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EXPERIMENTAL

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β-adrenergic receptor-dependent and -independent stimulation of adenylate cyclase is impaired during severe sepsis in humans

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Abstract *Objectives:* a) To investigate the functional consequences of sepsis on the β-adrenergic signal transduction in human circulating lymphocytes; b) to appreciate sepsis-associated catecholamine and cytokine release.
Design: Experimental, comparative study.
Setting: Research laboratory in a university hospital.
Subjects: Healthy controls ($n = 10$); critically ill patients who were not septic ($n = 7$); septic patients with severe sepsis or septic shock ($n = 11$).
Measurements and main results: Experiments were carried out using freshly isolated peripheral blood mononuclear cells (PBMC). We measured β-adrenergic receptor (βAR) number and affinity, and intracellular cAMP content at baseline and after the pharmacological stimulation of each component of the β-adrenergic complex: βAR with isoproterenol, Gs-protein with sodium fluoride (NaF), adenylate cyclase with forskolin. Catecholamine (adrenaline, noradrenaline) and cytokine (TNFα, IL-1α, IL-1β, IL-6) serum levels were measured. In both septic and non-septic patients we observed a similar 40 % down-regulation of βARs compared to controls, and a reduced basal and isoproterenol-stimulated cAMP accumulation ($p < 0.05$). The cAMP production elicited by NaF or forskolin was lower in septic patients than in the controls ($p < 0.01$). Forskolin-stimulated cAMP accumulation was significantly lower in septic patients than it was in non-septic ones ($p < 0.001$). Catecholamine serum concentrations were increased in the two patient groups without any significant difference. Elevated cytokine serum levels were detected in 45 % of the septic patients (versus 14 % of non-septic patients $p < 0.05$).
Conclusions: Patients presenting with severe sepsis or septic shock have extended postreceptor defects of the β-adrenergic signal transduction. This finding suggests a heterologous desensitization of adenylate cyclase stimulation.

Key words Adenylate cyclase · Cyclic adenosine monophosphate · β-adrenergic receptor · Lymphocyte · Cytokine · Sepsis

Introduction

The so-called "2nd messenger" – cyclic adenosine monophosphate (cAMP) – is synthetized from adenosine triphosphate (ATP) by the membranous enzyme adenylate cyclase. The intracellular level of cAMP regulates the function of protein kinase A and the subsequent phosphorylation reactions control a number of physiological functions, particularly in cardiovascular, metabolic and immune systems. The β-adrenergic re-

ceptor (βAR) system includes a membrane-bound receptor, regulatory G-proteins and adenylate cyclase and β-adrenergic stimulation increases intracellular cAMP content. Abnormal β-adrenergic signaling in myocardium [1, 2] or circulating lymphocytes [3] has been reported in several models of endotoxic shock. Although the mechanisms for alteration of the transduction pathway remain doubtful, catecholamine overload and/or host inflammatory mediators are supposed to participate in the impairment of βAR signaling [3]. Precisely, cytokine, as tumor necrosis factor (TNFα), has been demonstrated to mediate the uncoupling of the βAR and adenylate cyclase in cultured cardiac myocytes [4]. In human septic shock, data indicate that myocardial hyporesponsiveness to catecholamines is associated with a decreased stimulation of cAMP production in response to sodium fluoride (NaF), a compound that directly activates Gs transducing proteins [5]. Paradoxically, the functionality of adenylate cyclase was not addressed in these human investigations and data are lacking concerning the potential influence of sepsis on the ability of adenylate cyclase to increase cAMP content when stimulated with βAR-dependent or -independent agents.

The aims of the study were as follows: a) to analyse the functional consequences of severe sepsis at various levels of the lymphocyte β-adrenergic transduction pathway by measuring, in two groups of septic and non-septic critically ill patients, the number and the affinity of βARs and the intracellular cAMP accumulation at baseline and after pharmacological stimulation of each step of the transmembrane transduction process (i.e., βAR, G-protein, adenylate cyclase); b) to characterize the two patient populations in terms of catecholamine and cytokine serum concentrations. All experiments were carried out using peripheral blood mononuclear cells (PBMC).

