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Effect of glucocorticoids on 3-nitropropionic acid-induced oxidative stress in synaptosomes

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Abstract

The present study with rat striatal and cortical synaptosomes evaluated the effect of dexamethasone (300 μg/kg i.p./day) with and without simultaneous adrenalectomy on the oxidative stress induced by 3-nitropropionic acid (20 mg/kg/day for 4 days). Adrenalectomy enhanced the oxidative stress induced by 3-nitropropionic acid. These changes were prevented by previous and simultaneous administration of dexamethasone. Adrenalectomy alone induced oxidative stress with decreases in succinate dehydrogenase activity. Our results revealed that adrenal glucocorticoids, and especially dexamethasone (synthetic glucocorticoid), have a protective effect against oxidative stress induced by 3-nitropropionic acid in some brain regions of the Wistar rat.

Keywords: 3-Nitropropionic acid; Huntington's disease; Oxidative stress; Glucocorticoid

1. Introduction

Huntington's disease is a progressive neurodegenerative disorder associated with severe degeneration of basal ganglia neurons, especially the intrinsic neurons of the striatum, and is characterized by progressive dementia and involuntary abnormal choreiform movements (Martin and Gusella, 1986; Albin et al., 1990). Considerable advances have occurred since the publication of the paper by G. Huntington in 1872. Although the mechanisms of selective striatal damage in Huntington's disease are not known, the activation of excitatory amino acid receptors has been implicated (Beal, 1992; Davies and Ramsden, 2001; Mattson, 2003). Striatal neurons display a differential vulnerability under certain neurodegenerative conditions and in response to experimentally applied toxins. One such toxin that is gaining prominence for use in animal models of Huntington's disease is 3-nitropropionic acid.

3-Nitropropionic acid, a plant and fungal toxin, is reported to interrupt mitochondrial electron transport, resulting in a cellular energy deficit. This mycotoxin is an irreversible and suicide inhibitor of succinate dehydrogenase (E.C. 1.3.99.1), an enzyme located in the mitochondrial inner membrane and responsible for the oxidation of succinate to fumarate. The mitochondrial toxin inhibits both complex II–III of the respiratory chain and the tricarboxylic acid cycle (Kreb's cycle). Inhibition of succinate dehydrogenase interferes with the electron cascade and interrupts oxidative phosphorylation. This phenomenon induces a reduction in ATP production and oxidative stress. 3-Nitropropionic acid has also been reported to be a selective striatal neurotoxin in rodents provided that it is given in a sufficiently high dose and over a period of days. Generally, brain lesions caused by systemic 3-nitropropionic acid are highly specific to the striatum. Other studies using high doses of systemic 3-nitropropionic acid have identified the hippocampus and thalamus as other brain regions affected (Borlongan et al., 1997; Brouillet et al., 1999). Additionally, recent studies have suggested the involvement of reactive oxygen species and oxidative stress in 3-nitropropionic acid-induced neurotoxicity (Binienda et al., 1998; La Fontaine et al., 2000a,b, 2002). Excitotoxicity and oxidative stress are two conditions leading to cell death that are thought to be important in several neurodegenerative diseases and are relevant to the striatal cell loss seen in

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Huntington's disease (Petersen et al., 1999; Reddy et al., 1999; Gutekunst et al., 2000).

Glucocorticoids have different effects (Braughler, 1985; Mitchell et al., 1998; Ahlbom et al., 2000; Haynes et al., 2001, 2003; Méndez-Armenta et al., 2001, 2003). These agents can both enhance toxicity or protect against toxicity, and the direction of the effect depends on the dose and time of treatment, animal age, organ and tissue analyzed, and experimental model (Haynes et al., 2003; Méndez-Armenta et al., 2003).

Dexamethasone is a glucocorticoid that has a broad range of effects in the brain, although its actions occur predominantly but not exclusively in the hippocampus, a structure rich in corticosteroid receptors particularly sensitive to glucocorticoids (Méndez-Armenta, 2003). A recent study performed by Méndez-Armenta et al. has shown a protective effect of dexamethasone on the oxidative stress induced by cadmium in some brain regions (parietal cortex, striatum, hippocampus and cerebellum) (Méndez-Armenta et al., 2001, 2003). Also, different studies have shown an effect of dexamethasone against Huntington's disease in both clinical and experimental studies (Heuser et al., 1991; Nuti et al., 1991; Bonuccelli et al., 1992; Mitchell et al., 1998).

