Angiotensin Stimulates Bicarbonate Transport and Na+/K+ ATPase in Rat Proximal Straight Tubules

Jeffrey L. Garvin, Ph.D.

ABSTRACT
The effect of angiotensin on HCO$_3^-$ absorption, fluid absorption, and Na$^+$/K$^+$ ATPase activity in isolated rat proximal straight tubules was investigated. During the control period, tubules absorbed fluid at $0.66 \pm 0.12$ nL/mm-min and bicarbonate at $60.2 \pm 10.7$ pmol/mm-min. After 10$^{-10}$ M angiotensin was added to the bath, tubules absorbed fluid at $0.93 \pm 0.19$ nL/mm-min and bicarbonate at $77.4 \pm 15.2$ pmol/mm-min, indicating stimulation of both parameters. Time controls showed no significant change in the rate of bicarbonate or fluid absorption. To determine whether this stimulation was due to an increase in the maximum rate of transport, tubules were perfused at $20$ nL/mm-min. During the control period, tubules absorbed bicarbonate at $82.5 \pm 13.0$ pmol/mm-min. After 10$^{-6}$ M angiotensin was added to the bath, these same tubules absorbed bicarbonate at $75.9 \pm 11.9$ pmol/mm-min. Thus, angiotensin did not alter the maximum rate of transport. Angiotensin also had no effect on bicarbonate permeability, which was $1.1 \pm 0.2 \times 10^{-4}$ cm/s before treatment and $1.3 \pm 0.3 \times 10^{-4}$ cm/s afterward. Finally, the effect of angiotensin on Na$^+$/K$^+$ ATPase activity was measured in paired experiments. Na$^+$/K$^+$ ATPase activity of control tubules was $36 \pm 6$ pmol of ADP/mm-min; after angiotensin treatment, it was $47 \pm 6$ pmol ADP/mm-min. From these data it was concluded that: (1) angiotensin stimulates bicarbonate absorption in the rat proximal straight tubule; (2) this stimulation is the result of a change in $K_m$ rather than of an increase in the maximum rate of transport or permeability; and (3) angiotensin directly stimulates Na$^+$/K$^+$ ATPase activity in the proximal nephron.

Key Words: Na$^+$/H$^+$ exchange, sodium transport, ouabain, kidney, permeability

Both the proximal convoluted and straight tubules have receptors for angiotensin (1,2). When angiotensin, at physiological concentrations, occupies these receptors, it stimulates fluid and solute absorption in both segments (3–8). In vivo micropuncture experiments, Liu and Cogan (1,9) demonstrated that Na$^+$/H$^+$ exchange is stimulated by angiotensin, as measured by bicarbonate absorption in the proximal convoluted tubule of the rat. The increase in bicarbonate absorption appeared to be the result of a decrease in bicarbonate permeability and an increase in the affinity of the Na$^+$/H$^+$ exchanger. In contrast, in isolated, perfused rat proximal straight tubules, angiotensin stimulates Na$^+$/glucose cotransport by increasing the maximum rate of transport rather than affecting the $K_m$ or permeability (8). The effects of angiotensin on Na$^+$/H$^+$ exchange have not been studied in the rat proximal straight tubule; thus, it is unclear whether it stimulates Na$^+$/glucose and Na$^+$/H$^+$ transport in the proximal nephron by different means, or whether it enhances Na$^+$/H$^+$ exchange by affecting $K_m$ in the convoluted tubule and the maximum rate in the straight segment.

Because angiotensin stimulates transepithelial sodium transport, it must also enhance Na$^+$/K$^+$ ATPase activity, either directly or indirectly. Levin (10) reported that angiotensin was able to stimulate Na$^+$/K$^+$ ATPase activity in homogenates of the renal cortex. However, this stimulation amounted to ~8% and occurred only at concentrations of angiotensin >1 $\mu$M. Other investigators reported no effect of angiotensin on Na$^+$/K$^+$ ATPase at this or other concentrations (11–13). The fact that angiotensin had no or a small effect on Na$^+$/K$^+$ ATPase activity in these studies may be explained by the loss of cell integrity in homogenates.
The goals of this study are: (1) to demonstrate that angiotensin stimulates bicarbonate absorption in the rat proximal straight tubule; (2) to determine whether angiotensin stimulates bicarbonate transport by affecting the maximum rate of transport (as it does for glucose absorption in the proximal straight tubule) or by altering \(K_m\) (as it does for bicarbonate absorption in the proximal convoluted tubule); and (3) to investigate if angiotensin can directly stimulate \(Na^+/K^+\) ATPase activity.

