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Tumor Necrosis Factor-α Regulates Steroidogenesis, Apoptosis, and Cell Viability in the Human Adrenocortical Cell Line NCI-H295R

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TNF-α regulates the hypothalamo-pituitary-adrenal axis at several levels. It has been shown to modify adrenal steroidogenesis in many species, and it is supposed to act as an auto/paracrine factor. However, its significance in human adrenocortical function remains unclear. Therefore, we investigated the effect of TNF-α on adrenal steroidogenesis, expression of the key steroidogenic genes, apoptosis, and cell viability in the human adrenocortical cell line NCI-H295R. TNF-α treatment (1 nM for 48 h) decreased the basal production of cortisol, androstenedione, dehydroepiandrosterone sulfate (DHEAS), and aldosterone (14, 18, 35, and 52%, respectively), and the 8-bromo-cAMP-induced production of cortisol, androstenedione, dehydroepiandrosterone (DHEA), and DHEAS (44, 66, 58, and 48%, respectively). However, when the steroid production data were normalized by the cell number, TNF-α increased the basal production of cortisol, androstenedione, DHEA, DHEAS, and aldosterone (137, 121, 165, 73, and 28%, respectively), and the 8-bromo-cAMP-induced production of cortisol, DHEAS, and aldosterone (122, 121, and 256%, respectively). This was accompanied by a parallel increase in the expression of the genes encoding for the steroidogenic acute regulatory protein, 3β-hydroxysteroid dehydrogenase 2, and 17-hydroxylase/17,20-lyase (74, 200, and 50%, respectively; quantitative real-time RT-PCR analysis). TNF-α increased caspase 3/7 activity (an indicator of apoptosis) and decreased cell viability dose and time dependently. The effect of TNF-α on apoptosis was neutralized by a monoclonal TNF-α antibody. These findings indicate that TNF-α is a potent regulator of steroidogenesis and cell viability in adrenocortical cells. TNF-α may have physiological and/or pathophysiological significance as an endocrine and/or paracrine/autocrine regulator of adrenocortical function. (*Endocrinology* 148: 386–392, 2007)

TNF-α IS AN INFLAMMATORY cytokine of 157 amino acids produced mostly by activated monocytes and macrophages in response to tissue injury, infection, inflammation, and many other stimuli. It mediates its effects via two receptors (TNF-R1 and TNF-R2). Although TNF-R2 contributes to a number of TNF-α-induced responses, most effects of TNF-α are mediated by TNF-R1. TNF-α activates two major signaling pathways: the caspase cascade leads to apoptosis and the other pathway leads to the activation of two major transcription factors, activator protein 1 and nuclear factor κB (NF-κB), which in turn induces genes involved in inflammatory responses and suppression of apoptosis (1, 2). TNF-α and its receptors are expressed in the hypothalamus and the anterior pituitary gland, and TNF-α is involved in the cytokine-mediated communication between the immune system and the hypothalamic-pituitary-adrenal axis (3–10). TNF-α increases CRH release from the hypothalamus leading to increased ACTH production from the pituitary gland and subsequent stimulation of adrenal steroidogenesis (4, 6, 7, 11). TNF-α may also adjust the feedback control of the hypothalamic-pituitary-adrenal axis by regulating glucocorticoid receptor function (12).

TNF-α mRNA and protein are found in most mammalian endocrine tissues including the hypothalamus (13), pituitary (13), ovary (14), and testis (15). TNF-α mRNA and/or protein expression have been reported in rat (16–18), bovine (19, 20), as well as in human fetal (21) and adult adrenals (22). TNF-α has also been suggested for an important intraadrenal regulator of steroidogenesis (23–26). The resident macrophages, being located mainly at the corticomedullary junction, were previously thought to be the only site of TNF-α production in human adrenals (27). However, later reports demonstrated two separate sources of TNF-α within the adrenal gland: the resident macrophages and the adrenocortical cells themselves (22). Macrophages producing TNF-α are located in the fasciculate and reticular zones. Adrenocortical cells expressing TNF-α are also localized within the same zones, and the density of these cells increases toward the medulla (22). Circulating plasma concentrations of TNF-α seem to be too low to affect adrenal function (26, 28). These findings support the hypothesis that the local adrenal TNF-α system may be more important than circulating TNF-α in the regulation of adrenocortical function.

The ability of TNF-α to regulate directly steroidogenesis has been shown also in the ovary (14, 29–31) and testis (29, 32, 33). In the adrenal, the effects of TNF-α have been variable depending on the species or the developmental stage of the adrenal. TNF-α stimulated corticosterone production in normal rats in *in vivo* experiments (4, 6, 7), but inhibited ACTH-stimulated corticosterone and aldosterone release in *in vitro*

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Abbreviations: 8-Br-cAMP, 8-Bromo-cAMP; CYP17, 17α-hydroxylase/17,20-lyase; DHEA, dehydroepiandrosterone; DHEAS, dehydroepiandrosterone sulfate; 3β-HSD 2, 3β-hydroxysteroid dehydrogenase 2; NF-κB, nuclear factor κB; StAR, steroidogenic acute regulatory protein.

