0014-4754/96/040336-04$1.50 + 0.20

Effect of various stressors on the level of lipid peroxide, antioxidants and Na^+, K^+-ATPase activity in rat brain

A. A. Shaheen*, A. A. Abd El-Fattah and M. Z. Gad

Department of Biochemistry, Faculty of Pharmacy, Cairo University, Cairo (Egypt), Fax +20 2362 4105
Received 12 April 1995; received after revision 8 August 1995; accepted 29 August 1995

Abstract. The level of malondialdehyde (MDA), an index of lipid peroxidation, and the antioxidants superoxide dismutase (SOD) and glutathione (GSH), as well as the activity of Na^+, K^+-ATPase, were assessed in whole rat brain after immobilization, anemic hypoxia ($NaNO_2$) and 72 h starvation. The effect of these stressors on plasma glucose and corticosterone levels was also observed. Hypoxia and starvation stimulated the lipid peroxide formation in brain as indicated by an increase in the level of MDA, being higher after starvation than hypoxia. Brain SOD activity was also increased in response to hypoxia and starvation while GSH content was only diminished in hypoxia. However, neither MDA nor antioxidants were affected by immobilization. On the other hand, the activity of brain Na^+, K^+-ATPase was significantly increased by immobilization and hypoxia but decreased in starvation. A similar pattern of change was also observed in plasma glucose and corticosterone levels in response to these stressors. These results elucidate differences in the biochemical response of animals towards various types of stress, with increased lipid peroxide formation in hypoxia and starvation.
Key words. Stress; hypoxia; immobilization; starvation; lipid peroxide; antioxidants; Na^+, K^+-ATPase.

Hypoxia, immobilization and starvation are among the stressful physical stimuli applied to experimental animals. Acute exposure to a stressor produces a wide range of biochemical changes in an organism[1–4]. Oxygen free radicals and other reactive oxygen species are postulated by many investigators to be among the causal factors for those biochemical changes. One of the most common effects of an exacerbated free radical formation in living tissues is the peroxidation of polyunsaturated fatty acids. Increased lipid peroxide formation has been demonstrated in several tissues in stressed rats[5–9], suggesting that free radical generation is increased under stress. Unfortunately, the high lipid content of brain may render it highly susceptible to oxidative attack under stress. Concomitant disturbances in cellular antioxidant defense mechanisms and other normal cell functions were also demonstrated in hypoxia and other physical stress conditions[10–13]. Based on these findings, the aim of the present study was to investigate the effect of three models of stress (hypoxia, immobilization and starvation) on the level of antioxidants, superoxide dismutase (SOD) activity and glutathione (GSH), as well as the content of malondialdhyde (MDA) as an index of lipid peroxidation in rat-whole brain. The study was also extended to assess the degree of disturbance in neural activity under stress by measuring the whole brain Na^+, K + ATPase activity, an enzyme involved in the reuptake of physiologically released biogenic amines neurotransmitters[14]. Assessment of plasma glucose and corticosterone levels were also included to reflect the degree of stress achieved.

Materials and methods

Animals and experimental designs. Male Wistar albino rats (200–250 g) bred at the National Scientific Research Center Lab. Cairo, Egypt, were kept in groups of eight per cage under controlled environmental conditions (temperature 25 °C, lights on from 06.00–18.00). They had access to rat chow and water ad libitum. The animals were subjected to either of the following stress conditions:

a) *Hypoxia*: anemic hypoxia was induced chemically by injecting the rats subcutaneously with 15 mg $NaNO_2$/100 g body weight 20 min before decapitation[12]. MacMillan[15] had shown that chemical hypoxia provides an accurate model for hypoxic hypoxia.

b) *Immobilization*: the animals were subjected to immobilization stress by enclosing the rats in plastic meshed jars, fit to their individual body size, for one hour, so that their head, tail and limbs movements were minimized while breathing was not restricted[16].

c) *Starvation*: animals were deprived of all food but not water for 72 h[17]. The last group of animals was kept away from any stressful stimuli and served as control for the other groups.

Tissue sampling and methods. At the end of the experimental period of stress, the animals from the above groups were killed by decapitation at 10–12 a.m. The blood was collected in heparinized centrifuge tubes to separate plasma, in which glucose[18] and corticosterone[19]

* Corresponding author.