## Methods

Sprague-Dawley rats (Charles River Breeding Laboratories) weighing 130 to 180 g were kept on a reduced-sodium diet (0.2% Na and 1.1% K, Ralston Purina) for at least 5 days. Rats were anesthetized with ketamine (100 mg/kg; Parke-Davis). The peritoneal cavity was opened and flushed with cold physiological saline for 3 to 4 min to cool the kidneys while they were still perfused with blood. In preliminary studies, this procedure markedly improved yield, possibly by preventing anoxia when the kidney is removed (14). After removal, the left kidney was placed in chilled saline. Coronal slices were cut and transferred to a dissection dish containing physiological saline at 12°C. Medullary rays were dissected from the slices, and proximal straight tubules were dissected from the rays. Tubules were transferred to a perfusion chamber, mounted on concentric pipets (15), and perfused at 37°C. Only cortical segments of proximal straight tubules were studied.

### Solutions

The solutions used in this study are listed in Table 1. In isolated, perfused tubule studies, the bath and perfusion solutions had the same composition except when a bicarbonate gradient was created. The bath was changed at a rate of 0.5 mL/min; the volume was ~1 mL. The osmolality of all solutions was 290 ± 3 mosm as measured by freezing-point depression (Advanced Instruments, Needham Heights, MA). The pH of the bath was 7.4. Solutions were gassed with 95% \(O_2\)-5% \(CO_2\). Fifteen minutes elapsed before measurements were made after 10^{-10} M angiotensin was added to the bath. Angiotensin (Sigma Chemical Co., St. Louis, MO) was added to the bath only in perfusion studies.

In experiments designed to determine the effect of angiotensin on \(Na^+/K^+\) ATPase activity, tubules were dissected in a potassium-free dissection solution (solution 3). Two concentrated reaction buffers were used in the assay. To measure total ATPase activity solution 4 was used. To measure ouabain-insensitive ATPase, the KCl was replaced with NaCl and 4 mM ouabain was added (solution 5). The potassium concentration of the solution was approximately 0.3 to 0.4 mM before dilution. No KCl was added, but the enzymes are shipped in a solution containing potassium.
centration in the reaction buffer which used solution 5 was approximately 0.08 mM after all dilution because of the presence of potassium in the solution that contained pyruvate kinase and lactate dehydrogenase (Sigma). Both solutions were made fresh daily from a stock solution of salts to which NADH, ouabain, and ascorbic acid (Sigma) were added as dry reagents. ATP and phosphoenol pyruvate were added from concentrated stocks that were stored at -80°C. The pH of both reaction solutions were 7.0 after all dilutions.

**Measurements**

**Fluid Absorption.** Fluid absorption ($J_v$) was measured using raffinose as a volume marker. Raffinose was measured in the perfusate and collected fluid using a previously described enzymatic assay (16) in a continuous-flow ultramicrofluorometer. The estimated error in measuring fluid absorption using this technique is less than 4%. The perfusion rate ($V_0$) was calculated from the equation

$$V_0 = \frac{C_L}{C_0} V_L$$

where $C_L$ is the concentration of raffinose in the collected fluid, $C_0$ is the concentration of raffinose in the perfused fluid, and $V_L$ is the collection rate per unit of tubule length. The rate of fluid absorption was calculated as

$$J_v = V_0 - V_L$$

**Bicarbonate Absorption.** The bicarbonate concentration in samples of the bath, perfusate, and collected fluid was measured using a fluorometric assay (17). $J_{bicarb}$ was calculated according to the equation

$$J_{bicarb} = C_{bicarb} V_0 - C_{Lbicarb} V_L$$

where $C_{bicarb}$ is the bicarbonate concentration in the perfusate, $C_{Lbicarb}$ is the bicarbonate concentration in the collected fluid, and $V_0$ and $V_L$ have the same meaning as above. In experiments with rapid flow rates (>20 nL/mm-mm), $V_L$ was assumed to equal $V_0$ (18).