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conditions (34). On the other hand, TNF-α stimulated corticosterone production in both *in vivo* and *in vitro* conditions in cholestatic rats (35). In cultured human fetal adrenal cells, TNF-α decreased basal and ACTH-stimulated cortisol production (21, 23). It also inhibited basal and ACTH-induced IGF-II expression, indicating its potential role in the regulation of growth and differentiation of fetal adrenals (24, 25, 36). However, TNF-α increased cortisol release from adult human adrenal cells *in vitro* (4). These contradictory data leave the significance of TNF-α in the regulation of human adrenal function unclear.

Our goal was to clarify further the role of TNF-α in human adrenal function. We examined its effects on steroidogenesis, adrenocortical cell apoptosis, cell viability, and on the expression of three key genes involved in steroidogenesis [steroidogenic acute regulatory protein (StAR), 17α-hydroxylase/17,20-lyase (CYP17), 3β-hydroxysteroid dehydrogenase 2 (3β-HSD 2)] in the human adrenocortical cell line NCI-H295R, of which the parent cell line NCI-H295 previously has been described as an appropriate model for studying adrenocortical steroidogenesis (37, 38).

Materials and Methods

Cell culture

NCI-H295R cells, obtained from the American Type Culture Collection (Rockville, MD), were maintained in 1:1 mixture of DMEM:F12 (Life Technologies, Inc., Paisley, Strathclyde, UK) supplemented with 2 mM L-glutamine (Life Technologies, Inc.), 2% Ultroser (Biosepra, Marlborough, MA), and antibiotics (125 μg/ml streptomycin and 125 IU/ml penicillin; Orion Pharmaceutical Co., Espoo, Finland) at 37 C in a 5% CO_2 humidified atmosphere. Cells were counted and seeded at a density of 1×10^6 cells per well on six-well plastic cell culture dishes (Nalge Nunc International, Rochester, NY) and grown for 48 h. In dose response experiments, the cultures were treated with or without 1 pM to 100 nM recombinant human TNF-α (R&D Systems Inc., Minneapolis, MN) and 1 mM 8-bromo-cAMP (8-Br-cAMP) (Sigma Chemical Co., St. Louis, MO) for 24–48 h. In time course experiments, the cells were treated with or without 1 nM TNF-α and 1 mM 8-Br-cAMP for 3–48 h. In immunoneutralization experiments, monoclonal antihuman TNF-α antibody (R&D Systems Inc.; catalog no. MAB610) (0.01–30 μg/ml) was added with TNF-α. The conditioned culture media were collected, frozen immediately, and stored at −70 C for subsequent steroid analyses.

Enzyme immunoassays for steroid measurements

Steroid concentrations in the conditioned culture media were determined by commercial ELISA kits according to the instructions of the manufacturers. Cortisol was measured by a cortisol ELISA kit (Milenia Biotec GmbH, Bad Nauheim, Germany; catalog no. MDKCO1). Androstenedione, DHEA, DHEAS, and aldosterone were determined by ELISA kits from Diagnostic System Laboratories Inc. (Webster, TX; catalog nos. DSL-10-3800, DSL-10-9000, DSL-10-3500, and DSL-10-8600, respectively).

RNA extraction, RT, and quantitative real-time PCR

Total RNA was isolated from cultured cells using TRIzol reagent according to the manufacturer's protocol (Life Technologies, Inc., Rockville, MD). Total RNA concentration was measured spectrophotometrically at the wavelength of 260 nm. For the RT-PCR analyses, total RNAs originally from parallel triplicate wells were pooled, and the trace amounts of genomic DNA were removed from pooled RNA samples with DNA-free DNase treatment and removal kit (Ambion, Austin, TX) according to the manufacturer's instructions. In short, 10 μg of total RNA was incubated with 2 U of DNase I and 2.5 μl of 10-fold DNase buffer in the total volume of 25 μl at 37 C for 30 min. Divalent cations and DNase were removed by incubating samples with 5 μl inactivation reagent in ambient temperature for 2 min.

Reverse transcription was performed using High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. Reactions were made in the total volume of 20 μl containing 2 μg DNase-treated total RNA, 1-fold reaction buffer, dNTP mixture and random primers, and 50 U MultiScribe reverse transcriptase. Reaction mixtures were incubated at 25 C for 10 min followed by 2 h of incubation at 37 C. Quantitative real-time PCR was carried out in the Applied Biosystems 7500 Real Time PCR System (Applied Biosystems) using TaqMan Gene Expression Assays (Applied Biosystems) for StAR (assay ID Hs00264912_m1), steroid 17α-hydroxylase (CYP17; assay ID Hs 00164375_m1), 3β-HSD 2 (assay ID Hs00605123), and glyceraldehyde-3-phosphate dehydrogenase (assay ID Hs99999905_m1). Glyceraldehyde-3-phosphate dehydrogenase was chosen for endogenous control because it showed the least variation after several different treatments of the cell line in TaqMan Human Endogenous Control Plate (Applied Biosystems). Standard series of five dilutions containing 96, 24, 12, 3, and 1 ng template cDNA were prepared from pooled sample cDNAs. Sample dilutions were comprised of 12 ng template cDNA. All standards and samples were run in the total volume of 20 μl in triplicate.

