Angiotensin II Receptors on Human Platelets

Thomas J. Moore and Gordon H. Williams

From the Endocrine-Hypertension Unit, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts

SUMMARY. The investigation of the interaction between angiotensin II and its receptors in human subjects has been hampered by the inaccessibility of human tissue containing angiotensin II receptors. In order to find a more accessible angiotensin II-binding tissue, we studied angiotensin II binding to platelets in normal human volunteers. Platelet preparations purified on ficoll: Isopaque gradients were incubated with 125I-angiotensin II (30 pm), with and without unlabeled angiotensin at 22°C, separating bound from free hormone by microcentrifugation. Binding was linearly related to the number of platelets incubated, and, at 8 x 10^6 cells/μl, specific binding ranged from 0.8 to 10%. Scatchard analysis indicated a binding site with a Kd of 2.4 ± 0.3 x 10^-10 M which agreed well with the Kd, by displacement analysis (3.1 x 10^-10 M). The relative binding potencies for angiotensin II and analogues were: angiotensin II = des-Asp1 angiotensin II > [Sar1, Ala8] angiotensin II > des-Asp1-[Ile8] angiotensin II > angiotensin I. The effect of high and low sodium (Na) intake (200 vs. 10 mEq/day) on platelet angiotensin II binding was studied in nine subjects. Compared to low Na, high Na intake produced an 80% increase in the angiotensin II-binding capacity (P < 0.01) with no significant change in binding affinity. We conclude that human platelets possess angiotensin receptors whose binding characteristics and modulation by dietary sodium resemble the properties of the receptors on "classical" animal angiotensin target tissues. The platelet may provide an accessible source of angiotensin receptors for a detailed study of angiotensin-receptor interaction in human tissue. (Circ Res 51: 314-320, 1981)

ANIMAL studies have shown that the effect of angiotensin II on target tissues is mediated by the interaction between the homone and receptor sites on the cell surface (Douglas et al., 1976). Direct studies of these receptors suggest that some changes in tissue responsiveness to angiotensin (such as occur with changes in dietary electrolyte intake) are mediated by changes at the receptor level (Aguilera et al., 1978; Douglas, 1980; Gunther et al., 1980a). Unfortunately, no similar direct receptor studies have been performed in man, due to the inaccessibility of classical angiotensin target tissues (e.g., adrenal, vascular smooth muscle) in human subjects. However, circulating blood cells have been shown to possess receptor sites for several other hormones, such as insulin and catecholamines (Gavin et al., 1973; Alexander et al., 1978). The demonstration of these blood cell receptors not only permitted detailed studies of ligand-receptor interaction in human tissue, but also lead to the recognition of functions for these hormones in tissues where previously none were suspected. Therefore, we examined the possibility that human blood cells could possess angiotensin II receptors. Our studies have demonstrated angiotensin receptors on platelets which, similar to animal target tissues, are regulated by dietary sodium intake.

Methods

Materials

125I-Angiotensin II (1500-1800 μCi/g) was obtained from New England Nuclear. This material was resuspended in sterile, distilled water and frozen in 20-μl aliquots in the dark at -10°C until used (always within 30 days of resuspension). Under these conditions, this material is stable as assessed by reverse-phase high performance liquid chromatography [micro-Bondapack C-18 column (Waters Associates); 18% acetonitrile in 0.1 M phosphoric acid].

Other compounds used included [Ile8]angiotensin II (Sigma Chemical Co.); [Val8]angiotensin II amide (Ciba Pharmaceutical Co.); des-Asp1 angiotensin II and angiotensin I (Bachem Inc.); [Sar1, Ala8]angiotensin II and des-Asp1-[Ile8]angiotensin II (Norwich-Eaton Pharmaceuticals); disodium ethylenediaminetetraacetate (EDTA, Fisher Chemicals); metrizoate (Isopaque, Accurate Chemical and Scientific Corp.); diisopropylfluorophosphate (DFP, Calbiochem-Behring Corp.); dibutyl phthalate (Aldrich Chemical Co.); medium-199 (Grand Island Biological Co.); and bovine serum albumin (Miles Lab).