**Na*/K* ATPase Assay.** Na*/K* ATPase activity was measured according to methods adapted from O'Neil and Dubinsky (19). Four proximal straight tubules from the same medullary ray and cortical level were dissected in potassium-free medium (solution 3). One tubule was used to measure each of the following: total and ouabain-insensitive ATPase activity and total and ouabain-insensitive ATPase activity in the presence of angiotensin. Each tubule was transferred to a 1.5-ml microcentrifuge tube in 5 μL of dissection medium. To this was added 30 μL of 0.7% octylglucoside and either 5 μL of 10^-9 M angiotensin or 5 μL of diluent. The tubes were then placed on ice for 20 min, after which 13 μL of the appropriate 4X reaction medium was added. The reaction medium was then covered with mineral oil and incubated at 37°C for 30 min. Subsequently, 53 μL of 0.5 N HCl was added and the tubes were incubated at 37°C for an additional 15 min. The tubes were removed from the bath, 750 μL of 6 N KOH was added to each tube, and the tubes were then incubated at 60°C for 20 min in the dark and then allowed to cool to room temperature (~20 min). The solution was transferred to glass tubes, and fluorescence was measured. Na*/K* ATPase activity was taken as the difference between total and ouabain-insensitive ATPase activity and expressed as picomoles of ADP produced per minute. A standard curve was generated for each experiment by adding 0 to 2,400 pmol of ADP to those tubes without tubules. A combination of 1 mM ouabain and low K* was used to determine ouabain-insensitive ATPase, because the rat proximal nephron is relatively insensitive to ouabain (20) and higher concentrations quench fluorescence.

**Protocols**

**Maximum Rate of Transport.** To determine the maximum rate of bicarbonate transport, perfusion rates greater than 20 nL/mm-mm were used; because fluid absorption averaged ~0.7 nL/mm-mm, $V_L$ approximated $V_0$. Under these conditions, $J_{bicarb}$ is maximized because the luminal bicarbonate concentration does not fall substantially. This prevents absorption from being limited by (1) substantial backflux because of generation of a large bicarbonate gradient across the tubular epithelium; and (2) luminal bicarbonate becoming too low to support the maximum rate of transport.

**Permeability.** The effect of angiotensin on bicarbonate permeability was investigated by bathing tubules with 25 mM bicarbonate and perfusing them with a bicarbonate-free solution while measuring fluid flux with and without angiotensin. Tubules were perfused at rapid flow rates to minimize the effects of fluid absorption. Permeability was calculated according to the equation

$$P = \frac{J}{[A (C_B - (C_I/2))]$$

where $P$ is the permeability, $J$ is the bicarbonate flux, $A$ is the area, $C_B$ is the bicarbonate concentration of the bath, and $C_I$ is the bicarbonate concentration of the collected fluid. The area was calculated by assuming a smooth regular cylinder and measuring tubule diameter.

**Statistics**

Values are reported as mean ± standard error. Student’s paired $t$ test was used to test for significant differences. All experiments in which at least two
measurements of $J_c$ and $J_{bicarb}$ were made during each period were reported.

RESULTS

Figure 1 shows the effect of $10^{-10}$ M angiotensin on bicarbonate and fluid absorption by rat proximal straight tubules. During the control period, five tubules absorbed bicarbonate (left panel) at a rate of $60.2 \pm 10.7$ pmol/mm·min and fluid (right panel) at a rate of $0.66 \pm 0.12$ nL/mm·min when perfused at $6.52 \pm 0.73$ nL/mm·min. The mean length was $0.61 \pm 0.05$ mm. After $10^{-10}$ M angiotensin was added to the bath, the tubules absorbed bicarbonate at a rate of $77.4 \pm 15.2$ pmol/mm·min and fluid at a rate of $0.93 \pm 0.19$ nL/mm·min ($P < 0.05$). The paired difference in bicarbonate flux was $17.4 \pm 4.7$ pmol/mm·min (Table 2). Time controls showed no significant change in bicarbonate flux ($67.4 \pm 3.6$ versus $66.7 \pm 5.1$ pmol/mm·min) or fluid absorption ($0.91 \pm 0.14$ versus $0.88 \pm 0.13$ nL/mm·min).

