cAMP-dependent protein kinase is in an active state in rat small arteries possessing a myogenic tone

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Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1145–H1155, 1999.—The hypothesis that cAMP-dependent protein kinase (protein kinase A; PKA) is in an active state in small arteries possessing a myogenic tone was investigated in pressurized rat tail small arteries. At a pressure of 80 mmHg, these vessels constricted to 71.6 ± 1.0% (n = 32) of the diameter of the fully relaxed state. The PKA inhibitors Rp-8-(4-chlorophenylthio)-adenosine 3',5'-cyclic monophosphothioate (Rp-CPT-cAMPS) and N-(2-[3-(4-bromophenyl)-2-propenyl]aminoethyl)-5-isouquinolinesulfonamide HCl (H-89) CONSTRICTED these vessels dose dependently. For example, 300 µM Rp-CPT-cAMPS and 9 µM H-89 reduced vessel diameter by 11.0% (n = 6) and 14.3 ± 3.6% (n = 5), respectively. The cGMP-dependent protein kinase (protein kinase G; PKG) inhibitor Rp-8-bromo-β-phenyl-1,N'-etheno-guanosine 3',5'-cyclic monophosphothioate (Rp-8-Br-PET-cGMP) did not alter vessel diameter up to a concentration of 10 µM. Neither endothelium removal nor inhibition of neural transmission affected the action of Rp-CPT-cAMPS. The effect of 300 µM Rp-CPT-cAMPS was reduced by 82% after pretreatment of the vessel with 100 nM iberiotoxin, a blocker of calcium-activated potassium (KCa) channels. However, the effect of 300 µM Rp-CPT-cAMPS was not altered after pretreatment with 1 mM 4-aminoypyridine, a blocker of delayed rectifier potassium channels, or 10 µM ryanodine, a blocker of ryanodine receptor-generated calcium sparks. In inside-out patch-clamp experiments on cells isolated from rat tail small arteries, 10 U/ml of the catalytic subunit of PKA together with 100 µM MgATP increased KCa channel activity 30.1 ± 9.8-fold (n = 8). Additionally, neither inhibition of PKA or PKG nor moderate activation of PKA or PKG altered the vessel response to a pressure step from 80 to 120 mmHg. These results suggest that in rat tail small arteries possessing a myogenic tone 1) PKA is in an active state modulating the level of the myogenic tone, and 2) KCa channels mediate, at least partly, this effect of PKA.

Vascular smooth muscle; calcium-activated potassium channel; protein kinase A; myogenic response

THE MYOGENIC PROPERTIES of small arteries are functionally very important for the regulation of blood flow distribution into different vascular beds. A permanently applied constant transmural pressure produces a maintained constriction state of small arteries caused by the development of a myogenic tone, which represents the basal or spontaneous tone of a small vessel when no vasoactive substances are applied. This myogenic tone has been found in, for example, cerebral (7), coronary (13), skeletal (4), and mesenteric (32) small arteries.

Recently, considerable effort has been focused on the investigation of mechanisms determining the level of the myogenic tone of small arteries (for review, see Refs. 3, 14). It was found that the level of the myogenic tone depends on membrane potential (7), the activity of voltage-dependent calcium channels (35), phospholipase C and G proteins (22), and probably chloride channels (20). Furthermore, it was suggested that changes in inositol 1,4,5-trisphosphate production (18), the level of the intracellular calcium concentration (15) from extracellular (12) and sarcoplasmic reticulum (11, 33) calcium sources, the activity of protein kinase C (21), and the production of 20-hydroxyeicosatetraenoic acid via the cytochrome P-450 pathway (9) are involved. All previously mentioned investigations were concerned with mechanisms generating vessel constriction. Recently, the first mechanism was discovered that is involved in regulating the level of the myogenic tone by producing vasodilation. It was observed that calcium-activated potassium (KCa) channels are active in cerebral vessels (1, 11). This finding leads to the question of whether further vasodilating mechanisms participate in the regulation of the level of the myogenic tone. Thus there is a report demonstrating that stretch of cultured vascular smooth muscle cells can decrease adenyly cyclase activity (16), suggesting a role of the adenyly cyclase-cAMP-protein kinase A (cAMP-dependent protein kinase; PKA) cascade. Therefore, the aim of the present study was to test whether PKA is in an active state in rat tail small arteries possessing a myogenic tone. In the case of an active PKA, a further aim was to test whether KCa channels are mediating the effect of PKA, because these channels have been shown to be involved in the regulation of the level of the myogenic tone (1) and to be activated by PKA in vascular smooth muscle cells (27). These results appeared previously in abstract form (8, 28).

