Bromocriptine Regulates Angiotensin II Response on Sodium Pump in Proximal Tubules

Tahir Hussain, Renee Abdul-Wahab, Dharmi K. Kotak, Mustafa F. Lokhandwala

Abstract—Dopamine and angiotensin II (Ang II) receptors have been reported to exhibit an interaction in renal proximal tubules. The present study was designed to investigate the regulation by a D2-like dopamine receptor of Ang II–mediated stimulation of Na,K-ATPase activity in the renal proximal tubules. Ang II (10^{-13} to 10^{-8} mol/L) stimulated Na,K-ATPase activity in the proximal tubules that was completely abolished when the tubules were pretreated with the D2-like receptor agonist bromocriptine (1 μmol/L) for 30 minutes. The effect of bromocriptine on Ang II response was prevented by domperidone (1 μmol/L), a D2-like dopamine receptor antagonist. Similarly, the inhibition of forskolin (1 μmol/L)–induced cAMP accumulation caused by Ang II (10 pmol/L) was also abolished in bromocriptine-pretreated tubules. Basal and forskolin-stimulated cAMP was not significantly different in bromocriptine-treated tubules compared with the control. [3H]-Ang II binding sites (angiotensin type 1 [AT1] receptors) were reduced by ∼65% in bromocriptine-treated proximal tubules, a result that was further substantiated by Western blot analysis revealing a 50% decrease in AT1 receptors in bromocriptine-treated tubules compared with the control. Western blot analysis of G proteins revealed a 2-fold increase in Gsα and a 20% decrease in Gsα1 and Gsα2 in the bromocriptine-treated proximal tubules. Bromocriptine (1 μmol/L) alone stimulated Na,K-ATPase activity during the first 30 minutes of incubation, and thereafter the stimulation fell to the basal level. Similarly, bromocriptine-mediated inhibition of cAMP lasted only up to 20 minutes. The data suggest that preactivation of D2-like dopamine receptors abolishes Ang II–mediated stimulation of Na,K-ATPase activity and inhibition of cAMP accumulation. This phenomenon may be a consequence of a decrease in AT1 receptors and alterations in G protein levels in the proximal tubules. We propose that such a regulation of Ang II response by bromocriptine is the result of heterologous desensitization of the D2-like receptor system. (Hypertension. 1998;32:1054-1059.)

Key Words: receptor, dopamine ■ receptor, angiotensin ■ sodium pump ■ G protein ■ kidney tubules, proximal

Angiotensin (Ang) II and dopamine are 2 important regulators of sodium and water transport across the renal proximal tubules. Ang II receptors, mainly angiotensin type 1 (AT1) subtypes, have been shown to exist on both basolateral and brush border membranes. The activation of AT1 receptors by Ang II (picomolar) causes an increase in sodium reabsorption by proximal tubules. The activation of dopamine receptors, also present on the proximal tubules, leads to an inhibition of the tubular transport of sodium and thus promotes sodium and water excretion. The opposing actions of Ang II and dopamine on sodium transport result from the opposite effects of these hormones on sodium-transporting enzymes, ie, Na,H-exchanger on the brush border and Na,K-ATPase on the basolateral membranes. Whereas Ang II (pmol/L) causes activation of Na,H-exchanger and Na,K-ATPase activities, dopamine produces inhibition of these enzyme activities by activating D2-like dopamine receptors. Because of the opposite actions on tubular sodium transport of these 2 hormones, intrarenally produced Ang II has been shown to oppose the natriuretic response to D2-like receptor agonist in rats. Dopamine and the agonists at D1-like and D2-like receptors alone have been reported to antagonize the actions of Ang II on 22Na uptake by brush border vesicles and to decrease the Ang II binding sites on the vesicles. Cheng et al reported that dopamine, by activating D1-like receptors, decreases the expression of AT1 receptors in cultured proximal tubule cells, an effect likely mediated by increased intracellular cAMP.

