Simvastatin reverses impaired regulation of renal oxygen consumption in congestive heart failure

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Received 4 May 2001; accepted in final form 29 June 2001

Simvastatin reverses impaired regulation of renal oxygen consumption in congestive heart failure. Am J Physiol Renal Physiol 281: F802–F809, 2001. First published July 12, 2001; 10.1152/ajprenal.00138.2001.—Nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) is decreased in the heart and kidney of dogs with heart failure (CHF). Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl-CoA reductase, incompletely restored eNOS expression in the endothelium. Therefore, we studied whether simvastatin treatment could restore the regulation of renal O2 consumption by NO in dogs with CHF. Renal O2 consumption was measured after stimulation of NO production with bradykinin, ramiprilat, or amlodipine or the NO donor S-nitroso-N-acetylpenicilliamine (SNAP). Simvastatin delayed the time to euthanasia in dogs with CHF (35 ± 1.0 vs. 29 ± 1.2 days; P < 0.01). In normal dogs, bradykinin (10−6 M), ramiprilat (10−4 M), amlodipine (10−6 M), and SNAP (10−4 M) significantly reduced O2 consumption in the renal cortex (−31.8 ± 0.9% of baseline) and renal medulla (−29.7 ± 2.1%) but were partially or completely restored by simvastatin. Responses to SNAP were unaffected. These data demonstrate that treatment with simvastatin improves renal production of NO in CHF, restoring the normal regulation of renal O2 consumption by NO.

nitric oxide; renal physiology; endothelial nitric oxide synthase

NITRIC OXIDE (NO), in addition to being an important vasodilator and signaling molecule, regulates O2 consumption in several tissues, including cardiac and skeletal muscle and the kidney (13, 18, 20, 28, 32). It has been shown to be a physiological regulator of myocardial O2 consumption and may play a role in the disordered myocardial metabolism of congestive heart failure (CHF) (26, 29). NO production and endothelial nitric oxide synthase (eNOS) are decreased in the heart during CHF, and drugs that improve cardiac function, such as angiotensin-converting enzyme inhibitors (ACE-I), increase NO production and help restore regulation of myocardial O2 consumption by NO (19, 33). NO also regulates O2 consumption at the level of whole kidney in vivo (18). In vitro studies using both isolated cortical and medullary slices and isolated renal tubules have confirmed this effect (14, 18). We have previously shown that in the kidney, as in the heart, regulation of renal O2 consumption by NO is reduced in the presence of CHF (3). This is accompanied by an apparent decrease in NO production in response to several agonists and may have adverse effects on the renal circulation, especially medullary blood flow (3).

Expression of eNOS in endothelial cells is abnormal in several disease processes, including atherosclerosis and diabetes (9). Production of NO is decreased in patients with chronic renal insufficiency (CRI), and it has been suggested that this contributes to hypertension and progression of CRI (27). Inhibitors of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase, or “statins,” improve survival in patients with atherosclerosis and also retard progression of CRI in several different models of renal disease (12, 31). In addition to their cholesterol-lowering ability, these drugs ameliorate endothelial dysfunction, possibly through increases in expression of eNOS in endothelial cells secondary to inhibition of Rho A kinase and stabilization of eNOS mRNA (11, 31).

In isolated renal cortex, we have previously shown that the main source of the NO that plays a role in regulation of renal O2 consumption is eNOS (4). Because statins can increase endothelial expression of eNOS, and because regulation of renal O2 consumption by NO is abnormal in CHF, we tested the hypothesis that treatment with an HMG-CoA reductase inhibitor would improve the responsiveness of renal tissue to stimuli of NO production in dogs with pacing-induced CHF.

METHODS

Reagents. S-nitroso-N-acetylpenicilliamine (SNAP), bradykinin, nitro-L-arginine methyl ester (L-NAME), succinate, and sodium cyanide were purchased from Sigma. Ramiprilat...
was a gift from Hoechst Marion Roussel, (New Brunswick, NJ), and amlodipine was a gift from Pfizer (Groton, CT).

