, M., Ogita, K., and Yoneda, Y. (1997) Neurosci. Lett. 31, 73–82
29. Nakamura, Y., Tsuji, K., Shuto, M., Ogita, K., Yoneda, Y., Shimamoto, K., Shibata, T., and Kataklo, K. (1997) Neuroscience 66, 459–466
30. Luzzatto, L. (1987) Biochem. Biophys. Acta 146, 18–25
31. Eggleston, L. V., and Krebs, H. A. (1974) Biochem. J. 138, 425–435
32. Spolarics, Z. (1998) J. Leukocyte Biol. 63, 534–541
33. Pescarmona, G. P., Bosia, A., Arrese, P., Sartori, M. L., and Ghigo, D. (1982) Int. J. Biochem. 14, 245–245
34. Riganti, C., Aldieri, E., Bergandi, L., Fenoglio, I., Costamagna, C., Fubini, B., Bosia, A., and Ghigo, D. (2002) Free Radic. Biol. Med. 32, 938–949
35. Rieger, D., Loskutoff, N. M., and Betteridge, K. J. (1992) Reprod. Fertil. Dev. 4, 547–557
36. Vandeputte, C., Guizzoni, I., Genestie-Denis, I., Vannier, B., and Lorenzen, G. (1994) Cell Biol. Toxicol. 10, 415–421
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