Captopril Ameliorates the Decreased Na\textsuperscript{+},K\textsuperscript{+}-ATPase Activity in the Retina of Streptozotocin-Induced Diabetic Rats

Anna Ottlecz and Thouria Bensaoula

**Purpose.** To examine the effect of captopril, an angiotensin-converting enzyme (ACE) inhibitor, on the activity of retinal sodium–potassium ATPase (Na,K-ATPase) and the activity of ACE in the serum and retina of streptozotocin (STZ)-induced diabetic rats.

**Methods.** Experimental diabetes was induced in male Long-Evans rats by a single intraperitoneal injection of STZ (55 mg/kg body weight). Some groups of normal and diabetic animals were treated with captopril (10 mg/kg per day) added to the drinking water for either a week or a month. After 2 and 4 months of diabetes, the specific activity of retinal total Na,K-ATPase was determined. The components of the activity of Na,K-ATPase caused by the α\textsubscript{1} and α\textsubscript{3} isoforms were pharmacologically separated by their different sensitivity to ouabain. The activity of ACE in the serum and retina was measured by radioassay using benzoyl-gly-gly-gly as substrate (10\textsuperscript{3} cpm, 5 mM).

**Results.** The total Na,K-ATPase activity was decreased significantly after 2 (16%, \( P < 0.02 \)) and 4 months (15%, \( P < 0.02 \)) of diabetes. At both time points examined, the activities of the α\textsubscript{1}-low-ouabain-affinity isoform and the α\textsubscript{3}-high-ouabain-affinity isoform of retinal Na,K-ATPase were significantly reduced compared to those of age-matched controls (α\textsubscript{1}, 9% to 14%, \( P < 0.05 \); α\textsubscript{3}, 14% to 19%, \( P < 0.05 \) and \( P < 0.02 \), respectively). After 1 month of captopril administration, the activities of both Na,K-ATPase isoforms were at control level in 2-month diabetic rats, whereas they were restored only partially in 4-month diabetic rats. In age-matched normal animals, 1 month of captopril treatment did not alter the specific activities of either Na,K-ATPase isoform. One week or 1 month of captopril administration to diabetic rats did not change the activities of retinal Na,K-ATPase isoforms. Serum ACE activity was elevated significantly in both groups of untreated STZ rats (55% and 40%, respectively). One month of captopril administration further increased the ACE levels in 2- and 4-month diabetic rats (101% and 94%, respectively) and also enhanced significantly the serum ACE activity in normal animals (131%) versus the basal values. In contrast, retinal ACE activity was decreased significantly in both groups of untreated STZ rats (~37%). Captopril exerted a significant inhibitory effect on the retinal ACE activity in 2- and 4-month diabetic rats (37% and 31%, respectively) compared to untreated diabetic animals as well as in normal rats (29%).

**Conclusions.** These data suggest that stimulation of retinal Na,K-ATPase activity in diabetes is most likely one of the mechanisms through which captopril can improve retinal complications. The effect of captopril seems to be related to local effects in the retina. Whether the inhibition of retinal ACE is part of the mechanism of action of captopril requires further study. Invest Ophthalmol Vis Sci. 1996;37:1633-1641.

Captopril (D-3-mercapto-2-methylpropanoyl-L-proline, SQ 14, 225) and other non-sulfhydryl angiotensin-I-converting enzyme (ACE) inhibitors are used widely in the treatment of hypertension and heart failure. Recently, captopril was found to be protective against retinopathy and nephropathy in insulin-dependent patients with diabetes regardless of the presence or absence of hypertension. It has been reported that ACE inhibitors improve the leaky blood–retina barrier, delay or reverse diabetic retinopathy,\textsuperscript{1-3} and reduce the deterioration of renal function.\textsuperscript{4,5} ACE inhibitors also ameliorate increased vascular leakage in the retina of experimentally diabetic rats.\textsuperscript{6} The mechanism of these protective effects of ACE inhibitors in the retina is unknown.

