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Influence of cAMP on cerebrospinal fluid opioid concentration: role in cAMP-induced pial artery dilation

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Abstract

Previously, it has been observed that cGMP analogs and agents that elevate cGMP levels markedly increase the concentration of the opioids [Met^5]enkephalin and [Leu^5]enkephalin in cortical periarachnoid cerebrospinal fluid (CSF) of the newborn pig. However, such agents had no effect on CSF dynorphin-(1-13) concentration. The present study was designed to: (1) investigate the influence of cAMP on the CSF concentration of the opioids [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13); and (2) determine the role of these opioids in cAMP-induced pial artery vasodilation. Piglets equipped with closed cranial windows were used to measure pial artery diameter and collect cortical periarachnoid CSF for assay of opioids. The cAMP analog, 8-Bromoadenosine-3′,5′-cyclic monophosphate (8-Bromo cAMP) elicited pial dilation that was blunted by a cAMP antagonist, Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate (10^{-5} M) (11 ± 1 and 19 ± 1 vs. 1 ± 1 and 1 ± 1 for 10^{-8} M, 10^{-6} M 8-Bromo cAMP before and after Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate, respectively). The dilation produced by 8-Bromo cAMP was accompanied by modest increases in CSF [Met^5]enkephalin and co-administration of Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate with 8-Bromo cAMP blocked these increases in CSF opioid concentration (1179 ± 48, 1593 ± 92 and 2079 ± 88 vs. 1054 ± 32, 1038 ± 15 and 1071 ± 17 pg/ml for control, 10^{-8} M and 10^{-6} M 8-Bromo cAMP before and after Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate, respectively). The release of CSF [Leu^5]enkephalin by 8-Bromo cAMP was also blocked by Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate. In contrast, 8-Bromo cAMP produced marked increases in CSF dynorphin-(1–13) (38 ± 3, 61 ± 3 and 88 ± 6 vs. 27 ± 3, 28 ± 3 and 30 ± 4 pg/ml for control, 10^{-8} M and 10^{-6} M 8-Bromo cAMP before and after Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate, respectively). Similar blunted vascular and biochemical responses were observed with the co-administration of Sp 8-Bromoadenosine-3′5′-cyclic monophosphorothioate, another analog of cAMP, with Rp 8-Bromoadenosine-3′,5′-cyclic monosphorothioate. The opioid receptor antagonist naloxone (1 mg/kg i.v.) attenuated 8-Bromo cAMP-induced dilation (9 ± 1 and 17 ± 1 vs. 5 ± 1 and 8 ± 1 for 10^{-8} M, 10^{-6} M 8-Bromo cAMP before and after naloxone). These data show that cAMP contributes to the release of the CSF opioids [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13), and suggest that, while cGMP is more important relative to cAMP in elevating CSF [Met^5]enkephalin and [Leu^5]enkephalin concentration, the converse is true for dynorphin-(1–13). Further, these data indicate that opioids contribute to cAMP-induced pial artery vasodilation.

Keywords: Cyclic nucleotide; Cerebral circulation; Newborn

1. Introduction

Opioids contribute to the regulation of cerebral hemodynamics. Opioid receptor binding has been demonstrated on cerebral microvessels (Peroutka et al., 1980). Enkephalin and dynorphin immunoreactivity has been shown in large cerebral arteries of the pig (Thureson-Klein et al., 1989). Moreover, cerebrospinal fluid (CSF) opioid concentrations are in the vasoactive range in the newborn pig during resting conditions (Armstead et al., 1991). Previously, opioids, such as [Met^5]enkephalin and [Leu^5]enkephalin, have been observed to produce pial artery vasodilation, whereas dynorphin-(1–13) produces tone-dependent effects (dilation during normotension; vasoconstriction during hypotension) (Armstead et al., 1991).

