0031-3998/00/4703-0369
PEDIATRIC RESEARCH

Vol. 47, No. 3, 2000
Printed in U.S.A.

Maturational Differences in Soluble Guanylate Cyclase Activity in Ovine Carotid and Cerebral Arteries

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ABSTRACT

Basal cGMP concentrations are greater in immature than in mature cranial arteries, which may help explain why cerebrovascular resistance is lower in neonates than in adults. The present studies explore the hypothesis that this difference derives from age-related differences in soluble guanylate cyclase (sGC) activity. Maturation depressed ($p < 0.01$) maximal sGC activity (pmol cGMP/mg/min) in both carotid (from 11.10 ± 0.50 to 3.60 ± 0.20) and cerebral (from 3.10 ± 0.31 to 1.45 ± 0.08) arteries. Western blot analysis of relative sGC abundance (relative to sGC expression in adult kidney) found that sGC abundance was significantly greater ($p < 0.05$) in newborn carotid (0.38 ± 0.04) and cerebral arteries (0.37 ± 0.06) than in adult arteries (0.25 ± 0.05 and 0.17 ± 0.03, respectively). Basal K_m values in carotid and cerebral arteries did not differ significantly between newborns (3- to 7-d old) and adults. Activation of sGC with nitrosylated heme significantly reduced K_m values 3- to 5-fold in both types of artery and in both age groups. Within artery type, maturation had no significant effect on activated K_m. Between artery types, activated K_m values were greater ($p < 0.05$) in cerebral (200 ± 40 μM) than in carotid (80 ± 10 μM) arteries. Together, these data suggest that variations in sGC substrate affinity contribute to observed differences in sGC activity between artery types but not those between age groups. In contrast, variations in enzyme abundance, and possibly also enzyme-specific activity, appear responsible for differences in sGC activity associated with both age and artery type. **(*Pediatr Res* 47: 369–375, 2000)**

Abbreviations

cGMP, guanosine 3′,5′-cyclic monophosphate
sGC, soluble guanylate cyclase
GTP, guanosine 5′-triphosphate
DTT, D,L-dithiothreitol
NO-heme, nitrosylated-heme
PKC, protein kinase C
OD, optical density

One of the most important pathways for vasorelaxation is that mediated by cGMP. Increases in cGMP synthesis mediate the vasodilatory responses to atrial natriuretic peptide, nitrovasodilators, and nitric oxide released from either perivascular nerves (1) or vascular endothelium (2, 3). We have previously reported that basal levels of cGMP differ in cerebral and extracranial arteries and are elevated in the newborn, relative to the adult (4). Because basal levels of cGMP influence resting vascular tone, age-related differences in cGMP may contribute to the neonate's predisposition for vasorelaxation (4, 6). In turn, elevated basal levels of cGMP in the newborn could result from either depressed degradation or elevated synthesis of cGMP. Although degradation is a key factor in the determination of basal vascular cGMP levels, we have previously shown that maximum phosphodiesterase activity is actually significantly greater in newborn rather than in adult arteries and that rates of cGMP degradation are probably limited by the rate of cGMP synthesis (5). Therefore, age-related differences in cGMP synthesis appear responsible for the observed differences between newborn and adult basal levels of cGMP.

Guanylate cyclases [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] are a well-characterized family of enzymes that catalyze the cyclization of GTP to cGMP and occur in two biochemically distinct forms. The membrane-bound form consists of a single transmembrane protein that is activated by small peptide ligands such as atrial natriuretic peptide. The soluble intracellular form (sGC) is a heme-containing enzyme consisting of two similar protein subunits. Basal sGC activity is independent of the bound heme group, whereas activated sGC activity is heme dependent (6). The binding of free radicals such as nitric oxide to the heme group of sGC results in the subsequent activation of the enzyme. Although both forms are found in the mammalian vasculature, sGC is the form responsible for vasodilation responses to nitrovasodilators and nitric oxide. Despite extensive study of the physical

Received August 25, 1999; accepted October 7, 1999.
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This study was supported by USPHS grants PO1-HD31226 and RO1-HL54120.

characteristics and enzymology of sGC in vascular smooth muscle, little is known of its contribution to maturational changes in vascular tone and reactivity.

