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ORIGINAL ARTICLE

Negative immunoregulatory effects of noradrenaline through α2-adrenoceptor activation

Michael Maes,[1,2,3] Aihua Lin,[2] Gunter Kenis,[1] Belinda Egyed[1] & Eugene Bosmans[1,4]

1. Department of Psychiatry & Neuropsychology, University Hospital of Maastricht, the Netherlands.
2. Clinical Research Center for Mental Health (CRC-MH), Antwerp, Belgium.
3. Department of Psychiatry, Vanderbilt University, Nashville, TN, USA.
4. Eurogenetics, Tessenderlo, Belgium.

Correspondence to: Michael Maes, M.D., Ph.D., Professor and Chairman
Department of Psychiatry & Neuropsychology,
University Hospital of Maastricht, PO Box 5800,
AZ 6202 Maastricht, the Netherlands.
FAX (the Netherlands): +31-43-3875444
TEL (the Netherlands): +31-43-3877443
FAX (Belgium): +32-89-723531
E-MAIL crc-mh@online.be

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Abstract

BACKGROUND: There are some reports that catecholamines and β-adrenoceptor agonists may suppress some immune functions.
OBJECTIVES: The present study was carried out in order to examine the effects of noradrenaline (10^{-5} M, 10^{-6} M and 10^{-7} M), yohimbine (10^{-5} M, 10^{-6} M and 10^{-7} M), an α2-adrenoceptor antagonist, and clonidine (10^{-5} M, 10^{-6} M and 10^{-7} M), an α2-adrenoceptor agonist, on the stimulated production of interferon-γ (IFNγ), a pro-inflammatory cytokine, and interleukin-10 (IL-10), an immuno-inhibitory cytokine.
DESIGN: We measured the in vitro production of IFNγ and IL-10 by stimulated, diluted whole blood of 16 normal volunteers. The IFNγ/IL-10 production ratio was computed since this ratio reflects the pro- versus anti-inflammatory capacity of the cultured whole blood.
RESULTS: We found that 1) noradrenaline, 10^{-5} M, 10^{-6} M, and 10^{-7} M, significantly suppressed the production of IFNγ and that noradrenaline, 10^{-5} M, significantly enhanced the production of IL-10. Clonidine, 10^{-5} M and 10^{-7} M, significantly suppressed the production of IFNγ. Noradrenaline, 10^{-5} M and 10^{-6} M, and clonidine, 10^{-5} M, significantly suppressed the IFNγ/IL-10 production ratio. There were no significant effects of yohimbine on IFNγ or IL-10 production.
CONCLUSIONS: 1) noradrenaline has significant negative immunoregulatory effects in humans through enhancing the production of IL-10 and suppressing that of IFNγ; and 2) the suppression of the production of IFNγ is in part related to α2-adrenoceptor activation.

Introduction

Experimental and real life psychological stressors have been reported to suppress some immune functions such as mitogen- (e.g. phytohemaglutinin-PHA or concanavalin A- Con-A) induced lymphocyte proliferation and natural killer cell activity (NKCA) [1]. There is also a relative consistent literature that the effects of psychological stress on these immune functions are mediated predominantly through an enhanced activity of the autonomic system. For example, immobilization stress rapidly increases noradrenaline concentrations to ±800% of baseline in conjunction with suppressed NKCA in the spleen [2]. Mental arithmetic plus noise stress in humans increases plasma noradrenaline and adrenaline concentrations and decreases the blastogenic responses to Con-A [3]. The modulation of the immune response following psychological stress by the autonomic system [4] may be mediated by adrenoceptors located on immunocytes, which modulate the production rate of pro-inflammatory cytokines, e.g. interferon-γ (IFNγ) versus immuno-inhibitory cytokines, e.g. interleukin-10 (IL-10).

IFNγ is mainly produced by activated T-helper-1 (Th-1) immune cells; IL-10 is produced by Th-0, Th-1, Th-2 and B lymphocytes and by cells of the monocyte/macrophage lineage [5]. IL-10 inhibits the production of IFNγ by Th-1-like cell clones and is a major macrophage deactivating factor, which downregulates the production of monocytic cytokines [5]. Therefore, the production ratio of IFNγ to IL-10 is of critical importance in determining the pro- or anti-inflammatory capacity of the produced cytokines [6].

