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THE ROLE OF PREDNISOLONE AND EPINEPHRINE ON GASTRIC TISSUE AND ERYTHROCYTE ANTIOXIDANT STATUS IN ADRENALECTOMIZED RATS

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It has been believed that overproduction of free radicals and/or deficiency of antioxidant systems, and stress hormones may play a role in etiopathogenesis of many diseases, including gastric ulcer. This study evaluated whether there was an effect of adrenalectomy on lipid peroxidation [malondialdehyde (MDA)] and antioxidant [superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione (GSH) levels] systems in gastric tissue and erythrocyte in rats. As well, the impacts of administration of prednisolone and epinephrine on these systems in adrenalectomized rats were investigated. Thirty-three rats were randomly grouped as sham-operated (group I), adrenalectomized (group II), adrenalectomized + prednisolone (group III) and adrenalectomized + epinephrine (group IV). After experimental procedures, blood and gastric tissues samples were taken from each animal in all groups. Colorimetric assays were employed to determine gastric tissue and erythrocyte levels of MDA and GSH, and SOD and GPX activities. Adrenalectomy in group II rats caused a marked decrease of SOD and GPX activities and MDA levels, and an increase of GSH levels in gastric tissue and erythrocyte, when compared to sham-operated rats. However, especially epinephrine injection after adrenalectomy resulted in a significantly increase of measured antioxidant enzyme activities and GSH levels in both gastric tissue and erythrocyte. These results indicate that adrenalectomy appeared to alter the levels of antioxidants and lipid peroxidation product in gastric tissue and erythrocyte. Thus, the present study provides a physiological regulatory role of adrenal gland in the maintenance of oxidant/antioxidant balance in gastric tissue and erythrocyte.

Key words: *adrenalectomy, antioxidants, lipid peroxidation, epinephrine, prednisolone*

INTRODUCTION

The antioxidant enzymes and molecules with free radical scavenger properties in gastric tissue have an important role in the protection of gastric mucosal integrity in addition to the mucus, bicarbonate and prostaglandins secreted from surface epithelial cells of gastric tissue (1). The overproduction of free radicals and/or shortage of antioxidant system, and stress hormones may play a role in the etiopathogenesis of many diseases, including gastric ulcer (2).

Adrenal gland is known to be crucial in continually maintaining body homeostasis. Catecholamines and glucocorticoids, secreted by the adrenal gland, are important hormones in the preservation of physiological functions in the digestive tract, especially of the stomach (3,4). Recently, the corruptive roles of adrenalectomy in stress-induced gastric ulceration in the rat experiments have also been reported (5-9). However, the mechanism of adrenalectomy-related gastric injury has not been completely understood. Free radicals have been previously stated to be involving in gastric ulcers (10,11). In addition, various antioxidant molecules and antioxidant enzymes defend the gastric mucosa from oxidative stress. Significant decreases of superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities were declared in marginal mucosa of human gastric ulcers (12,13) and experimental rat ulcers (14). For this reason, we think that hormones secreted by the adrenal glands may have a regulatory function on the antioxidant system and induction of lipid peroxidation in gastric tissue. Although the importance of adrenal hormones on antioxidant cellular functions in a number of cells such as macrophages, neuronal and endothelial cells has previously been reported (15-17), their actions on antioxidant molecules or enzyme activities in gastric tissue have not been investigated. Therefore, we planned to investigate whether there was an effect of adrenalectomy on antioxidant molecules [e.g., SOD, GPX and glutathione (GSH)] and lipid peroxidation product (e.g., malondialdehyde, MDA) in rat gastric tissue and erythrocyte. As well, the impacts of administration of prednisolone and epinephrine on these oxidative stress parameters in adrenalectomized rats were investigated.

MATERIALS AND METHODS

Thirty three male Wistar rats, aged three months and weighing 210-250 g were operated in this conduct experiments. All animals were provided by the Center of Experimental Research and Practice in Ataturk University, and the experiments were performed according to the approved ethical rules. All chemicals used were purchased from Sigma Chemical Co. (St. Louis, MO., USA) unless otherwise stated.