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Prolonged hyperglycemia has been widely accepted as a primary factor in the pathogenesis of vascular and neural complications in diabetes.\(^7\) Diabetic retinopathy involves several functional and morphologic alterations in the retinal capillaries; however, the biochemical mechanisms critically responsible for this diabetic complication remain unclear.\(^8\) Diabetic animals have reduced activity of Na,K-ATPase in vascular tissues, including the retina.\(^{10\text{-}13}\) Decreased retinal Na,K-ATPase activity was demonstrated in galactose-fed rats that develop diabetic-like complications.\(^{14,15}\) Na,K-ATPase is involved fundamentally in the maintenance of sodium and potassium gradients that drive the cotransport of amino acids and sugars, regulate cell volume, and contribute to part of the membrane potential.\(^{16,17}\) Whether impairment of Na,K-ATPase activity has a pivotal role in the pathogenesis of diabetic retinopathy must be clarified.

Abnormalities of the renin-angiotensin system (RAS) have been observed involving patients with insulin-dependent (type I) and non-insulin-dependent (type II) diabetes, suggesting that the circulating RAS might play a significant role in the development of diabetic complications.\(^{18\text{-}21}\) ACE (kininase II; EC 4.3.15.1), as an essential component of RAS, generates the potent vasoconstrictor angiotensin II from angiotensin I and inactivates the vasodilator bradykinin by peptidylpeptidase action; it also is involved in the metabolism of several other peptides.\(^{22,23}\)

In the streptozotocin (STZ)-induced diabetic rat, a model of type I diabetes, abnormalities of both the circulating and local RAS have been described.\(^{24,25}\) The current studies were conducted to determine the pattern of changes in the activity of retinal Na,K-ATPase, including its \(\alpha_1\) and \(\alpha_3\) isoforms, the activities of serum and retinal ACE in STZ-diabetic rats, and the effect of captopril treatment.

**MATERIALS AND METHODS**

**Materials**

Ultrapure chloride salts of calcium, magnesium, potassium, and sodium were from Alfa Products (Danvers, MA). Captopril, ethylenediaminetetraacetic acid (EDTA), HEPES, sodium sulfate (\(\text{Na}_2\text{SO}_4\)), Tris-adenosine triphosphate (ATP, Vanadium free), Tris–adenosine triphosphate (ATP, Vanadium free) were from Sigma Chemical, (St. Louis, MO). \([\text{H}^3]\text{benzoyl-gly-gly-gly}\) and Ventrex #1 cocktail were from Hycor Biomedical (Ven- trex Division, Portland, MA). Protein assay kits were from Bio-Rad Laboratories (Richmond, CA). The buffers for ATPase assays were 305 ± 5 mOsm, as determined by a Wescor 5100C vapor pressure osmometer (Wescor, Logan, UT). Concentrations of all media, substrates, and chemicals are presented as final concentrations.

**Animal Protocols**

Male Long–Evans hooded rats (each weighing 150 to 175 g) were purchased from Charles River Breeding Laboratories (Wilmington, MA). All experimental and animal care procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. NIH 85-23, revised 1985) and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Rats were housed in stainless steel cages, were given standard Rat Chow (Ralston Purina, Richmond, IN) and tap water ad libitum, and were on a 12-hour light–12-hour dark cycle in a room with an ambient temperature of 22°C ± 1°C.

Rats were randomized to receive an intraperitoneal streptozotocin (STZ) injection (55 mg/kg body weight in 0.05 M citrate buffer, pH 4.5) or citrate buffer alone (control) after a 20-hour fast. Diabetes was verified 3 days later by estimating hyperglycemia using a glucose oxidase kit (Sigma Chemical). Diabetic rats with blood glucose levels greater than 350 mg/dl were divided into six groups. Groups 1 and 2 were untreated for 2 and 4 months. Groups 3 and 4 were given captopril for 1 week during the last week of 2 and 4 months of diabetes. Groups 5 and 6 received captopril for 1 month during the second and fourth months of diabetes, respectively. Age-matched normal rats received captopril according to the above regimen. Captopril (10 mg/kg) was administered in the drinking water, the volume of which averaged 35 ± 5 ml/day for normal animals and 190 ± 10 ml/day for diabetic animals. All group was composed of at least eight rats. Body weights were measured, and animals were killed by decapitation after 2 or 4 months of diabetes. Blood samples were collected for the determination of blood glucose levels and serum ACE activity. Retinas were removed within 1 minute of death for biochemical studies.

