Metabolism of cAMP to adenosine: role in vasodilation of rat pial artery in response to hypotension

KI WHAN HONG, HWA KYOUNG SHIN, HYUNG HWAN KIM,
JAE MOON CHOI, BYUNG YONG RHIM, AND WON SUK LEE
Department of Pharmacology, College of Medicine, Pusan National University,
Pusan 602-739; and Center for Biofunctional Molecules, Pohang University
of Science and Technology, Pohang 790-600, Korea

Hong, Ki Whan, Hwa Kyoung Shin, Hyung Hwan Kim, Jae Moon Choi, Byung Yong Rhim, and Won Suk Lee. Metabolism of cAMP to adenosine: role in vasodilation of rat pial artery in response to hypotension. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H376–H382, 1999.—The purpose of this experiment was to examine whether the cAMP-adenosine pathway is implicated in the autoregulatory vasodilation in response to hypotension. Suffusion with cAMP (1–100 µmol/l) or adenosine (0.01–10 µmol/l) caused a sustained vasodilation of the resting pial arteries in a concentration-dependent manner. In contrast, N8,2′-O-dibutyryl-cAMP and 8-bromo-cAMP exerted a weak dilation at high concentration (100 µmol/l). The vasodilation to cAMP (1–100 µmol/l), adenosine (0.01–10 µmol/l), and hypotension was significantly reduced by pretreatment with 3,7-dimethyl-1-propargylxanthine (1 µmol/l), an A2 receptor antagonist, as well as 3-isobutyl-1-methylxanthine (3 µmol/l), an inhibitor of endo- and ectophosphodiesterase, 1,3-dipropyl-8-p-sulfophenylxanthine (100 µmol/l), an inhibitor of ecto-5′-phosphodiesterase, or α,β-methylene-adenosine 5′-diphosphate (100 µmol/l), an inhibitor of ecto-5′-nucleotidase. However, 8-cyclopentyltheophylline (1 µmol/l), an A1 antagonist, did not elicit a similar response. The increased release of adenosine when the cortical surface was suffused with cAMP (100 µmol/l) was significantly reduced by 3-isobutyl-1-methylxanthine, 1,3-dipropyl-8-p-sulfophenylxanthine, and α,β-methylene-adenosine 5′-diphosphate (each 100 µmol/l). These results indicate that the cAMP-adenosine pathway as a viable metabolic mechanism is implicated in the production of adenosine in the rat pial artery and contributes to the regulation of vasodilation in response to hypotension.

A1 and A2 receptors; cerebral autoregulation; calcitonin gene-related peptide

THE AUTOREGULATION of cerebral blood flow has been explained in connection with metabolic and myogenic mechanisms involved in cerebral microcirculation (3, 17). Edwards et al. (5) suggested that calcitonin gene-related peptide (CGRP) stimulated adenyl cyclase activity in the small parenchymal cerebral arterioles in vitro. Recently, the autoregulatory vasodilation of rat pial arteries in response to hypotension has been demonstrated to be mediated by CGRP, which is released from the perivascular sensory fibers (8); furthermore, the CGRP-induced vasodilation is mediated by the formation of cAMP via activation of CGRP1 receptors (9).

The relationship between adenosine concentration and oxygen supply was documented in rat brain, in that adenosine is a mediator of the metabolic regulation of cerebral blood flow during normoxia and hypoxia (1, 19). A number of studies have reported that adenosine serves an important role in vascular biology through multiple actions that are mediated primarily by adenosine A1 and A2 receptors. Ngai and Winn (16) reported that the dilator response of intracerebral arterioles to adenosine was predominantly mediated by the A2 receptors.

On the other hand, Mi and Jackson (13) proposed a metabolic cAMP-adenosine pathway, in that perfusion with cAMP significantly increased AMP and adenosine. Reportedly, AMP is transported to the extracellular space when it is formed intracellularly (2) and then converted to AMP and, hence, to adenosine by the enzymes endo- and ectophosphodiesterase (PDE) and endo- and ecto-5′-nucleotidase, respectively (13). However, whether the cAMP-adenosine metabolic pathway is implicated in the cerebral vasodilation is uncertain.

