Effect of naproxen on the hypothalamic–pituitary–adrenal axis in healthy volunteers

Agnes M. M. Eijsbouts, Marlies J. E. Kempers,¹ Renske S. A. Kramer,² Maria T. E. Hopman,² Frank H. J. van den Hoogen, Ronald F. J. M. Laan,³ Ad R. M. M. Hermus,¹ Fred C. G. J. Sweep⁴ & Leo B. A. van de Putte³

Department of Rheumatology, Sint Maartenskliniek and Departments of ¹Endocrinology, ²Physiology, ³Rheumatology and ⁴Chemical Endocrinology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands

WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- In patients with rheumatoid arthritis (RA) the activity of the hypothalamic–pituitary–adrenal (HPA) axis is decreased.

WHAT THIS STUDY ADDS

- It is not known how the frequent use of nonsteroidal anti-inflammatory drugs (NSAIDs) in patients with RA influences the HPA axis.
- In this study the effect of the NSAID naproxen on HPA axis activity was tested in healthy volunteers.
- Two weeks' use of naproxen did not influence the activity of the HPA axis in healthy volunteers under basal circumstances, nor in response to physical stress.

Correspondence

Dr Agnes M. M. Eijsbouts, MD, PhD,
Department of Rheumatology, Sint
Maartenskliniek, PO Box 9011, 6500 GM,
Nijmegen, the Netherlands.
Tel: + 31 0 24 365 9911
Fax: + 31 0 24 365 9204
E-mail: a.eijsbouts@maartenskliniek.nl

Keywords

Hypothalamic–pituitary–adrenal axis,
naproxen, NSAID

Received

26 February 2008

Accepted

2 October 2008

AIM

To study the effect of the nonsteroidal anti-inflammatory drug naproxen on the activity of the hypothalamic–pituitary–adrenal (HPA) axis in healthy volunteers.

METHODS

A double-blind, randomized study in two groups of 20 healthy volunteers was performed. The activity of the HPA axis was measured before and after the use of naproxen or placebo during a period of 2 weeks. Basal plasma adrenocorticotrophic hormone (ACTH) and cortisol, 24-h urinary cortisol, and circadian cortisol rhythm in saliva were determined. Plasma ACTH and cortisol were also measured during submaximal physical exercise.

RESULTS

There were no significant differences between the placebo and naproxen groups in basal plasma ACTH [09.00 h 3.1 pmol l⁻¹, 95% confidence interval (CI) 2.0, 4.2, and 2.8 pmol l⁻¹, 95% CI 1.9, 3.7, respectively], cortisol levels (09.00 h 0.45 µmol l⁻¹, 95% CI 0.39, 0.51, and 0.40 µmol l⁻¹, 95% CI 0.35, 0.44, respectively), 24 h urinary cortisol excretion (67.5 nmol 24 h⁻¹, 95% CI 54.3, 80.7, and 86.8 nmol 24 h⁻¹, 95% CI 54.4, 119.2, respectively), circadian cortisol rhythm measured in salivary samples, or ACTH and cortisol concentrations after physical exercise. After the use of placebo or naproxen for 2 weeks, no significant change in any of the parameters occurred (ACTH 09.00 h 3.0 pmol l⁻¹, 95% CI 2.0, 3.9, and 3.0 pmol l⁻¹, 95% CI 2.2, 3.8, respectively; cortisol 09.00 h 0.45 µmol l⁻¹, 95% CI 0.37, 0.52, and 0.39 µmol l⁻¹, 95% CI 0.34, 0.44, respectively; cortisol urine 79.5 nmol 24 h⁻¹, 95% CI 59.5, 99.4, and 81.7 nmol 24 h⁻¹, 95% CI 64.0, 99.4, respectively), and no significant differences were found in these parameters between the placebo and naproxen groups.

CONCLUSIONS

The use of naproxen does not influence the activity of the HPA axis in healthy volunteers under basal circumstances or in response to physical stress.

Introduction

The hypothalamic–pituitary–adrenal (HPA) axis plays an important role in immune responses [1–3], and it has been suggested that a decreased response of the HPA axis, resulting in insufficient cortisol secretion, is a pathogenic factor in the development of autoimmune diseases, in particular rheumatoid arthritis (RA) [4–6]. The HPA axis is a neuroendocrine pathway that consists of the hypothalamus, the pituitary gland and the adrenal glands. In the hypothalamus, corticotrophin-releasing hormone (CRH) is secreted, which stimulates the synthesis and secretion of adrenocorticotrophic hormone (ACTH) by the pituitary gland into the circulation. ACTH induces the secretion of the glucocorticoid cortisol, which is produced by the adrenal glands. This glucocorticoid has strong anti-inflammatory properties and is vital in the organism's response to inflammatory challenge and stress [7].

