Nephrotoxicity of Allopurinol Is Enhanced in Experimental Hypertension

Howard Trachtman, Elsa Valderrama, and Stephen Futterweit

Hyperuricemia is present in 20–40% of pediatric and adult patients with essential hypertension. This metabolic abnormality may represent an additional risk factor for the development of cardiovascular disease. Therefore, we performed the following studies to determine 1) whether hyperuricemia is more prevalent in the spontaneously hypertensive rat (SHR) and 2) whether allopurinol treatment has a beneficial effect on the development of hypertension in this strain, based on its capacity to lower the serum uric acid concentration and to act as an antioxidant agent. SHR and control Wistar-Kyoto (WKY) rats were assigned to two groups, one given tap water to drink and the other provided water containing allopurinol (400 mg/l) to furnish an approximate daily dose equal to 100 mg/kg body wt. This treatment was maintained for 15 weeks. The serum uric acid levels were similar in untreated SHR and WKY rats (1.85±0.10 versus 1.66±0.14 mg/dl; p=0.28). In the control WKY rat strain, allopurinol therapy did not adversely affect weight gain or hematocrit and did not cause an increase in mortality. It resulted in a moderate decrement in kidney function (creatinine clearance: allopurinol-treated group 0.32±0.09 versus control group 0.46±0.04 ml/min/100 g body wt, in conjunction with mild-to-moderate tubulointerstitial inflammation (allopurinol-treated group 0.9±0.4 versus control group 0). In contrast, administration of allopurinol to SHR resulted in failure to thrive, marked anemia, severe azotemia (creatinine clearance: allopurinol-treated group 0.04±0.01 versus control group 0.39±0.04 ml/min/100 g body wt; p<0.001), and severe tubular atrophy and interstitial fibrosis (allopurinol-treated group 2.2±0.2 versus control group 0; p<0.001). These findings indicate that hyperuricemia is not more prevalent in the SHR. Furthermore, allopurinol administration is associated with markedly increased nephrotoxicity characterized by severe tubulointerstitial injury, azotemia, and impaired growth. (Hypertension 1991;17:194–202)

It has been noted that 20–40% of pediatric and adult patients with essential hypertension have hyperuricemia.1–3 In this setting, the metabolic abnormality has been attributed to alterations in renal tubular function and, in particular, to enhanced proximal urate reabsorption.4 Thus, hyperuricemia may reflect the primary role of the kidney in the pathogenesis of hypertension.5 It has been suggested that hyperuricemia may represent an additional risk factor for the development of cardiovascular disease.6

Excessive oxidant stress may be instrumental in the onset and maintenance of experimental hypertension. Recent data indicate that oxygen free radicals are involved in changes in vascular permeability during acute hypertension.7 In addition, aortic rings removed from spontaneously hypertensive rats (SHR) manifest increased contraction in vitro after exposure to reactive oxygen molecules.8 There have been no previous laboratory investigations of uric acid metabolism in experimental models of hypertension. Hyperuricemia arising from excessive production or enhanced renal reabsorption of this organic acid can be readily corrected by the administration of allopurinol, a xanthine oxidase inhibitor. Furthermore, this drug has antioxidant properties that may exert a beneficial in vivo effect on the development of hypertension.9,10 Therefore, we performed the following experiments to address these questions: 1) Is hyperuricemia more common in the SHR compared with control Wistar-Kyoto (WKY) rats? and 2) Does the administration of allopurinol in doses designed to lower the serum uric acid concentration have a beneficial effect on the development of hypertension?
Methods

Animals

Male SHR and WKY rats weighing 100–150 g were purchased from Taconic Farms, Germantown, N.Y. They were housed three animals per cage and were fed standard rodent chow (Purina, St. Louis, Mo.). They were maintained in an animal facility with a 12-hour light/dark cycle that was kept at 25°C.

