Straznicka, Michaela, Gary Gong, James Tse, Peter M. Scholz, and Harvey R. Weiss. cGMP level that reduces cardiac myocyte O2 consumption is altered in renal hypertension. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1949–H1955, 1997.—We tested the hypothesis that cardiac myocytes from hypertensive (one kidney, one clip; 1K,1C) cardiac hypertrophied rabbits require higher guanosine 3',5'-cyclic monophosphate (cGMP) to similarly lower O2 consumption than control myocytes and that this effect is caused by differences in guanylate cyclase activity. Using isolated myocytes from control and 1K,1C New Zealand White rabbits, we obtained O2 consumption (nl O2·min⁻¹·10⁵ cells) and cGMP (fmol/10⁵ cells) levels after stimulation of guanylate cyclase with nitroprusside, CO, or guanylin (10⁻⁸–10⁻⁵ M). Soluble guanylate cyclase activity was also determined. Basal cGMP was elevated in 1K,1C vs. control (176 ± 28 vs. 85 ± 13) myocytes. cGMP increased in 1K,1C and control myocytes after stimulation with nitroprusside, CO, and guanylin. Guanylate cyclase activity in 1K,1C vs. control myocytes was not statistically different. Basal O2 consumption in 1K,1C vs. control myocytes was comparable (307 ± 1 vs. 299 ± 22). O2 consumption was similarly decreased when guanylate cyclase was stimulated. Control regression equations correlating cGMP and O2 consumption were O2 consumption = 1.46·[cGMP] + 444.65 (r = 0.96) for CO, O2 consumption = 0.58·[cGMP] + 328.48 (r = 0.82) for nitroprusside, and O2 consumption = 1.25·[cGMP] + 389.15 (r = 0.88) for guanylin. The 1K,1C regression equations were O2 consumption = 1.36·[cGMP] + 537.81 (r = 0.97) for CO, O2 consumption = 0.23·[cGMP] + 307.30 (r = 0.88) for nitroprusside, and O2 consumption = 1.27·[cGMP] + 502.91 (r = 0.89) for guanylin. These data indicate that 1K,1C hypertrophic myocytes had higher cGMP than controls at every level of O2 consumption. This effect was not caused by differences in basal or maximal guanylate cyclase activity.

cardiac hypertrophy; guanosine 3',5'-cyclic monophosphate; guanylate cyclase; oxygen consumption

THE SECOND MESSENGER guanosine 3',5'-cyclic monophosphate (cGMP) has been shown to exert a significant influence on myocardial cells, including negative metabolic and functional effects (12, 14, 21). cGMP can cause reductions in local myocardial metabolism, inotropy, and force development (12, 21, 22). These effects have been found both in vivo and in vitro in a variety of species, including humans (12). The actions of cGMP may be mediated through protein phosphorylation, cGMP-stimulated or -inhibited adenosine 3',5'-cyclic monophosphate (cAMP) phosphodiesterases, and direct or indirect inhibition of L-type calcium channels (12, 13, 21–23). Intracellular levels of cGMP can be raised by either stimulating guanylate cyclase to increase production of cGMP or inhibiting cGMP phosphodiesterase to prevent its breakdown. In vascular smooth muscle, increases in cGMP have been shown to induce vasodilatation (14, 22). In the rabbit heart, previous in vivo work has demonstrated that increases in cGMP lead to decreases in myocardial O2 consumption (26, 27). The relationship between cGMP and myocardial consumption has not been fully elucidated.

In the hypertrophied myocardium, there are changes in the intracellular level and/or effects of second messengers such as cGMP (16, 18). Myocardial cGMP levels are reported to be elevated in some types of pressure-load hypertrophy as well as in some forms of heart failure (8, 16, 18). This is not true of all forms of cardiac hypertrophy (17, 26). In vivo, it has been shown that elevated cGMP was associated with significant reductions in myocardial O2 consumption in both control and hypertrophic hearts, although larger increases of cGMP were required in renal hypertension (one kidney, one clip; 1K,1C)-induced cardiac hypertrophic animals to produce similar reductions in O2 consumption (17). These results suggested that the response of myocardial O2 consumption to cGMP was reduced in this type of hypertrophy. However, the numerous cell types present in an intact heart make it difficult to determine the source of the cGMP and to assess cell-to-cell interactions. In the present study, we performed all of the experiments on myocytes isolated from control and 1K,1C rabbit hearts.

