Decreased Cardiac Concentration of cGMP Kinase in Hypertensive Animals
An Index for Cardiac Vascularization?

Thomas Ecker, Claus Göbel, Roger Hullin, Rainer Rettig, Gerhard Seitz, and Franz Hofmann

Cyclic GMP (cGMP) kinase is intimately involved in the regulation of vascular smooth muscle tone. Its tissue concentration was determined in normotensive and hypertensive rats by use of monospecific anti-cGMP kinase antibodies. Hearts of spontaneously hypertensive rats and renovascular (Goldblatt II) hypertensive rats contained half the concentration of cGMP kinase than those of the respective normotensive animals. The increase in blood pressure and the resulting left ventricular hypertrophy were correlated inversely with the left ventricular cGMP kinase concentration. This decrease was specific for the left ventricle and was not observed in other tissues. In addition, the cardiac concentration of cGMP kinase was unchanged in hyperthyroid animals that had comparable left ventricular hypertrophy and mild hypertension. This suggested that in severe renovascular hypertension the decrease in cardiac cGMP kinase concentration is caused by a relative lack of cardiac vessel growth during the development of hypertrophy. In agreement with this conclusion, immunohistochemistry of cardiac cross sections showed that cGMP kinase was exclusively located in cardiac vessels. In support of this localization, the maximal arterial blood flow of heart, liver, skeletal muscle, and kidney correlated excellently with the cGMP kinase content of the respective organ. These results suggest that the cGMP kinase concentration of nonsmooth muscle tissues depends on the amount of organ-specific vascular smooth muscle and may be used as an index for the vascularization of these organs. (Circulation Research 1989;65:1361-1369)

Cyclic GMP (cGMP) is a major relaxing factor in vascular and nonvascular smooth muscle.1-4 The cytosolic cGMP level is stimulated by atrial natriuretic peptides, endothelium-derived relaxation factor, and several cardiovascular therapeutics, including sodium nitroprusside and nitroglycerin. The drug- or hormone-induced increase in cGMP is associated with a decrease in the cytosolic calcium concentration in smooth muscle.5-7 Recent experiments indicate that activation of cGMP kinase8 or introduction of active cGMP kinase7 induces relaxation of smooth muscle strips and/or decreases cytosolic calcium.

The molecular mechanism of these cellular effects of cGMP kinase is unknown. cGMP kinase is a dimer of two identical 75 kDa subunits and is activated in vitro by cGMP at concentrations below 1 μM.8,10 The enzyme has an in vitro substrate specificity that is similar to that of cyclic AMP kinase11 but also phosphorylates specifically a number of proteins. These include the G substrate of cerebellum12 and 250, 130, 85, and 75 kDa membrane proteins from the particulate fraction of the aortic medial layer.13 It was suggested that phosphorylation of these proteins activates the plasmalemmal calcium-ATPase14,15 and stimulates calcium extrusions. It is unlikely that calcium extrusion is regulated by a direct phosphorylation of the calcium-ATPase since 1) cGMP kinase does not phosphorylate purified calcium-ATPase in vitro16 and 2) the 130 kDa membrane protein phosphorylated specifically by cGMP kinase in aortic smooth muscle membranes is not identical with the calcium-ATPase.17 However, it is possible that cGMP kinase regulates calcium extrusion through phosphorylation of other membrane components, that is, phosphatidylinositol kinase.16 Regardless of the mechanism, it appears quite clear that cGMP and cGMP kinase are primary...
regulators of smooth muscle tone. In agreement with these functional data, high concentrations of cGMP kinase have been found in smooth muscle cells, pericytes, platelets, Purkinje cells, lung, and heart. The enzyme approaches a micromolar concentration in isolated adult smooth muscle cells; this finding again supports its possible importance as a regulatory enzyme. Other cells and tissues such as endothelial and epithelial cells or liver and skeletal muscle synthesize cGMP but contain minimal or no cGMP kinase. Presumably, regulatory effects of cGMP in these cells are mediated by activation or inhibition of specific phosphodiesterases (for a recent discussion see Reference 4). The potential physiological importance of cGMP kinase was strengthened by the observation of Kuo and coworkers \(^{23}\) that the activity of cGMP kinase is lower in hearts of spontaneously hypertensive rats than in those of normotensive animals. Hypertension is a multifactorial disease that reportedly has an altered responsiveness of the cardiovascular system to various stimuli, that is, an increased sensitivity to vasoconstrictors and an attenuated responsiveness to vasodilators.\(^ {24-27}\) The regulation of smooth muscle tone and the handling of cytosolic calcium would be severely affected if the concentration of cGMP kinase is not only decreased in hypertensive hearts but also in vascular smooth muscle. Such a decrease could be a major factor that contributes to the development and/or maintenance of hypertension. To elucidate a possible causative role of cGMP kinase in hypertension and to verify the previous report, the concentration of the enzyme was measured by use of affinity-purified antibodies in the heart and other tissues of hypertensive rats.

