Selective Consumption of Thyroxine-Binding Globulin During Cardiac Bypass Surgery

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A study of serum thyroid hormone binding proteins and thyroid hormone concentrations during and after coronary artery bypass graft (CABG) surgery shows a marked difference in the response of thyroxine binding globulin (TBG) and transthyretin (TTR). The effects of CABG on TBG and TTR were compared in 32 patients during the day of surgery. In a few of these patients, additional determinations were performed to 5 days. When corrected for dilution, TTR concentrations decline gradually after surgery, with no significant decrease over the first 24 hours. In contrast, a rapid decrease of TBG to a mean level of 60% of the preoperative control at 12 hours after the start of surgery appears to account for the concomitant decrease of serum T_4. The rate at which the TBG concentration decreased far exceeds the reported fractional clearance of TBG and therefore implies accelerated consumption rather than inhibition of production. TBG is a member of the serine protease inhibitor (SERPIN) superfamily. We propose that its rapid consumption is due to protease cleavage at inflammatory sites. This may explain the previously observed accumulation of thyroxine iodine at such sites.

LIKE CORTICOSTEROID binding globulin (CBG), thyroxine binding globulin (TBG) is a member of the serine protease inhibitor (SERPIN) superfamily of proteins.[1] The classic action of SERPINS such as α1-antiprotease, the complement C1 inhibitor, and antithrombin III is to complex and inhibit proteolytic enzymes. However, CBG does not inhibit proteolytic activity and releases cortisol upon being cleaved by neutrophil elastase.[2-4] This appears to be an evolutionary adaptation of the SERPIN interaction with proteases that promotes localized delivery of cortisol at sites of inflammation. If TBG delivers thyroid hormones by a similar mechanism, one would expect that, like CBG,[5,6] TBG would be consumed during inflammation. Although the weakened serum thyroxine (T_4) binding that occurs in illness has been attributed at least in part to circulating inhibitors of binding or, alternatively, to partial desialylation of TBG,[7-9] immunoassay demonstrates a significant decrease in TBG.[10,11]

To differentiate an accelerated consumption of TBG from an inhibition of TBG production, we sought to observe the response of the TBG concentration to an acute inflammation with a defined onset. Coronary artery bypass graft (CABG) is associated with a massive but relatively brief inflammatory response and development of the euthyroid sick syndrome.[12-14] This temporally defined inflammatory response affords an opportunity to focus on the early effects of inflammation on the thyroid hormone binding proteins.

SUBJECTS AND METHODS

Short-term studies of TBG, transthyretin (TTR), T_4, free T_4, T_4 uptake, triiodothyronine (T_3), thyrotropin (TSH), and albumin during CABG and until day 1 after surgery were performed in 32 patients. Blood samples were obtained at 15 minutes before incision and at 15 minutes and 4.25, 12.4, and 24 hours after incision. Further follow-up data were obtained in 9 of these patients on day 2, 16 on day 3, 9 on day 4, and 11 on day 5. Informed consent was obtained from all subjects. Statistical significance was determined by paired *t* test using Sigmaplot for Windows (Jandel Scientific, San Rafael, CA). TBG concentrations were determined by the GammaDab [125I] TBG radioimmunoassay (RIA) kit (Incstar, Stillwater, MN). This is a sandwich assay in which serum TBG is bound by immobilized TBG antibody and measured by its binding of ^{125}I T_4. To confirm RIA results, the TBG level was also measured by a radial immunodiffusion (RID) kit (BIND A RID; Binding Site Limited, Birmingham, England). RID kits from the same source were used to measure TTR, α1-antitrypsin, and α2-macroglobulin concentrations. Thyroid hormone and TSH concentrations and T_4 uptake were determined with the Axsym system (Abbott Laboratories, Chicago, IL) in the clinical immunochemistry laboratory. With this method, T_4 uptake is determined by total rather than unoccupied T_4 binding capacity and free T_4 is estimated by the rate of uptake on immobilized T_4 antibody.[15] Serum albumin levels were measured in the clinical chemistry laboratory using Vitros (Johnson & Johnson, Rochester, NY).

