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A glucocorticoid receptor-independent mechanism for neurosteroid inhibition of tumor necrosis factor production

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Abstract

We investigated the effect of two neurosteroids, pregnenolone and dehydroepiandrosterone sulfate on lipopolysaccharide-induced tumor necrosis factor (TNF) production in vivo and in vitro. Dehydroepiandrosterone sulfate (0.3–30 mg/kg, i.p.) inhibited serum TNF induced by lipopolysaccharide (2.5 μg/mouse, i.p.), without affecting the induction of serum corticosterone. Intracerebroventricular (i.c.v.) administration of dehydroepiandrosterone sulfate (0.2–5 μg/mouse) also inhibited brain TNF induced by i.c.v. lipopolysaccharide (2.5 μg/mouse). Dehydroepiandrosterone sulfate and pregnenolone (10^{-6}–10^{-4} M) inhibited TNF production in vitro by lipopolysaccharide-stimulated human peripheral blood mononuclear cells or by the human THP-1 cell line, suggesting that this action might also be relevant in humans. We obtained two lines of evidence that neurosteroids do not inhibit TNF via the glucocorticoid receptor. (1) Dehydroepiandrosterone sulfate and pregnenolone did not activate the α_1-acid glycoprotein promoter, a typical effect of glucocorticoids mediated by the glucocorticoid receptor, while strong activation of this promoter was observed with dexamethasone. (2) The inhibitory effect of dehydroepiandrosterone sulfate and pregnenolone on TNF production was not reversed by the glucocorticoid receptor antagonist, mifepristone (RU38486). On the contrary the inhibitory effect of dexamethasone, a classical glucocorticoid and inhibitor of TNF synthesis, was completely reversed by RU38486.

Keywords: Neurosteroid; TNF (tumor necrosis factor); Glucocorticoid; Dehydroepiandrosterone sulfate; Pregnenolone

1. Introduction

Glucocorticoids, including endogenous corticosterone, are among the most potent inhibitors of the synthesis of tumor necrosis factor (TNF) and other pro-inflammatory cytokines including interleukin-1 and TNF (Fantuzzi and Ghezzi, 1993; Chrousos, 1995); they are protective in animal models of cytokine-mediated pathologies such as endotoxin (lipopolysaccharide)-induced lethal shock. Injection of lipopolysaccharide, interleukin-1 or TNF causes an elevation of serum glucocorticoids due to activation of the hypothalamus-pituitary-adrenal axis (Besedovsky et al., 1986; Fantuzzi and Ghezzi, 1993). This constitutes an important feedback mechanism that limits cytokine production and lipopolysaccharide toxicity, as demonstrated by the high sensitivity of adrenalectomized mice to these agents (Bertini et al., 1988).

Inhibition of TNF is mediated by the classical glucocorticoid receptor, since glucocorticoid inhibition of TNF synthesis in vitro is reversed by the glucocorticoid receptor antagonist, mifepristone (RU38486), which also increases the lethal effect of lipopolysaccharide (Hawes et al., 1992; Lazar et al., 1992).

The steroid hormones are all derived from cholesterol and are converted through a series of biochemical modifications to a great number of metabolites only some of which are biologically functional.

Steroids can also be synthetized de novo in the central nervous system (CNS). The steroids produced in the CNS are termed 'neurosteroids' reviewed in (Paul and Purdy, 1992; Majewska, 1992). These steroids include pregnenolone and dehydroepiandrosterone and their sulfates (pregnenolone sulfate and dehydroepiandrosterone sulfate). Not only can neurosteroids be produced and accumulate in

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the brain, but they also have a role as endogenous modulators of the $GABA_A$ (γ-aminobutyric acid) receptor (Paul and Purdy, 1992; Majewska, 1992).

Although neurosteroids can be produced by the CNS and are detected in the brain at higher concentrations than in plasma (Corpéchot et al., 1983, 1981), they are also synthetized by the adrenals and the gonads, and are intermediates in the synthesis of sex hormones.

The purpose of this study was to investigate the effect of two neurosteroids, dehydroepiandrosterone sulfate and pregnenolone, on TNF production in vivo and in vitro. In these experiments, the effect of the two steroids was compared with that of dexamethasone phosphate, a reference glucocorticoid and well known inhibitor of TNF synthesis (Beutler et al., 1986).