There is some evidence that catecholamines may suppress the production of IFNγ and enhance that of IL-10. In vitro incubation of Listeria Monocytogenes immune system cells with noradrenaline and adrenaline inhibits the PHA-induced production of IFNγ [7]. In HIV-1 infected peripheral blood mononuclear cells (PBMNs) activated with polyclonal activators, noradrenaline reduces the production of IFNγ [8]. This catecholamine-induced inhibition of IFNγ may be mediated by β-adrenoceptors. Thus, Con-A-induced IFNγ mRNA synthesis is dose-dependently inhibited by fenoterol, a selective β2-adrenoceptor agonist, and isoproterenol, a nonselective β-adrenoceptor agonist [9]. Salmeterol, a β2-adrenoceptor agonist, inhibits the PHA-induced production of IFNγ by PBMNs [10].

Noradrenaline and adrenaline both dose-dependently increase the LPS-induced production of IL-10 by human whole blood [11]. Accidental and iatrogenic brain trauma induces a rapid systemic release of IL-10 in patients with a sympathetic storm due to the acute trauma [12]. In vitro studies show that, within minutes, catecholamines trigger the secretion of IL-10 through a β-adrenoceptor-related mechanism [12]. Isoproterenol increases IL-10 mRNA levels and IL-10 release from LPS-activated mouse peritoneal macrophages in a dose-dependent manner [13]. Propanolol, a β-adrenoceptor antagonist, blocks the stimulatory effects of noradrenaline and adrenaline on the LPS-induced production of IL-10 by human whole blood [11]. These findings show that catecholamines enhance IL-10 production through β-receptor-mediated mechanisms [13]. It is suggested that α-adrenoceptor-related mechanisms are not involved in catecholamine-induced changes in IL-10 production. Thus, the catecholamine-induced secretion of IL-10 by macrophages, which are infected with Mycobacterium Avium, is blocked by propranolol but not by phentolamine, an α-adrenoceptor antagonist [14].

α2-Adrenoceptor activation may, however, be involved in the rat adrenergic suppression of peripheral blood T cell reactivity to Con-A [15]. In mice, the LPS-induced production of some pro-inflammatory cytokines, i.e. tumor necrosis factor (TNF), is significantly diminished by selective α2-adrenoceptor antagonists [16]. α2-adrenoceptors are present on human lymphocytes [17]. The total number of [^{3}H]-yohimbine sites is 19.9 ±5.3 fmol/10^7 lymphocytes. Adrenergic agonists compete for [^{3}H]-yohimbine binding sites with an order of potency: clonidine greater than (-)adrenaline greater than (-)noradrenaline [17]. To the best of our knowledge, however, the effects of α2-adrenoceptor agonists and antagonists and of noradrenaline on the LPS+PHA stimulated production of IFNγ versus IL-10 by human whole blood have not been studied yet.

The specific aims of the present study were to examine the effects of noradrenaline, clonidine, an α2-adrenoceptor agonist, and yohimbine, an α2-adrenoceptor antagonist, on the production of IFNγ and IL-10 by human whole blood stimulated with polyclonal activators.

Subjects and methods

Subjects

Blood samples for the assay of IFNγ and IL-10 were collected from 16 normal volunteers. The mean age of the subjects was 34.2 ±7.0 years (range: 24–48 years). The male/female ratio was 7/9. All subjects had a negative present, past and family history of axis-I psychiatric illnesses. None of the subjects suffered from an axis-II diagnosis, was a regular drinker or had ever been taking psychotropic drugs. All normal volunteers were free of any medical ill-

ness and acute infections, inflammatory or allergic reactions for at least two weeks prior to the study. All volunteers were free of any drugs for at least three months prior to these studies and none had ever been taking drugs which are known to modify immune or endocrine functions. All volunteers abstained from alcohol and caffeine for at least 12 hours prior to these studies. All subjects gave written informed consent after the study design was fully explained.