We tested the hypothesis that myocytes isolated from renal hypertension (1K,1C)-induced cardiac hypertrophic rabbits require higher levels of cGMP to similarly lower O2 consumption compared with controls and that this difference would be related to differences in guanylate cyclase activity. We used nitroprusside, CO, and guanylin, in increasing doses, to stimulate guanylate cyclase. The cells were activated by 2 mM calcium, and O2 consumption measurements, intracellular cGMP levels, and soluble guanylate cyclase activity were determined.

MATERIALS AND METHODS

New Zealand White rabbits (2–3 kg) were used for all experiments. Animals were prepared as a 1K,1C renal hypertensive model (2) under sterile, anesthetized conditions (30 mg/kg pentobarbital sodium iv). A left flank incision was used to expose the left kidney, and the renal artery was carefully dissected. A sterile silver clip (0.5-mm gap opening) was threaded around the artery and folded over itself to secure it in place. The incision was closed. The right kidney was then exposed through a right flank incision, and the ureter, renal
artery, and renal vein were ligated. The kidney was removed, and the incision was closed. The animals were allowed to recover for 35 days. All experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (DHHS Publication No. 85–23, Revised 1985) and were approved by our Institutional Animal Care and Use Committee. A total of 8 1K,1C and 11 control rabbits were used.

Cell dissociation. Cardiac ventricular myocytes were prepared as previously described (6), with the following modifications. The rabbits were anesthetized (35 mg/kg pentobarbital sodium) and then heparinized (10 U/g body wt) using the circumflex coronary vein. The heart was rapidly removed after an overdose of pentobarbital (60 mg/kg). Retrograde aortic perfusion of the heart was immediately begun at 70-mmHg constant pressure with N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES; pK 7.5) buffered with minimal essential medium (MEM). This solution contained (in mM) 117 NaCl, 5.7 KCl, 11 NaHCO₃, 1.5 NaH₂PO₄, 1.7 MgCl₂, 21.1 HEPES, and 11.7 glucose and amino acids and vitamins. We added 2 mM L-glutamine and 10 mM taurine, and the pH was adjusted to 7.2 with NaOH. This low-Ca²⁺-MEM solution had an osmolality of 296 mosM and a free-Ca²⁺ activity of 2–5 µM. After 5 min of perfusion with low-Ca²⁺ MEM, the heart was perfused at 50 mmHg with a 60-ml volume of low-Ca²⁺ MEM supplemented with 0.1% collagenase (Worthington type II). All perfusion solutions were maintained at 37°C and equilibrated with a water-saturated gas mixture (85% O₂-10% N₂-5% CO₂). After 30 min of collagenase perfusion with recirculation, the heart was removed from the perfusion apparatus and cut into 8–10 pieces in MEM containing 1.0 mM CaCl₂ and 0.5% bovine serum albumin (fraction V; Sigma, St. Louis, MO). This Ca²⁺-MEM was supplemented with 0.1% collagenase. The tissue suspension was gently swirled in 50-ml centrifuge tubes at 37°C by a wrist-action shaker (2 cycles/s, Multi-Mixer, Lab-Line Inst., Melrose Park, Ill.) for 5 min. A slurry containing isolated heart cells was decanted from the tissue suspension. The isolated cells were washed three times with the aid of a low-speed centrifuge (34 g) to completely remove the collagenase and some subcellular debris and then were resuspended in low-Ca²⁺ MEM solution. Incubation of the remaining tissue with collagenase was repeated at least two more times. The combined, washed cells were then maintained at room temperature. Immediately before the start of each experiment, the cells were placed in a high-Ca²⁺ (2 mM) MEM solution. The viability of the myocytes in the MEM suspension averaged between 70 and 80%. Yields were typically 10–14 × 10⁶ rod-shaped cells/heart. Using a Zeiss Axiovert 25 inverted microscope, we measured the length and width of 50 cells/heart from both control and 1K,1C myocardium. The average of these measurements was taken and represents the myocyte size for each animal.

Myocyte O₂ consumption measurements. Steady-state O₂ consumption was recorded continuously using O₂ electrodes and a two-channel oximeter (Univ. of Pennsylvania) fitted into a customized glass recording chamber (19). All experiments were performed under normoxic conditions. The recording started with a Po2 of ~115 mmHg and ended at a Po2 of 25 mmHg. Anaerobic metabolism occurs at Po2 levels <5–10 mmHg. Gradients in Po2 were not likely in the chamber, because a Teflon-coated stirring rod was used to keep the cells in suspension.