The purpose of the present study was to provide functional evidence in support of the working hypothesis that CGRP receptor-coupled cAMP production may be implicated in the autoregulatory vasodilation in response to acute hypotension. Thus experiments were designed to evaluate whether adenosine was formed by means of the cAMP-adenosine pathway, which would contribute to the autoregulatory vasodilation of the pial microvessels in response to hypotension. For this purpose, we first determined the vasodilating effects of cAMP and adenosine when the microvessels were pretreated with the A2-receptor antagonist 8-cyclopentyltheophylline (CPT) and the A2 receptor antagonist 3,7-dimethyl-1-propargylxanthine (DMPX). Second, we estimated the effects of the inhibitors of PDE and 5′-nucleotidase on the vasodilation induced by cAMP or hypotension. Thereafter, we measured the releases of AMP, adenosine, and inosine when the cortical surface was suffused with artificial cerebrospinal fluid (CSF) containing cAMP in the absence and presence of enzyme inhibitors.

METHODS

Preparation of animals. Male Sprague-Dawley rats (250–300 g) were anesthetized with urethan (1 g/kg ip) and placed on a heating pad to maintain a constant body (rectal) temperature (37 ± 0.5°C). After a tracheostomy, the trachea was cannulated, and the rat was mechanically ventilated with...
room air by a respirator (model 683, Harvard) after immobilization with 5 mg/kg gallamine triethiodide. Catheters were placed in the carotid artery for measurement of systemic arterial blood pressure (Statham P23 D pressure transducer) and in the femoral artery for sampling of arterial blood. Arterial blood was collected before and after installation of a cranial window for blood gas and pH determination (STAP Profiles, Nova Biomedicals). The mean arterial blood gases and pH were within normal limits as follows: pH 7.39 ± 0.02, arterial Pco2 34.7 ± 1.8 mmHg, arterial Po2 95.0 ± 1.2 mmHg. Rectal temperature was kept constant with a heating pad.

Measurement of vessel diameter. Pial microvessels were visualized through an implanted closed cranial window (8). Briefly, the head was fixed in the prone position with a stereotaxic apparatus (Stoelting, Wood Dale, IL), and a square (5 × 5 mm²) craniotomy was made over the right parietal cortex. The dura was carefully resected. Pial precapillary microvessels [64.0 ± 2.5 µm (large) and 31.0 ± 2.3 µm (small) arterioles] were visualized through the implanted cranial window, where prewarmed artificial CSF (37°C) saturated with 95% O2-5% CO2 was constantly perfused at 0.3 ml/min. Cerebral microvessels were allowed to equilibrate for 60 min after installation of the cranial window. The image of the pial vessels was captured with a CCD video camera (model VDC 3900, Sanyo) through a stereomicroscope (model SMZ-2T, Nikon) and fed to a television monitor for direct observation, and the caliper was measured using a width analyzer (model C3161, Hamamatou Photonics). The composition (in mmol/l) of the artificial CSF was as follows: 132 NaCl, 2.9 KCl, 1.4 MgCl2, 24.6 NaHCO3, 1.2 CaCl2, 6.7 urea, and 3.7 D-glucose (pH 7.4). The intracranial pressure was maintained within the normal range (105.5 ± 2.4 mmHg, n = 47). The basal pial arterial diameters were 64.0 ± 2.5 and 31.0 ± 2.3 µm for the larger and smaller arterioles, respectively; these values remained constant throughout the experiment, unless there was bleeding or an infusion of blood.