In patients with RA, normal or low cortisol levels have been observed under normal circumstances or in response to stress [8–10], whereas higher levels would be expected during active inflammation. Although this might point to an insufficient HPA response in patients with RA, most studies concern patients using nonsteroidal anti-inflammatory drugs (NSAIDs), which raises the question whether NSAIDs are able to reduce the activity of the HPA axis. Only a few studies have investigated the influence of NSAIDs on the HPA axis in humans, most of which have reported decreased activity of the HPA axis after the use of NSAIDs [11–16], although unchanged [17] and increased [18] activity have also been reported. NSAIDs inhibit the cyclooxygenase (COX) enzyme, the key enzyme for the conversion of arachidonic acid to prostaglandin (PG) G_2 and PGH_2 , which are subsequently converted to a variety of eicosanoids including a number of different PGs and thromboxane. Prostaglandins have proinflammatory effects, but also play a role in many different physiological processes, including the sensation of pain, induction of fever, kidney function, wound healing, blood vessel tone, and blood clotting. Inhibition of PG production through inhibition of COX accounts for the anti-inflammatory and pain-relieving effect of NSAIDs, but also for their side-effects [19]. Two isoforms of COX have been identified, COX1 and COX2, and the latter has been found to be mostly involved in inflammation, although its role in normal physiology still has to be defined [20]. The demonstration that ACTH and cortisol response to proinflammatory cytokines are blunted by inhibiting COX suggests the involvement of PG in the neurohormonal regulation of HPA activity [21, 22].

Naproxen is a relatively selective NSAID that inhibits both COX1 and COX2 and is frequently used in the treatment of RA. Previously, we have found a reduction of plasma cortisol levels and total cortisol excretion in 24-h urine in patients with recent-onset RA, after 2 weeks' use of naproxen [23]. This decreased HPA axis activity in RA

patients could be caused by a direct or an indirect effect of naproxen on the HPA axis. In animal studies PGs have been shown to be able to activate the HPA axis [24–26]. If NSAIDs decrease the activity of the HPA axis directly by inhibition of PG production, this would also be found in healthy volunteers. If, however, in RA patients the effect of naproxen is indirect by reducing inflammation and/or pain itself, thereby reducing stress and lowering cortisol levels, this might not be found in healthy volunteers.

To investigate whether naproxen has a direct or indirect effect on the activity of the HPA axis, we performed a double-blind, randomized study in 40 healthy volunteers. The activity of the HPA axis was studied both under basal circumstances as well as after stimulation by a standardized form of stress, induced by physical exercise on a bicycle ergometer. The basal activity measurements and the exercise test were performed twice in each subject, before and after 2 weeks' treatment with placebo or naproxen. In order to detect subtle differences that might not be detected after maximal exercise, we studied the reactions of ACTH and cortisol to submaximal exercise at 80%. An intermittent schedule of exercise at a load of 50–80% was chosen, which can be more easily sustained by untrained persons than a constant work load at 80%.

Methods

Subjects

We performed a double-blind, randomized study, which was approved by the hospital's ethics committee, and all participants voluntarily signed an informed consent form. Inclusion criteria were: self-reported health, age 18–47 years, normal physical examination and electrocardiogram. Exclusion criteria were: pregnancy, abnormal medical history or physical examination, medication use (including hormonal contraceptives), endocrine diseases, psychiatric diseases and the use of >7 units of alcohol per day. Well-trained athletes were not included, as elevated basal cortisol levels and an increased cortisol response to stress have been described in these subjects [27, 28]. Forty volunteers were randomized into two groups of 20 participants each.

Medication

One group received naproxen 500 mg orally, twice daily during a period of 2 weeks, and the other group received placebo during this period. Naproxen and placebo were given in a double-blinded fashion in the form of capsules, with equal appearance and smell, manufactured by the pharmacy of the university hospital.

