

# Increased Chromogranin A Levels Indicate Sympathetic Hyperactivity in Patients with Rheumatoid Arthritis and Systemic Lupus Erythematosus

SILVIA CAPELLINO, TORSTEN LOWIN, PETER ANGELE, WERNER FALK, JOACHIM GRIFKA, and RAINER H. STRAUB

**ABSTRACT. Objective.** Sympathetic hyperactivity is an unfavorable disease consequence in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) due to an increased risk of cardiovascular events. We aimed to identify a serum marker of the sympathetic nervous system, the adrenal chromogranin A (CHGA), in order to study sympathetic hyperactivity in RA and SLE.

**Methods.** Serum levels of CHGA were measured by radioimmunoassay in healthy subjects and patients with RA and SLE. CHGA immunofluorescence was performed in synovium of patients with RA and controls with osteoarthritis (OA). CHGA levels were measured in plasma, synovial fluid, and synovium superfusate in RA and OA controls.

**Results.** In healthy subjects, systemic CHGA levels correlated positively with age and plasma nor-epinephrine, indicating the sympathetic origin ( $p < 0.01$ ). Serum CHGA levels were higher in RA and SLE than in healthy subjects ( $p < 0.001$ ), which was particularly evident in female patients. Immunofluorescence revealed double-staining of CHGA and elastase-positive neutrophils in the synovium (but not with macrophages, T cells, fibroblasts, B cells, or tyrosine hydroxylase-positive cells). Density of CHGA+ cells was higher in RA synovium compared to OA controls. In OA controls and RA, CHGA levels were similar in plasma and synovial fluid, but levels in synovial tissue superfusate were markedly lower, which indicates that most of the CHGA is of systemic adrenal origin.

**Conclusion.** Increased level of CHGA is a good marker of systemic sympathetic hyperactivity. (First Release Dec 1 2007; J Rheumatol 2008;35:91-9)

*Key Indexing Terms:*

RHEUMATOID ARTHRITIS                      OSTEOARTHRITIS                      CHROMOGRANIN A  
SYSTEMIC LUPUS ERYTHEMATOSUS                      SYMPATHETIC NERVOUS SYSTEM

Previous reports found an increased risk of atherosclerosis in patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE)<sup>1-15</sup>. The reasons for this phenomenon are currently being investigated, and it seems that the proinflammatory load is an important triggering factor<sup>13,16</sup>. A traditional risk factor is sympathetic hyperactivity, which in the form of hypertension belongs to the classical symptoms of the metabolic syndrome<sup>17,18</sup>. Such a sympathetic hyperactivity, particularly in relation to a relatively normal functioning

hypothalamic-pituitary-adrenal (HPA) axis, has recently been claimed to be an unfavorable factor in patients with RA and SLE<sup>19-24</sup>. Sympathetic marker proteins such as neuropeptide Y, a neurotransmitter of the sympathetic nerve terminal, were used in order to detect an increased sympathetic activity by a simple radioimmunoassay<sup>25</sup>. However, no further adequate serum markers were tested to substantiate these findings and to facilitate diagnosis of sympathetic hyperactivity in patients with rheumatic diseases.

Chromogranin A (CHGA) is a marker for sympatho-adrenal activity, which has been described since the 1960s<sup>26,27</sup>. The CHGA serum level is an excellent indicator of sympathetic activity as demonstrated in healthy subjects<sup>28-30</sup>. CHGA is a 438 amino acid protein located in dense-core secretory granules of neuroendocrine cells necessary for catecholamine storage, and assembly and biogenesis of vesicles (as reviewed<sup>31</sup>). The CHGA precursor gene product is post-translationally processed yielding the biologically active peptides CHGA, vasostatin, pancreastatin, and parastatin<sup>32</sup>. CHGA has also been used as a diagnostic tool in neuroendocrine tumors<sup>33</sup>. However, the presence or relevance of CHGA has not been studied in patients with RA and SLE.

*From the Department of Internal Medicine I, the Department of Trauma Surgery, and the Department of Orthopedic Surgery, University Hospital Regensburg, Regensburg, Germany.*

*Supported by the Deutsche Forschungsgemeinschaft (DFG Research Unit FOR696).*

*S. Capellino, PhD; T. Lowin, PhD; W. Falk, PhD, Professor of Medicine; R.H. Straub, MD, Professor of Experimental Medicine, Rheumatologist, Department of Internal Medicine I; P. Angele, MD, Consultant of Trauma Surgery, Department of Trauma Surgery; J. Grifka, MD, Professor of Medicine, Chair, Department of Orthopedic Surgery, University Hospital Regensburg.*

*Address reprint requests to Dr. R.H. Straub, Department of Internal Medicine I, University Medical Center, D-93042 Regensburg, Germany. E-mail: rainer.straub@klinik.uni-regensburg.de*

*Accepted for publication September 4, 2007.*

Personal non-commercial use only. The Journal of Rheumatology Copyright © 2008. All rights reserved.

The aim of our study was to determine serum CHGA in patients with RA and SLE and to compare these values with healthy subjects. Since we also found CHGA-positive (CHGA+) cells in synovial tissue of patients with RA and osteoarthritis (OA), we investigated whether local production of CHGA in the synovium might contribute to elevated serum levels in RA, and we used patients with OA as controls. In addition, we studied CHGA+ cells in the synovium to uncover the cell type producing CHGA in the local proinflammatory microenvironment.

## MATERIALS AND METHODS

**Healthy subjects and patients.** In the first study, we investigated 92 healthy subjects [mean age  $\pm$  SEM: 44.0  $\pm$  1.7 yrs (range 18–75), women/men: 38/54], and health status was thoroughly verified by means of a 33-item questionnaire. The questionnaire addressed known diseases in the past and at present, current symptoms of diseases, current medication, prior vaccination, alcohol intake, smoking habits, family history, and surgical history. The questionnaire was adapted to the SENIEUR protocol<sup>34</sup>. This first study in healthy subjects was carried out to demonstrate the interrelation between serum levels of CHGA and a typical marker of the sympathetic activity (namely norepinephrine). We wanted to show that CHGA is a good marker of sympathetic activity when measured in the serum.