Apoptosis assay

Commercial luciferase based Caspase-Glo 3/7 Assay kit (Promega, Madison, WI) was used to detect dose- and time-dependent apoptotic effect of human recombinant TNF-α in the NCI-H295R cell line. A series of preliminary experiments were performed to determine optimum conditions (cell number per well, treatment and reading time) for this analysis with NCI-H295R cells. For all apoptosis experiments, 10,000 cells per well were subcultured on white flat-bottom 96-well plates (Nalge Nunc International) and incubated for 24 h to allow cell attachment. After that, the culture medium was removed, the cells were washed with PBS, and fresh medium with or without TNF-α (1 nM for time course and 1 pM to 100 nM for dose response experiments) and its antibody (0.01–30 μg/ml) was added. The experiments were terminated after 1, 3, 6, and 24 h (time course experiments) and 24 h (dose response experiments) of incubation by adding 100 μl of the Caspase-Glo 3/7 reagent. The contents of the wells were mixed on a plate shaker for 30 sec and incubated at room temperature for 1 h, and the luminescence was measured by a luminometer (BioPhotometer; Eppendorf, Hamburg, Germany).

Cell viability assay

Commercial CellTiter-Glo Luminescent Cell Viability Assay kit (Promega) was used to estimate the effect of TNF-α on the metabolic activity of the cells, based on the amount of cellular ATP. A series of preliminary experiments were performed to determine optimum conditions (the cell number, treatment and reading time) for this analysis with NCI-H295R cells. Five thousand cells per well were seeded on a flat-bottom 96-well plate (Nalge Nunc International). After 24 h of incubation, the culture medium was removed, the cells were washed with PBS, and fresh medium with or without TNF-α (1 pM–1 nM) was added. After 24 h of treatment, 100 μl of prepared reagent was added to each well. The contents of the wells were mixed on a plate shaker for 2 min and incubated at room temperature for 10 min, and then the luminescence was measured by a luminometer (Eppendorf).

Statistical analyses

Each experiment was repeated at least three times. Single experiments consisted of several treatments. All results are given as mean ± SEM of at least three separate experiments, each performed in triplicate, with the control mean adjusted to 1 arbitrary unit or 100%. The significance of the differences was assessed by Kruskal-Wallis and Mann-Whitney tests. The level of significance was chosen as $P < 0.05$. Data were analyzed using the statistical program SPSS for Windows, release 11.5 (SPSS Inc., Chicago, IL).

Results

The effect of TNF-α and 8-Br-cAMP on steroidogenesis

We used the human adrenocortical cell line NCI-H295R as a model to investigate the effects of TNF-α on adrenocortical function. NCI-H295R cells and their parent cell line (37, 38) respond poorly to ACTH, probably due to low ACTH receptor expression. Therefore, we used 8-Br-cAMP to mimic the ACTH signal pathway. 8-Br-cAMP (1 mM) increased significantly cortisol, androstenedione, DHEA, DHEAS, and aldosterone production from cultured NCI-H295R cells (Figs. 1–3). Similarly, 8-Br-cAMP induced the expression of three key genes involved in steroid production, StAR, 3β-HSD 2, and CYP17 (Fig. 4).

In our preliminary dose response experiments with TNF-α, the 1 nM concentration decreased cortisol production nearly maximally. Thus, this dose was chosen for most subsequent experiments. TNF-α treatment (1 nM) for 48 h decreased significantly the basal production of cortisol, androstenedione, DHEAS, and aldosterone (14, 18, 35, and 52%, respectively, $P < 0.05$ for all). TNF-α decreased also the 8-Br-cAMP-induced production of cortisol, androstenedione, DHEA, and DHEAS (44, 66, 58, and 48%, respectively, $P < 0.05$ for all) and tended to decrease that of aldosterone (25%, $P = 0.275$) (Fig. 1). Figure 2 shows the dose-dependent effect of TNF-α on basal and 8-Br-cAMP-induced cortisol production. However, when the steroid results were normalized according to the RNA content of the wells (reflecting the cell number at the end of the experiments), TNF-α increased the basal production of cortisol, androstenedione, DHEA, DHEAS, and aldosterone (137, 121, 165, 73, and 28%, respectively, $P < 0.05$ for all), and the 8-Br-cAMP-induced production of cortisol, DHEAS, and aldosterone (122, 121, and 256%, respectively, $P < 0.05$ for all) (Fig. 3, aldosterone data not shown). In time-course experiments (3, 12, 24, and 48 h), it took about 24 h before the stimulatory effect of 8-Br-cAMP on steroid production became clearly visible. However, DHEA production seemed to be induced somewhat earlier than that of cortisol, androstenedione, and DHEAS (Fig. 3). The stimulatory effect of TNF-α (1 nM) on basal or 8-Br-cAMP-induced steroid production (normalized data) did not become visible before 48 h of treatment (Fig. 3). Aldosterone was measured only at the 48-h time point; 8-Br-cAMP increased aldosterone production up to 7.1-fold ($P < 0.05$, normalized data).