Methods

After an overnight fast, blood for the assays of IFNγ and IL-10 was taken at 9 a.m. (±30 minutes). The effects of noradrenaline, clonidine and yohimbine on the production of IFNγ and IL-10 were examined by stimulating diluted (1/10) whole blood with PHA (1 μg/ml; Murex Diagnostics Ltd, Dartford, England; Lot: F568810) and LPS (5 μg/ml; E.Coli 026:B6; lyophylized and sterilized by gamma-irradiation; Sigma, Belgium; Lot: 107H4091). PHA + LPS-stimulated, diluted whole blood offers an appropriate and reproducible culture condition in order to measure the production of various cytokines [18–20]. Diluted whole blood cultures reflect the in-vivo immune cellular and humoral interactions and may be employed to examine the effects of drugs on cytokine secretion [18–22]. 1.8 ml of RPMI-1640 medium with L-glutamine (Gibco BRL, Life Technologies; Lot: 3011105) and 100 IU/mL penicillin and 100 μg/mL streptomycin (Gibco BRL, Life Technologies; Lot: 3011086) with PHA + LPS were placed into 24 well cell culture plates (Falcone 3047, Becton Dickinsen). (±)Noradrenaline-hydrogentartrate (Sigma; Lot: 107H1250), clonidine hydrochloride (Sigma; Lot: 28H3272) and yohimbine hydrochloride (Sigma; Lot: 48H1003) were dissolved in sterile water, whereas sterile water alone served as the corresponding control. 20 μl of each drug solution was added to the wells and gently mixed with the medium. 0.2 ml of whole blood from each of 16 subjects was cultured with (±)noradrenaline-hydrogentartrate (10^{-5} M, 10^{-6} M, and 10^{-7} M), clonidine hydrochloride (10^{-5} M, 10^{-6} M, and 10^{-7} M), and yohimbine hydrochloride (10^{-5} M, 10^{-6} M, and 10^{-7} M). The viability of the immune cells was checked by ethidium-bromide-dye exclusion. The samples were incubated for 72 hours in a humidified atmosphere at 37°C, 5% CO2. Supernatants were taken off carefully under sterile conditions, divided into eppendorf tubes, and frozen immediately at –75°C. IFNγ and IL-10 were quantified by means of ELISA methods based on appropriate and validated sets of monoclonal antibodies as described by us previously [21, 22]. All assays of IFNγ or IL-10 were carried out on the same day in one run by the same operator (BE). The intra-assay CV values for both analytes were less than 8%. In our laboratory, the detection limits are 0.9 U/mL for IFNγ and 9 pg/mL for IL-10. In order to examine the production ratio of pro-inflammatory (IFNγ) versus negative immunoregulatory (IL-10) cytokines, the IFNγ/IL-10 ratio was computed as: z transformed IFNγ - z transformed IL-10 [21, 22].

Statistics

Repeated measure design analyses of variance (RM ANOVAs) were used to examine the 1) within-subject variability with the effects of the drugs (i.e. the positive control versus noradrenaline, clonidine and yohimbine) and their concentrations as temporal conditions; and 2) between-subject variability with gender or age groups (divided according to the mean age of the subjects, i.e. 34.2 years) as factors. Tests on simple effects were employed to clarify main effects or significant interactions. A simple effect is the effect of one variable at one level of the other variable. Differences among treatment means were ascertained by means of the Dunn test, used after p-correction was made for multiple comparisons. The results of RM design ANOVAs were always corrected for sphericity. Differences among treatment means were ascertained by means of the Dunn test. IFNγ and IL-10 as well as the IFNγ/IL-10 production ratio were processed in Box-Cox transformation.

Results

RM design ANOVA performed on the IFNγ data with the drug status (control versus the three drugs conditions) and the three different concentrations as within-subject factors and gender as between-subject factor showed no significant differences in IFNγ production between males and females ($F=0.5$, $df=1/14$, $p=0.5$) and no significant interaction patterns between concentrations X gender ($F=0.03$, $df=1/12$, $p=0.9$), drugs X gender ($F=2.7$, $df=1/18$, $p=0.1$) or concentrations X drugs X gender ($F=0.6$, $df=3/37$, $p=0.6$). RM design ANOVA performed on the IL-10 data with the drug status and the three different concentrations as within-subject factors and gender as between-subject factor showed no significant differences in IL-10 production between males and females ($F=1.9$, $df=1/14$, $p=0.2$) and no significant interaction patterns between concentrations X gender ($F=0.4$, $df=1/12$, $p=0.5$), drugs X gender ($F=1.4$, $df=1/18$, $p=0.2$) or concentrations X drugs X gender ($F=0.4$, $df=3/36$, $p=0.8$). RM design ANOVA performed on the IFNγ data with the drug status (control versus the three drugs conditions) and the