Protocol of in vivo experiments. To examine the vasodilating responses of the resting pial arteries to cAMP and adenosine, the cortical surface was suffused with artificial CSF containing cAMP (1–100 µmol/l) or adenosine (0.01–10 µmol/l). The inhibitory effects of CPT (1 µmol/l) and DMPX (1 µmol/l) were observed on the cAMP- and adenosine-induced vasodilation, respectively. The inhibitors were applied beginning 30 min before suffusion of cAMP or adenosine. Furthermore, we examined the normal autoregulatory vasodilator function of the pial artery to a stepwise hypotension and its reverse to infusion of blood from the reservoir when the cortical surface was suffused with artificial CSF containing vehicle. Thereafter, we retested the autoregulatory responses when the vessels were pretreated with CPT or DMPX.

Analysis of adenine nucleoside. AMP, adenosine, and inosine levels in the suffusate were analyzed with an HPLC system. Briefly, in the first experimental series, the cortical surface was suffused with artificial CSF while vasodilation was monitored. After the suffusate exiting the cranial window for 2 min was collected as a basal sample, cAMP was added to the artificial CSF to a final concentration of 100 µmol/l and the suffusate was collected in small polycarbonate tubes at 1, 3, 5, 10, 15, 20, and 30 min of suffusion. A second experimental series was conducted in the same fashion, with addition of 3-isobutyl-1-methylxanthine (IBMX), α,β-methyl-adenosine-5′-diphosphate (AMP-CP), or 1,3-dipropyl-8-p-sulfophenylxanthine (DPSPX), inhibitors of enzymatic metabolism, 20 min before application of cAMP.

The suffusate samples were placed immediately on ice, and 20 µl of each sample were injected into an HPLC system consisting of a model 510 HPLC pump, a model 680 gradient programmer, an automated gradient controller, a series 440 absorbance detector, and a 2.0 × 300-mm µBondapak C18 column with 10-µm particle size (all from Waters). The procedure was conducted as described by Mi and Jackson (13) with mobile phase A of 0.1 mol/l KH2PO4 (pH 6.1) and mobile phase B of 80% 0.01 mol/l KH2PO4 (pH 3.5) and 20% methanol. The eluent was determined at a wavelength of 254 nm, and the levels of AMP, adenosine, and inosine were measured as the area under the chromatographic peak. The detection limit for AMP, adenosine, and inosine was ~2 pmol injected onto the column, and the amount of each substance in the samples is presented as micromoles per liter.

RESULTS

Under control conditions, mean arterial blood pressure was within the normal range (105.5 ± 2.4 mmHg, n = 47). The basal pial arterial diameters were 64.0 ± 2.5 and 31.0 ± 2.3 µm for the larger and smaller arterioles, respectively; these values remained constant throughout the experiment, unless there was bleeding or an infusion of blood.

Differences in the vasodilating effect of cAMP from cAMP analogs. The vasodilating effect of cAMP was compared with DBcAMP and 8-BrcAMP (Fig. 1). DBcAMP and 8-BrcAMP exerted weak vasodilation with relatively low maximal dilation at 100 µmol/l. In contrast, cAMP showed a sustained vasodilation in a concentration-dependent manner. The agonist concentrations required to cause 25% of observed maximum dilations (EC50) in the larger and smaller arterioles for cAMP-induced vasodilation were 43.0 ± 9.1% of the basal diameters, respectively. These findings were not significantly different between the larger and smaller arterioles.

Effects of adenosine receptor antagonists. Suffusion of the cortical surface with artificial CSF containing cAMP (1, 10, or 100 µmol/l) or adenosine (0.01, 0.1, 1, or 10 µmol/l) caused a sustained concentration-dependent vasodilation (Fig. 2).

Suffusion with artificial CSF containing CPT (1 µmol/l), a selective adenosine A1-receptor antagonist,
resulted in little alteration in adenosine- and cAMP-induced vasodilation (Fig. 2, Table 1). Suffusion of the cortical surface with 1 µmol/l CPT from 30 min before the experiment did not affect the basal pial arterial diameter.