RESULTS

Thyroid Hormone Binding Proteins

TBG, TTR, and albumin decreased to varying degrees during the procedure (Fig 1). Most of the initial decrease in albumin and TTR was attributable to dilution and shifts in the protein distribution space, as demonstrated by the recovery of albumin and TTR concentrations immediately after surgery (fourth observation at an average of 12.4 hours after incision). Interoperatively (third observation at 4.25 hours after incision), the mean decrease of serum albumin and TTR was to 69% and 73% of the respective preoperative concentration. At the first postoperative observation, albumin and TTR concentrations had recovered to 88% and 89% of their preoperative values. TBG concentrations measured by RIA decreased much more steeply, reaching a nadir at 48% of the preoperative values during the procedure, and showed no significant recovery at the first postoperative observation, remaining at 52% of the preoperative concentration. At the first postoperative day, TBG was at 60% of the preoperative concentration. In 19 cases, the preoperative and first postoperative day TBG determinations were repeated using RID. Although absolute TBG concentrations

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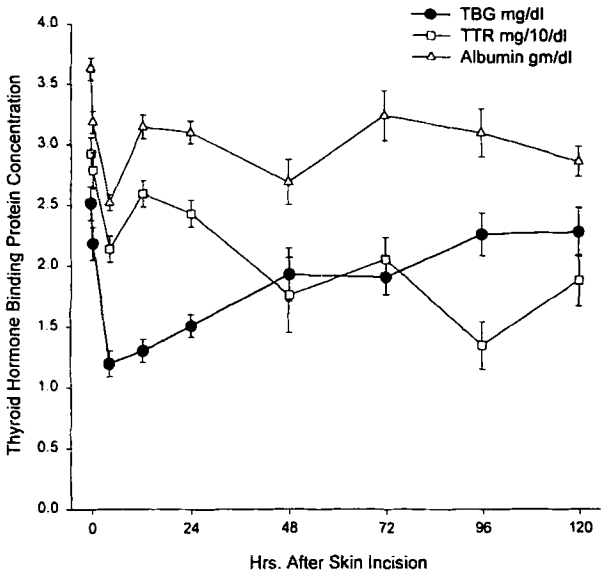

Fig 1. Concentration of thyroid hormone binding proteins during and after CABG. Values are the mean ± SEM here and in subsequent figures. Note that the units vary to allow representation on the same graph.

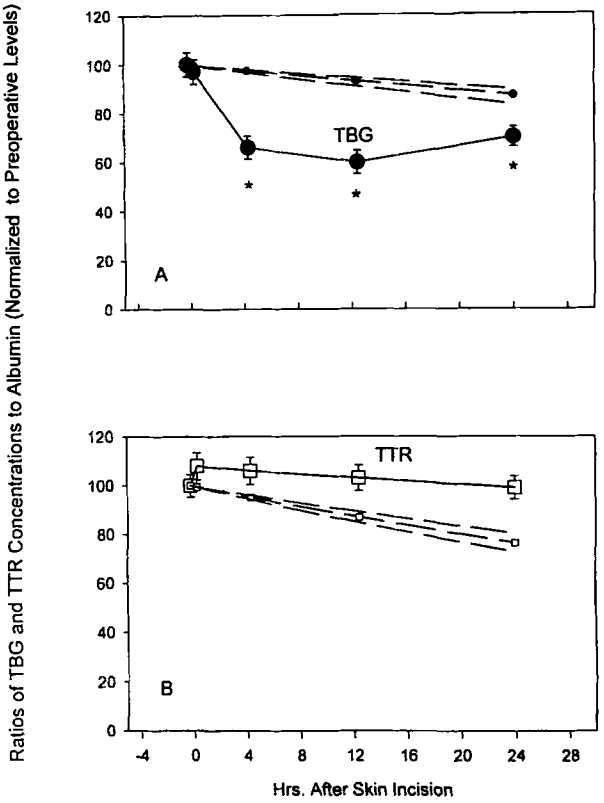

Fig 2. Rate of TBG and TTR decline during and immediately following CABG compared with their normal fractional clearance. Individual TBG and TTR concentrations were corrected for dilution and nonspecific changes in plasma protein distribution by dividing by the albumin concentration and then normalized to the preoperative concentrations. TBG (A) decreases much more rapidly than TTR (B). Broken lines represent the expected concentrations of TTR and TBG assuming that synthesis is completely stopped, given the published clearance rates.[17,18] TBG decreased more rapidly and TTR more slowly than this predicted rate. *$P < .001$.