In the second study, we compared healthy controls to patients with RA and SLE. This study was carried out to demonstrate serum levels of CHGA in patients with inflammatory diseases in comparison to healthy subjects. Diagnosis of RA and SLE was based on the established criteria according to the American College of Rheumatology<sup>35,36</sup>. Clinical and laboratory data for subjects in the second study are recorded in Table 1, and C-reactive protein (CRP) was measured according to standard techniques in the Department of Clinical Chemistry, University Hospital Regensburg.

In the third study, we compared patients with RA and OA (as controls) in order to investigate CHGA+ cells in synovial tissue, synovial fluid levels of CHGA, and serum levels of CHGA. This study was carried out in order to demonstrate the origin of CHGA because inflamed tissue might be a strong producer of CHGA. If CHGA is derived from the inflamed tissue, it would not

be a sign of sympathetic activity. The included patients underwent elective knee joint replacement surgery, and they were included without further selection. Clinical and laboratory data of the third study are recorded in Table 2, and erythrocyte sedimentation rate was measured by standard techniques.

All healthy subjects and patients were informed about the purpose of the study and gave written consent. The study was approved by the Ethical Committee of the University of Regensburg.

**Collection of material.** For the first and second study, healthy subjects and patients were investigated in the outpatient clinic and blood was drawn between 8:00 and 10:00 in the morning. In the third study, blood and synovial fluid were drawn during the procedure of arthroplastic surgery in the morning hours. Material was immediately centrifuged and stored at  $-80^{\circ}\text{C}$ . Synovial tissue samples were obtained immediately after opening the knee joint capsule. The preparation of the tissue for histology was as described<sup>37</sup>. Briefly, a piece of synovial tissue of up to 9 cm<sup>2</sup> was dissected. Fat tissue and tissue with a large number of blood vessels were removed. Four pieces of about 20 mm<sup>2</sup> of every patient were loaded into 4 superfusion chambers (superfusion technique see below), and 8 pieces of roughly 0.8 cm<sup>2</sup> were used for histology. Samples were fixed with 3.7% formaldehyde and then treated with sucrose 20% overnight at 4°C. Samples were then placed in protective freezing medium (Tissue Tek, Sakura Finetek, Zoeterwoude, The Netherlands) and quick-frozen floating on liquid nitrogen. All tissue samples were stored at  $-80^{\circ}\text{C}$ .

**Detection of CHGA+ cells, and double-staining with other cell types in RA and OA.** Cryosections (8  $\mu\text{m}$ ) of at least 2 different formaldehyde-fixed synovial tissue samples from each patient were air-dried for 1 h and then rehydrated in phosphate buffered saline (PBS). Unspecific binding sites were blocked with PBS containing 10% fetal calf serum, 10% bovine serum albumin, and 10% normal goat serum for 1 h at room temperature. After 10 min washing with PBS, the sections were incubated with rabbit polyclonal antibodies against CHGA (Chemicon, Hampshire, UK) and with a mouse monoclonal antibody against macrophages (CD163; DakoCytomation, Carpinteria, CA, USA), T lymphocytes (CD3; DakoCytomation), fibroblasts (prolyl-4 hydroxylase; DakoCytomation), B lymphocytes (CD19; DakoCytomation), neutrophils (Neomarkers, Fremont, CA, USA), or tyrosine hydroxylase (the key enzyme for norepinephrine production in sympathetic nerve endings; Chemicon, Temecula, CA, USA). The samples were incubated 3 h at room temperature and then washed 3  $\times$  5 min with PBS added with 0.3% Triton-

Table 1. Characteristics of healthy subjects and patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) in the second study. Data are given as means  $\pm$  SEM (%).

Characteristic	First Study	RA	Second Study	SLE
	Healthy Subjects		Healthy Subjects	
Number	50	24	37	24
Age, yrs	53.6 $\pm$ 1.5	54.0 $\pm$ 2.0	38.4 $\pm$ 2.0	36.6 $\pm$ 2.8
Women/Men	26/24	14/10	26/11	20/4
Swollen joints	NA	8.1 $\pm$ 1.6	NA	NA
Tender joints	NA	8.4 $\pm$ 1.5	NA	NA
Pain (patient)	NA	4.0 $\pm$ 0.5	NA	NA
SLEDAI	NA	NA	NA	11.7 $\pm$ 2.0
C-reactive protein, mg/l	NM	25.0 $\pm$ 6.4	NM	7.1 $\pm$ 1.2
Medication				
Prednisolone, n (%)	NA	14 (58)	NA	17 (71)
Daily prednisolone, mg	NA	6.1 $\pm$ 1.5	NA	17.1 $\pm$ 10.2
Methotrexate, n (%)	NA	13 (54)	NA	NA
Hydroxychloroquine, n (%)	NA	3 (13)	NA	1 (4)
Sulfasalazine, n (%)	NA	3 (13)	NA	NA
Azathioprine, n (%)	NA	1 (4)	NA	14 (58)
Cyclophosphamide, n (%)	NA	0 (0)	NA	0 (0)
NSAID, n (%)	NA	13 (55)	NA	3 (13)

NA: not applicable; NM: not measured; NSAID: nonsteroidal antiinflammatory drugs; SLEDAI: SLE Disease Activity Index.

Table 2. Characteristics of patients with rheumatoid arthritis (RA) and osteoarthritis (O) in the third study. Data are given as means  $\pm$  SEM (%).

Characteristic	OA	RA
Number	8	10
Age, yrs	71.8 $\pm$ 1.5	66.1 $\pm$ 2.3
Women/Men	7/1 (87/13)	7/3 (70/30)
Erythrocyte sedimentation rate, 1st h	12.8 $\pm$ 3.6	31.4 $\pm$ 13.0
Medication		
Prednisolone, n (%)	0 (0)	6 (60)
Daily prednisolone, mg	0	4.0 $\pm$ 1.2
Methotrexate, n (%)	0 (0)	2 (20)
Hydroxychloroquine, n (%)	0 (0)	0 (0)
Sulfasalazine, n (%)	0 (0)	1 (10)
Azathioprine, n (%)	0 (0)	1 (10)
Leflunomide, n (%)	0 (0)	1 (10)
Opioid, n (%)	0 (0)	2 (20)
NSAID, n (%)	2 (25)	7 (70)

NSAID: non-steroidal antiinflammatory drugs.