To determine how angiotensin increases bicarbonate transport, its effect on the maximum rate of absorption and permeability was investigated.

Maximum Rate of Transport

Figure 2 shows the effect of angiotensin on the maximum rate of bicarbonate transport. The maximum rate of transport was measured by perfusing tubules at rates >20 nL/mm·min to minimize the fall in luminal bicarbonate concentration and, consequently, backflux. Tubules absorbed bicarbonate at $71.7 \pm 14.3$ pmol/mm·min during the control period and $70.2 \pm 10.8$ pmol/mm·min after angiotensin was added to the bath (Table 3). The mean tubule length was $0.55 \pm 0.05$ mm. Thus, angiotensin appeared to have no effect on the maximum rate of transport.

Permeability

Angiotensin has been reported to alter bicarbonate permeability in the rat proximal convoluted tubule (1). To examine whether this is also true in the rat proximal straight tubule, a bath-to-lumen gradient was created and passive bicarbonate flux was measured (Table 4). The mean tubule length was $0.51 \pm 0.04$ mm. During the control period, the bicarbonate flux was $117.5 \pm 23.6$ pmol/mm·min and the calculated permeability was $1.1 \pm 0.2 \times 10^{-4}$ cm/s. After the tubules were treated with $10^{-10}$ M angiotensin,

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TABLE 2. Effect of $10^{-10}$ M angiotensin on bicarbonate absorption

<table>
<thead>
<tr>
<th></th>
<th>Perfusion Rate (nL/mm·min)</th>
<th>Collection Rate (nL/mm·min)</th>
<th>Perfusion (bicarbonate) (mM)</th>
<th>Collected fluid (bicarbonate) (mM)</th>
<th>$J_{bicarb}$ (pmol/mm·min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>$6.52 \pm 0.73$</td>
<td>$5.87 \pm 0.64$</td>
<td>$24.7 \pm 0.6$</td>
<td>$17.4 \pm 0.5$</td>
<td>$60.2 \pm 10.7$</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>$7.19 \pm 1.19$</td>
<td>$6.29 \pm 1.01$</td>
<td>$24.7 \pm 0.6$</td>
<td>$16.2 \pm 0.8$</td>
<td>$77.4 \pm 15.2$</td>
</tr>
<tr>
<td>Paired difference</td>
<td>$0.66 \pm 0.58$</td>
<td>$0.39 \pm 0.49$</td>
<td>$0.0 \pm 0.0$</td>
<td>$1.4 \pm 0.5$</td>
<td>$17.2 \pm 4.8$</td>
</tr>
<tr>
<td>$P$ value</td>
<td>$&gt;0.20$</td>
<td>$&gt;0.20$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
<td>$&lt;0.05$</td>
</tr>
</tbody>
</table>

*Values are reported as the mean ± SE for five tubules. Mean tubule length was $0.61 \pm 0.05$ mm.
Angiotensin II Increases HCO$_3^-$ Flux

TABLE 3. Effect of angiotensin on the maximum rate of bicarbonate absorption$^a$

<table>
<thead>
<tr>
<th></th>
<th>Collection Rate (nL/mm/min)</th>
<th>Perfusate (bicarbonate) (mM)</th>
<th>Collected fluid (bicarbonate) (pM)</th>
<th>J$_{bicarb}$ (pmol/mm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>20.90 ± 1.53</td>
<td>25.5 ± 0.3</td>
<td>21.9 ± 0.8</td>
<td>71.7 ± 14.3</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>21.79 ± 2.29</td>
<td>25.5 ± 0.3</td>
<td>22.1 ± 0.5</td>
<td>70.2 ± 10.8</td>
</tr>
<tr>
<td>Paired difference</td>
<td>0.88 ± 1.25</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.4</td>
<td>-1.4 ± 6.2</td>
</tr>
<tr>
<td>$P$ value</td>
<td>&gt;0.20</td>
<td></td>
<td>&gt;0.20</td>
<td>&gt;0.20</td>
</tr>
</tbody>
</table>

$^a$ Values are reported as the mean ± SE for five tubules. Mean tubule length was 0.55 ± 0.05 mm.