three different concentrations as within-subject factors and the age groups as between-subject factor showed no significant differences in IFNγ production between younger and older subjects (F=0.2, df=1/14, p=0.6) and no significant interaction patterns between concentrations X age groups (F=1.2, df=1/12, p=0.3), drugs X age groups (F=0.2, df=1/18, p=0.6) or concentrations X drugs X age groups (F=0.7, df=3/36, p=0.6). RM design ANOVA performed on the IL-10 data with the drug status and the three different concentrations as within-subject factors and the age groups as between-subject factor showed no significant differences in IL-10 production between the two age groups (F=0.07, df=1/14, p=0.8) and no significant interaction patterns between concentrations X age groups (F=0.4, df=1/12, p=0.6), drugs X age groups (F=0.1, df=1/18, p=0.7) or concentrations X drugs X age groups (F=0.7, df=3/36, p=0.6). Since there were no significant effects of gender and age on the production rate of IFNγ and IL-10, we have combined the data obtained in the male and female groups and in both age groups and performed all subsequent analyses in these groups combined.

Figure 1 shows the effects of noradrenaline, clonidine and yohimbine on the production of IFNγ and IL-10 and on the IFNγ/IL-10 production ratio. RM design ANOVA performed on the IFNγ measurements and with the 3 different concentrations and the 4 drug conditions as time factors showed significant effects of the drug concentrations (F=34.8, df=1/74, p=0.00001), and the drugs (F=88.1, df=1/74, $p<10^{-5}$) and a significant interaction pattern between drug X drug concentrations (F=13.4, df=3/74, p=0.00001). Analyses on simple effects showed significant effects of noradrenaline (F=63.2, df=2/165, $p<10^{-5}$) and clonidine (F=9.9, df=2/165, p=0.0002), but not yohimbine (F=1.4, df=2/165, p=0.2), on IFNγ production. Dunn's test showed significantly suppressant effects of clonidine (t=6.17, p=0.000003) and noradrenaline (t=13.89, $p<10^{-6}$), but not yohimbine (t=0.18, p=0.8), on the production of IFNγ.

RM design ANOVA performed on the IL-10 data showed significant effects of the drugs (F=5.0, df=1/72, p=0.02) but no significant drug X drug concentration interaction (F=0.5, df=3/72, p=0.7). Dunn's test showed significant enhancing effects of noradrenaline (t=3.75, p=0.0005), but not clonidine (t=1.17, df=0.2) or yohimbine (t=1.31, p=0.2), on the production of IL-10.

RM design ANOVA performed on the IFNγ/IL-10 ratio showed significant effects of the drugs (F=13.5, df=1/43, p=0.0009) but no significant interaction between drugs X drug concentrations (F=1.5, df=2/43, p=0.2). Dunn's tests showed significant suppressant effects of clonidine (t=2.72, p=0.007) and noradrenaline (t=6.10; $p<10^{-5}$), but not yohimbine (t=1.51, p=0.1), on the IFNγ/IL-10 ratio.

A RM design ANOVA preformed on the IFNγ values with the concentrations of noradrenaline as repeated measures showed a significant effect of noradrenaline (F=72.3, df=3/45, $p<10^{-5}$). Dunn's test showed significant suppressant effects of noradrenaline on IFNγ production at 10^{-5} M (t=13.33, $p<10^{-5}$), 10^{-6} M (t=8.68, $p<10^{-5}$) and 10^{-7} M (t=2.59, p=0.012). RM design ANOVA performed on IL-10 showed a significant effect of noradrenaline (F=3.4, df=2/37, p=0.04). Dunn's test showed a significant enhancing effect of noradrenaline 10^{-5} M (t=3.05, p=0.004) but not 10^{-6} M (t=2.42, p=0.01) or 10^{-7} M (t=1.84, p=0.07). RM design ANOVA showed a significant effect of noradrenaline on the IFNγ/IL-10 ratio (F=7.21, df=2/33, p=0.003). Dunn's test showed significant effects of noradrenaline 10^{-5} M (t=4.26, p=0.0002) and 10^{-6} M (t=3.55, p=0.001), but not 10^{-7} M (t=1.89, p=0.06).

