Increased Platelet Angiotensin II Receptor Number in Pregnancy-Induced Hypertension

Steven W. Graves, Thomas J. Moore, and Ellen W. Seely

Women with pregnancy-induced hypertension (PIH) are characterized by relatively greater blood pressure sensitivity to exogenous angiotensin II (Ang II) than normotensive pregnant women. Evidence suggests that this is due to an alteration in Ang II receptor sites. However, the question of whether this represents an increase in receptor number or affinity remains unanswered. To answer this question Ang II receptors on platelets from normotensive women during each trimester of pregnancy and the postpartum period were studied and compared with platelet Ang II binding in third trimester women with PIH and in postpartum women who had had a recent pregnancy complicated by PIH. We also measured plasma renin activity, Ang II, and aldosterone in blood samples from these women and sodium and creatinine in 24-hour urine collections. Normotensive pregnant women had significantly less platelet Ang II binding than nonpregnant, postpartum women (0.85±0.19 versus 2.87±0.83%, p=0.003), reflecting a reduction in receptor number but not affinity. This probably reflects the significant increase in Ang II during pregnancy. Urinary sodium excretion was equivalent and could not explain these changes. Comparisons of third trimester women with PIH against those without PIH documented a significantly higher Ang II binding in the women with PIH (2.23±0.42 versus 0.85±0.19%) that was caused by an increase in receptor number (6.0±1.3 versus 3.0±0.8 fmol Ang II per 5.6×10⁶ platelets, p=0.047) but similar Ang II binding affinity. This reflected significantly lower Ang II levels. There was no difference in urinary sodium excretion between the groups. In the postpartum period there were no differences in Ang II receptor binding and plasma hormone levels between women with previously normotensive versus hypertensive pregnancies. These results suggest that the enhanced Ang II sensitivity found in pregnant women with PIH is due to increased Ang II receptor number, which reflected relative reductions in Ang II levels. (Hypertension 1992;20:627–632)

KEY WORDS • angiotensin II • renin-angiotensin system • preeclampsia

Although the cause of pregnancy-induced hypertension (PIH) is unknown, it has been reported that women in whom this disorder develops have a more pronounced response to the potent vasoconstrictor angiotensin II (Ang II) relative to normotensive pregnant women.1 This enhanced sensitivity to Ang II precedes the development of high blood pressure and may reflect mechanisms that are in part responsible for the resulting PIH.2,3 However, current data suggest that after PIH is established, therapeutic use of an Ang II antagonist does not reduce blood pressure.4 In animals, vascular smooth muscle7 and kidney8 Ang II receptor number change inversely with the circulating Ang II concentrations. Thus, increased plasma Ang II causes a feedback “downregulation” of receptor number. These same changes occur in human platelets: high salt intake upregulates and low salt downregulates the number of Ang II receptors. It has also been shown that subjects with reduced Ang II receptor number have less pressor response to infused Ang II.9

In the normal pregnant state, Ang II is part of a hormonal system involved in the homeostasis of salt and water. Ang II is a secretagogue for the salt-conserving hormone aldosterone.5 As sodium intake decreases, plasma Ang II, and consequently aldosterone, increases.6 The converse is also true, and Ang II can be suppressed by increased salt intake.6 In animals, vascular smooth muscle7 and kidney8 Ang II receptor number change inversely with the circulating Ang II concentrations. Thus, increased plasma Ang II causes a feedback “downregulation” of receptor number. These same changes occur in human platelets: high salt intake upregulates and low salt downregulates the number of Ang II receptors. It has also been shown that subjects with reduced Ang II receptor number have less pressor response to infused Ang II.9

In the normal pregnant state, there is an increase in circulating levels in plasma renin activity (PRA), Ang II, and aldosterone.10 At the same time, vascular Ang II sensitivity is greatly reduced as early as 14 weeks gestation.1 In women in whom PIH develops, there is a reduction in Ang II sensitivity early in pregnancy, but this reduction is lost after 22 weeks gestation and predates the development of PIH.1 Whether this is due to an alteration in salt intake or volume status, a reduction in circulating Ang II levels, factors that control Ang II synthesis or degradation, or other factors is not well established. Only recently has it been reported that there is reduced Ang II binding to human platelets in normal pregnancy11 and a relatively increased Ang II binding in PIH.11 However, this latter study did not determine whether this increased “binding” was due to an increase in Ang II receptor number

From the Division of Endocrinology and Hypertension, Brigham and Women’s Hospital, Harvard Medical School, Boston, Mass.