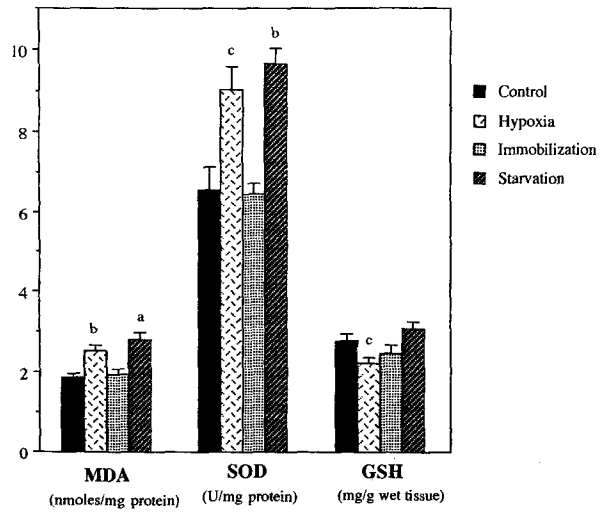

Figure 1. Malonaldehyde (MDA) and antioxidant (SOD and GSH) levels in rat brain after hypoxia, immobilization and starvation. Values shown are means ± SE for 6–7 animals in each group. Significant difference from control at $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.05$ (using Student's t-test).

levels were estimated. Meanwhile, whole brains were rapidly removed on an ice-cooled mixture ($CaCl_2$, NaCl and ice at −55 °C). The weighed brain was homogenized in ice-cold bidistilled water (20% w/v). Each brain homogenate was divided into 3 portions and treated differently. The first portion was mixed with phosphate buffer (50 mmol/L, pH 7.8) to reach a final concentration of 5% w/v. This was centrifuged at 600 × g for 10 min at 4 °C. In the supernatant, lipid peroxide was ascertained by measuring malondialdehyde (MDA) content[20] which was expressed as nmol MDA/mg protein using 1,1,3,3-tetraethoxypropane as a standard. The second centrifugation was done at 105,000 × g for 15 min at 4 °C to separate the cytosolic fraction in which the activity of SOD was estimated[21,22]. The activity was expressed as units/mg protein (one unit is the amount of enzyme that causes half maximal inhibition of nitroblue tetrazolium reduction). The second portion of brain homogenate was mixed with ice-cold 0.32 mol/L sucrose buffered with 50 mmol/L Tris HCl pH 7.4 at a concentration of 4% w/v. It was then centrifuged at 600 × g for 10 min at 4 °C. In the supernatant, the ouabain-sensitive Na^+, K^+-ATPase was assayed using the method of Post and Sen[23] and Lowry and Lopez[24]. Na^+, K^+-ATPase activity was calculated by subtracting the ATPase activity assayed with ouabain from that assayed without ouabain. Enzyme activity is expressed as μmoles P_i released/hour/mg protein. The protein content of the above supernatants was estimated by the method of Lowry et al.[25]. The third portion of brain homogenate was deproteinized with ice-cold 6% (w/v) m-phosphoric acid. The supernatant after centrifugation was used for estimation of GSH level[26].

Statistics. Statistical analysis of data was carried out using Student's t-test.

Results and discussion

In the present study, lipid peroxidation was increased in rat brain after hypoxia and 72 h starvation, as indicated by the significant increase in brain MDA level. The highest increase was observed after starvation (fig. 1). These observations are in agreement with previous reports which demonstrated an increase in the level of lipid peroxide in whole-brain homogenates, synaptosomal and mitochondrial fractions of hypoxic rats[27,28], as well as in the other tissues of starved rats[6,29]. In hypoxia and other related disorders, free radicals have been implicated as important pathogenic factors in their pathogenesis[30]. Hypothetically, the mechanisms by which the free radicals are generated during hypoxia are complex and depend on multiple interacting factors: a) shortage of O_2 at the cytochrome oxidase step may give rise to leakage of partially reduced oxygen species; b) rapid fall in cellular ATP, due to diminished aerobic oxidation, may result in the alteration of ionic transport with cytosolic calcium overload. This elevates AMP concentration and increases its catabolism to inosine and hypoxanthine, a substrate for xanthine oxidase[31,32]. Free radicals generated by those disorders attack the membrane phospholipids, causing their peroxidation. Peroxidation of membrane phospholipids is considered to be a fundamental aspect of free radical damage in brain due to its high lipid content[30]. These peroxidative processes in the brain are surely contributory to the inactivation of many membrane-bound biomolecules such as enzymes, since phospholipids are important for the optimum activity of many enzymes. On the other hand, increased catabolic processes in starvation appear to be the main mechanism underlying the generation of free radicals and lipid peroxidation in brain of starved rats.