**Materials and Methods**

**Materials**

Goat anti-rabbit immunoglobulin G (IgG) peroxidase conjugate was from Medac, Hamburg, FRG. Goat anti-rabbit IgG alkaline phosphatase conjugate was from Promega, Madison, Wisconsin. \(^ {125}\)I-sodium was obtained from NEN Chemicals, Dreieich, FRG. Thyroxine was from Sigma, St. Louis, Missouri. Immobilon transfer membranes were from Millipore, Bedford, Massachusetts. All other chemicals were of the highest purity available. Male Wistar rats were purchased from Charles River Wiga GmbH, Sulzfeld, FRG. Female stroke-prone spontaneously hypertensive rats and age-matched Wistar-Kyoto control rats were bred and provided by Dr. U. Ganten, Pharmacologisches Institut der Universität Heidelberg.

**Antibodies and Immunolabeling of Tissue cGMP Kinase**

cGMP kinase was purified from bovine lung.\(^ {28}\) Antibodies were induced in a goat and in several rabbits by repeated intradermal injection of 0.1 mg enzyme in the presence of Freund’s incomplete adjuvant. The animals were bled repeatedly, individual sera were kept at 4°C in the presence of 0.01% sodium azide. The antibodies were put on a cGMP kinase-Sepharose column by use of pH step from 7.0 to 3.0 for elution. The affinity-purified antibodies were stored at pH 7.0 at 4°C in the presence of 0.01% sodium azide. Immunolabeling of tissue cGMP kinase was tried out with these monospecific antibody described by Burnette with the following modifications: Tissue extracts were separated on 7.5% sodium dodecyl sulfate gel. The separate proteins were transferred to an Immobilon membrane. The blotting time was 16 hours at 10 V/m. The antibodies bound to cGMP kinase were detected by \( {^{125}}\)I-protein A or by goat anti-rabbit IgG antibody conjugated with alkaline phosphatase.

**Determination of cGMP Kinase**

The tissue concentration of cGMP kinase determined either by a radioimmunoassay (RIA) by an enzyme-linked immunosorbent assay (ELISA) with affinity-purified anti-cGMP kinase antibody from different rabbits or a goat.

**RIA of cGMP kinase**

Two hundred microliters of the tissue extract or of pure cGMP kinase (5,000-10,000 dilutions) were mixed with various amounts of pure cGMP kinase or tissue extracts that were diluted in buffer A. Purified rabbit or goat antibodies (5 diluted 1:1,500 in buffer A were added, and mixture was incubated for 16 hours at 4°C. Antibody \( {^{125}}\)I-cGMP kinase complex was precipitated by 50 μl Staphylococcus aureus suspending with 50 μg. The washed pellet was counted in a gamma counter.