The EC25 of cAMP-induced vasodilation was 6.6 ± 2.5 µmol/l in the smaller arterioles. Suffusion with 1 µmol/l DMPX markedly inhibited the vasodilation evoked by cAMP, with significantly increased EC25 values (65.6 ± 13.9 µmol/l with a dose ratio of 9.9) and decreased maximal dilation (Fig. 3, Table 1). Suffusion with artificial CSF containing 1 µmol/l DMPX from 30 min before the experiment did not affect the basal pial arterial diameter.

Table 1. Comparisons of EC25 and dose ratio in smaller arterioles after pretreatment with inhibitors

<table>
<thead>
<tr>
<th>Drugs</th>
<th>n</th>
<th>EC25, µmol/l</th>
<th>Dose Ratio</th>
<th>Maximum Dilation, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>cAMP</td>
<td>4</td>
<td>9.7 ± 3.9</td>
<td></td>
<td>77.2 ± 9.4</td>
</tr>
<tr>
<td>CPT (1 µmol/l) + cAMP</td>
<td>4</td>
<td>8.8 ± 4.3</td>
<td>0.9</td>
<td>77.6 ± 4.3</td>
</tr>
<tr>
<td>cAMP</td>
<td>6</td>
<td>6.6 ± 2.5</td>
<td></td>
<td>76.6 ± 8.7</td>
</tr>
<tr>
<td>DMPX (1 µmol/l) + cAMP</td>
<td>6</td>
<td>65.6 ± 13.9*</td>
<td>9.9</td>
<td>35.0 ± 5.2†</td>
</tr>
<tr>
<td>Adenosine</td>
<td>4</td>
<td>0.2 ± 0.1</td>
<td></td>
<td>77.5 ± 8.1</td>
</tr>
<tr>
<td>CPT (1 µmol/l) + adenosine</td>
<td>4</td>
<td>0.8 ± 0.3</td>
<td>3.8</td>
<td>76.0 ± 4.1</td>
</tr>
<tr>
<td>Adenosine</td>
<td>6</td>
<td>0.8 ± 0.2</td>
<td></td>
<td>80.6 ± 6.3</td>
</tr>
<tr>
<td>DMPX (1 µmol/l) + adenosine</td>
<td>6</td>
<td>11.5 ± 3.7*</td>
<td>13.9</td>
<td>49.0 ± 4.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of experiments. Potency of cAMP and adenosine was expressed as agonist concentration required to cause 25% of observed maximum dilations (EC25), since some of concentration-response curves for these agents did not plateau. Each EC25 was averaged from values calculated from individual concentration-response curves. Maximum effects were calculated as percentage of control diameter. CPT, 8-cyclopentyltheophylline; DMPX, 3,7-dimethyl-1-propargylxanthine. *P < 0.05; †P < 0.01 vs. corresponding control.
before the experiment had little effect on the basal diameter.

Likewise, the vasodilation evoked by adenosine was significantly suppressed by 1 µmol/l DMPX, with significantly increased EC25 values (dose ratio 13.9) and decreased maximal diameter. There was little difference between the two groups in the maximally dilating responses to cAMP and adenosine when the arterioles were pretreated with DMPX (Table 1).

Effects of enzyme inhibitors on cAMP-induced vasodilation. We observed the vasodilating effect of cAMP when the arterioles were pretreated with IBMX (3 µmol/l), an inhibitor of endo- and ecto-PDE, DPSPX (100 µmol/l), an inhibitor of ecto-5′-PDE, and AMP-CP (100 µmol/l), an inhibitor of ecto-5′-nucleotidase (Fig. 4). IBMX at >10 µmol/l caused vasodilation. Thus in the present study we employed IBMX at 3 µmol/l to inhibit PDE. Pretreatment with IBMX, DPSPX, or AMP-CP exerted a potent inhibitory effect on the cAMP-induced vasodilation of the pial arterioles, suggesting that endo- and ecto-PDE, and also ecto-5′-nucleotidase, are involved in the cAMP hydrolysis turnover.