Experimental Groups and Design

Animals from each rat strain were randomly assigned to a control group given tap water to drink and an experimental group that received water containing allopurinol (400 mg/l, Sigma Chemical Co., St. Louis, Mo.). Fresh solutions of allopurinol were prepared every 48 hours. Water was provided ad libitum to all four groups of animals. No attempt was made to pair feed the control and allopurinol-treated rats in each strain.

Animals were weighed, a serum sample and microhematocrit determination were obtained, and a 24-hour urine collection was completed before commencing the study. These urine collections were performed by placing the rats in individual metabolic cages (Nalge Co., Rochester, N.Y.). During this period, the rats were provided the appropriate drinking water but were denied access to food. Finally, blood pressure was measured in awake conscious rats. These studies were repeated at 4-week intervals during the course of the 15-week treatment period.

At the termination of the study, after completing the above-described evaluation, the rats were killed, and the kidneys were removed, weighed, and placed in a 10% formalin fixative solution for histological examination.

Blood Pressure Measurements

The rats were placed in a restraining cage that was maintained at 37°C with a built-in heating element. After the rats had been allowed to acclimatize to the apparatus, blood pressure was measured with a tail-cuff plethysmograph (Narco Bio Systems-HealthDyne, Houston, Tex.). Each determination represented the average of 3–4 individual readings.

Analytical Methods

Serum and urine creatinine concentrations were measured using a modified Jaffe reaction in an automated analyzer (Beckman Instruments, Inc., Fullerton, Calif.). Serum uric acid concentration was measured with a Technicon RA 500 autoanalyzer (Tarrytown, N.Y.), based on the uricase-catalyzed oxidation of uric acid to allantoin.11 Urinary Na⁺ excretion and osmolality were determined with an ion-selective electrode in an E4A analyzer (Beckman) and a vapor pressure osmometer (Wescor, Inc., Logan, Utah), respectively. Urinary protein concentration was measured using the Coomassie blue staining method (Quantimetrix Medical Industries, Hawthorne, Calif.).

Histological Assessment

After removal, the kidneys were cut in half longitudinally and placed in formalin solution. The tissue was sectioned and stained with periodic acid–Schiff (PAS) and silver stain (Jones) reagents. The sections were examined for the extent of tubular atrophy/interstitial fibrosis, interstitial inflammation, and arterial thickening. The findings were graded on the following semiquantitative scale: 0, no evident abnormalities; 1+, less than 25% of the interstitium affected; 2+, 25–50% of the interstitium affected; and 3+, more than 50% of the renal parenchyma demonstrated the abnormality. Although at least 100 glomeruli were examined in each case, the severity of the glomerular damage was much less than the tubulointerstitial injury, and therefore, it was not quantitated. All histopathological assessments were performed by a single pathologist who was unaware of the animal strain or the experimental group assignment.

Statistical Methods

Results are provided as mean±SEM. Differences between strains and treatment groups were evaluated with an analysis of variance followed by appropriate t tests. Differences in survival rates were analyzed using the Fisher exact test. The findings were considered statistically significant if the value of p was <0.05.

Results

At the onset of the study, there were no differences in creatinine clearance, protein excretion, or urinary osmolality between the two rat strains. The SHR were initially smaller than the WKY rats in body size (110±6 versus 180±3 g; p<0.01); however, blood pressure levels were comparable in the SHR and WKY rats (126±2 versus 124±4 mm Hg). The daily urinary Na⁺ excretion was reduced in the SHR compared with that in the WKY rats before assignment to the tap water or allopurinol-treatment group (2.6±0.5 versus 8.1±0.9 meq/day; p<0.01). A recent study of somatic growth and body fluid volumes in young SHR has also indicated that the hypertensive strain is smaller than normotensive WKY rats at an age comparable with the initial value of the animals used in this study.12 The decreased daily urinary Na⁺ excretion in the SHR may account for the relative expansion of the total body water and extracellular fluid compartments during the developmental phase of hypertension.12