Experimental procedure

All animals were alimented same laboratory chow and water *ad libitum* for a week before the experiments. Afterwards, the animals were randomly divided into 4 groups. In group I (sham-

operated, n=6), rats underwent laparatomy via dorsal approach but adrenals were not removed. In group II (ADX, n=8), rats were performed bilateral adrenalectomy. In group III (ADX + Prednisolone, n=8), bilateral adrenalectomy were performed in animals, and in the 8th day after adrenalectomy, prednisolone (Fako Co. Istanbul, Turkey) was administered intraperitoneally (i.p.) in a dose of 5 mg/kg body weight /12 hour for 1 day. In group IV (ADX + Epinephrine, n=8), rats were operated bilateral adrenalectomy, and in the 8th day after operation, epinephrine (1 mg/kg body weight, Biofarma Co. Istanbul, Turkey) was i.p. injected into the animals once an hour for 6 h. The injected drug doses were chosen according to the plasma half-lives of the drugs.

At the end of the treatments, blood of all animals was drawn from the heart following anesthesia. After the rats were sacrificed, gastric tissues were rapidly excised and washed with cold 0.9 % NaCl solution. Adrenalectomy and sham-operation were carried out via dorsal incisions, using aseptic procedures. Surgical operations were achieved under anesthesia created by i.p. given thiopental sodium at a dose of 25 mg/kg body weight. After operation, the animals were housed in their cages. While the rats in group I were subsequent to fed with standard commercial rat chow and tap water, rats in other groups were maintained with rat chow and 1 % NaCl solution after surgery.

Sample preparations

Blood samples were collected in glass tubes containing EDTA and centrifuged at 2500 x g for 5 min at 4 °C. Plasma was pulled out and the erythrocytes were washed (using cold 0.9 % NaCl solution) and centrifuged three-times. The hemolysate was prepared with double distilled water and were stored at -80 °C prior to assays.

For assays of GPX, SOD, GSH and MDA in gastric tissue of analyzed groups, the tissue homogenates were produced. All the processes were handled at 4°C throughout. Gastric tissue was cut into three small pieces and then weighed (about 200 mg for each). The tissues were homogenized (OMNI-TH International homogenizer) using the appropriate buffer, depending upon the variable to be measured. After centrifugation at 18 000 x g for 15 min at 4 °C, the supernatant was extracted, and kept at -80 °C in advance of assays.

Determination of GPX activity

GPX activity in tissue and hemolysate was measured by the method of Paglia and Valentine (18). This process is based on the oxidation of NADPH in the presence of H_2O_2 as substrate and monitored spectrophotometrically at 340 nm. The results were expressed as units per gram of protein (U/g protein, for tissue) or hemoglobin (U/g Hb, for erythrocyte).

Determination of SOD activity

SOD activity was detected spectrophotometrically at 560 nm using the previously described method (19). SOD estimation is determined by the inhibition of the reduction of nitrobluetetrazolium by superoxide radicals, which are produced by the XO system. Enzyme activity was measured by the degree of inhibition of this reaction, and was stated as units per gram of protein (U/g protein, for tissue) or hemoglobin (U/g Hb, for erythrocyte).

Measurement of MDA level

The levels of MDA in gastric tissue and erythrocyte were used as the indicator of lipid peroxidation. MDA level was determined according to the method described by Okhawa (20). In this method, MDA reacts with thiobarbituric acid to form a colored complex that has maximum

absorbance at 532 nm. MDA levels as micromole per milligram of wet tissue (μmol/mg wt) or hemoglobin (μmol/g Hb) were expressed.

Measurement of GSH level

Total glutathione (GSH) quantities were determined spectrophotometrically at 412 nm by measuring a yellow colored 5-thio-2-nitrobenzoic acid (TNB), as described by Tietze (21). In this procedure, the sulfhydryl group of GSH reacts with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and produces TNB. The rate of TNB production is directly proportional to the concentration of GSH in the sample. Results were communicated as nanomole per milligram of wet tissue (nmol/mg wt, for tissue) or hemoglobin (nmol/g Hb, for erythrocyte).