**ELISA of cGMP kinase**

Microtiter plates coated for 16 hours at 4°C with pure cGMP kinase. The coating solution was removed by aspiration and residual binding sites were saturated for 1 hour at room temperature with buffer A containing 0.01% BSA, pH 7.0. The microtiter plates were washed twice with buffer A containing 0.01% BSA. Affinity-purified rabbit antibodies were preincubated for 16 hours at 4°C with several dilutions of extracts or of pure cGMP kinase. These plates were transferred to the plates and incubated for 16 hours at 4°C. Thereafter, the plates were washed three times, and then a goat anti-rabbit IgG peroxidase conjugate was added. The microtiter plates were incubated 1 hour at room temperature, and washed four times. The substrate solution applied and contained 0.4 mg/ml 1,2 phenylene diamine and 0.001% H₂O₂ in a phosphate buffer at pH 5.0. The reaction was stopped after minutes with 4 M H₂SO₄. The reaction product was measured at 492 nm.

**Calculation of cGMP kinase concentration**

Calculation of cGMP kinase concentration. cGMP kinase (0.2–30 ng) standard displacement curve was constructed. The tissue concentration of cGMP kinase was calculated only from those
FIGURE 1. Immunoblots of various tissues of Wistar rat and displacement curves. Panel A: Extracts of aorta (250 µg), liver (800 µg), lung (340 µg), cerebellum (290 µg), kidney (800 µg), heart (470 µg), and pure cGMP kinase separated on 7.5% sodium dodecyl sulfate gel and transferred to nitrocellulose. Bound anti-cGMP kinase antibodies were detected with [125I]protein A. Panel B: Immunoblot of cardiac tissue extracts of Wistar rat (lane B), Goldblatt II hypertensive rat (lane C), Wistar-Kyoto rat (lane D), and stroke-prone spontaneously hypertensive rat (lane E) and 20 ng pure cGMP kinase (lanes A and F). Cardiac protein (200 µg each) was separated on a 7.5% sodium dodecyl sulfate gel and transferred to a Immobilon transfer membrane. Bound anti-cGMP kinase antibodies were detected with a goat-anti-rabbit alkaline phosphatase conjugate. Panel C: Displacement curves for pure cGMP kinase and various tissues. Enzyme-linked immunosorbent assay was carried out as described in "Materials and Methods." B, absorption at 492 nm after preincubation of anti-cGMP kinase antibodies with test samples; Bo, absorption at 492 nm after preincubation of anti-cGMP kinase antibodies with buffer A; Δ, lung; ×, heart; ○, kidney; □, liver; ⊗, skeletal muscle; ●, pure cGMP kinase. Tissues are from a normotensive Wistar rat.

Displacement curves in which serial dilutions of the tissue extracts ran parallel to the standard curve (Figure 1C). The concentration was calculated from that part in which displacement was between 75% and 25%. A molecular weight of 150,000 was used to calculate the molar concentration of cGMP kinase.

Preparation of tissue samples. Animals were decapitated and bled. Hearts and other organs were excised immediately, rinsed thoroughly in buffer B (20 mM KPO4, pH 7.0, and 150 mM NaCl) to remove blood and coagules, trimmed of fat and connective tissue, and stored at −70°C. Individual organs were weighed, minced with scissors, and homogenized in 6 vol buffer C (20 mM KPO4, pH 7.0, and 2 mM benzamidine) with a Waring blender at 4°C. The homogenate was centrifuged at 15,000g for 30 minutes, and the supernatant was stored at −20°C or used immediately. Only small amounts of cGMP kinase were detected in the pellets of most tissues even after reextraction of the pellets with buffer C containing 1% Triton X-100. The aortas of individual animals were homogenized in 10 vol buffer C containing 1% Triton X-100 by use of a glass/glass homogenizer. Brain microvessels were prepared from 10-week-old stroke-prone spontaneously hypertensive rats and age-matched Wistar-Kyoto controls according to Huang and Drummond.30