Recently, we have shown that Ang II (pmol/L) stimulated Na,K-ATPase activity in the proximal tubules through a pertussis toxin–sensitive G protein (likely Gi) and the inhibition of the cAMP pathway. Similarly, D2-like receptor agonist bromocriptine produced stimulation of Na,K-ATPase activity through a pertussis toxin–sensitive G protein and the inhibition of the adenylyl cyclase–linked pathway in the proximal tubules and in murine cells transfected with D2Long receptor cDNA. These findings demonstrate that the stimulatory action of Ang II and bromocriptine on Na,K-ATPase activity share the adenylyl cyclase–linked cellular signaling mechanism as a common pathway. We hypothe-
ized that the effect of D₂-like dopamine receptor agonist on the attenuation of Ang II response may be because of a mechanism related to heterologous desensitization process. Therefore, in the present study, Ang II–mediated stimulation of Na,K-ATPase activity was examined in the bromocriptine-pretreated proximal tubules, and subsequently Ang II binding, AT₁ receptors, G proteins, and cAMP were measured to investigate the mechanism of AT₁, Ang II and D₂-like dopamine receptor interaction.

Methods
Male Sprague-Dawley rats of ∼200 g weight (Harlan Sprague-Dawley, Inc, Indianapolis, Ind) were purchased and housed in plastic cages in an air-conditioned animal care facility. The animals were fed with standard rat chow (Purina Mills) and given tap water ad libitum.

Isolation and Enrichment of Renal Proximal Tubules
Rats were anesthetized with sodium pentobarbital (50 mg/kg IP). Renal cortical tubular suspensions were prepared according to the method of Gesek et al. with slight modifications adopted in our laboratory. Protein was assayed by use of a kit from Pierce, and bovine serum albumin was used as standard.

Na,K-ATPase Assay
The proximal tubular suspension (1 mg protein/mL) was divided into 2 sets: one was incubated with bromocriptine (1 μmol/L) and the other served as control. After incubation at 37°C for 30 minutes in a shaking water bath, Ang II was added (10⁻¹⁰ to 10⁻⁸ mol/L) to both sets, and the incubation was continued for another 30 minutes (a total of 60 minutes of bromocriptine presence). In another set of experiments, the tubules were incubated with bromocriptine (1 μmol/L) for varying time periods (10 to 60 minutes) to examine the effect of time on Na,K-ATPase stimulation by bromocriptine. After incubation, the tubules were made permeable by rapid freezing in dry ice/acetone and thawing. Subsequently, Na,K-ATPase activity was measured by the method of Quigley and Gotterer adopted in our laboratory with slight modifications. Na,K-ATPase activity was expressed as nanomoles P₃ per milligram of protein per minute.

cAMP Assay
The treatment of proximal tubular suspension (7 to 8 mg/mL protein) was performed in a way similar to that described in the previous section. Two sets of tubules were incubated with bromocriptine (1 μmol/L) for 30 minutes at 37°C in a shaking water bath; then Ang II was added and the incubation was continued for another 30 minutes. All the incubations were performed in the presence of forskolin (1 μmol/L). The tubules were also incubated with bromocriptine for varying periods of time (5 to 60 minutes). The reaction was terminated by putting the samples in a boiling water bath for 3 to 5 minutes. The samples were centrifuged, and the supernatant was used for cAMP measurement as we have described earlier. The values of cAMP were calculated and expressed as picomoles per milligram of protein by use of the cAMP standard curve (0.25 to 8 pmol) run in parallel.

Receptor-Ligand Binding
The proximal tubules were treated with bromocriptine for 60 minutes, which was the total amount of bromocriptine exposure time in Na,K-ATPase and cAMP assay protocols. The samples were homogenized in 10 mmol/L Tris-HCl buffer containing 1 mmol/L MgCl₂ and 2 mmol/L PMSF (buffer A) and centrifuged at 34 000 × g for 30 minutes followed by 2 washes with buffer A. Finally, the pellets as the proximal tubular membranes were collected and suspended in Tris-HCl buffer, 50 mmol/L, pH 7.4, containing 1 mmol/L MgCl₂ (buffer B) and used for ligand binding as well as for Western blot analysis of AT₁ receptors and G proteins (described below).