Investigations

After inclusion, a maximal exercise test was performed in order to determine the individual maximal power (W_{max}), from which the required load for the submaximal exercise

test of each individual could be calculated. On a separate day, basal activity of the HPA axis was determined as follows: blood was drawn at 09.00 h (fasting) and at 16.00 h for determination of plasma cortisol and ACTH levels. Urine was collected for determination of total cortisol excretion in 24 h, and on the same day salivary samples were collected every 4 h for a period of 24 h starting at 08.00 h, for the determination of the circadian rhythm of cortisol. These samples were collected by means of an absorbent swab, which was taken in the mouth for 5 min. It was then placed in a tube and kept in the refrigerator, and the next day all samples of 24 h were brought to the laboratory, where they were centrifuged and the saliva frozen until they were analysed. On the next day, a submaximal exercise test was performed as described below. Thereafter, subjects received naproxen or placebo during 2 weeks, and at the end of this period measurements of basal activity and submaximal exercise testing were repeated.

Laboratory measurements

Blood was collected in pre-chilled K3-ethylenediamine tetraacetic acid tubes and centrifuged for 10 min at 1500 *g* (4°C) within 1 h. The plasma obtained was aliquoted in polystyrene tubes containing 250 kIU mL⁻¹ plasma Trasyol® (aprotinine; Bayer, Leverkusen, Germany), frozen and stored at -20°C.

ACTH ACTH in plasma was measured by an immunoradiometric assay (IRMA) based on two polyclonal antibodies (EuroDiagnostics, Arnhem, the Netherlands). The catching antibody was directed against the C-terminal part of the ACTH molecule and coupled via a sheep antirabbit antibody to a polystyrene tube. The detecting antibody was directed against the N-terminal part of ACTH and radioiodinated. Standard curves were prepared by spiking ACTH-free plasma with ACTH₁₋₃₉ (MRC 74/555). The assay was performed as follows. Two hundred microlitres of sample or standard (0–220 pmol L⁻¹) was added to the coated tubes and subsequently iodinated ACTH antiserum (250 000 dpm 200 µL⁻¹) was added. The mixture was incubated for 24 h at room temperature. The supernatant was decanted and the tubes washed twice with 0.9% NaCl. Radioactivity in the tubes was counted using an automatic gamma-counter (1470 Wizard™; Wallac, Turku, Finland). The sensitivity of the assay was 0.5 pmol L⁻¹ and the within- and between-assay coefficients of variation of the IRMA procedure were 4.4 and 7.2%, respectively. All samples were measured in duplicate. The IRMA specifically detects ACTH₁₋₃₉. Cross-reactivity with ACTH₁₋₂₄, corticotropin-like intermediate lobe peptide (CLIP) and β-endorphin was <0.1%. Normal range at 08.00 h: 1.3–9.2 pmol L⁻¹.

Cortisol Plasma cortisol levels were measured by radioimmunoassay using an antiserum raised in rabbit against a cortisol-21-hemisuccinate-bovine serum albumin conjugate. The following cross-reactivities (expressed as % on

mass basis) were obtained: desoxy-cortisol, 3.1%; cortisol, 0.5%; dexamethason, 0.2%; prednisolone, 7.1%. Standard and samples (50 µL) were diluted 1 : 100 with ethanol/water (5/95, v/v) and corticosteroid binding globulin was denatured by incubation at 70°C for 1 h. Subsequently, tracer ([1,2,6,7-³H]cortisol) and antibody were added, and bound and free hormone were separated by dextran-coated charcoal after incubation for 18 h at 4°C. After centrifugation the supernatants were decanted and radioactivity was measured. The sensitivity of the assay was 0.02 µmol L⁻¹. The within- and between-assay coefficients of variation were 4.5 and 6.6% at 0.21 µmol L⁻¹ and 4.0 and 3.8% at 1.04 µmol L⁻¹, respectively. Normal range at 08.00 h: 0.19–0.55 µmol L⁻¹.

Maximal exercise test

The subject was seated on a bicycle ergometer (Excalibur; Lode, Groningen, the Netherlands), and electrodes were placed on the chest in order to monitor ECG. Heart rate during the test was measured by means of the polar vantage NV. A well-fitting mouthpiece was taken in the mouth by the subject in order to measure respiratory quotient (RQ), which was measured by an automated gas analysing system, the oxicon IV (Mijnhardt Oxicon Systems, Bunnik, the Netherlands). Work load was started at 20 W min⁻¹ with an increase of 20 W every minute until exhaustion. Two minutes after the end of exercise, a capillary blood sample was taken to determine capillary base excess (BE). The test was considered adequate if two of the following three criteria were met: maximal heart rate (Hf_{max}) > 220 – age ± 10, RQ > 1.0, and BE < -10.0 meq L⁻¹.