The main aim of the present study is to evaluate the effects of glucocorticoids on oxidative stress induced by 3-nitroptopionic acid. In order to reach this objective, we examined the effects of adrenalectomy (experimental model in which a lack of endogenous glucocorticoids is induced) with or without administration of dexamethasone, on the severity of oxidative stress induced by 3-nitropropionic acid in rat brain synaptosomes. We analyzed biochemical parameters indicative of oxidative stress, such as the content of protein carbonyl groups and changes in the activity of superoxide dismutase (E.C. 1.15.1.1) and succinate dehydrogenase. Under the same experimental conditions, we performed the Alamar Blue assay and measured serum levels of corticosterone.

2. Materials and methods

2.1. Chemical reagents and administered products

3-Nitropropionic acid and other reagents were purchased from Sigma (St. Louis, MO, USA). Dexamethasone (Fortecortin®) was supplied by Merck Farma y Quimica (Spain).

2.2. Animals

All animal care and procedures were in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the School of Medicine. Three-month-old male Wistar rats weighing between 250 and 300 g at the beginning of the study were purchased from Charles River of Barcelona, Spain. They were subjected to controlled conditions of temperature (20–23 °C), illumination (12-h light/12-h dark cycle, lights on at 08:00 h) and were provided with food (Purina®, Barcelona, Spain) and water ad libitum.

2.3. Experimental procedure

To carry out this study, 54 rats were used. These rats were divided into nine groups of six animals as follows: (i)

Table 1
Changes in the levels of protein carbonyl groups and enzyme activity in the striatal and brain cortex synaptosomes of rats treated with 3-nitropropionic acid alone or in combination with adrenalectomy and/or dexamethasone

	Protein carbonyl groups (nmol/mg protein)		Superoxide dismutase (U/mg protein)		Succinate dehydrogenase (U/mg protein)	
	Brain cortex	Estriatum	Brain cortex	Estriatum	Brain cortex	Estriatum
Control	0.83 ± 0.07	1.36 ± 0.18	6.73 ± 0.11	11.01 ± 0.27	23.61 ± 0.71	33.43 ± 0.94
Sham operated	0.85 ± 0.09	1.40 ± 0.21	6.57 ± 0.14	11.41 ± 0.18	23.75 ± 0.54	34.04 ± 0.78
Adrenalectomy	1.36 ± 0.15[a]	2.34 ± 0.11[a]	8.46 ± 0.11[b]	12.59 ± 0.27[a]	17.97 ± 0.34[a]	26.72 ± 0.32[a]
Dexamethasone	0.67 ± 0.07	1.11 ± 0.06	5.20 ± 0.07	9.04 ± 0.33	21.72 ± 0.62	32.57 ± 1.47
Adrenalectomy + dexamethasone	0.94 ± 0.07[c]	1.87 ± 0.19[c]	6.60 ± 0.32[d]	10.22 ± 0.16[d]	19.61 ± 0.30[d]	30.37 ± 0.80[d]
3-Nitropropinic acid	3.40 ± 0.33[b]	8.24 ± 0.42[b]	9.90 ± 0.69[b]	12.44 ± 0.21[b]	5.88 ± 0.39[b]	8.71 ± 0.41[b]
3-Nitropropionic acid + adrenalectomy	4.30 ± 0.39[e]	9.60 ± 0.28[e]	10.81 ± 0.36	13.19 ± 0.17	4.77 ± 0.15[e]	7.55 ± 0.42[e]
3-Nitropropionic acid + dexamethasone	1.68 ± 0.13[f]	4.86 ± 0.43[g]	7.47 ± 0.27	9.41 ± 0.16[g]	8.84 ± 0.26[f]	13.88 ± 1.09[g]
3-Nitropropionic acid + adrenalectomy + dexamethasone	2.92 ± 0.13[h]	6.96 ± 0.18[h]	7.30 ± 0.32[i]	10.03 ± 0.34	7.55 ± 0.43[h]	10.88 ± 0.32[h]

