Stress Mediators Regulate Brain Prostaglandin Synthesis and Peroxisome Proliferator-Activated Receptor-γ Activation after Stress in Rats

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Stress exposure leads to oxidative/nitrosative and neuroinflammatory changes that have been shown to be regulated by antiinflammatory pathways in the brain. In particular, acute restraint stress is followed by cyclooxygenase (COX)-2 up-regulation and subsequent proinflammatory prostaglandin (PG) E2 release in rat brain cortex. Concomitantly, the synthesis of the antiinflammatory prostaglandin 15d-PGJ2 and the activation of its nuclear target the peroxisome proliferator-activated receptor (PPAR)-γ are also produced. This study aimed to determine the possible role of the main stress mediators: catecholamines, glucocorticoids, and excitatory amino acids (glutamate) in the above-mentioned stress-related effects. By using specific pharmacological tools, our results show that the main mediators of the stress response are implicated in the regulation of prostaglandin synthesis and PPARγ activation in rat brain cortex described after acute restraint stress exposure. Pharmacological inhibition (predominantly through β-adrenergic receptor) of the stress-released catecholamines in the central nervous system regulates 15d-PGJ2 and PGE2 synthesis, by reducing COX-2 overexpression, and reduces PPARγ activation. Stress-produced glucocorticoids carry out their effects on prostaglandin synthesis through their interaction with mineralocorticoid and glucocorticoid receptors to a very similar degree. However, in the case of PPARγ regulation, only the actions through the glucocorticoid receptor seem to be relevant. Finally, the selective blockade of the N-methyl-D-aspartate type of glutamate receptor after stress also negatively regulates 15d-PGJ2 and PGE2 production by COX-2 down-regulation and decrease in PPARγ transcriptional activity and expression. In conclusion, we show here that the main stress mediators, catecholamines, GCs, and glutamate, concomitantly regulate the activation of proinflammatory and antiinflammatory pathways in a possible coregulatory mechanism of the inflammatory process induced in rat brain cortex by acute restraint stress exposure. (Endocrinology 149: 1969–1978, 2008)
Whereas NO and other free radicals have been demonstrated to have an important role on stress-induced neurodegeneration (12), a more complex regulatory role has been demonstrated for cyclooxygenases (COXs) and its by-products in these conditions (13, 14). PGs are a family of autocrine and paracrine molecules implicated in the regulation of numerous physiological and pathophysiological processes (15). In the CNS, PGs have been related to the control of neuroinflammatory processes, synaptic transmission, brain plasticity, regulation of fever, HPA axis activation, and sleep (16). After inflammatory/neuroendocrine challenges, different brain cellular types (neurons; glia; and vasculature-associated cells such as endothelial, leukocyte, and perivascular cells) have been identified as responsible for the prostaglandin synthesis (17).

All these compounds are generated via a complex multienzymatic pathway: PGs are produced by metabolism of arachidonic acid by COXs, this generating the intermediate substrate PGH2. COXs are bifunctional enzymes, with fatty acid cyclooxygenase and hydroperoxidase activities that are present in three isoforms: COX-1, COX-2, and COX-3, a splice variant of COX-1 (17, 18). Whereas COX-1 is constitutively expressed as a housekeeping enzyme mediating physiological responses in nearly all tissues, COX-2 is rapidly and transiently induced and is primarily responsible for the synthesis of prostanoids involved in pathological inflammatory processes. COX-2 is constitutively expressed in only certain tissues (such as the brain cortex) but is up-regulated in a wide variety of cells by cytokines, mitogens, growth factors, and bacterial lipopolysaccharide (19). Subsequently PGH2 is further metabolized by tissue-specific prostaglandin synthases into different prostanoids, such as PGE2, PGD2, PGI2, and thromboxane A2. In the case of PGE2 synthesis, microsomal PGE synthase-1 (μ-PGES-1) is the most important and studied E synthase-type enzyme in brain cortex (20), and for 15d-PGJ2 synthesis, lipocalin-type PGD synthase (L-PGDS) is the main one (21).

COX-2 up-regulation in different brain areas after stress exposure was first described by Yamagata et al. (22). We have shown that the PG synthesis pathway is activated by simple exposure (6 h) to restraint stress in rat cerebral cortex through COX-2 overexpression and subsequent PGE2 production in a NFkB-dependent mode (13). This up-regulation has been described to occur in different physical, psychological, or mixed stress protocols, such as crowding, immobilization, or cold restraint (23–25). Restraint stress also induces a COX-2-dependent increase of the antiinflammatory prostaglandin 15d-PGJ2 and in the expression and activity of its nuclear target, the PPARγ, in brain cortex (26, 27).