Previous studies have been designed to characterize the role of second messengers in opioid release. For example, isoproterenol or 8-Bromoadenosine-3′,5′-cyclic monophosphate (8-Bromo cAMP) cause the release of opioids from glial cells and ventricular cardiac muscle cells (Shinoda et

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al., 1989; Springhorn and Claycomb, 1992), suggesting the involvement of cAMP in that release. However, these and other studies were performed in vitro. Little is known about the mechanisms involved in opioid release in the intact animal.

The role of peptides in contributing to the response of substances that mediate vascular signal transduction has been investigated previously. For example, calcitonin gene-related peptide (CGRP) has been observed to contribute to sodium nitroprusside-induced vasodilation in feline cerebral arteries, suggesting its involvement in nitric oxide (NO)-induced dilation (Wei et al., 1992). Alternatively, it has recently been reported that CGRP is not a necessary intermediate in NO-induced dilation in parenchymal vessels of the rat hippocampus (Fergus et al., 1995). The role of opioids in cAMP-induced vasodilation has not been previously studied.

Therefore, the present study was designed to investigate the influence of cAMP on the CSF concentration of opioids and determine the role of these opioids in cAMP-induced pial artery vasodilation.

2. Materials and methods

All experiments were approved by the Institutional Animal Care and Use Committee. Newborn pigs (1–4 days old) of either sex were used in these experiments. The pigs were first sedated with ketamine hydrochloride/acepromazine (33 mg/kg i.m.). Anesthesia was maintained with α-chloralose (30–50 mg/kg initially, supplemented with 5 mg/kg i.v.). The trachea was cannulated and the animals were ventilated with room air. A femoral artery and femoral vein were cannulated and a catheter was inserted in each. The venous line was used for the injection of drugs, while the arterial catheter was used to record blood pressure and to sample for blood gases and pH. The pigs' body temperature was maintained at 37–38°C with a heating pad.

For insertion of the cranial window, the scalp was removed and an opening was made in the skull above the parietal cortex. The dura was cut and retracted over the cut bone edge. The cranial window was situated in the hole and cemented in position with dental acrylic. The space under the window was filled with artificial CSF of the following composition (mg/l): 220 KCl, 132 $MgCl_2$, 221 $CaCl_2$, 7720 NaCl, 402 urea, 665 dextrose and 2066 $NaHCO_3$, pH 7.30–7.36, pCO_2 42–49 mm Hg and pO_2 40–45 mm Hg.

Pial arterioles were observed with a dissecting microscope, a television camera mounted on the microscope and a video monitor. Vascular diameter was measured with a video micro scaler (model VPA 550; For-A-Corp, Los Angeles, CA, USA).

2.1. Protocol

Pial artery diameter (small artery 120–160 μm; arteriole 50–70 μm) was determined every min for a 10-min exposure period after injection under the window of artificial CSF alone or with an agent. Diameters were also measured 10–15 min after the highest concentration of a drug was flushed off the cerebral cortical surface with CSF containing no drug. Typically, 1–2 ml of CSF were flushed through the window over a 30-s period. Needles incorporated into the side of the window allowed the injection of CSF under the window and run off of the excess CSF. The peak responses were measured and a CSF sample for opioid analysis was collected at the end of the 10-min exposure period. The cerebral cortical periarachnoid CSF (300 μl) was collected slowly by infusing artificial CSF into one side of the window and allowing the CSF under the window to drip freely into a collection tube on the opposite side.