Immunohistochemistry

Animals were exsanguinated. Organs were cleansed of blood and connective tissue and frozen in isopropanol. Frozen sections (4 µm) were fixed in picric paraformaldehyde (pH 7.4) and immunohistochemically stained by use of affinity-purified anti-cGMP kinase antibodies diluted 1:10. Bound antibodies were detected by the avidin-biotin complex technique31 using nickel-intensified dianminobenzidine. Negative controls were obtained by deleting the primary antibody.
Treatment of Animals

Renovascular (Goldblatt II) hypertension was induced in male Wistar rats at the age of 8 weeks by narrowing the right arteria renalis with a silver clip (0.2 mm). Control animals were sham-operated. The blood pressure was measured in light ether anesthesia weekly for 6 weeks by tail plethysmography. Animals that had a stable systolic blood pressure above 160 mm Hg during the last 3 weeks were included in the hypertensive group. Animals that had a blood pressure below 160 mm Hg or a variable blood pressure during the last 2 weeks were only used to correlate blood pressure and cGMP kinase concentration.

Hyperthyroidism was induced in male Wistar rats at the age of 8 weeks. Hyperthyroid animals were injected daily subcutaneously with thyroxine (0.25 mg/kg in 0.25 ml saline) for 6 weeks. Control animals were injected daily with saline. The systolic blood pressure was measured weekly as described above. Control and experimental animals were killed at the age of 14 weeks.

Miscellaneous Methods

Protein A and cGMP kinase were iodinated with 125I either by the Jodo-Gen or the Bolton-Hunter procedure. All data shown are mean±SEM. Statistical differences were evaluated by Student's t test. Linear regression lines were calculated by a computer program. The cGMP kinase concentration of stroke-prone spontaneously hypertensive rats was determined by three independent persons who used different animals, primary antibody preparations (rabbit vs. goat), and techniques (RIA vs. ELISA) and worked in different laboratories (Heidelberg vs. Homburg) at different years.

Results

Specificity of Antibodies

Antisera raised against pure cGMP kinase reacted with several cardiac proteins in immunoblots and yielded nonparallel dilution curves. In contrast, monospecific anti-cGMP kinase antibodies affinity-purified from several antisera detected only a 75-kDa band in various rat tissues (Figure 1A) including hearts from normotensive and hypertensive animals (Figure 1B). The 75-kDa band was indistinguishable from purified cGMP kinase. The monospecific antibodies yielded parallel tissue dilution curves in the cGMP kinase RIA or ELISA (Figure 1C). The purified antibodies did not cross-react with the pure subunits of type I and type II cyclic AMP kinase added at a 1,000-fold higher concentration than the cGMP kinase. These antibodies detected cGMP kinase in all tested rat tissues in concentrations that are close to the reported ones. The concentration varied from 0.54 pmol/mg in heart and lung to 0.07 pmol/mg in skeletal muscle and liver (Table 1). This tissue distribution supports the specificity of the antibodies since the concentration of cyclic AMP kinase is about 0.32 pmol/mg in heart, liver, kidney, and skeletal muscle whereas the concentration of cGMP kinase varies from 0.54 to 0.07 pmol/mg in these tissues. These antibodies recognized the active fragment of cGMP kinase that contains the catalytic part of the enzyme. Recent cloning of the two isozymes Iα and Iγ of cGMP kinase shows that the two isozymes have an identical amino acid sequence in this part of the enzyme. Therefore, the used antibodies detect both isozymes if rat tissues have the same two isozymes of cGMP kinase as bovine aorta.

