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Modulation of Tumor Necrosis Factor-α, Interleukin-1β, Interleukin-6, Interleukin-8, and Granulocyte/Macrophage Colony-Stimulating Factor Expression in Human Monocytes by an Endogenous Anxiogenic Benzodiazepine Ligand, Triakontatetraneuropeptide: Evidence for a Role of Prostaglandins

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SUMMARY

Triakontatetraneuropeptide (TTN) is the major processing product of the endogenous anxiogenic peptide ligand of the benzodiazepine receptor, diazepam binding inhibitor. In the present study, we demonstrated by Northern blot analysis that the mRNA levels for tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, granulocyte/macrophage colony-stimulating factor, IL-6, and IL-8 were significantly increased after 4 hr of incubation of human monocytes with lipopolysaccharide (LPS) and TTN (10^{-11} M), compared with cells incubated with LPS alone. Exposure of monocytes for 20 hr to LPS and TTN (10^{-11} M) also stimulated TNF-α, IL-1β, and granulocyte/macrophage colony-stimulating factor release by 80%, 110%, and 98%, respectively, relative to the response elicited by LPS alone. Smaller stimulatory effects were observed using the prototypic pharmacological peripheral benzodiazepine Ro5-4864 (10^{-11} M) (55%, 72%, and 62%, assessed by means of specific enzyme immunoassays). In contrast, TTN and Ro5-4864 did not modulate LPS-induced IL-6 and IL-8 production. Treatment with the cyclooxygenase inhibitor indomethacin increased IL-1β and TNF-α secretion but not that of IL-6 or IL-8. The observed stimulatory effects of TTN and indomethacin were not additive. Taken together, these findings suggest a common mechanism of action for TTN and indomethacin, involving PG formation. In this respect, TTN inhibited prostaglandin (PG) E2 production by 30%. The fact that the observed modulatory effects correlated with PG levels suggests the existence of a second-messenger pathway associated with the peripheral-type benzodiazepine receptor. These results indicate that human TTN differentially modulates the LPS-induced expression of proinflammatory cytokines, and they further support the concept that this endogenous psychoactive peptide could be involved in physiological control of the inflammatory response.

DBI is an 11-kDa peptide with anxiogenic and proconflict activities. High concentrations of DBI have been detected in the cerebrospinal fluid of patients suffering from major depression but not in schizophrenics or Alzheimer patients (1). DBI was isolated from rat and human brain on the basis of its ability to displace diazepam from neuronal γ-aminobutyric acid-linked BZD receptors and is thought to be the main endogenous ligand for the BZD receptor (1, 2). DBI mRNA has also been detected in glial cells, as well as in adrenal, splenic, and hepatic tissues known to be rich in PBR (3, 4). The latter was recently cloned from rat adrenal and human mononuclear cells (5, 6).

TTN (rat DBI 17-50) is one of the two main biologically active products (also including ODN-DBI, i.e., rat DBI 33-50) derived from DBI processing in the rat brain. In endocrine organs, TTN appears to be more potent than ODN-DBI and as potent as full length DBI in stimulating steroid biosynthesis (7). Moreover, TTN appears to be the most abundant of the naturally occurring DBI-derived peptides (8). Rat TTN has been shown to bind more avidly to PBR than to central receptors (8).

We have previously demonstrated, both *in vivo* and *in vitro*, that PBR is involved in immune reactions, in particular in murine and human host defense mechanisms involving phagocyte-derived oxidative metabolism, as well as in cytokine pro-

ABBREVIATIONS: DBI, diazepam binding inhibitor; TTN, triakontatetraneuropeptide; ODN-DBI, octadecaneuropeptide; GM-CSF, granulocyte/macrophage colony-stimulating factor; BZD, benzodiazepine; PBR, peripheral-type benzodiazepine receptor; CO, cyclooxygenase; IL, interleukin; PG, prostaglandin; SDS, sodium dodecyl sulfate; SSC, standard saline citrate; SN, supernatant(s); Kb, kilobase(s); LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell(s); TNF, tumor necrosis factor.

duction (9–14). In the present study, we examined the *in vitro* effects of a synthetic human sequence of TTN (15), with close homology to rat TTN (DBI 17-50) and corresponding to human DBI 35-68, on both the secretion and mRNA levels of the main proinflammatory cytokines produced by LPS-treated human monocytes, i.e., TNF-α, IL-1β, IL-6, IL-8, and GM-CSF. We further demonstrate that the mechanism of TTN action involves the participation of metabolites of the arachidonic acid pathway, i.e., PG.

Experimental Procedures

Materials. Human TTN (DBI 35-68) was obtained from Neosystem Laboratory (Strasbourg, France), dissolved in sterile water (10^{-3} M), and further diluted in RPMI 1640 medium (GIBCO, Paisley, Scotland). LPS from *Escherichia coli* (055:B5) was purchased from Sigma Chemical Co. (St. Louis, MO). Ro5-4864 [7-chloro-1,3-dihydro-1-methyl-5-(*p*-chlorophenyl)-2*H*-1,4-benzodiazepine-2-one] from Fluka (Buchs, Switzerland) was dissolved in absolute ethanol (10^{-2} M) and further diluted in RPMI 1640 medium.