Values are means ± S.E.M.; $n = 6$ rats per group.
[a] $P < 0.01$ versus control.
[b] $P < 0.001$ versus control.
[c] $P < 0.01$ versus adrenalectomy.
[d] $P < 0.05$ versus adrenalectomy.
[e] $P < 0.05$ versus 3-nitropropionic acid.
[f] $P < 0.01$ versus 3-nitropropionic acid.
[g] $P < 0.001$ versus 3-nitropropionic acid.
[h] $P < 0.01$ versus 3-nitropropionic acid + adrenalectomy.
[i] $P < 0.001$ versus 3-nitropropionic acid + adrenalectomy.

injected with vehicle saline (control), (ii) treated with dexamethasone, (iii) sham operated: dissection of the adrenalectomy, (iv) adrenalectomized, (v) adrenalectomized plus dexamethasone, (vi) 3-nitropropionic acid, (vii) 3-nitropropionic acid plus dexamethasone, (viii) 3-nitropropionic acid plus adrenalectomized and (ix) 3-nitropropionic acid plus dexamethasone and adrenalectomized.

3-Nitropropionic acid was administered intraperitoneally (i.p.) at a dose of 20 mg/kg for 4 consecutive days, whereas dexamethasone was injected i.p. at 300 μg/kg i.p., daily for 8 days. Adrenalectomy was performed in the rats under deep anesthesia (pentobarbital 40 mg/kg, i.p.). The animals were bilaterally adrenalectomized according to the technique described by Poumeau-Delille (1953); saline drinking solution (0.9%) was given to adrenalectomized rats. Dexamethasone and adrenalectomy, both alone and combined with 3-nitropropionic acid, were given for a period of 8 days, beginning 4 days before and continuing for 4 days after the first injection of 3-nitropropionic acid.

2.4. Striatum and brain cortex studies

At the end of study and under ether anesthesia, the animals were killed. The whole brain was removed and brain cortex and striatum were isolated and suspended in 2 ml ice-cold isolation buffer (0.32 M sucrose, 20 mM HEPES, 4 μg/ml leupeptin, 4 μg/ml pepstatin, 5 μg/ml aprotinin, 20 μg/ml type II-S soybean trypsin inhibitor, 0.2 mM phenylmethylsylfonyl fluoride, 2 mM EGTA, 2 mM EDTA, pH 7.2). The homogenate was centrifuged at $450 \times g$ for 10 min at 4 °C, and the supernatant was transferred to a new tube. The remaining pellet was resuspended in 1.5 ml homogenization buffer and centrifuged as before. The two supernatant fractions were combined and centrifuged at $20{,}000 \times g$ for 10 min at 4 °C, and the resulting crude synaptosomal pellet was resuspended in 2 ml of Locke's buffer (154 mM NaCl, 5.6 mM KCl, 2.3 mM $CaCl_2$, 1.0 mM $MgCl_2$, 3.6 mM $NaHCO_3$, 5 mM glucose, 5 mM HEPES, at a pH 7.2) (Springere et al., 1997).

2.5. Alamar Blue assay

Alamar Blue is a redox indicator that exhibits both fluorescent and colorimetric changes in response to metabolic activity. Alamar Blue is a supposed to be reduced at a site in mitochondrial respiration downstream of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). The redox potential of Alamar Blue allows for the respiratory chain to function to near completion, which provides a sensitive indication of mitochondrial function. Synaptosomes (300 μg) were pipetted into tube, Alamar Blue was added (10% final volume), and the tubes were incubated at 37 °C, as described by Springere et al. (1997). Alamar Blue fluorescence levels were measured at 0, 15, 30, 45 and 60 min (530 nm excitation and 590 nm emission).

2.6. Protein carbonyl groups measurements

The protein carbonyl content was evaluated using of the Levine et al. method (1990). Sample (500 μg) was incubated with 500 μl of a 10 mM solution of 2,4 dinitrophenylhydrazine in 2 N HCl for 60 min. Subsequently, the proteins were precipitated from the solutions with the use of 500 μl of 20% trichloroacetate. Then the protein were washed three times with a solution of ethanol and ethylacetate (1:1, vol/vol) and dissolved in 1 ml of 6M guanidine (containing 20 mM phosphate buffer, pH 2.3, in trifluoroacetic acid) at 37 °C. The carbonyl content was evaluated in a spectrophotometer at wavelength 360 nm. The results are presented in nmol/mg protein.