Patients and methods

Study patients

Three groups of subjects were investigated. A healthy control group (HC) consisted of ten volunteers (6 men and 4 women). A septic group (S) comprising 11 consecutive patients (8 men and 3 women) who were admitted onto the ICU with diseases fulfilling the definitions of severe sepsis or septic shock [6]. A third "non-septic" group (NS) included seven patients presenting with diseases not related to sepsis. Patients with chronic heart failure due to valvular or ischemic diseases and those receiving β-blocking agents were excluded from the study. PBMC were prepared from each subject for the following studies: measurement of βAR density and affinity, and evaluation of basal and stimulated intracellular cAMP accumulation. Plasma catecholamine levels and plasma levels of various cytokines (TNFα, IL-1α, IL-1β, IL-6) were determined in the same blood sample. This protocol was approved by the institutional Human Investigation Committee and informed consent was obtained from each patient or next of kin.

PBMC preparation

PBMC were isolated from blood according to the method of Boyüm [7]. Fresh whole blood (40 ml on 0.129 M sodium citrate) was diluted with an equal volume of 10 mM phosphate-buffered saline (PBS) containing 120 mM NaCl, 2.7 mM KCl. Aliquots of 20 ml were carefully layered onto 10 ml of Ficoll-hypaque and then centrifuged (500 × g, 30 min, 18 °C). The opaque interface mononuclear cell layer (containing approximately 90 % lymphocytes and 10 % monocytes) was removed and washed with PBS. After centrifugation (350 × g, 10 min 4 °C), the pellet was resuspended in 20 ml ice-cold HHA buffer (Hank's solution without phenol red containing 20 mM *Hep*es and 1 mM L-ascorbic acid, pH 7.4 at 25 °C). After centrifugation (225 × g, 10 min, 4 °C) the pellet was resuspended in 4 ml of ice-cold HHA buffer for cell-counting.

cAMP accumulation in PBMC

Baseline levels of intracellular cAMP were measured as well as cAMP accumulation following activation of βAR, Gs-protein or adenylate cyclase with isoproterenol, NaF and forskolin, respectively.

Triplicate cell samples (10^6 cells) were incubated for 10 min at 37 °C in a light-protected shaking water bath in a final volume of 500 µl of HHA buffer containing 0.5 mM 3-isobutyl-1-methylxanthine and various concentrations of (l)-isoproterenol (10^{-9} M, 10^{-7} M, 10^{-5} M) or 10^{-1} M NaF or 5.10^{-5} M forskolin. Inhibition of isoproterenol-induced adenylate cyclase stimulation was assayed in the presence of propranolol at a final concentration of 2.10^{-5} M. All compounds were from Sigma Chemical Co. (St. Louis, MO). The reaction was stopped by plunging the reaction tubes into boiling water for 5 min. The tubes were centrifuged (2500 × g, 10 min, 4 °C) and 50 µl of supernatant was collected for the cAMP assay which was based on a competitive protein-binding method (Amersham Corp., Arlington Heights, IL). The accumulation of cAMP was expressed as the number of pmol accumulated in 10^6 cells. The detection threshold of the method was 3 pmol/10^6 cells. When the cAMP level was undetectable in individuals, the value of the detection threshold was utilized to calculate the mean value of cAMP cellular content for patient groups.

β-adrenergic receptor binding assay

Aliquots of 10^6 cells were incubated in triplicate for 60 min at 37 °C in a final volume of 1 ml of HHA buffer containing 0.1 % bovine serum albumin and increasing concentrations (between 3.5 pm and 120 pM) of 3-[^{125}I] iodocyanopindolol (^{125}I-ICYP; 2080 Ci/mmol, Amersham, UK). The reactions were stopped by filtration on GF-F glass filters (Whatman Inc., Clifton, NJ) followed by four rapid washes with 4 ml of ice-cold PBS. The radioactivity of filters was counted in a gamma-counter (Compugamma 1282, LBK Wallac, Turku, Finland). Non-specific binding was determined in the presence of 1 µM (l)-propranolol. Binding data were analysed by the method of Scatchard [8] to determine the maximal number of binding sites (B_{max}) and the equilibrium dissociation constant (K_d) of ^{125}I-ICYP.