For binding, we incubated 10 to 12 nmol/L (Kₐ of Ang II for AT₁ receptor) of [³H]-Ang II with 50 μg of proximal tubular membrane in buffer B. To determine the nonspecific binding, we added cold Ang II (1 μmol/L) to the binding assay. The binding was terminated by rapid filtration under vacuum using GF/C glass fiber filters followed by 3 washes of 4 mL each with buffer B. The filter papers were extracted in scintillation fluid, and the radioactivity was counted in liquid scintillation counter at an efficiency of ∼60%. Nonspecific binding was 20% of the total [³H]-Ang II binding.

Western Blot Analysis of AT₁ Receptors and G Proteins
The proteins were solubilized in Laemml buffer, and 25 μmol/L proteins (control and treated) were resolved by SDS–polyacrylamide gel (10%) electrophoresis. The resolved proteins were electro-photographically transferred onto immobilin P membrane (blot). The blot was incubated with polyclonal affinity-purified antibodies for AT₁ receptor, Gα₃, Gα₂₅, or Gα₁₁, for 1 hour. After 3 washes in Tris-buffered saline, the blot was incubated with anti-rabbit IgG-horseradish peroxidase conjugate. The signal was detected using chemiluminescent substrate and recorded on x-ray film. Density of the bands was analyzed using a Sharp color scanner. Prestained markers were used for calculating the molecular weight of the bands and to ascertain the transfer of proteins from the gel.

The antibodies are polyclonal, purified, and antipeptides. The amino acid sequence of the peptides, which is specific to the respective proteins, is as follows: QDDCPKAGRHC corresponding to 15 to 24 position on AT₁ receptor; RHMRLRQYELL corresponding to C-terminal position 385 to 394 on Gα₁₁; KNNLKDCCGLF corresponding to C-terminal common positions 345 to 154 and 346 to 355 on Gα₁₁ and Gα₁₁; and QLNLKEYNLV corresponding to C-terminal position on Gα₁₁. The AT₁ antibodies do not cross-react with AT₂ receptors. Similarly, the specificity of G protein antibodies was confirmed with the lysates from separate cultures of bacteria transformed with cDNA for various G protein α-subunits.

Data Analysis
The values were presented as mean±SEM and subjected to 2-way ANOVA for concentration response curves; 1-way ANOVA was used for 2 variables and for t test for the single concentration responses. A value of P<0.05 was considered statistically significant.

Chemicals
Bromocriptine was a gift from Sandoz Pharmaceuticals (E Nanover, NJ). Domperidone was purchased from Research Biochemicals Intl. Forskolin was purchased from Sigma Chemical Co. The kit for cAMP assay was purchased from DuPont NEN. [³H]-Ang II was purchased from Amersham. The kit for Western blot analysis was purchased from Alpha Diagnostic Intl. Other chemicals for various buffers were of the highest purity available and were purchased either from Sigma or Fisher Scientific Co. The antibodies for AT₁ receptors and G proteins were purchased from Santa Cruz Biotechnology, Inc and Calbiochem-Novabiochem, respectively.

Results
Effect of Bromocriptine Pretreatment on Ang II–Induced Stimulation of Na,K-ATPase Activity
Ang II produced a concentration-dependent (10⁻⁵ to 10⁻⁹ mol/L) stimulation in Na,K-ATPase activity. The maximal stimulation was observed at 10 pmol/L of Ang II (Figure 1). Pretreatment of the tubules with 1 μmol/L bromocriptine for 30 minutes abolished the stimulatory effect of Ang II (Figure 1). Domperidone (1 μmol/L), a D₂-like receptor antagonist, prevented the effect of bromocriptine on Ang II response.
Bromocriptine alone did not influence Na,K-ATPase activity after the total incubation time of 60 minutes (30 minutes of pretreatment and 30 minutes of further incubation with Ang II). The basal values of Na,K-ATPase activities in control and bromocriptine-pretreated tubules were 230±12 and 239±19 nmol Pi/mg protein per minute, respectively. Simultaneous addition of bromocriptine (1 μmol/L) and Ang II (10 pmol/L, ECmax) to the tubules and incubation (for 30 minutes) stimulated Na,K-ATPase activity in a way similar to the stimulation produced by 10 pmol/L of Ang II alone (data not shown).