Preparation and incubation of human monocytes. Human PBMC were isolated from heparinized blood of healthy donors, who had not taken benzodiazepines for at least 3 months, by centrifugation on a Ficoll-Paque density gradient. For cytokine determination, PBMC (5×10^4/well) were incubated at 37° for 1 hr in flat-bottomed 96-well microtiter plates and nonadherent cells were removed as described previously (13). Adherent cells (approximately 90% monocytes, as determined by May-Grünwald-Giemsa and nonspecific esterase staining) were incubated for 20 hr with LPS (1 μg/ml) in RPMI 1640 supplemented with 5% heat-inactivated fetal calf serum (Flow Laboratories, Irvine, Scotland), 100 units/ml penicillin, 100 μg/ml streptomycin, and 2 mM L-glutamine (GIBCO). In some experiments, monocytes were pretreated with indomethacin (0.5 μM; Sigma) for 30 min before LPS induction.

For the assay of PGE_2, PBMC (1×10^6/well) were incubated for 1 hr in flat-bottomed 24-well microtiter plates. Adherent cells were stimulated with LPS for 5 hr in RPMI 1640 containing 1% heat-inactivated AB serum. SN were washed in RPMI 1640 by centrifugation ($400 \times g$ for 10 min) and were stored at −70° until assay.

TNF-α, IL-1β, IL-6, IL-8, and GM-CSF assays. Monocytes culture SN were assayed in duplicate using specific enzyme-linked immunosorbent assay kits (Cistron Biotechnology, Pine Brook, NJ, for TNF-α and IL-1β; Quantikine, R & D Systems, Minneapolis, MN, for IL-6, IL-8, and GM-CSF). The lowest detectable concentrations were 15–20 pg/ml for TNF-α, IL-1β, and IL-6, 20–50 pg/ml for IL-8, and 3 pg/ml for GM-CSF. In some cases, the biological activity of the cytokines was also assessed as described previously (10, 13).

PGE_2 assay. Eicosanoid levels were quantified in monocytes culture SN by means of a radioimmunoassay, using a commercially available PGE_2 kit (Du Pont de Nemours/NEN Products, Boston, MA). Test samples were prepared in culture medium and assayed in duplicate. The limit of detection of this assay was 5 pg of added PGE_2.

Isolation of RNAs and Northern blot analysis. After 1 hr of adherence in flat-bottomed six-well microtiter plates, monocytes (2×10^6/well) were incubated in the presence or absence of LPS (1 μg/ml) and/or TTN (10^{-11} M) at 37° for 4 hr. To extract total RNA, cells were directly lysed in the wells with 400 μl of 4 M guanidinium isothiocyanate and were processed as described elsewhere (16). For Northern analysis, equal amounts of total cellular RNA (8–10 μg/lane) were electrophoresed through 1% agarose-formaldehyde gels and transferred to nylon membranes (Hybond-N; Amersham, UK). The membranes were prehybridized at 42° for 14 hr in buffer consisting of 50% formamide, 2× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 0.1% SDS, 5× Denhardt's reagent, and 200 μg/ml salmon sperm DNA. Hybridization was carried out for an additional 16 hr in the same buffer with 2×10^6 cpm/ml labeled probe. After hybridization, the blots were washed twice in 0.1% SDS/2× SSC for 30 min at room temperature and then washed twice more with 0.1% SDS/0.1× SSC at 55°. The blots were then dried and exposed to X-ray film for 12–24 hr at −80°, with an intensifying screen.

The following cDNA fragments were used: the 1.1-kb *Pst*I fragment of PE4 plasmid containing human TNF-α cDNA (17), the 1.3-kb IL-1β insert obtained by *Pst*I digestion of pSP64 vector, the 0.8-kb GM-CSF *Xho*I-digested fragment from pMT2 vector, the 1.2-kb IL-6 fragment obtained by *Eco*RI digestion of pMT2T vector (kindly provided by Dr. S. Clark, Genetics Institute, Cambridge, MA), the 0.3-kb *Pst*I/*Eco*RI fragment of IL-8 cDNA (18), and the chicken β-actin cDNA purified from pA1 plasmid (19). All the probes were labeled with [^{32}P] dCTP using the Multiprime DNA labeling system (Amersham, UK), as described by Feinberg and Vogelstein (20). Quantitative evaluation of the specific transcripts was carried out by laser densitometric scanning of the autoradiograms (LKB 2202 Ultrascan).

Statistical analysis. Data are expressed as means ± standard errors. Significance was assessed using Student's paired *t* test. Differences were considered significant when p was 0.05 or less.