The in vivo effect of one neurosteroid on serum TNF levels was studied in mice treated i.p. with lipopolysaccharide. To investigate the possible inhibition of neurosteroids on brain TNF production, in some experiments lipopolysaccharide and neurosteroids were injected intracerebroventricularly (i.c.v.), since previous experiments had indicated that central lipopolysaccharide administration is required to induce detectable TNF levels in the brain (Mengozzi et al., 1994). In these experiments we also measured the serum levels of glucocorticoids, to evaluate a possible interference of neurosteroids with the activation of the hypothalamus-pituitary-adrenal axis, evaluated as increase in plasma corticosterone. To better understand the mechanism of the inhibition of TNF production, we studied the in vitro effect of neurosteroids on TNF production by human peripheral blood mononuclear cells and by the THP-1 human monocytic leukemia cell line. In some of these experiments, we used mifepristone (RU38486) as a tool to investigate whether the effect of neurosteroids was mediated by the glucocorticoid receptor.

To further check whether the effect of dehydroepiandrosterone sulfate and pregnenolone was actually independent of the glucocorticoid receptor, we studied the effect of these two neurosteroids, and that of dexamethasone, using a construction in which a reporter gene, chloramphenicol acetyltransferase (CAT), was under the control of the α_1-acid glycoprotein (AGP) promoter, which is typically activated via the glucocorticoid receptor.

Also we used THP-1 cells, to investigate whether the inhibitory effect of dehydroepiandrosterone sulfate takes place at the transcriptional level. Measurement of TNF-α mRNA was applied for the purpose.

2. Materials and methods

2.1. Materials

Lipopolysaccharide (phenol-extracted, from *Escherichia coli* 055:B5) was from Sigma (St Louis, MO, USA). Human recombinant TNFα (specific activity 0.6×10^7 U/mg) was a kind gift of BASF/Knoll, Ludwigshafen, Germany. Dexamethasone was a gift from Laboratorio Farmacologico Milanese (Varese, Italy). Dehydroepiandrosterone sulfate (5-androsten-3β-ol-17-one sulfate sodium salt) and pregnenolone (5α-pregnan-3β-ol-20-one) were from Sigma. Dehydroepiandrosterone sulfate was dissolved in saline. Pregnenolone was dissolved in dimethylsulfoxide (DMSO); for this reason, only dehydroepiandrosterone sulfate was used in vivo, due to aspecific effects of DMSO, particularly in i.c.v. injections. Mifepristone (RU38486) was a kind gift from Roussel Uclaf, Roumainville, France.

2.2. Animals and treatments

Male CD-1 mice (25 g body weight) from Charles River Italia, Calco, Como, Italy, were used. The mice were housed five per cage and fed ad libitum. Lipopolysaccharide was injected i.p. or intracerebroventricularly (i.c.v.) at the dose of 2.5 μg/mouse. I.c.v. injections in a final volume of 20 μl were made via a 28-gauge needle into ether-anesthetized mice (Lipton et al., 1991; Haley and McCormick, 1957). Dehydroepiandrosterone sulfate was administered i.p. (in 0.2 ml of saline) or i.c.v. at the doses indicated in the text, simultaneously with lipopolysaccharide.

Blood was obtained from the retro-orbital plexus under light ether anesthesia and serum was prepared. Blood was collected at 1.5 h after treatment. When indicated, mice were killed by cervical dislocation and brains were removed 1.5 h after lipopolysaccharide for TNF determination, and homogenized with an Ultra Turrax in 4 volumes (w/v) of ice-cold saline. The homogenate was then centrifuged 10 min at 13000 rpm in a microfuge and the supernatant was used for TNF assay (Mengozzi et al., 1994). Preliminary experiments indicated that these were the optimal time points for the determination of TNF in lipopolysaccharide-treated mice (Mengozzi et al., 1994; Gnocchi et al., 1992; Sironi et al., 1992).

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, December 12, 1987; NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.3. TNF determination

Serum or brain TNF was measured by the degree of cytotoxicity on L929 cells in the presence of 1 μg/ml of actinomycin D, as previously described (Aggarwal et al., 1985), using human recombinant TNF as standard. The sensitivity of the assay was about 50 pg/ml. An anti-murine TNF antiserum was used to check the specificity of the TNF bioassay.