cGMP Kinase and Hypertension

The cardiac concentration of cGMP kinase is lower in spontaneously hypertensive rats than in normotensive age-matched Wistar-Kyoto rats (Table 2). This difference was highly significant regardless of whether the cGMP kinase concentration was calculated per milligram extracted protein or per gram wet weight. The change in kinase concentration was independent of the batch of animals, of the determination method, and of the antibody. The same result was obtained when a goat antibody or an ELISA was used. The decrease in cGMP kinase concentration was not caused by a translocation of cGMP kinase from the soluble to the particulate fraction. Twenty-eight percent of the total cGMP kinase sedimented with the particulate fraction. Twenty-eight percent of the total cGMP kinase sedimented with the particulate fraction. However, the concentration of particulate cGMP kinase did not differ between the two animal groups (Figure 2). The same protein of 75 kDa was detected in immunoblots of cardiac proteins (Figure 1B); thus, the possibility that hypertensive and normotensive animals express grossly different proteins was ruled out.

These results are compatible with the idea that hypertensive animals have a low vascular content of cGMP kinase. Since it is difficult to determine the enzyme content of resistance vessels, the cGMP kinase content of aorta and brain microvessels was

<table>
<thead>
<tr>
<th>Organ</th>
<th>n</th>
<th>cGMP kinase (pmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>5</td>
<td>0.54±0.03</td>
</tr>
<tr>
<td>Lung</td>
<td>9</td>
<td>0.54±0.03</td>
</tr>
<tr>
<td>Kidney</td>
<td>9</td>
<td>0.32±0.03</td>
</tr>
<tr>
<td>Ileum</td>
<td>5</td>
<td>0.20±0.04</td>
</tr>
<tr>
<td>Aorta</td>
<td>9</td>
<td>0.25±0.03</td>
</tr>
<tr>
<td>Testis</td>
<td>5</td>
<td>0.14±0.02</td>
</tr>
<tr>
<td>Liver</td>
<td>5</td>
<td>0.08±0.02</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>5</td>
<td>0.07±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The cGMP kinase concentration was measured by enzyme-linked immunosorbent assay as described in "Materials and Methods." Lung was homogenized in buffer C containing 1% Triton X-100. In the absence of Triton X-100, 46% of total cGMP kinase sedimented with the particulate fraction. Skeletal muscle was from the musculus gluteus maximus.
TABLE 2. Hypertension-Related Decrease in the cGMP Kinase Concentration in Rat Hearts

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Age (wk)</th>
<th>Blood pressure (mm Hg)</th>
<th>Relative heart weight (g/kg body wt)</th>
<th>Cardiac protein (mg/g wet wt)</th>
<th>cGMP kinase (pmol/mg protein)</th>
<th>cGMP kinase (pmol/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>24</td>
<td>104±4</td>
<td>2.5±0.1</td>
<td>38±2</td>
<td>0.24±0.03</td>
</tr>
<tr>
<td>Spontaneous hypertension</td>
<td>8</td>
<td>24</td>
<td>165±7*</td>
<td>3.4±0.06*</td>
<td>43±2</td>
<td>0.12±0.01*</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>9</td>
<td>108±3</td>
<td>3.0±0.3</td>
<td>40±2</td>
<td>0.49±0.03</td>
</tr>
<tr>
<td>Goldblatt II</td>
<td>14</td>
<td>9</td>
<td>189±8*</td>
<td>4.3±0.4*</td>
<td>37±2</td>
<td>0.30±0.04*</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>12</td>
<td>109±3</td>
<td>3.1±0.2</td>
<td>40±2</td>
<td>0.52±0.03</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>14</td>
<td>12</td>
<td>138±4*</td>
<td>4.1±0.2*</td>
<td>36±2</td>
<td>0.60±0.05</td>
</tr>
<tr>
<td>Left ventricle and septum</td>
<td>14</td>
<td>9</td>
<td>111±2</td>
<td>2.2±0.1</td>
<td>33±2</td>
<td>0.55±0.03</td>
</tr>
<tr>
<td>Goldblatt II</td>
<td>14</td>
<td>9</td>
<td>191±5*</td>
<td>3.0±0.2*</td>
<td>36±2</td>
<td>0.35±0.02*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The amount of cGMP kinase was measured by a specific radioimmunoassay (Wistar-Kyoto controls and spontaneously hypertensive animals) using two affinity-purified antibodies raised in a rabbit and a goat. These values were confirmed by an enzyme-linked immunosorbent assay, which was used for all other measurements.