2.7. Superoxide dismutase activity assay

Total superoxide dismutase activity was assayed by a method based on the inhibition of nitrite formation from

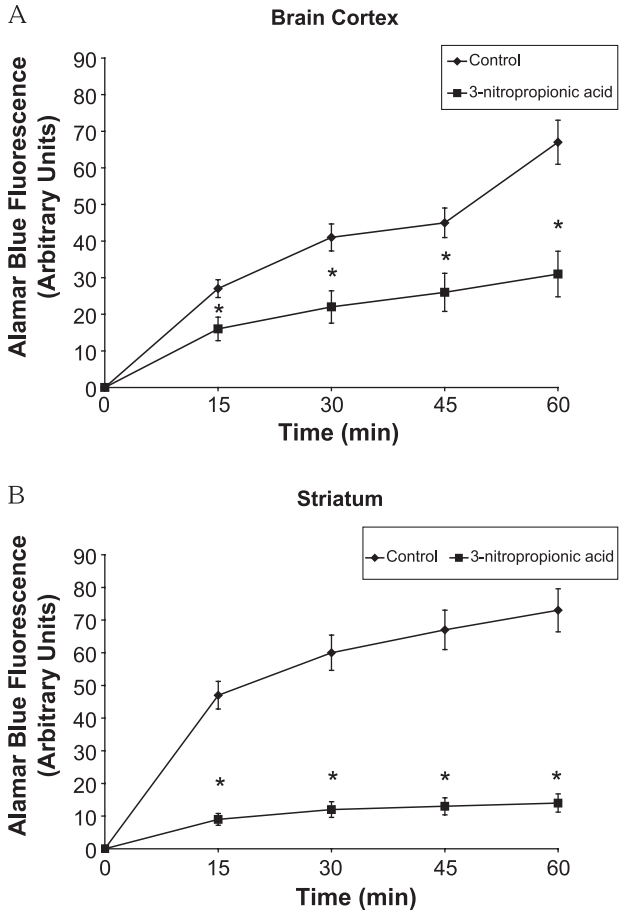

Fig. 1. Time course of the effect of the of succinate dehydrogenase inhibitor, 3-nitropropionic acid, on Alamar blue reduction in brain cortex (panel A) and striatal (panel B) synaptosomes. Values are means ± S.E.M. *$P < 0.001$ versus control.

hydroxylammonium in the presence of O_2^- generators (Pattichis et al., 1994). Sample (0.01 ml) or phosphate buffer (65 nM, pH 7.8) as blank was incubated with 1.490 ml phosphate buffer, 0.1 ml xanthine (15 mM in 25% NaOH, 0.1 M) and 0.1 ml hydroxylammonium chloride (20 mM). The reaction was initiated by the addition of 0.3 ml of xanthine oxidase (50 μg protein/0.3 ml) and kept at 25 °C for 20 min. Then, 0.5 ml of incubation mixture was incubated with 0.5 ml of sulfanilic acid (0.03 mM, in 25% glacial acetic acid) for 5 min at room temperature. Thereafter, 0.5 ml of α-naphthylamine (0.3 mM) was added, the contents were stirred using a cuvette stirrer, and absorbance at 530 nm was recorded.

2.8. Analysis of the succinate dehydrogenase activity

Each assay reaction contained the following solutions: 650 μl phosphate buffer solution (containing 0.3 M D-mannitol and 5.0 mM magnesium chloride, pH 7.03), 125 μl of 0.04 M sodium azide, 125 μl of 0.50 mM dichloroindophenol, 125 μl of 0.2 M succinate and 400 μl of a gradient interface. The gradient interface was added last to initiate the reaction. These reactions were allowed to proceed at room temperature and the discoloration caused by reduction of dichloroindophenol was monitored over a 40-min period at 600 nm (Strack et al., 2001).