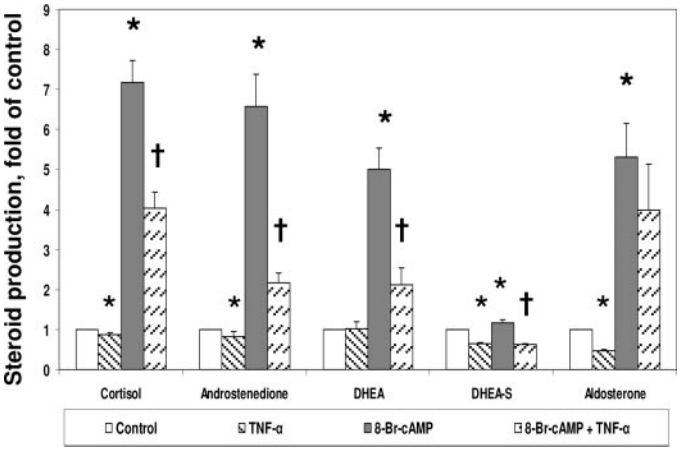

FIG. 1. The effect of TNF-α and 8-Br-cAMP on cortisol, androstenedione, DHEA, DHEAS, and aldosterone production by cultured NCI-H295R cells after 48 h of treatment. One million cells per well were plated and incubated for 48 h with or without 1 nM TNF-α, 1 mM 8-Br-cAMP, or their combination. The results (steroid concentration in the conditioned medium; mean ± SEM) are expressed as fold of control (without any treatment), and they originate from three separate experiments, each performed in triplicate wells. The steroid production data in these experiments were not normalized by the cell number at the end of the experiment. *, $P < 0.05$ compared with the control; †, $P < 0.05$ compared with the 8-Br-cAMP-treated wells.

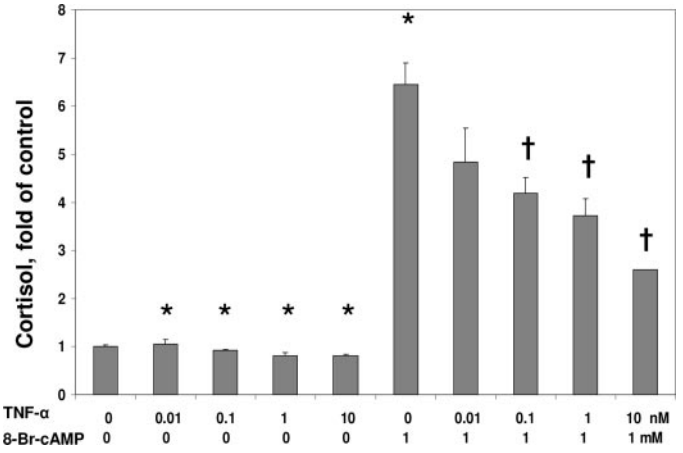

FIG. 2. The dose-dependent effect of TNF-α on basal and 8-Br-cAMP-induced cortisol production by cultured NCI-H295R cells after 48 h treatment. One million cells per well were plated and incubated for 48 h with or without 0.01–10 nM TNF-α, 1 mM 8-Br-cAMP, or their combination. The results (steroid concentration in the conditioned medium; mean ± SEM) are expressed as fold of control (without any treatment), and they originate from three separate experiments, each performed in triplicate wells. The steroid data are not normalized by the cell number at the end of the experiment. *, $P < 0.05$ compared with the control; †, $P < 0.05$ compared with the 8-Br-cAMP-treated wells.

The effect of TNF-α and 8-Br-cAMP on StAR, 3β-HSD 2, and CYP17 mRNA expression

As expected, 8-Br-cAMP (1 mM) increased the expression of the mRNAs for StAR, 3β-HSD 2, and CYP17 after 24–48 h of treatment. However, the time course for the induction of these genes was not identical. Although StAR and CYP17 mRNAs increased already after 3 h of 8-Br-cAMP treatment, 3β-HSD 2 mRNA increased only after 24 h (Fig. 4). Actually, 3β-HSD 2 mRNA decreased during the first hours of 8-Br-cAMP treatment. TNF-α (1 nM) increased the basal expression of the mRNAs for StAR, 3β-HSD 2, and CYP17 (74, 200, and 50% after 48 h, respectively; $P < 0.05$ for all), with some variation in the time course and magnitude of the induction. TNF-α increased also the 8-Br-cAMP-induced expression of these genes, although this was not significant in the case of CYP17 (Fig. 4).