PPARγ is a transcription factor that belongs to the superfamily of hormone nuclear receptors (28) widely expressed in brain cortex (29), exerting antiinflammatory activities in brain cells by reducing the release of proinflammatory cytokines, inducible NO synthase (NOS)-2, and COX-2 expression or interfering with inflammatory transcription pathways, such as activator protein-1 or NFkB (30–32). Thus, activation of PPARγ is considered as a mechanism able to halt the inflammatory response. Indeed, in the past 2 yr, an increasing number of studies have reported on the protective effects of PPARγ agonists in animal models of inflammatory-related neurologic diseases (33). The compensatory, antiinflammatory role of the COX-2-derived 15d-PGJ2-PPARγ pathway has been demonstrated to occur in brain prefrontal cortex by using natural, endogenous, and pharmacological ligands of PPARγ in rats acute or chronically stressed by restraint (26, 27, 34).

Taking into account the inherent limitations of any systemic pharmacological manipulation over the complex actions of the stress mediators in vivo, the present study was aimed to determine the possible role of CATs, GCs and GLUT in the regulation of prostaglandin synthesis and PPARγ activation previously observed in a single exposure to restraint stress model in rat brain cortex, a brain area with high levels of COXs and PPARγ proteins and very susceptible to the neuroinflammatory process elicited by restraint stress exposure.

Materials and Methods

Animals

Adult male Wistar: Hann (Harlan, Barcelona, Spain) rats weighing 225–250 g were used. All experimental protocols adhered to the guidelines of the Animal Welfare Committee of the Universidad Complutense following European legislation (November 24, 1986; DC 86/609/EEC). The rats were housed individually under standard conditions of temperature and humidity and a 12 h light, 12-h dark cycle (lights on at 0800 h) with free access to food and water. All animals were maintained under constant conditions for 7 d before stress.

RestRAINT Stress

Rats were exposed to stress between 0900 and 1500 h in the animal homecage. The restraint was performed using a plastic rodent restrainer that allowed for a close fit to rats during 6 h (35) in their home cages. Restraint is a stress model classified as physical-psychological predictable, and no escapeable suitable to study neurochemical effects of stress on brain function. It is a very complex challenge in which multiple elements such as heat, novelty, and forced immobility are minimized (reviewed in Ref. 36). In our model some of these elements were controlled: 6 h of restraint stress did not produce significant changes in body temperature, body weight, and respiration (35). Stressed animals were killed immediately after restraint (still in the restrainer) using sodium pentobarbital (320 mg/kg ip). Control animals were not subjected to stress but were handled at 0900 h for few seconds; food and water were removed and were killed at the same time of the stressed animals (1500 h). Blood for plasma determinations was collected by cardiac puncture and anticoagulated in the presence of trisodium citrate [3.15% (wt/vol), 1 vol citrate per 9 vol blood]. After decapitation, the brain was removed from the skull, and after careful removing of meninges and blood vessels, the prefrontal cortical areas from both brain hemispheres were excised and frozen at −80 C until assayed.

This single stress exposure was chosen according with the time course of activation of pro- and antiinflammatory pathways in brain cortex after stress: previous studies using this protocol have shown that soon after the onset of stress (20–60 min), GLUT and cytokines (TNFα and IL-1β) increase in many brain areas including cortex; NFkB and COX2 after 4 h; and inducible NOS after 6 h (reviewed in Ref. 10). The levels of proinflammatory PGE2 increase after 4 h from the onset of stress (4 h: 128%; 6 h: 208% from control, 27.8 ± 2.6 pg/mg protein). The time course of 15d-PGJ2 release after single restraint exposure also shows an increase after 4 h from the onset in parallel with COX-2 activation (4 h: 153%; 6 h: 205% from control, 489.89 ± 62.5 pg/mg protein).