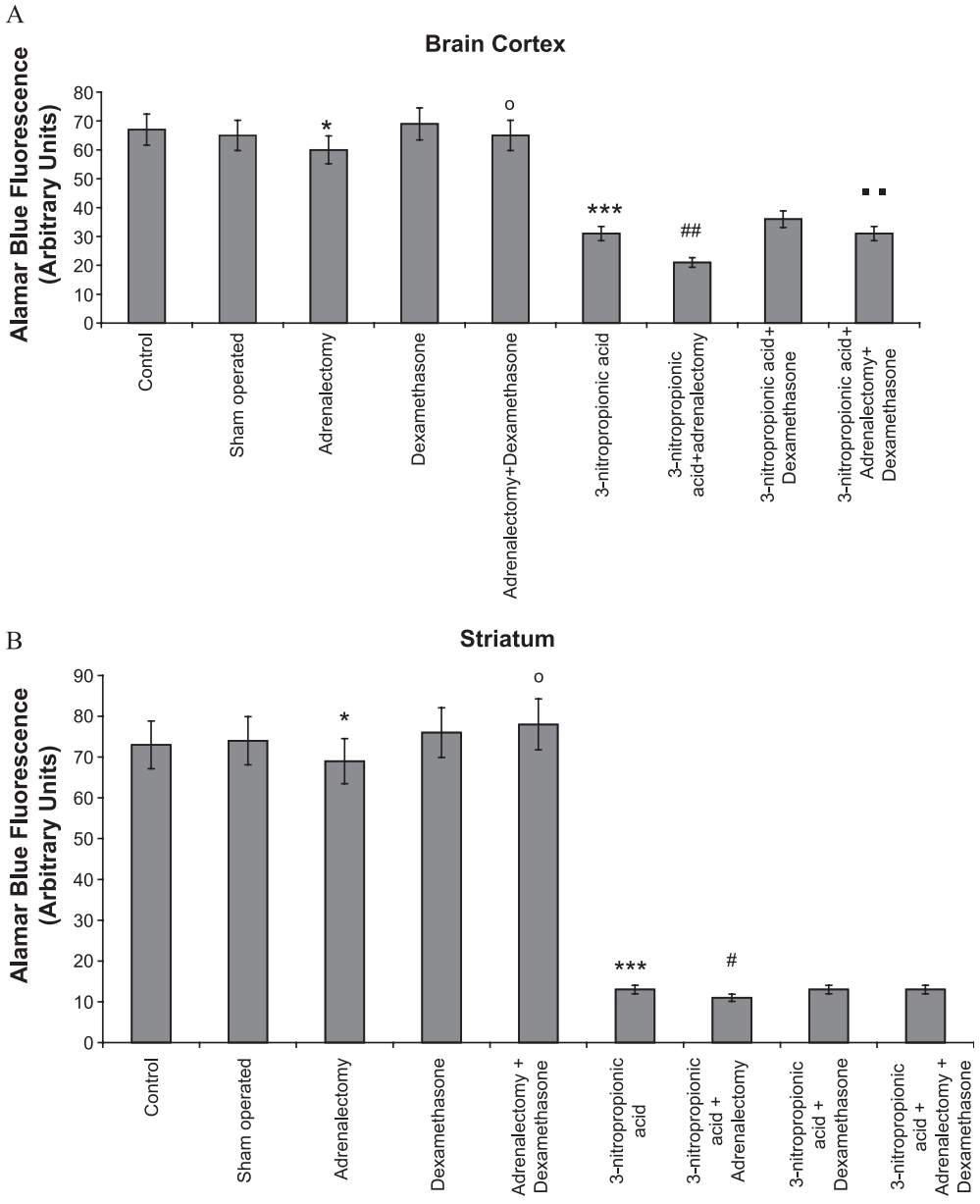

Fig. 2. Effects of 3-nitropropionic acid, adrenalectomy and dexamethasone on Alamar blue reduction in brain cortex (panel A) and striatal (panel B) synaptosomes. ***$P<0.001$ versus control; *$P<0.05$ versus control; $^{\circ}P<0.05$ versus adrenalectomy; $^{\#\#}P<0.01$ versus 3-nitropropionic acid; $^{\#}P<0.05$ versus 3-nitropropionic acid; $^{\blacksquare\blacksquare}P<0.01$ versus 3-nitropropionic acid + adrenalectomy.

2.9. Protein estimation

The protein concentration was determined by the Lowry method (Lowry et al., 1951), using bovine serum albumin as a standard.

2.10. Measurement of corticosterone

On the 8th day and under ether anesthesia, the animals were killed and trunk blood was collected. Corticosterone level in serum was measured by radioimmunoassay using [^{125}I]*-corticosterone as a tracer kit, specific for rat, supplied by IBL (Hamburg, Germany).

2.11. Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by Student's *t*-test with Bonferroni correction for multiple comparisons. Results are expressed as means ± S.E.M. The level of statistical significance was set at $P<0.05$.