**Effect of Bromocriptine Pretreatment on Ang II–Mediated Inhibition of cAMP**

Ang II (10 pmol/L) produced significant inhibition in cAMP accumulation stimulated by 1 μmol/L forskolin (Figure 2). Bromocriptine (1 μmol/L) pretreatment for 30 minutes completely prevented the inhibitory effect of angiotensin on cAMP accumulation (Figure 2). Bromocriptine alone over an incubation period of 60 minutes did not significantly affect the basal cAMP level (Figure 2, open columns).

**Effect of Incubation Time on Bromocriptine-Mediated Inhibition of cAMP Accumulation and Stimulation of Na,K-ATPase Activity**

Bromocriptine had no effect on Na,K-ATPase activity or on cAMP levels after an incubation period of 60 minutes (Figures 1 and 2). To investigate the effect of incubation time, we incubated the proximal tubules with bromocriptine (1 μmol/L) for varying periods (5 to 60 minutes), and the inhibition in the levels of cAMP accumulation and the stimulation of Na,K-ATPase activity were measured. Figure 3 shows that the inhibition of cAMP accumulation is maximal at 20 minutes of incubation, and it falls dramatically at 30 minutes (>10%) with no inhibition at 60 minutes of incubation. The stimulation of Na,K-ATPase activity has a similar pattern. However, the maximal stimulation of Na,K-ATPase activity by bromocriptine is at 30 minutes and falls sharply at 40 minutes (>10%); negligible stimulation occurs at 50 and 60 minutes of incubation.

**Effect of Bromocriptine Pretreatment on [3H]-Ang II Binding and Quantity of AT1 Receptor**

In this set of experiments, we chose a concentration of 10 to 12 nmol/L [3H]-Ang II, which has been reported as its Kd at AT1 receptors.14 Figure 4A shows that bromocriptine treatment produced an ≈65% decrease in specific binding of [3H]-Ang II (control 745±77 versus treated 264±24 fmol/mg protein) in proximal tubular membranes. The reduced Ang II binding was also confirmed by Western blot analysis of AT1 receptor using specific antibody. We detected 1 major band of ∼49 kDa and another minor band of 55 kDa (Figure 4B). The density of the 49-kDa band was reduced by 50% in the membranes of bromocriptine-treated proximal tubules. The density of the minor 55-kDa band was similar in the control and the treated tubules (Figure 4B). The 49-kDa protein represents glycosylated AT1 receptor.22

**Quantification of G Proteins**

Prolonged incubation of tissues/cells with receptor agonists may lead to an alteration in the levels of G proteins and...
signaling systems and may alter the receptor function. Because the incubation of proximal tubules with bromocriptine for 60 minutes abolished its own effects on both Na,K-ATPase activity and cAMP and also abolished the effects of Ang II, the levels of G proteins were measured by Western blot analysis in the proximal tubules treated with bromocriptine. Figure 5 shows that 60 minutes of incubation with bromocriptine increased the amount of Gs by 2-fold (in both Gs_short and Gs_long) and decreased Gi2 and Gi20. Gs and Gi regulate adenylyl cyclase in a stimulatory and inhibitory manner, respectively. Another protein, Gq/11, which does not regulate adenylyl cyclase but is present in the proximal tubules, was also measured as a control. The amount of Gq/11 was not altered in the proximal tubules incubated with bromocriptine compared with control (Figure 5).

**Discussion**

Dopamine and Ang II are 2 important regulators of sodium and water absorption in the kidney.\(^1,2\) During salt depletion, Ang II production and AT\(_1\) receptor expression increase in the proximal tubules, which subsequently causes an increased reabsorption of sodium and water.\(^2,3,4\) Conversely, sodium loading causes increased production of dopamine that, through the activation of D\(_2\)-like dopamine receptors, promotes renal sodium and water excretion.\(^5\) It has been shown that dopamine can downregulate sodium uptake in response to Ang II in renal brush border membrane vesicles.\(^14\) Preactivation of D\(_2\)-like receptors decreases expression of the AT\(_1\) receptors in the proximal tubules, possibly due to increased production of cAMP.\(^13\) The present study, which was conducted to examine the interaction between D\(_2\)-like receptors and Ang II, shows that bromocriptine, a D\(_2\)-like receptor agonist, downregulates Ang II binding sites and causes imbalance in the G/Gi proteins. This phenomenon may contribute to the abolished response of Ang II on the stimulation of Na,K-ATPase activity and the inhibitory effect on cAMP accumulation in the proximal tubules treated with bromocriptine.