**Preparation of Retinal Homogenate**

**ATPase Assay.** Retinas were placed in ice-cold Tris–EGTA buffer (150 mM NaCl, 5 mM KCl, 2 mM MgCl\(_2\), 1 mM EGTA, 30 mM Tris-base, pH 7.4) and were cleaned. When both retinas were used simultaneously (ouabain–inhibition curves), they were placed in 3.5 ml of ice-cold Tris–EDTA buffer (1 mM EDTA adjusted to pH 7.4 with Trizma base solution). If only one retina was used, it was placed in 1.75 ml of the same buffer. Retinas were stored at −85°C until used. On the day of experiment, retinas were thawed and homogenized by 20 strokes with a Teflon–glass homogenizer (Thomas Scientific, Swedesboro, NJ). Cellular debris was removed by centrifugation (1000g for 5 minutes), plasma membranes were permeabilized by freezing and thawing (three cycles), and aliquots of supernatants were used for ATPase determinations.

**ACE Assay.** Two retinas from each rat were placed into ice-cold Hanks’ balanced salt solution, cleaned, and transferred to 1 ml of HEPES buffer (0.05 M, pH 7.4, containing 0.1 M NaCl). They were frozen
Captopril Stimulates Retinal Na\(^{+},\)K\(^{+}\)-ATPase in Diabetes

### TABLE 1. Alterations of Body Weight and Blood Glucose Levels in Diabetic (STZ) Rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Months of Diabetes</th>
<th>n</th>
<th>Body Weight (g)</th>
<th>Blood Glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>—</td>
<td>18</td>
<td>437 ± 11.3</td>
<td>102 ± 0.9</td>
</tr>
<tr>
<td>STZ</td>
<td>2</td>
<td>12</td>
<td>312 ± 7.2</td>
<td>443 ± 21.1</td>
</tr>
<tr>
<td>STZ + Captopril</td>
<td>2</td>
<td>12</td>
<td>351 ± 9.6</td>
<td>409 ± 18.7</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>8</td>
<td>569 ± 14.8</td>
<td>106 ± 4.82</td>
</tr>
<tr>
<td>STZ</td>
<td>4</td>
<td>8</td>
<td>344 ± 6.5</td>
<td>503 ± 23.7</td>
</tr>
<tr>
<td>STZ + Captopril</td>
<td>4</td>
<td>8</td>
<td>339 ± 4.9</td>
<td>488 ± 22.0</td>
</tr>
</tbody>
</table>

STZ = streptozotocin.

immediately and stored at —85°C until used. On the day of experiment, retinal homogenates were prepared as described above.

### ATPase Assay

ATPase activity was determined in retinal homogenates by measuring the inorganic phosphate (p\(_i\)) liberated from ATP as previously described. Briefly, the assay mixture contained 130 mM NaCl, 5 mM KCl, 2 mM MgCl\(_2\), 1 mM EGTA, 30 mM Tris-HCl (pH 7.7), 0.1 ml retinal homogenate (40 to 45 µg of protein) in either the absence or presence of ouabain (10\(^{-2}\) to 10\(^{-3}\) M) in a final volume of 0.5 ml. The activities of the α1 and α3 isoforms of Na,K-ATPase (called α1 and α3 Na,K-ATPase) were determined in the presence of the half-maximal inhibitory concentrations (IC\(_{50}\)) of ouabain (2 × 10\(^{-5}\) M and 5 × 10\(^{-8}\) M, respectively). The reaction was initiated by the addition of ATP (2 mM final concentration) and proceeded for 30 minutes at 37°C. The total Na,K-ATPase activity was the difference between the activities recorded in the absence and presence of 10\(^{-3}\) M ouabain. The specific activity of the total Na,K-ATPase and its α1 and α3 isoforms is expressed as pmol P\(_i\)/hour\(^{-1}\)·mg protein\(^{-1}\).