Brain 15d-PGJ2 and PGE2 levels

By using enzyme immunoassay kits (Biolink, Barcelona, Spain, and Amersham, Buckinghamshire, UK, respectively). Samples were purified using polypropylene minicolumns (Amprep C18 octadecyl; Amersham). Cortices were homogenized by sonication (Branson sonifier 250);
(VibraCell); American Laboratory, Groton, CT) in an ice-cold buffer (pH 7.4) containing Tris-HCl (50 mM), sucrose (320 mM), dithiothreitol (1 mM), leupeptin (10 μg/ml), soybean trypsin inhibitor (10 μg/ml), and aprotinin (2 μg/ml), followed by centrifugation at 2500 g for 2 min at room temperature. Enzyme immunoassay isolation and prostaglandin quantification were carried out according to manufacturer’s instructions.

**Western blot analysis**

To determine COX-1, COX-2, L-PGDS, m-PGES-1, and PPARγ protein expression levels, tissues were homogenized in the same buffer used for quantification of prostaglandins (see above), except for microsomal protein expression levels, tissues were homogenized in the same buffer used for microsomal peroxidase assay (50 mM Tris-HCl, 50 mM sucrose, 1.5% sodium deoxycholate, 0.1 mM EDTA, 0.1 mM EGTA, 10 mM NaCl, pH 7.4) containing Tris-HCl (50 mM), sucrose (320 mM), dithiothreitol (1 mM), leupeptin (10 μg/ml), soybean trypsin inhibitor (10 μg/ml), and aprotinin (2 μg/ml), followed by centrifugation at 2500 g for 2 min at room temperature. Enzyme immunoassay isolation and prostaglandin quantification were carried out according to manufacturer’s instructions.

**Preparation of nuclear extracts**

A modified procedure based on the method of Schreiber et al. (37) was used. Tissues (brain frontal cortex) were homogenized with 300 μl buffer [10 mM/liter N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid (HEPES) 7.9; 1 mM/liter EDTA, 1 mM/liter EGTA, 10 mM/liter KCl, 1 mM/liter dithiothreitol, 0.5 mM/liter phenylmethylsulfonl fluoride, 0.1 mg/ml aprotinin, 1 mg/ml leupeptin, 1 mg/ml Na-p-tosyl-l-lysine-chloromethyl ketone, 5 mM/liter NaF, 1 mM/liter NaVO4, 0.5 mol/liter sucrose, and 10 mM/liter Na2MoO4]. After 15 min, Nonidet P-40 (Roche, Mannheim, Germany) was added to reach a 0.5% concentration. The tubes were gently vortexed for 15 sec, and nuclei were collected by centrifugation at 8000 × g for 5 min. Supernatants were considered as the cytosolic fraction. The pellets were resuspended in 100 μl buffer supplemented with 20% glycerol and 0.1 mol/liter KCl and gently shaken for 30 min at 4°C. Nuclear protein extracts were obtained by centrifugation at 13500 × g for 5 min, and aliquots of the supernatant were stored at −80°C. All steps of the fractionation were carried out at 4°C.

**PPARγ transcription factor assay**

PPARγ transcription factor activity was determined on nuclear extracts by using an ELISA-based kit, which allows to detect and quantify the specific transcriptional activity of PPARγ (Cayman Chemicals). Briefly, nuclear extracts were incubated in a multwell plate coated with specific peroxisome proliferator response element response probes, and PPAR bound to the peroxisome proliferator response element probe was detected using a specific antibody against the γ-isofrom. Horseradish peroxidase-labeled secondary antibody was added and the binding was detected by spectrophotometry. Measurement was performed according to the manufacturer’s instructions. This assay is specific for PPARγ activation, and it does not cross-react with other PPAR isomers such as PPARα or PPARβ-δ.

**Plasma corticosterone levels**

Plasma was obtained from blood samples (see above) by centrifuging the sample at 1000 × g for 15 min immediately after stress. All plasma samples were stored at −20°C before assay by using a commercially available kit by RIA of 125I-labeled rat corticosterone (Diagnostic Products Corp., Los Angeles, CA). A γ-counter was used to measure radioactivity of the samples. The values obtained in control animals (192 ± 7 ng/ml) are in accordance with the kit manufacturer’s expected values in adult male Wistar rats at the time of blood extraction (1500 h).