It was also noticeable that the elevation of MDA level in the brain of hypoxic and starved rats was accompanied by a significant increase in SOD activity, with a significant decrease in brain GSH only after hypoxia. An unexpected finding was the lack of a significant change in GSH content in the brain of starved rats (fig. 1). The observed increase in brain SOD activity seems to be an adaptive response to conditions of increased peroxidative stress. Reactive oxygen species such as O_2^-. have been reported to act as inducers of tissue SOD[33]. However, the decrease in GSH content after hypoxia was interpreted as being caused by its extensive utilization in scavenging the free radicals. In contrast to hypoxia and starvation, immobilization of rats for one hour affected neither the MDA content nor the measured antioxidants (fig. 1). Although our results failed to provide evidence for increased free radical

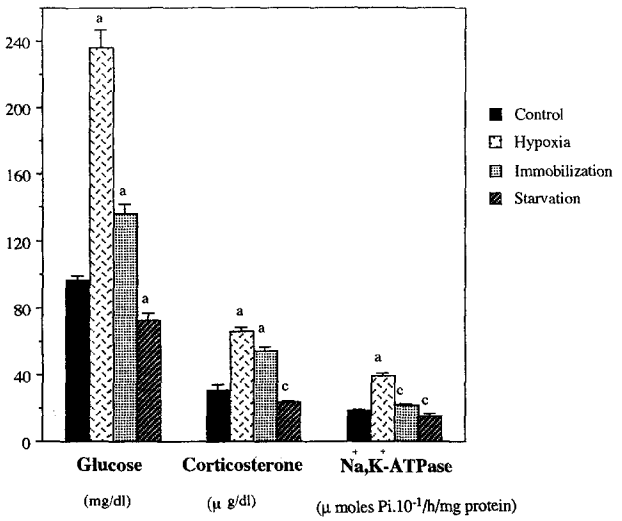

Figure 2. Plasma glucose and corticosterone levels, and brain Na^+, K^+-ATPase activity of rats after hypoxia, immobilization and starvation. Values shown are means ± SE for 6–8 animals in each group. Significant difference from control at $^a p < 0.001$, $^c p < 0.05$ (using Student's t-test).

production in immobilization, in contrast to previous reports[34,35], they do not exclude the possibility that increased amounts of free radicals may have been generated, but scavenged by high concentrations of brain catecholamines evoked by immobilization[36]. Monoamines and related metabolites have previously been shown to scavenge free radicals, inhibit lipid peroxidation, and chelate metal ions, slowing their participation in lipid peroxide reactions[37].

The results of the present study also revealed that both hypoxia and immobilization caused significant increases in plasma glucose and corticosterone levels (fig. 2). These results clearly showed that the corticosterone response to stress was related to the intensity of the stressor to which the animals were subjected. Thus, plasma corticosterone evoked in response to hypoxia is greater than to immobilization. The increase in plasma corticosterone of hypoxic rats was in agreement with the previous results that showed an elevation of blood glucocorticoids in rabbit[38] and humans[39] subjected to hypoxic conditions. The elevation of plasma corticosterone in response to immobilization or hypoxia could be explained by a stimulation of the hypothalamopituitary-adrenal axis, that will trigger the release of corticotropic releasing factor from hypothalamus with a subsequent increase in ACTH secretion[40]. The results also showed that plasma glucose is strongly influenced by the intensity of the stressor. It is higher in hypoxia than in immobilization, since plasma glucose is strongly influenced by glucocorticoids[41]. In contrast to the rise in plasma glucose and corticosterone levels of hypoxic and immbolized rats, starvation of rats for 72 h lowered these two parameters significantly (fig. 2). Such a response of animals to food deprivation is an adaptive mechanism to suppress the stress-induced rise in catabolic hormones, saving energy expenditure[42].