Measurement of catecholamine levels

Plasma catecholamine concentrations were determined using a high performance liquid chromatography (HPLC) with electrochemical detection after batch alumina extraction, as described previously (22, 23). In short, 20 μl of stabilization solution (0.3 M EGTA and 0.28 M GSH in 0.5 M NaOH) was transferred to a tube containing 1 ml of blood sample, and pH was adjusted to 7.4 with 2 N NaOH. Final volume was completed to 10 ml with distilled water, mixed and centrifuged. Then 1 ml plasma, 0.5 ml extraction buffer and 50 μl internal standard (dihydroxybenzylamine) were added to a sample clean up cartridge and mixed for 10 min. After centrifugation, wash step and elution process, 50 μl of the eluate was injected onto the HPLC column.

Protein and hemoglobin concentrations in homogenate or hemolysate were respectively determined by Bradford and Fairbanks methods (24,25). All photometrical measurements were performed with a DU 530 spectrophotometer (Beckman Instruments, Fullerton, CA) in a quartz cuvette.

Statistical analysis

Results were expressed as mean ± SD. Groups were compared using nonparametric Mann-Whitney test. A value of $P<0.05$ was considered as significant. For these procedures, SPSS 11.5 version for Windows was used.

RESULTS

Plasma levels of epinephrine

Effectiveness of adrenalectomy was assessed by plasma epinephrine determination using a HPLC analyzer. As shown in *Table 1*, while the average plasma epinephrine concentration of shame-operated rats was 3.41 ± 0.83 pmol/ml, and that of ADX rats was 0.18 ± 0.06 pmol/ml.

GPX and SOD activities

In the gastric tissue and erythrocyte of ADX rats (group II), both GPX and SOD activities were relatively low compared with sham-operated rats (group I) (*Fig. 1* and *2*). Injection of prednisolone (5 mg/kg body weight, i.p.) in adrenalectomized rats (group III) caused a marked rise of GPX and SOD

Table 1. Plasma catecholamine levels in sham-operated and adrenalectomized male Wistar rats were measured on blood samples taken from the tail vessels in the 7th day after surgical operation. Samples were analyzed by the HPLC column, and effectiveness of adrenalectomy was assessed by plasma epinephrine concentration. Data are expressed as mean ± SD.

	Control (Sham-operated, n=6)	**Adrenalectomy** (n=24)	**P**
Epinephrine (pmol/ml)	3.41 ± 0.83	0.18 ± 0.06	< 0.001
Norepinephrine (pmol/ml)	0.126 ± 0.018	0.092 ± 0.013	< 0.05
Dopamine (pmol/ml)	0.063 ± 0.020	0.024 ± 0.007	< 0.05