Results

Effect of the endogenous ligand TTN on cytokine production by human monocytes. Monocytes obtained from several healthy donors were incubated in the presence of LPS (1 μg/ml) and TTN or the PBR ligand Ro5-4864. A dose-response curve (10^{-15} to 10^{-5} M) was established for TTN (data not shown) and revealed optimal stimulatory concentrations of 10^{-11} M TTN and Ro5-4864 for LPS-induced cytokine production. Stimulation by TTN (10^{-11} M) was significant at doses of LPS ranging from 1 ng/ml to 10 μg/ml, with a plateau between 0.1 and 10 μg/ml LPS (data not shown). Therefore, all experiments were performed at the intermediate dose of 1 μg/ml LPS. Fig. 1 shows the effect of TTN (10^{-11} M) on cytokine production measured in culture SN of LPS-stimulated human monocytes, relative to that of the pharmacological ligand Ro5-4864. TTN induced a significant increase in LPS-induced TNF-α, IL-1β, and GM-CSF production, by comparison with LPS alone. TTN exerted a stimulatory effect as potent as or, for TNF-α and IL-1β, more potent than that of the synthetic peripheral ligand Ro5-4864. With cells from four different donors, the stimulatory effects of TTN and Ro5-4864 varied from 50% to 150%, with a mean of 80% and 55% for TNF-α, 110% and 72% for IL-1β, and 98% and 62% for GM-CSF, respectively. As shown in Fig. 1, both TTN and Ro5-4864 failed to enhance the production of IL-6 and IL-8 by LPS-stimulated monocytes. Similar results were obtained using biological assays for TNF-α, IL-1β, and IL-6 (data not shown). In the absence of LPS, neither TTN nor Ro5-4864 induced cytokine production. TTN showed no interference with immunoreactive or biological assays. The survival index in monocytes cultures was unaffected by TTN.

Effect of TTN on cytokine mRNA expression. We examined whether TTN affected cytokine transcription by measuring mRNA levels in monocytes (Fig. 2) after 4 hr of culture in the absence of LPS, in the presence of TTN alone, in the presence of LPS, or in the presence of both LPS and TTN. Northern blot analysis showed that, as described previously, unstimulated adherent cells cultured in the presence of serum contained low levels of TNF-α, IL-1β, IL-6, IL-8, and GM-CSF mRNAs (21–23). This representative experiment (one of four donors) also shows that TTN alone had no detectable enhancing effect on mRNA expression, whereas LPS increased TNF-α, IL-1β, GM-CSF, IL-6, and IL-8 mRNA accumulation ap-

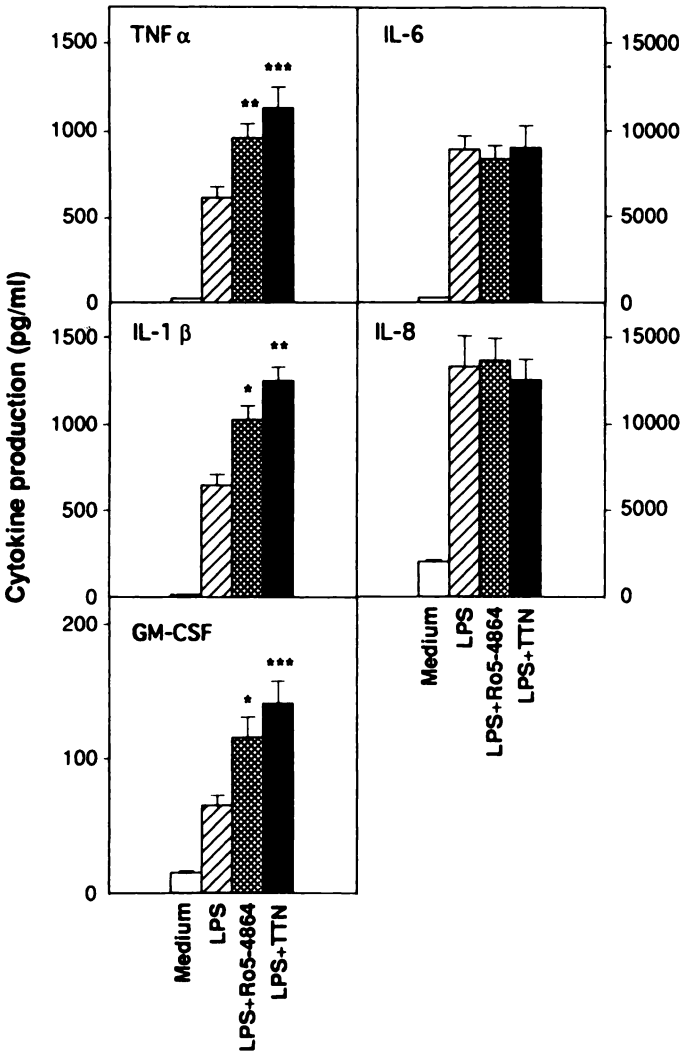

Fig. 1. Effect of TTN and Ro5-4864 on cytokine production by LPS-treated human monocytes. Human monocytes were exposed to control medium, LPS (1 μg/ml), LPS (1 μg/ml) plus Ro5-4864 (10^{-11} M), or LPS (1 μg/ml) plus TTN (10^{-11} M) for 20 hr. TNF-α, IL-1β, GM-CSF, IL-6, and IL-8 productions were assessed by specific enzyme-linked immunosorbent assay and results obtained from four different donors are expressed as pg/ml mean ± standard error. Statistical significance was assessed by paired Student's t test (★, $p < 0.05$; ★★, $p < 0.02$; ★★★, $p < 0.01$) versus control (response to LPS alone). TTN or Ro5-4864 did not modulate response to medium alone.

proximately 2–3-fold. TTN (10^{-11} M) increased LPS-induced TNF-α and IL-1β mRNA expression by about 60% and GM-CSF, IL-6, IL-8 mRNA expression by about 30%. The observed increase in mRNA levels was not related to differences in RNA levels, because Northern blot analysis of actin, hybridized under the same conditions, were unaffected by LPS alone and with TTN.

