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Potentiation of cyclic AMP and cyclic GMP accumulation by p38 mitogen-activated protein kinase (p38MAPK) inhibitors in rat pinealocytes

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Abstract

The effects of p38 mitogen-activated protein kinase (p38MAPK) inhibitors on the adrenergic-stimulated cyclic nucleotide production in rat pinealocytes were investigated. Treatment with SB202190 [4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)IH-imidazole] and SB203580 [4-(4-fluoropheny)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)IH-imidazole] (1–100 μM), two pyridinyl imidazole compounds that inhibit p38MAPK, as well as SB202474 [4-(ethyl)-2-(4-methoxyphenyl)-5-(4-pyridyl)IH-imidazole], an inactive analog, was effective in potentiating norepinephrine- and isoproterenol-stimulated cyclic AMP (cAMP) and cyclic GMP (cGMP) accumulation in a concentration-dependent manner. All three compounds caused a greater increase in the cGMP than the cAMP response, with SB202474 being substantially more potent than the two active analogs. At 100 μM, SB202474 potentiated the isoproterenol-stimulated cAMP and cGMP accumulation by 65 and 500%, respectively. Pharmacological studies indicated that the potentiating effect of SB202474 was independent of protein kinase C activation, intracellular calcium elevation, or serine/threonine phosphatase inhibition, three pathways known to potentiate the β-adrenergic-stimulated cyclic nucleotide responses in rat pinealocytes. In contrast, the potentiating effect of SB202474 was abolished in the presence of a phosphodiesterase inhibitor, isobutylmethylxanthine. At 100 μM, all three compounds inhibited cAMP- and cGMP-phosphodiesterase activities by 50 and 80%, respectively. These results suggest that the commonly used p38MAPK inhibitors can modulate cyclic nucleotide responses through phosphodiesterase inhibition, a mechanism that appears to be independent of p38MAPK inhibition.

Keywords: SB202474; p38MAPK inhibitors; cAMP; cGMP; Phosphodiesterase; Pinealocytes

1. Introduction

p38MAPK, a conserved subfamily of MAPKs, is involved in stress responses in eukaryotic cells [1,2]. It is activated by diverse stimuli that include ultraviolet light, irradiation, heat shock, osmotic stress, and proinflammatory cytokines, and appears to play a role in cellular processes such as apoptosis, transcriptional regulation, and cytoskeletal reorganization [1,2]. A group of pyridinyl imidazole compounds, which have been found to be highly specific inhibitors of p38MAPK, have contributed significantly in identifying the physiological function of p38MAPK [3]. Among these compounds, SB203580 [4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)IH-imidazole] and SB202190 [4-(4-fluorophenyl)-2-(4-hydroxyphenyl)-5-(4-pyridyl)IH-imidazole] have been shown to inhibit p38MAPK but have no effect on related kinases such as extracellular signal regulated protein kinase and c-Jun amino-terminal kinase [3]. In contrast, SB202474 [4-(ethyl)-2-(4-methoxyphenyl)-5-(4-pyridyl)IH-imidazole], which has no effect on p38MAPK [4], is commonly included as a negative control when SB203580 and SB202190 are used to investigate the involvement of p38MAPK.

The production of cAMP and cGMP in rat pinealocytes requires activation of adenylyl or guanylyl cyclases by stim-

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Abbreviations: MAPK, mitogen-activated protein kinase; cAMP and cGMP, cyclic AMP and cyclic GMP, respectively; PKC, protein kinase C; IL-1β, interleukin-1β; IBMX, isobutylmethylxanthine; ISO, isoproterenol; PMA, 4β-phorbol 12-myristate 13-acetate; NE, norepinephrine; RIA, radioimmunoassay; DMEM, Dulbecco's modified Eagle's medium; PDE, phosphodiesterase; and CM, calmodulin.