Submaximal exercise test

The subject reported to the laboratory at 09.00 h after an overnight fast, and at 09.30 h a catheter was inserted in an antecubital vein and kept patent by saline solution. Electrodes were placed on the chest in order to monitor heart rate during the test. After a 30-min rest in a supine position, the subject was seated on a bicycle ergometer. At 10.00 h exercise was started with power set at 50% of the subject's *W*_{max}. After 5 min, a 15-min period followed in which power was intermittently set at 50 and 80% of *W*_{max}, changing every 30 s. This was followed by a recovery period of 3 min at 30%, and then the subject rested in a supine position (see Figure 1). Blood samples were drawn just before exercise and at 20, 30, 40 and 60 min after start of exercise.

Statistical analysis

Results are expressed as the mean [95% confidence interval (CI)]. Statistical comparisons within groups were made with paired *t*-tests or by Wilcoxon rank sum test if data were not normally distributed. Comparisons between groups were made by unpaired *t*-test or by the Mann-Whitney test if data were not normally distributed. Because of multiple comparisons a significance level of *P* < 0.01 was applied. The areas under the curve (AUCs) were calculated

Figure 1

Schedule of submaximal exercise. ▲, insertion of venous canule; ↓, time points of blood sampling; A, work load at 50% of individual maximal workload; B, intermittent work load at 50–80%; C, workload at 30%

Table 1

Demographic variables of the study subjects and results of maximal exercise test

	Placebo (<i>n</i> = 20)	Naproxen (<i>n</i> = 20)
Age (years)	36.2 (32.6, 39.8)	37.3 (34.9, 39.7)
Male : female	7 : 13	8 : 12
Weight (kg)	73.7 (66.2, 81.1)	73.2 (68.9, 77.5)
HR ₀ (bpm)	85 (80, 91)	80 (74, 85)
HR _{max} (bpm)	187 (182, 193)	184 (180, 188)
RQ ₀	0.77 (0.74, 0.81)	0.75 (0.70, 0.80)
RQ _{max}	1.21 (1.14, 1.27)	1.22 (1.18, 1.27)
BE _{max} (mEq l ⁻¹)	-11.0 (-9.9, -12.0)	-10.9 (-9.5, -12.3)
W _{max} (W)	243 (213, 272)	250 (218, 281)

Values are mean (95% CI). HR₀, heart rate before exercise; HR_{max}, heart rate at end of maximal exercise; RQ₀, respiratory quotient before exercise; RQ_{max}, respiratory quotient at end of maximal exercise; BE_{max}, base excess at end of maximal exercise; W_{max}, maximal reached workload.

with the trapezoid method from the individual hormone levels at the various time points, using the absolute hormonal values.

Results

The demographic variables of the two study groups and the results of their maximal exercise test are shown in Table 1. There were no significant differences between the two groups. The placebo group consisted of seven men and 13 women with a mean age of 36.2 years (95% CI 32.6, 39.8). The naproxen group consisted of eight men and 12 women with a mean age of 37.3 years (95% CI 34.9, 39.7). W_{max} was 243 W (95% CI 213, 272) in the placebo group, and 250 W (95% CI 218, 281) in the naproxen group, with maximal heart rate 187 bpm (95% CI 182, 193) and 184 bpm (95% CI 180, 188), respectively.

Basal values

Before medication basal values of plasma ACTH and cortisol did not differ significantly between the groups

(Table 2). After the use of placebo or naproxen, no significant change in basal values had occurred, and no significant differences were seen between the two groups (Table 2).

Total cortisol excretion in 24-h urine before medication was 67.5 nmol 24 h⁻¹ (95% CI 54.3, 80.7) in the placebo group, and 86.8 nmol 24 h⁻¹ (95% CI 54.4, 119.2) in the naproxen group, which was not significantly different. After the use of placebo or naproxen, these values were 79.5 nmol 24 h⁻¹ (95% CI 59.5, 99.4) and 81.7 nmol 24 h⁻¹ (95% CI 64.0, 99.4), respectively, with no significant difference between the two groups, and no significant change in either of the groups after the use of placebo or medication (Table 2).