Platelet Preparation

The subjects for these studies were normal volunteers taking no medications. The protocol was approved by the Human Subjects Committee of our hospital. Platelets were prepared from whole blood anticoagulated with EDTA or heparin. Fifty to 60 ml of blood provided enough platelets for a complete competition curve (see below). Buffy coats were diluted 1:4 with medium-199-containing EDTA (5 mEq/ml) and then centrifuged through a ficoll:isopaque solution (specific gravity 1.077) for 20 minutes at 200 g. The mononuclear/platelet layers then were aspirated and washed twice with normal saline. After the second wash, the suspensions were centrifuged for 5 minutes at 100 g, pelleting the mononuclear cells but leaving the platelets in suspension. Prepared this way, the platelets contained less than 2% red and white cell contamination. The platelets then were collected at 1000 g for 10 minutes and resuspended in medium-199 containing 3.6 mEq/liter potassium, 0.5% bo-
vine serum albumin, and 5 mM EDTA and DFP. The resultant suspensions then were used in the binding assays. Platelets were counted manually by standard techniques. These manual counts correlated well (±5%) with automated counts (Coulter counter).

**Binding Assay**

The techniques used for binding studies were modifications of those described by Lin and Goodfriend (1970) and Douglas and Catt (1976). Aliquots of the platelet suspension (400 µl) were incubated in siliconized glass tubes with increasing concentrations of 125I-angiotensin II (0.05-2.5 nM). Alternatively, binding was analyzed by competitive displacement, incubating tracer amounts of 125I-angiotensin II with increasing concentrations of unlabeled angiotensin II or its analogues (0.03-10 nM; final volume 500 µl). Incubations were performed at 22°C in a Dubnoff metabolic shaker. To separate bound from free hormone, duplicate 200-µl aliquots of the incubates were microcentrifuged (Microfuge B; Beckman) for 90 seconds through dibutyl phthalate oil (200 µl) in 500-µl plastic tubes [a modification of the methods of Gambhir et al. (1978)]. The platelet pellet then was cut off and counted as “bound” hormone; the supernate was counted as free in a Packard gamma counter (Packard Instrument Co., Inc.). The nonspecific binding was defined as the amount of angiotensin bound to platelets in the presence of excess (10^-6 M) unlabeled angiotensin II. In studies with tracer amounts (30 pm) of 125I-angiotensin II, nonspecific binding amounted to less than 1% of added label and ranged from 10 to 30% of total binding. In studies with varying concentrations of labeled angiotensin II, nonspecific binding represented a constant fraction of the total hormone concentration in the incubation.

**Red and White Cell Binding**

Because the platelet suspensions contained small amounts of red cell and mononuclear leukocyte contamination, we assessed binding to purified red and white cells in six subjects (all sodium restricted) to assure that the observed binding was to platelets and not to those contaminating cells. For these studies, platelet suspensions were prepared in the usual way and red and white cell contamination was calculated by cell counts. Purified red cells then were obtained from the cell pellets of EDTA-anticoagulated whole blood of the same subjects and were washed twice with 0.9% NaCl before binding assay. Mononuclear white cells (lymphocytes and monocytes) were obtained by ficoll-Isoopaque centrifugation and then were pelleted at 100 g for 5 minutes. The red and white cells were resuspended in medium-199, and the cell concentrations adjusted to match those of the contaminants in the platelet suspension. Thus, for each of these six subjects, there were three cell suspensions: platelets (including red and white cell contaminants), red cells, and mononuclear white cells. These suspensions were then incubated at 22°C for 90 minutes with 30 pm 125I-angiotensin II with and without 10^-6 M unlabeled angiotensin II to assess specific and non-specific binding.

**Effect of Dietary Sodium**

For these studies, nine normal subjects were studied as inpatients in the Clinical Research Center of the Brigham and Women’s Hospital. They consumed constant diets containing 100 mEq potassium per day with either 10 or 200 mEq sodium. A 200 mEq sodium intake is equivalent to 12 g of NaCl [by comparison, the average American adult consumes 10 g/day (Dahl, 1977)]. Each subject was studied on both sodium intakes, and the order of diets was randomized. When urinary electrolyte determinations indicated that metabolic balance had been achieved (usually after 4-5 days on the diets), blood was drawn for platelet collection, and binding assays were performed as outlined above.