The effect of TNF-α on apoptosis and cell viability

TNF-α treatment (1 nM) resulted in a time-dependent increase (up to 5.5-fold at 12–24 h) in the apoptotic index as measured by the luciferase based caspase 3/7 assay. This increase was detectable within 3 h of TNF-α treatment and

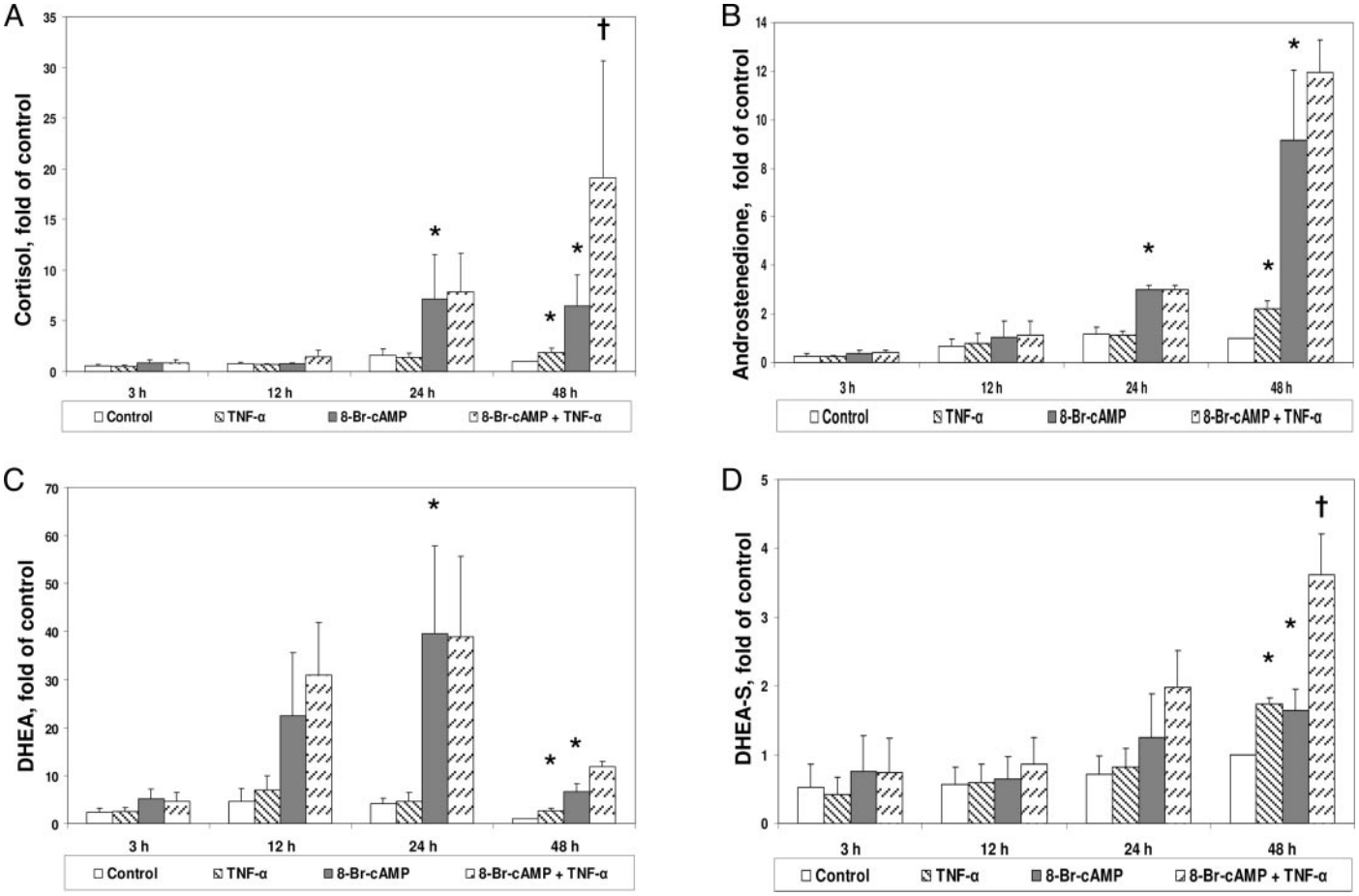

FIG. 3. The time-dependent effect of TNF-α and 8-Br-cAMP on cumulative cortisol (A), androstenedione (B), DHEA (C), and DHEAS (D) production by cultured NCI-H295R cells. One million cells per well were plated and incubated for 3–48 h with or without 1 nM TNF-α, 1 mM 8-Br-cAMP, or their combination. The results (steroid concentration in the conditioned medium; mean ± SEM) are expressed as fold of control (without any treatment) after 48 h of incubation. The data originate from three separate experiments, each performed in triplicate wells. The steroid production data in these experiments were normalized by the total RNA content of the wells at each time point (reflecting the cell number). *, $P < 0.05$ compared with the control of the same time point; †, $P < 0.05$ compared with the 8-Br-cAMP-treated wells at the same time point.

continued to rise until 12 h (Fig. 5A). The effect of TNF-α on the apoptotic index was dose dependent: a significant increase was detectable at 1 pM and the maximal effect was evident at 10 nM concentration (Fig. 5B). The apoptotic effect of TNF-α was efficiently neutralized by the monoclonal antihuman TNF-α antibody (Fig. 6). TNF-α also decreased dose-dependently (10 pM to 1 nM) the number of metabolically active cells (down to 63.3% of control; Fig. 7).