*Significant at p≤0.005.
†Significant at p≤0.05.
‡Significant at p≤0.01.

compared (Figure 2). Triton X-100 extracts of aorta or brain microvessels prepared from both animal groups contained identical concentrations of cGMP kinase (Figure 2).

These results corroborate those of Kuo et al but are inconclusive in themselves since they do not rule out either of the following possibilities: 1) Hypertension changes the concentration of cGMP kinase only in resistance vessels, and this decrease is involved in the development or in the maintenance of high blood pressure. 2) The low level of cardiac cGMP kinase is a genetic variability of stroke-prone spontaneously hypertensive rats and is unrelated to the development and maintenance of hypertension. The second possibility can be tested by using experimentally induced renovascular hypertension. Hypertension was induced in 8-week-old rats by a clip on the right arteria renalis (Goldblatt II hypertension). Forty percent of the operated animals developed severe stable hypertension within 6 weeks with a systolic blood pressure of 189±8 mm Hg. These animals developed the expected cardiac hypertrophy. Their cardiac concentration of cGMP kinase decreased from 0.49 to 0.30 pmol/mg protein (Table 2). This decrease is also seen when the cGMP kinase concentration is calculated per gram wet weight.

The cGMP Kinase Content of Other Organs

The above results support the possibility that the decrease in cardiac cGMP kinase is causally connected with the development of renovascular hypertension. To test this further, the cGMP kinase content of lung, aorta, and both kidneys was determined. Each organ contains a relatively high level of cGMP kinase (see Table 1), which should be mainly localized in the vascular smooth muscle. However, these organs are perfused at quite different blood pressures, which should affect their cGMP kinase concentration differently. The lung and the clipped right kidney are exposed to low blood pressure whereas aorta and the left kidney are perfused with a high blood pressure. Figure 3 shows that the cGMP kinase content of lung and right and left kidney did not differ between hypertensive and normotensive animals. Furthermore, no
difference was measured between the right and left kidney of hypertensive animals although each kidney was perfused at a different blood pressure. The aorta of hypertensive animals contained slightly higher concentrations of cGMP kinase; the difference was not significant. Apparently, the change in cGMP kinase concentration is organ specific and does not reflect a general decrease in the concentrations of cGMP kinase in hypertensive animals.

cGMP Kinase and Cardiac Hypertrophy

It is well established that renovascular hypertension induces left ventricular hypertrophy, which was also present in the animals used in this study (see Table 2). Therefore, it was possible that the cGMP kinase concentration decreased only in the hypertrophied left ventricle and remained unchanged in atria and right ventricle; these changes were observed (Table 2, Figure 3). The blood pressure as well as the relative left ventricular weight was inversely correlated with the cardiac cGMP kinase content (Figure 4); these findings suggested that either the pressure itself or the pressure-induced hypertrophy was responsible for the decrease in cardiac cGMP kinase concentration. These two possible causes can be differentiated experimentally by inducing ventricular hypertrophy by a high cardiac output. Hyperthyroidism is associated with an elevated cardiac output, cardiac hypertrophy, and mild hypertension.37,38 The extent of cardiac hypertrophy induced by hyperthyroidism was comparable with that of hypertensive animals, but no change in cardiac cGMP kinase concentration was measured (Table 2).