Serum levels of catecholamines and cytokines

Catecholamine levels were measured in triplicate using an HPLC-electrochemical method. Normal values in subjects, at rest for at least 15 min, were within the range of 1–2 nmol/l for noradrena-

Table 1 Patient clinical and microbiological data (Group: *(NS)* non-septic, *(S)* septic; *MV:* presence *(Y)* /absence *(N)* of mechanical ventilation; *Dp* dopamine, *Db* dobutamine, *Na* noradrenaline; Blood culture: positive *(P)* /negative *(N);* Shock: presence *(Y)* /absence *(N)* of shock)

No.	Group	Age	Primary diagnosis	Infecting organism	Blood culture	Shock	Circulatory support	MV	Outcome
1	NS	52	Heart failure			Y	Dp Db (5)*	Y	alive
2	NS	53	Digestive bleeding			Y		N	alive
3	NS	50	Digestive bleeding			N		N	alive
4	NS	23	Status asthmaticus			N	β2 agonist (1)	N	alive
5	NS	49	Toxic renal failure			N	Dp (3)	N	alive
6	NS	71	Status epilepticus			N		Y	alive
7	NS	28	Heroin overdose			Y		N	alive
8	S	54	Cholecystitis		N	Y	Dp Db Na (8)	Y	alive
9	S	71	Pneumonia	A. baumanii	N	Y	Dp Db (5)	Y	dead
10	S	79	Pyelonephritis	E. Coli	P	Y	Dp Db Na (1)	Y	dead
11	S	49	Enteric fever	S. enteritidis	P	Y	Dp Db (4)	N	dead
12	S	27	Neck cellulitis		N	N		N	alive
13	S	44	Leg cellulitis	Streptococcus	N	N		N	alive
14	S	69	Pneumonia	A. baumanii	N	Y	Dp (4)	Y	alive
15	S	35	Pneumonia	S. pneumoniae	P	Y	Db (1)	N	alive
16	S	35	Pneumonia	S. pneumoniae	N	N		N	alive
17	S	77	Pneumonia	K. pneumoniae	N	Y	Dp Db (6)	Y	dead
18	S	67	Aspiration pneumonia		N	Y	Dp (3)	N	alive

* Number of days of therapy before study

line, and 0.2–0.5 nmol/l for adrenaline. Cytokines levels (TNFα, IL-1α, IL-1β, IL-6) were measured using an enzyme-linked immunoassay. The detection limits were 100 ng/l for TNFα, 60 ng/l for IL-1α, 20 ng/l for IL-1β, 100 ng/l for IL-6.

Statistical analysis

The results are presented as means ± SD. The chi-squared test was used to analyse the distribution of qualitative characteristics among the patient groups. Correlation between catecholamine serum level and βAR number was performed using simple regression analysis. The comparison of mean values between the three groups was performed using ANOVA and a probability value of less than 0.05 was considered significant. Specific differences were identified by an unpaired Student's *t*-test corrected for multiple comparisons with the Bonferroni inequality adjustment. To ensure that the overall risk of committing a type I error was held at 0.05, the critical value of 0.0167 (risk divided by the number of inter-group comparisons) was used as the significance threshold for individual comparisons (Statview 4.5 statistical software, Abacus concept, Berkeley, CA).

Results

Characteristics of patients

The main clinical and bacteriological features of patients are listed in Table 1. Of the 11 septic patients, 8 presented with ongoing circulatory failure at the time of blood sampling (shock symptoms and/or need for hemodynamic support with vasopressors and inotropic drugs). Of the seven non-septic patients, three patients presented with circulatory shock. There was no significant difference between S and NS groups, respectively, for age (55 ± 18 years vs 47 ± 16 years) and gravity score (Simplified Acute Physiologic Score II [9] was 43 ± 15 vs 34 ± 10). The mortality rate was 36% for S group whereas all non-septic patients were discharged alive from the ICU. The HC group was significantly younger than the S group (35 ± 7 years, $p < 0.05$).