3. Results

3.1. Changes in protein carbonyl group content and superoxide dismutase activity (Table 1)

3-Nitropropionic acid produced a significant increase in the content of protein carbonyl groups in both striatal nucleus ($P<0.001$) and brain cortex synaptosomes ($P<0.001$) (Table 1). A similar effect, but with less intensity, was detected in adrenalectomized rats ($P<0.05$). The combination of adrenalectomy and 3-nitropropionic acid further increased the protein carbonyl groups content in the cerebral areas studied ($P<0.001$). These changes were partially prevented by the simultaneous administration of dexamethasone, whereas the single administration of dexamethasone did not induce significant changes.

3-Nitropropionic acid and adrenalectomy enhanced superoxide dismutase activity in the striatum and brain cortex ($P<0.001$). These changes were reversed by dexamethasone administration. Nevertheless, administration of dexamethasone to the control animals did not have a significant effect on the activity of this enzyme.

3.2. Changes in Alamar Blue assay (Figs. 1 and 2) and succinate dehydrogenase activity (Table 1)

3-Nitropropionic acid produced a significant decrease in Alamar Blue fluorescence ($P<0.001$) and succinate dehydrogenase activity ($P<0.001$) in both striatum and brain cortex, effects that were modestly intensified by adrenalectomy (Figs. 1 and 2). Adrenalectomy alone caused a slight reduction in Alamar blue fluorescence and succinate dehydrogenase activity. Moreover, the administration of dexamethasone partially prevented the effects induced by 3-nitropropionic acid and adrenalectomy on succinate dehydrogenase activity in both the cortex and striatum. In addition, this glucocorticoid slightly and irregularly prevented the effect of 3-nitropropionic acid on the changes in Alamar Blue fluorescence.

The administration of a single dose of dexamethasone did not induce changes in these parameters.

3.3. Changes in serum corticosterone (Fig. 3)

Adrenalectomy and dexamethasone separately produced a significant decrease in corticosterone levels ($P<0.001$), and this effect was more intense when adrenalectomy and

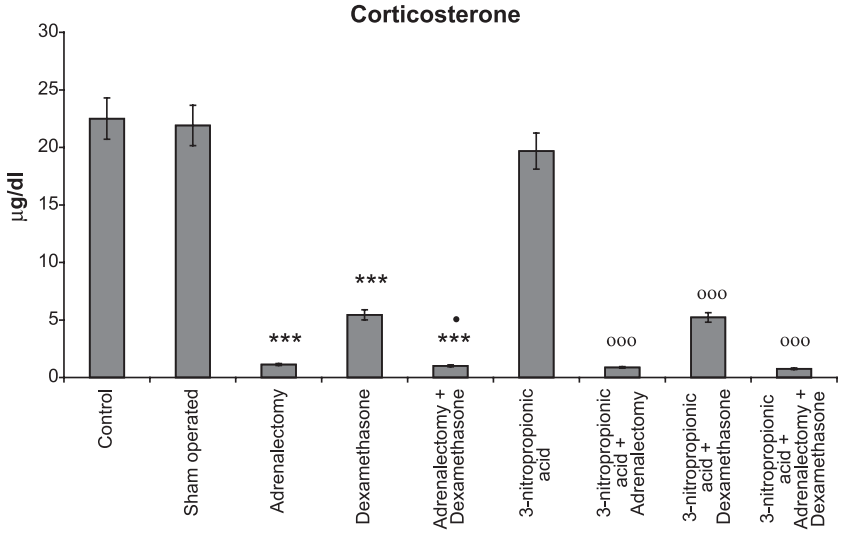

Fig. 3. Serum corticosterone levels in all study groups. ***$P<0.001$ versus control; $^{ooo}P<0.001$ versus 3-nitropropionic acid; $^{\blacksquare}P<0.05$ versus adrenalectomy; $^{\#}P<0.005$ versus 3-nitropropionic acid + adrenalectomy.

dexamethasone were combined (Fig. 3). 3-Nitropropionic acid did not change these levels. These results indicate that treatment with dexamethasone and adrenalectomy was performed correctly.