RM design ANOVA showed a significant effect of clonidine (F=15.0, df=2/25, p=0.0001) on the IFNγ production. Dunn's test showed significant suppressant effects of clonidine at 10^{-5} M (t=6.55, p=0.000005) and 10^{-7} M (t=3.52, p=0.001), but not 10^{-6} M (t=2.21, p=0.03). RM design ANOVA did not show any significant effects of clonidine on the production of IL-10 (F=1.2, df=2/32, p=0.3). RM design ANOVA showed a significant effect of clonidine on the IFNγ/IL-10 ratio (F=4.2, df=1/22; p=0.049). Dunn's test showed a significant suppressant effect of clonidine at 10^{-5} M (t=3.52, p=0.001), but not 10^{-6} M (t=1.70, p=0.09) or 10^{-7} M (t=2.17, p=0.03). RM design ANOVAs did not show any significant effects of yohimbine on the production of IFNγ (F=2.2, df=2/36, p=0.1) and IL-10 (F=1.1, df=2/33, p=0.3) and on the IFNγ/IL-10 production ratio (F=1.7, df=2/26, p=0.2).

Discussion

The results of the present study show that, in humans, noradrenaline 10^{-5}, 10^{-6}, and 10^{-7} M, and clonidine, 10^{-5} and 10^{-7} M, significantly suppressed the stimulated production of IFNγ and that noradrenaline, 10^{-5} M, significantly enhanced the production of IL-10. Noradrenaline, 10^{-5} M and 10^{-6} M, and clonidine 10^{-5} M, significantly diminished the IFNγ/IL-10 production ratio. These results show that noradrenaline has significant negative immunoregulatory effects through an enhanced production of IL-10 and suppression of the production