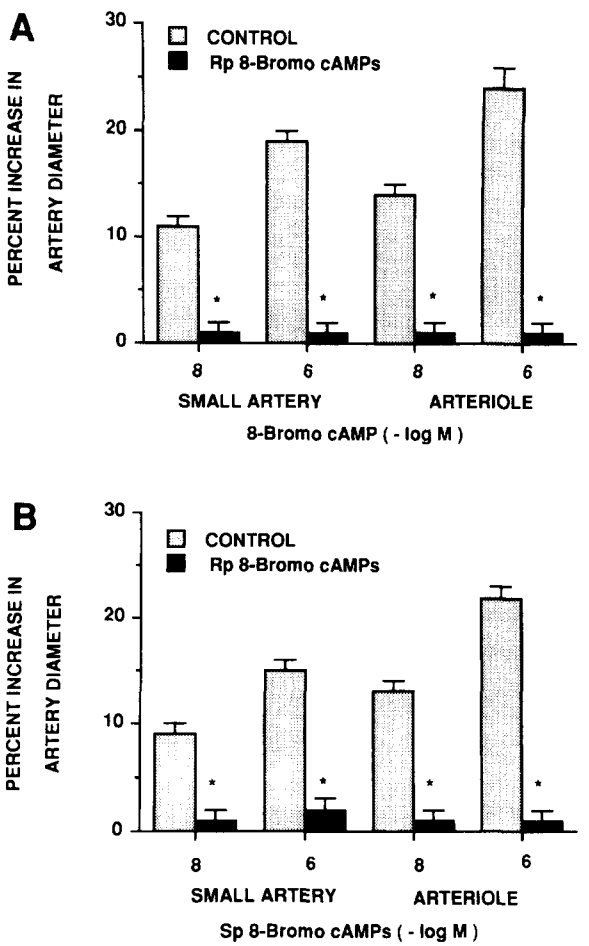

Fig. 1. (A) Influence of 8-Bromoadenosine-3′,5′-cyclic monophosphate (8-Bromo cAMP) (10^{-8} M, 10^{-6} M) on small pial arteries and arterioles in the absence (control) and presence of Rp 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp 8-Bromo cAMPS) (10^{-5} M). (B) Influence of Sp 8-Bromo adenosine-3′,5′-cyclic monophosphorothioate (Sp 8-Bromo cAMPs) (10^{-8} M, 10^{-6} M) on small pial arteries and arterioles in the absence (control) and presence of Rp 8-Bromo cAMPs (10^{-5} M), $n = 6$. $^{*}P < 0.05$ compared to corresponding control.

Responses were observed after administering 8-Bromo cAMP (10^{-8} M, 10^{-6} M) (Research Biochemicals, Natick, MA, USA) and SP 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Sp 8-Bromo cAMPs) (10^{-8} M, 10^{-6} M) (Biolog Life Science Institute, La Jolla, CA, USA) both analogs of cAMP, in the absence and presence of Rp 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp 8-Bromo cAMPs) (10^{-5} M) (Biolog), a purported cAMP antagonist. Responses to 8-Bromo cAMP and Sp 8-Bromo cAMPs were also obtained in the absence and presence of naloxone (1 mg/kg i.v.; Sigma, St. Louis, MO, USA). To confirm opioid receptor blockade, responses to [Met^5]enkephalin (10^{-8} M, 10^{-6} M, Sigma) were obtained in the absence and presence of naloxone and then again at the end of the protocol so as to bracket the responses to the cAMP analogs obtained in the presence of naloxone. Animals received a maximum of three agonists each at two concentrations, administered in an ascending concentration fashion before and after the antagonist.

Time control experiments were designed such that responses were obtained initially (defined at 1st) and then again 60 min later (defined as 2nd in tables).

The vehicle for Rp 8-Bromo cAMPs (ethanol) had no effect on pial artery diameter when added to CSF in a 1 : 1000 dilution. Appropriate aliquots of the vehicle for all other agents (0.9% saline) were also added to CSF infused under the window. This CSF vehicle also had no effect on pial artery diameter. All working solutions of agents were made fresh the day of their use.

2.2. Opioid analysis

The CSF samples collected were immediately acidified with 1 N acetic acid to prevent peptide degradation, rapidly frozen and stored at −20°C. Radioimmunoassay (RIA) kits for [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13) are commercially available (Incstar, Stillwater, MN, USA; Peninsula Lab, Belmont, CA, USA). The RIA used simultaneous addition of sample, rabbit antiopioid antibody and the I^{125} derivative of the opioid. After an overnight incubatin at 4°C, the free opioid was separated from the opioid bound to the antibody by the addition of saturated ammonium sulfate in the presence of rabbit carrier γ globulin. Following centrifugation at 760 × *g* for 10 min, the supernate was decanted and the pellet was counted using a gamma scintillation counter. All samples and standards were assayed in duplicate. Data were calculated as % B/B_0 vs. concentration, where % B/B_0 = mean cpm of sample − mean cpm of non-specific binding tube/B_0 × 100. B_0 = mean cpm of the total binding tube − mean cpm of the non-specific binding tube.