TABLE 4. Effect of 10$^{-10}$ M angiotensin on bicarbonate permeability$^a$

<table>
<thead>
<tr>
<th></th>
<th>Length (mm)</th>
<th>Collection Rate (nL/mm/min)</th>
<th>Collected fluid concn (mM)</th>
<th>J$_{bicarb}$ (pM)</th>
<th>$P_{bicarb}$ (10$^{-10}$ cm/s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.51 ± 0.04</td>
<td>37.00 ± 3.39</td>
<td>3.4 ± 0.7</td>
<td>117.5 ± 23.6</td>
<td>1.1 ± 0.2</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>0.51 ± 0.04</td>
<td>34.92 ± 2.65</td>
<td>4.5 ± 1.2</td>
<td>140.4 ± 32.3</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

$^a$ Values are reported as the mean ± SE for five tubules.

flux was 140.4 ± 32.3 pmol/mm/min and permeability was 1.3 ± 0.3 × 10$^{-4}$ cm/s, not significantly different from the control value.

**Na$^+/K^+$ ATPase Activity**

Angiotensin stimulates transepithelial sodium transport in the proximal nephron. Thus, Na$^+/K^+$ ATPase activity must be stimulated either indirectly by an increase in intracellular sodium concentration or directly by a second messenger of angiotensin. To investigate whether angiotensin could directly stimulate Na$^+/K^+$ ATPase activity, it was measured in tubules dissected from the same medullary ray in the presence and absence of 10$^{-10}$ M angiotensin (Figure 3). In 9 of 10 experiments, angiotensin stimulated Na$^+/K^+$ ATPase activity. In control tubules, Na$^+/K^+$ ATPase activity was 36 ± 6 pmol of ADP/mm-min, ~40% of total ATPase activity. Angiotensin-treated tubules had significantly greater Na$^+/K^+$ ATPase activity: 47 ± 6 pmol of ADP/mm-min. The paired difference was 11 ± 3.7 (P < 0.02). Thus, angiotensin directly stimulated Na$^+/K^+$ ATPase activity by 30%.

**DISCUSSION**

Angiotensin stimulates fluid and sodium absorption in the proximal nephron of the rat. Recent evidence suggests that angiotensin either enhances Na$^+/glucose$ and Na$^+/H^+$ transport processes in different ways or else enhances Na$^+/H^+$ transport by different means in the proximal convoluted and

![Figure 3](image-url)
it is likely that angiotensin stimulates this process in the proximal straight tubule as it does in the proximal convoluted tubule (1,9). The increase in bicarbonate transport caused by angiotensin appears to be due to a change in the affinity of the Na+/H+ exchanger, rather than either an increase in the maximum rate of transport or a decrease in permeability. Bicarbonate transport was stimulated by 30% in the initial experiments. In contrast, in experiments with rapid perfusion rates designed to test if the maximum rate of transport had changed, angiotensin had no effect. The change in affinity is likely to occur at the proton rather than the sodium binding site. The \( K_m \) for sodium of the Na+/H+ exchanger is 5 to 10 mM (21,22), whereas the luminal sodium concentration is ~150 mM. Thus, the sodium concentration is 15 to 30 times the \( K_m \) and the exchanger should be saturated with respect to sodium. It can be shown that, under the condition of rapid perfusion, a given change in \( K_m \) of the proton site caused by angiotensin produces a much smaller increase in bicarbonate transport than does the same change in the maximum rate induced by the hormone, assuming that Michaelis-Menten kinetics can be used to describe proton transport and literature values for other parameters. Liu and Cogan (1) have also concluded that angiotensin stimulates bicarbonate absorption in the proximal convoluted tubule by altering the \( K_m \) of the Na+/H+ exchanger rather than the maximum rate of transport, which remained unchanged.