**Statistics**

Results are presented as mean values ± SEM. Binding was analyzed by the method of Scatchard (1949). Curvilinear Scatchard plots were analyzed with a computer-assisted curve-fitting program (MLAB) in the PROPHET computer system. In addition, the dissociation constant (Kd) was estimated from displacement studies as the total ligand concentration required to inhibit 125I-angiotensin II binding by 50% (IC50). Comparison of the relative potencies of unlabeled peptides are presented as dose ratios (i.e., IC50 angiotensin II/IC50 peptide). For group comparisons, we used the paired t-test or, for non-paired or non-parametric data, the Fisher exact test and the Wilcoxon signed rank tests. Linear regression was assessed with the least squares technique (Snedecor and Cochrane, 1967).

**Results**

**Binding Characteristics**

Using the outlined methodology, we found reproducible specific binding of labeled angiotensin to platelets. The intras assay coefficient of variation was 7.3%.

**Time Course of Binding and Effect of Cell Number**

In assessing the time course of binding, we incubated labeled hormone with platelets at 22°C and stopped the equilibration by centrifugation at varying time periods. Specific binding increased rapidly for 30 minutes, then more slowly between 30 and 60 minutes (Fig. 1). There was a constant plateau between 60 and 120 minutes where binding remained relatively constant. Non-specific binding achieved 90% of its maximal level within the first 5 minutes and remained constant throughout the full 120 minutes. At 37°C, specific binding increased more rapidly and, in paired experiments, achieved slightly greater levels compared to 22°C (3.4 ± 0.9 vs. 2.8 ± 0.9), but the plateau phase was short and inconsistent. All subsequent experiments were performed at 22°C.

When labeled angiotensin II was incubated with a broad range of platelet concentrations (5 × 10⁹ to 2.0 × 10¹⁰ cells/µl), we found a direct, significant relationship between binding and cell number (Fig. 2). In each of nine experiments, the correlation coefficient relating these two variables was 0.96 or greater. Non-specific binding also correlated with cell number although less consistently than did specific binding, and, at cell concentrations greater than 1 × 10⁶ per µl, the increase in non-specific binding was disproportionately steepened. Consequently, in subsequent experiments, we used platelet concentrations between 6 and 8 × 10⁹ cells per µl, giving specific binding in the 0.8-10% range.

**Effect of Contaminating Red and White Blood Cells**

When we examined the contaminating red and white blood cells contained in the platelet suspensions
in six subjects, we found that these cells amounted to 1% of the total cell number (Table 1). When we assessed binding of angiotensin II to these contaminants, 0.09 ± 0.02% of $^{125}$I-angiotensin II bound nonspecifically in both the red cell and mononuclear leukocyte suspensions, but there was no detectable specific binding to either cell type. The non-specific binding in platelet suspensions was 0.48 ± 0.04%. These data suggest that the contaminating cells in the 

<table>
<thead>
<tr>
<th>Cells/µl incubation mixture</th>
<th>% Specific binding*</th>
<th>% Non-specific binding*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets (including contaminating cells)</td>
<td>620,000 ± 7000</td>
<td>1.12 ± 0.28</td>
</tr>
<tr>
<td>Red cells alone†</td>
<td>3700 ± 1600</td>
<td>N.D.†</td>
</tr>
<tr>
<td>Mononuclear leukocytes alone†</td>
<td>3100 ± 800</td>
<td>N.D.†</td>
</tr>
</tbody>
</table>

* Binding is presented as percent of $^{125}$I-angiotensin II added.
† Red and white cell concentrations were adjusted to match the number of contaminating cells in the platelet preparations.
‡ N.D. = none detected.

platelet preparations do not contribute to the specific binding observed, but may account for approximately 35% of the non-specific binding.