4. Discussion

In this study, our group shows for first time the effect of endogenous and exogenous glucocorticoids in the model of oxidative stress induced by 3-nitropropionic acid. The results demonstrate that: (i) 3-nitropropionic acid induces oxidative stress (increased levels of protein carbonyl groups and induction of superoxide dismutase activity) and interrupts mitochondrial electron transport (decrease in Alamar Blue fluorescence and inhibition of succinate dehydrogenase). (ii) The effects of 3-nitropropionic acid are enhanced by adrenalectomy. (iii) The administration of dexamethasone partially improves the changes induced by 3-nitropropionic acid and adrenalectomy.

The data obtained are in agreement with the reports from La Fontaine et al. (2000a,b, 2002), Wüllner et al. (1994), Binienda et al. (1998), Binienda and Ali (2001) and Binienda (2003), who found that the neurotoxin increased levels of protein carbonyl groups in synaptosomes enhances the activity of superoxide dismutase and induced an energy deficit. Superoxide dismutase reduces concentrations of reactive oxygen species, preventing cell damage. Increased enzyme activity is indicative of oxidative stress (Binienda et al., 1998; Binienda and Ali, 2001). Furthermore, our findings show that, in developing rats, the changes caused by 3-nitropropionic acid can be partially prevented by dexamethasone administration, indicating a possible protective effect of dexamethasone in the rat brain. Previous reports have shown apoptosis, cellular damage and oxidative stress in developing rats treated with dexamethasone (Mitchell et al., 1998; Haynes et al., 2001, 2003). Nevertheless, other authors show the possible protective effect of glucocorticoids, especially dexamethasone (Braughler, 1985; Kumagai et al., 1997; Méndez-Armenta et al., 2001, 2003). According to Harvey et al. (1994), glucocorticoids can both enhance toxicity or protect against toxicity, and the direction of the effect depends on the target organ, animal strain and age, and the temporal administration regimen. Moreover, and continuing in this line, Hayne's group found that the dose of 0.7 mg/kg i.p. of dexamethasone was the minimal dose that induced significant changes in the level of lethal and sublethal damage in rats of approximately 60 days old (200–250 g) (Haynes et al., 2003). Also, recent studies performed by Mendez-Armenta et al. (2001, 2003) have shown the protective effect of dexamethasone against the oxidative stress induced by cadmium in some brain regions, namely, the parietal cortex, striatum, hippocampus and cerebellum. This effect is characterized by a decrease in lipid peroxidation and an increase in metallothionein levels in the groups treated with cadmium. In the same study, dexamethasone increased significantly lipid peroxide levels in the parietal cortex, striatum and cerebellum in Wistar rats of 13 days old (15–20 g) and treated with dexamethasone (2 mg/kg i.p.) for 5 days (Méndez-Armenta et al., 2003). In the present study, we did not measure significant changes in control animals injected with dexamethasone. This difference is due to differences between the two experimental models. However, we found that adrenalectomy increased oxidative stress (Table 1) and decreased succinate dehydrogenase activity (Table 1) and Alamar Blue fluorescence (Fig. 2). In addition, adrenalectomy intensified the effects of 3-nitropropionic acid. In both cases, dexamethasone administration prevented the induced changes. All data support the possible beneficial effect of glucocorticoids, especially dexamethasone (Table 1 and Fig. 2).

Although it was not the main target of the present study, the results seem to indicate that glucocorticoids may affect succinate dehydrogenase activity. In our study, both 3-nitropropionic acid and adrenalectomy reduced succinate dehydrogenase activity. In addition, adrenalectomy intensified the change caused by 3-nitropropionic acid. However, dexamethasone partially improves succinate dehydrogenase activity in experimental models of adrenalectomy, whereas it does not induce changes when administered to control animals. These data seem to support the hypothesis that glucocorticoids can regulate succinate dehydrogenase activity. This effect depends on the experimental model, dose and oxidative stress.

In summary, our study proves that: (i) 3-nitropropionic acid induces both oxidative stress and changes in succinate dehydrogenase activity; (ii) the absence of endogenous glucocorticoids (adrenalectomy) produces a state similar to that induced by 3-nitropropionic acid although of smaller intensity; (iii) adrenalectomy intensifies the changes induced by 3-nitropropionic acid; and (iv) dexamethasone at low doses (300 μg/kg i.p.) decreases oxidative stress and increases succinate dehydrogenase activity. These data indicate the beneficial effect of glucocorticoids, especially dexamethasone, against oxidative stress induced by 3-nitropropionic acid. However, more investigations are required to evaluate the participation of succinate dehydrogenase activity in the protective effect of dexamethasone.

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