The present studies address the hypothesis that basal, as well as activated, cGMP levels are elevated in newborn relative to adult arteries as a result of enhanced sGC activity. To explore this hypothesis, we have examined and compared the kinetics and relative abundance of sGC in ovine arteries as a function of age and artery type. Given that basal cGMP values vary among artery types, we studied the cerebral arteries that comprise the circle of Willis and, for comparison, the large extracranial common carotid artery. Whereas basal sGC activity has important implications for resting vascular tone, enhanced sGC activity during active vasodilation may also contribute to low vascular resistance in newborn arteries. Therefore we have examined the kinetics of sGC under both basal and activated conditions. Because enzyme concentration is a key determinant of total tissue sGC activity, we also determined the relative abundance of sGC in all experimental groups, using Western blot analyses.

METHODS

All procedures and protocols used in the present studies were approved by the Animal Research Committee of Loma Linda University and followed all guidelines put forth in the *National Institutes of Health Guide for the Care and Use of Laboratory Animals*. We obtained common carotid and cerebral arteries from young nonpregnant adult sheep (age 18–24 mo) and newborn lambs (age 3–5 d). Vessels from adult animals were obtained from a local slaughterhouse and were kept packed on ice until dissection. Vessels from newborn animals were obtained from lambs brought into the facility and killed with a lethal injection of sodium pentobarbital. All artery segments were cleaned of adipose and connective tissue and were flushed with isotonic Krebs solution to remove coagulated blood (7). The artery segments were then flash frozen in liquid nitrogen and stored at −80°C until they were assayed.

Because of the very small amounts of cerebral artery tissue available from newborn animals (30–50 mg total artery wet weight per animal), sGC activity and abundance measurements were conducted with homogenates prepared from tissues collected from multiple animals for both artery types and age groups. The term "basal" is used throughout this study to denote the activity of sGC observed in the presence of exogenous GTP but in the absence of exogenous NO or heme. The term "activated" is used to denote the activity of sGC obtained after the addition of both NO-heme and GTP.

Semi-crude artery homogenate preparation. For each enzyme preparation, frozen artery segments from four to six animals (~0.3 g of cerebral or ~1.5 g of carotid), were homogenized together in 1 mL of 50 mM Tris-HCl buffer (pH 7.5) containing 4 mM $MgCl_2$, and 2 mM DTT (8, 9). The homogenization buffer also contained a mixture of protease inhibitors including 76.8 nM aprotinin, 83 mM benzamidine, 1 mM iodoacetamide, 1.1 μM leupeptin, 7 μM pepstatin A, and 0.23 mM phenylmethanesulfonyl fluoride (10). The addition of the protease inhibitors stabilized sGC activity to the extent that it did not change significantly over the 24-h period typically needed to complete the activity assays. For homogenization, a motor-driven ground-glass pestle and mortar (Lurex, Vineland, NJ) were used. To separate particulate guanylate cyclase and other proteins from soluble guanylate cyclase, we centrifuged the homogenate for 60 min at 100,000 × *g*, then removed an aliquot of supernatant fluid for protein determination (see below). The remaining supernatant fluid was diluted to the desired protein concentration with homogenization buffer (see below). A fresh homogenate was prepared for each activity measurement.

During preliminary studies to confirm the removal of particulate guanylate cyclase from the supernatant extract, cGMP synthesis was monitored before and after the addition of 100 nM atrial natriuretic peptide and GTP to the samples. Atrial natriuretic peptide had no effect on cGMP synthesis, an observation which was taken as evidence that our procedure effectively reduced particulate guanylate cyclase to negligible levels.

General sGC assay procedure and optimization. The activity of sGC was determined by measuring the formation of cGMP from GTP in 200-μL aliquots of the diluted semi-crude artery homogenate. To remove endogenous cGMP and GTP from the preparation, we warmed each tube in a 38°C water bath for a minimum 30-min "clearing period." Versions of this protocol without the clearing period produced varying starting levels of cGMP (5). To prevent the hydrolysis of cGMP during the experimental procedure, a solution containing 1 mM 3-isobutyl-1-methylxanthine, a wide-spectrum phosphodiesterase inhibitor) and 0.1 mM zaprinast (a selective inhibitor of cGMP phosphodiesterase, PDE V) was added to each tube after the clearing period and allowed to warm for an additional 30 min. Reactions were initiated by the addition of GTP ("basal" sGC activity), or GTP plus preformed NO-heme ("activated" sGC activity). The final reaction volume was 350 μL. The samples were incubated for 5 or 15 min, after which we added 2 mL of ice-cold 6% trichloroacetic acid to each tube to halt the reaction. The samples were then centrifuged, ether extracted, lyophilized, and assayed for cGMP by RIA.