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ulation of the β-adrenoceptors [5,6]. The magnitude of these responses can be potentiated by other signaling pathways not directly involved in the activation of the cyclases. Pathways that can potentiate the β-adrenoceptor-stimulated cyclic nucleotide responses include elevation of intracellular calcium, activation of PKC, and inhibition of serine/threonine phosphatases or tyrosine kinases [6–12]. The initial objective of this study was to investigate the role of p38MAPK on adrenergic-stimulated cAMP and cGMP accumulation by using specific p38MAPK inhibitors, SB203580 and 202190, with SB202474 as the negative control [3]. However, our results show that all three pyridinyl imidazole compounds including SB202474, the putative inactive compound, can modulate cyclic nucleotide responses through a mechanism that appears to be independent of their effects on p38MAPK inhibition.

2. Materials and methods

2.1. Materials

SB203580, SB202190, SB202474, ionomycin, and calyculin A were obtained from Calbiochem. Human recombinant IL-1β, IBMX, ISO, PMA, prazosin, NE, culture medium, and fetal bovine serum were obtained from the Sigma Chemical Co. [^{125}I]cAMP and [^{125}I]cGMP were obtained from ICN Immunobiologicals. All other chemicals were of the purest grade available and were obtained commercially. Antibodies for the RIAs of cAMP and cGMP were gifts from Dr. A. Baukal (National Institute of Child Health and Human Development, NIH).

2.2. Preparation and treatment of rat pinealocytes

Pinealocytes were prepared from male Sprague–Dawley rats (150 g, University of Alberta Animal unit) by trypsinization as previously described [8,13]. The cells were suspended in DMEM containing 10% fetal bovine serum and maintained at 37° for 24 hr in a gas mixture of 95% air and 5% CO_2 before experimental treatment.

Aliquots of cells (2×10^4/0.4 mL) were treated with drugs that had been prepared in concentrated solutions in water or DMSO. The final concentration of the latter never exceeded 1.0%. At this concentration, DMSO had no effect on NE- or ISO-stimulated cAMP or cGMP accumulation. The duration of the drug treatment was 15 min, unless indicated otherwise. At the end of the treatment period, cells were collected by centrifugation (2 min, 10,000 *g*, at room temperature), the supernatant was aspirated, and the tube was placed on solid CO_2. The frozen cell pellets were lysed by the addition of 5 mM acetic acid (100 μL) and boiling (5 min). The lysates were stored frozen at –20° until analysis.

2.3. Cyclic nucleotide assay

The lysates were centrifuged (12,000 *g*, at 10 min, 4°), and samples of the supernatant were used to determine cellular cAMP and cGMP contents, using an RIA procedure in which samples were acetylated prior to analysis [8,14]. Since there was a small batch-to-batch variation of the cyclic nucleotide responses between cell preparations, all comparisons were performed within the same batch of cells.

2.4. PDE assay

Cytosolic cAMP- and cGMP-PDE activities were assayed by a modification of the method of Thompson and Appleman as described previously [15,16]. Briefly, freshly dissected pineal glands or dissociated pinealocytes were homogenized in an ice-cold buffer containing 0.25 M sucrose, 50 mM Tris–HCl, 1 mM EDTA, 1 mM EGTA, 1 mM dithiothreitol, 10 μM phenylmethylsulfonyl fluoride, 2 μg/mL of leupeptin and pepstatin, pH 7.4. The homogenate was centrifuged for 20 min (13,000 *g*, 4°), and the supernatant was used as an enzyme source. The reaction mixture contained 40 mM Tris–HCl, 4 mM 2-mercaptoethanol, 0.5 mM EGTA, 5 mM $MgCl_2$, 100 μM [^{3}H]cAMP or cGMP (~ 20,000 cpm), pH 7.5. To determine the background, 1 mM IBMX was included in some assay tubes. Compounds to be tested for their effects on PDE activities were added in the incubation mixture. In some experiments, 1 mM Ca^{2+} and 10 μM CM were included. The reaction was initiated by adding 10–20 μg of pineal protein and incubated for 20 min at 30°. Snake venom 5′-nucleotidase (100 μg, incubated for 20 min at 37°) was used to convert 5′-AMP or 5′-GMP to adenosine or guanosine, which was then isolated by chromatography, using Dowex AG 1-X8 resin, and eluted by water.

2.5. Statistical analysis

Data are presented as the mean ± SEM of the amount of cyclic nucleotide or PDE activity in four aliquots of cells. Each experiment was repeated at least twice with different cell preparations. Data were analyzed by ANOVA and the Newman-Keuls test, with statistical significance set at $P < 0.05$.