Before medication, circadian rhythm of cortisol in saliva showed a normal cortisol rhythm in both groups with a peak at 08.00 h, and AUCs in both groups did not differ significantly (*P* = 0.893, 95% CI of the difference -14.8, +16.9). After the use of placebo or naproxen, AUCs in both groups increased slightly (Figure 2), but without significant differences between the two groups (Figure 2, *P* = 0.608, 95%CI of the difference -16.9, +28.4).

Submaximal exercise test

All subjects completed both tests. A significant rise of ACTH was seen (*P* < 0.001 and *P* = 0.003 before and after placebo, respectively; *P* < 0.001 before and after naproxen) in response to physical exercise with the maximum of ACTH reached at 20 min (Figure 3A), and a significant rise of cortisol (*P* < 0.001 before and after placebo; *P* = 0.006 and 0.001 before and after naproxen, respectively), with its peak at 30 min (Figure 3B). Before medication, no significant differences were seen in AUC of either ACTH (*P* = 0.514) or cortisol (*P* = 0.652) between the groups, nor were any significant changes seen in these AUCs after the use of placebo (ACTH *P* = 0.319, cortisol *P* = 0.949) or naproxen (ACTH *P* = 0.145, cortisol *P* = 0.165) in the groups or between the groups (ACTH *P* = 0.572, cortisol *P* = 0.159).

Discussion

In healthy, non-athletic subjects, levels of plasma ACTH and cortisol, 24-h urinary cortisol excretion, and circadian cortisol rhythm in salivary samples under basal circumstances did not change after the use of placebo or naproxen. Plasma levels of ACTH and cortisol in response to physical exercise on a bicycle ergometer, which caused significant rises in ACTH and cortisol, did not change either after the use of placebo or naproxen. On the basis of these results we conclude that the use of the NSAID naproxen does not influence the activity of the HPA axis in healthy volunteers.

PGs are known to be involved in the regulation of ACTH and cortisol secretion [29, 30]. Reports in the literature about the effect of NSAIDs on the HPA axis in healthy humans have been few and have yielded conflicting

Table 2

Basal values of plasma adrenocorticotrophic hormone (ACTH), plasma cortisol and cortisol in 24-h urine collection

	Before placebo <i>n</i> = 20	Before naproxen <i>n</i> = 20	Difference	After placebo* <i>n</i> = 20	After naproxen** <i>n</i> = 20	Difference
ACTH (pmol l⁻¹)						
09.00 h	3.1 (2.0, 4.2)	2.8 (1.9, 3.7)	-0.31 (-1.67, +1.05)	3.0 (2.0, 3.9)	3.0 (2.2, 3.8)	0.04 (-1.2, +1.2)
16.00 h	2.2 (1.6, 2.7)	2.5 (1.8, 3.2)	+0.38 (-0.45, +1.20)	2.0 (1.5, 2.5)	2.3 (1.7, 2.9)	0.30 (-0.46, +1.06)
Cortisol (μmol l⁻¹)						
09.00 h	0.45 (0.39, 0.51)	0.40 (0.35, 0.44)	-0.06 (-0.12, +0.01)	0.45 (0.37, 0.52)	0.39 (0.34, 0.44)	-0.06 (-0.14, +0.03)
16.00 h	0.25 (0.21, 0.29)	0.23 (0.21, 0.25)	-0.02 (-0.07, +0.03)	0.23 (0.19, 0.27)	0.23 (0.19, 0.28)	-0.01 (-0.05, +0.06)
Cortisol urine (nmol 24 h⁻¹)	67.5 (54.3, 80.7)	86.8 (54.4, 119.2)	19.3 (-11.7, +50.4)	79.5 (59.5, 99.4)	81.7 (64.0, 99.4)	2.23 (-23.4, +27.8)

Values are mean (95% CI). Cortisol urine = total cortisol excretion in 24-h urine collection. *Difference of paired samples (mean and 95% CI) before and after placebo: ACTH: 09.00 h -0.34 (-1.20, +0.50), *P* = 0.408, 16.00 h -0.15 (-0.69, +0.38), *P* = 0.558; cortisol: 09.00 h 0.00 (-0.05, +0.05), *P* = 0.916, 16.00 h -0.03 (-0.08, +0.02), *P* = 0.273; cortisol: urine 11.1 (-11.4, +33.6), *P* = 0.309. **Difference of paired samples (mean and 95% CI) before and after naproxen: ACTH: 09.00 h 0.22 (-0.48, +0.92), *P* = 0.516, 16.00 h -0.21 (-0.64, +0.22), *P* = 0.323; cortisol: 09.00 h -0.01 (-0.05, +0.04), *P* = 0.806, 16.00 h 0.00 (-0.04, +0.04), *p* = 0.900; cortisol: urine -13.2 (-46.0, +19.5), *P* = 0.399.