As shown by numerous investigators (11–13), preformed NO-heme maximally activates sGC. In the present study, NO-heme was prepared immediately before use by the addition of 10 mM DTT to hematin ($Fe3^+$) to produce reduced heme ($Fe2^+$). A small aliquot of the reduced heme was mixed into a large volume of assay buffer containing 1.4 mM *S*-nitroso-*N*-acetyl-penicillamine (an exogenous source of nitric oxide) to produce NO-heme.

To determine the optimum ratio of Tris-HCl soluble protein to NO-heme for our assay conditions, we diluted aliquots of adult common carotid and cerebral artery homogenates to 0.3, 0.6, 3.0, or 6.0 mg/mL in Tris buffer. The activated sGC activity for each protein concentration was determined in the presence of 1 mM GTP and 0.02, 0.2, 2, or 20 μM NO-heme. Maximal sGC activation occurred at 2 μM NO-heme, independent of Tris-soluble protein concentration or artery type. This heme concentration was well below the 100 μM concentration reported to inhibit sGC activity (14) and was used for

sGC activation throughout all subsequent experimental procedures.

sGC activity in newborn and adult carotid and cerebral arteries. We conducted all activity assays in parallel under basal and activated conditions in paired homogenate samples. Carotid artery homogenates were diluted to a final protein concentration of 3.0 mg/mL in Tris buffer. Because of the relatively low protein yield for cerebral arteries, homogenates from cerebral arteries were used at a final protein concentration of 1.5 mg/mL. Final concentrations of the GTP substrate used in both basal and activated preparations ranged from 0.02 to 5.0 mM.

Western blot analysis of sGC abundance. Arteries designated for Western blot analysis were frozen in liquid nitrogen, pulverized, then incubated on ice for 30 min at pH 7.4 in a lysis buffer containing 150 mM NaCl, 50 mM Tris, 10 mM EDTA, 0.1% Tween-20, 0.1% β-mercaptoethanol, 1 mM benzamide, 1 mM phenylmethylsulfonyl fluoride, 7.3 μM pepstatin A, 5 μM leupeptin, and 0.7 μM aprotinin. Next, the samples were centrifuged at 10,000 $\times$ *g* for 10 min at 4°C, after which the supernatant fluids were loaded onto 8% polyacrylamide gels and resolved at 100 volts for 2 h. The resolved proteins were transferred onto nitrocellulose membranes over a 90-min period in Towbin buffer at room temperature with a constant current of 25 mA. The membranes were blocked overnight at 4°C in 5% dry milk, then exposed to the primary antibody (Cayman Laboratories, Ann Arbor, MI) at a 1:1000 titer for 2 h at room temperature. The Cayman antibody used was a polyclonal prepared from a synthetic peptide constructed from a highly conserved region of the α and β subunits. The membranes were then washed in 5% dry milk with 0.1% Tween-20 in TBS, and were exposed to the secondary antibody at a 1:3000 titer in the presence of 5% dry milk for 1 h at room temperature. The membranes then were rinsed and visualized by enhanced chemiluminescence (Immunostar, Amersham). The resulting films were scanned and analyzed with a Bio-Rad model GS-700 imaging densitometer.

To standardize measurements of protein abundance, each gel used to blot for sGC included at least three lanes loaded with varying amounts of a standard reference homogenate prepared from sheep kidney. All reference standards were prepared from a single starting homogenate, then were frozen in multiple aliquots, one of which was used with each gel. In addition, each sample homogenate was also loaded and analyzed at multiple protein concentrations on each gel, and the slope of the relation between OD and protein mass loaded was used to determine abundance relative to that of the kidney reference. The relations between OD and standard mass loaded per lane were analyzed for each gel to obtain a regression curve with which OD values obtained from artery homogenates could be converted into μg-equivalent standard protein. Using this approach, all measures of sGC abundance were expressed relative to the abundance in the kidney homogenates with units of μg-equivalent kidney protein per μg vascular protein loaded.