The results also showed that immobilization of the rats for one hour or their exposure to anemic hypoxia caused a significant increase in the activity of brain Na^+, K^+-ATPase. The rise was more pronounced in response to hypoxia than to immobilization (fig. 2). In stressful conditions, such as immobilization and hypoxia, there is an increase in the release and turnover of biogenic amines and excitatory amino acid neurotransmitters[36,43,44]. Biogenic amines have previously been shown to stimulate brain Na^+, K^+-ATPase activity both in vitro[45] and in vivo[46]. The increased neural excitability associated with marked changes in electrolyte distribution in the brain of stressed rats may account for the increased activity of brain Na^+, K^+-ATPase in this study. In contrast, starvation for 72 h caused a decrease in brain Na^+, K^+-ATPase activity (fig. 2). In starvation, the increased lipid peroxide formation (fig. 1) could disturb the anatomical integrity of the biomembrane and diminish its fluidity leading to inhibition of several membrane-bound enzymes including Na^+, K^+-ATPase. Ohta et al.[47] demonstrated the role of lipid peroxidation and the decrease of membrane fluidity in inhibition of membrane bound Ca^{2+}-ATPase activity of the intestinal brush-border membrane. Thus, the inhibition of ion channel conductance in brain of starved animals has a significant effect on metabolic cost and may be an important mechanism to reduce energy expenditure during food deprivation.

In conclusion, the present study revealed quite distinct variations in the biochemical response of the animal toward various types of stress. The starved animal metabolism is directed toward minimizing energy expenditure as indicated by reduced catabolic hormone and blood sugar levels as well as decreased brain Na^+, K^+-ATPase activity. Hypoxia and immobilization, on the other hand, are associated with enhancement of metabolic processes as reflected by elevation of catabolic hormones and blood sugar levels as well as increased brain Na^+-K^+-ATPase activity. It is also noteworthy that no correlation exists between the elevation of lipid peroxide level and other parameters in the brain of hypoxic and starved rats.

1 Kopin, I. J., Lacke, C. R., and Ziegler, M., Ann. intern. Med. *88* (1978) 671.
2 Thurston, J. H., Hauhart, R. E., and Nelson, J. S., Metab. Brain Dis. *2* (1987) 223.
3 Adell, A., Garcia-Marquez, C., Armario, A., and Gelpi, E., J., Neurochem. *50* (1988) 1678.
4 Armario, A., Montero, J. L., and Balasch, J., Physiol. Behav. *37* (1986) 559.
5 Alessio, H. M., and Goldfarb, A. H., J. appl. Physiol. *64* (1988) 1333.
6 Hidalgo, J., Gampmany, L., Barras, M., Garvey, J. S., and Armario, A., Am. J. Physiol. *255* (1988) ES 18.

