Losartan and Its Metabolite E3174 Modify Cardiac Delayed Rectifier K⁺ Currents

Ricardo Caballero, BPharm; Eva Delpón, BPharm, PhD; Carmen Valenzuela, BSc, PhD; Mónica Longobardo, BPharm; Juan Tamargo, MD, PhD, FESC

Background—The effects of type 1 angiotensin II receptor antagonist losartan and its metabolite E3174 on transmembrane action potentials, hKv1.5, HERG, and I_K1 currents were analyzed.

Methods and Results—Guinea pig ventricular action potentials were recorded with microelectrode techniques and hKv1.5 and HERG currents with the whole-cell patch-clamp technique. I_K1 was recorded in guinea pig ventricular myocytes with the perforated-nystatin-patch configuration. Losartan and E3174 transiently increased the hKv1.5 current by 8.0±1.4% and 7.4±1.6%, respectively. Thereafter, they produced a voltage-dependent block, E3174 being more potent than losartan (P<0.05) for this effect. Losartan decreased HERG currents elicited at 0 mV (23.3±4.8%), whereas E3174 increased the current (30.5±6.2%). Both drugs shifted the midpoint of the activation curve of HERG channels to more negative potentials. In ventricular myocytes, losartan and E3174 inhibited the I_K1 (18.4±3.2% and 6.5±0.7%, respectively). Losartan-induced block was voltage-independent, whereas E3174 shifted the midpoint of the activation curve to more negative potentials. Losartan lengthened the duration of the action potentials at both 50% and 90% of repolarization, whereas E3174 slowed only the final phase of the repolarization process.

Conclusions—These results demonstrated that at therapeutic concentrations, both losartan and E3174 modified the cardiac delayed rectifier hKv1.5, HERG, and Ks currents. (Circulation. 2000;101:1199-1205.)

Key Words: losartan ■ E3174 ■ ion channels ■ myocytes ■ electrophysiology

Transmembrane Action Potentials

Transmembrane action potentials were recorded in guinea pig (250 to 300 g) papillary muscles driven at 1 Hz through conventional microelectrode techniques as previously described. Experiments were performed at 34°C.

Isolation of Single Guinea Pig Ventricular Myocytes and Cell Culture

Single ventricular myocytes were isolated by use of collagenase and protease digestion as described previously. Stably transfected Ltk⁻ cells were cultured in DMEM with 10% horse serum and 0.25 mg/mL G418 (Gibco) in a 5% CO₂ atmosphere as previously described. Chinese hamster ovary (CHO) cultures were grown in Ham’s-F12 medium with 10% FBS and transiently transfected with the cDNA encoding the HERG channel (4 μg/mL) and cDNA encoding the CD8 antigen (0.5 μg/mL) by use of lipofectamine. Before experimental use, cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450, Dynal). Most of the cells that were beaded also had channel expression.

Solutions

To measure I_K1 in guinea pig ventricular myocytes, the external solution contained (mmol/L) NaCl 136, KC1 5.4, CaCl₂ 1.0, MgCl₂ 0.7, K₂HPO₄ 1.0, KH₂PO₄ 0.4, and glucose 10.0. The internal solution contained (mmol/L) KCl 150, MgCl₂ 1.0, CaCl₂ 2.0, EGTA 0.5, HEPES 10, and glucose 10.0.

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1.0, CaCl₂ 1.20, tetrodotoxin 0.03, glucose 10, and HEPES 10 (pH adjusted to 7.4 with NaOH). Under these conditions, sodium and calcium currents were blocked by tetrodotoxin and CoCl₂, respectively. To analyze the effects on $I_{Ca}$, the solution was supplemented with 30 μmol/L LaCl₃ to block Kr channels.⁹ Papillary muscles were perfused with the Co²⁺- or the Co²⁺-La³⁺-containing solution, in which tetrodotoxin was omitted. Ltk⁻ and CHO cells were perfused with an external solution containing (mmol/L) NaCl 130, KCl 4, CaCl₂ 1, MgCl₂ 1, Hepes 10, and glucose 10 (pH adjusted to 7.4 with NaOH). The internal solution contained (mmol/L) potassium aspartate 80, KCl 42, KH₂PO₄ 10, MgATP 5, phosphocreatine 3, and EGTA 5 (pH adjusted to 7.2 with KOH). Losartan and E3174 (Merck Sharp & Dohme España) were dissolved in methanol to make 1 mmol/L stock solution.