**Pharmacological tools**

To modulate GC effects, various groups of animals were ip injected with the glucocorticoid synthesis blocker metyrapone [2-methyl-1,2-di-3-pyridyl-1-propanone (MET)] (100 mg/kg), the glucocorticoid receptor-(GR)-type II antagonist mifepristone [11β-(4-dimethylaminophenyl)-17β-hydroxy-17α-(prop-1-ynyloxy)estra-4,9-dien-3-one] (RU38486 (RU), 100 mg/kg), and the mineralocorticoid receptor (MR; type I) antagonist spironolactone (SP; 50 mg/kg). The dose of MET used was chosen based on previous studies showing that this dose is sufficient to block GC synthesis during 24 h after injection; however, a higher single dose of MET (200 mg/kg) causes long-term dysregulation of the HPA axis (ACTH permanent overrelease) in the rat (38, 39). The dose of RU used completely blocks GR classical actions (glucocorticoid negative feedback) and was chosen in base of other preceding works (40–42). The dose of SP is in the optimal range to block MR and was selected in base of several previous studies in rats (43, 44).

To modulate CAT effects, various groups of animals were ip injected with propranolol [1-(isopropylamino)-3-(1-naphthyloxy)-2-propanol hydrochloride (PRO) (5 mg/kg)], a liposoluble, nonselective β-adrenergic blocker that readily crosses the blood-brain barrier (45) or with the nonselective α-adrenergic blocker phenolamine (PHE) (2.5 mg/kg). The PRO dose range chosen has proven to block adrenergic effects in brain by various studies focused on stress released catecholamines (45, 46). As in the case of PRO, the dose range of PHE used is sufficient to block catecholamine α-adrenergic receptor-dependent actions in brain (47–49).

To block N-methyl-d-aspartate (NMDA) receptor-dependent GLUT effects, two groups of animals were ip injected with dizocilpine [(+)-5-methyl-10,11-dihydroxy-5H-dibenzo-(a,d) cyclohepten-5,10-imine (MK), a specific noncompetitive NMDA antagonist (MK; 0.1 mg/kg)]. This low dose was chosen to avoid possible neurotoxic, hallucinogenic, and HPA axis disturbing effects of this compound when it is used at higher doses (0.5–3 mg/kg) and was based in preceding studies from our laboratory and others (13, 50, 51).

MK was dissolved in saline, MET, and RU in 10% propylylglycol and SP, PHE, and PRO in 5% ethanol. All drugs and vehicles were injected ip 1 h before the onset of stress (0800 h). None of the parameters studied were modified in the three different vehicle-treated groups of rats when compared with noninjected animals. To simplify figures, these three groups were unified in only one vehicle-injected group in control (C+VEH) and stress (S+VEH) conditions. Each experimental group included at least six animals.

**Adrenalectomy**

A number of animals were also bilaterally adrenalectomized (ADX) under general anesthesia using isoflurane. A small incision was made along the midline of the back just below the rib cage, connective tissue and fat was displaced, and a small hole was made through the muscle using blunt cut scissors. The adrenals were excised with curved forceps. The integrity of each removed gland was examined after its excision. Incisions were closed and treated with surgical antiseptic solution. After surgery, all ADX animals were immediately given 0.9% saline to drink to maintain their salt balance. Surgery for sham animals was identical, but the adrenals were not removed. Animals were allowed 5 d postoperative recovery.
Protein assay

Proteins were measured using the bicinchoninic acid method (52).

Chemicals and statistical analyses

Unless otherwise stated, the chemicals were from Sigma (Madrid, Spain). Data in text and figures are expressed as mean ± SEM of the indicated number of experiments. For multiple comparisons, a one-way ANOVA followed by the Newman-Keuls post hoc test to compare all pairs of means between the control group and the rest of the groups. Furthermore, all the different stress + treatments groups were compared with the stress + vehicle group using the same statistical analysis. P < 0.05 was considered statistically significant.

Results

Regulation of cortical 15d-PGJ2 and PGE2 production in control and stress conditions by GCs, CATs, and GLUT

Single exposure (6 h) to restraint produces a marked increase in 15d-PGJ2 (P < 0.05 vs. control, 427.83 ± 70 pg/mg protein) and PGE2 (P < 0.05 vs. control, 38 ± 5 pg/mg prot) levels in rat cortex, compared with control + vehicle conditions (Fig. 1, A and B).

Preadministrations (1 h before the onset of stress) of the glucocorticoid synthesis inhibitor MET, the GR antagonist RU, the MR antagonist SP, the β-blocker PRO, or the NMDA glutamate receptor blocker MK prevents the overproduction of 15d-PGJ2 observed after single-exposure stress (6 h) (Fig. 1A). Blockade of the α-receptor with PHE did not prevent this effect (Fig. 1A).