Alterations in autoregulatory responses. The alteration in autoregulatory vasodilation in response to a stepwise hypotension was observed after treatment with the adenosine A1- and A2-receptor antagonists CPT and DMPX (Figs. 5 and 6). In the vehicle group the pial arterial diameters increased during stepwise hypotension and quickly decreased on recovery of the arterial pressure. Changes in pial arterial diameter were plotted as a function of changes in mean arterial blood pressure (MABP). All slopes of regression lines showed statistical significance. No difference was observed between vehicle- and CPT-treated groups.

![Fig. 4. Percent changes in diameter of smaller pial arterioles in response to cAMP (1–100 µmol/l) in absence and presence of 3-isobutyl-1-methylxanthine (IBMX, 3 µmol/l, n = 5), 1,3-dipropyl-8-sulfophenylxanthine (DPSPX, 100 µmol/l, n = 4), and α,β-methylene-adenosine 5′-diphosphate (AMP-CP, 100 µmol/l, n = 4). Cortical surface was suffused with artificial CSF containing each inhibitor 30 min before and throughout experiment. Values are means ± SE.](http://ajpheart.physiology.org/)

![Fig. 5. Autoregulatory vasodilation and vasoconstriction in response to stepwise hypotension after pretreatment with CPT (1 µmol/l) in comparison with vehicle. Changes in pial arterial diameter were plotted as a function of changes in mean arterial blood pressure (MABP). All slopes of regression lines showed statistical significance. No difference was observed between vehicle- and CPT-treated groups.](http://ajpheart.physiology.org/)

![Fig. 6. Autoregulatory vasodilation and vasoconstriction without and with DMPX (1 µmol/l). After autoregulation was confirmed, cortical surface was suffused with artificial CSF containing DMPX (1 µmol/l, n = 7) 30 min before and throughout stepwise hypotension. DMPX significantly attenuated autoregulatory vasodilation and vasoconstriction. Significant difference (P < 0.05) was identified between blood pressure-diameter relationship of vehicle- and DMPX-treated groups by 2-way repeated-measures ANOVA.](http://ajpheart.physiology.org/)
μmol/l DMPX (Table 2). Furthermore, with IBMX (3 μmol/l) and AMP-CP (100 μmol/l) pretreatment, the slopes of vasodilator and vasoconstrictor responses to hypotension and to reverse of blood pressure were significantly less steep (Fig. 7, Table 2).

CAMP metabolism in the suffusate. When the artificial CSF containing 100 μmol/l CAMP was suffused over the cortical surface, the levels of AMP, adenosine, and inosine increased significantly with time: 7.7 ± 0.5, 1.1 ± 0.1, and 0.7 ± 0.4 μmol/l, respectively, at 5 min (Fig. 8). In the group not treated with CAMP, these nucleosides were not detectable.

Otherwise, in arterioles treated with IBMX, DPSPX, or AMP-CP, the production of AMP, adenosine, and inosine was measured 5 min after suffusion of 100 μmol/l CAMP. IBMX (100 μmol/l) significantly inhibited the formation of AMP (1.2 ± 0.5 μmol/l, n = 5, P < 0.01) and adenosine (0.5 ± 0.2 μmol/l, n = 5, P < 0.05). DPSPX (100 μmol/l) also inhibited CAMP metabolism to AMP (2.6 ± 0.3 μmol/l, n = 4, P < 0.01), with no detectable adenosine. However, when arterioles were treated with IBMX and DPSPX, there was little difference in inosine level from control. When the arteriole was pretreated with AMP-CP (100 μmol/l), an inhibitor of ecto-5’-nucleotidase, the release of adenosine and inosine was below the assay detection limits, whereas the AMP level was high (17.8 ± 4.8 μmol/l, P < 0.05; Fig. 9).