**Recording Techniques**

hKv1.5 and HERG currents were measured with the whole-cell patch-clamp technique. In ventricular myocytes, $I_{Ks}$ was recorded with the perforated-nystatin-patch configuration to avoid the rundown of the current.⁸ Recordings were performed at 24°C to 25°C with 200B patch-clamp amplifiers and pClamp 6.1 software (Axon Instruments). Pipettes had a tip resistance <3 MΩ when filled with the internal solution. Cell capacitance and access resistance were calculated for each cell. Thereafter, capacitance and series resistance compensation were optimized, and ~80% compensation was usually obtained. Maximum hKv1.5 current amplitudes at +60 mV averaged 1.5±0.1 nA, mean uncompensated access resistance was 3.2±0.5 MΩ, and cell capacitance was 10.2±0.9 pF (n=22). Thus, no significant voltage errors (<5 mV) were expected with the electrodes used. In ventricular myocytes, the effective access resistance calculated was 13.4±0.8 MΩ (n=8), and the larger currents recorded were <1 nA (278±33 pA); thus, the mean value of voltage error has an upper limit of 2.7 mV. The current records were sampled at 3 to 10 times the antialias filter setting.

The activation curves were constructed by plotting tail current amplitudes elicited as a function of the membrane potential and were fitted with a Boltzmann distribution:

\[ y = \frac{A}{1 + \exp((V - V_m)/k)} \]

where A is the amplitude, $V_m$ is the midpoint of activation, $V_h$ is the test potential, and k represents the slope factor. Under some circumstances, a Boltzmann distribution with 2 terms was needed to fit the experimental data, the equation being

\[ y = \frac{A}{1 + \exp((V_h - V_m)/k_1)} + \frac{A}{1 + \exp((V_h - V_m)/k_2)} \]

To determine the voltage dependence of hKv1.5 current block, the leak-corrected current in the presence of drug was normalized to the test potential and compensated for the fractional block at each voltage. The voltage dependence of block was fitted to

\[ f = \frac{[D]}{[D] + K_{D}^* \times \exp(-z \delta FE/RT)} \]

where z, F, R, and T have their usual meaning, $\delta$ represents the fractional electrical distance, and $K_{D}^*$ represents the apparent dissociation constant at the reference potential (0 mV).

Time-dependent drug effects on $I_{Ks}$ were quantified by measurement of the amplitudes of tail currents elicited by depolarizing pulses from -40 to +50 mV of variable duration (0.25 to 10 seconds). The relation between the amplitude tails and the pulse duration was fitted by a monoexponential time function. To describe the time course of the tail currents on repolarization, exponential analysis was used. Results are expressed as mean±SEM. Data were compared by ANOVA followed by Newman-Keuls test. A value of $P<0.05$ was considered significant.

**Results**

**Effects on hKv1.5 Currents**

Figure 1 shows hKv1.5 currents recorded in 2 cells in the absence of and 4 minutes after perfusion of 1 μmol/L losartan (A) or E3174 (B). At the beginning of the infusion, losartan and E3174 increased the amplitude of hKv1.5 currents elicited by 250-ms pulses from -80 to +60 mV by...
increased the time constant of tail current decline from 63.5±9.6 to 117.7±16.8 ms (n=8, P<0.01). E3174 markedly decreased the peak tail amplitude and increased the time constant of tail current decline from 56.4±6.4 to 111.2±13.4 ms (n=16, P<0.01), inducing the “crossover” of the tail currents.