The stress-induced PGE2 production was reduced by all treatments (Fig. 1B).

In control conditions (nonstressed animals), pretreatment with MET produced an increase in 15d-PGJ2 and PGE2 levels (Fig. 1, A and B). PHE also increased the PGE2 control levels (Fig. 1B). Preadministration of MK significantly decreased the basal concentration of 15d-PGJ2 (Fig. 1A).

COX expression in control and stress conditions by GCs, CATs, and GLUT

To test whether the above-mentioned effects in PG synthesis were correlated with changes in COX protein expression levels, Western blot studies were made on cortical samples from control and stressed animals:

- Single exposure to restraint stress produces an increase of COX-2 protein levels in rat brain cortex. However, previous administration of MK and PRO prevented this increase (Fig. 2A). Cortical COX-1 protein levels were not affected by stress or any particular drug pretreatments (Fig. 2B).

L-PGDS and m-PGES-1 expression in control and stress conditions by GCs, CATs and GLUT

To check whether the aforementioned effects in PG synthesis were correlated with modifications in the expression levels of the respective PG synthases, Western blot analysis was carried out. Both stress and the respective pretreatments do not modify the protein levels of the particular synthases, m-PGES-1 and L-PGDS (Fig. 3), suggesting that modification in 15d-PGJ2 and PGE2 levels (Fig. 1) are mainly due to the effect of the drugs on COX-2 protein.

PPARγ expression in control and stressed rat brain cortex by GCs, CATs, and GLUT

To further investigate the effects of the respective treatments in the COX-2/L-PGDS/15d-PGJ2/PPARγ antiinflammatory pathway, PPARγ protein expression was studied in brain cortex. Single exposure to restraint stress enhanced PPARγ protein expression (Fig. 4). Furthermore, all the treatments except for SP and PHE completely blocked this stress-induced PPARγ overexpression (Fig. 4). None of the treatments modified PPARγ expression in control, nonstressed animals.

Regulation of PPARγ activity in control and stressed rat brain cortex by GCs, CATs, and GLUT

As in the case of PPARγ expression, single exposure to restraint stress (S6) produced a significant up-regulation of PPARγ transcriptional activity in nuclear extracts obtained from rat brain cortex samples. This increase was prevented
by the respective pretreatment with MK, MET, RU, or PRO (Fig. 5). Interestingly, all these pharmacological tools prevented the stress-induced increase in PPARγ activity. In stress conditions, MK, MET, RU, and PRO also decreases basal (vehicle-treated control animals) transcriptional activity (Fig. 5). Similarly to PPARγ expression, SP and PHE did not modify PPARγ activity. Basal PPARγ transcriptional activity remained unchanged in all groups when compared with vehicle-treated control group (Fig. 5).

**Effects of adrenalectomy in prostaglandin levels and COX-2 and PPARγ expression in control and stressed rat brain cortex**

To confirm the results obtained with the pretreatment of MET (pharmacological adrenalectomy), a complementary study with ADX rats in control and acute stress conditions was carried out. Adrenalectomy (S6+ADX) prevented the overproduction of 15d-PGJ2 and PGE2 observed after single exposure stress (S6 SHAM) (Fig. 6A). Furthermore, the surgical ablation of the adrenal glands blocked the stress-induced increase in COX-2 and PPARγ expression, analyzed by Western blot (Fig. 6B).

Interestingly, neither prostaglandin levels (Fig. 6A) nor COX-2 and PPARγ expression (Fig. 6B) were modified by adrenalectomy (C+ADX) in control conditions (CONTROL SHAM).

**Effects of the drugs used on plasma corticosterone levels**

To determine the interactions between the aforementioned pharmacological tools and the stress response, corticosterone levels were measured in the plasma obtained from rats treated with the different drugs. As shown in Fig. 7, stress exposure caused an increase in corticosterone as expected by such stimulus. The administration of the GR antagonist RU resulted in increased corticosterone concentration after stress when compared with the value obtained in the group of animals stressed in the absence of RU (Fig. 7). The inhibition of glucocorticoid synthesis by MET had the opposite effect, causing a reduction of about 50% in stress-induced corticosterone levels (Fig. 7), indicating that, in the conditions used, MET-induced reduction of glucocorticoids is able to interfere with the HPA pathway and the subsequent synthesis of corticosterone. Similarly, the blockade of β-adrenergic receptors by PRO reduced the stress-induced corticosterone levels.