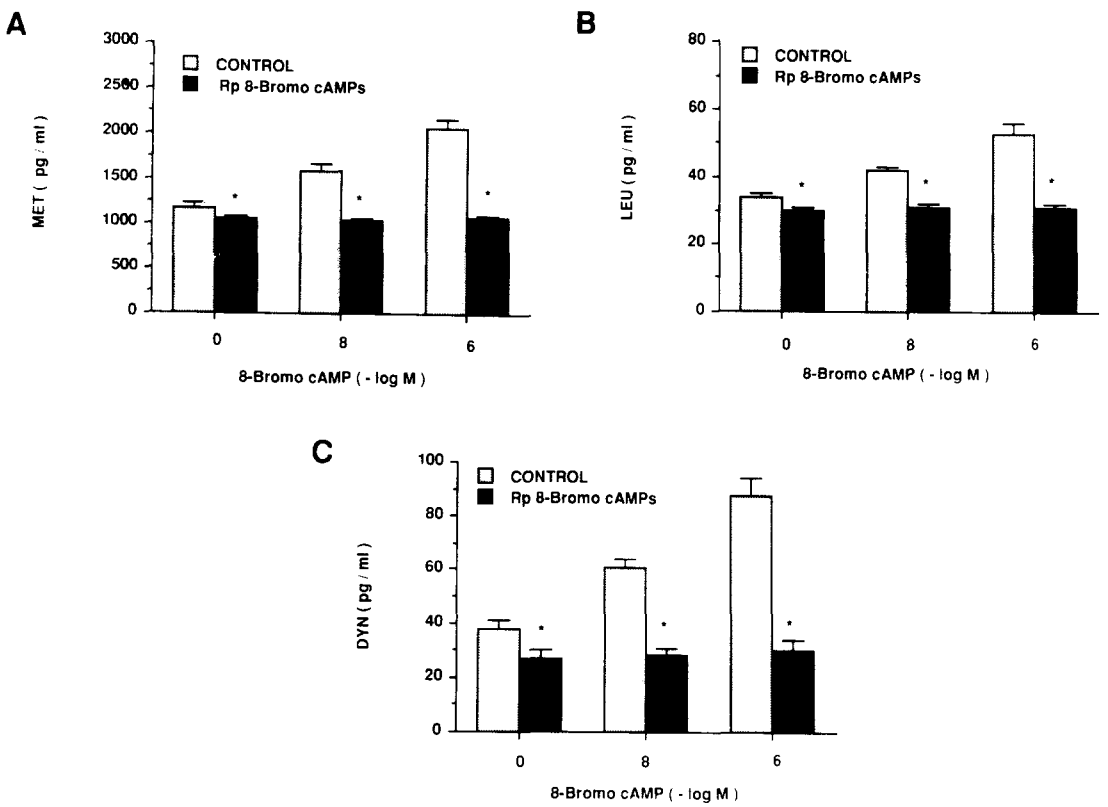

Fig. 2. Influence of 8-Bromoadenosine-3′,5′-cyclic monophosphate (8-Bromo cAMP) (10^{-8} M, 10^{-6} M) on CSF opioid concentration (pg/ml) in the absence (control) and presence of Rp 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp 8-Bromo cAMPs) (10^{-5} M). Panel A, [Met^5]enkephalin; panel B, [Leu^5]enkephalin; panel C, dynorphin-(1–13); $n = 6$ for each opioid. * $P < 0.05$ compared to corresponding control.

2.3. Statistical analysis

Pial arteriolar diameter, systemic arterial pressure and opioid levels were analyzed using analysis of variance for repeated measures. If the values were significant, the Fisher test was performed. An α level of $P < 0.05$ was considered significant in all statistical tests. The n values reflect data for one vessel in each animal. Values are represented as means ± S.E. of absolute values or as percentages of change from control values. Data presented as percentage change were compared by non-parametric means using the Wilcoxin signed rank test.