were slightly lower by RID than by RIA, the percent decrease at postoperative day 1 was virtually identical (Table 1). Postoperatively, TBG concentrations gradually increased through day 5. At day 5, they were significantly above the postoperative day 1 levels ($P < .001$, n = 11). As expected, the changes in T_4 uptake (not shown) were a mirror image of the changes in TBG concentration. During the same period, TTR declined, decreasing to the lowest level at 4 days after surgery, when it was significantly below the day 1 level ($P < .05$, n = 9). The decreases in albumin that occur during cardiopulmonary bypass are almost entirely attributable to dilution and shifts in the protein distribution space rather than protein metabolism.[16]

To compare the acute effects of CABG on serum T_4 binding proteins, serum TBG and TTR concentrations through postoperative day 1 were first corrected for dilution and shifts in the protein distribution space by dividing by the simultaneously determined albumin concentration and then normalized as a percent of their preoperative value (Fig 2). These are subsequently referred to as normalized values. At the first postoperative observation (fourth observation) when normalized TBG was decreased slightly more than 40%, normalized TTR was not decreased below preoperative levels. The observed decreases in normalized TBG and TTR concentrations were compared with their known fractional clearance rates.[17,18] TBG decreased much more rapidly than could be accounted for by its fractional clearance rate. In contrast, TTR concentrations did not decline during the first 24 hours despite a faster fractional clearance rate than that of TBG. Intraoperatively, the normalized concentrations of 2 other SERPINS, α1-antitrypsin and α2-macroglobulin, decreased similarly to TBG (Fig 3). As would be expected, α1-antitrypsin, a positive acute-phase reactant, increased rapidly after surgery.

Table 1. Comparison of RIA and RID Measurement of TBG in 19 Patients

Variable	TBG (mg/dL)	
	RIA	RID
Preoperative	2.45 ± .18	2.20 ± .24
Postoperative day 1	1.45 ± .15	1.29 ± .09
Decrease (%)	40	41
P	<.001	<.001

Thyroid Hormones

Total T_4 concentrations decreased rapidly, reaching their lowest point during the surgery (observation 3), and did not decline further at 24 hours, a pattern similar to that of TBG. T_3, on the other hand, continued to decline through the first postoperative day. Although decreasing markedly, T_4 concentrations remained within the normal range, while the mean T_3 eventually decreased to slightly below normal as a result of its continued decline to postoperative day 1. Free T_4 was unchanged except for a possible small increase during the procedure. This apparent small peak of free T_4 during surgery (third observation) is statistically significant ($P = .05$) com-

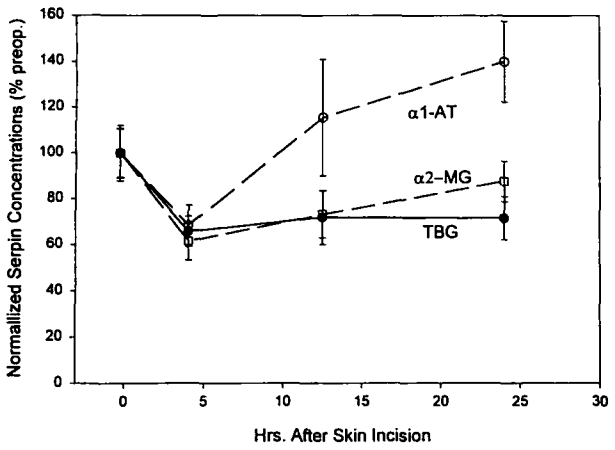

Fig 3. Comparison of the effect of CABG on TBG, α1-antitrypsin (α1-AT), and α2-macroglobulin (α2-MG) in 8 patients. The 3 SERPINs show the same initial decrease in concentration.

pared with the preceding observation at 15 minutes after skin incision and with the subsequent observation at 24 hours ($P = .04$), but it is not significant relative to baseline (Fig 4).