When TNF was measured in human peripheral blood

mononuclear cells and THP-1 cells, a sandwich ELISA method was applied as described (Corti et al., 1992). Anti-TNF monoclonal antibodies were from Pharmacia-Farmitalia-Carlo Erba (Nerviano, Italy) and rabbit polyclonal antibodies anti-TNF were a gift from Sclavo (Siena, Italy). Recombinant TNF (specific activity 6.6×10^6 U/mg, kindly provided by BASF-Knoll, Ludwigshafen, Germany) was used as a standard. The sensitivity of the ELISA was 30 pg/ml.

2.4. TNF mRNA

Total RNA was extracted from 10^7 cells by centrifugation through 5.7 M CsCl as previously described (Chirgwin et al., 1979). A 10-μg aliquot of each sample was electrophoresed through denaturing gels (containing 1.7% formaldehyde) in 3-morpholinopropane sulfonic acid (MOPS) buffer (MOPS 40 mM, Na-acetate 10 mM, EDTA 1 mM, pH 8) for 5 h at 70 V. The Gels were blotted on nylon membranes (Gene ScreenTM Plus, DuPONT, Dreieich, Germany) in 10 × standard saline citrate (SSC) (NaCl 1.5 M, sodium citrate trisodium salt dihydrate 150 mM, pH 7). A cDNA fragment (kind gift of Dr. Emanuela Palla, Siena, Italy) corresponding to nt 220–1003 of human TNFα sequence (Wang et al., 1985), was used as a probe. Membrane hybridization was carried out with 106 cpm/ml in 50% formamide, 10% dextran sulfate, 1% sodium dodecyl sulfate, and 250 μg/ml salmon sperm DNA at 45°C for about 15 h. Membranes were washed to a final stringency of 0.1 × SSC, 0.1% SDS at 65°C and exposed to KODAK X-OMAT AR-5 film (Eastman Kodak, Rochester NY) with an intensifying screen at −70°C for 24 h.

2.5. Corticosterone determination

Serum corticosterone was measured by radioimmunoassay (RIA), using an antiserum obtained from Sigma (C-8784) and following the manufacturer's indications. [^{3}H]Corticosterone was purchased from Amersham.

2.6. Human peripheral blood mononuclear cell preparation

Human peripheral blood mononuclear cells were obtained as previously described (De Boer et al., 1981) from the buffy coats of blood donations from normal volunteers. Cells were suspended in RPMI 1640 medium with 10% FCS (HyClone, Logan, UT, USA) at the concentration of 2.5×10^6/ml and plated (0.2 ml/well) in 96-well tissue culture plates (Falcon, Lincoln Park, NJ) with 10 ng/ml of lipopolysaccharide in the presence and absence of the test compounds at the concentration indicated. Since pregnenolone was dissolved in DMSO, control samples with DMSO alone were included. After a 4-h culture, cells were lysed by two cycles of freezing and thawing and total (secreted + cell-associated) TNF was determined with an ELISA for human TNF (Corti et al., 1992) using a monoclonal anti-human TNFα antibody kindly donated by Pharmacia-Farmitalia (Nerviano, Italy) and a rabbit polyclonal antibody kindly donated by Sclavo (Siena, Italy).

2.7. THP-1 cells

THP-1 cells were maintained in a humidified incubator under an atmosphere of 5% CO_2 in air, in RPMI 1640 (Seromed, Berlin, Germany) supplemented with 2 mM L-glutamine, 100 U/ml penicillin G, 100 μg/ml streptomycin sulfate (all from Gibco, Paisley, Scotland, UK), 10% fetal bovine serum (FBS, Hyclone Laboratory, Logan, UT, USA), and 1.5 g/l $NaHCO_3$, and 12 mM Hepes buffer (Carlo Erba, Milano, Italy). THP-1 cells were used for the TNF synthesis experiments at a density of 10^6/ml in fresh medium in 24-well plates, 1 ml/well (Falcon Becton Dickinson, Lincoln Park, NJ, USA) and incubated for 4 h in the presence of 1 μg/ml lipopolysaccharide and concentrations of the test compounds as indicated.