The serum uric acid concentrations were similar in both groups of SHR and WKY rats before the onset of the study (Table 1). The mean values obtained by pooling all samples collected before group assignment together with final specimens drawn from the tap water groups were also similar in the SHR and WKY rats (1.85±0.10 versus 1.66±0.14 mg/dl, respectively). These values are consistent with previously published normative data for various strains of rats.13 Furthermore, none of the hypertensive rats had serum uric acid concentrations that were more than 3 mg/dl.
TABLE 1. Serum Uric Acid Levels

<table>
<thead>
<tr>
<th></th>
<th>SHR</th>
<th></th>
<th>WKY</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Allopurinol</td>
<td>Control</td>
<td>Allopurinol</td>
<td>Control</td>
</tr>
<tr>
<td>Baseline</td>
<td>1.79±0.16</td>
<td>1.92±0.34</td>
<td>1.58±0.17</td>
<td>1.95±0.37</td>
</tr>
<tr>
<td>Final</td>
<td>0.80±0.18*</td>
<td>1.68±0.09</td>
<td>1.22±0.18</td>
<td>1.45±0.12</td>
</tr>
</tbody>
</table>

Values are in milligrams per deciliter. *p<0.02 versus spontaneously hypertensive rat (SHR) control group and Wistar-Kyoto (WKY) control group.

The serum uric acid concentration declined in response to the administration of allopurinol in both rat strains (Table 1). However, the magnitude of the hypouricemic effect was significantly greater in the SHR.

All animals assigned to the allopurinol treatment regimen drank a daily amount of water that was comparable with paired strain members given tap water. Each month, the volume of water ingested in relation to body weight was estimated during the 24-hour urine collection period and was found to be similar in all four groups of rats. The amount of allopurinol-treated water consumed provided approximately 100 mg/kg/day of the drug to both the SHR and WKY rats.

In the control WKY strain, allopurinol treatment did not interfere with somatic growth or cause alterations in blood pressure; however, drug administration to WKY rats did result in mild anemia (Table 2).

The survival rate was 100% in the tap water and allopurinol-treated groups of WKY rats. However, administration of the drug did cause renal dysfunction compared with rats given tap water. The renal injury was manifested by a higher serum creatinine concentration (0.9±0.3 versus 0.5±0.1 mg/dl) and a reduction in creatinine clearance (0.32±0.09 versus 0.46±0.04 ml/min/100 g body wt) (Table 3). The nephrotoxicity of allopurinol in the WKY rats involved the tubulointerstitium, based on an increase in urine flow rate from 12±2 to 28±7 μl/min and a decrease in the urine osmolality from 502±116 to 210±50 mosm/kg H2O, p<0.05. Urinary Na+ excretion was unaffected by the administration of allopurinol (WKY rats given tap water, 8.3±1.9 versus allopurinol-treated WKY rats, 7.8±1.4 meq/day).

The alterations in renal function in WKY rats given allopurinol compared with rats given tap water were paralleled by changes in renal histopathology. Gross examination of the kidneys removed from WKY rats given allopurinol did not reveal any macroscopic abnormalities (Figure 1). However, allopurinol treatment compared with tap water administration was associated with moderate increases in the semiquantitative scores for tubular atrophy/interstitial fibrosis (0.9±0.4 versus 0) and for interstitial inflammation (1.4±0.7 versus 0; p<0.01) (Table 4). Representative photographs of the light microscopic examination of renal tissue from WKY rats given tap water and allopurinol-treated water are provided in Figures 2 and 3, respectively.

TABLE 2. General Animal Data

<table>
<thead>
<tr>
<th>Group</th>
<th>Wt (g)</th>
<th>Hct (vol%)</th>
<th>BP (mm Hg)</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=11)</td>
<td>157±13*</td>
<td>-14±4*</td>
<td>31±5†</td>
<td>5/11†</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>226±6</td>
<td>3±1</td>
<td>47±7†</td>
<td>7/10</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=6)</td>
<td>258±24</td>
<td>-1±1‡</td>
<td>-4±4‡</td>
<td>6/6</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>301±13</td>
<td>5±1</td>
<td>-19±4</td>
<td>6/6</td>
</tr>
</tbody>
</table>

Data for weight (Wt), hematocrit (Hct), and blood pressure (BP) are given as the change between final and initial values. SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

\*p<0.01 versus all other groups.