Figure 2

Circadian cortisol rhythm measured in saliva samples of two groups of healthy volunteers, before and after 2 weeks treatment with placebo (*n* = 20) or naproxen (*n* = 20). The boxes represent the 95% CI. before placebo (○); after placebo (●); before NSAID (□); after NSAID (■)

results [11–15, 17, 18]. In these studies different methods of stimulating the HPA axis were used [hypoglycaemia-induced stress, stimulation with naloxone or arginine vasopressin (AVP) and water-immersion], and various NSAIDs were studied in different time courses, which perhaps accounts for the contradictory results. In a study of five normal men, indomethacin had no effect on basal ACTH and cortisol levels, but decreased the ACTH response to hypoglycaemia-induced stress [11]. Treatment with indomethacin and acetylsalicylic acid in a single dose did not alter ACTH and cortisol levels, but treatment with indomethacin for 4 days increased the response of ACTH

to hypoglycaemia while decreasing cortisol response [12], and treatment with acetylsalicylic acid for 4 days decreased ACTH response and delayed the cortisol response to hypoglycaemia [12]. An i.v. infusion of sodium salicylate in six healthy men increased ACTH and cortisol responses to hypoglycaemia [18]. Three days' treatment with indomethacin or meclofenamate in seven healthy subjects did not influence ACTH and cortisol responses to CRH [17]. A single oral dose of acetylsalicylic acid reduced ACTH and cortisol responses to the pituitary corticotrophic stimulator, AVP [14], and three doses of 500 mg acetylsalicylic acid significantly increased ACTH but not cortisol after exercise [31]. In placebo-controlled studies in 12 healthy volunteers, treatment with acetylsalicylic acid for 3 days was found to inhibit ACTH and cortisol response to physical stress induced by treadmill exercise [15], and acetylsalicylic acid for 10 days reduced morning plasma cortisol [16]. However, in contrast to our study, these studies investigated male athletes, who may respond differently from our untrained and moderately trained subjects. Patients with RA receiving daily NSAID therapy were found to have lower ACTH levels under basal circumstances than patients without NSAID therapy, but no differences in cortisol levels were found [13]. Water immersion did not change the ACTH or cortisol levels in either of the groups.

The strength of our study is that the number of subjects was larger than in any of the above-mentioned studies, and that it had a randomized, placebo-controlled design. Furthermore, study medication was given during 2 weeks, allowing also for possible slow changes of the HPA axis resetting at a new threshold. A limitation of our study is that the only NSAID tested was naproxen, and we should therefore be cautious not to generalize our conclusion to all NSAIDs. However, since naproxen is a nonselective COX inhibitor, our conclusion might hold also for more selective COX inhibitors.

Our findings in healthy volunteers suggest that the lowering of cortisol levels we found after the use of naproxen in RA patients, and the relatively low cortisol

Figure 3

(A) Plasma adrenocorticotrophic hormone (ACTH) levels of two groups of healthy volunteers during submaximal exercise test, before and after 2 weeks' treatment with placebo ($n = 20$) or naproxen ($n = 20$). The boxes represent the 95% CI. (B) Plasma cortisol levels of two groups of healthy volunteers during submaximal exercise test, before and after 2 weeks' treatment with placebo ($n = 20$) or naproxen ($n = 20$). The boxes represent the 95% CI. before placebo (—○—); after placebo (—●—); before NSAID (—□—); after NSAID (—■—)

levels in RA patients reported by others in the literature, are not the result of a direct effect of NSAIDs on the activity of the HPA axis. At least, no interaction with circadian stimulation or altered response to exercise was observed. However, responses to other 'stresses' might be reduced significantly. If ACTH responses to pain, for example, were PG sensitive the possibility of an indirect effect of the use of NSAIDs through reduction of pain and/or inflammation, resulting in lower cortisol levels, remains. However, other factors may contribute to these low cortisol levels, which may be characteristic of the disease itself, or the result of adaptive mechanisms in response to constant challenge of the HPA axis by chronic inflammation and/or pain.