2.8. Activation of the α_1-acid glycoprotein promoter

HTC(JZ-1) cells were stably transfected with the −215 + 1 α_1-acid glycoprotein promoter fragment inserted into the vector pSVOCAT using the lipofectin method (Ingrassia et al., 1994). A single pool of the transformed −215 + 1 AGP-HTC(JZ-1) cells (Ingrassia et al., 1994), was cultured in RPMI with 1% glutamine and 10% fetal calf serum (FCS). Before harvesting, three flasks of cells for each treatment were incubated with the various steroids (dehydroepiandrosterone sulfate and pregnenolone; 10^{-6} M) for 24 h. Cell lysis and CAT protein determination were performed using the CAT-ELISA enzyme immunoassay kit (Boehringer) (Ingrassia et al., 1994). Total protein concentration in the nuclear extracts was determined by the Bradford method.

3. Results

3.1. Dehydroepiandrosterone sulfate inhibits serum and brain TNF production in mice

Fig. 1 shows the effect of i.p. administration of dehydroepiandrosterone sulfate (0.03, 0.3, 3 or 30 mg/kg) on serum TNF levels induced by lipopolysaccharide (2.5 μg/mouse, i.p.). Significant inhibition of TNF production was obtained with a dose as low as 0.3 mg/kg. At the highest dose (30 mg/kg), 80% inhibition of TNF production was observed. It should be noted that, in the same model, dexamethasone inhibited TNF production by more than 95% at a dose as low as 0.3 mg/kg (Sironi et al., 1992).

Fig. 2 shows the effect of dehydroepiandrosterone sul-

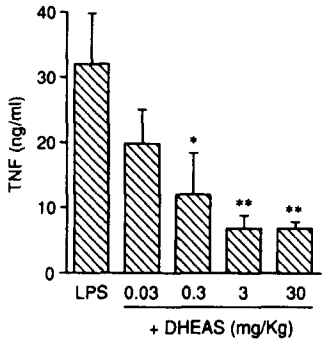

Fig. 1. Effect of dehydroepiandrosterone sulfate on serum TNF production in mice. Mice were treated simultaneously with lipopolysaccharide (LPS) (2.5 μg/mouse, i.p.) and dehydroepiandrosterone sulfate (DHEAS) at the doses indicated. Serum TNF was measured 90 min later. No TNF was detectable in mice injected with saline or dehydroepiandrosterone sulfate without lipopolysaccharide. Data are means ± S.E. (five mice/group). * $P < 0.05$; ** $P < 0.01$ versus LPS alone by Dunnett's test.

fate on brain TNF production. In these experiments dehydroepiandrosterone sulfate (at the doses of 0.2, 1 and 5 μg/mouse) and lipopolysaccharide (2.5 μg/mouse) were administered simultaneously i.c.v., and TNF was measured in the brain homogenate 90 min later. Dehydroepiandrosterone sulfate inhibited central TNF production at a dose as low as 0.2 μg/mouse. In these experiments, dexamethasone also inhibited brain TNF production.

3.2. Effect of dehydroepiandrosterone sulfate and dexamethasone on serum corticosterone after lipopolysaccharide in mice

As shown in Table 1, lipopolysaccharide (2.5 μg/mouse, i.p.) caused an 8-fold elevation of serum corticosterone levels 90 min after treatment. Dehydroepiandrosterone sulfate, even at the highest dose of 30 mg/kg, i.p., did not affect this increase. On the contrary, dexamethasone at the same doses completely suppressed

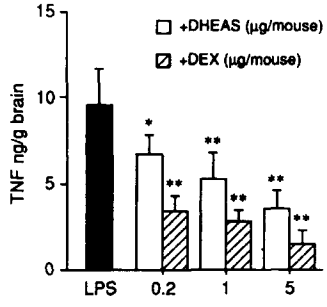

Fig. 2. Effect of dehydroepiandrosterone sulfate on brain TNF production in mice. Mice were treated simultaneously with lipopolysaccharide (LPS) (2.5 μg/mouse, i.c.v.) and dehydroepiandrosterone sulfate (DHEAS) at the doses indicated. The effect of dexamethasone (DEX) as a reference inhibitor is also shown. Brain TNF was measured 90 min later. Data are means ± S.E. (five mice/group). * $P < 0.05$; ** $P < 0.01$ versus LPS alone by Dunnett's test.