The plasma corticosterone levels of control and stressed
adrenalectomized rats are under the detection limits of the RIA kit (data not shown), validating the surgical ablation of the adrenal glands in these two groups of animals.

**Discussion**

The synthesis of prostaglandins and the activation of PPARγ detected in the cortices of stressed animals are regulated by CATs, GCs, and GLUT. The data presented here indicate that the blockade of CAT actions in the CNS after a single stress exposure can inhibit the overexpression of

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**Figure 3.** A, L-PGDS protein expression in brain cortex of control and stressed rats (S6) treated with vehicle (VEH), MK (0.1 mg/kg), MET (100 mg/kg), RU (100 mg/kg), SP (50 mg/kg), PRO (5 mg/kg), or PHE (2.5 mg/kg). The inset panel is representative of the mean of three independent experiments ± SEM. B, Western blot analysis of m-PGES-1 expression in the same groups of animals as in A. Each band is representative of the mean of three independent experiments ± SEM.

**Figure 4.** Western blots analysis showing the effects vehicle (VEH), MK (0.1 mg/kg), MET (100 mg/kg), RU (100 mg/kg), SP (50 mg/kg), PRO (5 mg/kg), or PHE (2.5 mg/kg) respective pretreatments in PPARγ protein expression in control and stressed (S6) rats brain cortex. Each band is representative of the mean of three independent experiments ± SEM. *, P < 0.05 vs. control; #, P < 0.05 vs. S6+VEH (Newman-Keuls test).

**Figure 5.** PPARγ transcriptional activity in brain cortex of control and acute stressed rats (S6) after the respective preadministration of vehicle (VEH), MK (0.1 mg/kg), MET (100 mg/kg), RU (100 mg/kg), SP (50 mg/kg), PRO (5 mg/kg), or PHE (2.5 mg/kg). Data represent the mean ± SEM of six rats. *, P < 0.05 vs. C+VEH; #, P < 0.05 vs. S6+VEH (Newman-Keuls test).

**Figure 6.** A, 15d-PGJ2 and PGE2 levels in brain cortex of control (C+ADX) and stressed adrenalectomized (S6+ADX) rats and their respective controls without adrenalectomy (sham). Data represent the mean ± SEM of six rats. *, P < 0.05 vs. C; #, P < 0.05 vs. S6 (Newman-Keuls test). B, Western blots and densitometric analyses of the protein COX-2 and PPARγ in control and acute stressed rats with (+ADX) and without adrenalectomy (SHAM). Each band is representative of the mean of three independent experiments ± SEM. *, P < 0.05 vs. control SHAM; #, P < 0.05 vs. S6 SHAM (Newman-Keuls test).
COX-2, which leads to a reduction of 15d-PGJ$_2$ and PGE$_2$ synthesis and the activation of PPAR$_\gamma$. This is mainly a $\beta$-adrenergic-dependent process, and in the case of PGE$_2$, it is also $\alpha$-adrenergic receptor dependent. Whereas glucocorticoids binding to both MR and GR type of receptors acts as a stimulus for the synthesis of PGs, only the actions through GR seem to be relevant for the regulation of PPAR$_\gamma$. Finally, the key role of excitatory amino acids in stress-induced consequences in brain is also demonstrated here: the overactivation of the NMDA glutamate receptor caused by stress participates in the induction of COX-2 and PPAR$_\gamma$ and the production of 15d-PGJ$_2$ and PGE$_2$.

During the early phases of stress response, catecholamines have been identified as regulators of the production of proinflammatory-related molecules, such as cytokines (TNF-$\alpha$, IL-1$\beta$), NfkB, and NO (reviewed in Ref. 3). Our results are in agreement with previous studies showing that CATs (nor-epinephrine) stimulate the synthesis of PGE$_2$ via mechanisms dependent on both types of adrenergic receptors (53–55), although the opposite role of $\alpha$-adrenergic receptors in control conditions remains controversial. Certainly further work is necessary to explain this unexpected emerging role of $\alpha$-adrenergic receptors, but individual differences explain similar findings of the $\alpha$-blocker prazosin on corticosterone levels in control and stressed animals (56). The different levels of other inflammatory-related molecules as NfkB, cytokines, or GCs in control and after stress conditions could be a possible explanation.