Supported by grants HD-24499 and HL-36568 from the National Institutes of Health, Bethesda, Md. Support for portions of this project were provided through a General Clinical Research Center (RR 02635).

Address for correspondence: Dr. Steven Graves, Division of Endocrinology and Hypertension, Brigham and Women’s Hospital, 221 Longwood Avenue, Boston, MA 02115.

Received April 14, 1992; accepted in revised form July 20, 1992.
or affinity. In addition, there was no assessment of sodium intake in any of their study groups, and other significant hormones (e.g., PRA and aldosterone) were not measured, precluding an explanation of why and in what way Ang II binding was altered.

To answer the questions of how Ang II might interact with its receptor in PIH and whether there are changes in receptor number or affinity, we measured specific Ang II binding to its receptor in platelets from normotensive pregnant women in each trimester of pregnancy and postpartum. We also studied potential differences in specific Ang II binding in third trimester women with PIH and in those without PIH. By constructing concentration-binding profiles, we were able to calculate the receptor capacity and affinity. PRA, Ang II, and aldosterone and urinary sodium and creatinine were assessed in these same women.

Methods

Patient Population

These were cross-sectional studies. Pregnant women during each trimester of pregnancy and other women at least 6 weeks postpartum (6 weeks to 18 months) were recruited from the antenatal clinics and obstetrical wards of Brigham and Women’s Hospital. All women gave voluntary consent to participate in investigational protocols previously approved by the Human Subjects Committee of the hospital. Blood pressures were measured at random times of day while the subject was in a seated position. All women termed normotensive were normotensive at the outset of pregnancy and had normotensive blood pressure measurements throughout the pregnancy and the postpartum period. Women were considered to have PIH if they showed increases in diastolic blood pressure of ≥15 mm Hg or in systolic blood pressure of ≥30 mm Hg over first trimester values and had an absolute blood pressure of at least 140 mm Hg systolic or 85 mm Hg diastolic on at least two measurements 6 hours apart. Women with PIH were considered to have proteinuria (preeclampsia) if urinary protein excretion exceeded 300 mg/24 hr at the time of blood sampling. Pregnant women in the first trimester were between 10 and 12 weeks of gestation; those in the second trimester were between 20 and 22 weeks gestation; those in the third trimester were 30 weeks or greater. None of the women had major intercurrent disease; none were taking medications (excluding antenatal vitamins) except one woman with PIH who was taking aspirin (80 mg b.i.d.). Among the third trimester women, three of 18 normotensive women and three of 13 women with PIH were black. Blood was drawn at random times of day with women in either the sitting position or in the left lateral decubitus position. In a subset of women PRA measured in both positions was the same (n = 7: sitting, 7.1 ± 2.4 versus LLD, 7.3 ± 1.8 ng angiotensin I • ml⁻¹ • min⁻¹). A 24-hour urine collection was also begun the day before the blood was drawn. Specimens for Ang II receptor analysis were drawn in EDTA tubes and kept at 4°C until assayed. Specimens for PRA, Ang II, and aldosterone were drawn and kept on ice until centrifugation; plasma was then aliquotted, quickly frozen, and maintained that way until assay.

Assays

Plasma renin activity, angiotensin II, and aldosterone and urinary sodium and creatinine. PRA and Ang II were measured by radioimmunoassay (RIA) following previously published procedures. Plasma aldosterone was quantitated by a commercially available RIA (Diagnostic Products, Los Angeles, Calif.). The concentration of sodium in urine was determined by ion selective electrode (NOVA I, Nova Biomedical, Newton, Mass.). Urinary creatinine was measured by autoanalyzer (Beckman Instruments, Irvine, Calif.).