Table 1
Effect of dehydroepiandrosterone sulfate on serum corticosterone levels in mice

Treatment	CS (ng/ml)
Control	35 ± 5
LPS	280 ± 46 [a]
LPS + DHEAS (3 mg/kg)	237 ± 34
LPS + DHEAS (30 mg/kg)	307 ± 85
LPS + DEX (3 mg/kg)	20 ± 4 [b]
LPS + DEX (30 mg/kg)	43 ± 5 [b]

Mice were treated simultaneously with lipopolysaccharide (LPS) (2.5 μg/mouse, i.p.) and dehydroepiandrosterone sulfate (DHEAS) at the doses indicated. DEX = dexamethasone. Control mice received saline alone. Serum corticosterone (CS) was measured 90 min later. Data are means ± S.E. (five mice/group). [a] $P < 0.01$; versus control by Dunnett's test. [b] $P < 0.01$; versus LPS alone by Dunnett's test.

the increase of serum corticosterone by lipopolysaccharide, as previously described (Fantuzzi et al., 1994).

3.3. Inhibition of in vitro TNF production in human peripheral blood mononuclear cells by dehydroepiandrosterone sulfate, pregnenolone and dexamethasone

Fig. 3 shows the effect of various doses of dehydroepiandrosterone sulfate, pregnenolone and dexamethasone on TNF production by human peripheral blood mononuclear cells cultured for 4 h with 10 ng/ml of lipopolysaccharide. All three steroids inhibited TNF production. Dexamethasone and dehydroepiandrosterone sulfate had a comparable effect, but the maximal inhibition obtained with dexamethasone was higher than that obtained with dehydroepiandrosterone sulfate (76% and 63% inhibition, respectively). Pregnenolone was the least active of the three steroids (minimal inhibitory dose 10^{-4} M; maximal inhibition attained, 35%).

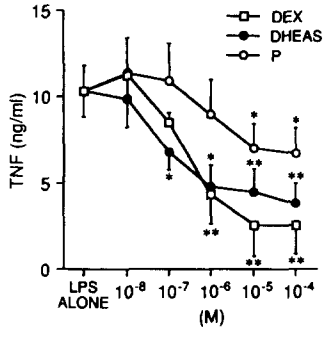

Fig. 3. Effect of dehydroepiandrosterone sulfate, pregnenolone and dexamethasone on TNF production by human peripheral blood mononuclear cells in vitro. Human peripheral blood mononuclear cells were cultured 4 h with lipopolysaccharide (LPS) (10 ng/ml) and various doses of dehydroepiandrosterone sulfate (DHEAS), pregnenolone (P) or dexamethasone (DEX). Data are the means ± S.E. from six independent experiments with different donors. * $P < 0.05$; ** $P < 0.01$ versus LPS alone by Dunnett's test.