Cyclic nucleotide and protein determinations. Total protein concentrations were determined as previously described (5), using Coomassie brilliant blue protein dye (Bio-Rad, cat# 500-0006). Identically treated BSA served as the reference standard. For cGMP analysis, supernatant extracts, obtained after trichloroacetic acid precipitation and centrifugation, were washed with water-saturated diethyl ether a minimum of three times to remove the trichloroacetic acid. Any remaining ether was then allowed to evaporate, and aliquots of the aqueous phase were lyophilized and stored at 4°C until assayed. The cGMP content of each sample was determined with a commercially available RIA kit (RPA 525, Amersham, Piscataway, NJ), as previously described (4).

Statistical analysis. All values are given throughout the text as mean $\pm$ SEM. All reported values of N refer to the number of homogenate preparations used in activity or Western blot determinations. Unless otherwise indicated, statistical significance was taken at the $p < 0.05$ level. To compare rates of sGC activity, corresponding newborn and adult variances were compared with an F-ratio test. In cases in which newborn and adult variances were not significantly different, significant differences between means were calculated by Student's *t* test. When newborn and adult variances were significantly different, differences between means were calculated by Behren's-Fisher analysis with pooled variance. A two-way ANOVA with age and artery type as the factors was used to compare sGC abundances. For posthoc comparisons, Duncan's multiple range analysis was used.

RESULTS

Determination of the NO-heme yielding maximum activation. Multiple concentrations of NO-heme were used to activate sGC from two concentrations of semi-crude homogenates from adult carotid (6.0 and 0.6 mg/mL Tris buffer) and cerebral (3.0 and 0.3 mg/mL Tris buffer) arteries. NO-heme and 1 mM GTP were added simultaneously to each reaction tube, and cGMP accumulation was measured between 5 and 15 min of incubation. Irrespective of absolute protein concentration or artery type, peak sGC activation occurred at $\approx$2 μM NO-heme (Fig. 1). Our optimum NO-heme concentrations were far less

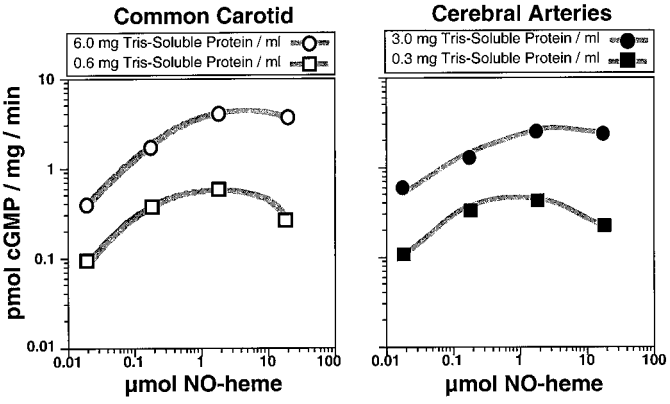

Figure 1. Soluble guanylate cyclase activation with multiple concentrations of NO-heme in homogenates of adult common carotid and cerebral arteries. In the presence of 1 mM GTP, multiple concentrations of NO-heme were used to activate sGC at two protein concentrations from adult carotid *(left panel)* and adult cerebral *(right panel)* semi-crude homogenates. Accumulation of cGMP was measured between 5 and 15 min of incubation. Maximum sGC activation occurred at approximately 2.0 μM NO-heme, independent of protein concentration or artery type.

than those reported to inhibit sGC activity (14).

Validation of the steady state assumption in semi-crude homogenates. Consistent with the Briggs-Haldane steady state assumption (15), activated sGC activity in semi-crude homogenates from both artery types displayed a linear relationship between enzyme concentration and initial velocity with an r^2 value of 0.99 (data not shown). Because less than 1% of substrate was converted to product over 10 min of incubation under activated conditions, the free substrate concentration could be regarded as remaining invariant and saturating. Accordingly, simple first-order Michaelis-Menten kinetics were used to calculate the K_m and V_{max} for sGC from semi-crude homogenates.