Human platelet preparation. This followed our previously published procedure. In brief, approximately 30–40 ml EDTA blood was obtained the morning of the experiment. Red blood cells were removed from the specimen by centrifugation at 2000 x g for 10 minutes at room temperature. The platelet-rich plasma was then transferred to separate tubes and the platelets collected by a second centrifugation step (1,200 x g for 10 minutes at room temperature). The platelet-poor plasma was aspirated away and discarded, and the platelet pellet was gently resuspended in 2.0 ml Medium 199 containing 5.0 mM EDTA, 0.1% diisopropylfluorophosphate, and 0.5% bovine serum albumin (Fraction V), pH 7.40 (incubation buffer). The cell number was determined with a Reichert Hemacytometer and adjusted to 7 x 10⁸ platelets per milliliter by adding incubation buffer.

Platelet angiotensin II binding assay. The method used has been detailed previously. Binding was measured by incubating 400-μl aliquots of platelet suspension with tracer amounts of iodine-125-labeled Ang II (New England Nuclear, Billerica, Mass.) and graded concentrations of unlabeled Ang II (Sigma Chemical Co., St. Louis, Mo.) (final volume 500 μl, final platelet concentration 5.6 x 10⁹ platelets per milliliter incubate). Cells were incubated for 90 minutes at 22°C in an Eberbach shaker bath. Separation of platelet-bound Ang II from unbound Ang II was accomplished by microcentrifugation of duplicate 200-μl aliquots of the incubation mixture (Microfuge B. Beckman) for 90 seconds through oil (dibutyl phthalate/dinonyl phthalate, 8:1). The platelet pellet in the apex of the microfuge tube, representing bound Ang II, was cut away and counted as “bound” hormone, whereas the supernatant, representing free Ang II, was collected and counted separately. Ang II binding data are reported as specific binding of Ang II (B), after subtraction of any radioactivity bound in the presence of an excess (1.0 μM) of Ang II (nonspecific binding) from the total Ang II bound. Platelet Ang II affinity (Kd) and binding capacity were determined by Scatchard analysis. For some women, specific platelet Ang II binding was too low to allow meaningful Scatchard analysis (i.e., <0.5% specifically bound Ang II). For these subjects we report only percentage of specific binding.

Statistical Analysis

Analysis of variance was used to evaluate changes that occurred in each trimester of normal pregnancy and in the postpartum period. Student’s t test was used for comparison of third trimester pregnant women both with and without PIH and for comparison of some specific variables before delivery and postpartum. Assessment of the potential interrelation of two parame-
TABLE 1. Longitudinal Studies in Normotensive Pregnant and Postpartum Women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1st trimester (n=2)</th>
<th>2nd trimester (n=11)</th>
<th>3rd trimester (n=18)</th>
<th>Postpartum (n=7)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uo&lt;sub&gt;s&lt;/sub&gt; (mg/day)</td>
<td>89±31</td>
<td>102±5</td>
<td>98±6</td>
<td>96±38</td>
<td>NS</td>
</tr>
<tr>
<td>U&lt;sub&gt;Crea&lt;/sub&gt; (mg/day)</td>
<td>955±26</td>
<td>1,178±60</td>
<td>1,205±47</td>
<td>986±172</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng Ang I • ml&lt;sup&gt;-1&lt;/sup&gt; • min&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8.9±1.1</td>
<td>5.6±1.1*</td>
<td>9.3±1.5*</td>
<td>1.3±0.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Ang II (pg/ml)</td>
<td>31.0±4.0</td>
<td>39.7±3.5*</td>
<td>39.5±2.4*</td>
<td>13.5±2.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aldosterone (ng/dl)</td>
<td>42.5±6.5</td>
<td>63.8±5.7*</td>
<td>121.0±9.7</td>
<td>21.8±8.9</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

U<sub>o</sub>s, urinary sodium excretion; U<sub>Crea</sub>, urinary creatinine excretion; PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II.