Effect of the endogenous (TTN) and synthetic (Ro5-4864) PBR ligands on cytokine production in the presence of a CO inhibitor. It has been reported that CO inhibitors, including indomethacin, can increase cytokine synthesis by inhibiting the autocrine suppressive effect of PG (24, 25). We therefore examined whether the stimulatory effect of TTN on LPS-induced cytokine expression might result from the inhibitory action of TTN on PGE_2 synthesis. Monocytes were preincubated for 30 min with indomethacin (0.5 μM) and then

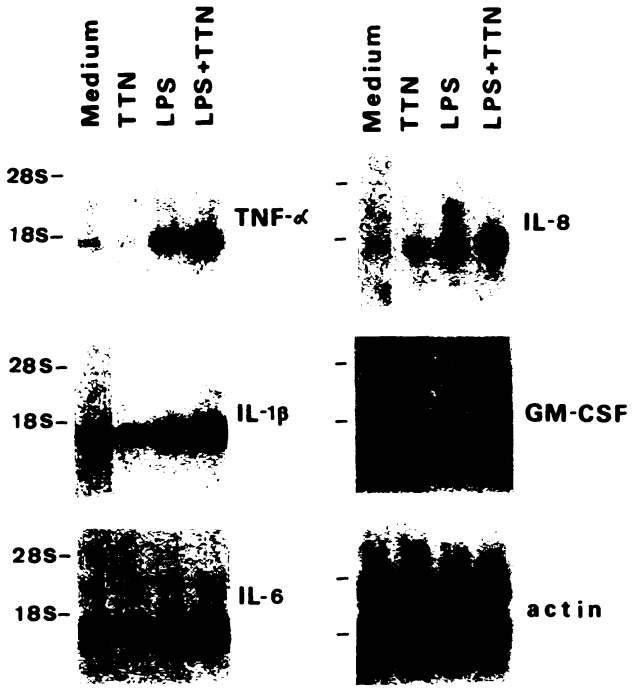

Fig. 2. Effect of TTN on cytokine mRNA accumulation in LPS-stimulated human monocytes. Total cellular RNA was extracted from adherent mononuclear cells after 4 hr of incubation without LPS (control medium), with TTN alone (10^{-11} M), with LPS (1 μg/ml), or with both LPS and TTN and was hybridized with specific ^{32}P-labeled cDNA probes for TNF-α, IL-1β, IL-6, IL-8, GM-CSF, and β-actin.

treated with LPS (1 μg/ml) and TTN (10^{-11} M) or with Ro5-4864 (10^{-11} M). Fig. 3 shows that indomethacin increased IL-1β and TNF-α secretion, relative to LPS-stimulated control cells. Indomethacin and TTN had identical but noncumulative stimulatory effects on IL-1β and TNF-α production. Indomethacin and TTN, alone or together, did not enhance LPS-induced production of IL-6 or IL-8. Indomethacin had no effect on LPS-induced GM-CSF production and did not abrogate the stimulatory effect of TTN. In the absence of LPS, there was no significant increase in cytokine production by monocytes incubated with indomethacin, TTN, or Ro5-4864, either separately or together.

Effect of TTN on PGE_2 formation. To confirm that the action of TTN interfered with the PG pathway, we investigated the effect of the endogenous BZD ligand on PGE_2 production by LPS-stimulated human monocytes. SN were collected 5 hr after cell activation, a time reported to correspond to half-maximal production of PGE_2 by LPS-stimulated human monocytes (24). As shown in Table 1, TTN and Ro5-4864 exerted a significant inhibitory effect on PGE_2 formation (mean of 33% and 32%, respectively) in cells obtained from four different blood donors. In the presence of indomethacin, PGE_2 production was almost abolished and neither TTN nor Ro5-4864 had any effect (data not shown).

Discussion

We have previously found that the PBR is strongly expressed in macrophages (26). PBR is involved in immune regulation *in vitro* and *in vivo*, given the capacity of BZD ligands to modulate cytokine production in both murine macrophages and human monocytes (10, 13, 14).