We conclude that the use of naproxen does not influence the activity of the HPA axis in healthy volunteers. Further studies will be needed to investigate the effect of NSAIDs in chronic inflammatory states and in chronic pain, in order to determine its effect on the HPA axis in these circumstances, and increase our understanding of abnormalities of the HPA axis in patients with chronic inflammatory diseases.

Competing interests

None to declare.

The authors are grateful for the financial support by the Dutch League against Rheumatism ('Het Nationaal-Reumafonds') and by a 'Sandoz Rheumatology Grant', which made this work possible.

REFERENCES

- 1 Besedovsky H, Sorkin E, Keller M, Muller J. Changes in blood hormone levels during the immune response. *Proc Soc Exp Biol Med* 1975; 150: 466–70.
- 2 Bateman A, Singh A, Kral T, Solomon S. The immune–hypothalamic–pituitary–adrenal axis. *Endocr Rev* 1989; 10: 92–112.
- 3 Wilder RL. Neuroendocrine–immune system interactions and autoimmunity. *Annu Rev Immunol* 1995; 13: 307–38.
- 4 Sternberg EM, Hill JM, Chrousos GP, Kamilaris T, Listwak SJ, Gold PW, Wilder RL. Inflammatory mediator-induced hypothalamic–pituitary–adrenal axis activation is defective in streptococcal cell wall arthritis-susceptible Lewis rats. *Proc Natl Acad Sci USA* 1989; 86: 2374–8.
- 5 Sternberg EM, Young WS III, Bernardini R, Calogero AE, Chrousos GP, Gold PW, Wilder RL. A central nervous system defect in biosynthesis of corticotropin-releasing hormone is associated with susceptibility to streptococcal cell wall-induced arthritis in Lewis rats. *Proc Natl Acad Sci USA* 1989; 86: 4771–5.
- 6 Panayi GS. A defect in the neuroendocrine axis in rheumatoid arthritis: pathogenetic implications. *Clin Exp Rheumatol* 1993; 11 (Suppl. 8): S83–5.
- 7 Elenkov IJ, Chrousos GP. Stress system – organization, physiology and immunoregulation. *Neuroimmunomodulation* 2006; 13: 257–67.
- 8 Chikanza IC, Petrou P, Kingsley G, Chrousos G, Panayi GS. Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis Rheum* 1992; 35: 1281–8.
- 9 Gudbjornsson B, Skogseid B, Oberg K, Wide L, Hallgren R. Intact adrenocorticotrophic hormone secretion but impaired