*p<0.05, **p<0.01 vs. postpartum values.

We then studied other third trimester women with a diagnosis of PIH. The general demographic information for this population and the third trimester, normotensive women are displayed in Table 3. Both groups had equivalent maternal and gestational ages and similar racial composition. Blood pressures differed significantly by protocol design. Six of 13 (46%) women with PIH were nulliparous, whereas eight of 18 (44%) normotensive, third trimester women were nulliparous. This did not represent a significant difference. Comparison of the plasma PRA, Ang II, and aldosterone values are also found in Table 3. Third trimester women with PIH showed markedly lower PRA (2.2±0.6 versus 9.3±1.5 ng angiotensin I • ml<sup>-1</sup> • min<sup>-1</sup>, p=0.0003), Ang II (27.8±1.5 versus 39.5±2.4 pg/ml, p=0.0006), and aldosterone (43.9±10.7 versus 121.0±9.7 ng/dl, p=0.0001) values compared with those of normotensive third trimester women. Twenty-four-hour urinary sodium and creatinine values, however, were very similar. The platelet Ang II binding studies for third trimester women with PIH and without PIH as well as postpartum data are summarized in Table 4. Platelet Ang II binding was substantially greater in women with PIH compared with that in their normotensive counterparts (2.23±0.42 versus 0.85±0.19*, p=0.003) (Figure 1). This increased specific binding reflected an increase in capacity (6.0±1.3 versus 3.0±0.8 fmol Ang II per 5.6×10<sup>8</sup> platelets, p=0.047) as opposed to changes in Ang II binding affinity (2.26±0.23 versus 2.03±0.19×10<sup>-6</sup> M, p=0.47). Only three of 13 women with PIH had specific binding so low (<0.5%) as to make Scatchard analysis of capacity and affinity unreliable, whereas nine of 18 of the normotensive, third trimester women had Ang II binding <0.5% (p=0.16). Postpartum, the women with a previously normotensive pregnancy looked similar to those women who had had PIH for all parameters measured (Table 4).

Women with PIH, when classified by the presence (preeclampsia) or absence of proteinuria, showed simi-
Ang II in particular showed an activation of the PRA, Ang II, and aldosterone. This would explain the previously reported refractoriness to exogenous Ang II as previously found in normotensive pregnant women, hypertensive pregnant and postpartum women. We found that specific binding of Ang II during pregnancy was similar in women with previous normotensive or hypertensive pregnancies. Binding was similar in women with previous normotensive or hypertensive pregnancies. We found no significant differences between the two groups in terms of maternal age, gestational age, racial composition, and parity. When we measured the plasma and urine values, we found that urinary sodium excretion was quite similar between the two groups. However, PRA, Ang II, and aldosterone were all significantly lower in the women with PIH. This has been a frequent but not an invariable finding, particularly for the lower Ang II levels.

Characterization of the interaction of Ang II with its platelet receptor demonstrated that women with PIH had significantly higher specific binding of Ang II to its receptor than third trimester, normotensive women. There were nine of 18 third trimester, normotensive women whose binding was so low as to preclude calculating affinity or specificity. This contrasts with only three of 13 for the group with PIH. In the nine normotensive and 10 PIH women whose Scatchard analysis was possible, the affinity constants were similar between the two parameters was similar to that found previously in normotensive women.