**Voltage- and Frequency-Dependent Effects of Losartan and E3174 on hKv1.5 Currents**

Figure 2A shows the current-voltage relationship (I-V curves) obtained in the absence and in the presence of losartan (left) or E3174 (right). At negative potentials (−30/−20 mV), both drugs increased the current amplitude, whereas at depolarized potentials, an inhibitory effect was observed. To quantify this voltage dependence, the ratio \( I_{\text{drug}}/I_{\text{control}} \) for each experiment was plotted as a function of the membrane potential, together with the activation curve obtained in control conditions (Figure 2B). For both drugs, the blockade reached a maximum at 0 mV and thereafter decreased with a shallow voltage-dependence. Figure 2B shows that the blockade induced by losartan and E3174 was significantly higher at +10 than +60 mV (P<0.05). Fitting the experimental data to Equation 3, the \( z \) value averaged −0.27±0.04 and −0.14±0.01 in the presence of losartan and E3174, respectively. Moreover, losartan and E3174 dramatically modified the voltage-dependence of channel activation. Under control conditions, the averaged values for \( V_h \) and \( k \) of the activation curve were −19.5±1.2 and 4.9±0.3 mV (n=16), respectively (Figure 2C). In the presence of losartan or E3174, the activation curve displayed 2 components, the steeper one being responsible for ≈75% of the activation process. The solid lines illustrate a fit with a sum of 2 Boltzmann components, and the \( V_h \) and \( k \) values for each component were summarized in Table 1. Both losartan and E3174 significantly shifted the midpoint of the steeper component to more negative potentials.

In another group of experiments, trains of 200-ms pulses to +10 or +60 mV at 1 or 2 Hz were applied. Trains were separated from each other by 2-minute intervals. Under control conditions, the current amplitude decreased by 7.0±0.5% and 16.3±1.2% when trains of pulses to +10 mV were applied at 1 and 2 Hz, respectively. The percentages of block obtained from the ratio of current amplitudes in the absence and in the presence of either losartan or E3174 during application of the trains were averaged (Table 2). A certain amount of block was apparent from the first depolarization applied, ie, “ionic block.” Thereafter, during the train, the

### Table 1. Effects of Losartan and E3174, 1 μmol/L, on the Voltage-Dependence of hKv1.5 Channel Activation

<table>
<thead>
<tr>
<th></th>
<th>( V_{h1} )</th>
<th>( k_1 )</th>
<th>Amplitude, ( % )</th>
<th>( V_{h2} )</th>
<th>( k_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>−19.5±1.2</td>
<td>4.9±0.3</td>
<td>100</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>Losartan</td>
<td>−29.1±2.0*</td>
<td>4.3±0.3</td>
<td>64.1±5.6</td>
<td>31.6±11.2</td>
<td>22.6±6.3</td>
</tr>
<tr>
<td>E3174</td>
<td>−27.8±1.7*</td>
<td>4.0±0.2</td>
<td>74.6±1.7</td>
<td>23.8±5.8</td>
<td>16.4±2.0</td>
</tr>
</tbody>
</table>

Values represent the midpoint of activation (\( V_h \)) and slope factor (\( k \)) of the activation curves. Data are mean±SEM of 16 experiments.

*P<0.01.
TABLE 2. Frequency-Dependent Effects Induced by Losartan and E3174

<table>
<thead>
<tr>
<th>Test Potential</th>
<th>Tonic block</th>
<th>Frequency-dependent block</th>
</tr>
</thead>
<tbody>
<tr>
<td>+10 mV</td>
<td>9.4±3.3</td>
<td>20.0±6.1</td>
</tr>
<tr>
<td></td>
<td>17.1±1.0</td>
<td>36.8±8.2*</td>
</tr>
<tr>
<td></td>
<td>2 Hz</td>
<td>36.7±2.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>52.8±5.6*</td>
</tr>
<tr>
<td></td>
<td>+60 mV</td>
<td>7.5±1.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.2±2.6</td>
</tr>
<tr>
<td></td>
<td>1 Hz</td>
<td>19.5±3.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>34.3±9.1*</td>
</tr>
<tr>
<td></td>
<td>2 Hz</td>
<td>28.2±3.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>48.9±7.5*</td>
</tr>
</tbody>
</table>

Percentage of tonic block and frequency-dependent block induced by losartan and E3174 at 1 μmol/L when applying trains of 200-ms depolarizing pulses to +10 or +60 mV at 1 or 2 Hz. Data are mean±SEM of 16 experiments.

*p<0.05 vs losartan.

blockade increased until a new steady-state level was reached, ie, the blockade was frequency-dependent. Moreover, the frequency-dependent block produced by E3174 was more marked than that induced by losartan (Table 2).