X100. Staining was achieved with a secondary anti-rabbit Alexa Fluor 488 antibody (Molecular Probes, Invitrogen, Eugene, OR, USA) against CHGA and with a secondary anti-mouse Alexa Fluor 546 antibody against the second staining antibody (Molecular Probes). The sections were incubated 1 h 45 min in the dark at room temperature, and then washed 3  $\times$  5 min with PBS with 0.3% Triton-X100.

Control staining was performed with unspecific rabbit IgG instead of the above-mentioned primary antibodies against CHGA. In a further analysis without double-staining, the density of CHGA+ cells was averaged from 17 randomly selected high-power fields of view (400 $\times$ ) and expressed per square millimeter.

*Superfusion of synovial tissue.* As described<sup>37</sup>, we used a microsuperfusion chamber apparatus to superfuse slices of synovial tissue with culture medium (RPMI 1640, 25 mM HEPES, without FCS, 1% Pen/Strep, 30  $\mu$ M mercaptoethanol, 0.57 mM ascorbic acid, 1.3 mM calcium, all additions from Sigma, Germany). These superfusion chambers had a volume of approximately 80  $\mu$ l. Superfusion was performed for 120 min at a temperature of 37°C and a flow rate of 66  $\mu$ l/min (1 piece per chamber, 4 chambers in parallel). Synovial tissue pieces had a standard size of 5 mm in diameter using a precision biopsy punch (Stiefel, Offenbach, Germany). Using 4 chambers, we were able to investigate 4 slices in one experiment of one synovial tissue sample. At 120 min, superfusate was collected in order to measure CHGA in a fraction of approximately 1 ml (collected over 15 min).

*Measurement of CHGA and norepinephrine.* CHGA in serum, synovial fluid, or superfusate was measured by a commercial radioimmunoassay (Asbach Medical Products, Obrigheim, Germany). The detection limit for the assay was 1.5 ng/ml. Interassay and intraassay coefficients of variation were below 10%.

The amount of norepinephrine in serum was measured by radioimmuno-metric assay (IBL, Hamburg, Germany). The high-sensitive protocol used with this kit has a detection limit of 10 pg/ml. Test samples analyzed with HPLC showed that this radioimmunometric technique produced comparable results. Interassay and intraassay coefficients of variation were below 10%.

*Presentation of the data and statistical analysis.* All data are given as mean  $\pm$  SEM. Correlations were calculated by correlation analysis (SPSS/PC, Advanced Statistics, v12.0.0, SPSS Inc., Chicago, IL, USA) and graphically demonstrated by a linear regression line. Group means were compared by the nonparametric Mann-Whitney test (SPSS).  $p < 0.05$  was the significance level.

## RESULTS

*CHGA as a marker of the sympathetic nervous system in healthy subjects.* In order to investigate whether serum CHGA

is a marker of sympathetic activity, we correlated serum CHGA with age and plasma norepinephrine. Age was shown earlier to positively correlate with an increased sympathetic activity<sup>38</sup>. In our study, age correlated positively with serum CHGA (Figure 1A). In addition, plasma norepinephrine correlated positively with serum CHGA (Figure 1B), which indicates that CHGA measured with our radioimmunoassay is a marker of sympathetic activity.

*Serum CHGA in patients with RA and SLE.* Serum CHGA was increased in all patients with RA and SLE compared to healthy subjects (Figures 2A, 2B). The subdivision into female and male patients revealed that particularly women with RA and SLE demonstrated increased levels (Figure 2). However, men also tended to have increased serum CHGA as compared to healthy men, which was most probably not significant due to the lower number of male subjects investigated (type II error; Figure 2). Interestingly, serum CHGA was higher in women with RA as compared to men with RA, which delineates a sex-specific difference (Figure 2A). This was not observed in patients with SLE (Figure 2B).

*CHGA+ cells in synovial tissue of patients with RA and OA.* In a further analysis, we wanted to clarify whether CHGA is present in inflamed tissue. It might well be that the presence of CHGA in inflamed tissue might contribute substantially to systemic CHGA levels. Indeed, CHGA+ cells existed in synovium of patients with RA and OA (Figure 3). Double immunofluorescence revealed that CHGA+ cells did not double-stain with CD163+ macrophages, CD3+ T lymphocytes, prollyl-4-hydroxylase-positive fibroblasts, CD19+ B lymphocytes, and tyrosine hydroxylase-positive cells (Figure 3). However, CHGA+ cells double-stained with elastase-positive neutrophils (Figure 3).

A further analysis of patients with RA and OA demonstrated that patients with RA had a higher synovial density of CHGA+ cells as compared to patients with OA (Figure 4A). Further, the systemic inflammation marker CRP correlated positively with synovial density of CHGA+ cells in patients with RA (Figure 4B). This indicates that these cells might be involved in systemic inflammation. The correlation was also positive in OA but it did not reach the significance level (Figure 4B).

*Comparison of CHGA levels in serum, synovial fluid, and synovium superfusate in RA and OA.* In order to investigate whether the source of CHGA might be the inflamed tissue and not the sympathetic nervous system (particularly the adrenal glands), we investigated CHGA levels in serum, synovial fluid, and synovium superfusate of the same patient. Although density of CHGA+ cells was lower in OA than in RA (Figure 4A), systemic serum levels and synovial fluid levels of CHGA were similar in both patient groups (Figure 4C, indicated as nonsignificant). If CHGA is locally produced, one would have expected a higher synovial fluid level in RA because of the markedly higher density of CHGA+ cells (Figure 4A). In addition, superfusate levels of CHGA were lower by a factor