TSH

Serum TSH increased briefly during surgery and then decreased significantly below baseline ($P < .001$), returning to normal by day 5 (Fig 4).

Mock Bypass

To examine the possibility that extraction by the bypass apparatus was responsible for the observed changes in T_4 binding proteins and thyroid function tests, a 50% dilution of pooled fresh frozen plasma was circulated through a mock CABG consisting of the reservoir, oxygenator, filters, pump, and connecting tubing for 2 hours. Thirty minutes after commencement, 5,000 U heparin was added (proportional to the usual addition of about 20 to 25,000 U per 5 to 6 L blood vol).

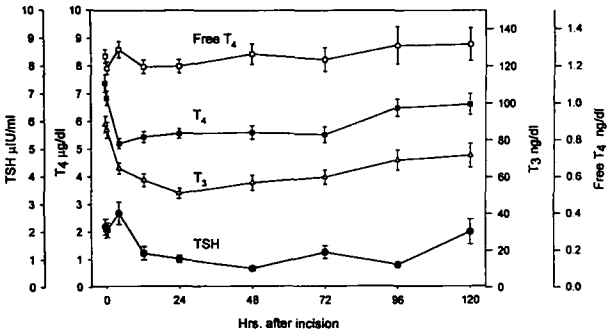

Fig 4. Effect of CABG on thyroid hormone concentrations. There is a slight peak of free T_4 at the 2-hour time point that is just significant ($P = .05$) in comparison to the immediately preceding and following values. The remaining free T_4 concentrations are not significantly different from the −15-minute and +15-minute samples. Total T_4 and T_3 decreased steeply during surgery. The decline in T_3 continued through postoperative day 1. TSH decreased significantly ($P < .001$) at 8 hours postsurgery and continued below control levels at 24 ($P < .001$), 48 ($P < .001$), and 72 hours ($P < .01$), with a return to baseline by 96 hours.

Circulation through the bypass apparatus did not affect TBG, TTR, total T_4, free T_4, or TSH concentrations. The initial/final concentrations in the dilute serum were as follows: T_4 4.9/4.6 μg/dL, T_3 47/50 ng/dL, TSH .5/.4 μlU/mL, albumin 1.5/1.3 g/dL, TBG 1.6/1.6 mg/dL, and TTR 9/9 mg/dL.

DISCUSSION

The contrast between the stability of normalized TTR and the steep decrease of normalized TBG during surgery (Fig 3) supports the method of calculation, since inadequate correction for dilution and shifts in protein distribution would have shown an apparent decrease of TTR. The measurement of TBG by RIA is validated by the close correspondence to the RID measurement which is independent of T_4 binding (Table 1). The mean preoperative TBG of 2.5 ± .8 mg/dL was the same as that of 11 normal subjects (2.5 ± .5 mg/dL). The observed decrease of normalized TBG to 60% of the preoperative concentration is comparable to the decrease of TBG (58% of normal) observed by Chopra et al[10] in the low-T_3, low-T_4 syndrome of nonthyroidal illness. Our results are consistent with the rapid decrease of immunoassayable TBG during CABG previously observed by Holland et al,[14] although the latter data were not corrected for changes in volume or distribution space. The abrupt loss of 40% of TBG during CABG is far too rapid to reflect an inhibition of synthesis. The normal TBG half-life of 5.01 ± 1.21 days,[17] or 14% per day, cannot account for the acute decrease of TBG during surgery, which is equivalent to a half-life of .3 days, or 230% per day (Fig 3). The bypass apparatus does not extract TBG. Therefore, it is reasonable to conclude that the rapid decrease of TBG is predominantly due to an accelerated consumption that is limited to the inflammatory phase. If there is also an inhibition of TBG synthesis, this could account for only a minor part of the total decrease in the TBG concentration. The rate of recovery seems consistent with at least a normal rate of replacement. On the other hand, the gradual decrease of the TTR concentration over the 5 postoperative days is consistent with the expected partial inhibition of the synthesis of this negative acute-phase protein.[19]