Non-competitive and Competitive Estimates of Affinity

Using increasing concentrations of $^{125}$I-angiotensin II, the mass of specifically bound hormone increased in a dose-related fashion and plateaued at a concentration of 1 nM angiotensin II, indicating that the binding site was saturable (Fig. 3). Non-specific bind-
ing represented a constant percentage of hormone incubated over a broad range of hormone concentrations (0.05-2.5 nM). Estimating the affinity of the receptors from direct analysis of the binding curve (i.e., ligand concentration at 50% receptor occupancy), the $K_D$ was $2.4 \times 10^{-10}$ M. The $K_D$ by Scatchard analysis of these data was $2.3 \times 10^{-10}$ M.

Unlabeled [Ile]$^5$angiotensin II competed with $^{125}$I-angiotensin II in a dose-related fashion (Fig. 4). Significant displacement of label was noted at concentrations of unlabeled hormone as low as $6 \times 10^{-11}$ M and $10^{-9}$ M concentrations displaced 85% of the label. The $K_D$ estimated from these competition curves was $3.1 \times 10^{-10}$ M. The Scatchard plots were linear in most cases, but occasionally they were concave upward, suggesting a second, low affinity, "non-specific" site in some subjects. The $K_D$ of the saturable binding site by Scatchard analysis was $2.4 \pm 0.3 \times 10^{-10}$ M. Thus, there was good agreement in the affinities of the platelet receptor as calculated by Scatchard and displacement analyses.

Ligand Specificity

To determine the ligand specificity of this receptor, competition curves were prepared using several angiotensin peptides as the unlabeled hormone (Fig. 5). In this analysis, [Ile]$^5$ angiotensin II and [Val]$^8$ angiotensin II-amide demonstrated identical potencies. Des-Asp$^1$ angiotensin II (angiotensin III) was equipotent with angiotensin II, followed by [Sar$^1$, Ala$^8$] angiotensin II and then des-Asp$^1$-[Ile]$^8$ angiotensin II. Angiotensin I was the least potent peptide in this series; its IC$_{50}$ was nearly 50-fold greater than angiotensin II. The dose ratios for these compounds were: angiotensin II and III, 1.0; [Sar$^1$, Ala$^8$] angiotensin II, 0.89; des-Asp$^1$-[Ile]$^8$ angiotensin II, 0.10; angiotensin I, 0.03. Non-angiotensin peptides (insulin, bradykinin, vasopressin) did not displace 50% of labeled hormone even at concentrations of $10^{-6}$ M.

Effect of Dietary Sodium

Urinary electrolyte excretion indicated appropriate metabolic balance on both 10 and 200 mEq sodium diets (11 ± 3 vs. 190 ± 14 mEq Na/day). The supine PRA (4.4 ± 0.7 vs. 1.5 ± 0.5 ng/ml per hr) and plasma angiotensin II levels (49 ± 5 vs. 29 ± 3 pg/ml) were also consistent with the dietary sodium intakes. In any given subject, the platelet concentrations used in the binding assay were adjusted to be equivalent on both diets (6.0 ± 0.1 x 10$^5$ cells/µl on low salt and 6.1 ± 0.1 x 10$^5$ cells/µl on high salt). The binding of tracer amounts of angiotensin to platelets increased on the high salt intake compared to low salt. The mean specific binding of 30 pM $^{125}$I-angiotensin II for the group was 1.76 ± 0.94% on the 10 mEq Na diet and 3.94 ± 1.23% on the 200 mEq Na diet ($P < 0.001$). Non-specific binding was the same on both diets (0.80 ± 0.06% on low salt vs. 0.77 ± 0.1% on high salt). Scatchard analysis of these data showed that the change in binding was related to an increase in receptor capacity with no significant change in affinity (Fig. 6). The mean receptor capacity of the high affinity binding site was 4.9 ± 2.4 fmol/10$^9$ cells on the 10 mEq sodium diet and increased to 8.8 ± 3.7 fmol/10$^9$ cells on the 200 mEq diet (Fig. 7). This increase in receptor capacity was observed in eight of nine subjects studied ($P < 0.01$). The affinity of the receptor was slightly higher on the high salt intake ($K_D = 1.8 \pm 0.2 \times 10^{-10}$ M on high salt vs. 2.6 ± 0.4 x 10$^{-10}$ M on low salt diet), but the difference did not achieve statistical significance (0.10 > $P > 0.05$).
Discussion