3. Results

3.1. Influence of Rp 8-Bromo cAMPs on 8-Bromo cAMP and Sp 8-Bromo cAMPs-induced pial artery dilation and increased CSF opioid concentration

8-Bromo cAMP and Sp 8-Bromo cAMPs (10^{-8} M, 10^{-6} M) elicited reproducible pial small artery (120–160 μm) and arteriole (50–70 μm) vasodilation (Table 1). Dilation in response to both agonists was blunted with the

Table 1
Influence of 8-Bromo cAMP, Sp 8-Bromo cAMPS and [Met^5]enkephalin on pial artery diameter

	Time control			
	Time 1		Time 2	
	Small artery	Arteriole	Small artery	Arteriole
8-Bromo-cAMP (−log M)				
0	146±1	60±2	146±1	61±2
8	161±1 [a]	68±2 [a]	162±1 [a]	69±2 [a]
6	179±1 [a]	75±3 [a]	179±1 [a]	77±2 [a]
Sp 8-Bromo cAMPS (−log M)				
0	145±1	60±2	146±1	60±3
8	161±1 [a]	68±2 [a]	161±1 [a]	68±3 [a]
6	177±2 [a]	76±3 [a]	177±1 [a]	76±3 [a]
[Met^5]enkephalin (−log M)				
0	142±3	52±3	142±4	52±3
8	157±3 [a]	59±3 [a]	159±4 [a]	59±3 [a]
6	166±4 [a]	63±3 [a]	166±4 [a]	63±4 [a]

$n = 5$. Values are in μm.
[a] $P < 0.05$ compared to corresponding control value (0).