METHODS

Dissection and mounting of vessels. Male Wistar-Kyoto rats, 16–25 wk old, were killed by stunning and subsequent decapitation. The rat tail was removed and placed into a physiological salt solution (PSS) containing (in mM) 145 NaCl, 4.5 KCl, 1.2 NaH2PO4, 1.0 MgSO4, 0.1 CaCl2, 0.025 EDTA, and 5 HEPES, pH 7.4 at 4°C. Small tail arteries with an internal diameter of 250–320 µm in the fully relaxed state...
(first-order branches of the main ventral tail artery) were dissected free and cleaned of connective tissue. A 3- to 5-mm-long piece was then transferred to the experimental chamber containing an ice-cold experimental solution consisting of (in mM) 120 NaCl, 4.5 KCl, 1.2 NaH2PO4, 1.0 MgSO4, 1.6 CaCl2, 0.025 EDTA, 5.5 glucose, 26 NaHCO3, and 5 HEPES at pH 7.4. In the chamber, the vessel was fitted onto two fire-polished glass cannulas pulled from borosilicate glass and secured with 10-0 ophthalmological suture. The experimental chamber with the mounted vessel was placed onto the stage of an inverted microscope (Carl Zeiss Jena). The vessel was transilluminated with fiber optics. The microscope image was viewed with a charge-coupled device camera (Kappa Messtechnik), and the video signal of the horizontally oriented vessel was stored on a videocassette recorder (VCR) (Panasonic) and sent to an IBM-compatible AT 80486 personal computer equipped with a frame-grabber board (FG-30; HaSoTec). With the use of a ×10 objective and a ×3.2 projective, a vessel image resolution of 2 μm/pixel was obtained. The on-line video signal from the camera or the off-line signal from the VCR was digitized by the frame grabber, and a special data analysis program measured the internal diameter of the vessel in micrometers, after calibration with a microscope stage micrometer, and stored the diameter data in an ASCII file (S).

Experimental protocol. After the experimental chamber was mounted on the microscope stage, the chamber (2 ml vol) was continuously perfused with the experimental solution at a rate of 2 ml/min with a peristaltic pump (Petro-Gas). One cannula was connected to a reservoir, and the desired intravascular pressure was produced by elevating the reservoir to the appropriate height; the other cannula was connected to a pressure monitor (RFT). A thin, horizontally placed glass capillary tube filled with an air bubble was inserted into the pressure line coming from the reservoir. Controlling the movement of this air bubble after pressurizing the vessel allowed us to determine even very small leaks in the vessel during the experiment. Leaking vessels were discarded at any stage of the experiment to ensure complete nonflow conditions. The pressure was then increased to 80 mmHg, and the vessel was lengthened approximately two times, up to its in vivo length. This removed all buckling of the vessel appearing during pressurization. The vessel was allowed to stabilize for 15 min. Thereafter, the temperature was raised from room temperature to 37 ± 0.5°C with a heating pump (Gössner). Probes for temperature and pH were placed in the experimental chamber for permanent control of these two parameters. The pH was set to 7.4 ± 0.05. A PO2 of ~150 mmHg and a PCO2 of ~40 mmHg of the PSS in the experimental chamber were measured with a blood gas analyzer (AVL Medizintechnik).