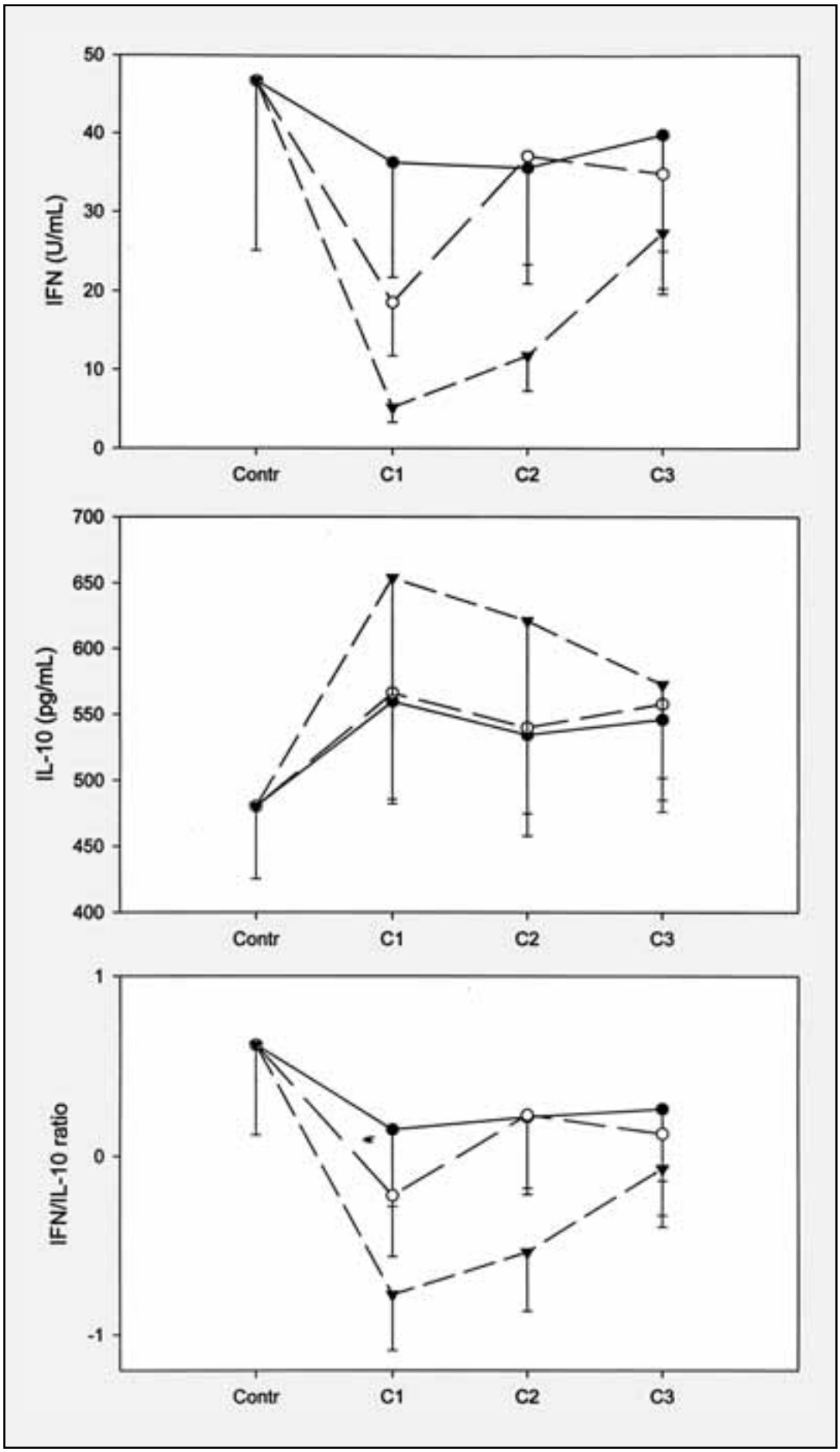

Fig 1. Effects of noradrenaline (10^{-5} M, 10^{-6} M and 10^{-7} M), clonidine (10^{-5} M, 10^{-6} M and 10^{-7} M) and yohimbine (10^{-5} M, 10^{-6} M and 10^{-7} M) on the stimulated production of interferon-γ (IFN), interleukin-10 (IL-10) by whole blood of normal volunteers and on the IFNγ/IL-10 production ratio.

▼—-▼—-▼: noradrenaline

❍—-❍—-❍: clonidine

●— ●—●: yohimbine

Contr: the positive control condition
C1:10^{-5} M
C2:10^{-6} M
C3:10^{-7} M

of IFNγ and that the latter may, in part, be related to α2-adrenoceptor activation.

The results of the present study that noradrenaline inhibits the production of IFNγ and extend those of previous reports which showed that noradrenaline inhibits the stimulated production of IFNγ by Listeria Monocytogenes immune system cells and by HIV-1 infected PBMCs [7, 8]. Our finding that noradrenaline enhances the stimulated production of IL-10 is in agreement with previous reports that catecholamines increase the stimulated production of IL-10 by human whole blood [11]. Catecholamines interact with macrophages which are infected with Mycobacterium Avium resulting in the induction of IL-10 mRNA and protein [14]. Woiciechowsky et al. [12] have found that, in vitro, catecholamines trigger the secretion of IL-10 from unstimulated monocytes. There is a substantial body of evidence showing that the above effects are related to activation of β-adrenoceptors. Thus, β-adrenoceptor agonists such as salmeterol, proterenol and fenoterol dose-dependently inhibit the stimulated production of IFNγ mRNA and IFNγ [9, 10]. β-Adrenoceptor antagonists, such as propanolol, prevent the inhibitory effects of noradrenaline on the stimulated production of IFNγ by immune spleen cells of Listeria Monocytogenes [7]. Also, β-adrenoceptor agonists, such as isoproterenol, enhance the release of IL-10 and increase IL-10 mRNA levels in a dose-dependent manner [13, 23, 24]. β-Adrenoceptor antagonists, such as propanolol, block the effects of noradrenaline on the LPS-induced production of IL-10 by human whole blood or by macrophages [11, 14].