**Surgical procedures.** Mongrel male dogs (n = 14), weighing 26–31 kg, were sedated with acepromazine maleate (1 mg/kg im, Ferneta Animal Health), anesthetized with pentobarbital sodium (Nembutal, 25 mg/kg iv, Abbott Laboratories), and ventilated with room air using a Harvard respirator (Harvard Apparatus, Holliston, MA). A thoracotomy was performed in the left fifth intercostal space. Catheters (Tygon) were placed in the descending thoracic aorta for arterial pressure measurement. A solid-state manometer (P 5.6, Konigsberg Instruments, Pasadena, CA) was inserted into the left ventricle (LV) through the apex. A human, screw-type, unipolar myocardial pacing lead was placed on the LV. Wires and catheters were run subcutaneously to the interscapular region, the chest was closed in layers, and the pneumothorax was reduced. Antibiotics were administered for 7 days after surgery (Amoxicillin, 400 mg/day im, Amoxi- inject, SmithKline Beecham Animal Health), and dogs were allowed to fully recover. The protocols were approved by the Institutional Animal Care and Use Committee of New York Medical College and conform to the Guiding Principles for the Care and Use of Laboratory Animals (of the American Physiological Society and the National Institutes of Health). Dogs were allowed to recover from surgery for 7–10 days. Experiments were conducted when animals were afebrile and had been trained to lie quietly without restraint on the laboratory table.

**Induction of CHF.** Hearts were paced at 210 beats/min for 3 wk. After this 3-wk period, the pacing rate was increased to 240 beats/min until overt CHF was observed. We used external pacemakers (model EV4543, Pace Medical, Waltham, MA) carried by the dogs in a vest. Dogs were euthanized after 29 days after initiation of pacing and lasting to the day of euthanasia. After euthanasia, the left kidney was removed, decapsulated, and weighed.

**Drug treatment.** Dogs selected at random from among the dogs with chronic cardiac pacing (n = 8) were treated with simvastatin (20 mg·kg⁻¹·day⁻¹, given orally) beginning 10 days after initiation of pacing and lasting to the day of euthanasia. Nontreated dogs with cardiac pacing were euthanized after 29 ± 1.2 days, whereas simvastatin-treated dogs were euthanized after 35 ± 1.0 days.

**Measurement of hemodynamics.** On the day of study, the aortic catheter was connected to a pressure transducer (P23XL, Spectramed, Oxnard, CA) and a preamplifier to measure arterial blood pressure. LV pressure was measured using the solid-state pressure gauge. The first derivative of LV pressure with respect to time, dP/dt, was obtained using an operational amplifier (National Semiconductor LM 324, Santa Clara, CA), and triangular wave signals with known slopes were substituted for the pressure signals to calibrate the differentiator directly. Heart rate was measured using a cardiotachometer (model 9857B, Beckman Instruments, Fullerton, CA) from the LV pressure pulse interval. All signals were recorded continuously throughout the experimental protocol on a chart recorder (model 2800S, Gould, Valley View, OH). Mean arterial blood pressure was derived using a 2-Hz low-pass filter.

**Preparation of kidney tissue slices and measurement of O₂ consumption.** Thin slices (~1 mm, weight 15–25 mg) were prepared from cortex and medulla of six normal dogs, six dogs with pacing-induced CHF, and eight dogs with pacing-induced CHF treated with simvastatin (CHF+simvastatin). Tissue was incubated in Krebs bicarbonate solution (containing (in mmol/l) 118 NaCl, 4.7 KCl, 1.5 CaCl₂, 25 NaHCO₃, 1.2 KH₂PO₄, and 1.1 MgSO₄, pH 7.4) bubbled with 21% O₂-5% CO₂-74% N₂ at 37°C for 2 h. At the end of incubation, each piece of tissue was placed in a stirred chamber with 3 ml of air-saturated Krebs bicarbonate solution containing 10 mmol/l HEPES and 5.6 mmol/l glucose (pH 7.4), and the chamber was sealed with a Clark-type platinum O₂ electrode (Yellow Springs Instruments, Yellow Springs, OH). O₂ consumption was measured polarographically using an O₂ monitor (model YSI 5300) connected to a linear chart recorder (model 1202, Barnstead/Termolyne, Dubuque, IA). Dose-response curves of the effect of different agonists on kidney O₂ consumption were then measured. Succinate (10⁻⁶ mol/l) was added at the end of each incubation to verify that there was adequate O₂ remaining to support succinate-stimulated mitochondrial respiration. This was followed by addition of sodium cyanide (10⁻³ mol/l) to confirm the involvement of mitochondrial respiration in O₂ consumption.