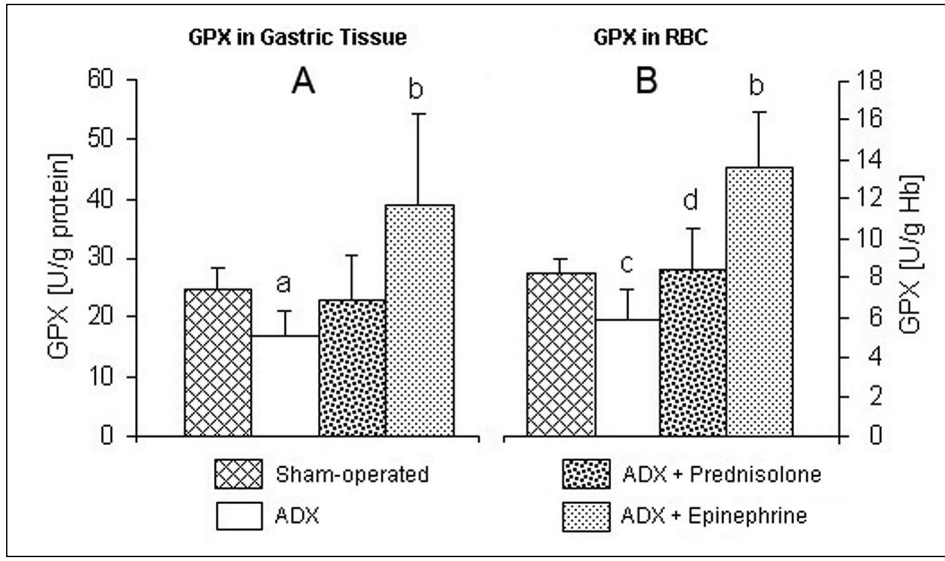

Fig. 1. GPX activities in gastric tissue **(A)** and erythrocyte **(B)** after different experimental procedures: sham-operation (n=6); adrenalectomized (ADX, n=8); (ADX + prednisolone, n=8) after 1 week adrenalectomy, prednisolone was injected (i.p.) 5 mg/kg b.w./12 hour for 1 day; (ADX + epinephrine, n=8) after 1 week adrenalectomy, epinephrine was treated (i.p.) 1 mg/kg b.w./an hour for 6 h. Results are mean ± SD. Statistically significant differences as compared to sham-operated (a, P<0.05; c, P<0.01) and as compared to ADX (b, P<0.001; d, P<0.05). RBC; red blood cell.

activities comparison to ADX-alone rats (group II); however, a statistically significant difference did not exist between these groups. Compared with ADX rats, epinephrine injected in a dose of 1 mg/kg body weight (i.p.) significantly increased both GPX and SOD activities in group IV rats (*Fig. 1* and *2*).

Concentration of GSH

Total GSH content was apparently augmented in erythrocyte and gastric tissue after adrenalectomy (*Fig. 3*). GSH levels in gastric tissue were notably higher in

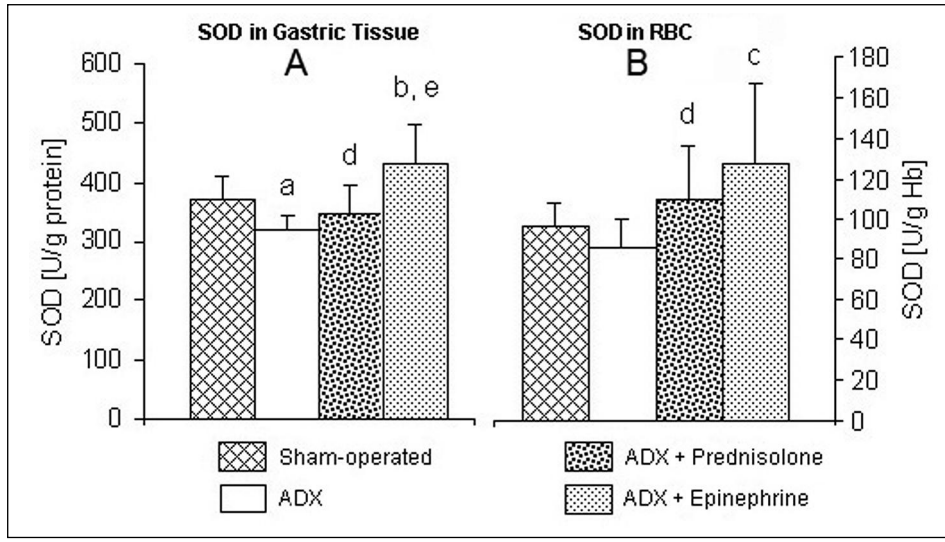

Fig. 2. SOD activities in gastric tissue **(A)** and erythrocyte **(B)** after different experimental procedures: sham-operation (n=6); adrenalectomized (ADX, n=8); (ADX + prednisolone, n=8) after 1 week adrenalectomy, prednisolone was injected (i.p.) 5 mg/kg b.w./12 hour for 1 day; (ADX + epinephrine, n=8) after 1 week adrenalectomy, epinephrine was treated (i.p.) 1 mg/kg b.w./an hour for 6 h. Results are mean ± SD. Statistically significant differences as compared to sham-operated (a, $P<0.05$), as compared to ADX (b, $P<0.001$; c, $P<0.01$; d, $P>0.05$) and as compared to ADX + prednisolone (e, $P<0,01$). RBC; red blood cell.

both prednisolone- (group III) and epinephrine-treated rats (group IV) than in ADX animals (group II). Compared with ADX-alone rats, epinephrine injection (1 mg/kg i.p.) after adrenalectomy resulted in a significant increase of erythrocyte GSH concentrations (*Fig. 3*).