In contrast to the role of the D\(_1\)-like receptors, the physiological role of D\(_2\)-like receptors is not yet well defined in the kidney. However, the activation of D\(_2\)-like receptors has been proposed to synergize the effect of D\(_1\)-like receptors on Na,K-ATPase activity in the proximal tubules,\(^26\) to inhibit norepinephrine release from postganglionic sympathetic nerves,\(^27\) and to produce antidiuretic effects.\(^28\) Recently, we have shown that the activation of D\(_2\)-like receptors stimulated Na,K-ATPase activity and inhibited cAMP accumulation in the proximal tubules.\(^17\) These effects of bromocriptine, as observed in this study, disappear after \(\approx 40\) minutes of incubation of the proximal tubules with the agonist, which suggests the existence of some regulatory mechanisms related to prolonged activation of D\(_2\)-like receptors. Such mechanisms may include desensitization or internalization of the D\(_2\)-like receptor. In the present study, we found that such a regulation of D\(_2\)-like receptors by pretreatment of proximal tubules with bromocriptine also abolished the stimulatory effect of Ang II on Na,K-ATPase activity and the inhibitory effect on cAMP accumulation. Furthermore, we found a decrease in Ang II binding sites, a 2-fold increase in the amount of G\(_2\), protein, and a decrease in G\(_i\) proteins in bromocriptine-treated tubules, which may have contributed to the failure of Ang II to stimulate Na,K-ATPase activity. The responses of Ang II and bromocriptine on Na,K-ATPase activity share at least 1 common signaling pathway, ie, pertussis toxin–sensitive G protein (likely G\(_i\)) linked inhibition of cAMP.\(^17\) Adenylyl cyclase/cAMP is regulated by G\(_s\) and G\(_i\) proteins in a stimulatory and inhibitory manner. An imbalance in the amount of G\(_s\) and G\(_i\) proteins may alter regulation of the adenylyl cyclase pathway. It is possible that prolonged activation of D\(_2\)-like receptors by bromocriptine decreased G\(_i\) and increased G\(_s\) to compensate for the inhibi-
ory pathway. Because AT$_1$ receptors are coupled to G$_i$, a decrease in the levels of G$_i$ and increased opposing basal tone of G$_s$ might have affected the response of Ang II. Alterations in the levels of G proteins have been shown in tissues/cells exposed to receptor agonist and in pathophysiological conditions such as hypertension and aging. 29–32 For example, coronary arteries exposed to A$_2$ adenosine receptor agonist produced a decrease in G$_s$ and an increase in G$_i$ proteins. 29 On the other hand, A$_1$ adenosine receptor agonist exposure produced an increase in G$_s$ and a decrease in G$_i$ proteins. 30 Such alterations in the levels of G proteins have been suggested to contribute to a change in the response not only of the receptors with which the agonist interacts but also of those receptors that are linked to these proteins. Such a phenomenon is referred to as heterologous desensitization.