Renal cortical or medullary O₂ consumption is calculated as the rate of decrease in O₂ concentration, assuming an initial O₂ concentration of 224 nmol/ml (1), and is expressed as nanomoles O₂ consumed per minute per gram of tissue. The effects of drugs used on O₂ consumption are expressed as a percent change from baseline O₂ consumption. Baseline O₂ consumption was measured in the cortex and medulla in the absence and presence of L-NAME (10⁻⁴ mol/l) in groups of six normal dogs, six dogs with CHF, and eight dogs with CHF+simvastatin.

**Effect of agonists on O₂ consumption.** Bradykinin or ramiprilat at concentrations of 10⁻⁷–10⁻⁴ mol/l or amlodipine at concentrations of 10⁻⁷–10⁻⁵ mol/l were added in a cumulative concentration-dependent manner. The response to these drugs was also examined after preincubation with the NOS inhibitor L-NAME (10⁻⁴ mol/l) to determine the role of NO in the regulation of O₂ consumption. Each drug, with and without L-NAME, was used in groups of six normal, six CHF, and eight CHF+simvastatin dogs.

**Effect of NO donor on O₂ consumption.** SNAP, at concentrations of 10⁻⁷–10⁻⁴ mol/l, was added in a cumulative concentration-dependent manner to assess the effects of exogenous NO on renal O₂ consumption. The response to SNAP was also examined after preincubation with L-NAME (10⁻⁴ mol/l). Each condition was tested in three groups of six normal, six CHF, and eight CHF+simvastatin dogs.

**Statistical analysis.** All data are expressed as means ± SE. Statistical analysis of baseline O₂ consumption was performed using Student’s t-test. Changes in O₂ consumption caused by drug treatment were analyzed using two-way ANOVA followed by multiple comparisons using the Tukey test (Sigma-Stat, SPSS-Science, Chicago, IL). Statistical significance was achieved at P < 0.05.

**RESULTS**

**Hemodynamic changes during chronic pacing.** Hemodynamic data from normal dogs and dogs with CHF, either untreated or treated with simvastatin, are shown in Table 1. The time until euthanasia of dogs treated with simvastatin was significantly longer than that of untreated dogs with pacing-induced CHF (35 ± 1.0 vs. 29 ± 1.2 days, P < 0.01). This relates to less severe symptoms of CHF in simvastatintreated dogs. There were significant increases in LVEDP after 28 days of cardiac pacing in normal dogs (23 ± 1.6 mmHg)
and at both 28 and 35 days of pacing in dogs treated with simvastatin (19 ± 1.6 and 21 ± 1.4 mmHg respectively) compared with normal animals (8.0 ± 1.3 mmHg, P < 0.05 for each comparison). LVEDP was similar in simvastatin-treated dogs after 28 days (19 ± 1.6 mmHg) compared with untreated dogs with CHF (23 ± 1.6 mmHg; P = 0.09). LVEDP did not rise significantly during a further week of pacing at 240 beats/min in the simvastatin-treated dogs. There was also a significant reduction in LV contractility, as evidenced by a fall in LV dp/dt in dogs with CHF at 28 days (1,648 ± 270 mmHg/s) and in those treated with simvastatin at 28 and 35 days (1,559 ± 158 and 1,578 ± 101 mmHg/s respectively; P < 0.05 for all comparisons vs. controls).