\(\triangle p<0.05\) versus allopurinol-treated WKY rat group and control WKY rat group.

\(\wedge p<0.01\) versus control WKY rat group.

TABLE 3. Renal Function at Completion of Study

<table>
<thead>
<tr>
<th>Group</th>
<th>(U_{\text{prot}}) (mg/24 hr)</th>
<th>(S_{\text{creat}}) (mg/dl)</th>
<th>(C_{\text{cr}}) (ml/min/100 g body wt)</th>
<th>Urine flow (μl/min)</th>
<th>(U_{\text{osm}}) (mosm/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=11)</td>
<td>74±19*</td>
<td>3.4±0.7*</td>
<td>0.04±0.01*</td>
<td>24±4†</td>
<td>145±19</td>
</tr>
<tr>
<td>Control (n=10)</td>
<td>19±2</td>
<td>0.5±0.1</td>
<td>0.39±0.04</td>
<td>14±2</td>
<td>215±29</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=6)</td>
<td>31±14</td>
<td>0.9±0.3</td>
<td>0.32±0.09</td>
<td>28±7</td>
<td>210±50‡</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>29±4</td>
<td>0.5±0.1</td>
<td>0.46±0.04</td>
<td>12±2</td>
<td>502±116</td>
</tr>
</tbody>
</table>

Values given are mean±SEM. \(U_{\text{prot}}\), urinary protein; \(S_{\text{creat}}\), serum creatine; \(C_{\text{cr}}\), creatinine clearance; urine flow, urine flow rate; \(U_{\text{osm}}\), urine osmolality; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

\(\ast p<0.01\) versus all other groups.

\(\wedge p<0.01\) versus SHR control group.

\(\triangledown p<0.03\) versus SHR control group.

\(\triangle p<0.05\) versus WKY rat control group.
The SHR given allopurinol did not tolerate drug therapy very well; they did not thrive as well as comparably treated WKY rats or SHR given tap water. SHR receiving allopurinol gained significantly less weight and displayed marked anemia compared with the other three rat groups (Table 2). The survival rate among SHR given allopurinol was 45%, significantly lower than the survival rate observed in WKY rats given allopurinol (Table 2). The administration of allopurinol did not prevent the development of hypertension.

The SHR given allopurinol manifested severe nephrotoxicity compared with SHR given untreated tap water. The derangements in renal structure and function were much worse in the SHR that received allopurinol than similarly treated WKY rats. Thus, compared with SHR given tap water, hypertensive rats given allopurinol that survived for the entire treatment period had significant proteinuria (74±19 versus 19±2 mg/24 hr; p<0.01), an elevated serum creatinine concentration (3.4±0.7 versus 0.5±0.1 mg/dl; p<0.01), and a decreased creatinine clearance (0.04±0.01 versus 0.39±0.04 ml/min/100 g body wt; p<0.01). The renal injury affected tubular function, evidenced by a higher urine flow rate (24±4 versus 14±2 µl/min; p<0.03) and a reduced urinary osmolality (145±19 versus 215±29 mosm/kg H₂O) (Table 3). Urinary Na⁺ excretion was comparable and significantly increased above the initial values in both groups (SHR given tap water, 6.1±1.6 versus allopurinol-treated SHR, 6.2±1.5 meq/day).