Our understanding of the interaction between angiotensin II and its receptor site has improved rapidly in recent years, in parallel with improvements in receptor methodology. Receptor studies have clarified our understanding of the affinity and analogue specificity of the angiotensin receptor (Douglas et al., 1980) and have demonstrated that dietary sodium or potassium intake may modify angiotensin receptor binding (Douglas and Catt, 1976; Aguilera et al., 1978; Aguilera and Catt, 1978; Devynck et al., 1978; Douglas, 1980; Gunther et al., 1980). These studies provide a probable mechanism explaining the regulatory effects of dietary electrolytes on tissue responses to angiotensin II seen in earlier pharmacological studies (Laragh et al., 1980; Blair-West et al., 1970; Dluhy et al., 1972; Oelkers et al., 1974; Hollenberg et al., 1974). Unfortunately, our understanding of the angiotensin receptor is based almost solely on animal studies. Studies in humans necessarily have been hampered by the inaccessibility of human target tissues. Thus, our knowledge of the human angiotensin receptor is based on indirect, pharmacological evidence: measurement of responses to the administration of angiotensin, angiotensin antagonists, and inhibitors of angiotensin-converting enzyme. Despite their limitations, these indirect methods have provided increasing evidence that tissue responsiveness to angiotensin II is altered in several disease states. In various studies, the adrenal, vascular, and/or renovascular responses have been found to be altered in essential hypertension, "low-renin" hypertension, and primary aldosteronism (Pearl and Brown, 1961; Kaplan and Silah, 1964; Kisch et al., 1976; Moore et al., 1977; Wisgerhof and Brown, 1978; Wisgerhof et al., 1978). These studies suggest the possibility of an altered angiotensin receptor in these conditions. The goal of our study, then, was to determine whether human blood cells might possess angiotensin receptors, affording us an opportunity for a more direct assessment of these receptors in man.

Our results indicate that human platelets possess specific, saturable binding sites for angiotensin II. The fact that preparations of red blood cells and mononuclear leukocytes displayed no specific binding at the concentrations tested confirms the fact that platelets were the site of the observed binding. [An earlier report had described the presence of angiotensin binding to human mononuclear leukocytes (Shimada and Yazaki, 1978), but we were unable to duplicate those findings.] We used 125I-angiotensin II as our radiolabeled ligand rather than the tritiated peptide because of the higher specific activity of the iodinated compound. The fact that receptor affinity was identical for both 125I-angiotensin II and the native hormone (as evidence in the 125I-angiotensin II saturation curve vs. competitive displacement studies) suggests that iodination of the hormone does not alter its binding to the platelet receptor site and thus justifies its use as a radiolabeled tracer in our subsequent studies. In this regard, the platelet receptor resembles the angiotensin receptors on animal target tissues which also bind iodinated and native angiotensin equally (Glossmann et al., 1974; Gunther et al., 1980a). The affinity of the platelet receptor ($K_D = 3 \times 10^{-10}$ M) also compared favorably with the affinities reported for animal adrenal ($0.9-5 \times 10^{-10}$ M) (Douglas and Catt, 1976; Aguilera et al., 1978) and vascular smooth muscle ($5.6-9 \times 10^{-10}$) (Gunther et al., 1980a).
In addition, the ligand specificity of the platelet receptor for angiotensin and related peptides resembled that described for the receptors on classical animal target tissues (Douglas et al., 1980). Of particular interest, the affinity was equal for angiotensin II and angiotensin III. This seems to disagree with the affinities expected from pharmacological studies: during angiotensin II and III infusions in man, the heptapeptide evokes only 15-30% of the pressor and sympathetically mediated effects of angiotensin II (Carey et al., 1978). Utilizing receptor methodology, however, several groups have demonstrated similar affinities for these two peptides in adrenal and smooth muscle receptors in dogs, rabbits, and cattle (Capponi and Catt, 1979; Simpson et al., 1980; Gregory and Aguilerà, 1981). In addition, recent evidence has indicated that the different responses to infusion of these peptides may be related to differences in their degradation rates (GREGORY and Aguilerà, 1981).