3. Results

3.1. Effects of p38MAPK inhibitors on adrenergic-stimulated cAMP and cGMP accumulation

Stimulation of rat pinealocytes with the β-adrenergic agonist ISO (1 μM) caused an 8- and 5-fold increase in cAMP and cGMP accumulation, respectively (Fig. 1). Co-treatment with SB202190 or SB 203580 (1–100 μM), two active inhibitors of p38MAPK [3], caused a significant

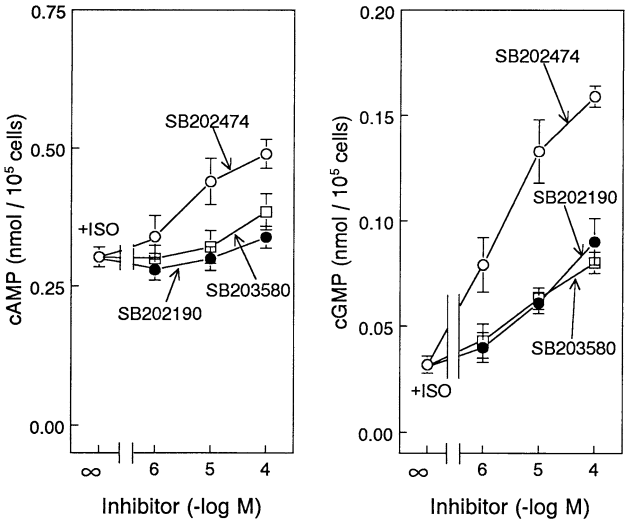

Fig. 1. Effects of p38MAPK inhibitors on ISO-stimulated cAMP and cGMP accumulation. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with ISO (1 μM + 1 μM prazosin) for 15 min in the presence or absence of various concentrations of SB202474, SB203580, or SB202190. Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. For further details, see "Materials and methods."

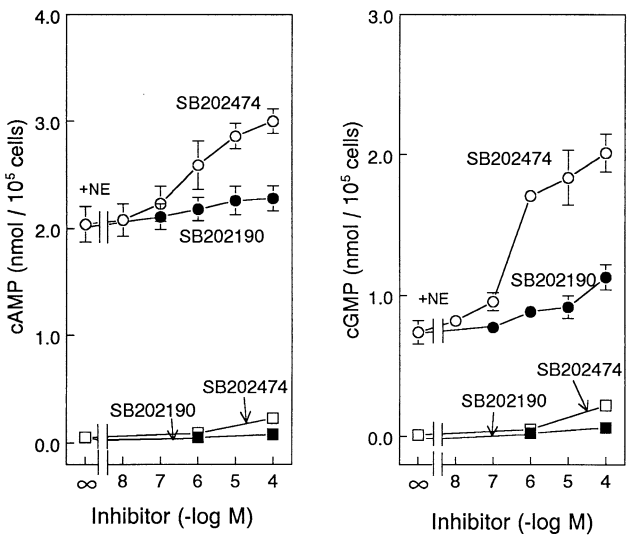

Fig. 2. Effects of p38MAPK inhibitors on NE-stimulated cAMP and cGMP accumulation. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with NE (10 μM) for 15 min in the presence or absence of various concentrations of SB202474 or SB202190. Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. The absence of an error bar indicates that the error was smaller than the size of the symbol.

increase in the ISO-stimulated cAMP accumulation at 100 μM ($P < 0.05$) and the cGMP accumulation at concentrations above 10 μM ($P < 0.05$). However, co-treatment with SB202474 (1–100 μM), the inactive p38MAPK inhibitor [3], caused a greater potentiation of the ISO-stimulated cAMP and cGMP accumulation with a significant increase observed at 1 μM ($P < 0.05$) (Fig. 1). At 100 μM, SB202474 potentiated the ISO-stimulated cAMP accumulation by about 65% and the corresponding cGMP accumulation by 5-fold (Fig. 1). When pinealocytes were stimulated with NE (10 μM), a mixed α- and β-adrenergic agonist, SB202190 and SB202474 (10 nM to 100 μM) also potentiated the cAMP and cGMP accumulation in a concentration-dependent manner, with SB202474 being more potent than SB202190 (Fig. 2). Although SB202190 (100 μM) alone had no effect on basal cyclic nucleotide contents, SB202474 (100 μM) caused a small increase in basal cAMP and cGMP accumulation ($P < 0.05$) (Fig. 2).