Discussion

As expected, 8-Br-cAMP increased steroid production and the expression of genes involved in steroid synthesis in the adrenocortical NCI-H295R cell line. Furthermore, TNF-α had remarkable time- and dose-dependent effects on steroidogenesis and cell viability in these cells. Initially, TNF-α seemed to inhibit steroid production in the cell line. However, when the steroid data were normalized with the total RNA amount (reflecting the cell number in the culture wells) at the end of the experiments, it turned out that TNF-α actually increased steroidogenesis (per cell number). This discrepancy was explained by the finding that TNF-α increased apoptosis and reduced the number of viable cells in the cultures.

In principle, our findings of the effects of TNF-α on steroidogenesis are in agreement with those of Darling *et al.* (4), who found that TNF-α increases cortisol production in human adult adrenocortical cell cultures. Similarly, Swain and Maric (35) reported that TNF-α increases corticosterone production by incubated adrenal slices from cholestatic rats. However, in cultured human fetal adrenal cells TNF-α suppressed ACTH-induced steroid production and the expression of steroidogenic enzyme genes (18, 21, 23, 34).

In our cell culture experiments with NCI-H295R cells, TNF-α increased the expression of genes involved in steroid synthesis (StAR, 3β-HSD 2, CYP17). This is again opposite to the results obtained in primary cultures of human fetal adrenal cells where TNF-α decreased ACTH-induced expression of steroidogenic P450 genes (25). In our current time course experiments, up-regulation of the steroidogenic genes by TNF-α and 8-Br-cAMP was detectable earlier than the increase in steroid production, as expected. The time courses for the induction of cortisol, androstenedione, and DHEAS

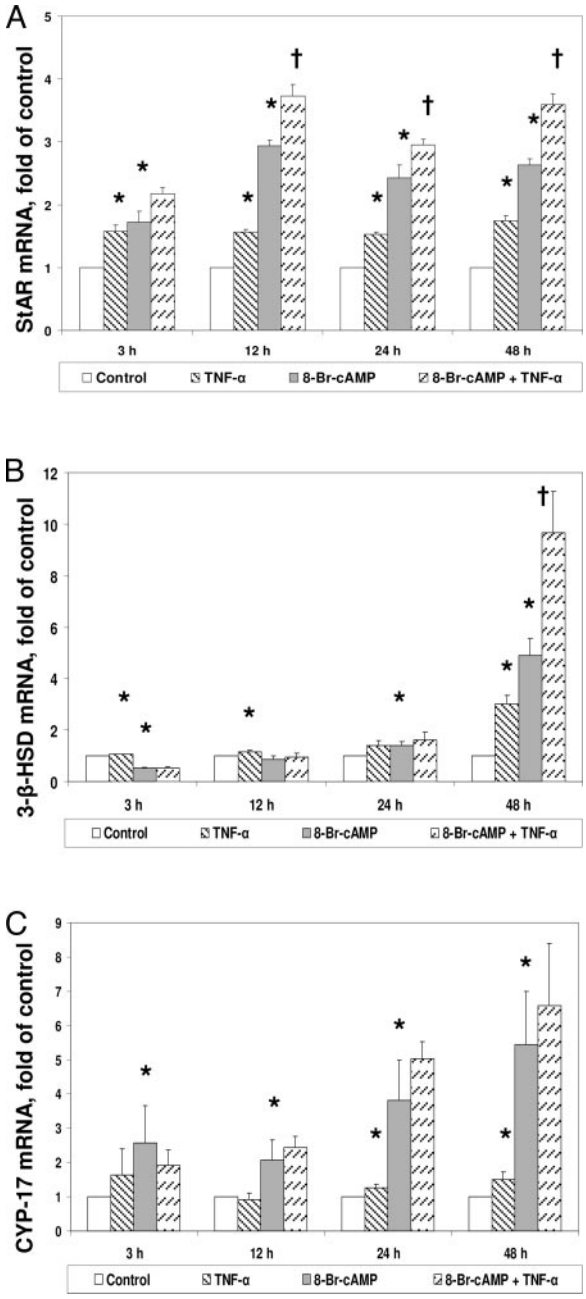

FIG. 4. The time-dependent effect of TNF-α (1 nM), 8-Br-cAMP (1 mM), and their combination on the expression of StAR (A), 3β-HSD 2 (B), and CYP17 (C) mRNAs in cultured NCI-H295R cells. The cell culture experiments were the same as presented in Fig. 3 for steroid production. RNA was isolated at each time point, and RT-PCR was performed as described in *Materials and Methods*. The results (mean ± SEM) are expressed as fold of control (without any treatment) at each time point. The data originate from three separate experiments, each performed in triplicate wells. *, $P < 0.05$ compared with the control of the same time point; †, $P < 0.05$ compared with the 8-Br-cAMP-treated wells at the same time point.