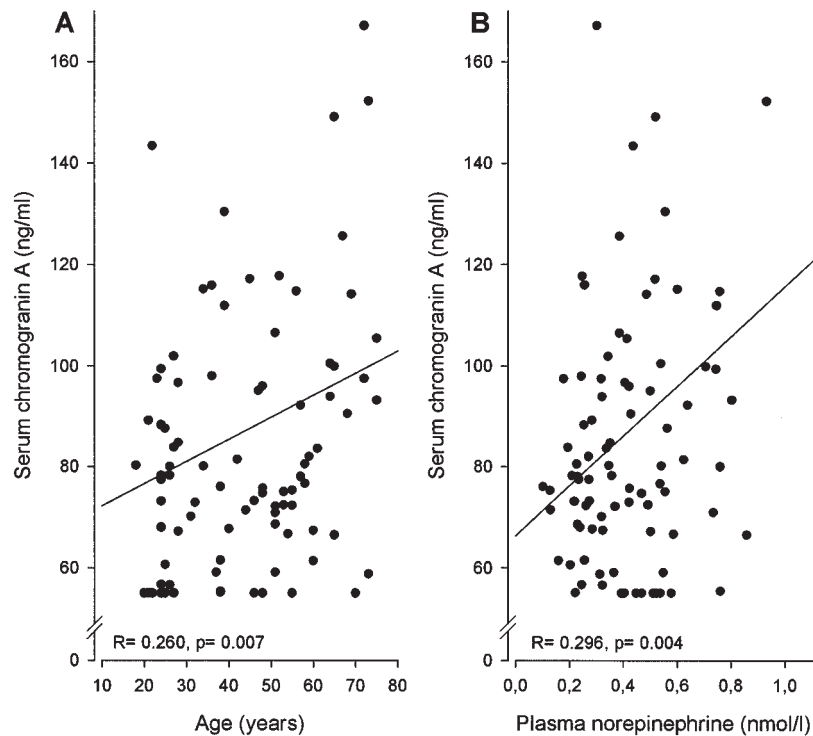


Figure 1. Correlation between age or plasma norepinephrine and serum chromogranin A. Graph depicts the linear regression line, correlation coefficient, and p value. Each symbol represents 1 healthy person.

of 100 as compared to serum and synovial fluid levels (Figure 4C). Despite the lower density of CHGA+ cells in OA than RA, no differences were observed in serum, synovial fluid, and superfusate levels between RA and OA (Figure 4C).

## DISCUSSION

Our study demonstrates elevated systemic levels of CHGA in RA and SLE compared to healthy subjects. It also demonstrates CHGA+ cells in inflamed tissue of patients with RA, but these cells are most probably not the source for systemic CHGA in these patients.

By using a different sympathetic marker molecule (CHGA), our study attempted to corroborate other studies, which demonstrated sympathetic hyperactivity in patients with inflammatory diseases<sup>19-24</sup>. In our study, we used CHGA as a marker of adrenal origin from vesicles in sympatho-adrenal cells of the adrenal medulla (as reviewed<sup>31</sup>). In our recent studies, we demonstrated sympathetic hyperactivity using the sympathetic marker neuropeptide Y<sup>25,39</sup>, which is mainly released from sympathetic nerve terminals and much less from adrenomedullary cells. Both markers from a different sympathetic source, adrenal medulla (CHGA) versus sympathetic nerve endings (neuropeptide Y), demonstrate sympathetic hyperactivity in patients with RA and SLE.

The question remained whether previously identified CHGA+ cells in inflamed tissue in RA might be a substantial source of elevated serum CHGA. If this would be the case,

sympathetic hyperactivity would not be the reason for elevated CHGA serum levels in patients with RA. In our study, we tried to answer this question by comparing material from patients with RA and OA controls.

The density of CHGA+ cells was markedly higher in RA as compared to OA controls. Thus, we thought that elevated CHGA serum levels can be derived from exaggerated local production of CHGA from synovial cells in inflamed tissue in patients with RA. However, since OA controls compared to RA demonstrated a markedly lower density of CHGA+ cells and serum levels, synovial fluid levels, and superfusate levels were similar in RA versus OA, most of the CHGA should come from another source outside the joint. As mentioned above, this source is most probably the adrenal medulla because CHGA is released from this particular organ (as reviewed<sup>31</sup>). The detection of relatively high levels of CHGA in synovial fluid in RA and OA controls is thus only a sign of spillover into the joint cavity. Nevertheless, there is little production of local CHGA as substantiated in superfusion experiments. The role of the local production remains to be determined. Double immunofluorescence revealed that at least some neutrophils expressed CHGA, which probably is responsible for vesicle biogenesis in these cells similar to that in sympatho-adrenal cells of the adrenal medulla (as reviewed<sup>31</sup>). However, most CHGA+ cells do not double-stain with neutrophils, which indicates that other presently unidentified cells produce this sympathetic marker. It must be