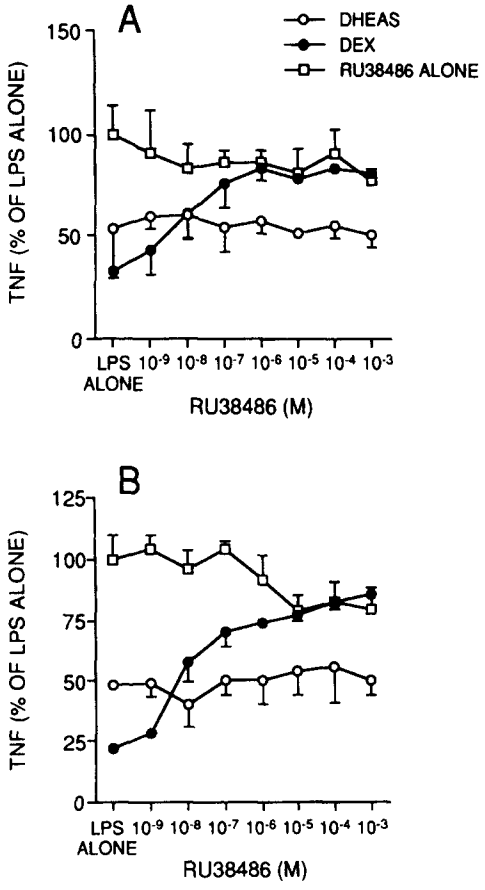

Fig. 4. Effect of various concentrations of mefipristone (RU38486) on the inhibition of TNF production in human peripheral blood mononuclear cells by dehydroepiandrosterone sulfate or dexamethasone. Human peripheral blood mononuclear cells were cultured 4 h with lipopolysaccharide (LPS) (10 ng/ml) and 10^{-5} M dehydroepiandrosterone sulfate (DHEAS) or dexamethasone (DEX). Mifepristone (RU38486) was added at the concentrations indicated. A control group (LPS alone, open squares) was included in which various doses of RU38486 were added to lipopolysaccharide-stimulated cells without dehydroepiandrosterone sulfate or dexamethasone, to evaluate the effect of RU38486 alone. Data are the means ± S.D. ($n = 2$) of TNF produced and are expressed as % of LPS alone. Panels A and B show the results from two donors.

3.4. Inhibition of TNF production by dehydroepiandrosterone sulfate or pregnenolone was not reversed by mifepristone (RU38486)

Fig. 4 shows the effect of various doses (10^{-9}–10^{-3} M) of RU38486 on the inhibition of TNF production by 10^{-5} M dexamethasone or dehydroepiandrosterone sulfate in human peripheral blood mononuclear cells from two different donors.

It can be seen that 10^{-5} M dexamethasone inhibited TNF production by 75–80% for boths donors. Its inhibitory effect was reversed by increasing concentration of RU38486 with an IC_{50} of about 10^{-8} M. On the other hand, the inhibitory effect of dehydroepiandrosterone sulfate was not reversed by RU38486 even at the highest concentration tested (10^{-3} M). RU38486 was not toxic (by trypan blue exclusion) at these concentrations; however, at

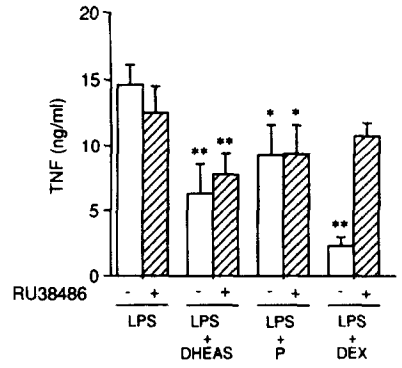

Fig. 5. Effect of mifepristone (RU38486) on the inhibition of TNF production in human peripheral blood mononuclear cells by dehydroepiandrosterone sulfate, pregnenolone and dexamethasone. Peripheral blood mononuclear cells (PBMC) were cultured 4 h with lipopolysaccharide (LPS) (10 ng/ml) and 10^{-5} M dehydroepiandrosterone sulfate (DHEAS), pregnenolone (P) or dexamethasone (DEX). When indicated (+), RU38486 was added at a concentration of 10^{-4} M. Data are the means ± S.E. from four experiments with different donors. * $P < 0.05$; ** $P < 0.01$ versus LPS alone by Dunnett's test.

the highest concentrations tested it slighly inhibited TNF production, so that it was impossible to use it at concentrations higher than 10^{-3} M.

The inhibitory effect of pregnenolone (10^{-5} M) also was not modified by 10^{-4} M RU38486, as shown in Fig. 5, showing results obtained from experiments with four donors.

3.5. Dehydroepiandrosterone sulfate or pregnenolone did not activate the α_1-acid glycoprotein promoter

As shown in Fig. 6, dexamethasone (10^{-6} M) markedly activated the transcription of the CAT reporter gene indicating that it activates the glucocorticoid receptor-dependent α_1-acid glycoprotein promoter. On the other hand, dehydroepiandrosterone sulfate or pregnenolone were inactive in this model.

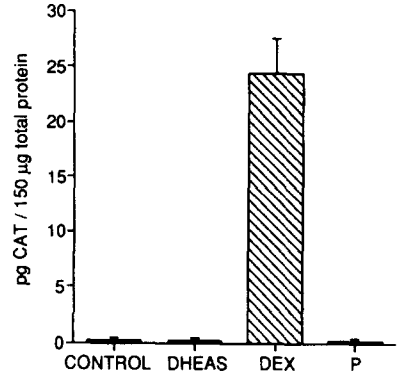

Fig. 6. Effect of dehydroepiandrosterone sulfate, pregnenolone and dexamethasone on the α_1-acid glycoprotein promoter. The −215 + 1AGP-HTC (JZ-1) cells were treated with 10^{-6} M dexamethasone (DEX), dehydroepiandrosterone sulfate (DHEAS) or pregnenolone (P) for 24 h. Data are expressed as pg of CAT produced/150 µg of total proteins (means ± S.E. of triplicate cultures).