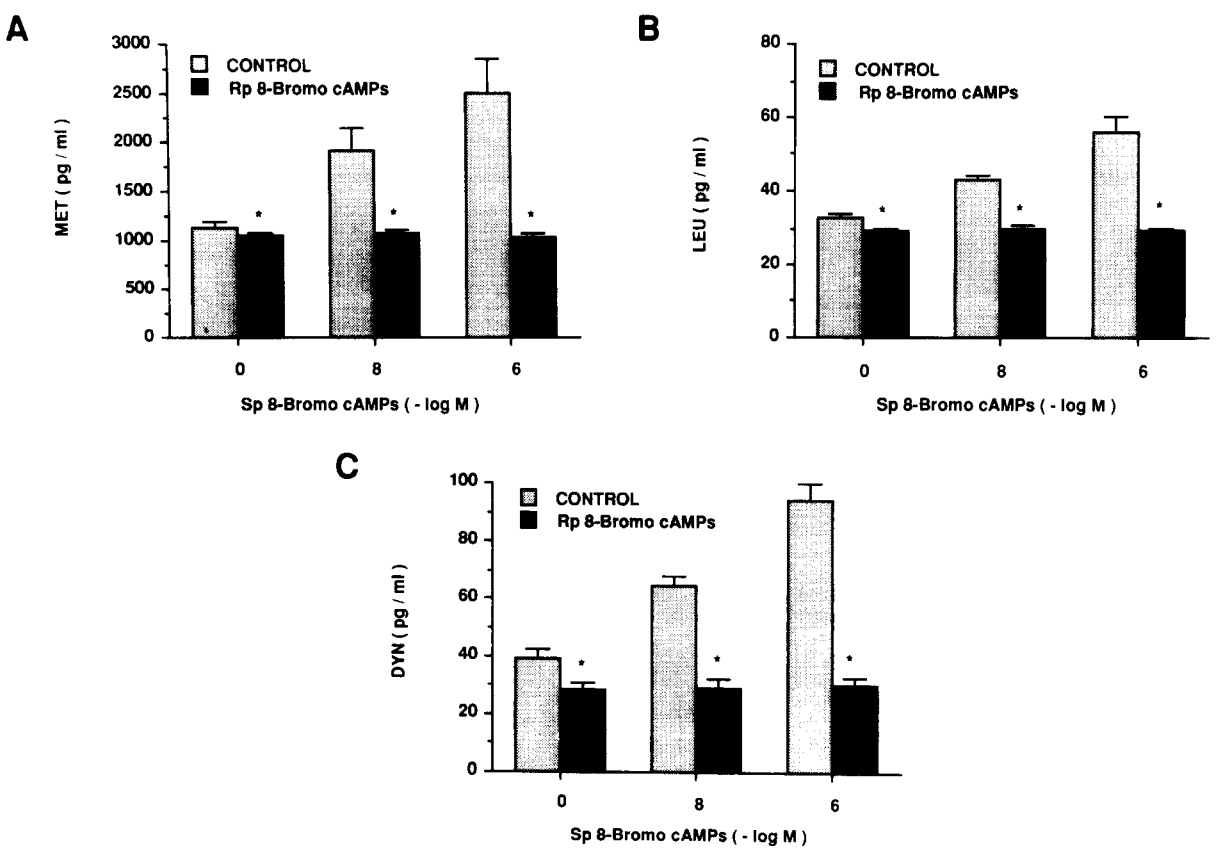

Fig. 3. Influence of Sp 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Sp 8-Bromo cAMPs) (10^{-8} M, 10^{-6} M) on CSF opioid concentration (pg/ml) in the absence (control) and presence of Rp8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp 8-Bromo cAMPs) (10^{-5} M). Panel A, [Met^5]enkephalin; panel B, [Leu^5]enkephalin; panel C, dynorphin-(1–13). * $P < 0.05$ compared to corresponding control.

co-administration of the antagonist, Rp 8-Bromo cAMPs (10^{-5} M) (Fig. 1a,b). The responses of 8-Bromo cAMP and Sp 8-Bromo cAMPs were also associated with modest increases in cortical periarachnoid CSF [Met^5]enkephalin and [Leu^5]enkephalin (Figs. 2 and 3, panels A and B). These changes in CSF opioid concentration reflect 1.4 ± 0.1- and 1.8 ± 0.1-fold increases in [Met^5]enkephalin for 8-Bromo cAMP (10^{-8} M, 10^{-6} M) and 1.7 ± 0.2- and 2.3 ± 0.3-fold increases in [Met^5]enkephalin for Sp 8-Bromo cAMPs (10^{-8} M, 10^{-6} M), respectively. Similarly, there were 1.3 ± 0.1- and 1.6 ± 0.1-fold increases in [Leu^5]enkephalin for 8-Bromo cAMP and 1.3 ± 0.1- and 1.8 ± 0.2-fold increases in [Leu^5]enkephalins for Sp 8-Bromo cAMPs (10^{-8} M, 10^{-6} M), respectively. In contrast, these two agonists markedly increased CSF dynorphin-(1–13) concentration (Figs. 2 and 3, panel C). These changes in CSF dynorphin-(1–13) reflect 1.7 ± 0.2- and 2.4 ± 0.2-fold increases for 8-Bromo cAMP (10^{-8} M, 10^{-6} M) and 1.7 ± 0.2- and 2.5 ± 0.3-fold increases for Sp 8-Bromo cAMPSs (10^{-8} M, 10^{-6} M), respectively. Rp 8-Bromo cAMPs blocked the 8-Bromo cAMP and Sp 8-Bromo cAMPs-induced changes in CSF opioid concentration for all three opioids (Figs. 2 and 3). Rp 8-Bromo cAMPs had no effect on pial artery diameter (150 ± 10 vs. 142 ± 8 μm). However, Rp 8-Bromo cAMPs modestly decreased the resting control values for all three opioids (Figs. 2 and 3).