Interestingly, CATs also regulate the antiinflammatory prostaglandin 15d-PGJ$_2$ overrelease previously shown in this model of stress (26) through a $\beta$-adrenergic-related mechanism. Emerging literature suggests a predominant antiinflammatory role of CATs in several models of neurotoxicity, a role that seems to be exerted through $\beta$-adrenergic-type receptors, further confirming our results (55). In this way, CATs could control COX-2-dependent proinflammatory and antiinflammatory prostaglandin synthesis in a balanced mechanism. In support of this idea, some authors recently identified 15d-PGJ$_2$ as a potent blocker of m-PGES-1 mRNA induction by IL-1$\beta$ or lipopolysaccharide in chondrocytes, synovial fibroblasts, and macrophages (57–59). In this vein, we previously demonstrated that the administration of supraphysiological doses of 15d-PGJ$_2$ can prevent the release of PGE$_2$ and COX-2 up-regulation after a single exposure to restraint stress (26).

The regulation of the different prostaglandins synthesis by GCs during a single exposure to restraint stress seems to be a relatively complex process. Although the data presented here represent an advance over *ex vivo* or *in vitro* studies, it is necessary to address the limitations of the *in vivo* approaches to interpret the results. For instance, the pharmacological modulation of GRs after systemic administration of drugs may cause changes in the stress system (GR-dependent negative feedback on the HPA axis) (60). Furthermore, all these changes may affect the prostaglandin synthesis when trying to extrapolate these results to chronic stress exposure or chronic treatments.

With these limitations in mind, we report here that the glucocorticoids secreted after restraint stress exposure seem to up-regulate the synthesis of the proinflammatory prostaglandin PGE$_2$ in brain via both types of receptors. This is a paradoxical result, taking into account the classical therapeutic use of GCs in diverse pathologies (autoimmune diseases, chronic inflammation, or transplant rejection), acting as immunosuppressor and antiinflammatory (mainly due to their capacity to inhibit COX activity) in several body systems, including the brain (22, 61, 62).

In this vein, we have demonstrated that, in the presence of high GCs levels, a strong inflammatory response is induced in brain along with the accumulation of oxidative and nitrative mediators (11). This inflammatory response is characterized by the activation of proinflammatory nuclear factors (NfkB), up-regulation of NOS-2 and COX-2 with the subsequent NO and PGE$_2$ production, and the release of proinflammatory cytokines (TNF-$\alpha$ and IL-1$\beta$). All these effects decisively contribute to an increase in the susceptibility to cerebral damage after exposure to high GC levels (63).

Adding to the complexity of the GC effects on inflammatory processes, we demonstrate here that the same stress protocol is able to enhance the synthesis of antiinflammatory mediators as 15d-PGJ$_2$ and its nuclear target, PPAR$_\gamma$ (26, 27), through a mechanism related to both types of glucocorticoid receptors.

An interesting hypothesis derived from our results is that the glucocorticoids secreted after stress exposure could regulate the balance between proinflammatory (PGE$_2$) and antiinflammatory mediators (15d-PGJ$_2$ and PPAR$_\gamma$) in a possible endogenous mechanism of brain plasticity against the cellular damage caused by excessive inflammation (27). Further studies are necessary to demonstrate the status of this balance in chronic stress conditions and other stress protocols.

Unexpectedly, in our model, the pharmacological inhibition of the synthesis of GCs and the blockade of MR- and GR-dependent actions inhibit prostaglandin synthesis after stress in the brain without affecting COX-2 or specific prostaglandin synthases expression, results that indirectly indicate possible effects of GCs in the catalytic activity or protein stability of these enzymes (64). Using a different model, Garcia-Fernandez et al. (65) identified the synthetic glucocor-
some authors have found certain neuroprotective action for this adrenaline and its effect of RU on the GRs (61). The interference between noradrenaline and its concentration after stress may be attributed to the blockade of the medullar catecholamines release.

Interestingly, adrenalectomy prevents the COX-2 overexpression observed after acute stress exposure. In line with the results obtained with the other pharmacological modulators of the GC pathway used in our study, we can speculate that this differential effect shown after adrenalectomy could be related with the stronger suppression of GC synthesis, compared with the treatment with MET and the synergic inhibition of the medullar catecholamines release.