At the end of the warm-up period, a myogenic tone (basal tone, spontaneous tone) developed. If the diameter after the establishment of the myogenic tone was <85% of the diameter in the fully relaxed state, which was obtained in a low-calcium solution, the vessel was used for the experiments. This criterion was used because vessels with a diameter in the range of 85–100% of the diameter of the fully relaxed state showed a decreased sensitivity to vasoconstrictor agonists, suggesting altered functional properties. With this criterion, ~10% of the vessels were discarded. The established myogenic tone was allowed to equilibrate for 60 min. Thereafter, vessel viability was tested by short applications of 1 μM acetylcholine to test endothelial cell function and 100 μM adenosine to test smooth muscle cell dilatory function. These drugs produced almost complete dilations of the vessel. In addition, 0.1 μM norepinephrine was applied to test smooth muscle cell contractile function resulting in an ~25% reduction of vessel diameter. All drugs were applied only to the adventitial side of the vessels. The drugs were injected with a syringe into the perfusion line with a syringe pump working at 0.01 times the rate of the peristaltic pump of the perfusion line. Thereafter, the desired experiments were performed and were always finished by a 20-min perfusion with a modified experimental (relaxation) solution from which calcium was omitted, and 1 mM EGTA was added to determine the fully relaxed diameter of the vessel at 80 mmHg.

In the experiments investigating the role of PKA, the specific PKA inhibitors N-(2-[3-(4-bromophenyl)-2-propenyl] amino-ethyl)-5-isouquinolinesulfonamide HCl (H-89) and Rp-8-(4-chlorophenylthio)-adenosine 3’,5’-cyclic monophosphothioate (Rp-CPT-cAMPS), the potent and specific PKA activator Sp-5,6-dichloro-1-β-ribofuranosylbenzimidazol-3’,-5’-cyclic monophosphothioate (Sp-5,6-DCI-cBIMPS), the potent and specific protein kinase G (cGMP-dependent protein kinase; PKG) inhibitor Rp-8-bromo-β-phenyl-1,N6-etheno-guanosine 3’,5’-cyclic monophosphothioate (Rp-8-Br-PET-cGMPS), and the potent and specific PKG activator 8-(4-chlorophenylthio)-guanosine 3’,5’-cyclic monophosphate (CPT-cGMP) were applied at different concentrations for 10–15 min to achieve a new diameter steady state. Rp-8-Br-PET-cGMPS is a recently developed PKG inhibitor with improved properties in that it is 1) potent and selective for PKG with an apparent inhibitory constant 300 times smaller for PKG than for PKA, 2) resistant to degradation by phosphodiesterase V, and 3) highly membrane permeant. In functional studies on rat tail arteries, the selectivity of Rp-8-Br-PET-cGMPS was demonstrated by its inhibitory action on 3-morpholinosydnonimine-induced relaxations, whereas forskolin-induced relaxations remained unchanged (2). The properties of H-89, Rp-CPT-cAMPS, Sp-5,6-DCI-cBIMPS, and CPT-cGMPS are well established (see discussion in Refs. 25, 29).

In the experiments investigating the role of the endothelium in the PKA-mediated modulation of the myogenic tone, the endothelium was removed by passing air through the lumen of the vessel for ~1 min. After this procedure 10 μM acetylcholine, which produced a complete relaxation of endothelium-intact vessels, did not affect vessel diameter. This observation was considered evidence for a successful functional removal of the endothelium. However, this procedure of endothelium removal preserved smooth muscle cell contractile and dilatory function. Thus 0.1 μM norepinephrine decreased vessel diameter by 26.6 ± 3.8% (n = 5) in endothelium-denuded vessels, which is not significantly different from the 26.8 ± 2.9% (n = 5) decrease in diameter in endothelium-intact vessels (P = 0.97). Furthermore, 100 μM adenosine increased vessel diameter to 84.3 ± 3.8% of the fully relaxed diameter (n = 3) in endothelium-denuded vessels and to 89.7 ± 6.1% of the fully relaxed diameter (n = 3) in endothelium-intact vessels, which are not significantly different (P = 0.50).