**DISCUSSION**

The major findings of this study are as follows: 1) CAMP, but not DBcAMP and 8-BrCAMP, showed a potent vasodilation; 2) vasodilation induced by adenosine or CAMP and vasodilation in response to hypotension were markedly attenuated when the cortical surface was suffused with artificial CSF containing DMPX, an A2-receptor antagonist; but not by CPT, an A1-receptor antagonist; 3) when the arteriole was pretreated with IBMX (3 μmol/l, an inhibitor of endo- and ecto-PDE), DPSPX (100 μmol/l, an inhibitor of ecto-5’-PDE), and AMP-CP (100 μmol/l, an inhibitor of ecto-5’-nucleotidase), the vasodilating effect of CAMP (1–100 μmol/l) was significantly decreased; 4) the autoregulatory vasodilation and release of AMP and adenosine from the suffusate containing CAMP were significantly decreased by IBMX, DPSPX, and AMP-CP, inhibitors of enzymatic metabolism of CAMP.

In the present study, DBcAMP and 8-BrCAMP showed weak vasodilation in comparison with CAMP, although their hydrolytic stability and membrane permeability were high in comparison to CAMP. Consistent with the report of Johnson et al. (11), suffusion of CAMP provided large amounts of metabolites, such as adenosine. Rosenblum (18) demonstrated the existence of an adenyl cyclase-CAMP system in mice for dilating cerebral arterioles, suggesting that the cerebral surface arterioles contain the enzyme system for producing the dilator. However, the CAMP analogs DBcAMP and 8-BrCAMP did not produce the metabolites. Nevertheless, the nonmetabolizable analogs of CAMP exerted vasodilating effects at 100 μmol/l. This fact may indicate an involvement of a mechanism other than adenosine-mediated cascade. Clarification requires further study.

Kalaria and Harik (12) demonstrated with specific tritiated ligands for A1 (cyclohexyladenosine) and A2 receptors (5’-N-ethylcarboxamide adenosine) that human pial vessels have specific high-affinity 5’-N-ethylcarboxamide adenosine binding to A1 receptors and few, if any, A2 receptors. In agreement with their results, our study showed that CPT, an A1 receptor antagonist, did not exert any inhibitory effect on the autoregulatory vasodilation in response to hypotension or on the CAMP-induced vasodilation.

Jackson (10) and Mi and Jackson (13) recently proposed the CAMP-adenosine pathway for adenosine pro-
production in vascular smooth muscle cells. This pathway begins with activation of adenyl cyclase. Within the cell, the cAMP produced is metabolized to AMP by the catalyzing enzyme cytosolic PDE, and AMP is metabolized to adenosine by cytosolic 5'-nucleotidase. Then the adenosine is transported to the extracellular space by way of facilitated transport (13). The egress of cAMP from metazoon cells has been demonstrated on stimulation of adenyl cyclase (2). Moreover, vascular smooth muscle cells have an abundant ecto-5'-nucleotidase, which efficiently metabolizes AMP to adenosine (6). The ecto-5'-nucleotidase is tethered to the extracellular face of the plasma membrane via a lipid-sugar linkage (14).

In the present in vivo study, there is no way to measure the intracellular adenosine concentration. Instead, in the HPLC study, AMP, adenosine, and inosine were produced by suffusion of cAMP (100 µmol/l) over the cortical surface. These metabolites were significantly increased with time, implying a viable metabolic cAMP-adenosine pathway in the extracellular space of the cortical surface. Other aforementioned reports, together with our findings that IBMX and DPSPX significantly inhibited the metabolism of cAMP to AMP and, hence, to adenosine, further support the speculation that the cAMP-adenosine pathway may play an important role in mediating vasodilation in response to hypotension. The AMP metabolism to adenosine was completely inhibited by AMP-CP, whereas AMP secretion remained unchanged. These findings were consistent with the results of Dubey et al. (4), who observed an inhibitory effect of cAMP-derived adenosine on vascular smooth muscle cell growth. Therefore, it can be inferred that if a relatively modest increase in cAMP production occurs in the pial artery, a significant amount of adenosine can be formed by the intracellular and extracellular enzyme reactions.