Cardiac Localization of cGMP Kinase

These results are not compatible with the hypothesis that cardiac hypertrophy and the concentration of cGMP kinase are causally related to each other. Cardiac tissue is not homogeneous and contains several cell types. It was possible that the content of one cardiac cell type that contained a high concentration of cGMP kinase changed relative to the other cell types during the development of hypertension-induced hypertrophy. The coronary vessels could contain this cell type since the density of capillaries decreases during pressure39-42 and increases during thyroxine-induced hypertrophy.37,38 In addition, the minimal coronary resistance increases only in pressure-induced hypertrophy due to a decreased cross-sectional area of the resistance vessels (see Reference 39 and references cited therein). Previous studies suggested also that cardiac muscle cells contain no or very low concentrations of cGMP kinase.19 The organ-selective decrease in cGMP kinase content could be explained if cGMP kinase were mainly present in cardiac vascular smooth muscle since a relative decrease of cardiac
vascularization would lead to a selective decrease in cGMP kinase. This possibility was tested by immunohistochemistry of cross sections of hearts from normal and hypertensive animals (Figure 5). Anti-cGMP kinase antibodies stained exclusively large and small coronary arteries. The distribution and the intensity of the immunoreactive stain did not differ between normal and hypertensive animals. No difference was observed between genetic and experimental hypertensive rats. Cardiac muscle cells were not stained at all or only minimally. Microscopic inspection of the cross sections at higher magnification suggested that cGMP kinase was absent from vascular endothelium and present in high concentration in the medial layer. This staining pattern is strong evidence that the organ-selective decrease in cGMP kinase may be caused by a rarification of coronary smooth muscle cells during the development of pressure-induced left ventricular hypertrophy.

This interpretation is supported further by a recalculation of the total cardiac cGMP kinase content per kilogram body weight. This calculation is based on the known fact that the cardiac weight depends on the body weight, which is reduced in growing, hypertensive animals. The total cardiac cGMP kinase content per kilogram body weight should remain constant in pressure-induced hypertrophy if the vascular smooth muscle does not grow together with the cardiac muscle and should increase if the vascular smooth muscle grows to the same extent as the cardiac muscle. As shown in Table 3, exactly the predicted changes occurred in the two hypertrophy models. The total cGMP kinase content per kilogram body weight remained constant in hypertensive animals and increased in hyperthyroid rats. That is, the hearts of hypertensive rats have a decreased cGMP kinase concentration since their vascular smooth muscle content does not increase in accordance with the cardiac muscle whereas the hearts of hyperthyroid animals have a regular cGMP kinase concentration since their vessels do grow together with the muscle.

The validity of the above conclusion can be tested further. The amount of smooth muscle present in nonsmooth muscle organs such as skeletal muscle, liver, heart, and kidney should be determined by the number of organ vessels that perfuse these tissues. The arterial blood flow through these organs would depend then on the number of the arterial vessels and that number should correlate with the number of smooth muscle cells. If cGMP kinase is mainly present in the vascular smooth muscle of these organs as suggested previously, its concentration should correlate with the maximal arterial flow. The values for the maximal arterial blood flow have been reported for normotensive and hypertensive (Goldblatt II) rats. Figure 6 shows that the cGMP kinase concentration of liver, skeletal muscle, kidney, and heart correlates excellently with the maximal arterial blood flow; this correlation suggests that the cGMP kinase content of these organs may be a suitable index of vascularization.

### Table 3. Relative Cardiac cGMP Kinase Content

<table>
<thead>
<tr>
<th>Group</th>
<th>Cardiac cGMP kinase (pmol/g cardiac wet wt)</th>
<th>Relative heart weight (g cardiac wet wt/kg body wt)</th>
<th>Total cGMP kinase/body weight (pmol/kg body wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td>11.8±1.5</td>
<td>4.3±0.4</td>
<td>51±4</td>
</tr>
<tr>
<td>Control</td>
<td>19.5±1.4</td>
<td>3.1±0.3</td>
<td>58±4</td>
</tr>
<tr>
<td>Hyperthyroid</td>
<td>20.9±1.4</td>
<td>4.1±0.2</td>
<td>84±4</td>
</tr>
<tr>
<td>Control</td>
<td>20.4±1.2</td>
<td>3.1±0.2</td>
<td>62±3</td>
</tr>
</tbody>
</table>

Values are mean±SEM. The cGMP kinase is calculated per kilogram body weight since hyperthyroid and hypertensive animals have a lower body weight than control animals.