In the experiments investigating the role of nerve endings in the PKA-mediated modulation of the myogenic tone, the influence of nerve endings was blocked by the application of 10 μM phentolamine. This procedure was chosen because of the following observations. The influence of nerve endings on vessel diameter was tested with electrical field stimulation (EFS; frequency 20 Hz; pulse duration 0.1 ms; current density 40 mA/mm2) of vessels that had been denuded of the endothelium to prevent the release of endothelium-dependent substances by EFS. EFS decreased vessel diameter by 12.0 ± 2.2% (n = 6). However, EFS increased vessel diameter by 0.2 ± 3.9% (n = 6) after pretreatment of the vessel with 1 μM
tetrodotoxin and by 4.0 ± 4.1% (n = 6) after pretreatment of the vessel with 10 µM phentolamine; the latter two effects were not significantly different (P = 0.51). This finding shows that phentolamine-sensitive receptors on the smooth muscle cells mediate the action of transmitters released from nerve endings by EFS. Thus phentolamine was used to remove the influence of nerve endings on smooth muscle cells.

In the experiments investigating the involvement of potassium channels, either iberotoxin or 4-aminoypyridine was added for 5–15 min to achieve a new diameter steady state, and then, during the continued application of iberotoxin or 4-aminoypyridine, the vessels were additionally exposed to Rp-CPT-CAMPS for 10 min. In the experiments investigating the involvement of ryanodine receptor-generated calcium sparks, ryanodine was added for 5–8 min to get a new diameter steady state, and then, during the continued application of ryanodine, the vessels were additionally exposed to Rp-CPT-CAMPS for 10 min. Control diameter values were obtained by applying only the experimental solution for a period of time equal to the application time of the drugs and were expressed as percentage of the initial diameter, i.e., of the vessel diameter before application.

Cell isolation. After dissection, a piece of artery was placed into a microtube containing 1 ml of an enzyme solution and stored there overnight at 4°C. The enzyme solution contained 110 mM NaCl, 5 KCl, 0.16 mM CaCl2·2H2O, stored there overnight at 4°C. The enzyme solution contained 10 Na-HEPES, 0.16 CaCl2·2H2O, and 10 Na-HEPES. The enzyme solution contained in the whole cell experiments was 110 mM NaCl, 5 KCl, 0.16 CaCl2·2H2O, 10 Na-HEPES, 10 NaHCO3, 0.5 KH2PO4, 0.5 NaH2PO4, 10 glucose, 0.49 EDTA, and 10 taurine at pH 7.0, as well as 1.5 mg/ml papain, 1.6 mg/ml albumin, and 0.4 mg/ml DL-dithiothreitol. On the next day, the microtube with the vessel was incubated for 5–10 min at 37°C. Thereafter, the vessel was removed from the enzyme solution and stored in the low-calcium solution at 4°C. Single cells were released by trituration with a polyethylene pipette into the experimental bath solution. The experimental bath solution contained in the whole cell experiments (in mM) 135 NaCl, 6 KCl, 1 MgCl2, 1 CaCl2, 3 EGTA (purity 96%), and 10 HEPES at pH 7.4 (giving free calcium concentration < 10−7 M) and in the inside-out experiments (in mM) 140 KCl, 1 MgCl2, 3 EGTA (purity 96%), 10 HEPES, and an appropriate amount of CaCl2 to get a free calcium concentration of 300 nM at pH 7.4. The free calcium concentrations were determined at the end of the current traces and determined after the calculation of the mean of three consecutive traces. Single-channel data were stored on a DTR-1800 data recorder (Biologic) and replayed for analysis, where they were filtered at 1 kHz with use of an eight-pole Bessel filter (model 902, Frequency Devices) and digitized at 5 kHz. Thereafter, they were analyzed off-line with the software package ASCD (G. Droogmans, Lab. Fysiologie, Katholieke University, Leuven, Belgium) on an 80486 computer. The single-channel amplitudes were determined by fitting Gaussian distributions to the amplitude histograms of the closed and the open state, respectively. The activity of the channel in a patch was determined as NP, where P is the open probability of one channel and N is the number of channels in the patch, which could not be determined in most cases because of the low level of activity. NP was calculated as the sum of the times spent at current levels corresponding to 1, 2,..., N channels open multiplied by the number of open channels, and this sum was then divided by the total registration time. The registration time was 3–5 min. All potentials are expressed as membrane potentials.