Recently, we reported in vivo evidence that the autoregulatory vasodilation of rat pial arterioles in response to a stepwise hypotension is mediated by CGRP, which is released from the perivascular sensory fibers (8), and that the CGRP-induced cerebral vasodilation is mediated by the formation of cAMP via activation of CGRP1 receptors (9). These results are consistent with the in vitro results of Edwards et al. (5), who showed that CGRP stimulates adenylyl cyclase in the cerebral arteries. Under the hypothesis that CGRP receptor-coupled CAMP production is implicated in the autoregulatory vasodilation in response to acute hypotension, it is speculated that adenosine may be formed by the means of the CAMP-adenosine pathway.

Winn et al. (20) described the role of adenosine in autoregulation of cerebral blood flow as a metabolic factor, in that brain adenosine concentrations are rapidly increased within 5 s of the onset of systemic hypotension and parallel the changes in pial vessel diameter. In line with this report, the autoregulatory vasodilator adjustment in response to hypotension was significantly suppressed by pretreatment with the A1-receptor antagonist DMPX and the inhibitors of cAMP metabolism IBMX, DPSPX, and AMP-CP. These findings provide strong evidence to support the hypothesis that a rapid increase in extracellular adenosine concentration is implicated in the autoregulatory vasodilation in response to hypotension.
that the extracellular cAMP-adenosine pathway may be implicated in the CGRP-induced neurogenic vasodilation of the pial arteries during acute hypotension. In the present study, the role of inosine, however, remained unexplained. Ngai et al. (15) reported that inosine alone does not affect pial vessel diameter, but it potentiated the response of pial arterioles to exogenous adenosine as an adenosine uptake inhibitor.

The extracellular concentration of adenosine in the rat brain measured by means of brain dialysis (22) and the freeze-blow technique (21) was reported to be 1–2 μM. In our study, vasodilation of the pial arterioles was elicited by 0.01–0.1 μmol/l adenosine under normoxic conditions. Thus the concentrations used in this study appear to be lower than those measured in the parenchymal tissue. However, an important issue is whether concentrations of cAMP in the interstitial fluid surrounding pial arterioles are high enough to support adenosine formation. Even though interstitial levels of cAMP on the cortical surface are determined by dialysis or the freeze-blow technique, it is not easy to equate the interstitial levels with the concentrations of cAMP achieved by formation and transport into the extracellular sites surrounding the arterioles.

The present in vivo study does not allow identification of the cell types involved in the metabolism of cAMP to adenosine. Ecto-5′-nucleotidase was reported to be present in abundance in cultured aortic endothelial cells and aortic smooth muscle cells (6, 7). Nevertheless, it is not clear what cell type contributes to the conversion of cAMP to adenosine. It seems impossible to judge how many fractions of cAMP endogenously are produced, actually gain to access to the enzyme system of the cAMP-adenosine pathway, and are efficiently converted to adenosine.

It is suggested that the cAMP-adenosine pathway is implicated in the production of adenosine in the rat pial artery. Thus it is concluded that the contribution of the cAMP-adenosine pathway may be important in the regulation of cerebral vasodilation in response to hypotension. Further study is required to identify the cascade of the CGRP receptor-coupled cAMP production and its metabolism to adenosine in the cerebral arterioles.

This work was supported in part by the Research Fund for Basic Medicine of the Korea Ministry of Education and by the Center for Biofunctional Molecules (Pohang, Korea) to K. W. Hong.

Address for reprint requests: K. W. Hong, Dept. of Pharmacology, College of Medicine, Pusan National University, 10 Ami-Dong 1-Ga, Seo-Gu, Pusan 602-739, Korea.

Received 29 May 1998; accepted in final form 23 September 1998.

REFERENCES