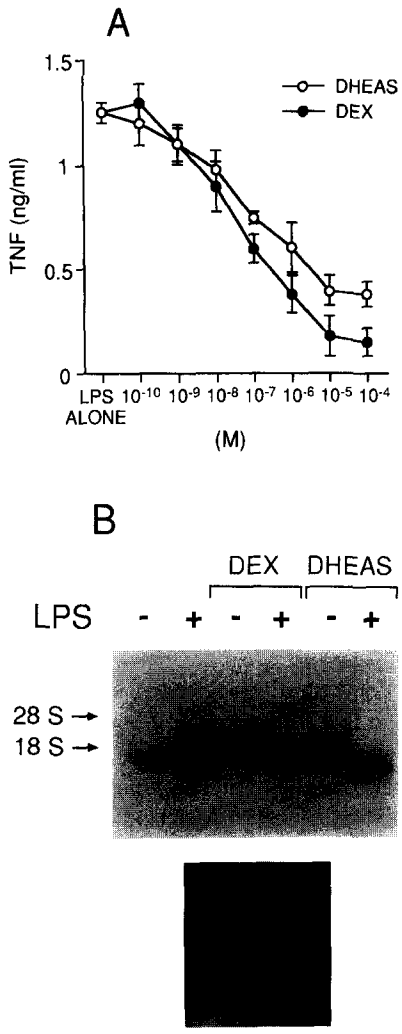

Fig. 7. Effect of dehydroepiandrosterone sulfate and dexamethasone on TNF production (panel A) and mRNA levels (panel B) in THP-1 cells. Cells were stimulated with 1 μg/ml lipopolysaccharide (LPS) as described in Materials and methods. Panel A: dehydroepiandrosterone sulfate (DHEAS) or dexamethasone (DEX) was added at the concentrations indicated; TNF production was expressed as ng/ml (means ± S.D. from triplicate assays). Panel B: DHEAS or DEX was added at 10^{-5} M. Top, TNF mRNA; bottom, ethidium bromide staining of the gel to show RNA loading.

3.6. Effect of dehydroepiandrosterone sulfate on TNF production and TNF mRNA levels in THP-1 cells

The effect of dehydroepiandrosterone sulfate and dexamethasone on TNF production and of its mRNA levels was studied in THP-1 cells. As shown in Fig. 7, dehydroepiandrosterone sulfate and dexamethasone both inhibited TNF production (evaluated by ELISA, panel A) and reduced TNF mRNA levels (panel B).

4. Discussion

We now showed that administration of dehydroepiandrosterone sulfate inhibits the increase in serum TNF levels in lipopolysaccharide-treated mice in a dose-dependent fashion. Since neurosteroids are present in the brain at concentrations up to 20–30-fold higher than in plasma (Corpéchot et al., 1981, 1983), we also studied the effect of centrally administered dehydroepiandrosterone sulfate on local TNF production in the brain. The results show that dehydroepiandrosterone sulfate also inhibits brain TNF produced in response to a central injection of lipopolysaccharide. In both models, dexamethasone is also a potent inhibitor of TNF production, as previously reported (Beutler et al., 1986; Sironi et al., 1992), and is more active than dehydroepiandrosterone sulfate. These data are in agreement with the previous report of a protective effect of dehydroepiandrosterone in endotoxic shock (Danenberg et al., 1992).

On the other hand we showed that dehydroepiandrosterone sulfate does not affect the lipopolysaccharide-induced activation of the hypothalamus-pituitary-adrenal axis. Thus, while dehydroepiandrosterone sulfate inhibits TNF production, it does not interfere with the autocrine inhibitory feedback represented by the hypothalamus-pituitary-adrenal axis. On the contrary, dexamethasone abolishes the increase of serum corticosterone induced by lipopolysaccharide. This phenomenon was described earlier (Fantuzzi et al., 1994) and is due to the well-known inhibitory feedback by which glucocorticoids inhibit the hypothalamus-pituitary-adrenal axis at the hypothalamic level (Haynes, 1990). The lack of effect of dehydroepiandrosterone sulfate on post-lipopolysaccharide serum corticosterone levels also indicates that endogenous corticosterone does not mediate the inhibitory effect of dehydroepiandrosterone sulfate. Together these data suggest that dehydroepiandrosterone sulfate and hypothalamus-pituitary-adrenal axis-derived corticosterone represent two independent pathways to inhibit TNF production.