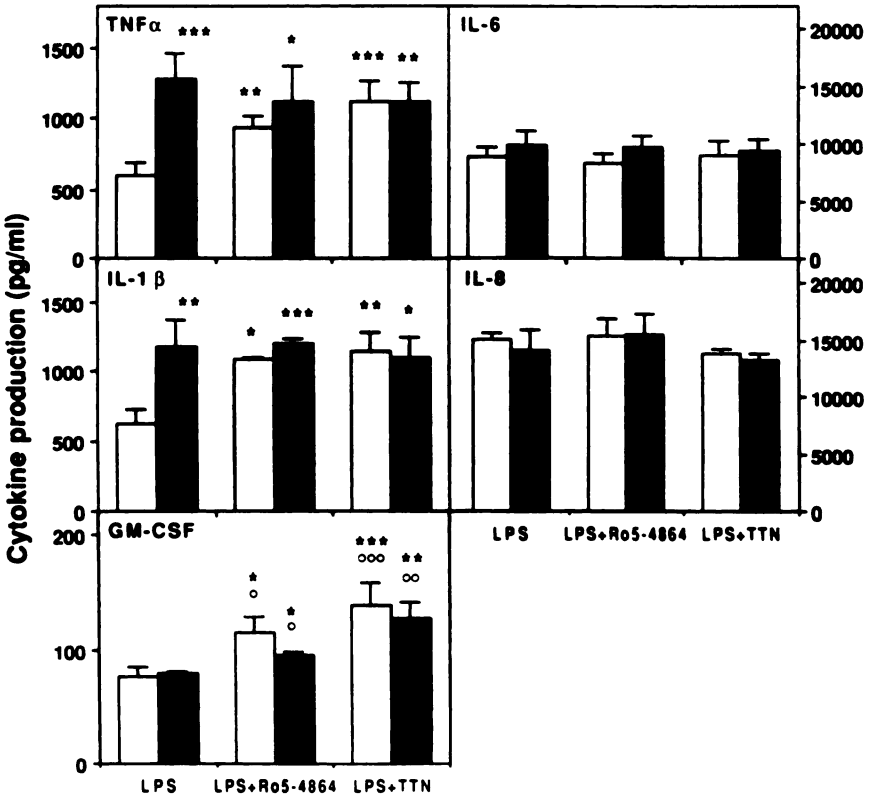

Fig. 3. Effect of human TTN and Ro5-4864 on cytokine production in the presence of indomethacin. Human blood monocytes were exposed to LPS (1 μg/ml), LPS (1 μg/ml) plus Ro5-4864 (10^{-11} M), or LPS (1 μg/ml) plus TTN (10^{-11} M) for 20 hr in the absence (□) or in the presence (■) of indomethacin (0.5 μM). TNF-α, IL-1β, GM-CSF, IL-6, and IL-8 productions were assessed by specific enzyme-linked immunosorbent assay and results obtained from four different donors are expressed as pg/ml mean ± standard error. Statistical significance was assessed by paired Student's *t* test (★, $p < 0.05$; ★★, $p < 0.02$; ★★★, $p < 0.01$ versus response to LPS alone; ○, $p < 0.05$; ○○, $p < 0.02$; ○○○, $p < 0.01$ versus response to LPS plus indomethacin).

TABLE 1

Effect of human TTN and Ro5-4864 on PGE_2 production by LPS-stimulated PBMc

The level of PGE_2 production in the absence of LPS was about 50 pg/1 × 10^6 cells; this level remained unchanged after treatment with TTN or Ro5-4864.

	PGE_2 production		
	LPS (1 μg/ml)	LPS + Ro5-4864 (10^{-11} M)	LPS + TTN (10^{-11} M)
	pg/1 × 10^6 cells		
Experiment 1	2947	2079 (30%)[a]	1973 (33%)
Experiment 2	1351	1019 (25%)	1094 (20%)
Experiment 3	4834	3501 (28%)	3401 (30%)
Experiment 4	1390	755 (46%)	702 (50%
		32 ± 5%[b]	33 ± 6%[b]

[a] Figures in parentheses express percentage of inhibition of control response (response to LPS alone).
[b] $p < 0.05$. Values are mean ± standard error.

DBI, the endogenous ligand of BZD receptors, has been shown to displace diazepam from central-type receptors and from PBR (27). TTN is one of the main biologically active and abundant post-translational processing products of DBI. It is able to displace the peripheral ligand [^{3}H]Ro5-4864 from its binding sites in olfactory bulb synaptosomal membranes with a potency higher than that of ODN-DBI (8). Moreover, TTN has been shown to stimulate steroid biosynthesis by adrenals with an efficacy similar to that of DBI and greater than that of ODN-DBI (7). This prompted us to investigate whether TTN might be an endogenous regulator of the production of the main proinflammatory cytokines in human monocytes.

We first showed that TTN enhances by approximately 2-fold the LPS-induced secretion of TNF-α, IL-1β, and GM-CSF but not that of IL-6 or IL-8. Compared with the prototypic synthetic PBR ligand Ro5-4864, TTN appeared to be more potent in enhancing TNF-α and IL-1β production. TTN also induced a small increase in LPS-induced expression of all the cytokine mRNAs investigated, including those of IL-6 and IL-8. This is the first demonstration that a specific endogenous PBR ligand, at picomolar concentrations, interferes with cytokine production at the level of gene expression. Whether TTN augments transcription or, rather, increases mRNA stabilization remains to be determined. Endotoxin contamination of this peptide would be very unlikely to account for the observed effects, because TTN was totally devoid of activity in the absence of LPS, whereas monocytes responded to LPS concentrations as low as 50 pg/ml under the same experimental conditions (data not shown). Moreover, the finding that TTN (10^{-11} M) still exerted stimulatory effects on the response triggered by LPS (1 μg/ml, a plateau dose) further demonstrates that TTN is not acting merely through endotoxin contaminants.