The renal functional derangements observed in the SHR given allopurinol were accompanied by severe renal parenchymal damage compared with SHR given tap water. Examination of the gross renal specimens removed from SHR treated with allopurinol demonstrated shrunken, scarred kidneys with substantial thinning of the cortical mantle (Figure 4). In addition, the semiquantitative scores for tubular atrophy/interstitial fibrosis were 2.2±0.2 versus 0; for interstitial inflammation, 1.8±0.3 versus 0; and arterial thickening, 0.8±0.3 versus 0. All of these changes were significantly worse in the allopurinol-treated SHR (p<0.02) compared with the three other experimental groups (Table 4 and Figures 5 and 6). Structural damage to the glomeruli was much less

<table>
<thead>
<tr>
<th>Group</th>
<th>Kidney wt (g)</th>
<th>TA/IF</th>
<th>II</th>
<th>Art</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHR</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=6)</td>
<td>0.76±0.10*</td>
<td>2.2±0.2†</td>
<td>1.8±0.3†</td>
<td>0.8±0.3†</td>
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<tr>
<td>Control (n=7)</td>
<td>1.09±0.03</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WKY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allopurinol (n=6)</td>
<td>1.45±0.12</td>
<td>0.9±0.4</td>
<td>1.4±0.7‡</td>
<td>0</td>
</tr>
<tr>
<td>Control (n=6)</td>
<td>1.59±0.06</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

TA/IF, tubular atrophy/interstitial fibrosis; II, interstitial inflammation; Art, arterial thickening; SHR, spontaneously hypertensive rats; WKY, Wistar-Kyoto rats.

* p<0.001 versus all other groups.
† p<0.02 versus all other groups.
‡ p<0.01 versus control group, same strain.
FIGURE 2. Light micrograph of renal tissue from Wistar-Kyoto rat given tap water. Glomeruli, tubules, and interstitium are normal. (Hematoxylin and eosin stain; original magnification ×113.)

than the corresponding alterations in the integrity of the tubulointerstitial regions. It consisted of rare crescents and lesions of segmental glomerulosclerosis observed only in the SHR given allopurinol. The frequency of these abnormalities was too low to enable accurate quantitative assessment.

Discussion

The results of our investigation indicate that, contrary to the clinical observation that 20–40% of patients with essential hypertension have hyperuricemia, genetically hypertensive rats have a similar serum uric acid concentration compared with control rats. Differences in the serum uric acid concentration between species may arise from differences in production or renal excretion of uric acid. Renal uric acid handling is regulated by a balance between secretion and absorption in the proximal tubule. Serum uric acid levels are lower in rats and other non-primate species compared with humans because of the presence of the enzyme uricase in the liver. Nonetheless, the proposed mechanism of hyperuricemia in essential hypertension centers on altered renal handling of uric acid. In patients with essential hypertension, serum urate levels are a marker of reduced renal cortical blood flow, a factor that would compromise uric acid excretion. In young SHR, basal renal vascular resistance and the reactivity to angiotensin and thromboxane are enhanced compared with normotensive WKY rats. Therefore, a difference might still have been anticipated in the serum uric acid concentration between SHR and WKY rats. Additional studies in other genetic strains such as the Milan hypertensive rat and in volume-mediated hypertension (deoxycorticosterone acetate–salt) are warranted to extend our findings. In the absence of data indicating differences in urate metabolism between SHR and WKY rats, our findings suggest that alterations in function of the proximal nephron segment are not involved in the pathogenesis of experimental hypertension in this strain.

The SHR displayed an enhanced sensitivity to the hypouricemic action of allopurinol compared with the WKY rats. This finding could be explained by interstrain differences in the metabolism and clearance of the drug, the level of xanthine oxidase in various tissues, or the kinetic characteristics of this enzyme. These issues need to be addressed in future investigations since no previous studies have examined uric acid metabolism or tissue xanthine oxidase activity in the SHR versus the WKY rat or other experimental models of hypertension.

The failure of allopurinol to favorably alter the natural history of hypertension in the SHR is worthy
of comment. Oxygen free radicals contribute to the vasculopathy in acute norepinephrine-induced hypertension. There are also data that implicate reactive oxygen molecules in the pathogenesis of genetic hypertension in the SHR. Aortic rings removed from SHR display augmented contraction in response to
oxygen-derived free radicals, and this abnormality in vascular contractility is reversed by the addition of allopurinol. However, contrary to expectation, chronic inhibition of xanthine oxidase with allopurinol did not prevent or blunt the rise in blood pressure in the SHR. These negative findings need to be interpreted in light of the antioxidant qualities of allopurinol exerted through a scavenging action on hydroxyl radicals. This effect is independent of the inhibition of xanthine oxidase. The role of oxygen-derived free radicals in the onset of experimental hypertension may be exaggerated in vitro where various compensatory antioxidant systems are removed.