Drugs and chemicals. Norepinephrine, acetylcholine, adenosine, papain, albumin, DL-dithiothreitol, cAMP, cGMP, MgATP, as well as the salts for the solutions were obtained from Sigma (Deisenhofen, Germany). H-89 was purchased from Calbiochem (Bad Soden, Germany), iberotoxin, ryanodine, and phentolamine from Research Biochemical International (Biotrend, Cologne, Germany), 4-aminoypyridine from Merck (Darmstadt, Germany), and tetrodotoxin from Molecular Probes (Leiden, The Netherlands). The catalytic and regulatory subunits of PKA and PKG were from Promega (Mannheim, Germany), and Sp-5,6-DCI-cBIMPS, Rp-CPT-CAMPS, Rp-8-Br-PET-cGMPS, and CPT-cGMP were from Biolog (Bremen, Germany).

Statistics. All data are means ± SE. Only one vessel was taken from each rat; thus n is the number of rats. In the patch-clamp experiments, n is the number of cells. Statistical analysis was performed using paired t-test, unpaired t-test, two-way analysis of variance, one-way analysis of variance followed by a Bonferroni test, or one-way repeated-measures analysis of variance, as appropriate (SPSS for Windows 7.5.1).

RESULTS

Rat tail small arteries exposed to a constant transmural pressure of 80 mmHg developed a myogenic tone when temperature was raised to 37°C. After a 60-min equilibration period and the standard viability tests (see METHODS), a vessel diameter reduction to 71.6 ± 1.0% (n = 32) of the diameter of the fully relaxed state, which was determined in a calcium-free solution, was observed. The diameter of the vessel in this constricted state is the basal diameter for the present experiments.

Inhibition of PKA. Application of Rp-CPT-CAMPS dose-dependently strengthened the myogenic tone at 80 mmHg, shown as a significant, reversible constriction of the vessel (P < 0.001) (Fig. 1, A and B). For example, a 11.0 ± 1.2% (n = 8) reduction of vessel diameter was observed at 300 µM Rp-CPT-CAMPS. Higher concentrations were not used because this substance was relatively expensive. Furthermore, application of H-89 also dose-dependently strengthened the myogenic tone at 80 mmHg, shown as a significant, reversible constriction of the vessel (P < 0.001) (Fig. 1C). For example, a 14.3 ± 3.6% (n = 5) reduction of vessel diameter was observed at 9 µM H-89.
concentrations were not used because they were not desirable to avoid interactions with PKG (29). In contrast to these observations, the PKG inhibitor Rp-8-Br-PET-cGMPS did not alter the myogenic tone at 80 mmHg in the concentration range tested (P = 0.35; Fig. 1D).

Functional localization of PKA. Application of Rp-CPT-cAMPS to endothelium-denuded vessels (for technical details see METHODS) dose-dependently strengthened the myogenic tone at 80 mmHg, shown as a significant, reversible constriction of the vessel. This effect was not significantly different from the Rp-CPT-cAMPS-induced constriction in endothelium-intact vessels (n = 6, P = 0.95; Fig. 2A). Application of Rp-CPT-cAMPS to vessels with blocked transmission from nerve endings (for technical details see METHODS) dose-dependently strengthened the myogenic tone at 80 mmHg, shown as a significant, reversible constriction of the vessel. This effect was not significantly different from the Rp-CPT-cAMPS-induced constriction in non-blocked vessels (n = 4, P = 0.22; Fig. 2B).