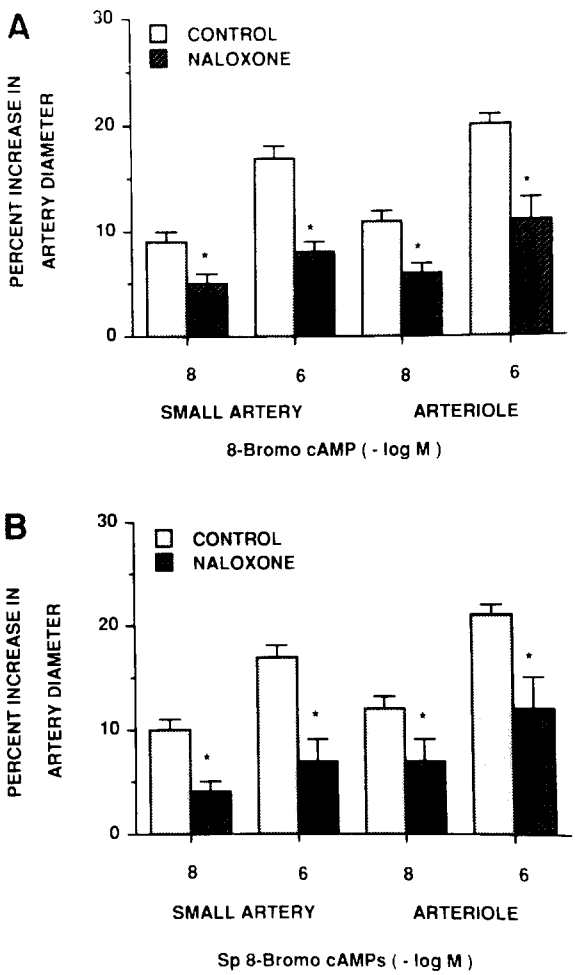

Fig. 4. (A) Influence of 8-Bromoadenosine-3′,5′-cyclic monophosphate (8-Bromo cAMP) on small pial arteries and arterioles in the absence (control) and presence of naloxone (1 mg/kg i.v.). (B) Influence of Sp 8-Bromoadenosine-3′,5′-cyclic monophosphorothioate (Sp 8-Bromo cAMPs) (10^{-8} M, 10^{-6} M) on small pial arteries and arterioles in the absence (control) and presence of naloxone (1 mg/kg i.v.), $n = 8$. * $P < 0.05$ compared to corresponding control.

3.2. Influence of naloxone on pial artery responses to 8-Bromo cAMP, Sp 8-Bromo cAMPs and [Met^5]enkephalin

Naloxone (1 mg/kg i.v.) attenuated responses to 8-Bromo cAMP and Sp 8-Bromo cAMPs (Fig. 4). [Met^5]enkephalin elicited reproducible pial small artery and arteriole vasodilation (Table 1). In contrast, responses to [Met^5]enkephalin were blocked by naloxone and these responses remained blocked at the end of the experiment.

3.3. Blood chemistry

Blood chemistry and mean arterial blood pressure values were obtained at the beginning and at the end of all the experiments. These values were unchanged at the end compared with values obtained at the beginning (7.40 ± 0.02, 31 ± 1, 94 ± 3 and 67 ± 2 vs. 7.43 ± 0.01, 30 ± 1, 92 ± 3 and 63 ± 2 mm Hg for pH, pCO_2, pO_2 and mean arterial blood pressure, respectively, $n = 31$).

4. Discussion

Results of the present study show that 8-Bromo cAMP and Sp 8-Bromo cAMPs, two stable analogs of cAMP (Schafer et al., 1994; Wang et al., 1991) elicited vasodilation and increased the cortical periarachnoid CSF concentration of [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13). Co-administration of the aforementioned agonists along with a cAMP antagonist, Rp 8-Bromo cAMPs, blunted the vascular and biochemical effects of these agents. Additionally, Rp 8-Bromo cAMPs modestly decreased the resting CSF values for all three opioids. Since Rp 8-Bromo cAMPs is a selective inhibitor of cAMP-dependent protein kinase (Schafer et al., 1994; Wang et al., 1991) and activation of cAMP kinase, but not cGMP kinase, is thought to be responsible for vascular smooth muscle relaxation by cAMP, Rp 8-Bromo cAMPs may be considered to be a cAMP antagonist. These results indicate that the cAMP second messenger system contributes to the release of CSF opioids. Further, these results suggest that cAMP has a small role in the tonic release of [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13). Finally, these data confirm and extend previous observations demonstrating the existence of an adenylate cyclase-cAMP dilating mechanism in pial arteries (Rosenblum, 1988).