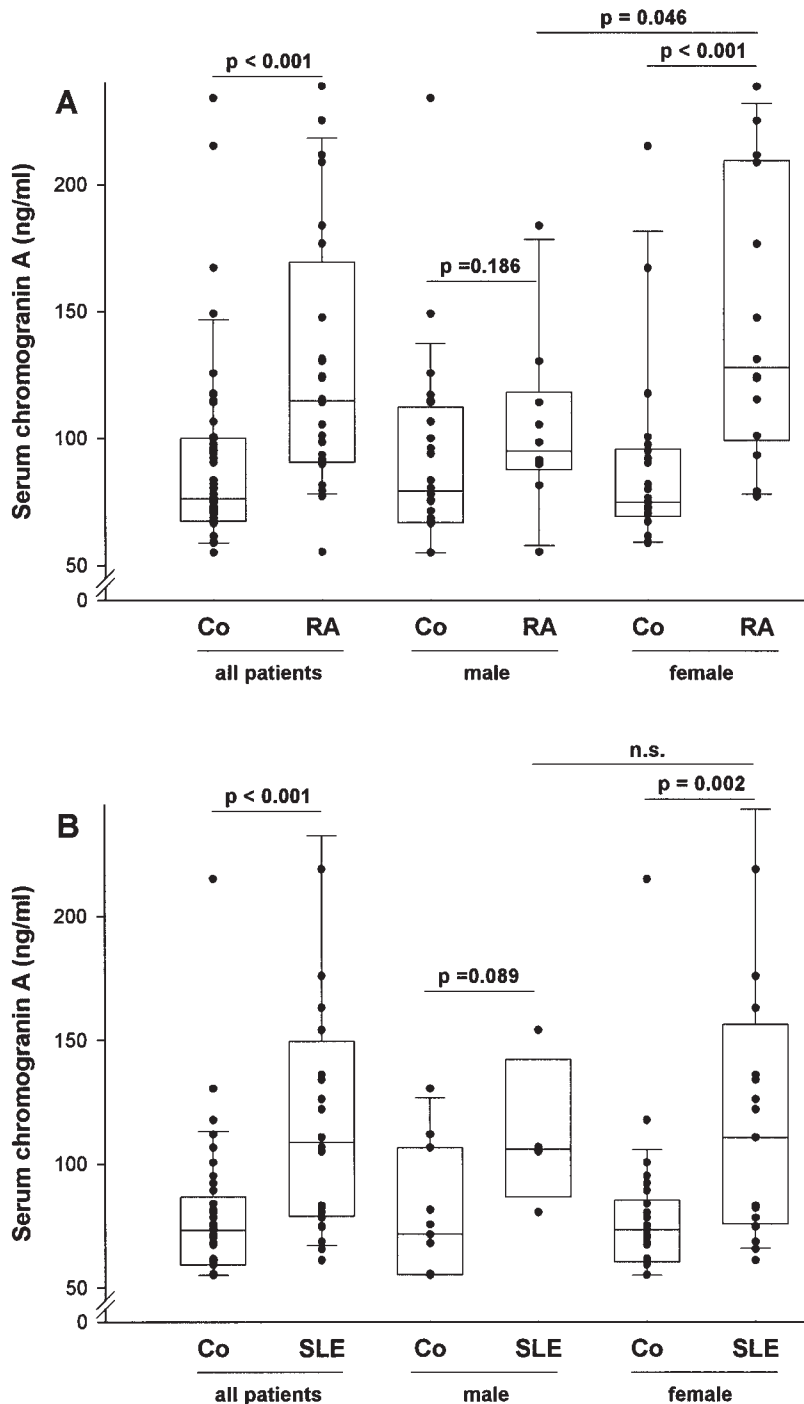


Figure 2. Serum levels of chromogranin A in patients with RA and SLE. Box plots show 10th, 25th, 50th (median), 75th, and 90th percentiles. Each symbol represents 1 person. The p values are derived from Mann-Whitney test. n.s.: not significant, Co: control.

the subject of further studies to identify the cellular source of CHGA in RA synovial tissue.

Since we did not investigate inflamed tissue of patients with SLE, we cannot answer whether or not CHGA is derived from cells in inflamed tissue in these patients. However, since we know that patients with SLE have elevated neuropeptide Y

serum levels, and thus an elevated sympathetic activity, we speculate that elevated serum CHGA levels in these patients are similarly derived from the adrenal medulla and not from inflamed tissue. We suggest that this needs to be substantiated by investigation of inflamed tissue of patients with SLE.