The chamber used was constructed of glass and contained a small Teflon-coated stirring bar. The cuvette was mounted on a magnetic stirrer. A ground glass stopper was used to eliminate the gas phase. This stopper also provided access to the assay medium via a central hole (1.3-mm internal diameter) for addition of agents during the experiment. The volume of the recording chamber was 1.5 ml. The myocytes were added to the chamber, and their number was determined. The total volume of all drugs added to the chamber was <100 µl; therefore, no significant dilution of the myocyte suspension occurred.

O₂ consumption was measured polarographically with a Clark-type electrode. The electrode was calibrated by placing it into a solution saturated with two known concentrations of O₂. When the electrode was calibrated, a 95% response could be obtained within 3–4 s. The rate of fall in O₂ tension within the chamber was used to determine the O₂ consumption of the myocytes over time. O₂ consumption was expressed as nanoliters of O₂ per minute per 10⁵ myocytes. O₂ consumption determinations were obtained in the same MEM solution used to resuspend the cells. The sample was stirred at a rate sufficient to keep the cells suspended and yet not rapidly enough to compromise their viability. We found that 70% of the cells were quiescent, rod-shaped cells at the completion of the experiment.

Protocol. The following protocol was used for the O₂ consumption recording for this study. Myocytes were suspended in the chamber with MEM at an appropriate myocyte concentration, and the cells were allowed to stabilize for 10–15 min. A 5-min recording was made for baseline measurements. CO, sodium nitroprusside, or guanylin dissolved in MEM was then added. A 5-min interval was allowed between reagent additions, during which time O₂ consumption was measured again. Reagents were added in increasing doses: CO, 1.5 × 10⁻⁸, 1.5 × 10⁻⁷, and 1.5 × 10⁻⁶ M; nitroprusside, 10⁻⁸, 10⁻⁷, and 10⁻⁶ M; and guanylin, 10⁻⁸, 10⁻⁷, 10⁻⁶, and 10⁻⁵ M. The two groups were treated as follows. Of the 11 controls, all were treated with CO and guanylin, and 5 were treated with nitroprusside. Of the eight 1K,1C rabbits, all were treated with CO and guanylin, and five were treated with nitroprusside. At the end of each series of recordings, 2,4-dinitrophenol (DNP; Sigma) was added (5 × 10⁻⁵ M) to the chamber. This always at least doubled the O₂ consumption from its baseline value. The same experimental manipulations were performed to additional myocytes incubated in a flask, following the exact same protocol (timing, dosage, temperature, stirring, etc.). These myocytes were frozen in liquid nitrogen within 15 s of completion of each respective drug treatment for later cGMP determinations.

cGMP measurements. To determine cGMP levels, myocytes were warmed to 0°C and homogenized in ethanol using a Brinkmann Polytron in an ice bath. The homogenate was centrifuged at 30,000 g for 15 min in a Sorvall RC-5B centrifuge. The supernate was recovered. The pellet was resuspended in 1 ml of 2:1 ethanol-water and centrifuged as before. The combined supernatants were evaporated to dryness in a 60°C bath under a stream of nitrogen gas. The final residue was dissolved in 1.5 ml of assay buffer (0.05 M sodium acetate, pH 5.8, containing sodium azide). cGMP levels were determined by radioimmunoassay using a scintillation proximity assay (Amersham). This assay measures the competitive binding of ¹²⁵I-labeled cGMP-specific antibody. After construction of the standard curve, we determined cGMP levels directly from the counts.

Guanylate cyclase activity measurements. Guanylate cyclase activity was determined in the cytosolic fraction of the isolated myocytes. The protein concentrations were measured using the Bio-Rad protein assay. The tissue samples were homogenized with 30 vols of ice-cold buffer (25 mM tris(hydroxymethyl)aminomethane (Tris)-HCl (pH 7.5)) with three bursts of 5 s using a Brinkmann Polytron at a speed of 28,000
RESULTS

The heart weight (7.58 ± 0.40 g) and heart weight-to-body weight ratio (3.22 ± 0.11 g/kg) of the control rabbits were significantly lower than the heart weight (12.76 ± 0.66 g) and heart weight-to-body weight ratio (4.08 ± 0.24 g/kg) of the 1K,1C rabbits. The control myocytes had an average length of 116 ± 4 µm and an average width of 20 ± 1 µm. The 1K,1C myocytes had an average length of 139 ± 3 µm and an average width of 22 ± 2 µm. This represents a significant difference in length but not width between control and hypertrophic myocytes.