Specificity of PKA inhibitors. A considerable inhibitory potency of 300 µM Rp-CPT-cAMPS and 9 µM H-89 on PKA in our preparation was suggested by a 88.1 ± 9.5% (n = 3) and 55.6 ± 6.9% (n = 6) attenuation, respectively, of the effect of 100 µM adenosine, the action of which is supposed to be mediated by PKA (30). The specificity of Rp-CPT-cAMPS was additionally tested by investigating its effect on the action of PKA and PKG on KCa currents, which are known to be activated by both protein kinases in smooth muscle cells. In whole cell experiments on smooth muscle cells from the main rat tail artery, an outward current was observed, which is mainly determined by KCa currents (for details of current isolation, see Ref. 27). All test substances, i.e., the protein kinases and the kinase blockers, were contained in the pipette solution, in which the effect on the KCa current developed slowly because of the diffusion time necessary for entering the cell. The catalytic subunit of PKA at 10 U/ml together with 10 U/ml of the regulatory subunit of PKA, 100 µM cAMP, and 100 µM MgATP increased the KCa current in the whole voltage range tested (Fig. 3). An increase of the KCa current was also observed with
PKG at 1 U/µl together with 100 µM cGMP and 100 µM MgATP. When the pipette solution contained PKA and 100 µM Rp-CPT-cAMPS, the increase of the KCa current was prevented completely. In contrast, 100 µM Rp-CPT-cAMPS did not affect the increase in KCa current induced by PKG, although the effect of PKG was completely inhibited when PKG and 1 µM Rp-8-Br-PET-cGMPS were included in the pipette solution (Fig. 3). A quantitative analysis was performed at a membrane potential of −70 mV (Table 1). This analysis showed that PKA as well as PKG increased the KCa current significantly compared with the time-control current. In the presence of PKA and Rp-CPT-cAMPS, the KCa current was not significantly different from the time control, i.e., Rp-CPT-cAMPS completely inhibited the effect of PKA. In contrast, in the presence of PKG and Rp-CPT-cAMPS the KCa current was increased significantly compared with the time control, i.e., Rp-CPT-cAMPS completely inhibited the effect of PKA.
The application of 10 µM ryanodine, which blocks ryanodine receptor-generated calcium sparks (19), resulted in a strengthening of the myogenic tone at 80 mmHg, reducing vessel diameter significantly by 20.7 ± 1.7% (n = 4, P < 0.01; Fig. 5, A and B). Application of 300 µM Rp-CPT-cAMPS to a vessel preconstricted with 10 µM ryanodine produced an overall (i.e., related to the basal diameter before ryanodine pretreatment) 29.5 ± 1.1% (n = 4) reduction of vessel diameter (Fig. 5, A and B). The vessel diameter reduction induced by Rp-CPT-cAMPS related to the diameter after ryanodine pretreatment was 11.7 ± 0.7% (n = 4) and was not significantly different compared with the vessel diameter reduction induced by Rp-CPT-cAMPS without ryanodine pretreatment, which amounted to 11.0 ± 1.2% (n = 8, P = 0.84) (Fig. 5E).

Effect of PKA on K\textsubscript{Ca} channels. In the inside-out configuration of the patch-clamp technique, the predominantly observed channel in single rat tail small artery smooth muscle cells showed a conductance of ~170 pS, a steep voltage dependence and a remarkable dependence of channel activity on intracellular calcium ion concentration. In the outside-out configuration tetraethylammonium and iberiotoxin blocked this channel (data not shown). All these properties are characteristic for the K\textsubscript{Ca} channel of high conductance ubiquitously found in all vascular smooth muscle cells investigated so far. Application of MgATP to the intracellular side of the K\textsubscript{Ca} channel did not change channel activity (Fig. 6A). The open probability NP\textsubscript{o} increased 1.2 ± 0.2-fold (n = 9) after the application of 100 µM MgATP in comparison to the control recording (Fig. 6B). The following additional exposure of the intracellular side of the channel to the catalytic subunit of PKA increased the channel activity remarkably (Fig. 6A). In comparison to the control recording, NP\textsubscript{o} increased significantly (30.1 ± 9.8-fold, P < 0.01; n = 9) after the additional application of 10 µM of the catalytic subunit of PKA, a concentration used in most other studies investigating PKA regulation of K\textsubscript{Ca} channels in smooth muscle cells (Fig. 6B). The channel amplitude did not change during the course of the experiments.

Myogenic response. The myogenic response, i.e., the reaction of the vessel to a change in transmural pressure, was evaluated with use of a pressure step from 80 to 120 mmHg. This pressure step was chosen because rat tail small arteries show similar myogenic responses in the pressure range from 40 to 160 mmHg (data not shown). Under all conditions tested, namely after application of 300 µM Rp-CPT-cAMPS, 10 µM Sp-5,6-DCl-cBIMPS (a specific PKA activator), 10 µM Rp-8-Br-PET-cAMPS and 100 µM MgATP, control 0.94 ± 0.05 (Fig. 6F). The open probability NP\textsubscript{o} increased significantly (30.1 ± 9.8-fold, P < 0.01; n = 9) after the additional application of 10 µM of the catalytic subunit of PKA, a concentration used in most other studies investigating PKA regulation of K\textsubscript{Ca} channels in smooth muscle cells (Fig. 6B). The channel amplitude did not change during the course of the experiments.