**Discussion**

We found that specific binding of Ang II during normotensive pregnancy was only 30% of the postpartum levels. Given the similar binding affinities during pregnancy and postpartum and the lower Ang II binding capacity during pregnancy for these subjects' platelets, we conclude that this reduced binding of Ang II during pregnancy is due to a reduction in receptor number. This would explain the previously reported refractoriness of pregnant women to administered Ang II. We also found, as had been reported previously, that there was an activation of the PRA, Ang II, and aldosterone system during pregnancy. Ang II in particular showed a markedly increased plasma level as early as the first trimester and by the second and third trimesters was approximately threefold higher than in the postpartum period. Aldosterone in contrast showed a graded increase from the first to the third trimester (fivefold to sixfold). Based on these findings, the reduction in platelet Ang II receptor number appears to be a feedback response to the increased circulating Ang II levels. Note, urine sodium excretion in each trimester of pregnancy and after delivery were similar. Thus, the differences in Ang II levels between pregnant and nonpregnant women are not explained by differences in their sodium balance.

When third trimester women with PIH were compared with third trimester, normotensive women, we found no significant differences between the two groups in terms of maternal age, gestational age, racial composition, and parity. When we measured the plasma and urine values, we found that urinary sodium excretion was quite similar between the two groups. However, PRA, Ang II, and aldosterone were all significantly lower in the women with PIH. This has been a frequent but not an invariable finding, particularly for the lower Ang II levels.

Characterization of the interaction of Ang II with its platelet receptor demonstrated that women with PIH had significantly higher specific binding of Ang II to its receptor than third trimester, normotensive women. There were nine of 18 third trimester, normotensive women whose binding was so low as to preclude calculating capacity or affinity. This contrasts with only three of 13 for the group with PIH. In the nine normotensive and 10 PIH women whose Scatchard analysis was possible, the affinity constants were similar between the two groups, whereas the capacity, i.e., the receptor number, was significantly greater in women with PIH. Again the higher receptor number found in women with PIH is probably a response to the significantly reduced plasma levels of Ang II. This increase in Ang II receptor number could then confer the relatively increased sensitivity to exogenous Ang II as previously found in pregnant women with PIH. Given the similarity of urinary sodium excretion between the two groups, the markedly reduced Ang II levels in women with PIH do not appear to be due to differences in sodium balance. It is of interest to note that postpartum platelet Ang II binding was similar in women with previous normotensive or hypertensive pregnancies.

**Table 3.** Subject Data for Third Trimester Normotensive and Pregnancy-Induced Hypertensive Women

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PIH</th>
<th>Normotensive</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 13)</td>
<td>(n = 18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>31.2±1.0</td>
<td>29.8±0.9</td>
<td>0.31</td>
</tr>
<tr>
<td>Gestational age (years)</td>
<td>35.2±0.9</td>
<td>35.5±0.4</td>
<td>0.75</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>139.2±3.3</td>
<td>105.2±2.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>95.8±1.8</td>
<td>67.2±1.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>U Na (meq/day)</td>
<td>104±14</td>
<td>98±6</td>
<td>NS</td>
</tr>
<tr>
<td>U cre (mg/day)</td>
<td>1,311±106</td>
<td>1,204±47</td>
<td>NS</td>
</tr>
<tr>
<td>PRA (ng Ang I·ml⁻¹·min⁻¹)</td>
<td>2.2±0.6</td>
<td>9.3±1.5</td>
<td>0.0003</td>
</tr>
<tr>
<td>Ang II (pg/ml)</td>
<td>27.8±1.5</td>
<td>39.5±2.4</td>
<td>0.0006</td>
</tr>
<tr>
<td>Aldo (ng/dl)</td>
<td>43.9±10.7</td>
<td>121.0±9.7</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

PIH, pregnancy-induced hypertension; SBP, systolic blood pressure; DBP, diastolic blood pressure; U Na, urinary sodium excretion; PRA, plasma renin activity; Ang I, angiotensin I; Ang II, angiotensin II; Aldo, aldosterone.