At this point the question arises why the sympathetic nerv-

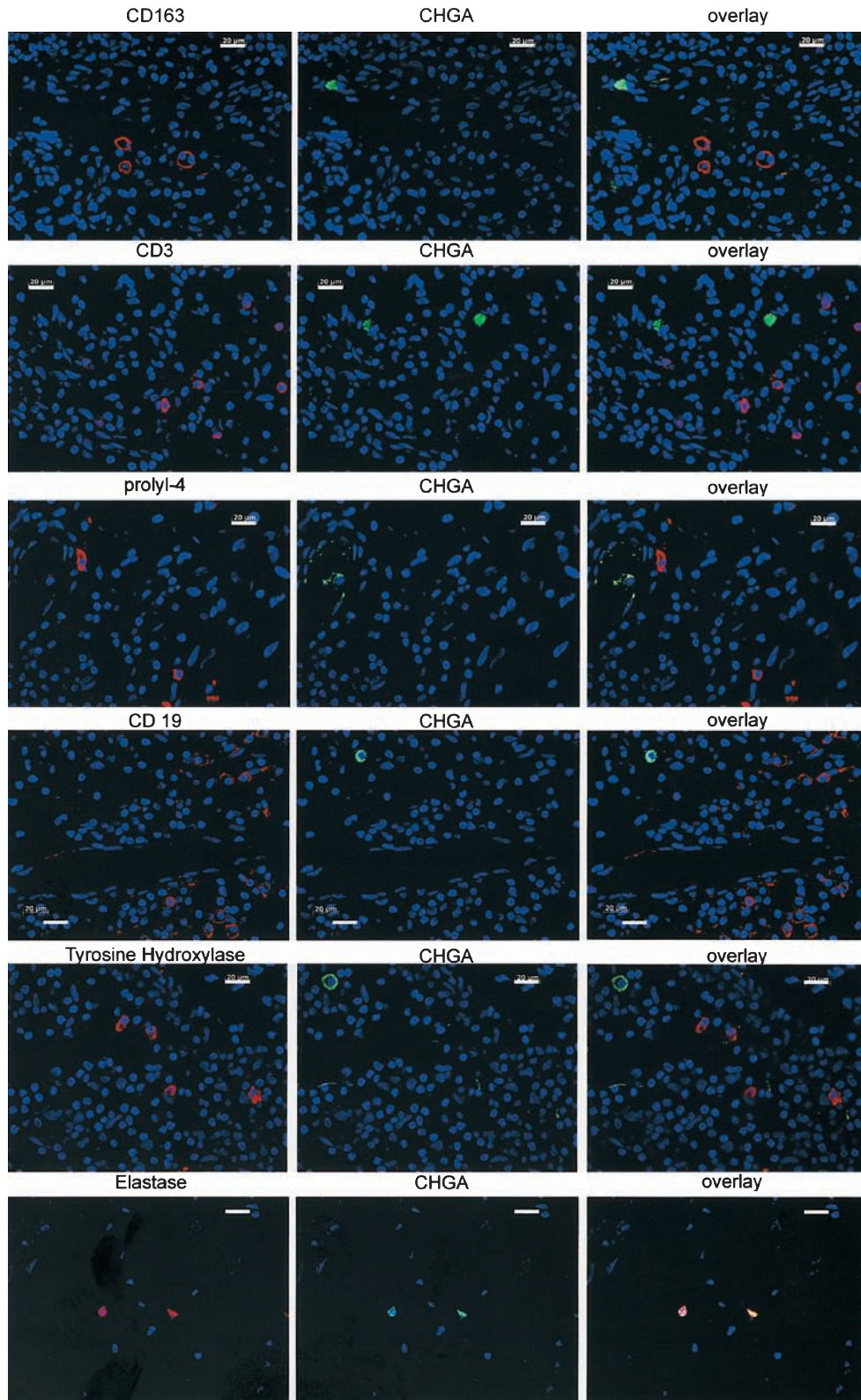


Figure 3. Double-immunohistochemistry of chromogranin A (CHGA)-positive cells and various synovial cells. In the left column, cells positive for CD163+ macrophages, CD3+ T lymphocytes, prolyl-4-positive fibroblasts, CD19+ B lymphocytes, tyrosine hydroxylase-positive cells, and neutrophil elastase are shown in the tissue of a patient with RA. In the middle column, CHGA+ cells of the same patient and the same synovial high-power field are shown. The right column shows the overlay of the 2 images to the left. Magnification 400 $\times$ . Bar = 20  $\mu$ m.

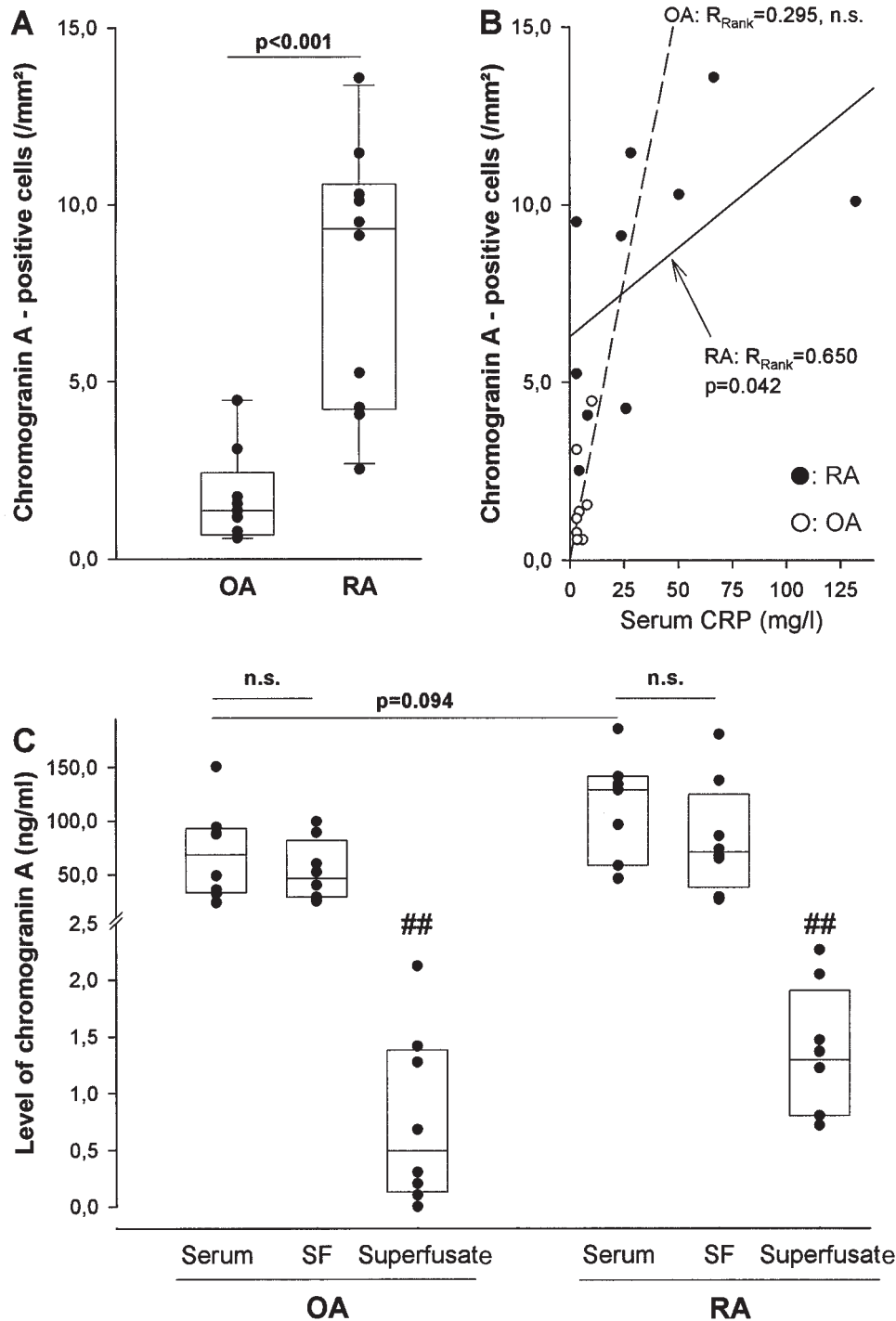


Figure 4. Density of chromogranin A (CHGA)-positive cells in synovium and CHGA levels in different body fluids. A. Density of CHGA-positive cells in OA versus RA. B. Correlation between serum level of CRP and density of CHGA-positive cells in RA and OA. The rank correlation coefficient and p value are given. C. Levels of CHGA in different body fluids of the same patients with OA and RA. Box plots show 10th, 25th, 50th (median), 75th, and 90th percentiles. Each symbol represents 1 person. The p values are derived from Mann-Whitney test or Spearman rank correlation analysis. ##  $p < 0.001$  vs serum and synovial fluid levels. n.s.: not significant; SF: synovial fluid.

ous system is activated in these patients. Sympathetic hyperactivity is found in the presence of a relatively low activity of the HPA axis, because serum cortisol levels are normal<sup>25</sup>.