Treatment with β-adrenergic agonists

β-adrenergic agonist treatment was required in three of the seven non-septic patients and in 8 of the 11 septic patients. In S group the mean doses were 1.9 ± 1.9 μg/kg per min for dopamine and 5.7 ± 7.3 μg/kg per min for dobutamine, and noradrenaline was added to the combination dopamine-dobutamine in two patients. The delay between admission and blood sampling for the experiments was similar in S and NS groups (2.8 ± 2.2 vs 2.4 ± 1.4 days, respectively). The duration of catecholamine administration before PBMC collection for individual patients is shown in Table 1.

Density and affinity of β-adrenergic receptors on PBMC

The data are presented in Fig. 1. The correlation value of Scatchard plots for all individual experiments ranged between 0.73 and 0.98 and was greater than 0.90 in more than 75% of the experiments. In the HC group

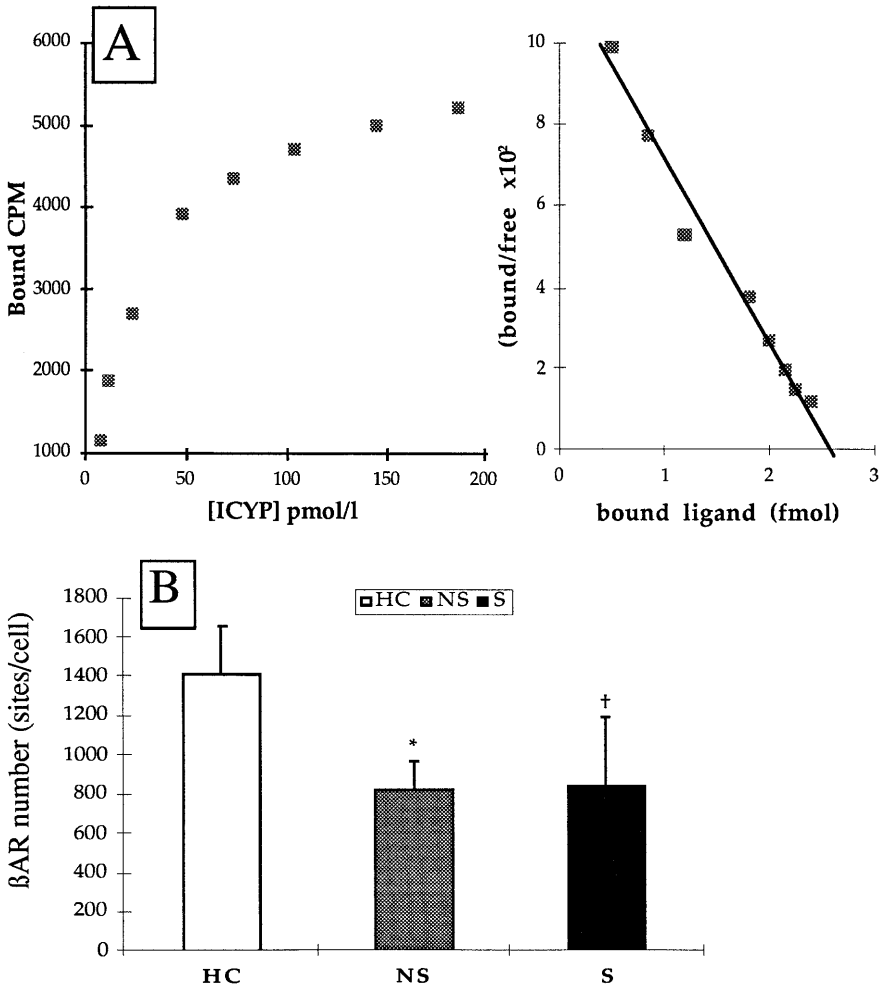

Fig. 1 Density of β-adrenergic receptors on PBMC. **A** Saturation binding isotherm of ICYP and Scatchard representation on intact PBMC of one patient. This figure is representative of all other binding studies on patients and healthy controls PBMC. **B** ICYP binding sites on patients and healthy controls PBMC (mean values ± SD).
* $p < 0.01$ between non-septic group and healthy control group
† $p < 0.01$ between septic group and healthy control group