**Table 4.** Comparisons of Normotensive Pregnant and Pregnancy-Induced Hypertensive Women During the Third Trimester and the Postpartum Period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Normotensive</th>
<th>PIH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 13)</td>
<td>(n = 18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd trimester</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₚ (%)</td>
<td>0.85±0.19</td>
<td>2.23±0.42</td>
<td>0.003</td>
</tr>
<tr>
<td>Capacity (fmol Ang II/5.6×10⁶ platelets)</td>
<td>3.0±0.8</td>
<td>6.0±1.3</td>
<td>0.047</td>
</tr>
<tr>
<td>Kₖ (×10⁻¹⁵ M)</td>
<td>2.03±0.19</td>
<td>2.26±0.23</td>
<td>0.47</td>
</tr>
<tr>
<td>Postpartum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bₚ (%)</td>
<td>2.87±0.83</td>
<td>3.81±1.80</td>
<td>0.65</td>
</tr>
<tr>
<td>Capacity (fmol Ang II/5.6×10⁶ platelets)</td>
<td>6.6±1.7</td>
<td>8.5±3.4</td>
<td>0.62</td>
</tr>
<tr>
<td>Kₖ (×10⁻¹⁵ M)</td>
<td>2.04±0.12</td>
<td>2.28±0.39</td>
<td>0.58</td>
</tr>
</tbody>
</table>

PIH, pregnancy-induced hypertension; Bₚ, specific binding; Ang II, angiotensin II; Kₖ, dissociation constant.
Ang II in parallel with the receptor in the vasculature as opposed to its regulation in the adrenal. The fact that Ang II binding to the platelet has been previously shown to cause changes in cytosolic calcium suggests that the human platelet Ang II receptor represents the type 1 subtype, which has been shown to be coupled to changes in cell calcium and phosphoinositide turnover (as opposed to the type 2 subtype, which is not). The lower plasma Ang II levels in our PIH subjects may be the result of reduced PRA, but this reduction in PRA, Ang II, and aldosterone is independent of salt balance. Clearly additional investigation of renin, PRA, and Ang II and their precise regulation and interrelation to volume and salt homeostasis in the mother, and perhaps the fetus, is still needed.

Acknowledgments
We express our gratitude to Derna DeMaggio, MD, for help with patient recruitment and to Sandra Cook, RN, for the excellent nursing support provided.

References

As indicated above, several previous studies have documented significantly lower PRA, Ang II, and aldosterone levels in women with PIH compared with those in their normotensive counterparts. A number of factors could potentially affect renin activity, including prostacyclin and progesterone. Whether alterations in these factors contribute to the reduced PRA seen in PIH has not yet been established, but regardless of the explanation, our studies suggest that the enhanced vascular sensitivity to exogenous Ang II seen in women with PIH is a consequence of increased Ang II receptor sites on target cells, which is a response to lower circulating Ang II. Previously, studies have suggested that the Ang II receptor on the platelet is regulated by the ban. All, angiotensin II.

FIGURE 1. Bar graphs show comparison of platelet angiotensin II (Ang II) binding in third trimester women with normotensive (NT) pregnancies (left) and those with pregnancy-induced hypertension (PIH) (right). Shown are the mean±SEM specific Ang II binding (B, top panel), capacity (middle panel), and the Ang II binding affinity (K, bottom panel) for both groups. The probability values appear between the bars. All, angiotensin II.

ACKNOWLEDGMENTS
We express our gratitude to Derna DeMaggio, MD, for help with patient recruitment and to Sandra Cook, RN, for the excellent nursing support provided.

REFERENCES
Increased platelet angiotensin II receptor number in pregnancy-induced hypertension.
S W Graves, T J Moore and E W Seely

_Hypertension_. 1992;20:627-632
doi: 10.1161/01.HYP.20.5.627

_Hypertension_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://hyper.ahajournals.org/content/20/5/627

_Permisssions:_ Requests for permissions to reproduce figures, tables, or portions of articles originally published in
_Hypertension_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial
Office. Once the online version of the published article for which permission is being requested is located, click
Request Permissions in the middle column of the Web page under Services. Further information about this
process is available in the Permissions and Rights Question and Answer document.

_Reprints:_ Information about reprints can be found online at:
http://www.lww.com/reprints

_Subscriptions:_ Information about subscribing to_Hypertension_ is online at:
http://hyper.ahajournals.org//subscriptions/