Calcitonin precursor (CTpr) levels are both markers and mediators of inflammation. The duration of their elevation after intravenous endotoxin challenge and the effects of anti-inflammatory therapies were studied in 52 subjects. CTpr levels maximized at 24 h in all subjects. At 7 days (n = 4), after levels of acute-phase cytokines and C-reactive protein had normalized, CTpr levels remained 2–4-fold above baseline levels. The elimination half-life of CTpr levels ranged from 26.9 to 45.7 h. At 24 h, endotoxin and ibuprofen (compared with endotoxin alone) increased CTpr levels ∼2-fold (P = .03), whereas soluble tumor necrosis factor receptor blunted the increase in CTpr levels by 2–3-fold (P = .0015). However, soluble interleukin-1 receptor failed to alter the increase in CTpr levels. Thus, the fact that anti-inflammatory agents may alter CTpr levels resulting from a single stimulus must be considered when CTpr is used as a clinical marker. Of importance, this study reveals that anti-inflammatory agents may modulate the CTpr level, which is a potential toxic mediator of inflammation.
tation, to define the temporal sequence of increase and decrease of CTpr levels. The remaining 3 groups were studied for 24 h after endotoxin administration. The second study group consisted of 12 subjects (10 men and 2 women) who were given either endotoxin with placebo or endotoxin with ibuprofen [8]. Ibuprofen is a cyclooxygenase inhibitor that attenuates fever and symptoms associated with endotoxin administration and that enhances TNF release [11]. These subjects were studied on 2 occasions (separated by 1 week) with administration of saline or intravenous endotoxin. Six subjects were randomized to receive ibuprofen (800 mg orally at -1.5, 0, and 3 h after being administered endotoxin or saline; Upjohn), and 6 were given placebo after being administered endotoxin or saline.

The third study group consisted of 18 subjects (14 men and 4 women). Six each of the subjects were given low (10 mg/m²) or high (60 mg/m²) doses of soluble TNF dimeric receptor (Entanercept, TNFR:Fc; Immunex) or intravenous placebo 30 min before endotoxin administration [10]. TNFR:Fc is a potent inhibitor of TNF effects on target cells [10].

The fourth study group consisted of 18 subjects (13 men and 5 women). Six each of the subjects were given low (1 mg/m²) or high (10 mg/m²) doses of soluble human type I IL-1 receptor (sIL-1R1; Immunex) or intravenous placebo 30 min before endotoxin administration [9]. sIL-1R1 inhibits a wide variety of inflammatory responses due to IL-1 [9].

Assay of CTpr. The RIA was done as described elsewhere [1, 3]. Polyclonal antiserum R2B7 specific for synthetic nProCT (50 μL; diluted 1:20,000) was preincubated with standards or unknowns (20-100 μL) and goat anti–rabbit IgG bound to iron particles (50 μL) for 1–2 days in assay buffer (0.2% gelatin in 0.13 M borate [pH, 7.5]). Antibody-bound material was separated from the supernatant by means of magnetic tube racks. The antibody-bound pellet then was rinsed with assay buffer, 50 μL of labeled [125I]-nProCT (25,000 cpm) was added, and incubation was continued for 3 h. Again, the bound and free hormone were separated by use of magnetic tube racks, and the bound pellet was rinsed twice more with assay buffer. This assay detects free nProCT, intact ProCT, and ProCT gene–related peptide. It has a sensitivity of 0.65 fmol/mL (4 pg/mL), and the 50% B/Bo is 22.5 fmol/mL (140 pg/mL) [1, 3]. C-reactive protein was measured by nephelometry (The Binding Site).

Statistical analysis. The elimination half-lives (t1/2) of CTpr and C-reactive protein were determined by using standard non-compartamental equations with the WinNonlin program (Scientific Consulting). A 4-way analysis of variance (ANOVA) based on endotoxin administration, treatment, subjects nested within treatment, and time was used to assess the impact of anti-inflammatory agents on CTpr levels (SAS System 6.09; SAS Institute). For all ANOVA models, normality of residuals was assessed by using a Shapiro-Wilk test, and, if necessary, data were log-transformed to improve the distribution of the residuals. Data are presented as mean ± SE.