Effects on K⁺ Currents in Ventricular Myocytes

Figure 3, A and B, shows current traces elicited in 2 guinea pig ventricular myocytes perfused with Co²⁺-containing solution when 5-second pulses were applied from −40 to +50 mV. Under these conditions, the outward current is the sum of the fast (I_K) and the slow (I_Ks) components of the delayed rectifier. At 1 μmol/L, losartan decreased the maximum outward current at the end of the pulse to +50 mV by 27.5±1.8%, (P<0.01, n=6). Steady-state block was voltage-independent; thus, blockade after pulses to −10 mV averaged 22.1±2.4% (n=6, P>0.05). Figure 3B shows that at 1 μmol/L, E3174 transiently increased the total maximum outward current elicited on depolarization by 9.8±1.8% (P<0.05), but after 10 to 15 minutes of perfusion, the current amplitude decreased by 8.2±1.7% (P<0.05, n=6). At 10 μmol/L, E3174 decreased the maximum outward current by 16.0±1.5% (P<0.05) without any significant modification of the tail current. Figure 3C shows the current traces elicited in the same cell as in 3B when the steady-state effects were achieved. After a 5-second depolarization to +50 mV, the cell was repolarized at 0 mV for 10 seconds. Under these conditions, the I_K was deactivated, and hyperpolarization to −50 mV elicited a tail current that is mainly due to the I_K because of its marked inward rectifier properties. E3174 at 1 μmol/L slightly decreased the maximum and the tail currents elicited by the first depolarizing pulse, but increased (Figure 3D) the tail amplitude obtained at −50 mV by 15.3±2.3% (P<0.05, n=3).

Effects on HERG Channels

Figure 4 shows the effects of losartan and E3174 on HERG currents. Depolarizations to potentials positive to −50 mV elicited an increasing outward current that was followed by a pronounced deactivating tail current on repolarization to −60 mV (Figure 4, A and B). The I-V curves were obtained by plotting the HERG current amplitude at the end of 5-second pulses as a function of the membrane potential in control conditions and in the presence of 1 μmol/L losartan (C) and E3174 (D). The current amplitude reached a maximum at ≈0 mV, decreasing at more positive voltages, consistent with the marked inward rectification observed for I_K. Losartan decreased HERG currents elicited at 0 mV by 23.3±4.8% (P<0.05), whereas E3174 increased the current by 30.5±6.2% (from 133±36 to 175±74 pA, P>0.05). Both drugs shifted the V₅₀ of the activation curve into the negative direction. In fact, V₅₀ in the absence and in the presence of losartan averaged −12.7±1.5 and −19.9±3.6 mV (n=5, P>0.05), respectively, whereas E3174 shifted the V₅₀ 10.8±2.1 mV in the hyperpolarizing direction (n=5, P<0.05). Deactivating tail currents at −60 mV after pulses to +60 mV exhibited a biexponential time course with a fast (230.9±30.5 ms) and a slow (1390.4±161.9 ms) time constant. Losartan did not modify the fast (217.2±39.4 ms, A through C, Dotted line represents zero current level.

Figure 3. A, Effects of 1 μmol/L losartan on I_K elicited by a 5-second pulse from −80 mV to +50 mV and on tail currents elicited on repolarization to −30 mV. B, Effects of 1 and 10 μmol/L E3174 on I_K. C, Current records obtained by applying pulse protocol shown at top in same cell as in B when effects of 1 μmol/L E3174 reached steady state. D, Expanded scale of tail currents elicited by latter hyperpolarization to −50 mV. A through C, Dotted line represents zero current level.
or the slow \(1285.6 \pm 279.6 \text{ ms, } n=5, P>0.05\) time constants of deactivation. E3174 also failed to modify the time course of the deactivating tails \(238.6 \pm 38.9 \text{ and } 1417.4 \pm 213.6 \text{ ms, } n=5, P>0.05\).