Coupling of the sympathetic nervous system and HPA axis is important to support the  $\beta$ -adrenergic and glucocorticoid receptor pathways<sup>40-47</sup>. This leads to stronger cooperative

effects than using one system alone. Cooperative activity of both axes is observed in asthmatics when these patients use local glucocorticoids and local  $\beta_2$ -adrenergic agents<sup>48,49</sup>. Cooperation increases the bronchodilatory effect of each substance alone. A similar cooperativity can be observed in patients with septic shock<sup>50</sup>. In septic shock, combined treatment with norepinephrine and cortisol leads to improved effects on circulation, blood pressure, and glucose allocation. In patients with chronic inflammatory diseases, a relative loss of HPA axis hormones in relation to proinflammatory cytokines may lead to deficient vasopressive activity of sympathetic neurotransmitters and reduced glucose allocation, which may consequently lead to upregulation of the sympathetic tone. From this point of view, sympathetic hyperactivity is the consequence of an inadequate activity of the HPA axis. The activity of the sympathetic nervous system is upregulated in order to sustain important bodily functions such as glucose allocation and systemic circulation.

By using an alternative sympathetic marker molecule (CHGA), our study confirms sympathetic hyperactivity in patients with RA, and possibly also in SLE. We confirm sympathetic hyperactivity, which is an unwanted symptom because it is an important risk factor for premature atherosclerosis in patients with chronic inflammatory diseases.

## REFERENCES

- Meller J, Conde CA, Deppisch LM, Donoso E, Dack S. Myocardial infarction due to coronary atherosclerosis in three young adults with systemic lupus erythematosus. *Am J Cardiol* 1975;35:309-14.
- Urowitz MB, Bookman AA, Koehler BE, Gordon DA, Smythe HA, Ogryzlo MA. The bimodal mortality pattern of systemic lupus erythematosus. *Am J Med* 1976;60:221-5.
- Fukumoto S, Tsumagari T, Kinjo M, Tanaka K. Coronary atherosclerosis in patients with systemic lupus erythematosus at autopsy. *Acta Pathol Jpn* 1987;37:1-9.
- Mandell BF. Cardiovascular involvement in systemic lupus erythematosus. *Semin Arthritis Rheum* 1987;17:126-41.
- Farhey Y, Hess EV. Accelerated atherosclerosis and coronary disease in SLE. *Lupus* 1997;6:572-7.
- George J, Harats D, Gilburd B, Levy Y, Langevitz P, Shoenfeld Y. Atherosclerosis-related markers in systemic lupus erythematosus patients: the role of humoral immunity in enhanced atherogenesis. *Lupus* 1999;8:220-6.
- Manzi S, Selzer F, Sutton-Tyrrell K, et al. Prevalence and risk factors of carotid plaque in women with systemic lupus erythematosus. *Arthritis Rheum* 1999;42:51-60.
- Bruce IN, Gladman DD, Urowitz MB. Premature atherosclerosis in systemic lupus erythematosus. *Rheum Dis Clin North Am* 2000;26:257-78.
- del Rincon ID, Williams K, Stern MP, Freeman GL, Escalante A. High incidence of cardiovascular events in a rheumatoid arthritis cohort not explained by traditional cardiac risk factors. *Arthritis Rheum* 2001;44:2737-45.
- Kumeda Y, Inaba M, Goto H, et al. Increased thickness of the arterial intima-media detected by ultrasonography in patients with rheumatoid arthritis. *Arthritis Rheum* 2002;46:1489-97.
- Park YB, Ahn CW, Choi HK, et al. Atherosclerosis in rheumatoid arthritis: morphologic evidence obtained by carotid ultrasound. *Arthritis Rheum* 2002;46:1714-9.
- Rodriguez G, Sulli A, Cutolo M, Vitali P, Nobili F. Carotid atherosclerosis in patients with rheumatoid arthritis: a preliminary case-control study. *Ann NY Acad Sci* 2002;966:478-82.
- Doria A, Sherer Y, Meroni PL, Shoenfeld Y. Inflammation and accelerated atherosclerosis: basic mechanisms. *Rheum Dis Clin North Am* 2005;31:355-62.
- Maradit-Kremers H, Crowson CS, Nicola PJ, et al. Increased unrecognized coronary heart disease and sudden deaths in rheumatoid arthritis: a population-based cohort study. *Arthritis Rheum* 2005;52:402-11.
- Maradit-Kremers H, Nicola PJ, Crowson CS, Ballman KV, Gabriel SE. Cardiovascular death in rheumatoid arthritis: a population-based study. *Arthritis Rheum* 2005;52:722-32.
- Hurlimann D, Forster A, Noll G, et al. Anti-tumor necrosis factor-alpha treatment improves endothelial function in patients with rheumatoid arthritis. *Circulation* 2002;106:2184-7.
- Grassi G, Dell'Oro R, Quarti-Trevano F, et al. Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. *Diabetologia* 2005;48:1359-65.
- Brunner EJ, Hemingway H, Walker BR, et al. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: nested case-control study. *Circulation* 2002;106:2659-65.
- Straub RH, Glück T, Zeuner M, Schölmerich J, Lang B. Association of pupillary parasympathetic hyperreflexia and systemic inflammation in patients with systemic lupus erythematosus. *Br J Rheumatol* 1998;37:665-70.
- Glück T, Oertel M, Reber T, Zietz B, Schölmerich J, Straub RH. Altered function of the hypothalamic stress axes in patients with moderately active systemic lupus erythematosus. I. The hypothalamus-autonomic nervous system axis. *J Rheumatol* 2000;27:903-10.
- Kuis W, de Jong-de Vos van Steenwijk C, Sinnema G, et al. The autonomic nervous system and the immune system in juvenile rheumatoid arthritis. *Brain Behav Immun* 1996;10:387-98.
- Perry F, Heller PH, Kamiya J, Levine JD. Altered autonomic function in patients with arthritis or with chronic myofascial pain. *Pain* 1989;39:77-84.
- Nakajima A, Sendo W, Tsutsumino M, et al. Acute sympathetic hyperfunction in overlapping syndromes of systemic lupus erythematosus and polymyositis. *J Rheumatol* 1998;25:1638-41.
- Dekkers JC, Geenen R, Godaert GL, Bijlsma JW, van Doornen LJ. Elevated sympathetic nervous system activity in patients with recently diagnosed rheumatoid arthritis with active disease. *Clin Exp Rheumatol* 2004;22:63-70.
- Härle P, Straub RH, Wiest R, et al. Increase of sympathetic outflow measured by NPY and decrease of the hypothalamic-pituitary-adrenal axis tone in patients with SLE and RA — Another example of uncoupling of response systems. *Ann Rheum Dis* 2005;65:51-6.
- Hopwood D. An immunohistochemical study of the adrenal medulla of the ox. A comparison of antibodies against whole ox chromaffin granules and ox chromogranin A. *Histochemie* 1968;13:323-30.
- De Potter WP, De Schaepdryver AF, Smith AD. Release of chromogranin A and dopamine-beta-hydroxylase from adrenergic nerves during nerve stimulation. *Acta Physiol Scand Suppl* 1970;357:8.
- Cryer PE, Wortsman J, Shah SD, Nowak RM, Deftos LJ. Plasma chromogranin A as a marker of sympathochromaffin activity in humans. *Am J Physiol* 1991;260:E243-6.
- Takiyuddin MA, Cervenka JH, Pandian MR, Stuenkel CA, Neumann HP, O'Connor DT. Neuroendocrine sources of chromogranin-A in normal man: clues from selective stimulation of endocrine glands. *J Clin Endocrinol Metab* 1990;71:360-9.
- O'Connor DT, Bernstein KN. Radioimmunoassay of chromogranin A in plasma as a measure of exocytotic sympathoadrenal activity in normal subjects and patients with pheochromocytoma. *N Engl J*