Effects of simvastatin therapy. At baseline, serum cholesterol was 148 ± 12 mg/dl (n = 7), similar to levels previously reported by us (22). After 18 days of treatment with simvastatin, cholesterol levels had fallen significantly to 77 ± 6 mg/dl on day 28 (n = 7; P < 0.01). This is similar to levels achieved in normal dogs after 2 wk of treatment with a similar dose of simvastatin (82 ± 7 mg/dl) (22).

O2 consumption in renal cortex and medulla of normal and paced dogs. Baseline renal cortical and medullary tissue rates of O2 consumption were not different in normal (cortex: 478 ± 22 nmol O2·min⁻¹·g⁻¹; medulla: 483 ± 16 nmol O2·min⁻¹·g⁻¹; n = 6); CHF (cortex: 476 ± 38 nmol O2·min⁻¹·g⁻¹; medulla: 482 ± 57 nmol O2·min⁻¹·g⁻¹; n = 5); and CHF + simvastatin animals (cortex: 461 ± 21 nmol O2·min⁻¹·g⁻¹; medulla: 471 ± 26 nmol O2·min⁻¹·g⁻¹; n = 8). Addition of the NOS inhibitor l-NAME (10⁻⁴ mol/l) did not significantly alter O2 consumption in control, CHF, and CHF + simvastatin animals, respectively (cortex: 498 ± 16 nmol O2·min⁻¹·g⁻¹; medulla: 523 ± 25 nmol O2·min⁻¹·g⁻¹, n = 6; cortex: 522 ± 59 nmol O2·min⁻¹·g⁻¹; medulla: 512 ± 60 nmol O2·min⁻¹·g⁻¹, n = 5; cortex: 454 ± 36 nmol O2·min⁻¹·g⁻¹; medulla: 447 ± 11 nmol O2·min⁻¹·g⁻¹, n = 8).

Effect of bradykinin on renal O2 consumption. Cumulative doses of bradykinin (10⁻⁷–10⁻⁴ mol/l) produced significant, dose-dependent decreases in renal cortical O2 consumption in all three groups of dogs (control: from 2.8 ± 1.4 to 31.8 ± 0.9%, n = 6; CHF: from 0.5 ± 0.5 to 19.9 ± 1.3%, n = 6; CHF + simvastatin: from 2.1 ± 0.8 to 25.6 ± 1.4%, n = 8) (Fig. 1, A, C, and E). Addition of l-NAME significantly attenuated the effect of bradykinin at most of the doses studied, demonstrating the role of NO synthesis in the bradykinin effect (Fig. 1, A, C, and E). The effect of l-NAME was lowest in the dogs with CHF (Fig. 1C), consistent with impaired NO production in the kidney in the presence of CHF. Dogs with CHF had significantly less depression of cortical O2 consumption than normal dogs at 10⁻⁶–10⁻⁴ mol/l of bradykinin (P < 0.05 for each comparison; Fig. 2). Treatment with simvastatin significantly improved responsiveness to bradykinin at 10⁻⁵–10⁻⁴ mol/l in the cortex, although it remained significantly different from results in normal animals (P < 0.05 vs. normal and CHF dogs at 10⁻⁵–10⁻⁴ mol/l of bradykinin) (Fig. 2).

The effect of simvastatin on depression of medullary O2 consumption by bradykinin was not as pronounced. Bradykinin again significantly decreased medullary O2 consumption in all groups (control: from 6.3 ± 1.2 to 29.7 ± 2.1%, n = 6; CHF: from 1.1 ± 0.7 to 19.2 ± 0.7%, n = 6; CHF + simvastatin: from 3.6 ± 0.6 to 23.1 ± 1.5%, n = 8; P < 0.05) (Fig. 1, B, D, and F). Addition of l-NAME significantly attenuated the effect of bradykinin, demonstrating the role of NO synthesis in the bradykinin effect (Fig. 1, B, D, and F), with the effect again being lowest in dogs with CHF (Fig. 1D). Dogs with CHF, both treated with simvastatin and untreated, had significantly less depression of medullary O2 consumption at all doses of bradykinin (P < 0.05 for both groups). Depression of medullary O2 consumption in CHF + simvastatin dogs, although showing a trend toward improved response, was not significantly different from that in CHF dogs (P > 0.05; Fig. 2).