Results

In the 4 subjects who were followed up for 7–14 days, CTpr levels increased by 3 h and attained peak values at 24 h (P < .001). For subjects 1–4, t1/2 for CTpr were 45.7, 30.3, 27.4, and 26.9 h, respectively. At 7 days, all 4 subjects had levels (45.3 ± 17.7 fmol/mL) that remained above the baseline (0 h) values, which were 10.4 ± 1.13 fmol/mL for all 52 subjects (figure 1A). Levels normalized in the 2 subjects for whom blood samples were obtained on days 10 (1) and 14 (1). The time course of C-reactive protein reached peak levels at 24 h and returned to basal levels by 7 days (figure 1B). For subjects 1–4, t1/2 values of C-reactive protein were 34.3, 33.4, 30.6, and 29.9 h, respectively.

In subjects given saline with either ibuprofen or placebo, no changes in baseline values of CTpr occurred over 24 h (0 h, 17.2 ± 4.0; 24 h, 16.0 ± 2.0 fmol/mL; P not significant [NS]). The combination of ibuprofen and endotoxin was associated with a 2-fold enhanced release of CTpr, compared with release in subjects given placebo or endotoxin alone (P = .03 from the interaction term; F value, 2.84; df, 4; df error, 61; figure 2A). In subjects given low-dose TNFR:Fc, the increase in CTpr levels was suppressed 2–3-fold, compared with that in subjects given placebo or high-dose TNFR:Fc (P = .0015 from the interaction term; F, 3.68; df, 8; df error, 59; figure 2B). sIL-1R1 had no effect on the acute increase of CTpr levels at 24 h (P NS; figure 2C).

Figure 1. Blood levels of calcitonin precursors (A) and C-reactive protein (B) over 7 days in 4 healthy subjects after intravenous administration of endotoxin. Dashed line reflects mean normal range.
Figure 2. Effects of anti-inflammatory agents on calcitonin precursor (CTpr) levels after intravenous administration of endotoxin. A. Blood levels of CTpr after administration of endotoxin plus placebo and endotoxin plus ibuprofen. Ibuprofen was associated with enhanced CTpr levels ($P = .03$). B. Changes in CTpr levels after administration of endotoxin with either low-dose (LD) or high-dose (HD) soluble tumor necrosis factor dimeric receptor (TNFR:Fc) or placebo. LD TNFR:Fc was associated with lower CTpr levels than were placebo or HD TNFR:Fc ($P = .0015$). C. Changes in CTpr levels after administration of endotoxin with either LD or HD soluble type I interleukin-1 receptor (sIL-1R1) or placebo. sIL-1R1 did not alter the increase in CTpr. Each treatment group had 6 subjects.

Discussion

The use of anti-inflammatory agents in sepsis has been hampered by difficulties in identifying patients who are likely to benefit from these interventions. This is, in part, due to differences in underlying disease, infecting microorganism, and variables that underlie host inflammatory responses. Because clinical parameters alone do not provide sufficient information about a patient’s inflammatory response, biologic markers, such as blood cytokines (i.e., TNF or IL-6), C-reactive protein, and CTpr, have been used to characterize risk and outcomes in patients with severe infections [1, 2]. Furthermore, although elevations in CTpr levels occur in a variety of clinical conditions associated with infection or systemic inflammation, the ability of these peptides to reflect the effects of anti-inflammatory interventions has not been described.

By using a model of systemic inflammation after intravenous endotoxin administration, we showed that CTpr levels in healthy subjects reached a maximum by 24 h and remained above normal for >7 days. In contrast, C-reactive protein, another marker sensitive to systemic and localized inflammation, had normalized in the same subjects by 7 days. A previous series found that the $t_{1/2}$ of CTpr production was independent of renal function, age, sex, and organ failure score [12]. Within 24 h of peak concentrations, the median $t_{1/2}$ was estimated to be 28.9 h, which suggests persistent CTpr production [12]. Our data show that, after a single short-lived inflammatory stimulus in healthy humans, $t_{1/2}$ ranges from 26.9 to 45.7 h, and that this rate appears to be independent of a continuous stimulus for CTpr production. Presumably, this reflects the finding that essentially every cell up-regulates the calcitonin gene to produce CTpr mRNA after septic challenge [13].