Interestingly, TTN modulated the mRNA levels of IL-6 and IL-8, but not their secretion. It should be noted that these cytokines are released in larger amounts than are TNF-α, IL-1β, and GM-CSF, but TTN remained ineffective even when smaller quantities of IL-6 and IL-8 were secreted, using sub-optimal concentrations of LPS. No modulation of IL-6 production was observed in either the biological or immunoreactive assays. Differential modulatory actions on cytokine gene expression have been previously reported by Hurme (28), who found that cAMP inhibited LPS-induced IL-1β synthesis without modifying the corresponding mRNA levels. Likewise, Chantry *et al.* (29) found that TGF-β1 stimulated IL-1β and TNF-α mRNA expression in monocytes but did not induce IL-1β or TNF-α protein secretion. The differential effects of TTN on IL-6 and IL-8 gene transcription and translation that we observed could relate to a similar mechanism. Although TTN influences only the signal for IL-6 and IL-8 production at the

transcriptional level, it may modulate both the transcription and translation of the IL-1β, TNF-α, and GM-CSF genes by exerting a priming effect. Indeed, we previously found that Ro5-4864 stimulates IL-1β and TNF-α production only when added at an early phase of activation (13).

It is noteworthy that the expression of some cytokines is regulated by arachidonic acid metabolites (24, 25, 30, 31). The second messenger pathways involved in signal transduction after PBR activation have not yet been elucidated. In the second part of this study, we showed that the effect of TTN and Ro5-4864 on the cytokine response is controlled, at least in part, by CO-derived metabolites. Pretreatment with indomethacin increased TNF-α and IL-1β synthesis to levels that could not be further modulated by TTN, thus suggesting that TTN acts as a CO inhibitor. In agreement with previous studies, indomethacin had no effect on IL-6 or IL-8 production (22, 32). Likewise, TTN did not modulate IL-6 or IL-8 production. These observations suggest that the effect of TTN may be controlled in part by PG at the post-transcriptional level. In keeping with this interpretation, our results demonstrate that TTN affects PG synthesis.

Our data further indicated that TTN modulates both mRNA levels and GM-CSF secretion, whereas the PG inhibitor indomethacin had no effect, in contrast to the case for IL-1β and TNF-α. Moreover, relative to IL-1β and TNF-α, GM-CSF production is very low and delayed in LPS-stimulated human monocytes (32). The observed stimulatory effect of TTN on GM-CSF expression may, therefore, proceed through an additional mechanism distinct from the PG pathway. In particular, whether mitochondrial steroidogenesis, which has been shown to be affected by TTN (7), has any direct or indirect influence on cytokine gene expression and/or secretion would be interesting to investigate.

All these cytokines have been shown to play a role in mediating the inflammatory responses of phagocytic cells stimulated by endotoxin. Moreover, they have a mutually stimulatory effect and act in synergy to develop a proinflammatory reaction. Indeed, IL-1β and TNF-α have been reported to induce GM-CSF, IL-6, and IL-8 production by human monocytes (21, 33, 34), whereas GM-CSF itself is able to increase IL-1β expression (35). GM-CSF and IL-8 display high activation potentials for neutrophils at inflammatory sites (33). The modulatory action exerted by the human endogenous anxiogenic peptide described here could, therefore, contribute to the recruitment and activation of blood-derived monocytes and neutrophils during inflammation.

In a previous *in vitro* study, we demonstrated that rat ODN-DBI (rat DBI 33-50) at picomolar concentrations significantly enhanced LPS-induced secretion of IL-1β and TNF-α by human monocytes (13). Like ODN-DBI, TTN displays anxiogenic and proconflict activities but, in contrast, these effects are blocked by PK11195, a PBR ligand with both antagonistic and agonistic actions on PBR (8). Moreover, TTN has been shown to bind to the Ro5-4864 subsite of the BZD receptor expressed in human monocytes. The fact that TTN exerted cytokine modulation at picomolar concentrations correlates well with its binding affinity for the Ro5-4864 site (8). Whether the mitochondrial PBR or a receptor localized at the monocyte plasma membrane (36, 37) is implicated in the observed effects of TTN remains to be ascertained.

The endogenous BZD receptor ligand DBI has been suggested to play a role in the pathogenesis of both severe depression and hepatic encephalopathy, based on its levels in cerebrospinal fluid (1, 38). We now provide evidence that its bioactive processing product, TTN, modulates the expression *in vitro* of the main proinflammatory cytokines by human monocytes. Although experimental *in vitro* systems similar to ours, in which TTN displayed modulatory capacities at a wide range of LPS doses, were used to demonstrate the regulatory properties of endogenous modulators of cytokine production (29, 35, 39), caution is needed for extrapolation from *in vitro* results to cytokine regulation *in vivo*. In particular, levels of circulating DBI (or its processing products) in healthy, anxious, or immunologically challenged subjects and their possible variations remain unknown at the present time. The possibility that DBI or TTN could be involved in the physiological regulation of the inflammatory response appears, nevertheless, an interesting hypothesis to investigate.