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<tr>
<th>Supplements to Internal Solution</th>
<th>x-fold Increase in Whole Cell Current</th>
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<tr>
<td>100 µM MgATP, control</td>
<td>0.94 ± 0.09</td>
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<tr>
<td>10 U/ml PKA catalytic subunit + 10 U/ml PKA regulatory subunit + 100 µM cAMP + 100 µM MgATP</td>
<td>2.24 ± 0.44†</td>
</tr>
<tr>
<td>1 µM Rp-CPT-cAMP + 100 µM cGMP + 100 µM MgATP</td>
<td>1.74 ± 0.24†</td>
</tr>
<tr>
<td>100 µM Rp-CPT-cAMP + 10 U/ml PKA catalytic subunit + 100 µM cAMP + 100 µM MgATP</td>
<td>1.05 ± 0.09</td>
</tr>
<tr>
<td>100 µM Rp-CPT-cAMP + 1 U/ml PKG + 100 µM cGMP + 100 µM MgATP</td>
<td>1.65 ± 0.20†</td>
</tr>
<tr>
<td>1 µM Rp-8-Br-PET-cGMPS + 1 U/ml PKG + 100 µM cGMP + 100 µM MgATP</td>
<td>1.00 ± 0.05</td>
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</table>

Values are means ± SE; n = 5 observations per group. Membrane potential (V\textsubscript{m}) = −70 mV, holding potential (V\textsubscript{h}) = −40 mV, 500-ms pulse. K\textsubscript{Ca}, calcium-activated potassium; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; Rp-CPT-cAMPS, Rp-8-(4-chlorophenylthio)-adenosine 3'5'-cyclic monophosphoioate; Rp-8-Br-PET-cGMPS, Rp-8-bromo-β-phenyl-1,3,4,7-etheno-guanosine 3',5'-cyclic monophosphoioate. *Significant change in comparison to control; †significant change in comparison to Rp-CPT-cAMPS + PKA (P < 0.05, ANOVA with subsequent Bonferroni test, no more significant differences observed).
Fig. 4. Effect of PKA inhibitor Rp-CPT-cAMPS on myogenic tone after block of K channels. A: application of 100 nM iberiotoxin (IBTX) resulted in a strengthening of the myogenic tone, shown as a decrease in vessel diameter. Addition of 300 µM Rp-CPT-cAMPS during continuous application of 100 nM iberiotoxin produced a further strengthening of the myogenic tone. B: summarized data of effect of 100 nM iberiotoxin and 300 µM Rp-CPT-cAMPS. *Significant difference (n = 4, a priori paired t-test). C: application of 1 mM 4-aminopyridine (4-AP) resulted in a strengthening of the myogenic tone, shown as a decrease in vessel diameter. Addition of 300 µM Rp-CPT-cAMPS during continuous application of 1 mM 4-aminopyridine produced a further strengthening of the myogenic tone. D: summarized data of effect of 1 mM 4-aminopyridine and 300 µM Rp-CPT-cAMPS. *Significant difference (n = 5, a priori paired t-test). E: summarized data of separate effect of 300 µM Rp-CPT-cAMPS after different pretreatment, i.e., with physiological salt solution (no pretreatment), iberiotoxin, or 4-aminopyridine. *Significant difference (1-way analysis of variance followed by Bonferroni test); n.s., not significant.
cGMPS, and 5–10 µM CPT-cGMP (a specific PKG activator), the myogenic response was not significantly different compared with the control myogenic response (Table 2). For all conditions tested, the diameter change due to the myogenic response was completely reversible after pressure was stepped back from 120 to 80 mmHg; the vessel diameter obtained after returning to 80 mmHg was not significantly different from the vessel diameter before the application of 120 mmHg (data not shown).