βAR density was 1412 ± 249 sites/cell and the mean K_d for ^{125}I-ICYP was 25 ± 18 pm. The number of βARs was significantly decreased in all patients by approximately 40%, but no difference was found between the S and NS groups. The βAR affinity for ^{125}I-ICYP was not different between the groups (K_d values were 25.1 ± 17.7 pmol/l, 29.8 ± 8.4 pmol/l and 30.5 ± 22.2 pmol/l for healthy controls, non-septic patients and septic patients, respectively).

cAMP accumulation in PBMC

Basal and stimulated cAMP accumulation data are presented in Fig. 2. In the HC group, basal intracellular cAMP increased in a dose-dependent manner after βAR stimulation with the agonist isoproterenol. Co-incubation of cells with the β-adrenergic antagonist propranolol completely prevented the isoproterenol-stimulated cAMP accumulation. Direct stimulation of Gs proteins with NaF and activation of adenylate cyclase with the diterpene forskolin resulted in a two- to three-fold increase over the basal cAMP level. In S and NS groups, both basal and isoproterenol-stimulated cAMP values were significantly lower than in the HC group ($p < 0.01$). In the S group, intracellular cAMP values after NaF and forskolin stimulation were lower than in healthy controls ($p < 0.01$). Importantly, forskolin-stimulated cAMP accumulation was significantly lower in septic than in non-septic patients ($p < 0.001$).

Regarding the individual data, a basal cAMP level was not detectable in 9 of the 11 septic and in only 1 of the 7 non-septic patients (82% vs 14%, $p < 0.05$). Interestingly, in the three septic patients without symptoms of circulatory shock who were free of β-adrenergic agonists, a cAMP content was undetectable and poorly responsive to pharmacological stimulation. For two septic patients, another set of intracellular cAMP content measured just before ICU discharge (7–10 days after β-adrenergic therapy weaning) showed a complete reversal of signal transduction alterations as indicated by basal and stimulated cAMP levels over the mean values found in HC.

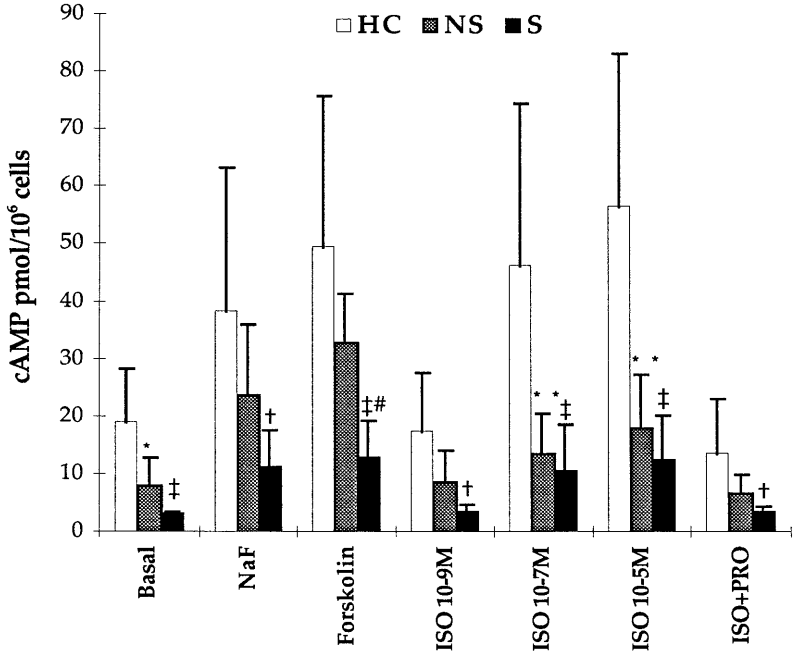

Fig. 2 Basal and stimulated cAMP accumulation in PBMC. Production of cAMP (mean ± SD) under basal condition and after stimulation with sodium fluoride (10^{-1}M, forskolin (5.10^{-5}M, isoproterenol (10^{-9}M, 10^{-7}M, 10^{-5}M) and the combination isoproterenol (10^{-5}M) + propranolol (2.10^{-5}M). Comparison between non-septic patients (NS) and healthy controls (HC): * $p < 0.0167$; ** $p < 0.01$. Comparison between septic patients (S) and healthy controls (HC): † $p < 0.01$; ‡ $p < 0.001$. Comparison between S and NS: # $p < 0.001$