Since TBG is a SERPIN, it seems likely that the acute decrease of TBG, like that reported for CBG,[6] α1-antitrypsin, and α2-macroglobulin[20] in CABG, is due to cleavage by inflammatory proteases. The susceptibility of TBG to proteolytic attack by elastase has been demonstrated previously.[3,21] However, the 2 studies differ as to whether elastase proteolysis diminishes the affinity of TBG for T_4. Recent studies in our laboratory confirm elastase cleavage of TBG and demonstrate that it results in an increased free to bound T_4 ratio. Incubation with polymorphonuclear cells also results in proteolysis of TBG and an increased proportion of free T_4.[22] The virtually identical acute decrease in the concentrations of TBG and the SERPINS α1-antitrypsin and α2-macroglobulin (Fig 3) is consistent with a shared mechanism of clearance. The subsequent increase in α1-antitrypsin is expected as part of the acute-phase response to inflammation and is due to increased synthesis. It differs from the slow recovery of TBG which seems consistent with normal synthesis in the absence of accelerated clearance.

A comparison of the time course of the decrease in TBG (Figs 1 and 2) and T_4 (Fig 4) is consistent with the hypothesis that T_4 is lost as TBG is consumed. As is the case for TBG, the decrease

in T_4 is too rapid to be explained by decreased secretion, since T_4 has a fractional clearance rate of about 12% per day[23] but decreases by a mean of 26% at the first postoperative observation without recovering at 24 hours. Since the decrease in TBG is approximately 40% and about 2 thirds of the serum binding power for T_4 is contributed by TBG,[24,25] the overall decrease in total serum T_4 binding power attributable to the decrease in TBG would be about 25%, corresponding to the acute decrease in T_4. The slow recovery of T_4 concentrations argues against a temporary shift of the distribution space as a cause of the decrease in T_4 concentration. Interestingly, the pattern of decline of T_3 differs from that of T_4, exhibiting a slow component that is consistent with decreased synthesis due to the known inhibition of 5′ T_4 deiodination in the euthyroid sick syndrome.[8]

Serum thyroid hormone binding has a distributive action.[26] However, this does not require the very strong T_4 binding exhibited by TBG, and patients with a congenital absence of TBG have no evidence of tissue hypothyroidism. CBG and TBG, the 2 SERPIN family hormonal transport proteins, maintain serum hormone concentrations (.3 and .1 mmol/L, respectively) that are orders of magnitude higher than those of the peptide hormones and other small protein-bound hormones such as estradiol. The maintenance of a high concentration of thyroid hormones on a protein that appears to be consumed during the inflammatory process suggests that TBG, like CBG, provides a mechanism for the discharge of its ligands at inflammatory sites. There is some evidence that this occurs, since T_4, or at least thyroxine iodine, has been shown to accumulate at sites of pulmonary infection.[27] Our failure to observe more than a very slight increase of serum free T_4 despite the marked decrease in TBG implies that the T_4 released by TBG consumption was metabolized before equilibrating with the total T_4 distribution space. These studies provide no direct evidence as to where such metabolism occurred. It may have been at the site where T_4 was freed from TBG. Rapid metabolism of T_4 by activated neutrophils contributes to local oxidative potential and antibacterial action via ether-bond cleavage and the generation of diiodotyrosine or oxidized forms of iodine.[28-32] This is a non–T_3-generating system. Since neutrophils metabolize T_4 and elastase expressed by neutrophils cleaves TBG,[3,21,22] it is possible that a coordinated release and oxidative metabolism of T_4 at inflammatory sites is a component of the response to infection. The tissue effects of this oxidative response may be of clinical relevance in the context of the noninfectious inflammatory response studied here.

We propose that the acute decrease of TBG observed in CABG is characteristic of the development of the euthyroid sick syndrome in response to inflammation. Clearly, further studies are needed to establish this as a general phenomenon and to localize and define the effects of TBG loss on the release and metabolism of T_4.

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