Rates of sGC activity. Exposure of newborn and adult carotid artery homogenates to varying concentrations of GTP with or without NO-heme increased accumulation of cGMP (Fig. 2). Even though activity was much lower in the absence than in the presence of NO-heme, basal activity was still progressively increased by exposure to increasing concentrations of GTP. Activation with NO-heme increased carotid artery activity by a maximum of 9-fold in newborn preparations, but only 5-fold in adult preparations. Activation with NO-heme also produced similar increases in newborn (8-fold) and adult (4-fold) cerebral arteries, although the absolute magnitudes of both basal and activated sGC activities were markedly less in cerebral than in carotid arteries.

Figure 3 summarizes the K_m and V_{max} results for each experimental group calculated by using Lineweaver-Burk double-reciprocal plots of the initial rate of cGMP formation as a function of GTP concentration. In carotid arteries, the apparent V_{max} for basal sGC activity (in pmol cGMP/mg/min) was significantly greater in newborn (1.22 ± 0.13, $n = 7$) than adult (0.79 ± 0.07, $n = 6$) preparations ($p < 0.03$). Similarly, the apparent V_{max} for activated sGC was significantly greater in newborn (11.1 ± 0.5, $n = 7$) than adult (3.6 ± 0.2, $n = 6$) carotid arteries ($p < 0.01$). No significant age differences were observed in the apparent K_m for carotid sGC under basal (newborn: 0.51 ± 0.06 and adult: 0.46 ± 0.07 mM GTP) or activated (newborn: 0.08 ± 0.01 and adult: 0.08 ± 0.01 mM GTP) conditions. In all groups, activation with NO-heme reduced K_m values at least 3-fold below basal values ($p < 0.01$).

In cerebral arteries, the apparent V_{max} (in pmol cGMP/mg/min) for basal sGC activity did not differ significantly between newborn (0.41 ± 0.08, $n = 9$) and adult (0.36 ± 0.05, $n = 14$) preparations. However, after activation, the apparent V_{max} values were significantly greater in newborn (3.10 ± 0.31, $n =$

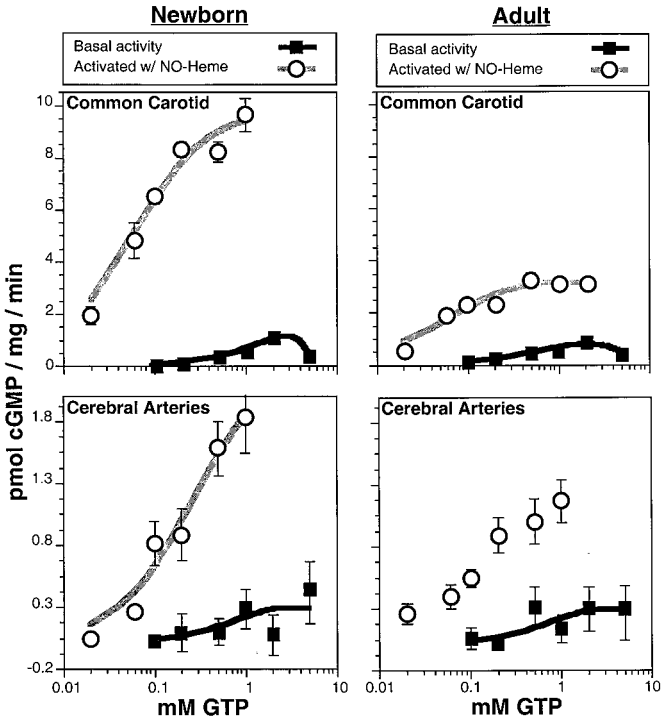

Figure 2. Effects of maturation on substrate-velocity relations in common carotid and cerebral arteries. Rates of basal *(squares)* and activated *(circles)* sGC activity are shown for homogenates from newborn *(left panels)* and adult *(right panels)* arteries, with common carotids in the *upper panels* and cerebral arteries in the *lower panels*. Basal and activated rates were measured at varying concentrations of GTP in the presence or absence of NO-heme, respectively. Rates are expressed in pmol cGMP/mg protein/min, calculated between 5 and 15 min of incubation with GTP. Values are given as means ± SEM.