Intracellular cGMP levels were significantly lower in control than in 1K,1C rabbit myocytes at baseline (Table 1). Stimulation with CO resulted in a dose-dependent increase in both control and 1K,1C intracellular cGMP levels. Increasing doses of nitroprusside increased cGMP; 10⁻⁵ M nitroprusside resulted in the greatest increase in control (154%) and in 1K,1C intracellular cGMP levels, and 10⁻⁶ M nitroprusside resulted in the greatest increase in 1K,1C (131%) intracellular cGMP levels. Stimulation with guanylin also resulted in a significant dose-related increase in both control and 1K,1C intracellular cGMP levels (Table 1).

Basal soluble guanylate cyclase activity was 3.7 ± 0.8 and 2.2 ± 0.5 pmol·mg protein⁻¹·min⁻¹ in control and 1K,1C myocytes, respectively. After addition of 0.1 mM nitroprusside, the stimulated guanylate cyclase activity was 99.3 ± 8.8 and 110.0 ± 27.0 pmol·mg protein⁻¹·min⁻¹ in control and 1K,1C myocytes, respectively. There were no statistical differences between the control and 1K,1C basal or stimulated soluble guanylate cyclase activity.

Table 1. Effect of stimulation of guanylate cyclase on level of cGMP in control and 1K,1C cardiac myocytes

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (n=11)</th>
<th>1K,1C (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>85.5 ± 129</td>
<td>158.5 ± 27.7</td>
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<tr>
<td>Carbon monoxide</td>
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<td></td>
</tr>
<tr>
<td>1.5 × 10⁻⁸ M</td>
<td>154.5 ± 23.4</td>
<td>189.8 ± 38.1</td>
</tr>
<tr>
<td>1.5 × 10⁻⁷ M</td>
<td>181.5 ± 29.3*</td>
<td>287.3 ± 45.9†</td>
</tr>
<tr>
<td>1.5 × 10⁻⁶ M</td>
<td>183.9 ± 45.6*</td>
<td>280.8 ± 35.9*</td>
</tr>
<tr>
<td>Guanylin</td>
<td></td>
<td></td>
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<tr>
<td>10⁻⁸ M</td>
<td>130.3 ± 18.0</td>
<td>180.5 ± 29.0</td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>168.9 ± 34.0*</td>
<td>180.5 ± 24.6</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>156.9 ± 13.9*</td>
<td>258.1 ± 32.2*</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>164.6 ± 26.2*</td>
<td>262.9 ± 24.8*</td>
</tr>
<tr>
<td>Nitroprusside</td>
<td></td>
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<tr>
<td>10⁻⁸ M</td>
<td>82.3 ± 9.5</td>
<td>282.7 ± 35.3†</td>
</tr>
<tr>
<td>10⁻⁷ M</td>
<td>91.2 ± 6.8</td>
<td>363.1 ± 62.7†</td>
</tr>
<tr>
<td>10⁻⁶ M</td>
<td>180.8 ± 30.7*</td>
<td>506.6 ± 65.2*</td>
</tr>
<tr>
<td>10⁻⁵ M</td>
<td>216.0 ± 60.1*</td>
<td>408.0 ± 59.1†</td>
</tr>
</tbody>
</table>

Values, in femtomoles per 10⁵ cells, are means ± SE; n, no. of cells. * Different from baseline, † different from control (P < 0.05).
The equations for the three control treatments were $O_2$ consumption $= -1.46 \cdot [\text{cGMP}] + 444.65$ ($r = 0.96$) for CO, $O_2$ consumption $= -0.58 \cdot [\text{cGMP}] + 328.48$ ($r = 0.82$) for nitroprusside, and $O_2$ consumption $= -1.25 \cdot [\text{cGMP}] + 389.15$ ($r = 0.88$) for guanylin. The regression equations for the three 1K,1C treatments were $O_2$ consumption $= -1.36 \cdot [\text{cGMP}] + 537.81$ ($r = 0.97$) for CO, $O_2$ consumption $= -0.23 \cdot [\text{cGMP}] + 307.30$ ($r = 0.88$) for nitroprusside, and $O_2$ consumption $= -1.27 \cdot [\text{cGMP}] + 502.91$ ($r = 0.89$) for guanylin. The regression lines for the CO and guanylin treatments were parallel for 1K,1C and control myocytes. However, the intercepts were significantly shifted for 1K,1C myocytes such that the cGMP level was higher at any $O_2$ consumption. The slope of the nitroprusside regression line was significantly steeper in control compared with 1K,1C myocytes.