The biologic effects of circulating CTpr after 24 h in healthy humans are unknown. Healthy hamsters given an infusion of human ProCT do not exhibit overt toxicity [4]. However, during sepsis, CTpr may be toxic to the host. Infusion of CTpr during bacterial peritonitis increases mortality, and antibody neutralization of CTpr under similar conditions improves survival [4]. Although these data suggest that CTpr may amplify inflammation initiated by infection, it is unknown whether the persistence of CTpr during sepsis will prime the host to more intense inflammatory responses after exposure to a second inflammatory stimulus. Subjects given 2 doses of endotoxin within 24–48 h of each other develop tolerance rather than enhanced inflammatory responses [6]. This suggests that other host factors interact with CTpr levels during infection to promote inflammation.

The mature peptide, calcitonin, is a key modulator of bone resorption via its suppressive action on the osteoclast and causes hypocalcemia when administered in pharmacologic doses. In sepsis, the high serum CTpr level is not accompanied by an increase in mature calcitonin, and the effect of CTpr on calcium physiology has not been studied. However, in 101 critically ill patients, the increase in serum CTpr levels correlated strongly with a decrease of ionized calcium, which suggests that there may be an impact of these precursors on calcium metabolism [14].

Our study shows that anti-inflammatory agents have a variable effect on CTpr levels. Ibuprofen, a cyclooxygenase inhibi-
tor, suppresses the fever and symptoms of experimental endotoxemia; it has no effect on the cardiovascular response but enhances neutrophil elastase release [6]. Furthermore, because of the loss of prostaglandin suppression of cytokine production, TNF-α levels are increased in subjects given ibuprofen and endotoxin, compared with those in subjects given endotoxin alone [11]. These enhanced mediator responses may have contributed to the increased CTpr levels after ibuprofen and endotoxin administration. However, the effects of prostaglandins on CTpr release from cells is unknown. ProCT suppresses TNF production by 27% in endotoxin-stimulated whole blood, but it had no effect on IL-1, IL-6, or IL-8 production [15]. ProCT also suppressed TNF in experimental sepsis [16]. CTpr levels may have a counterregulatory role in TNF release, although the physiologic importance of this effect is unknown.

The increase of CTpr levels after endotoxin was diminished by the anti-inflammatory effects of low-dose TNFR:Fc, yet this was only partly due to inhibition of blood TNF bioactivity. When either high- or low-dose TNFR:Fc is given during experimental endotoxemia, blood TNF bioactivity is undetectable, and secondary inflammatory responses (e.g., cytokines, stress hormones, and kallikrein activation) are diminished, whereas others (e.g. fever, symptoms, leukocytosis, and cardiovascular responses) are unaffected [6, 10]. However, high-dose TNFR:Fc has fewer anti-inflammatory effects than low-dose TNFR:Fc, which is possibly due to higher levels of TNFR:Fc-TNF complexes or extravascular sources of TNF [10]. Low-dose TNFR:Fc had more anti-inflammatory effects (e.g., suppressing cytokine, stress hormone, and kallikrein activation responses), which were reflected by lower CTpr levels. Although CTpr levels are altered by changes in TNF bioactivity, other non-TNF inflammatory pathways appear to likely contribute to the increased CTpr levels during endotoxemia.

In contrast, endotoxin-induced increases in CTpr were not altered by sIL-1R1. This may be due to several factors. Only low blood levels of IL-1β are detected in humans after endotoxin administration, whereas cell-associated IL-1β rises significantly [9]. sIL-1R1 decreases blood levels of both IL-1β and IL-1 receptor antagonist, resulting in enhanced levels of TNF, IL-8, and C-reactive protein after high-dose sIL-1R1 [9]. However, these enhanced cytokine levels were not reflected by differences in CTpr levels.

Our study demonstrates that CTpr has a prolonged t1/2 in healthy humans. Anti-inflammatory agents alter the peak levels of CTpr, due in part to TNF-α activity. As a marker, CTpr may offer a means to evaluate the potential efficacy of anti-inflammatory therapy. As a mediator susceptible to anti-inflammatory agents or immunoneutralization, its prolonged t1/2 might facilitate therapeutic interventions.

Acknowledgments

We gratefully acknowledge the statistical analysis provided by Steven Banks and elimination rate determinations performed by Steven Piscatelli.

References