Concentration of MDA

When compared to sham-operated rats (group I), MDA levels in ADX-alone rats (group II) were found significantly decreased (*Fig. 4*). In comparison with ADX-alone rats, MDA levels in gastric tissue were determined moderately increased in both prednisolone- (group III) and epinephrine-treated animals (group IV). However, injection of epinephrine (1 mg/kg, i.p.) in adrenalectomized rats resulted in a significant increase of erythrocyte MDA concentrations, as compared to ADX-alone rats (group II) (*Fig. 4*).

DISCUSSION

Several studies have been previously reported on the pathogenesis of stress-induced gastric ulcerations. Menguy (26) and Brodie (27) have previously stated

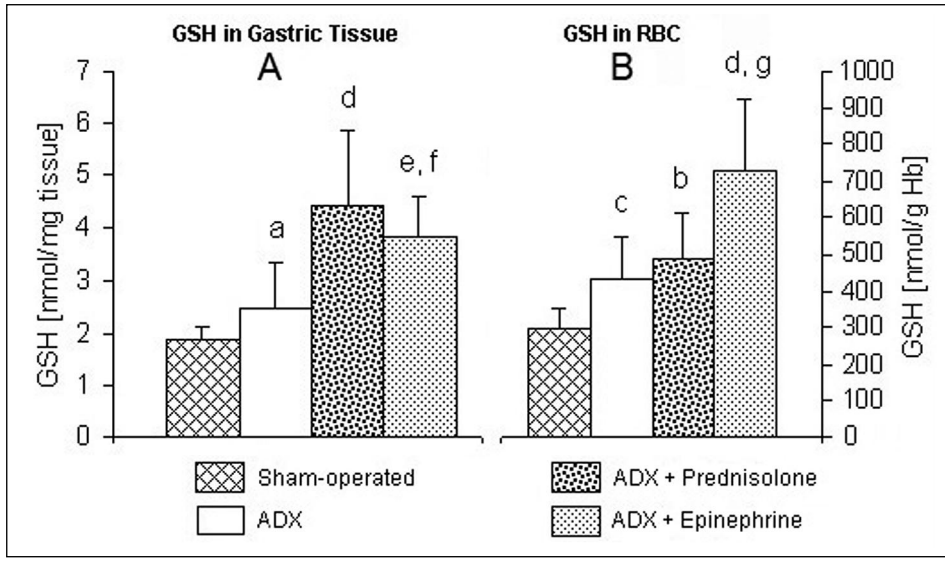

Fig. 3. GSH levels in gastric tissue **(A)** and erythrocyte **(B)** after different experimental procedures: sham-operation (n=6); adrenalectomized (ADX, n=8); (ADX + prednisolone, n=8) after 1 week adrenalectomy, prednisolone was injected (i.p.) 5 mg/kg b.w./12 hour for 1 day; (ADX + epinephrine, n=8) after 1 week adrenalectomy, epinephrine was treated (i.p.) 1 mg/kg b.w./an hour for 6 h. Results are mean ± SD. Statistically significant differences as compared to sham-operated (a, $P>0.05$; c, $P<0.005$), as compared to ADX (d, $P<0.005$; f, $P<0.01$; b, $P>0.05$) and as compared to ADX + prednisolone (e, $P>0,05$; g, $P<0.01$). RBC; red blood cell.

that restraint-stress ulcers are aggravated by adrenalectomy. In more recently literature, it was reported that ADX rats exhibited significantly greater incidence and severity of gastric ulcer after restraint-stress in a cold room and indomethacin or ethanol administration than intact control rats (5, 6, 9). These studies focused on the various damaging factors such as chemical agents and restraint- stress rather than the biological effects (i.e., free radical metabolism) of adrenal hormones on the gastric tissue. Although a relationship between the adrenal gland and gastric lesion has been postulated in different studies (5, 9), the influence of adrenalectomy on antioxidant status in the rat gastric tissue is unknown. In the present study, the possible influence of endogenous adrenal hormones on antioxidant status and lipid peroxidation in gastric tissue and erythrocyte has been examined in rats subjected to adrenalectomy. The erythrocyte system was selected to compare the potential alteration in gastric tissue antioxidant capacity or lipid peroxidation.