**DISCUSSION**

The major findings of the current study were that an inverse relationship existed between the level of cGMP and $O_2$ consumption in isolated cardiac myocytes from control and renal hypertension (1K,1C)-induced cardiac hypertrophic rabbits but that a greater level of cGMP was found at each level of $O_2$ consumption in the 1K,1C myocytes. Further data revealed that these results were not caused by differences in guanylate cyclase. cGMP levels were increased using three distinct guanylate cyclase stimulators, CO, nitroprusside, and guanylin. Baseline and maximal guanylate cyclase activity were comparable in control versus 1K,1C myocytes. Baseline cGMP levels were higher in 1K,1C than
control myocytes. cGMP levels were also greater in 1K,1C myocytes at the same doses of CO, nitroprusside, and guanylin. At similar intracellular levels of cGMP, myocyte O2 consumption was greater in the 1K,1C group than in controls. We demonstrated that guanylin increased cGMP levels in a dose-dependent manner in both control and hypertrophic cardiac myocytes. Because guanylin is reported to stimulate the particulate form of guanylate cyclase in various cell types (3, 24), it may be that particulate guanylate cyclase directly affects the level of cGMP in both control and hypertrophic cardiac cells.

Most studies with guanylin have focused on its effects in intestinal cells. Previous studies have not documented effects of guanylin on cardiac myocytes, although myocytes are known to contain particulate guanylate cyclase in their membranes (5, 10, 20). This laboratory is the first to demonstrate that guanylin produces a dose-dependent increase in intracellular cGMP in cardiac myocytes, presumably by activation of particulate guanylate cyclase.

We had high yields of healthy myocytes (70–80% viability) from both control and hypertrophic rabbits. The viability of the myocytes at the end of each experiment was confirmed by rechecking the percentage of rod-shaped myocytes and their morphology and by adding DNP to the O2 consumption measurement chamber. By using isolated myocytes, we established that the effects seen on O2 consumption and cGMP were accounted for entirely by myocytes. This claim could be disputed when intact heart preparations, with heterogeneous cell types present, were being used. Measuring errors with regard to O2 consumption and cGMP due to damaged cells should be small, with ~20% rounded cells in our cell preparations. Although they may still metabolize to an unknown extent, this would lead to a shift in the absolute values of O2 consumption and cGMP without altering the conclusions.

In the in vivo rabbit heart, increases in cGMP reduced myocardial O2 consumption (17, 26, 27). This change in consumption may be due to cGMP-induced changes in the contractile performance and/or direct effects on oxidative phosphorylation, or it may affect other factors that determine O2 consumption (17). The in vitro effect of cGMP as a negative metabolic agent is more pronounced under conditions of increased cellular metabolism (7, 15), as seen with electrical stimulation, or high Ca2+ (2.0 mM). In a previous study from our laboratory (7), it was shown that the levels of cGMP and O2 consumption were significantly higher in rabbit cardiac myocytes placed in a high-Ca2+ medium compared with a low-Ca2+ (0.5 mM) medium. In the current study, all of the experiments were carried out in a 2 mM Ca2+-MEM solution; therefore, the cells were in a noncontracting high metabolic state.

The cellular effects of cGMP have been studied in various tissues, including myocardium. Several studies have reported cGMP to have negative metabolic and functional effects on intact hearts and isolated myocytes, and it has also been postulated to be antagonistic to cAMP (12, 14, 21, 22, 27). The mechanisms of action include inhibition of L-type Ca2+ channels (ICa), protein phosphorylation by cGMP-dependent protein kinases, and activation or inhibition of cyclic nucleotide phosphodiesterases (11, 20, 22, 23). Mery et al. (13) suggested that the effects of cGMP on the inhibition of Ica are mediated by the activation of a cGMP-dependent protein kinase that phosphorylates a channel or regulatory protein. There may also be additional effects mediated by the hydrolysis of cAMP via a cGMP-stimulated cAMP phosphodiesterase (11, 20).