### ACE Assay

The assay mixture (150 µl) contained 75 µl Hepes buffer 0.05 M, pH 8, containing 0.1 M NaCl, 0.6 M Na\(_2\)SO\(_4\), 0.1 mM phenylmethylsulfonyl fluoride, 50 µl of serum (diluted 1:1 by the assay buffer) or crude retinal homogenate, and 25 µl of substrate (10\(^5\) cpm of [\(^{3}\)H]-labeled and 5 mM of unlabeled benzoyl-gly-gly-gly) according to Erdös et al. When retinal homogenates were assayed, the incubation mixture contained two additional protease inhibitors, 0.05 mM leupeptin and 7 µM aprotinin. Incubations were carried out at 37°C for 30 minutes for serum and 2 hours for retina samples. The enzyme reaction was stopped with 50 µl of 0.5 N HCl. The hydrolyzed substrate was extracted by Ventrex #1 cocktail and counted for 10 minutes in a Beckman (Fullerton, CA) LS 6000 liquid scintillation counter. Results are expressed as nmol benzoyl-gly released/minute\(^{-1}\)·ml\(^{-1}\) in undiluted serum and pmol [\(^{3}\)H]benzoyl-gly released/hour\(^{-1}\)·mg protein\(^{-1}\) in retinal homogenates after correction for blank activity (in the presence 1.5 µM of captopril).

### Protein Assay

Protein was estimated by Coomassie blue G binding as described in the Bio-Rad kit, using bovine serum albumin as a standard.

### Data and Statistical Analysis

Data are presented as means ± SEM of experiments performed using triplicate samples for each condition. Because the retina essentially contains only the α1 and α3 isoforms of Na,K-ATPase, a computer program based on a mathematical model and validated by Attewill and coworkers was used to distinguish quantitatively the activity of the α1 and α3 Na,K-ATPase. All group data were analyzed by an overall unweighted means analysis of variance. Statistical significance levels are based on post hoc multiple comparisons using Tukey's Honestly Significant Difference test (statistical package from SAS Institute, Cary, NC).

### RESULTS

#### Effect of Captopril Treatment on the Activity of Total Na,K-ATPase in the Retina

Diabetic animals of each STZ group showed fourfold to sixfold elevation in blood glucose level and markedly reduced body weight compared to age-matched controls. One-month captopril treatment failed to alter blood glucose levels and body weight in 2- and 4-month diabetic rats (Table 1).

The Na,K-ATPase activity was significantly lower in the retina of diabetic rats after 2 (16%) and 4 months (15%) of the disease compared to age-matched controls. One-month captopril treatment failed to alter blood glucose levels and body weight in 2- and 4-month diabetic rats (Table 1).
whether captopril can overcome the inhibitory effect was found previously, when retinas were used for AT-

The total Na,K-ATPase activity was the difference between the activities recorded in the absence and presence of 10^-3 M ouabain.

did not alter significantly the activity of Na,K-ATPase in diabetic (STZ) rats (Table 2). To determine whether captopril can overcome the inhibitory effect of diabetes, we treated STZ rats with this ACE inhibitor. One week of captopril administration to diabetic rats did not alter significantly the activity of Na,K-ATPase (data not shown). Captopril treatment for 1 month restored the activity to normal value in the retina of 2-month diabetic animals. To investigate whether captopril can stimulate Na,K-ATPase activity at a later stage of diabetes, rats diabetic for 3 months started to receive daily captopril treatment for 1 month, and they were killed after 4 months of diabetes. Captopril administration resulted in a significant increase in the Na,K-ATPase activity of diabetic rats compared to the untreated diabetic group. Captopril did not stimulate retinal Na,K-ATPase activity in normal rats (Table 2).

**Effect of Captopril on the α1 and α3 Na,K-ATPase Activities in the Retina**

The activities of the α1 and α3 Na,K-ATPase in the retina were measured to analyze the stimulatory effect of captopril treatment. We used the sensitivity to ouabain to differentiate between the activities of the α1 and α3 Na,K-ATPase. The ouabain-inhibition curves show a biphasic pattern with two distinct IC_{50} values. The α3 isoyme activity is the primary measurable component of the Na,K-ATPase activity between 10^{-9} to 10^{-5} M ouabain, and the activity of α1 is the primary measurable component at higher ouabain concentrations (10^{-8} to 10^{-3} M).