Effect of ramiprilat on renal O2 consumption. The ACE inhibitor ramiprilat (10⁻⁷–10⁻⁴ mol/l), which stimulates endogenous NO production, similarly caused concentration-dependent decreases in renal cortical O2 consumption in the three groups of dogs (control: from 5.4 ± 1.2 to 30.3 ± 1.1%, n = 6; CHF: from 0.8 ± 0.5 to 19.1 ± 2.0%, n = 6; CHF + simvastatin: from 4.1 ± 0.8 to 27.9 ± 1.1%, n = 8) (Fig. 3, A, C, and E). Addition of l-NAME significantly attenuated the effect of ramiprilat, although in dogs with CHF this effect was absent at some dosages (Fig. 3C). Dogs with CHF had significantly less depression of cortical O2 consumption at all doses of ramiprilat studied (Fig. 4). Treatment with simvastatin completely reversed the effect of CHF at 10⁻⁴ mol/l of ramiprilat. At lower doses of ramiprilat, the effect of simvastatin was to counteract the effect of CHF, although this
change was not significantly different from untreated CHF.

The effect of simvastatin on depression of O₂ consumption by ramiprilat in the medulla was again not as pronounced as in the cortex. Ramiprilat significantly reduced medullary O₂ consumption in all three groups (control: from 6.9 ± 1.0 to 33.0 ± 2.7%, n = 6; CHF: from 1.6 ± 1.0 to 17.8 ± 1.9%, n = 6; CHF+simvastatin: from 5.1 ± 1.5 to 24.6 ± 1.3%, n = 8) (Fig. 3, B, D, and F). Incubations were performed in the absence (○) or presence (○) of nitro-L-arginine methyl ester (L-NAME; 10⁻⁴ mol/l). Bradykinin caused dose-dependent decreases in renal cortical and medullary O₂ consumption in all groups. Addition of L-NAME significantly attenuated the effect of bradykinin in cortex and medulla. *P < 0.05 vs. incubation in the absence of L-NAME.

Effect of amlodipine on renal O₂ consumption. Amlodipine, which also stimulates renal NO production, decreased renal cortical O₂ consumption in the three groups (control: from 8.5 ± 0.8 to 30.1 ± 2.0%, n = 6; CHF: from 0.6 ± 0.6 to 14.2 ± 2.5%, n = 6; CHF+simvastatin: from 6.7 ± 1.4 to 25.4 ± 2.3%, n = 8) (Fig. 5, A, C, and E). Addition of L-NAME attenuated the effect of amlodipine, although this was less in dogs with CHF (Fig. 5, C and D). The response of cortical O₂ consumption to amlodipine was significantly less in dogs with CHF (Fig. 6). Treatment with simvastatin prevented the decreased responsiveness to amlodipine seen with CHF (P < 0.05 vs. CHF at 10⁻⁷ and 10⁻⁵ mol/l).

Amlodipine also significantly decreased O₂ consumption in the medulla (control: from 11.6 ± 1.4 to 30.8 ± 2.2%, n = 6; CHF: from 2.0 ± 1.2 to 15.6 ± 2.6%, n = 6; CHF+simvastatin: from 6.4 ± 1.1 to 24.3 ± 2.1%,
n = 8) (Fig. 5, B, D, and F). L-NAME significantly reversed this, and the effect was less in dogs with CHF.

Treatment with simvastatin restored responsiveness to amlodipine toward that seen in normal dogs, but this was only significant at the highest concentration of amlodipine (10^{-5} mol/l; P < 0.05).