The pressure-load model of cardiac hypertrophy (1K,1C) used in this study develops hypertrophy within a month (2). In vivo studies demonstrated basal myocardial O2 consumption to be greater in hypertrophied rabbits than controls (2). The hearts of 1K,1C rabbits were capable of significant increases in O2 consumption (2, 25). In this study, baseline myocardial O2 consumption was comparable between hypertrophic and control rabbits, which may be accounted for by the altered paracrine milieu of the isolated myocytes.

The myocardial level of cGMP has been reported to be elevated in some forms of pressure-load hypertrophy (16, 18). In a canine pressure-overload hypertrophy model, cGMP levels were significantly elevated (18). In some forms of heart failure, cGMP levels may be elevated (8). These results have not been consistent in all models studied. Dowell et al. (4) used rats with aortic constriction and reported that baseline cGMP levels were not elevated. We examined in vivo 1K,1C rabbit hearts and found that their cGMP levels were not significantly different from controls (17). However, the 1K,1C isolated myocytes had significantly higher levels of intracellular cGMP than controls. Other cell types or differences in cell sizes may have affected our in vivo results. This may also be caused by an alteration in the extracellular milieu, presence or absence of paracrine factors from neighboring cells, and lack of sympathetic activity in the isolated cells.

The effects of pressure-load cardiac hypertrophy on the relationship between O2 consumption and cGMP have not been fully elucidated. A previous study in this laboratory (17) using 1K,1C rabbits in vivo demonstrated that larger increases in cGMP were required to produce decreases of O2 consumption in 1K,1C hearts versus controls. This study demonstrates that the similar in vivo and in vitro results were myocyte specific. In the current study in 1K,1C myocytes, we found a shift in the relationship between cGMP and O2 consumption. A higher level of cGMP was required to produce the same O2 consumption in the 1K,1C myocytes compared with controls. Although basal levels of cGMP may not be responsible for metabolic control in 1K,1C cardiac myocytes, there may be a shift in the sensitivity of the heart for elevated levels of cGMP. This may indicate that renal hypertension-induced hypertrophy dampens the effects of cGMP by alterations in protein phosphorylation or Ca2+ currents or by other mechanisms downstream of this second messenger. There could also be changes in Ca2+ sensitivity in 1K,1C myocytes.
In both the control and hypertrophied myocytes, stimulation of guanylate cyclase to produce cGMP was associated with a significant decrease in O₂ consumption. Guanylate cyclase, which has been described in virtually all cell types, is composed of a group of enzymes that function to produce cGMP (5, 10, 24). The two major families of guanylate cyclase are the particulate-associated enzymes and the soluble-type enzymes. In our study, we used guanylin and noted a dose-dependent increase in cGMP and a corresponding decrease in O₂ consumption in both control and hypertrophic cardiac myocytes. From our data, we conclude that guanylin, which stimulates particulate guanylate cyclase, increases intracellular cGMP levels in rabbit cardiac myocytes. Carbon monoxide or nitroprusside can stimulate the soluble form of guanylate cyclase (1, 14). In our study nitroprusside, a nitric oxide donor, caused an increase in cGMP and a decrease in O₂ consumption in both control and 1K,1C myocytes. Using CO-saturated MEM solution, we observed increases in cGMP and corresponding decreases in O₂ consumption. With all the methods used to stimulate guanylate cyclase in the current study, the level of cGMP required to produce a given O₂ consumption was greater in the 1K,1C myocytes. This was not related to difference in activity of the soluble form of guanylate cyclase and appeared to be related to changes in the cell postproduction of this second messenger.

In summary, we found that isolated cardiac myocytes from control and 1K,1C renal-hypertrophic rabbits respond to stimulation of guanylate cyclase by increases in cGMP and decreases in O₂ consumption. Renal hypertension-induced hypertrophic myocytes had significantly higher levels of cGMP at any given level of O₂ consumption compared with controls. This is the first study to demonstrate, using guanylin, that the particulate form of guanylate cyclase has an important role in the production of cGMP and affects O₂ consumption of cardiac myocytes. We studied the soluble form of guanylate cyclase and found no qualitative or quantitative differences between the two groups. We conclude that although cGMP plays an important role in the control of metabolism of both control and hypertrophic cardiac myocytes, the relationship is altered by renal hypertension-induced hypertrophy.

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