Acknowledgments

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References

1. Barbaccia, M. L., A. Berkovich, P. Guarneri, and E. Slobodyansky. DBI (diazepam binding inhibitor), the precursor of a family of endogenous modulators of $GABA_A$ receptor function: history, perspectives, and clinical implications. *Neurochem. Res.* **15**:161–168 (1990).
2. Costa, E. The allosteric modulation of $GABA_A$ receptors: seventeen years of research. *Neuropsychopharmacology* **4**:225–235 (1991).
3. Gray, P. W., D. Glaister, P. H., Seeburg, A. Guidotti, and E. Costa. Cloning and expression of cDNA for human diazepam binding inhibitor, a natural ligand of an allosteric regulatory site of the γ-aminobutyric acid type A receptor. *Proc. Natl. Acad. Sci. USA* **83**:7547–7551 (1986).
4. Ahlo, H., R. T. Fremeau, H. Tiedge, J. Wilcox, P. Bovolin, J. Brosius, J. L. Roberts, and E. Costa. Diazepam binding inhibitor gene expression: localization in brain and peripheral tissue of rat. *Proc. Natl. Acad. Sci. USA* **85**:7018–7022 (1988).
5. Sprengel, R., P. Werner, P. H. Seeburg, A. G. Mukhin, M. R. Santi, D. R. Grayson, A. Guidotti, and K. E. Krueger. Molecular cloning and expression of cDNA encoding a peripheral-type benzodiazepine receptor. *J. Biol. Chem.* **264**:20415–20421 (1989).
6. Riond, J., M. G. Mattei, M. Kaghad, X. Dumont, J. C. Guillemot, G. Le Fur, D. Caput, and P. Ferrara. Molecular cloning and chromosomal localization of a human peripheral-type benzodiazepine receptor. *Eur. J. Biochem.* **196**:306–311 (1990).
7. Papadopoulos, V., A. Berkovich, K. E. Krueger, E. Costa, and A. Guidotti. Diazepam binding inhibitor and its processing products stimulate mitochondrial steroid biosynthesis via an interaction with mitochondrial benzodiazepine receptors. *Endocrinology* **129**:1481–1488 (1991).
8. Slobodyansky, E., A. Guidotti, C. Wambebe, A. Berkovich, and E. Costa. Isolation and characterization of a rat brain triakontatetraneuropeptide, a posttranslational product of diazepam binding inhibitor: specific action at the Ro-5-4864 recognition site. *J. Neurochem.* **53**:1276–1284 (1989).
9. Zavala, F., and M. Lenfant. Peripheral benzodiazepines enhance the respiratory burst of macrophage-like P388D1 cells stimulated by arachidonic acid. *Int. J. Immunopharmacol.* **3**:269–274 (1987).
10. Zavala, F., V. Taupin, and B. Descamps-Latscha. *In vivo* treatment with benzodiazepines inhibits murine phagocyte oxidative metabolism and production of interleukin-1, tumor necrosis factor and interleukin-6. *J. Pharmacol. Exp. Ther.* **255**:442–450 (1990).
11. Zavala, F., F. Veber, and B. Descamps-Latscha. Altered expression of neutrophil peripheral benzodiazepine receptor in X-linked chronic granulomatous disease. *Blood* **76**:184–188 (1990).
12. Zavala, F., F. Veber, V. Taupin, A. T. Nguyen, and B. Descamps-Latscha. Reconstitution of peripheral benzodiazepine receptor in X-linked chronic granulomatous disease by interferon-γ. *Lancet* **336**:758–759 (1990).
13. Taupin, V., A. Herbelin, B. Descamps-Latscha, and F. Zavala. Endogenous anxiogenic peptide, ODN-diazepam binding inhibitor, and benzodiazepines enhance the production of interleukin-1 and tumor necrosis factor by human monocytes. *Lymphokine Cytokine Res.* **1**:7–13 (1991).
14. Taupin, V., P. Jayais, B. Descamps-Latscha, J. B. Cazalaa, G. Barrier, J. F. Bach, and F. Zavala. Benzodiazepine anesthesia in humans modulates the interleukin-1β, tumor necrosis factor-α and interleukin-6 responses of blood monocytes. *J. Neuroimmunol.* **35**:13–19 (1991).
15. Costa, E., and A. Guidotti. Diazepam binding inhibitor (DBI): a peptide with multiple biological actions. *Life Sci.* **49**:325–344 (1991).