**Effect of NO donor (SNAP) on renal O₂ consumption.** Administration of cumulative doses of the NO donor SNAP (10^{-7}–10^{-4} mol/l) decreased renal cortical and medullary O₂ consumption in all dogs (control: cortex from 7.3 ± 1.5 to 46.9 ± 1.0%; medulla from 4.2 ± 1.0 to 46.8 ± 1.1%; CHF: cortex: from 3.3 ± 2.5 to 40.1 ± 2.6%; medulla: from 2.6 ± 1.8 to 43.8 ± 2.5%; CHF + simvastatin: cortex: from 6.9 ± 0.8 to 42.4 ± 1.8%; medulla: from 4.8 ± 0.5 to 44.8 ± 2.5%) (Fig. 7). There was no significant difference between any of the groups in responsiveness to SNAP in the cortex or medulla (P > 0.05). Addition of L-NAME had no effect on the response to SNAP (data not shown).

**DISCUSSION**

Similar to the situation in the failing heart, production of NO and regulation of O₂ consumption are abnormal in the kidneys of animals with CHF. The studies presented here confirm our previous observations in kidneys from dogs with CHF, namely, that the response to drugs that stimulate NO production and reduce O₂ consumption in the normal kidney is attenuated in kidneys in the presence of CHF (3). In normal kidneys in the present studies, NO stimulation directly by bradykinin or amlodipine, or indirectly by inhibition of bradykinin degradation with the ACE inhibitor ramiprilat, reduced renal cortical and medullary O₂ consumption by 30–35%. In CHF, the response to all
three drugs was significantly attenuated, with maximum inhibition of O$_2$ consumption of only 15–20%. L-NAME, which inhibits NO synthesis, significantly reversed this effect in normal kidneys, but the response to L-NAME was less or absent in CHF, again demonstrating that NO production is diminished in the kidney during CHF. The response to an NO donor was not different among groups, documenting that responsiveness to NO itself is maintained. Thus, after the development of CHF, production of NO in the kidneys...
appears to be reduced, with consequent changes in the regulation of renal \( O_2 \) consumption.

What is new in these studies is the demonstration that treatment with simvastatin partially or completely reverses the effect of CHF on responsiveness of renal \( O_2 \) consumption to stimulators of NO production. In animals treated with simvastatin beginning 10 days after the initiation of cardiac pacing, depression of renal cortical \( O_2 \) consumption after stimulation of NO production with the higher doses of bradykinin, ramiprilat, and amiodipine was significantly greater than in untreated CHF, returning toward the response seen in normal dog kidney. For ramiprilat and amloidipine, the response was not significantly different from that in normal dogs. Renal medullary \( O_2 \) consumption after exposure to stimulators of NO production also showed a trend toward greater depression, although, except for the highest dose of amloidipine studied, the results were not significantly different from those in CHF dogs. The time to euthanasia was also lengthened from 29 to 35 days, reflecting a failure of LVEDP to rise as much despite an extra week of pacing and fewer clinical symptoms in the simvastatin-treated dogs.

The effect of NO on \( O_2 \) consumption occurs via a direct effect on mitochondrial respiration (13, 28, 32). NO binds to and inhibits complexes I and II of the electron transport chain and also interacts with cytochrome oxidase (7, 10, 30). Its effect of inhibiting renal \( O_2 \) consumption has been demonstrated in the intact kidney in vivo and in isolated renal tissues (3, 4, 14, 18).

Previous work in the dog heart has demonstrated decreased NO production after the development of CHF in association with reduced expression of eNOS (26, 29). In the kidney, several NOS isoforms are present, including eNOS, inducible NOS (iNOS), and neuronal NOS (nNOS) (15, 16). However, we have previously demonstrated, using the eNOS-knockout mouse, that the eNOS isoform is the primary source of the NO that regulates renal \( O_2 \) consumption (4). Thus one possible mechanism of the action of simvastatin is through increases in eNOS gene expression despite the presence of CHF.