**Voltage- and Time-Dependent Effects on \(I_{Ks}\)**

Effects of losartan and E3174 on \(I_{Ks}\) were studied in ventricular cells perfused with an external solution supplemented with LaCl\(_3\). Figure 5A shows that 1 \(\mu\text{mol/L}\) losartan decreased both the maximum outward and the tail current amplitudes. This blockade was not voltage-dependent, averaging \(18.4 \pm 3.2\%\) and \(22.0 \pm 4.2\%\) when pulses to \(+50\) and to \(-10\) mV were applied \((n=6, P>0.05)\). The dotted representation is the ratio between the losartan-sensitive current \((I_{c}-I_{L})\) and the current in control conditions. Fitting this ratio to a monoeponential function yielded the time constant of development of block, which averaged \(481.1 \pm 64.7 \text{ ms } (n=6)\). Furthermore, losartan (Figure 5C) did not modify the \(V_{h}\) of the \(I_{Ks}\) activation curve \((26.7 \pm 2.5 \text{ versus } 26.3 \pm 2.4 \text{ mV, } n=6, P>0.05)\), but it increased the \(k\) value from \(22.4 \pm 1.5\) to 19.2 \(\pm 1.6\) mV \((n=6, P<0.05)\).

Figure 5B shows that E3174, 1 \(\mu\text{mol/L}\), was less potent than losartan at blocking \(I_{Ks}\) \((6.5 \pm 0.7\% \text{ at } +50 \text{ mV, } n=5, P>0.05)\). Moreover, this blockade was voltage-dependent, being apparent at potentials positive to 30 mV. Thus, currents elicited by pulses to \(-10\) mV were 1.2 \(\pm 0.1\) times higher than those obtained in the absence of drug \((P>0.05)\). E3174 did not modify the \(k\) value of the activation curve and shifted the \(V_{h}\) toward more negative potentials \((29.4 \pm 2.7 \text{ versus } 26.1 \pm 2.3 \text{ mV, } n=5, P<0.05)\), an effect that can account for the small increase in current amplitude observed at negative potentials (Figure 5D).
To study the effects on the activation kinetics of $I_{Ks}$, the tail amplitudes elicited on return to $-30$ mV after pulses to $+50$ mV of increasing duration (0.25 to 10 seconds) were fitted by a monoeXponential function of time. By use of this procedure, the dominant time constant of the activation process of $I_{Ks}$ (2615.6±236.0 ms, n=10) can be determined.9 Neither losartan nor E3174 modified the time course of $I_{Ks}$ activation.

Finally, tail currents were adjusted by a biexponential function, and neither the fast nor the slow time constants were modified in the presence of losartan. E3174 increased both the $\tau_f$ (299.4±35.0 versus 379.6±43.2 ms, n=5) and $\tau_s$ (2029.5±113.0 versus 2288.3±32.5 ms, n=5) values, but this increase did not reach statistical significance.

### Effects on Transmembrane Action Potentials

In guinea pig papillary muscles perfused with the 2 mmol/L Ca$^{2+}$-containing solution, losartan and E3174 (1 μmol/L) did not modify the resting membrane potential (−78.7±0.7 mV, n=12) or the amplitude of the action potential (106.7±2.2 mV, n=12). Losartan lengthened the activation duration at both 50% of repolarization (APD$_{50}$=157.3±2.9 versus 170.0±3.8 ms, n=6, P<0.05) and 90% of repolarization (APD$_{90}$=204.0±3.5 versus 225.0±3.8 ms, n=6, P<0.05). E3174 prolonged the APD$_{50}$ from 156.2±7.2 to 167.7±9.3 ms (n=6, P<0.05) without modifying the APD$_{90}$ (119.2±7.7 versus 125.0±10 ms, n=6, P>0.05). In muscles perfused with 2 mmol/L Ca$^{2+}$+30 μmol/L La$^{3+}$-containing solution, losartan did not modify the action potential characteristics, whereas E3174 shortened the APD$_{50}$ from 161.6±7.5 to 150.6±7.9 ms (n=6, P<0.05). Figure 6 shows superimposed action potentials recorded in the presence and in the absence of losartan or E3174 under each experimental condition.