Serum catecholamine and cytokine levels

In HC, adrenaline and noradrenaline levels were 0.11 ± 0.05 nmol/l and 1.27 ± 0.15 mmol/l, respectively. Thirteen of the 18 patients had high noradrenaline levels at entry into the study, but no difference was found between NS and S patients (2.8 ± 1.1 nmol/l vs 3.7 ± 2.1 nmol/l, respectively). Adrenaline concentrations ranged within normal values except in two septic patients. No correlation was found between noradrenaline concentration and βAR density.

In NS patients, TNFα, IL-1β and IL-6 were undetectable in all but one patient with an evolutive bronchial neoplasm. In the S group, TNFα was increased in three patients and IL-1β in four patients. Increased levels of IL-6 were found in four septic patients. Elevated serum concentration for at least one cytokine was found in 45 % of septic patients, compared to 15 % of non-septic patients ($p < 0.05$).

Discussion

An altered transduction of the β-adrenergic signal in PBMC of critically ill patients was demonstrated in this study. Compared to healthy controls, we observed a 40 % reduction of the βAR density with unchanged affinity for agonist and a low basal and isoproterenol-stimulated cAMP accumulation in the two groups of patients. cAMP production elicited by NaF or forskolin was significantly reduced in patients admitted with severe septic processes.

Mechanisms for altered β-adrenergic transduction

Our data indicate a heterologous desensitization of adenylate cyclase stimulation in PBMC of septic patients. The heterologous nature of this desensitization was confirmed by the poor cAMP response obtained after stimulation with either the β-adrenergic agonist (isoproterenol) or other compounds bypassing the βAR (e.g. NaF and forskolin). Such impairment of the βAR-independent stimulation of adenylate cyclase can result from alteration (quantitative or qualitative) of G proteins and/or adenylate cyclase enzymes [10].

The precise mechanism of these alterations remains unclear, but some etiopathogenic factors could be evoked. *Cell exposure to high catecholamine concentrations* (resulting from increased sympathetic stimulation and drug administration) can produce severe dysfunction of the β-adrenergic system. Reithmann and colleagues demonstrated that a 24 h culture of rat cardiomyocytes with noradrenaline leads to heterologous desensitization of adenylate cyclase stimulation [11]. The cAMP response to forskolin improved after pertussis toxin pretreatment, suggesting an involvement of the Gi pathway. An increase in the level of the α-subunits of inhibitory G protein was identified as the mechanism of the noradrenaline-mediated heterologous desensitization of adenylate cyclase stimulation in rat heart muscle cells [12]. In the same way, Xiao et al. demonstrated experimentally that the Gs-coupled β2AR can simultaneously activate a pathway that leads to functional inhibition in cardiac cells via a pertussis toxin-sensitive G protein [13]. Such opposite effects triggered by the β2

Table 2 Catecholamines and cytokine serum levels (Shock: presence *(Y)* or absence *(N)* of shock symptoms; *MD* missing data, *< dl* below detection limit)