Figure 1 illustrates the results of experiments performed using 10^{-9} M to 10^{-3} M ouabain to define the characteristics of ouabain-inhibition of Na,K-ATPase activity in the retina. A comparison of the ouabain inhibition curves demonstrates that the activity of α3 Na,K-ATPase was lowered in the 2-month diabetic group (14%, P < 0.05) compared to the control. In diabetic rats receiving daily captopril treatment during the entire second month of diabetes, the activity of α3 Na,K-ATPase returned to the control level. The activity of α3 Na,K-ATPase was not stimulated in the retina of captopril-treated, normal, age-matched rats.

Table 2 summarizes α3 Na,K-ATPase activity and its significant alterations in the diabetic groups compared to age-matched controls. The activity of α3 Na,K-ATPase was lowered in the 2-month diabetic group (14%, P < 0.05) compared to the control. In diabetic rats receiving daily captopril treatment during the entire second month of diabetes, the activity of α3 Na,K-ATPase returned to the control level. The activity of α3 Na,K-ATPase was not stimulated in the retina of captopril-treated, normal, age-matched rats. Table 2 summarizes α3 Na,K-ATPase activity and its significant alterations in the diabetic groups compared to age-matched controls. The activity of α3 Na,K-ATPase was lowered in the 2-month diabetic group (14%, P < 0.05) compared to the control. In diabetic rats receiving daily captopril treatment during the entire second month of diabetes, the activity of α3 Na,K-ATPase returned to the control level. The activity of α3 Na,K-ATPase was not stimulated in the retina of captopril-treated, normal, age-matched rats. Table 2 summarizes α3 Na,K-ATPase activity and its significant alterations in the diabetic groups compared to age-matched controls.
Na,K-ATPase was decreased by 19% (P < 0.02) in 4-month diabetic rats. After captopril treatment applied during the fourth month of diabetes, the activity of α3 Na,K-ATPase was improved compared to the untreated STZ group; however, it remained significantly lower than that of the untreated age-matched controls. Captopril administration during the last week of 2 months or 4 months of diabetes did not result in any stimulatory effect on this isoform (data not shown).

Between $10^{-6}$ and $10^{-3}$ M ouabain concentrations, the curve primarily represents the activity of the α1 low ouabain affinity Na,K-ATPase isoform. The activity of α1 Na,K-ATPase was diminished significantly in the 2-month diabetic group (9%, P < 0.05) and returned to the normal range after 1 month of captopril administration (Fig. 1). Table 2 also summarizes the α1 Na,K-ATPase activity and its significant alterations in each diabetic group compared to age-matched controls. In 4-month diabetic rats, the activity of α1 Na,K-ATPase showed a greater decrease (14%, P < 0.02) than that in the 2-month diabetic rats. After 1 month of captopril treatment, the activity of α1 Na,K-ATPase was moderately but significantly stimulated in the 4-month STZ rats compared to the untreated STZ group. One week of captopril administration to 4-month STZ rats did not improve significantly the activity of the α1 Na,K-ATPase.

**Effect of Captopril on Serum and Retinal ACE Activities**

To determine the possible alterations of ACE activity as a response to the diabetic state, captopril treatment, or both, ACE activity was measured in the serum and retina from 2- and 4-month diabetic rats and age-matched controls. Figure 2 demonstrates that serum ACE activity was significantly increased in 2-month diabetic rats (55%, P < 0.001) compared to the age-matched controls. Serum ACE activity was elevated by 40% in 4-month diabetic animals (68 ± 5.2 nmol [3H]bz-gly/minute⁻¹·ml⁻¹, P < 0.001 versus controls [44.5 ± 4.6]). Administration of captopril over 1 month increased serum ACE activity above the basal value in 2-month (101%, Fig. 2) and 4-month diabetic rats (90%, 129 ± 8.1 nmol [3H]bz-gly/minute⁻¹·ml⁻¹, P < 0.0005) and also in nondiabetic age-matched controls (131%, Fig. 2). A stimulatory effect on serum ACE activity was already observed after 1 week of captopril administration in diabetic (17%, P < 0.02) and normal age-matched rats (28%, P < 0.01).