Bromocriptine treatment of the proximal tubules also led to a reduction in Ang II binding sites. In an earlier study, both D$_1$-like and D$_2$-like agonists were shown to decrease Ang II binding sites without causing a change in the affinity and without reducing the stimulatory effects of Ang II on phospholipase A$_2$ and Na uptake by border brush membrane vesicles. 14 On the basis of the earlier study, 14 we propose that decrease in the Ang II receptor binding was not associated with any changes in the affinity. The reduction in [H]$^+$Ang II binding in the bromocriptine-treated tubules is further supported by Western blot analysis. Immunodetectable quantity of AT$_1$ receptors is reduced in the proximal tubules treated with bromocriptine compared with the controls. The decrease in AT$_1$ receptors by bromocriptine treatment may be because of (1) increased degradation of the receptor, (2) decreased synthesis of the receptor protein, or (3) both of these factors as contributors to this phenomenon. In another study, dopamine has been proposed to decrease AT$_1$ receptor expression in the proximal tubule cells, possibly due to a D$_1$-like receptor–mediated increase in cellular levels of cAMP. 15 The mechanism by which D$_2$-like receptor activation causes a decrease in Ang II binding sites and AT$_1$ receptors is not known. However, the regulatory mechanism of the D$_2$ receptor (a member of the D$_2$-like dopamine receptor family) and its signaling pathway have been studied in the transfected cell lines. 31 Quinpirole (1 $\mu$mol/L), a D$_2$-like dopamine receptor agonist, produced inhibition in forskolin-stimulated cAMP, 32 as we have found in the present study performed in the proximal tubules. Prolonged exposure of cells with quinpirole caused desensitization of the D$_2$-receptor and sensitization of adenylyl cyclase, leading to higher basal cAMP in the agonist-treated cells. 33 Although we did not detect significantly higher basal levels of cAMP in bromocriptine-treated proximal tubules, it is possible that a small increase in cAMP levels (15% to 20%) caused by prolonged exposure to bromocriptine may have contributed to a decrease in Ang II binding sites. The increase in cellular cAMP levels produced by dopamine (through the activation of D$_2$-like receptors) has been implicated as the mechanism by which D$_2$-like receptors regulate the AT$_1$ receptors. 15 On the basis of the above explanation, it may be speculated that both D$_1$-like and D$_2$-like receptor agonists decrease AT$_1$ receptors partly by a similar mechanism, despite the fact that D$_1$-like and D$_2$-like receptors are linked to adenylyl cyclase in an opposing (stimulatory and inhibitory, respectively) manner. Furthermore, it is suggested that regulation of AT$_1$ receptors may be linked with the changes in the G proteins. It is possible that the small increase in cAMP due to prolonged incubation with bromocriptine, which is implicated in the reduction of AT$_1$ receptors, may be the result of increased G$_s$ and decreased G$_i$ proteins.

The regulation of the Ang II response by the D$_2$-like receptors may be important under pathophysiological conditions. Recently, knockout of the D$_1$ receptor, a member of the D$_2$-like dopamine receptor family, increased renin-angiotensin activity and caused hypertension in mice, a condition that was reversed by the infusion of AT$_1$ receptor antagonist losartan. 14 This suggested that D$_2$-like receptors play a role in keeping the low tone of the renin-angiotensin system in the kidney. The D$_1$-like receptor that mediates natriuresis and diuresis is known to be defective in the proximal tubules of human primary hypertension and hypertensive animal models. 20,35,36 Under such a pathophysiological condition, the D$_1$-like receptor may not be able to antagonize the effects of Ang II as observed in normal animal models and in isolated proximal tubular preparations. 13–15 It remains to be investigated whether prolonged administration of D$_2$-like agonist can reduce the activity of Ang II by regulating the AT$_1$ receptor/signalizing pathway and promote sodium excretion in hypertensive animals, a response that may not be achieved by D$_1$-like agonist because of a defect in D$_1$-like receptor/signalizing system.

In summary, the present study has demonstrated that prior treatment of proximal tubules with bromocriptine abolished Ang II stimulation of Na,K-ATPase activity and inhibition of cAMP accumulation. The bromocriptine treatment of the proximal tubules was accompanied by a decrease in AT$_1$ receptors and alterations in G proteins, which may have contributed to the failure of Ang II to stimulate Na,K-ATPase activity and inhibit cAMP accumulation. We propose that heterologous desensitization of D$_2$-like receptors may be an underlying mechanism responsible for affecting the Ang II response on the sodium pump. The physiological significance of the D$_2$-like receptor regulation of the Ang II response in normal and disease states remains to be determined.

References

8. Hegde SS, Ricci A, Amenta F, Lokhandwala MF. Evidence from functional and autoradiographic studies for the presence of tubular...
Bromocriptine Regulates Angiotensin II Response on Sodium Pump in Proximal Tubules
Tahir Hussain, Renee Abdul-Wahab, Dharmi K. Kotak and Mustafa F. Lokhandwala

Hypertension. 1998;32:1054-1059
doi: 10.1161/01.HYP.32.6.1054

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1998 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/32/6/1054

Permissions: 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/