Our results also indicated that dietary sodium intake influenced platelet angiotensin binding. Sodium loading significantly increased the number of receptors without significantly changing receptor affinity. This pattern is similar to that observed in smooth muscle (both uterine and vascular) and brain angiotensin receptors from animal studies (Devynck et al., 1978; Aguilerà and Catt, 1978; Gunther et al., 1980b; Mann et al., 1980) where sodium loading also increases receptor capacity. Conversely, high sodium intake reduces the number of adrenal angiotensin II receptors in animal studies (Douglas and Catt, 1976; Devynck et al., 1978; Aguilerà and Catt, 1978). Thus, from the viewpoint of receptor regulation, the platelet appears more akin to animal smooth muscle or brain.

The concentration of angiotensin receptors on platelets deserves additional comment. On the 200 mEq sodium intake, the level of high affinity binding observed is equivalent to approximately 10 receptor sites per cell. It is possible to compare the density of the angiotensin receptors on platelets to that on other angiotensin target tissues if one corrects for differences in cell size by relating the number of binding sites to the mass of cell membrane protein. Assuming 20,000 mEq sodium intake, the level of high affinity binding observed is equivalent to approximately 10 receptor sites per cell. It is possible to compare the density of the angiotensin receptors on platelets to that on other angiotensin target tissues if one corrects for differences in cell size by relating the number of binding sites to the mass of cell membrane protein. Assuming 1 mg of platelet membrane protein per 10⁹ cells [a value established in our lab as well as in the literature (Alexander et al., 1978)], the platelet, with 10 fmol angiotensin bound/mg protein, has 50% of the receptor density reported in uterine smooth muscle (45 fmol/mg) and vascular smooth muscle (57 fmol/mg) (Aguilerà et al., 1978; Gunther et al., 1980b). All these tissues exhibit considerably less receptor density than the adrenal glomerulosa cell (> 1000 fmol/mg) (Aguilerà et al., 1981).

Whether the relatively low angiotensin receptor density observed in platelets would be sufficient to evoke some cellular response remains to be established. Such a role for angiotensin has been suggested by Poplowski (1971) who found that angiotensin II added to platelet suspensions enhanced the aggregation response to epinephrine. Further work is needed to confirm Poplowski’s results. However, even in the absence of a definite function for angiotensin II in the platelet, the presence of angiotensin receptors on platelets provides promising opportunities for further study. The platelet, bearing receptors with affinity, ligand specificity, and dynamic regulation by sodium intake, similar to angiotensin receptors on “classical” target tissues, may provide the first readily accessible human tissue for a detailed study of angiotensin II-receptor interaction in normal and hypertensive man.

We gratefully acknowledge the contributions of Deanna Kinney for her technical assistance and of Miss Diane Rioux for her help in preparing this manuscript. Data analysis was performed with the assistance of Dr. Bernard Ransil on the PROPHET system, a national computer resource sponsored by the Division of Research Resources, National Institutes of Health.

This work was presented in part at the 63rd meeting of the Endocrine Society, June, 1981.

Dr. Moore is the recipient of Clinical Investigator Award 5 K08 AM00594 from the NIAID. This work was also supported in part by Clinical Research Center Grants 5 MO1 RR00866 and RR-01032 from the Division of Research Resources of the National Institutes of Health and by Grant 5 RO1 HL16821-06 from the National Heart, Lung, and Blood Institute.

Address for reprints: Thomas J. Moore, M.D., Brigham and Women’s Hospital, 75 Francis Street, Boston Massachusetts 02115.

Received March 15, 1982; accepted for publication June 4, 1982.

References


Douglas JG, Catt KJ (1976) Regulation of angiotensin II receptors...
Scatchard G (1949) The attractions of proteins for small molecules and ions. Ann NY Acad Sci 51: 660-672

INDEX TERMS: Angiotensin II • Angiotensin II receptors • Receptors • Human platelets • Sodium balance
Angiotensin II receptors on human platelets.
T J Moore and G H Williams

Circ Res. 1982;51:314-320
doi: 10.1161/01.RES.51.3.314
Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/51/3/314

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/