- Med 1984;311:764-70.
31. Day R, Gorr SU. Secretory granule biogenesis and chromogranin A: master gene, on/off switch or assembly factor? *Trends Endocrinol Metab* 2003;14:10-3.
  32. HENDY GN, BEVAN S, MATTEI MG, MOULAND AJ. Chromogranin A. *Clin Invest Med* 1995;18:47-65.
  33. Ferrari L, Seregini E, Bajetta E, Martinetti A, Bombardieri E. The biological characteristics of chromogranin A and its role as a circulating marker in neuroendocrine tumours. *Anticancer Res* 1999;19:3415-27.
  34. Straub RH, Konecna L, Hrach S, et al. Serum dehydroepiandrosterone (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and DHEA inhibits IL-6 secretion from mononuclear cells in man in vitro: possible link between endocrinosenescence and immunosenescence. *J Clin Endocrinol Metab* 1998;83:2012-7.
  35. Arnett FC, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;31:315-24.
  36. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271-7.
  37. Miller LE, Weidler C, Falk W, et al. Increased prevalence of semaphorin 3C, a repellent of sympathetic nerve fibers, in the synovial tissue of patients with rheumatoid arthritis. *Arthritis Rheum* 2004;50:1156-63.
  38. Straub RH, Cutolo M, Zietz B, Schölmerich J. The process of aging changes the interplay of the immune, endocrine and nervous systems. *Mech Ageing Dev* 2001;122:1591-611.
  39. Straub RH, Herfarth H, Falk W, Andus T, Schölmerich J. Uncoupling of the sympathetic nervous system and the hypothalamic-pituitary-adrenal axis in inflammatory bowel disease? *J Neuroimmunol* 2002;126:116-25.
  40. Oikarinen J, Hamalainen L, Oikarinen A. Modulation of glucocorticoid receptor activity by cyclic nucleotides and its implications on the regulation of human skin fibroblast growth and protein synthesis. *Biochim Biophys Acta* 1984;799:158-65.
  41. Gruol DJ, Campbell NF, Bourgeois S. Cyclic AMP-dependent protein kinase promotes glucocorticoid receptor function. *J Biol Chem* 1986;261:4909-14.
  42. Nakada MT, Stadel JM, Poksay KS, Crooke ST. Glucocorticoid regulation of beta-adrenergic receptors in 3T3-L1 preadipocytes. *Mol Pharmacol* 1987;31:377-84.
  43. Dong Y, Aronsson M, Gustafsson JA, Okret S. The mechanism of cAMP-induced glucocorticoid receptor expression. Correlation to cellular glucocorticoid response. *J Biol Chem* 1989;264:13679-83.
  44. DiBattista JA, Martel-Pelletier J, Cloutier JM, Pelletier JP. Modulation of glucocorticoid receptor expression in human articular chondrocytes by cAMP and prostaglandins. *J Rheumatol* 1991;18 Suppl 27:102-5.
  45. Korn SH, Wouters EF, Wesseling G, Arends JW, Thunnissen FB. Interaction between glucocorticoids and beta 2-agonists: alpha and beta glucocorticoid-receptor mRNA expression in human bronchial epithelial cells. *Biochem Pharmacol* 1998;56:1561-9.
  46. Eickelberg O, Roth M, Lorx R, et al. Ligand-independent activation of the glucocorticoid receptor by beta 2-adrenergic receptor agonists in primary human lung fibroblasts and vascular smooth muscle cells. *J Biol Chem* 1999;274:1005-10.
  47. Schmidt P, Holsboer F, Spengler D. beta(2)-adrenergic receptors potentiate glucocorticoid receptor transactivation via g protein beta-gamma-subunits and the phosphoinositide 3-kinase pathway. *Mol Endocrinol* 2001;15:553-64.
  48. Motulsky HJ, Insel PA. Adrenergic receptors in man: direct identification, physiologic regulation, and clinical alterations. *N Engl J Med* 1982;307:18-29.
  49. Pauwels RA, Lofdahl CG, Postma DS, et al. Effect of inhaled formoterol and budesonide on exacerbations of asthma. Formoterol and Corticosteroids Establishing Therapy (FACET) International Study Group. *N Engl J Med* 1997;337:1405-11.
  50. Briegel J, Forst H, Haller M, et al. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999;27:723-32.