The second major finding of our study is that allopurinol administration to the SHR was associated with severe nephrotoxicity, directed primarily at the tubulointerstitial structures with relative sparing of the glomeruli. This adverse reaction to chronic allopurinol administration is in sharp contrast to the benefits of this drug in other organ systems (e.g., it attenuates ischemia and reperfusion-induced cerebral injury in this hypertensive strain).

We used the endogenous creatinine clearance as an index of the glomerular filtration rate and kidney function. There are inherent inaccuracies with this method due to incomplete bladder emptying and a variable contribution of tubular secretion to creatinine excretion. The latter difficulty is especially important in the context of tubulointerstitial injury. However, in these studies all rats demonstrated a high urine flow rate that minimized the error attributable to incomplete sample collection. In addition, the changes in the creatinine clearance were validated by large differences in the serum creatinine concentration. Finally, as in other recently published investigations, we are confident that consistent application of this methodology to all experimental groups indicates the presence of significant interstrain differences in kidney function in response to allopurinol treatment.

It is likely that the primary site of the nephrotoxicity of allopurinol in the SHR is the tubulointerstitium. This is based on the accumulated clinical evidence of tubular dysfunction, the disproportionate histological injury in this segment of the renal tissue, and the infrequent detection of glomerular abnormalities. A rare form of allopurinol nephrotoxicity directed at glomeruli has been described in patients and is characterized by a diffuse vasculitis and glomerular crescent formation. A similar pattern of renal parenchymal damage has been observed in a model of chronic renal failure after 5/6 nephrectomy, in which...
there is a better correlation between the level of renal function and the extent of tubulointerstitial injury than to the degree of glomerular damage. 25

An explanation for the enhanced toxicity of allopurinol in the hypertensive rats compared with normotensive controls is not apparent from these experiments. It is not a consequence of differences in drug dosing since both strains of rats consumed similar quantities of allopurinol-treated water in relation to body weight. In addition, the failure to pair feed the rats is unlikely to account for these findings since deficient caloric intake in the SHR should have protected renal function and preserved tubulointerstitial integrity. 25 It is likely that the heightened susceptibility of the SHR to allopurinol-induced renal injury is a quantitative rather than a qualitative difference between the two strains, since WKY rats also demonstrated evidence of drug-induced nephrotoxicity, albeit much less severe. The SHR may have increased metabolism of allopurinol to oxypurinol or diminished renal clearance of this more toxic metabolite. 26,27 The hypertensive rats may possess altered protective mechanisms against oxygen free radical damage that are perturbed by allopurinol treatment. 19 Differences in T cell–mediated immunopathological responses to allopurinol between these two strains may have augmented the toxicity of allopurinol in the hypertensive strain. 26,29 Finally, the SHR may be more susceptible to disruption of renal function and structure as a result of enhanced response to other actions of allopurinol (e.g., its function as a free radical scavenger or binding of various divalent ions). 9,10 Additional experiments are needed to address each of these possible explanations individually.

In summary, we have demonstrated that 1) SHR do not have hyperuricemia compared with control, normotensive WKY rats; 2) allopurinol therapy designed to lower the serum uric acid concentration does not prevent hypertension; and 3) allopurinol therapy is associated with profound nephrotoxicity, resulting in increased mortality, failure to thrive, decrement in renal function and severe, and irreversible tubulointerstitial injury. It is difficult to extrapolate from these experimental observations any recommendation regarding the therapy for hyperuricemia in patients with essential hypertension. However, we would suggest that extreme caution be exercised when prescribing allopurinol for any clinical indication in hypertensive patients.

References


KEY WORDS • hypertension • uric acid • allopurinol • nephrotoxicity
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