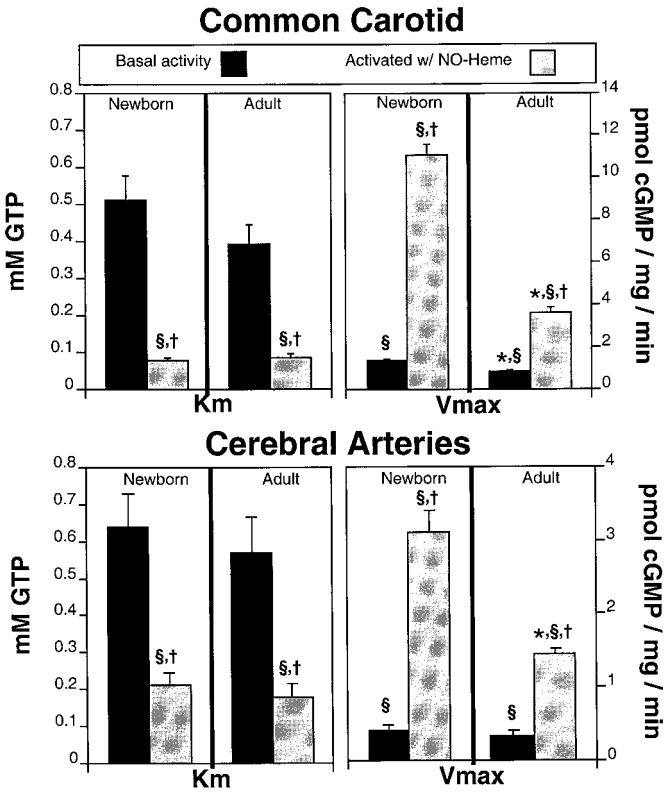

Figure 3. Effects of maturation on guanylate cyclase K_m and V_{max} values in common carotid and cerebral arteries. K_m and V_{max} values calculated from double-reciprocal plots of cGMP formation as a function of GTP concentration are given here as means ± SEM. At the top of each column, * indicates significant effects of age within an artery type, § indicates significant effects between corresponding artery types, and † indicates significant effects of activation for each artery type. Activation significantly reduced K_m values in all experimental groups, and maturation had no significant effect on activated or basal K_m within a given artery type. Between artery types, the K_m for activated sGC was significantly greater in cerebral compared with carotid arteries. Maturation significantly depressed activated V_{max} in carotid and cerebral arteries, but had a significant effect on basal V_{max} values only in carotid arteries.

9) than in adult (1.45 ± 0.08, $n = 14$) arteries ($p < 0.01$). No significant age differences were observed in the apparent K_m values (in mM GTP) under either basal (newborn: 0.64 ± 0.09; adult: 0.42 ± 0.08) or activated (newborn: 0.21 ± 0.04; adult: 0.19 ± 0.04) conditions.

When statistical comparisons were made between artery types, V_{max} values for basal and activated sGC were significantly greater in common carotid than in cerebral arteries ($p < 0.01$). Whereas basal K_m did not differ significantly between artery types, activated K_m varied significantly with artery type and was 2-fold greater in cerebral than in carotid arteries ($p < 0.05$).

Western blot analysis of sGC abundance. A representative blot comparing relative sGC abundances is shown in Figure 4A. Values of sGC abundance for all experimental groups were expressed relative to a single external pool of reference samples from homogenized adult sheep kidney, in units of μg-equivalent kidney protein per μg total vascular protein. In carotid arteries, sGC abundance was significantly greater in newborns (0.38 ± 0.04, $n = 9$) than adults (0.25 ± 0.05, $n = 7$) ($p < 0.05$) (Fig. 4B). In cerebral arteries, sGC abundance was also significantly greater in newborns (0.37 ± 0.06, $n = 8$) than adults (0.17 ± 0.03, $n = 10$) ($p < 0.05$).