The results of the present study show that α2-adrenoceptor activation by clonidine inhibits the production of IFNγ but does not affect the production rate of IL-10. Previously, it has been reported that in the rat α2-adrenoceptor activation is involved in the adrenergic suppression of peripheral blood T cell reactivity to Con-A [15]. The enhancing effects of catecholamines on the production of IL-10 by macrophages infected with Mycobacterium Avium are not inhibited by treating the cells with phentolamine, an α-adrenoceptor antagonist [14]. It has been shown that α2-adrenoceptors are present on human lymphocytes [17]. Human polymorphonuclear cell membranes contain α-adrenergic receptors which are measured by binding of the α2-adrenergic antagonist [^{3}H]-yohimbine [25]. The presence of a macrophage α2-adrenoceptor was established by demonstrating binding of yohimbine to membranes prepared from macrophages [26]. Thus, the results of the present and previous studies show that the negative immunoregulatory effects of noradrenaline are obtained through suppression of IFNγ production, which is related to β- as well as α2-adrenoceptor stimulation, and activation of the production of IL-10, which is related to β-, but not α2-, adrenoceptor stimulation.

Another finding of the present study is that yohimbine did not have any significant effects on the production rates of IFNγ and IL-10. In mice, on the other hand, the LPS-induced production of pro-inflammatory cytokines, i.e. tumor necrosis factor (TNF), is significantly blunted by CH-38083, a highly selective α2-adrenoceptor antagonist [16, 23]. α2-adrenoceptor antagonists also attenuate the production of TNF by murine peritoneal macrophages [26, 27], while α2-adrenoceptor agonists can augment the LPS-stimulated TNF production from elicited macrophages [27].

One of the mechanisms whereby noradrenaline exerts its negative immunoregulatory effect probably involves changes in the second messenger of the β-adrenoceptor, i.e. cAMP. Stimulation of β-adrenoceptors by noradrenaline increases the intracellular levels of cAMP by direct activation of adenylate cyclase. Intracellular cAMP levels play a pivotal role in the production of IFNγ and IL-10. Thus, in human T cells, cAMP elevating agents inhibit IFNγ production and IFNγ mRNA expression, and increase intracellular levels of IL-10 and IL-10 mRNA expression [28]. Also, in monocytes cAMP elevating drugs upregulate IL-10 mRNA [29]. The β2-adrenoceptor-mediated inhibition of IFNγ is related to the accumulation of intracellular cAMP [9]. The effects of catecholamines increasing the production of IL-10 mRNA and protein are mimicked by the addition of β2-adrenoceptor agonists and by cAMP [14]. Recent data suggest a role for protein kinase A (PKA) activation for catecholamine-induced IL-10 synthesis in human mononuclear cells [30]. Thus, (Rp)-cAMPS, a diastereomer of adenosine-3', 5'-cyclic phosphorothionate, which inhibits competitively the cAMP-induced activation of PKA, led to a reversal of the catecholamine-induced synthesis of IL-10 [30]. In vitro studies show that within minutes, catecholamines trigger the secretion of IL-10 from unstimulated monocytes through a β-adrenoceptor-mediated cAMP/PKA-dependent pathway [12]. PKA is involved in activating gene transcription aspects to produce IL-10 [31]. Another possible mechanism is that β2-agonists inhibit the monocytic production of IL-12, which plays a central role in the Th-1 versus Th-2 responsivity by skewing the immune response towards Th-1-type responses [32].

The mechanisms whereby α2-adrenoceptor activation may diminish IFNγ production, however,

have remained elusive. Indeed, an important consequence of α2-adrenoceptor activation is inhibition of adenylyl cyclase activity, which results in decreased cAMP formation [33]. Possible mechanisms are the importance of calcium and potassium channels [33] and activation of inositol phosopholipid metabolism [34] in the molecular pharmacology of the α2-adrenoceptor.

The results suggest that psychological stressors accompanied by significant elevated circulating plasma concentrations of noradrenaline may modulate the Th-1/Th-2 balance by causing a selective suppression of Th-1 functions and increasing the production of negative immunoregulatory cytokines, such as IL-10.

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