### Discussion

Our results demonstrated that losartan and E3174 directly affected hKv1.5, HERG, and Ks channels. This assertion is supported by the following evidence: (1) experiments were carried out in the absence of angiotensin II, and thus, the observed effects are not the consequence of the antagonisms of its effects at the AT$_1$ receptor level. (2) Effects of losartan and E3174 on each current differ in potency and in voltage- and time-dependence, which is not consistent with a common mechanism of action, ie, blockade of AT$_1$ receptors.

**Effects on hKv1.5 Currents**

Losartan and E3174 transiently increased the hKv1.5 current at voltages at which the activation curve of hKv1.5 channels reached saturation. The gating modification underlying this effect is unknown and needs to be studied at the single-channel level. Under steady-state conditions, E3174- and losartan-induced block increased concomitantly with the channel opening, suggesting that both drugs bind primarily to an open and/or inactivated state of hKv1.5 channels. When almost all channels are open, a shallow voltage-dependent unblock appeared. Because losartan and E3174 are weak acids (pK$_a$=5.6 and 4.2, respectively), this voltage dependence can be explained by the hypothesis that the binding site is within the transmembrane electrical field and that the anionic form of both drugs reached this receptor site from the inside by crossing ≈20% of the membrane electrical field. In the presence of losartan and E3174, the activation curve of hKv1.5 channels became biphasic, and both drugs shifted the midpoint of the steeper component toward more negative potentials, an effect that can account for the increase in current produced at negative potentials. Because hKv1.5 channels exhibited multiple open states,13 the second component of the activation curve could be the consequence of a selective affinity of these drugs for the first open state, whereas strong depolarizations, which promote the transition to the second open state, will produce drug unbinding. A second possibility is that they change the voltage- and time-dependence of transitions between open states. Losartan and E3174 slowed the time course of hKv1.5 tail-current decline. This result indicates that both drugs must dissociate from the receptor before the channel can close. Finally, the blockade induced by both drugs was frequency-dependent, increasing at relevant physiologically driven frequencies.

**Effects on HERG and Ks Channels**

The results obtained in guinea pig myocytes in the absence of LaCl$_3$, suggested that losartan blocked, whereas E3174 enhanced, Kr currents, and this was confirmed in human Kr (HERG) channels. Both losartan and E3174 modified the voltage-dependence of HERG channel activation, and as was observed on hKv1.5 channels, E3174 was more potent for this effect. The negative shift of the midpoint of activation can only partially account for the E3174-induced increase on HERG currents.

Losartan-induced block on $I_{Ks}$ developed faster than channel activation, which explains why it did not modify the time course of channel activation. This fast kinetics of block suggested that block already starts during conformational states that appear during transitions between the rested and the open state. Moreover, losartan apparently did not affect the time course of channel closing, which could be the consequence of its fast dissociation from the channel before it closes. In contrast, E3174-induced block on $I_{Ks}$ is very small, and it developed only after channels began to open (see Figure 5B). In the presence of LaCl$_3$, losartan and E3174 modified the voltage-dependence of $I_{Ks}$ channel activation. Indeed, losartan increased the slope factor, whereas E3174 shifted the $V_1$ of the activation curve. The E3174-induced
shift can be responsible for the small increase in the $I_{Ks}$ amplitude observed at negative potentials.

Clinical Implications
Preliminary results indicated that losartan reduced the QT dispersion in patients from the ELITE study.\textsuperscript{14} We demonstrated that at therapeutic concentrations,\textsuperscript{2} losartan blocks $I_{Ks}$, HERG, and hKv1.5 channels. Thus, a prolongation of the human atrial and ventricular action potentials would be expected. However, it is difficult to correlate K\textsuperscript{+} current blockade with action potential prolongation. In fact, hKv1.5 blockage could result in a shortening of the APD.\textsuperscript{15} In contrast, E3174 blocks hKv1.5, increases HERG, and shortens the APD$_{50}$ in the presence of LaCl$_3$, which suggests that it probably modified another current involved in ventricular repolarization. However, caution should be exerted before extrapolating the present results to explain the possible antiarrhythmic action of losartan, particularly when both losartan and E3174 are active compounds with different effects on K\textsuperscript{+} channels, so that the final result on APD is difficult to predict. Thus, further studies are needed to correlate our findings with their effects on human cardiac APD and to confirm its possible antiarrhythmic and/or proarrhythmic properties.

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References
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