**DISCUSSION**

The level of the myogenic tone of rat tail small arteries amounted to 71.6%, i.e., vessel diameter after development of the myogenic tone decreased to 71.6% of the fully relaxed diameter. In rat cerebral (21), femoral (34), and mesenteric (31) and pig coronary (13) small arteries, the level of the myogenic tone was between 60 and 85%. Thus the myogenic tone of rat tail small arteries is in the same range as in vessels from other vascular beds.

The two PKA inhibitors and the PKG inhibitor used in this study have been proven to be selective and potent for their respective protein kinases (see discussion in Refs. 2, 25, 29). In smooth muscle cells, the selective action of H-89 and Rp-8-Br-PET-cGMPS on PKA and PKG, respectively, has been demonstrated clearly in the literature (2, 17). The selective action of Rp-CPT-cAMPS on PKA in smooth muscle cells was shown in the present study by its ability to inhibit completely PKA-induced enhancement of KCa currents and its inability to affect PKG-induced enhancement of KCa currents. In addition, the following arguments support a selective action of Rp-CPT-cAMPS and H-89 on PKA in the present study. Both PKA inhibitors produced a similar degree of vessel constriction when
used in a concentration range reported to be selective for PKA. This was observed, although these substances belong to chemically different classes of compounds and have completely different mechanisms of action. Furthermore, these inhibitors blocked a large part of the relaxation of rat tail small arteries induced by adenine, a substance proposed to act via PKA (30). Moreover, Rp-8-Br-PET-cGMPS, a specific inhibitor of PKG, did not influence the diameter of rat tail small arteries. Therefore, a nonspecific action of the PKA inhibitors is very unlikely, because it seems that they are even not able to affect PKG, which possesses the structurally closest binding sites for these inhibitors in comparison to their native binding sites on PKA.

The present study reports the novel observation that two different PKA inhibitors, Rp-CPT-cAMPS and H-89, increased the level of the myogenic tone in rat tail small arteries in the absence of any exogenous substances known to activate PKA. Thus PKA may be in an active state in a vessel possessing a myogenic tone. An active PKA opens more possibilities for the regulation of the level of the myogenic tone, i.e., for blood flow regulation. Such a regulation can now be achieved with a variety of agonists not only by activating but also by inhibiting PKA. The presented data show not only that the level of the myogenic tone is determined by several contractile mechanisms (for review, see Refs. 3, 14) but also that these contractile mechanisms are counteracted by a dilatory mechanism involving PKA. However, PKA is probably only one of several dilating pathways, because the K

Table 2. Effect of activators and inhibitors of PKA and PKG on myogenic response

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<th>Vessel Treatment</th>
<th>% Change of Vessel Diameter for Testing</th>
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<tr>
<td></td>
<td>Myogenic tone at 80 mmHg</td>
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<tr>
<td>Control</td>
<td>−0.6 ± 0.6</td>
</tr>
<tr>
<td>300 µM Rp-CPT-cAMPS</td>
<td>−10.3 ± 2.36</td>
</tr>
<tr>
<td>Control</td>
<td>−0.7 ± 0.4</td>
</tr>
<tr>
<td>10 µM Sp-5,6-DCI-dBIMPS</td>
<td>+12.5 ± 1.25</td>
</tr>
<tr>
<td>Control</td>
<td>−0.9 ± 0.2</td>
</tr>
<tr>
<td>10 µM Rp-8-Br-PET-cGMPS</td>
<td>−2.1 ± 1.5</td>
</tr>
<tr>
<td>Control</td>
<td>−0.1 ± 0.7</td>
</tr>
<tr>
<td>5–10 µM cPT-cGMPS</td>
<td>+9.8 ± 0.75</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = no. of observations. Sp-5,6-DCI-dBIMPS, Sp-5,6-dichloro-1-β-β-ribofuranosylbenzimidazole-3,5'-cyclic monophosphothioate; CPT-cGMPS, 8-(4-chlorophenylthio)guanosine 3,5'-cyclic monophosphate. §Significant change in comparison to control (P < 0.05, paired t-test).
KCa channels may mediate, at least partly, the effect of... Furthermore, new data are shown that suggest that... tone. This PKA seems to be located in smooth muscle...

...modulate the myogenic tone, indicating that PKA may present the novel observation that PKA inhibitors...activity were evoked from different initial states...activity of the protein kinases altered vessel diameter,...