To our knowledge, there is no available data on gastric tissue lipid peroxidation in rats with adrenalectomy. In the present study, we measured gastric tissue and erythrocyte MDA concentrations. The removal of adrenal gland significantly reduced the content of MDA in gastric tissue and erythrocyte when compared to sham-operated rats, suggesting a factor presenting in the adrenal gland or being

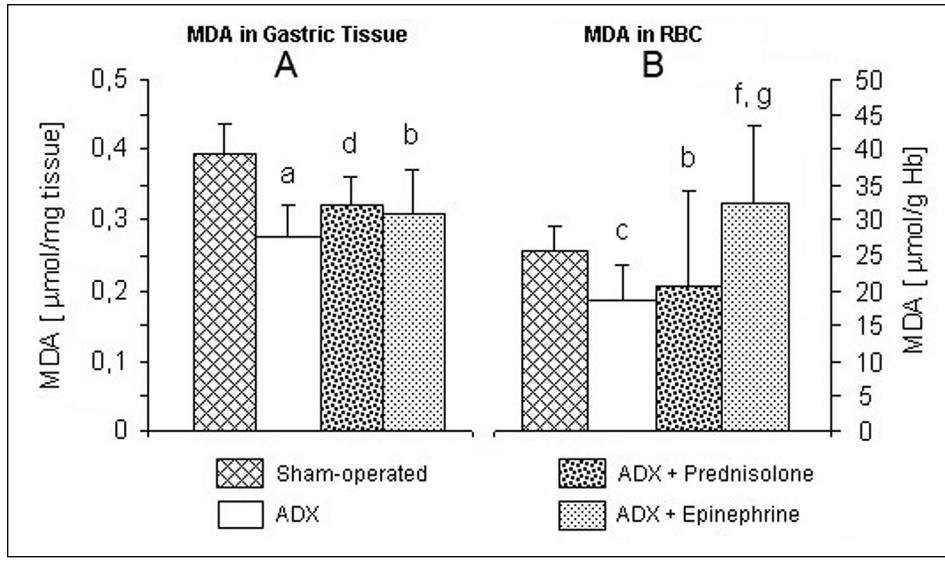

Fig.4. MDA levels in gastric tissue **(A)** and erythrocyte **(B)** after different experimental procedures: sham-operation (n=6); adrenalectomized (ADX, n=8); (ADX + prednisolone, n=8) after 1 week adrenalectomy, prednisolone was injected (i.p.) 5 mg/kg b.w./12 hour for 1 day; (ADX + epinephrine, n=8) after 1 week adrenalectomy, epinephrine was treated (i.p.) 1 mg/kg b.w./an hour for 6 h. Results are mean ± SD. Statistically significant differences as compared to sham-operated (a, $P<0.001$; c, $P<0.05$), as compared to ADX (d, $P<0.05$; f, $P<0.01$; b, $P>0.05$) and as compared to ADX + prednisolone (g, $P<0.05$). RBC; red blood cell.

controlled by some adrenal factors that might predispose the production of MDA. However, the data reported in the literature on lipid peroxidation associated with the effect of adrenalectomy in a number of tissues in the rats are controversial. Toleikis et al. (28) have determined that *in vitro* lipid peroxidation was decreased in liver, lung and kidney homogenates of Wistar rats after adrenalectomy. On the contrary, in an *in vivo* study made by Hidalgo et al. (29) it has been maintained that thiobarbituric acid-reactants (TBARs) in the liver tissue were higher in adrenalectomized group than in sham-operated group. In the same study, however, it has also been published that liver TBARs level was lower in adrenalectomy plus corticosterone-administrated group than in sham-operated group (29). In our research, it was also found that gastric tissue MDA levels in ADX rats treated with prednisolone were higher than in ADX-alone rats. In contrast, in ADX rats treated with epinephrine erythrocyte MDA concentration was higher than in ADX rats. These results suggested that an organ-specific effect of adrenal hormones on lipid peroxidation might be present, which deserve further attention.