We measured the enzyme activity in retinal homogenates from the same groups of animals. After 2 months of diabetes, the activity of retinal ACE was reduced significantly (38%, P < 0.01) compared to that of age-matched controls (Fig. 3). Similar reduction in the activity of retinal ACE (37%) also was observed in 4-month diabetic animals (175 ± 21 pmol [3H]bz-gly/hour⁻¹·mg protein⁻¹, P < 0.01 versus controls [277 ± 43]). One month of captopril treatment significantly lowered the retinal ACE activity below the basal value in 2-month diabetic rats (38%, Fig. 3), 4-month diabetic rats (32%, 119 ± 8.5 pmol [3H]bz-gly/hour⁻¹·mg protein⁻¹, P < 0.01) and also in normal animals (29%, Fig. 3).

**DISCUSSION**

Results presented here confirm our earlier observations that the activity of Na,K-ATPase is decreased significantly in the retina of STZ-induced diabetic rats. Our current data also demonstrate that decreased retinal Na,K-ATPase activity, including both the α1 and α3 isoforms, is reversed by 1 month of captopril treatment in 2-month diabetic rats and is stimulated without complete restoration in 4-month diabetic rats. Furthermore, our results are the first to demonstrate decreased ACE activity in the retina of diabetic rats.

**Retinal Na,K-ATPase**

Na,K-ATPase activity was found to be reduced in the outer plexiform and outer nuclear layers of retinas from diabetic rabbits, in whole homogenates of retinas from STZ-diabetic rats and Zucker diabetic fatty rats, and in retinal capillary endothelial cells and pericytes cultured in medium containing high concent-
Angiotensin-converting enzyme (ACE) activity in the retina of diabetic (STZ) rats and the effect of captopril. STZ 2m = 2-month diabetes; Cap 1m = captopril treatment for 1 month. Values are mean ± SEM of triplicate determinations (8 rats/group). *P < 0.01, **P < 0.005, difference versus control; †P < 0.01 versus STZ. STZ = streptozotocin.

The isoenzyme of Na,K-ATPase has been localized almost exclusively in the outer retina (photoreceptors, horizontal cells, and certain bipolar cells)—that hyperpolarize under the influence of light. The α1 isoform seems to be responsible for Na⁺,K⁺ exchange in other retinal cells. Fink and coworkers, using Western blot analysis, found a marked deficit in the α1 isoform, much more than in the α2 or α3 isoforms of Na,K-ATPase in the sciatic nerve of STZ-induced diabetic rats. Other investigators reported a 10% to 30% decrease in Na,K-ATPase enzymatic activity in different regions of the brain (supplied by all three isoforms) in STZ-diabetic rats. These reports suggest that cells are impaired nonselectively in diabetes, at least regarding the types of subunits they express. Our results demonstrate that long-term captopril treatment improved the activity of both the α1 and α3 isoforms without exerting a selective stimulatory effect on one of these two isoforms.

**Circulating and Retinal ACE**

Previous studies have suggested a potential role for the renin–angiotensin system in the development of diabetic retinopathy. We measured increased ACE activity in the serum and decreased ACE activity in the retina of diabetic rats. No correlations were found between elevated ACE activities and blood glucose levels. A number of diabetic rats with higher than 450 mg/dl blood glucose level had lower serum ACE levels than some of those with less than 390 mg/dl blood glucose (data not shown). Other studies reporting increased serum ACE activities in patients with either type I or type II diabetes concluded that serum ACE levels are independent of blood glucose and HbA1c levels. Increased serum ACE levels in STZ-diabetic rats can be reduced to normal by insulin administration. These suggest that serum ACE elevation is diabetes related, but, in addition to hyperglycemia, probably other factor(s) influence its value as well. Elevated circulating ACE in diabetes might be derived from increased release of ACE from endothelial cell damage, increased ACE gene expression in endothelial cells, and reduced catabolism of the enzyme.

One-week or 1-month captopril treatment did not inhibit ACE activity in the blood; instead, it markedly enhanced the enzyme level in diabetic and normal rats. One-month captopril treatment only improved retinal Na,K-ATPase activity in diabetic rats. The stimulatory effect of captopril on retinal Na,K-ATPase does not seem to be linked to circulating ACE levels. Chronic treatment with captopril, lisinopril, or perindopril (added to the drinking water) may lead to increased expression and release of ACE in the lungs, kidneys, and blood vessels. How ACE inhibitors increase circulating ACE levels is uncertain. It may result from elevated angiotensin I level (because of its reduced hydrolysis) accompanied by decreased angiotensin II in the plasma (reduced production) as a
feedback response. The antihypertensive effect of ACE inhibitors is unaltered when plasma ACE levels are increased. These observations and others indicate that the efficacy of ACE inhibitors is not caused by direct blockage of circulating ACE; rather, it is caused by inhibition of vascular ACE or other local mechanisms. Angiotensin peptides are synthesized and processed locally in the vascular wall and other numerous tissues, including the retina, leading to the assumption that local RAS plays an autocrine or paracrine role in the regulation of local tissue functions.