- cortisol response in patients with active rheumatoid arthritis. Effect of glucocorticoids. *J Rheumatol* 1996; 23: 596–602.
- 10** Eijsbouts AM, van den Hoogen FH, Laan RF, Hermus AR, Sweep CG, van de Putte LB. Hypothalamic–pituitary–adrenal axis activity in patients with rheumatoid arthritis. *Clin Exp Rheumatol* 2005; 23: 658–64.
- 11** Beirne J, Jubiz W. Effect of indomethacin on the hypothalamic–pituitary–adrenal axis in man. *J Clin Endocrinol Metab* 1978; 47: 713–6.
- 12** Cavagnini F, Di LA, Maraschini C, Invitti C, Pinto ML. Effect of two prostaglandin synthesis inhibitors, indomethacin and acetylsalicylic acid, on plasma ACTH and cortisol levels in man. *Acta Endocrinol (Copenh)* 1979; 91: 666–73.
- 13** Hall J, Morand EF, Medbak S, Zaman M, Perry L, Goulding NJ, Maddison PJ, O'Hare JP. Abnormal hypothalamic–pituitary–adrenal axis function in rheumatoid arthritis. Effects of nonsteroidal antiinflammatory drugs and water immersion. *Arthritis Rheum* 1994; 37: 1132–7.
- 14** Nye EJ, Hockings GI, Grice JE, Torpy DJ, Walters MM, Crosbie GV, Wagenaar M, Cooper M, Jackson RV. Aspirin inhibits vasopressin-induced hypothalamic–pituitary–adrenal activity in normal humans. *J Clin Endocrinol Metab* 1997; 82: 812–7.
- 15** Di Luigi L, Guidetti L, Romanelli F, Baldari C, Conte D. Acetylsalicylic acid inhibits the pituitary response to exercise-related stress in humans. *Med Sci Sports Exerc* 2001; 33: 2029–35.
- 16** Di Luigi L, Rossi C, Sgro P, Fierro V, Romanelli F, Baldari C, Guidetti L. Do non-steroidal anti-inflammatory drugs influence the steroid hormone milieu in male athletes? *Int J Sports Med* 2007; 28: 809–14.
- 17** Zacharieva S, Wippermann M, Mucha I, Andonova K, Jankova G. Indomethacin and meclofenamate do not alter ACTH and cortisol responses to corticotropin-releasing hormone (CRH) in normal subjects. *Clin Physiol Biochem* 1993; 10: 28–32.
- 18** Halter JB, Metz SA. Sodium salicylate augments the plasma adrenocorticotropin and cortisol responses to insulin hypoglycemia in man. *J Clin Endocrinol Metab* 1982; 54: 127–20.
- 19** DuBois RN, Abramson SB, Crofford L, Gupta RA, Simon LS, van de Putte LB, Lipsky PE. Cyclooxygenase in biology and disease. *FASEB J* 1998; 12: 1063–73.
- 20** Crofford LJ, Lipsky PE, Brooks P, Abramson SB, Simon LS, van de Putte LB. Basic biology and clinical application of specific cyclooxygenase-2 inhibitors. *Arthritis Rheum* 2000; 43: 4–13.
- 21** Parsadaniantz SM, Lebeau A, Duval P, Grimaldi B, Terlain B, Kerdellhue B. Effects of the inhibition of cyclo-oxygenase 1 or 2 or 5-lipoxygenase on the activation of the hypothalamic–pituitary–adrenal axis induced by interleukin-1beta in the male rat. *J Neuroendocrinol* 2000; 12: 766–73.
- 22** Marx C, Ehrhart-Bornstein M, Scherbaum WA, Bornstein SR. Regulation of adrenocortical function by cytokines – relevance for immune-endocrine interaction. *Horm Metab Res* 1998; 30: 416–20.
- 23** Eijsbouts A, vandenHoogen F, Laan R, Hermus A, Sweep F, vandePutte L. Effect of naproxen on pituitary and adrenal function in rheumatoid arthritis. *Arthritis Rheum* 1997; 40: 1310.
- 24** Watanabe T, Morimoto A, Sakata Y, Murakami N. ACTH response induced by interleukin-1 is mediated by CRF secretion stimulated by hypothalamic PGE. *Experientia* 1990; 46: 481–4.
- 25** Navarra P, Tsagarakis S, Faria MS, Rees LH, Besser GM, Grossman AB. Interleukins-1 and -6 stimulate the release of corticotropin-releasing hormone-41 from rat hypothalamus *in vitro* via the eicosanoid cyclooxygenase pathway. *Endocrinology* 1991; 128: 37–44.
- 26** Rivier C, Vale W. Stimulatory effect of interleukin-1 on adrenocorticotropin secretion in the rat: is it modulated by prostaglandins? *Endocrinology* 1991; 129: 384–8.
- 27** Luger A, Deuster PA, Kyle SB, Gallucci WT, Montgomery LC, Gold PW, Loriaux DL, Chrousos GP. Acute hypothalamic–pituitary–adrenal responses to the stress of treadmill exercise. Physiologic adaptations to physical training. *N Engl J Med* 1987; 316: 1309–15.
- 28** Fellmann N, Coudert J, Jarrige JF, Bedu M, Denis C, Boucher D, Lacour JR. Effects of endurance training on the androgenic response to exercise in man. *Int J Sports Med* 1985; 6: 215–9.
- 29** Besedovsky HO, Del RA. Immune–neuro-endocrine interactions: facts and hypotheses. *Endocr Rev* 1996; 17: 64–102.
- 30** Straub RH, Cutolo M. Involvement of the hypothalamic–pituitary–adrenal/gonadal axis and the peripheral nervous system in rheumatoid arthritis: viewpoint based on a systemic pathogenetic role. *Arthritis Rheum* 2001; 44: 493–507.
- 31** Przybylowski J, Obodynski K, Lewicki C, Kuzniar J, Zaborniak S, Drozd S, Czarny W, Garmulewicz M. The influence of aspirin on exercise-induced changes in adrenocorticotrophic hormone (ACTH), cortisol and aldosterone (ALD) concentrations. *Eur J Appl Physiol* 2003; 89: 177–83.