Results of the present study also show that the opioid receptor antagonist naloxone attenuated pial dilation to the cAMP analog, 8-Bromo cAMP and Sp 8-Bromo cAMPs. To confirm that adequate blockade of opioid receptors had been achieved and maintained throughout the balance of the protocol, responses to [Met^5]enkephalin were obtained initially after naloxone administration as well as at the end of the protocol and compared to responses obtained before naloxone, thereby bracketing the pial responses to the cAMP analogs. Since responses to [Met^5]enkephalin were blocked by naloxone, these data indicate that uniform blockade of opioid receptors was present throughout that portion of the protocol. Further, since responses to the cAMP analogs were attenuated by naloxone, these data indicate that opioids contribute to pial dilation produced by cAMP.

Previous studies have attempted to characterize the mechanisms involved in opioid release. For example, excitatory amino acids have been observed to release [Met^5]enkephalin from slices of the rat striatum and globus pallidus (Ruzicka and Jhamandas, 1991), suggesting the involvement of NO in that release. Previous in vitro studies have alternatively shown that isoproterenol or 8-Bromo cAMP causes the release of opioids from glial cells, adrenal chromaffin cells and ventricular cardiac muscle cells, suggesting the involvement of cAMP in such release (Quach et al., 1984; Shinoda et al., 1989; Springhorn and Claycomb, 1992). More recently, it has been observed that sodium nitroprusside and the stable cGMP analog 8-Bromo cGMP, produce large increases in CSF [Met^5]enkephalin and [Leu^5]enkephalin concentration (Wilderman and Armstead, 1996). These biochemical changes and the pial artery dilation induced by these agents were blocked by the cGMP antagonist, Rp 8-Bromo cGMPs. However, sodium nitroprusside and 8-Bromo cGMP had no effect on CSF dynorphin-(1–13) concentration (Wilderman and Armstead, 1996). In contrast, results of the present study show that the cAMP analogs, 8-Bromo cAMP and Sp 8-Bromo cAMPs, produce marked increases in CSF dynorphin-(1–13) concentration. Therefore, taken together, these studies suggest that, while cGMP is more important relative to cAMP in elevating CSF [Met^5]enkephalin and [Leu^5]enkephalin concentration, the converse is true for dynorphin-(1–13). Additionally, the above previous and the present pharmacologic approach support the use of Rp 8-Bromo cGMPs and Rp 8-Bromo cAMPs as probes for the relative roles of cGMP and cAMP second messenger systems in opioid release. Possible sources of these opioids include cortical vessels, nerves associated with these vessels, neurons or glia. However, the origin of the opioids cannot be determined from the present experiments.

Opioids contribute to the regulation of the piglet cerebral circulation. For example, opioids are detectable in CSF under resting conditions (Armstead et al., 1991). Additionally, hypoxic pial artery dilation is associated with elevated CSF [Met^5]enkephalin and [Leu^5]enkephalin concentration; the administration of pharmacologic antagonists of either opioid blunted hypoxic pial dilation (Armstead, 1995a,b). Data from the present study suggest that endogenous activators of adenylate cyclase, such as pituitary adenylate cyclase-activating polypeptide (Miyata et al., 1989; Tong et al., 1992), could contribute to the basal or stimulated release of opioids in CSF.

In conclusion, results of the present study show that cAMP contributes to the release of the opioids [Met^5]enkephalin, [Leu^5]enkephalin and dynorphin-(1–13). In the context of previous studies, these data suggest that, while cGMP is more important relative to cAMP in elevating CSF [Met^5]enkephalin and [Leu^5]enkephalin concentration, the converse is true for dynorphin-(1–13). Finally, these data indicate that opioids contribute to cAMP-induced pial artery vasodilation.

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