HMG-CoA reductase inhibitors, or statins, have been shown to prevent suppression of eNOS mRNA expression in response to oxidized low-density lipoprotein (LDL) in bovine aortic endothelial cells (11). Simvastatin and lovastatin increased eNOS mRNA and protein expression, as well as activity, in human saphenous vein endothelial cells treated with oxidized LDL through a specific stabilization of the mRNA (17). The levels of oxidized LDL used in these studies are only 1–2% of serum LDL cholesterol levels. Thus a similar effect of statins might occur in vivo, a possibility supported by the observation that clinical benefit occurs before plaque remodeling (31) and that as little as 4 wk of treatment improves acetylcholine-stimulated forearm blood flow in humans, an effect mediated via NO (24).

With a model of high-output CHF in the rat, normal to increased expression of eNOS mRNA and protein was demonstrated in the cortex and medulla (1, 2), along with normal levels of \( NO_2 \) and \( NO_3 \), the end products of NO metabolism, in the urine (1). Although the authors suggested that this argues against decreased NO production in the kidney in CHF as a cause of decreased renal \( O_2 \) blood flow, they also found decreased responsiveness to acetylcholine, an effect that would be expected if NO production were impaired. Furthermore, the presence and degree of severity of CHF in the rats were not well documented, whereas we have studied a well-defined, reproducible model of CHF and quantitated the degree of cardiac dysfunction. Our results argue that NO synthesis in the kidney is decreased in the presence of CHF and that it is restored toward normal by treatment with simvastatin.

Of note in our studies is the difference in responsiveness of renal cortex and medulla in terms of reversibility of the defect in NO-mediated suppression of \( O_2 \) consumption by simvastatin. The renal medulla contains higher levels of NOS activity than the cortex and has been demonstrated to have increased capacity to generate NO (16, 18). Even if one were to argue that only the levels of eNOS are important in the effect of agonists to suppress renal \( O_2 \) consumption, overall levels of eNOS in the medulla also appear to be higher than in the cortex (2). However, renal cortical blood vessels appear to be relatively enriched in NOS, mainly eNOS and nNOS (21), whereas there appears to be less or no iNOS in the renal vasculature (5, 21, 23). Most medullary production of NO is likely to come from iNOS constitutively expressed in tubules (6). Local effects of NO may depend not only on the isoform present but also on the specific cell or structure within which it is expressed. Thus our data might indicate a more important role of eNOS in regulation of \( O_2 \) consumption in the cortex versus the medulla due to a higher level of expression in cortical blood vessels. Alternatively, the effect of simvastatin to increase eNOS expression might be less in the medulla than in the cortex.

The level of NO production in the kidney appears to play an important role in regulation of renal blood flow, and this may be especially true in the medulla during ischemia or hypoxia (1, 2, 8). Decreased NO production in the kidney during CHF might be expected to aggravate the response to endogenous vasoconstrictors such as endothelin and angiotensin II. Inhibition of \( O_2 \) consumption by NO is magnified in the presence of hypoxia (14), and the normally hypoxic medulla would be expected to be especially sensitive to the effects of NO. Inhibition of NO production further augments medullary hypoxia and tubular injury (8). Drugs such as ACE inhibitors might thus also have beneficial effects on the kidney through increased NO production and decreased \( O_2 \) consumption, allowing for more \( O_2 \) availability to promote NO synthesis. If simvastatin increases renal eNOS, it might play a similar protective role.

Finally, our previous work suggested that decreased regulation of cortical and medullary \( O_2 \) consumption by stimulators of NO production in the kidney in CHF was probably due to decreased NO production, mainly
from eNOS. NO production by eNOS in thick ascending limb cells of the renal tubule has now been shown to mediate inhibition of chloride transport (25). Decreased levels of eNOS might thus impair salt excretion, and treatments that specifically increase eNOS levels might also play a role in improving CHF through effects on the kidney.

This work was supported by National Heart, Lung, and Blood Institute Grants PO-1 HL-43023, RO-1 HL-50142, and HL-61290 (T. H. Hintze) and the Westchester Artificial Kidney Center (S. Adler). Ramiprilat was the kind gift of Hoechst Marion Rousell.

REFERENCES