The observation that angiotensin does not alter the maximum rate of transport is at odds with the findings of Saccomani et al. (23). These authors reported that, although angiotensin stimulated Na+/H+ exchange, this was due to an increase in the maximum rate of transport rather than \( K_m \). This discrepancy may be based on (1) the different techniques used to measure Na+/H+ exchange activity; and/or (2) the failure of Saccomani et al. to determine whether the \( K_m \) for protons had changed. They reported that Na+/H+ exchange was stimulated and demonstrated that the \( K_m \) for sodium was unchanged. As stated above, it is unlikely that a change in sodium affinity could stimulate Na+/H+ exchange under physiological conditions, so these results would have been expected. However, it does not necessarily follow that the maximum rate has been enhanced, because a change in the \( K_m \) for protons could also explain the data. It must be noted that the experiments reported here do not absolutely rule out a change in the maximum rate of transport, only that its importance is small when compared with the role of changing the \( K_m \) of the proton site.

**Bicarbonate Permeability**

Angiotensin had no effect on the bicarbonate permeability of the proximal straight tubule. The lack of an effect on bicarbonate permeability is similar to previous results that show that glucose permeability is not affected by angiotensin in the proximal straight tubule (8). In contrast, Liu and Cogan (1) reported that angiotensin decreased bicarbonate permeability by 40%. Possible explanations of this disparity are that the straight and convoluted tubules respond differently to angiotensin with respect to changes in permeability or that brush-border receptors mediate these changes. (In the studies of Liu and Cogan, angiotensin may have reached the luminal receptors, because they infused angiotensin intravenously and it is filtered.)

**Fluid Absorption**

Angiotensin (10^{-10} M) enhanced fluid absorption by 41% in this study. Although these results are at odds with the findings of Liu and Cogan (1), they are qualitatively consistent with previous findings from this laboratory (8), as well as others that show that angiotensin stimulates fluid absorption in the proximal nephron (4,6). At present, there is no explanation for these disparate results.

**Na+/K+ ATPase Activity**

Angiotensin stimulates net sodium absorption. Therefore, Na+/K+ ATPase must be stimulated either indirectly through changes in intracellular sodium or directly through a second messenger of angiotensin. In this study, 10^{-10} M angiotensin increased Na+/K+ ATPase activity by 27%. This concentration stimulates fluid absorption by 30 to 40% (8; this study). Previously, Levin (10) demonstrated that angiotensin could stimulate Na+/K+ ATPase activity in cortical homogenates of the kidney. However, the stimulation amounted to only 8% and required 10^{-6} M angiotensin, a concentration which would be expected to inhibit fluid absorption (4). Other investigators have been unable to demonstrate any effect of angiotensin on Na+/K+ ATPase activity with similar (11-13) or much lower concentrations (11). The loss of cell integrity before the application of angiotensin may explain the lack of an effect in some studies.

The ability of angiotensin to enhance Na+/K+ ATPase activity is most likely due to direct action through a second messenger(s) of angiotensin rather than to an increase in intracellular Na+. The intracellular sodium concentration would be expected to be the same in control and angiotensin-treated tubules (approximately 60 mM) under the conditions of this assay, because the tubules were permeabilized before they were treated with angiotensin. Additionally, the stimulation is most likely due to an increase in the maximum rate of transport rather than due to an alteration of the \( K_m \) for sodium, potas-
sium, or ATP, because the concentration of each substrate in the medium was well above its $K_m$ (24–27). However, it is impossible to ascertain from these studies whether the increase in activity was due to an increase in the number of pumps or an increase in the turnover of individual pumps. It should be noted that these studies do not rule out the possibility that $K_m$ values have also changed in addition to the maximum rate.

Angiotensin-enhanced Na*/K* ATPase activity may partially explain the stimulation of some brush-border membrane transporters. This is especially true in those cases, such as glucose absorption, in which angiotensin stimulates transport by a change in maximum rate. A change in maximum rate would be consistent with an effect caused by reduced intracellular sodium. The direct action of angiotensin on Na/K ATPase may not have a significant effect on the maximum rate of Na/H exchange in the "steady state," because intracellular alkalization caused by angiotensin (23) also increases the gradient against which extrusion of protons must occur.

ACKNOWLEDGMENTS

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REFERENCES