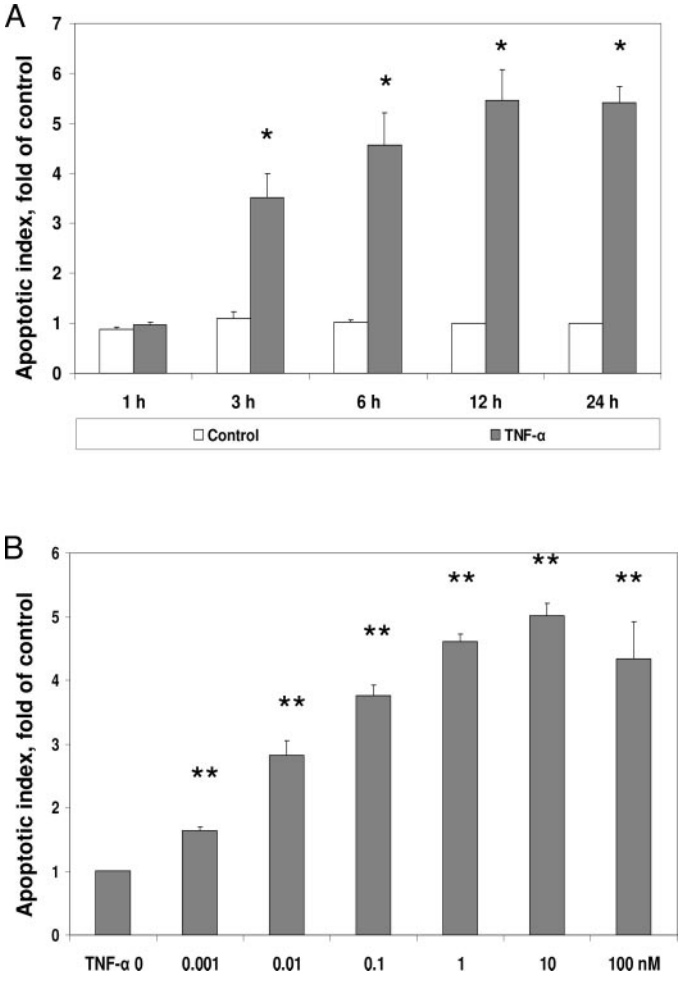

FIG. 5. The time- (A) and dose-dependent (B) effect of TNF-α on the apoptotic index in cultured NCI-H295R cells. Ten thousand cells per well on a 96-well plate were seeded and incubated for 1–24 h with 1 nM TNF-α in the time course experiments (A) or 24 h with 0.001–100 nM TNF-α in the dose response experiments (B). The results (mean ± SEM of caspase 3/7 activity) are expressed as fold of control (without TNF-α treatment) after 24 h incubation. The data originate from three (A) or seven (B) separate experiments, each performed in triplicate wells. *, $P < 0.05$, **, $P < 0.01$ compared with the respective control.

production paralleled with significant increases after 24–48 h of 8-Br-cAMP treatment. However, DHEA production increased already after 12–24 h of 8-Br-cAMP treatment. This time difference can be explained by the late induction of 3β-HSD 2 (mRNA increase visible only after 24 h of 8-Br-cAMP treatment) compared with that of StAR and CYP17 (mRNA increase detectable already after 3 h of 8-Br-cAMP treatment).

TNF-α is known as a potent regulator of cell viability (1, 2, 39–41). TNF-α increased time and dose dependently the apoptotic index (caspase activity) and decreased cell viability in cultured NCI-H295R cells. The effect of TNF-α was also specific, because the monoclonal antihuman TNF-α antibody neutralized the apoptotic effect of TNF-α efficiently and dose dependently. The apoptotic effect of TNF-α is not surprising on the basis of the ability of TNF-α to induce apoptosis of many cancer cell types. Our data are in line with the findings of Liu *et al.* (41) who demonstrated the involvement of different mechanisms in the activation of adrenocortical cell apoptosis. However, it is likely that the NCI-H295R cells are far more sensitive to the apoptotic effect of TNF-α than normal human adrenocortical cells. This view is supported by