16. Chomczynski, P., and N. Sacchi. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156–159 (1987).
17. Wang, A. M., A. A. Creasey, M. B. Ladner, L. S. Lin, J. Strickler, J. N. Van Arsdell, R. Yamamoto, and D. F. Mark. Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science (Washington D. C.)* **228**:149–154 (1985).
18. Matsushima, K., K. Morishita, T. Yoshimura, S. Lavu, Y. Kobayashi, W. Lew, E. Appella, H. F. Kung, E. J. Leonard, and J. J. Oppenheim. Molecular cloning of a human monocyte-derived neutrophil chemotactic factor (MDNCF) and the induction of MDNCF mRNA by interleukin 1 and tumor necrosis factor. *J. Exp. Med.* **167**:1883–1893 (1988).
19. Cleveland, D. W., M. A. Lopata, R. J. Mac Donald, N. J. Cowan, W. J. Rutter, and M. W. Kirschner. Number and evolutionary conservation of *alpha*- and *beta*-tubulin and cytoplasmic *beta*- and *gamma*-actin genes using specific cloned cDNA probes. *Cell* **20**:95–105 (1980).
20. Feinberg, A. P., and B. Vogelstein. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6–13 (1983).
21. Seitz, M., B. Dewald, N. Gerber, and M. Baggiolini. Enhanced production of neutrophil-activating peptide-1/interleukin-8 in rheumatoid arthritis. *J. Clin. Invest.* **87**:463–469 (1990).
22. Tovey, M. G., J. Content, I. Gresser, J. Gugenheim, B. Blanchard, J. Guymarho, P. Poupart, M. Gigou, A. Shaw, and W. Fiers. Genes for IFN-*beta*-2 (IL-6), tumor necrosis factor, and IL-1 are expressed at high levels in the organs of normal individuals. *J. Immunol.* **141**:3106–3110 (1988).
23. Fibbe, W. E., P. M. Kluck, N. Duinkerken, P. J. Voogt, R. Willemzel, and J. H. F. Falkenburg. Factors influencing release of granulocyte-macrophage colony-stimulating activity from mononuclear phagocytes. *Eur. J. Haematol.* **41**:352–358 (1988).
24. Kunkel, S. L., S. W. Chensue, and S. H. Phan. Prostaglandins as endogenous mediators of interleukin-1 production. *J. Immunol.* **136**:186–192 (1986).
25. Van Der Meer, J. W., S. Endres, G. Lonnemann, J. G., Cannon, T. Ikejima, S. Okusawa, J. A. Gelfand, and C. A. Dinarello. Concentrations of immunoreactive human tumor necrosis factor *alpha* produced by human mononuclear cells *in vitro*. *J. Leukocyte Biol.* **43**:216–223 (1988).
26. Zavala, F., J. Haumont, and M. Lenfant. Interaction of benzodiazepines with mouse macrophages. *Eur. J. Pharmacol.* **106**:561–566 (1984).
27. Guidotti, A., H. Alho, A., Berkovich, D. H. Cox, C. Ferrarese, E. Slobodyansky, M. R. Santi, and C. Wambebe. DBI processing: allosteric modulation at different GABA/benzodiazepine receptor subtypes, in *Allosteric Modulation of Amino Acid Receptors: Therapeutic Implications* (E. A. Barnard and E. Costa, eds.). Raven Press, New York, 100–123 (1989).
28. Hurme, M. Modulation of interleukin-1β production by cyclic AMP in human monocytes. *FEBS Lett.* **263**:35–37 (1990).
29. Chantry, D., M. Turner, E. Abney, and M. Feldmann. Modulation of cytokine production by transforming growth factor-β. *J. Immunol.* **142**:4295–4300 (1989).
30. Knudsen, P. J., C. A. Dinarello, and T. B. Strom. Prostaglandins posttranscriptionally inhibit monocyte expression of interleukin-1 activity by increasing intracellular cyclic adenosine monophosphate. *J. Immunol.* **137**:3189–3194 (1986).
31. Kunkel, S. L., M. Spengler, M. A. May, R. Spengler, J. Larrick, and D. Remick. Prostaglandin E2 regulates macrophage-derived tumor necrosis factor gene expression. *J. Biol. Chem.* **263**:5380–5384 (1988).
32. Sullivan, R., P. J. Gans, and L. A. McCarrol. The synthesis and secretion of granulocyte-monocyte colony-stimulating activity (CSA) by isolated human monocytes: kinetics of the response to bacterial endotoxin. *J. Immunol.* **130**:800–807 (1983).
33. Ruef, C., and D. L. Coleman. Granulocyte-macrophage colony-stimulating factor: pleiotropic cytokine with potential clinical usefulness. *Rev. Infect. Dis.* **12**:41–62 (1990).
34. Baggiolini, M., A. Walz, and S. L. Kunkel. Neutrophil-activating peptide 1/interleukin 8, a novel cytokine that activates neutrophils. *J. Clin. Invest.* **84**:1045–1049 (1989).
35. Smith, P. D., C. L. Lamerson, H. L. Wong, L. Wahl, and S. M. Wahl. Granulocyte-macrophage colony-stimulating factor stimulates human monocyte accessory cell function. *J. Immunol.* **144**:3829–3834 (1990).
36. Doble, A., J. Benavides, O. Ferris, P. Bertrand, J. Menager, N. Vaucher, M. C. Burgevin, and A. Uzan. Dihydropyridine and peripheral type benzodiazepine binding sites: subcellular distribution and molecular size determination. *Eur. J. Pharmacol.* **119**:153–167 (1985).
37. O'Beirne, G. B., M. J. Woods, and D. C. Williams. Two subcellular locations for peripheral-type benzodiazepine acceptors in rat liver. *Eur. J. Biochem.* **188**:131–138 (1990).
38. Rothstein, J. D., G. McKhann, P. Guarneri, and M. L. Barbaccia. Cerebrospinal fluid content of diazepam binding inhibitor in chronic hepatic encephalopathy. *Ann. Neurol.* **26**:57–62 (1989).
39. Essner, R., K. Rhoades, W. H. McBride, D. L. Morton, and J. S. Economou. IL-4 down-regulates IL-1 and TNF gene expression in human monocytes. *J. Immunol.* **142**:3857–3861 (1989).

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