Gastric mucosa possesses an effective antioxidant system, which scavenges reactive oxygen species (ROS) and prevents their destructive action on this mucosa (30). GSH, an endogenous antioxidant, is found at high concentrations in gastric mucosa and erythrocyte of humans and rats (31, 32). Recent studies

suggested that sulfhydryl compounds in the stomach might be important for the maintenance of gastric mucosal integrity (1, 33, 34). The results of the present experiment point out an increase in GSH concentration in ADX rats as compared to the sham-operated group. Our findings agree with the previous study reported by Toleikis et al. (28) who observed a significant increase in erythrocyte GSH levels in ADX rats compared to the control group. The augmentation of GSH levels after adrenalectomy may be associated with decreased levels of endogenous catecholamines. Moreover, administration of epinephrine caused an apparent increase of GSH concentrations in both gastric tissue and erythrocyte (*Fig.3*), as was also observed previously in the gastric mucosa (3). Epinephrine can stimulate H_2O_2 production via cAMP in macrophages. Also, H_2O_2 is converted to 2 mol H_2O by GPX reaction in which GSH is utilized (35). In condition without epinephrine, both GPX reaction and H_2O_2 production (indirectly MDA) may diminish, and this situation may lead to less consumption of GSH. This is probably because the increased GSH levels in gastric tissue and erythrocyte after adrenalectomy as mentioned above.

As previously stated above, the increased GSH and decreased MDA concentrations after adrenalectomy does not suggest that adrenalectomy has an antioxidative effect. In contrast, our results showed a decline in GPX and SOD activities in gastric tissue and erythrocyte of rats with adrenalectomy as compared to sham-operated group. Besides, the activities of GPX and SOD in these tissues were significantly elevated in epinephrine-treated rats. To our knowledge, a direct effect of altered catecholamine levels on the gastric tissue GPX and SOD activities in rats, however, has not been demonstrated, yet. Nonetheless, Mehta et al. (15) demonstrated that epinephrine increased superoxide generation and SOD expression and activity in human coronary artery endothelial cells. Pereira et al. (17) have announced that epinephrine may physiologically regulate the activities of GPX and Mn-SOD in resident peritoneal macrophages. In consideration of our results associated with activities of GPX and SOD, the hypothesis of Pereira et al. may be submitted for gastric tissue and erythrocyte, as well. Because, the various molecules with ortho hydroxyl groups on a benzene ring such as epinephrine are capable of releasing iron from ferritin (36), and readily reduce ferric iron (37). Iron catalyzes the generation of highly reactive hydroxyl radicals ($HO^{\cdot}$) from H_2O_2 by Fenton reaction. H_2O_2 into water by GPX is accompanied by the conversion of glutathione from reduced form (GSH) into oxidized form (GSSG) (30). The autooxidation of cathecolamines can produce ROS, too (38). As a result, if cathecolamines give rise to ROS generation (30, 37, 38), an effective antioxidant system (i.e., GPX and SOD), which scavenges generated ROS has to be activated concomitantly for metabolic stability. Therefore, the findings of an alteration in GPX and SOD activities due to adrenalectomy or administration of epinephrine led us to speculate that adrenal hormones in particular epinephrine, may play an important role for the regulation of these enzymes activities in the gastric tissue and erythrocyte. Also, although statistically significant difference did not exist

between the investigation groups, injection of steroid hormone (prednisolone) in ADX rats caused a marked rise of GPX and SOD activities when compared to ADX-alone rats in the present study. Similar findings were reported previously in the renal (39) and lung tissues (40). For example, Wistar rats treated with 6-methylprednisolone showed increased activity of GPX in renal glomeruli (39). Asayama et al. (40) found an increase in the activity of GPX in fetal lung tissue of rats treated with dexamethasone in a time- and dose-dependent manner.

In summary, the results of this work suggest that adrenalectomy resulted in a significant a decline in the levels of MDA and a rise in the amounts of GSH in gastric tissue and erythrocyte of Wistar rats, suggesting a factor presenting (possibly epinephrine) in the adrenal gland might predispose the production of free radicals. The results also suggest that injection of epinephrine markedly elevated the activities of SOD and GPX and the levels of GSH in both gastric mucosa and erythrocyte. Thus, the present study suggests a physiological regulatory role of adrenal gland in the maintenance of oxidant/antioxidant balance in such tissues (stomach and erythrocyte). However, further investigations will be required to delineate the mechanisms of adrenal hormones-induced alteration in SOD and GPX activities and GSH level in gastric tissue and erythrocyte.

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