DISCUSSION

Since its discovery more than two decades ago, soluble guanylate cyclase has been the topic of numerous studies that have revealed much about the physical characteristics and mechanisms of activation of this enzyme. Despite this progress, knowledge of the changing role of vascular sGC during maturation and development is limited to only a few indirect observations (16, 17). This lack of attention is surprising given the potential pathophysiologic importance of age-related differences in cGMPs ability to promote vasodilation, particularly in light of observations that basal levels of cGMP fall during maturation in sheep (4) due to mechanisms other than changes in GMP degradation (5). Because of these considerations, the present studies addressed the hypothesis that basal cGMP levels are elevated in newborn compared with adult arteries as a result of enhanced cGMP synthesis.

The main findings of the present study are that maturation decreased both maximum sGC activity and relative abundance in extracranial and cerebral arteries, without effect on apparent K_m values. The kinetic values we obtained were similar to those reported by others for both basal and activated V_{max} in newborn and adult semi-crude homogenates (18), and for basal and activated K_m in purified preparations (19, 20). Equally important, the finding that maximal rates of sGC activity in artery homogenates were approximately 2-fold greater in newborn than adult arteries, agrees well with our previous findings based on measurements of maximal rates of cGMP accumulation in intact ovine arteries (4, 5). Unique to the present study were the significant differences observed between artery types in apparent K_m (cerebral > carotid) and tissue activity (carotid > cerebral) for sGC.

Despite the limitations inherent in performing kinetic analyses on partially purified enzyme preparations, we specifically chose to perform our kinetic studies in semi-crude artery homogenates, to preserve as many of the factors that are unique to the tissue's maturational state as possible, yet enable a direct assessment of the effects of maturation on vascular sGC. This approach avoided the prohibitively large amounts of tissue required for enzyme purification from both large extracranial and very small cerebral arteries. It also maintained the relative tissue concentrations of sGC in both age groups and artery types. Although this approach may have introduced artifacts owing to the presence of factors not normally accessible to compartmentalized sGC in intact cells, such effects were probably modest, given that maturation attenuated rates of cGMP synthesis in the present homogenates to an extent similar to that observed in intact arteries (4, 5).

Consistent with previous studies of sGC (21), the present data reinforce the view that reconstitution with heme is necessary for maximal enzyme activation. As shown in Figure 1, optimal concentrations of NO-heme were determined for each experimental group and used throughout the study. This approach eliminated the possibility that differences in maximal sGC activity could be attributed to differences in the extent of activation. Thus, our finding that maturation significantly depressed activated V_{max} in both carotid and cerebral arteries strongly suggests that enzyme concentration and/or enzyme-specific activity is greater in newborn than adult arteries. The Western blot results (Fig. 4) further indicate that increased sGC abundance contributes significantly to the observed age-related variation in tissue sGC activity.

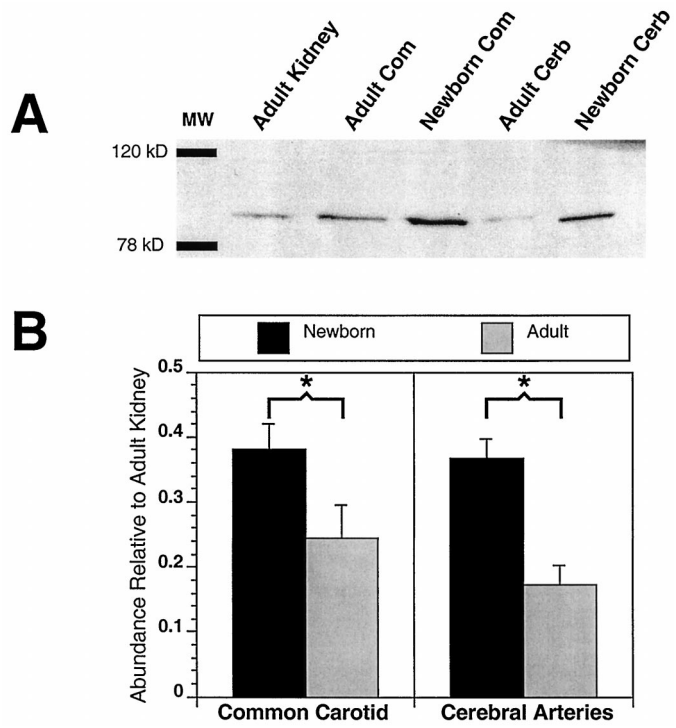

Figure 4. Western blot analysis of sGC abundance. *A*, the *upper panel* depicts a representative blot comparing relative sGC abundances in each of the four experimental groups. Identical amounts of total protein (50 μg) were loaded on each lane. *B*, the *lower panel* summarizes values of sGC abundance expressed relative to a single external pool of reference samples from homogenized adult sheep kidney in units of μg-equivalent kidney protein per μg total vascular protein. Values are given as means ± SEM. *Asterisks* indicate significant effects of age within an artery type ($p < 0.05$).