No.	Group	Shock	Adrenaline (nmol/l)	Noradrenaline (nmol/l)	TNFα (ng/ml)	IL-1α (ng/ml)	IL-1β (ng/ml)	IL-6 (ng/ml)
1	NS	Y	0.12	1.68	0.45	< dl	0.70	0.15
2	NS	Y	0.23	2.89	< dl	< dl	< dl	< dl
3	NS	N	0.12	3.45	< dl	< dl	< dl	< dl
4	NS	N	MD	MD	MD	MD	MD	MD
5	NS	N	0.31	1.56	< dl	< dl	< dl	< dl
6	NS	N	0.49	4.38	< dl	< dl	< dl	< dl
7	NS	Y	0.19	2.92	< dl	< dl	< dl	< dl
8	S	Y	0.24	4.28	< dl	0.11	0.53	< dl
9	S	Y	0.33	6.41	0.2	< dl	0.35	0.46
10	S	Y	0.42	3.45	< dl	< dl	< dl	< dl
11	S	Y	0.10	6.80	0.39	0.12	0.13	0.90
12	S	N	0.10	1.58	< dl	< dl	< dl	< dl
13	S	N	0.20	2.58	< dl	< dl	< dl	< dl
14	S	N	1.03	6.83	< dl	< dl	< dl	< dl
15	S	Y	0.25	2.08	< dl	< dl	< dl	< dl
16	S	N	0.60	2.31	< dl	0.10	0.56	0.74
17	S	Y	0.17	1.12	0.15	< dl	< dl	0.54
18	S	Y	0.32	3.30	< dl	< dl	< dl	< dl

stimulation could represent a homeostatic process to protect cardiac cells from Ca^{++} overload, rather than a mechanism of cardiac dysfunction [13].

Other mechanisms contribute to blunt the function of adenylate cyclase in cells exposed to high catecholamine concentrations. Receptor or voltage dependent Ca^{++} entry is susceptible by itself to inhibit adenylate cyclase activity and has been regarded as a potential molecular mechanism for the clinical observation that Ca^{++} administration blunts the cardiotonic effects of β-agonists in experimental animals and patients [14]. To date, there is convincing evidence that such a Ca^{++}-inhibitory form of adenylate cyclase (type 5 and 6) is widely expressed in various tissues, including myocytes and lymphocytes [14, 15], and it is thought to underlie – at least in part – the concept of calcium-induced catecholamine resistance. At this point, the crucial question of molecular consequences of septic shock therapy on the β-adrenergic transduction pathway is raised with regard to the marked desensitization of adenylate cyclase induced in vitro by noradrenaline (a strong βAR-stimulating agent) but not dopamine [16], and that is suspected to contribute to the tachyphylaxis observed in septic shock.

The role of a more aggressive β-adrenergic agonist therapy in the postreceptor dysfunction, that was specifically noted in septic patients, should certainly be investigated. However, although catecholamines were more often used in the S group, no difference between the S and NS groups was found in terms of catecholamine serum concentrations, and the direct consequences of catecholamine exposure – level of βAR downregulation and isoproterenol potency in cAMP production – were similar in the two groups. Furthermore, undetectable intracellular cAMP contents, which could be poorly stimulated, were also found in septic patients free of β-adrenergic agonists. This suggests that other factors apart from catecholamine therapy could have participated in the postreceptor alterations of β-adrenergic signaling. This has been nicely demonstrated in endotoxin-challenged dogs by Silverman et al. who showed that pretreatment with the β-blocking agent propranolol prevented a decrease in lymphocytic βAR density but not a decrease in the fluoride-stimulated cAMP [3]. Recently, the deleterious effect on the β-adrenergic function of associated factors (including catecholamine therapy and sepsis mediators) was suggested in a clinical study by Böhm et al. [17]. These investigators reported, in patients with catecholamine-refractory septic shock or septic multiorgan failure, an overexpression of myocardial Giα which was much more marked than that generally found in patients with end-stage dilated cardiomyopathy. Moreover, myocardial Giα remained elevated for at least 2–4 days after catecholamine withdrawal [17].