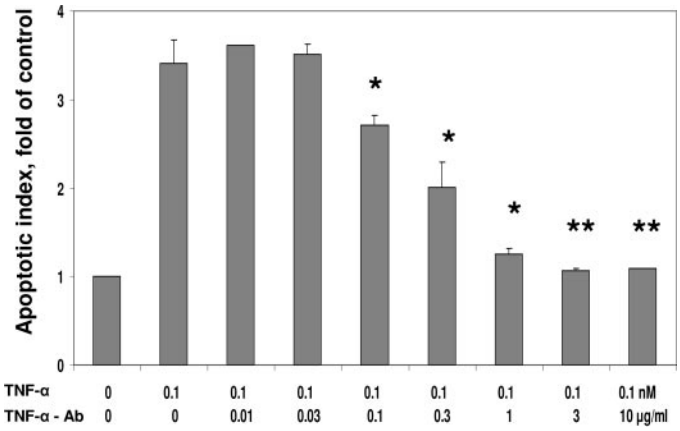

FIG. 6. The dose-dependent effect of a neutralizing monoclonal antihuman TNF-α antibody on the TNF-α-induced apoptosis in cultured NCI-H295R cells. Ten thousand cells per well on a 96-well plate were seeded and incubated for 24 h with or without 0.1 nM TNF-α and 0–10 μg/ml of the TNF-α antibody. The results (mean ± SEM of caspase 3/7 activity) are expressed as fold of control (without TNF-α or its antibody treatment). The data originate from seven separate experiments, each performed in triplicate wells. *, $P < 0.05$, **, $P < 0.01$ compared with the TNF-α treatment without any antibody.

previous experiments in which TNF-α had no effect on cell number or viability in human fetal (21, 23) or adult (4) adrenal cells.

Stimulation of adrenocortical cell steroidogenesis and apoptosis at the same time could be explained by the different pathways involved in these processes. Two receptors (TNF-R1 and TNF-R2), and two different bioactive forms of TNF-α—a membrane-bound and a soluble form—have been identified. Although membrane-bound TNF activates both TNF-R1 and TNF-R2, soluble TNF predominantly signals via TNF-R1 (1, 2, 42–44). We could assume that, in our experiments, the added (soluble) TNF-α activated mainly TNF-R1.

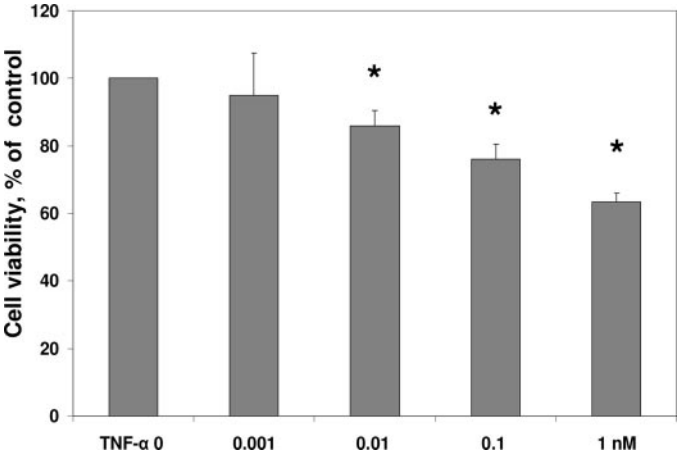

FIG. 7. The dose-dependent effect of TNF-α on cell viability in cultured NCI-H295R cells. Five thousand cells per well on a 96-well plate were seeded and incubated for 24 h with or without 0.001–1 nM TNF-α. The results (mean ± SEM of the luminescence reflecting the amount of cellular ATP and the number of metabolically active cells) are expressed as fold of control (without any TNF-α treatment) after 24 h of incubation. The data originate from three separate experiments, each performed in triplicate wells. *, $P < 0.05$ compared with the control.

The first protein associated with TNF-R1 is TNF-α receptor-associated death domain, which recruits three additional mediators, receptor-interacting protein 1, Fas-associated death domain, and TNF-receptor-associated factor 2 (2). Receptor-interacting protein 1 mediates inflammatory, proliferative, and gene expression activities of TNF-α via MAP and transcription factor activator protein 1 signaling pathways, while TNF-receptor-associated factor 2 initiates IκB kinase and NF-κB cascade (1, 2, 42–44). TNF-R1-mediated apoptosis typically develops only when the antiapoptotic NF-κB response is down-regulated (39). Thus, the crosstalk between the TNF receptors and their intracellular signaling pathways upon TNF-α exposure can simultaneously lead to induction of steroidogenesis and apoptosis. Variable expression of the components of this complex TNF signaling system can explain why the effects of TNF-α in different adrenocortical cell types (human *vs.* other species, fetal *vs.* adult, normal cells in primary cultures *vs.* permanent cell lines) can vary.

In conclusion, TNF-α is a potent regulator of steroidogenesis and apoptosis in adrenocortical cells. Its effects on steroidogenesis are mediated at least partly by altered expression of the genes regulating steroid synthesis. The numerous biological effects of TNF-α, as well as the presence of TNF-α and its receptors in human adrenocortical cells make this cytokine a potentially important auto- and paracrine modulator of adrenal steroidogenesis in various pathophysiological conditions.

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