The finding that activated K_m values did not change with age (Fig. 3) further supports the interpretation that age-related changes in abundance contributed to the observed differences in sGC activity. Typically, changes in an enzyme's tissue activity based solely on changes in protein concentration do not affect an enzyme's apparent K_m (15). In other studies, sGC expression and activity have also been shown to decrease with age in pulmonary smooth muscle and lung fibroblast cells (18, 22). Maturation also modulates abundance of the membrane-bound form of guanylate cyclase; the increased susceptibility of newborns to enterotoxin-mediated diarrheal disease has been linked to an increased concentration of the membrane-bound form of guanylate cyclase in the intestine (23–25). Together, these observation support our interpretation that maturation decreases sGC abundance and, thereby, activity in ovine carotid and cerebral arteries.

In addition to age-related changes in sGC abundance, the present data do not preclude the possibility that maturation involves differences in enzyme-specific activity. One mechanism that can shift specific activity for most enzymes is covalent posttranslational modification, such as that produced by changes in enzyme phosphorylation state. Protein kinase C, for example, can phosphorylate sGC in some intact cell preparations (26–28). Consistent with the apparent effects of maturation on sGC seen in the present study, PKC-mediated phosphorylation of sGC stably increases basal sGC activity without significantly altering basal K_m. Given that PKC activity has been shown to be greater in newborns than adults (29), it is possible that PKC-mediated phosphorylation of sGC acts as a mechanism to modulate the catalytic efficiency of sGC as a function of age. If and to what extent PKC-mediated phosphorylation of sGC contributes to maturational changes in sGC activity remains a promising topic for future investigation.

Aside from posttranslational changes, simultaneous differences in both substrate affinity and maximum velocity can also be brought about by differential expression of various enzyme isoforms. Age-dependent differential expression of at least two different sGC isoforms has been demonstrated during early development in the human and rat brain (16, 30). Tissue-specific variations in sGC isozyme expression have also been demonstrated (31, 32), and in the vasculature at least two specific isoforms of sGC have been identified (33, 34). Possible kinetic differences among these different developmental and tissue-specific isoforms of sGC have not been fully investigated, but the differences in K_m and V_{max} for sGC observed between large extracranial and cerebral arteries in the present studies support the possibility of artery-specific isozyme expression in vascular smooth muscle. For example, the present differences in activated K_m observed between cerebral and common carotid arteries for both age groups (Fig. 3) could easily be explained by the presence of an isoform in the carotid arteries that has a higher substrate affinity than the isoform predominant in the cerebral arteries.

Overall, the present studies support the hypothesis that maximal rates of cGMP synthesis are greater in neonatal than adult carotid and cerebral arteries. These differences in tissue activity appear attributable, at least in part, to corresponding age-related differences in the relative abundance of sGC. It remains possible that tissue-specific, but not age-specific, differences in enzyme isoform and/or in posttranslational modification are involved, particularly in light of the significant differences in apparent activated K_m values observed between different types of arteries. Superimposed on these basic kinetic differences are further possible differences in substrate and/or cofactor availability in the intact tissues, which could further influence artery-to-artery differences in sGC activity and the overall capacity for vasodilation. Finally, documented age-related differences in cGMP-specific phosphodiesterase activity (5) could also influence the balance between rates of cGMP synthesis and degradation in intact tissue and, thereby, modulate vasoreactivity to activators of sGC. Given that elevated cGMP levels in intact arteries may contribute to the lower hydraulic vascular resistance and greater cerebrovascular vulnerability characteristic of neonates, further studies of these age- and tissue-specific differences in sGC activity show promise for future investigation.

Acknowledgments. The authors thank Dr. John Farley and Dr. Peter Yuen for their valuable suggestions and comments regarding the work described in this manuscript.

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