The in vitro data for human peripheral blood mononuclear cells and THP-1 cells indicate that dehydroepiandrosterone sulfate inhibits TNF production directly, and that this effect is not restricted to mice. In the in vitro model, we could also test the effect of a water-insoluble neurosteroid, pregnenolone, that also inhibited TNF production in these cells, although at a lesser extent than dehydroepiandrosterone sulfate.

The use of the THP-1 cell line allowed us to study the effect of dehydroepiandrosterone sulfate, which was the neurosteroid that best inhibited TNF production, on the induction of TNF mRNA. The results indicated that dehydroepiandrosterone sulfate also exerts its inhibitory effect at the transcriptional level. In these experiments, dexamethasone also markedly inhibited TNF mRNA levels, although its inhibitory effect was less marked than on TNF protein levels. This is in agreement with previous data of Beutler et al. (1986), and suggests that dexamethasone inhibits TNF production at both the transcriptional and the post-transcriptional level.

Since dehydroepiandrosterone sulfate was suggested not

to interact with glucocorticoid receptor (Shafagoy et al., 1992), we investigated the effect of the glucocorticoid receptor antagonist, mifepristone (RU38486), on the inhibitory effect of dexamethasone, dehydroepiandrosterone sulfate and pregnenolone. The complete reversal of dexamethasone inhibition of TNF production by RU38486 confirms previous reports indicating that inhibition of TNF synthesis by glucocorticoids is mediated by glucocorticoids receptor (Hawes et al., 1992; Lazar et al., 1992). Our data indicate that RU38486 does not reverse dehydroepiandrosterone sulfate inhibition of TNF production even at concentrations 5 logs higher than those reversing the effect of dexamethasone.

The fact that the effect of dehydroepiandrosterone sulfate and pregnenolone was not reversed by RU38486 supports the concept that they do not act through the glucocorticoid receptor. The in vivo data showing that dehydroepiandrosterone sulfate, unlike dexamethasone, does not desensitize the hypothalamus-pituitary-adrenal axis suggest further that dehydroepiandrosterone sulfate does not act as a glucocorticoid. It should also be noted that a related neurosteroid, dehydroepiandrosterone, not only does not have an immunosuppressive action but was reported to prevent immunosuppression induced by dexamethasone (May et al., 1990; Blauer et al., 1991). On the other hand; dehydroepiandrosterone has some immunostimulatory activity (Daynes et al., 1990).

The lack of effect of dehydroepiandrosterone sulfate and pregnenolone on the glucocorticoid receptor-activated α_1-acid glycoprotein promoter (where dexamethasone had a strong transcriptional activity) confirms the lack of functional activation of the glucocorticoid receptor by these two neurosteroids.

Thus, the mechanism by which neurosteroids (at least dehydroepiandrosterone sulfate and pregnenolone) inhibit TNF production remains to be elucidated. One possibility is that this inhibitory effect is exerted through an interaction with the $GABA_A$ (γ-aminobutyric acid) receptor. In fact, neurosteroids were shown to modulate the activity of the $GABA_A$ receptor which is associated with a chloride channel (Paul and Purdy, 1992; Majewska, 1992). In this respect, it is of interest to note that benzodiazepines, which also interact with the $GABA_A$ receptor (Schofield et al., 1987), were reported to inhibit TNF production in mice (Zavala et al., 1990).

Several mediators originating in the central nervous system or the neuroendocrine system, such as glucocorticoids (through the hypothalamus-pituitary-adrenal axis) (Besedovsky et al., 1986; Fantuzzi and Ghezzi, 1993) and melanocyte stimulating hormone (Catania and Lipton, 1993), were shown to regulate cytokine production. These data suggest that neurosteroids might be listed among these mediators. The inhibitory effect of neurosteroids on TNF production might be important under physiological conditions such as stress and pregnancy, in which their levels are elevated (Majewska, 1992).

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