The fact that RU administration increased corticosterone concentration after stress may be attributed to the blockade of the self-inhibitory feedback of corticosterone due to the effect of RU on the GRs (61). The interference between norepinephrine and its β-receptors is known to have an inhibitory effect on not only the secretion of corticosterone or stress-associated behaviors (67) but also the synthesis of the GRs (68). According to this, the alteration of corticosterone levels by PRO described here confirms the interaction of the two systems and allows us to attribute some of the effects of PRO to an inhibition not only of the β-adrenergic-dependent pathway but also to some extent to other mechanisms regulated by corticoids.

Finally, the stress-induced stimulation of the NMDA type of glutamate receptor is implicated in prostaglandin synthesis, as demonstrated by using MK. Previously our group and others demonstrated the control of PGE2 production after stress by NMDA receptors (13). Here we show that the activation of NMDA receptors also up-regulates 15d-PGJ2 synthesis in control and stress conditions in a COX-2-dependent mechanism.

Several animal models of neurodegeneration, including single exposure to restraint stress, demonstrated that the up-regulation of COX-2 is implicated in the excitotoxic processes generated by NMDA excessive activation (13). The mechanisms through which COX-2 might exert its neurotoxic effects are still partially unknown, but they seem to be due to the large quantities of free radicals generated by cyclooxygenase activity and also to its principal products like PGE2, which are neurotoxic per se (69–71), although other authors have found certain neuroprotective action for this compound, depending on the type of PGE2 receptor activated (72, 73). In our single exposure to restraint stress paradigm, glutamate actions through its NMDA-type receptor regulate the brain synthesis of typically proinflammatory prostaglandins like PGE2 (74) and also antiinflammatory ones such as 15d-PGJ2 (26) in what might constitute a regulatory mechanism of the inflammatory process generated after NMDA excessive activation. In this vein we and other authors (34, 75, 76) found the administration of supraphysiological doses of 15d-PGJ2 to be neuroprotective against excitotoxic insults in in vivo and in vitro models.

As in the case of its endogenous ligand 15d-PGJ2, PPARγ expression and transcriptional activity are modulated by all stress mediators studied: glutamate (NMDA dependent), catecholamines (β-adrenergic type), and glucocorticoids (GR type). PPARγ regulation is not yet completely understood, but one of the main mechanisms may be the ligand availability (77). This kind of regulation could be important in our stress model because we found a 3- to 4-fold increase in 15d-PGJ2 levels, compared with control conditions. In addition to this, all the drugs that inhibit PPARγ activation also reduce 15d-PGJ2 synthesis (78).

A tight relation exists between GC type I receptor and PPAR because it has been demonstrated in in vitro studies in which GCs induce PPARα mRNA synthesis in isolated human adipocytes in a GR-dependent manner (as our data show) (79) and in vivo with the synthetic glucocorticoid dexamethasone as a potent inducer of PPARα mRNA binding GR in hepatic tissue (80).

Some authors have shown that norepinephrine is able to up-regulate PPARγ expression in a β- but not α-adrenergic receptor-type-dependent mechanism, an effect considered as antiinflammatory and neuroprotective in animal models of Alzheimer’s disease in which PPARγ expression is reduced (81).

To our knowledge, this is the first time that a relation between stress-induced NMDA receptor activation and PPARγ activation has been demonstrated. As in the case of its natural ligand 15d-PGJ2, this activation could represent a possible endogenous neuroprotective mechanism against the excitotoxic process generated. Of course, further studies are required to explore the precise mechanism implicated. Accordingly, recent studies from our group show that PPAR activation by 15d-PGJ2 or rosiglitazone provide neuroprotection through up-regulation of the main glutamate transporter in glia excitatory amino acid transporter-2 in animal models in which its expression and activity are reduced such as subchronic restraint stress (34) or ischemic preconditioning (82).

In conclusion, main stress mediators CATs, GCs, and GLUT concomitantly modulate the activation of proinflammatory (COX-2/m-PGES-1/PGE2) and antiinflammatory (COX-2/L-PGDS/15d-PGJ2/PPARγ) pathways in a possible complex mechanism regulating the inflammatory process induced by single exposure to restraint stress exposure in rat brain cortex. Furthermore, more detailed studies are necessary to explain the possible interaction among these three stress mediators in the regulation of brain cortex prostaglandin synthesis and to identify the origin (vascular or within brain parenquima) and the precise role of each cellular type implicated in our stress model.

The study of the balance and time course among these pro- and antiinflammatory pathways in specific brain areas related to pathological conditions, in which the inflammatory overreaction contributes to the cellular damage observed, seems to be critical to find new possible therapeutic strategies.

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