7 Hidalgo, J., Gasull, T., Garcia, A., Blanquez. A., and Armario, A., Horm. Metab. Res. *23* (1991) 104.
8 Halliwell, B., Drugs, *42* (1991) 570.
9 Halliwell, B., FASEB J. *1* (1987) 358.
10 Guliaeva, N. V., and Levshina, I. P., Biul. EK sp. Biol. Med. *106* (1988) 153.
11 Reuter, A., and Klinger, W., Expl Toxicol. Pathol. *44* (1992) 339.
12 Gibson, G. E., Shimada, M., and Blass, J. P., J. Neurochem. *31* (1978) 757.
13 Graham, E., Mishra, O. P., and Delivoria-Papadopoulos, M., Neuroscience Lett. *153* (1993) 93.
14 Tissari, A. H., Schonnoler, P. S., and Bogdanski, B. B., Molec Pharmac. *5* (1969) 593.
15 MacMillan, V., Brain Res. *121* (1977) 271.
16 Masatoshi, T., Yasuko, K., and Ryoichi, N., Neurosciences (Kobe, Japan) *5* (1979) 34.
17 Wohaieb, S. A., and Godin, D. V., Diabetes *36* (1987) 169.
18 Tinder, P., J. clin. Path. *22* (1969) 246.
19 Mattingly, D., J. clin. Path. *15* (1962) 374.
20 Uchiyama, M., and Mihara, M., Analyt. Biochem. *86* (1978) 271.
21 Winterbourn, C. C., Hawkins, R. E., Brain, M., and Carrell, R. W., J. Lab. clin. Med. *85* (1975) 337.
22 Maral, J., Pugget, K., and Michelson, A. M., Biochem. biophys. Res. Commun. *77* (1977) 1525.
23 Post, R. L., and Sen, A. K., Methods in Enzymology, Vol. 10, p. 762. Eds. S. P. Colowick and N. O. Kaplan. Academic Press, New York 1967.
24 Lowry, O. H., and Lopez, J. A., J. biol. Chem. *162* (1946) 421.
25 Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. L., J. biol. Chem. *193* (1951) 265.
26 Beutler, E., Duron, O., and Kelly, B. M., J. Lab. clin. Med. *61* (1963) 882.
27 Minyailenko, T. D., Pozharov, V. P., and Seredenko, M. M., Chem. Phys. Lipids *55* (1990) 25.
28 Dzhafarov, A. I., Magomedov, N. M., Babaev, K. F., Akhemedova, G. S., and Bekhbudova, Z. A., Vop. med. Khim *35* (1989) 51.
29 Asayama, K., Hayashibe, H., Dobashi, K., Niitsu, T., Miyao, A., and Kato, K., Diabetes Res. *12* (1989) 85.
30 Siesjo, B. K., Agardh, C. D., and Bengtsson, F., Cerebrovas Brain Metab. Rev. *1* (1989) 165.
31 Vanello, A., Di Giacomo, C., Sorrenti, V., Campisi, A., Castorina, C., Pinturo, R. Chiarenza, G., and Perez-Polo J. R., Pharmacology of Cerebral Ischemia, p. 311. Eds J. Krieglstein and M. Oberpichler. Wissenschaftliche Verlagsgesellschaft GmbH, Stuttgart 1990.
32 Siesjo, K., Lundgren, J., and Pahlmark, K., Pharmacology of Cerebral Ischemia, p. 319. Eds. J. Krieglstein and H. Oberpichler. Wissenschaftliche Verlagsgesellschaft GmbH, Stuttgart 1990.
33 Matkovics, B. Superoxide and Superoxide Dismutase, p. 501. Eds A. M. Michelso, J. M. McCord, and I. Fridovich. Academic Press, New York 1977.
34 Melkonyan, M. M., Araratyan, E. A., Mikaelyan, E. M., and Mkhitaryan, V. G., Zh. EK sp. Klin. Med. *18* (1978) 25.
35 Mikaelyan, E. M., Mkrtchyan, S. L., Melik-Agaeva, E. A., and Mkhitaryan, V. G., Zh. EK sp. Klin. Med. *23* (1983) 407.
36 Ida, Y., Tanaka, M., Tsuda, A., Tsujimaru, S., and Nagasaki, N., Life Sci. *37* (1985) 2491.
37 Liu, J., and Mori, A., Arcs Biochem. Biophys. *302* (1993) 118.
38 Sridharan, K., Patil, S. K. B., Upadhyay, T. N., and Mukherjee, A. K., Horm. Metab. Res. *23* (1991) 62.
39 Bobrovnitskii, I. P., Petrova, T. V., Yakovleva, I. P., and Kalinkin, S. V., Kosm. Biol. Aviakosm. Med. *25* (1991) 43.
40 Holzbauer, M., Mitchell, B., and Youdim, M. B. H., in: Catecholamines and Stress, p. 321. Eds E. Usidin, R. Kvetnansky, and I. J. Kopin. Pergamon Press, New York 1975.
41 Newsholme, E. A., Clin. Endocr. Metab. *5* (1976) 543.
42 Wronska, D., Niezgoda, J., Sechman, A., and Bobek, S., Physiol. Behav. *48* (1990) 531.
43 Broderick, P. A., and Gibson, G. E., Metab. Brain Dis. *4* (1989) 143.
44 Phillis, J. W., and Walter, G. A., Neuroscience Lett. *106* (1989) 147.
45 Logan, J. G., and O'Donovan, D. J., J. Neurochem. *27* (1976) 185.
46 Sastry, B. S., and Phillis, J. W., Can. J. Physiol. Pharmacol. *55* (1977) 170.
47 Ohta, A., Mohri, T., and Ohyashiki, T., Biochem. biophys. Acta *984* (1989) 151.