Beside catecholamines, host inflammatory mediators and particularly the cytokines TNFα and IL-1β can also impair β-adrenergic signaling. Probably due to both the transient release and the delayed measurement of cytokine concentrations after patients' admissions [18, 19], we found detectable cytokine levels in only 45 % of patients. Nonetheless, regarding the more frequent elevation of cytokine plasma concentrations observed in the S group, we can assume its potential role in the low cAMP production noted after stimulation with NaF and forskolin. Experimentally, IL-1β and TNFα have been shown to decrease cardiac cell contractility by disrupting the transmembrane β-adrenergic signal transduction [20]. Such impairment of transduction is due to an activation of the Gi pathway that mediates the uncoupling of the βARs from the adenylate cyclase [4,

12]. In contrast, a direct TNFα-related alteration of adenylate cyclase function is not supported by experimental studies, which demonstrated a preservation of forskolin or colforsin-stimulated cAMP accumulation in cardiac cells [20, 21] and lymphocytes [22]. The depressant effect of TNFα and IL-1β on myocardial cells previously demonstrated in various experimental models [23, 24] was confirmed by demonstration of the protective effect of anti-TNFα antibodies on cardiac contractility in endotoxinic animals [25, 26]. Improvement of the β-adrenergic function was suggested because the anti-TNFα therapy prevented the endotoxin-induced decrease in cAMP synthesis [25]. Such data should be linked with the clinical observation of Vincent et al., who noted that administration of anti-TNFα antibody improved left ventricular function in septic shock patients [27], and with that of Silverman et al. showing that these patients have a reduced intralymphocytic cAMP production associated with myocardial hyporesponsiveness to cathecholamines [5]. In the near future, catecholamine refractoriness might be a target for pharmacological intervention in order to improve the β-adrenergic function in septic shock patients. Investigations demonstrating that the administration of methylprednisolone was associated with both an increased cardiac performance and a βAR up-regulation in patients with circulatory shock receiving long-term catecholamine treatment certainly merit further consideration [28].

Limits of the study

This in vitro study presents some limitations. We did not measure adenosine triphosphate (ATP) concentration in lymphocytes at baseline and the responsibility of intracellular ATP deficit in the low cAMP accumulation might be suspected [29]. However, this possibility appears to be unlikely because we did not find recognized predisposing factors, such as hypophosphatemia or hypoglycemia, in our patients. Moreover preservation of intracellular ATP levels has previously been demonstrated in septic animal models characterized by a hyperdynamic state [30]. Investigation of another group of severely infected patients, manifesting signs of sepsis-related organ dysfunction or hypotension but prior to the initiation of β-adrenergic support, would seem to be a more straightforward approach for studying the influence of sepsis per se in altered signal transduction. However, this work could not be realized because of ethical considerations and the impossibility of delaying experiments which were performed immediately on freshly isolated PBMC.

We selected the β2AR of circulating lymphocytes to study the β-adrenergic transduction because this tissue is readily accessible and is a very sensitive model of βAR agonist-mediated desensitization and down-regulation [31]. The βAR-adenylate cyclase complex in lymphocytes closely resembles that of cardiac cells [32] and has been widely used to model myocardial β-adrenergic function [33, 34]. Finally, among the eight isoforms of adenylate cyclase identified so far, type V and VI are particularly abundant in the heart, and type VI is also found in lymphoid tissue [35]. However, whereas lymphocytes are useful tools for studying the mechanisms of transmembrane signaling and cyclic nucleotide regulation, extrapolation to in vivo cardiac function from in vitro data obtained from pharmacologically stimulated lymphocytes remains extremely hazardous. In particular, in this study we did not focus on cardiac performance and there was no attempt to correlate cAMP content with any contractility marker. Using our data, it can only be stated that serious dysfunction of β-adrenergic signaling was found in severely infected patients, i.e., in patients presenting with, at least, signs of organ dysfunction or poor peripheral perfusion and, at most, a patent circulatory shock; all these conditions suggest inadequate oxygen transport.

In conclusion, in the lymphocytes of septic ICU patients we found severe alterations of the transmembrane β-adrenergic signaling, including a down-regulation of βARs, and a profound dysfunction of the adenylate cyclase system. In this severe sepsis/septic shock population with high catecholamine serum concentrations, elevated cytokine serum levels were detected in 45% of patients. Although we cannot conclude a cause-and-effect relationship, these data enhance our suspicion concerning the deleterious role of catecholamine and cytokine release in the sepsis-induced β-adrenergic system dysfunction.

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