**Possible Mechanisms of Action of Captopril**

Captopril and other orally active inhibitors of ACE reduce the production of the vasoconstrictor angiotensin II and enhance the level of the vasodilator bradykinin in several tissues. The most prominent effect of ACE inhibitors is the antihypertensive action, which depends on lowering peripheral resistance and improving tissue blood flow. Because captopril has been found beneficial in hypertensive and normotensive patients with diabetes with retinopathy, its mechanism of action most probably is not restricted to its hypotensive effect.

Our data indicate that the probability of angiotensin II formation from angiotensin I is decreased in the retina of diabetic rats because of markedly reduced ACE activity. Because captopril treatment further inhibits renal ACE activity, we suggest that the stimulatory effect of captopril on retinal Na,K-ATPase activity is most probably not related to reduced angiotensin II formation.

How captopril and other ACE inhibitors can delay or reverse diabetic retinopathy is not well understood and might be the result of several mechanisms. One might be an increase in bradykinin content in the retina through lowered degradation by ACE. Bradykinin stimulates the formation of endothelium-derived relaxing factor(s) (EDRFs), such as nitric oxide, which, in turn, might increase the activity of Na,K-ATPase in the vascular wall. In addition to captopril, non-sulfhydryl ACE inhibitors also increase local kinin content in the vascular wall, leading to increased nitric oxide formation. How the kallikrein–kinin system is altered in the retina in diabetes must be clarified.

A second possibility is that captopril, as do other ACE inhibitors, improves insulin sensitivity as has been observed in patients with hypertension with and without diabetes. Insulin restores the activity of renal glomerular α1 isozyme obtained from STZ-induced diabetic animals and produces a selective stimulation of the activity of α2-α3 isozymes in rat adipocytes by increasing their affinity for sodium ion.

A third possibility is that captopril might act as a scavenger of the superoxide (O$_2^-$) free radical because of its sulfhydryl group and thereby might be able to protect EDRF–nitric oxide. In support of this hypothesis, it has been reported that captopril is able to increase the formation of EDRF(s) in isolated canine arteries and to evoke endothelium-dependent relaxation in rabbit aortic rings.

In contrast, captopril has been reported to inhibit the activity of ouabain-sensitive Na,K-ATPase in human erythrocyte ghost membranes, intact erythrocytes, or purified porcine cortex Na,K-ATPase preparations, as well as the K$^+$-induced relaxation of rat tail artery segments. Pharmacologic or even higher doses of captopril were tested. The inhibition by captopril seems to be a direct effect on the enzyme because it was observed not only on intact erythrocytes but on erythrocyte membrane preparations and purified porcine Na,K-ATPase as well. The doses of captopril used in these laboratories were 3 to 4 log units higher than required to inhibit ACE activity. In our laboratory, the ACE inhibitory dose of captopril (1.5 μM) did not change the activity of retinal Na,K-ATPase in vitro (data not shown). The above ouabain-like effect of captopril is evident only at pharmacologic or pathologic drug concentrations (hemolysis of erythrocytes) that never are achieved in clinical applications; therefore, it should be considered a nonspecific effect.

In conclusion, captopril, a sulfhydryl ACE inhibitor, restored the decreased retinal Na,K-ATPase activity in the early stage of STZ-induced diabetes. The stimulatory effect of this drug on retinal Na,K-ATPase activity does not seem to be related to its effect on circulating ACE level but to retinal ACE activity and/or other local mechanisms. Further studies are required to establish the variables of mechanisms of actions of captopril and other ACE inhibitors.

**Key Words**

ACE, captopril, Na$^+$,K$^+$-ATPase, retina, streptozotocin–diabetic rats

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**References**


Captopril Stimulates Retinal Na\(^{+},K\(^{-}\)ATPase in Diabetes