![Figure 6](http://circres.ahajournals.org/)

**Figure 6.** Graph showing correlation between maximal arterial blood flow and cGMP kinase content of various rat organs. cGMP kinase content was determined as described in legend to Table 1. The maximal arterial blood flow is taken from References 39 and 40 for liver (1), skeletal muscle (2), kidney (3), hearts of Goldblatt II hypertensive rats (4), and hearts of normotensive control rats (5). The maximal blood flow has been corrected for systolic blood pressure.
larization. This conclusion is in line with the previous demonstration\textsuperscript{7,18–22} that high concentrations of cGMP kinase are only present in platelets, smooth muscle, pericytes, and Purkinje cells.

**Discussion**

The points of this study are as follows:

1) Hypertension is associated with a selective decrease of the cardiac cGMP kinase concentration. 2) cGMP kinase is exclusively localized in cardiac vascular smooth muscle; this does not exclude the possibility that low concentrations of the enzyme are present in other cells. 3) The cGMP kinase content of nonsmooth muscle organs such as skeletal muscle, liver, kidney, and heart may be a suitable index for the vascularization of these organs.

The first and second point need no further discussion since they are supported by previous work that showed that the activity of cGMP kinase decreases in hearts of stroke-prone spontaneously hypertensive rats\textsuperscript{39} and that cGMP kinase is localized in these organs mainly in the vascular smooth muscle.\textsuperscript{19,20} The third point is based on the conclusion that the decreased cardiac cGMP kinase concentration is not caused by a decreased cGMP kinase concentration in individual vascular smooth muscle cells but represents a change in the ratio of vascular smooth muscle mass to cardiac muscle mass. This conclusion is based on the finding that neither arterial hypertension nor cardiac hypertrophy itself caused the decrease in cGMP kinase concentration (compare the values for hypertensive animals with those for hyperthyroid animals in Table 2).

These results are explained by morphometric studies\textsuperscript{37–41} in connection with the determination of the minimal coronary resistances in hypertrophied hearts.\textsuperscript{37,39,45,46} Both types of measurements suggest that cardiac hypertrophy caused by thyrotoxicosis is associated with an adequate proliferation of capillaries and that the cross-sectional area of the small arterioles (determined by the minimal coronary resistance) matches the increased ventricular weight.\textsuperscript{37,38} In contrast, pressure-induced cardiac hypertrophy is reportedly associated with a decreased density of capillaries and a relative decrease in the cross-sectional area of small arterioles.\textsuperscript{39,45,46} The latter finding does not directly prove that a decreased cross-sectional area is caused by a decreased proliferation of the arterioles since hypertrophy of the walls of the resistance vessels\textsuperscript{47} or extravasal compression\textsuperscript{48} might as well contribute to a decreased cross-sectional area of the resistance vessels. However, the wall-to-lumen ratio in the early stage of Goldblatt II hypertension was not changed by arterial hypertension.\textsuperscript{49} Furthermore, if the minimal coronary resistance in pressure-induced hypertrophy is not expressed per gram of ventricle but for the whole ventricle, no decrease occurred during cardiac hypertrophy.\textsuperscript{39}

The latter finding suggests that the cross-sectional area of cardiac vessels remains constant during hypertension-induced hypertrophy. In accordance with this conclusion, the total concentration of cGMP kinase remained constant in hypertensive-induced hypertrophy (Table 3). This supports the idea that a relative lack of coronary vessel proliferation is, at least in the early stages of the cardiac hypertrophy, the main reason for the decreased cross-sectional area and the decreased cGMP kinase. This conclusion is important since it opens the way to determine more closely the relation between cardiac hypertrophy and coronary vessel growth.

**Acknowledgments**

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**References**

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