The effects of various concentrations of NE on cAMP and cGMP accumulation were determined in the presence or absence of SB202474 (10 μM) (Fig. 3). SB202474 was effective in potentiating the NE (10 nM to 100 μM)-stimulated cAMP and cGMP accumulation ($P < 0.05$ for NE concentrations between 1 and 100 μM) (Fig. 3). SB202474 further increased the maximal NE (100 μM)-stimulated cAMP and cGMP responses by 30 and 150%, respectively ($P < 0.05$). The effect of p38MAPK inhibitors on the time-course of NE-stimulated cAMP production also was investigated. Treatment with NE (10 μM) resulted in 50- and 100-fold increases in cAMP and cGMP levels, respectively, and both responses peaked at around 10 min (Fig. 4). Concurrent treatment with SB202474 (10 μM) potentiated NE-stimulated cAMP and cGMP accumulation with a significant increase in cAMP accumulation occurring between 10 and 60 min and in cGMP accumulation between 5 and 60 min ($P < 0.05$). Compared with SB202474, the potentiating effects of SB202190 (10 μM) on the time-course of the

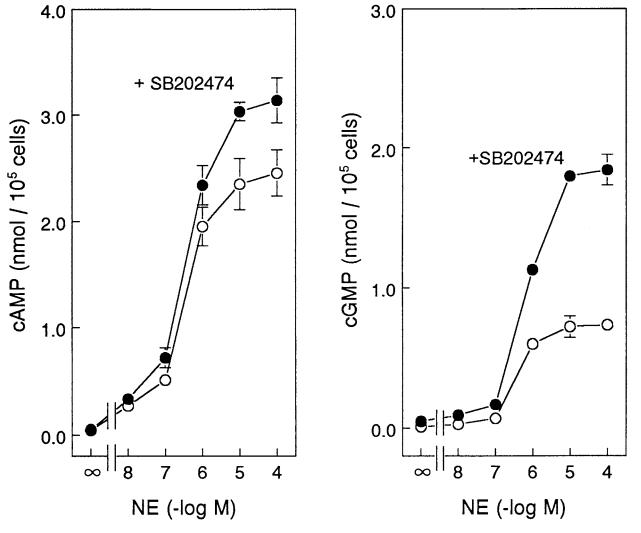

Fig. 3. Effects of p38MAPK inhibitors on the concentration–response of NE-stimulated cAMP and cGMP accumulation. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with various concentrations of NE in the presence or absence of SB202474 (10 μM). Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. The absence of an error bar indicates that the error was smaller than the size of the symbol.

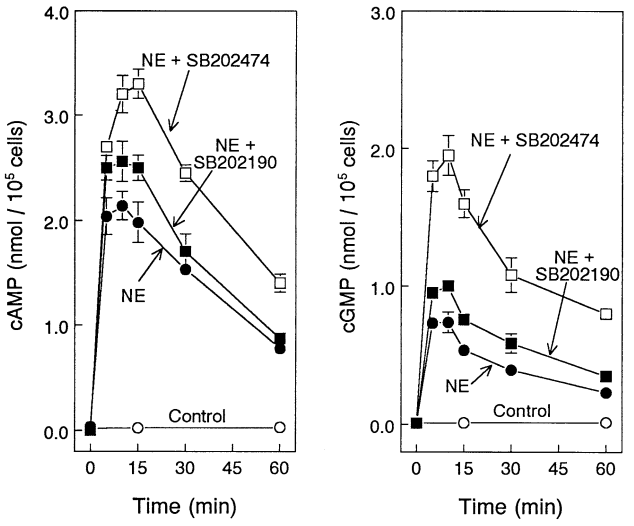

Fig. 4. Effects of p38MAPK inhibitors on the time-course response of NE-stimulated cAMP and cGMP accumulation. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with NE (10 μM) for the indicated period of time in the presence or absence of SB202474 (10 μM) or SB202190 (10 μM). Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. The absence of an error bar indicates that the error was smaller than the size of the symbol.

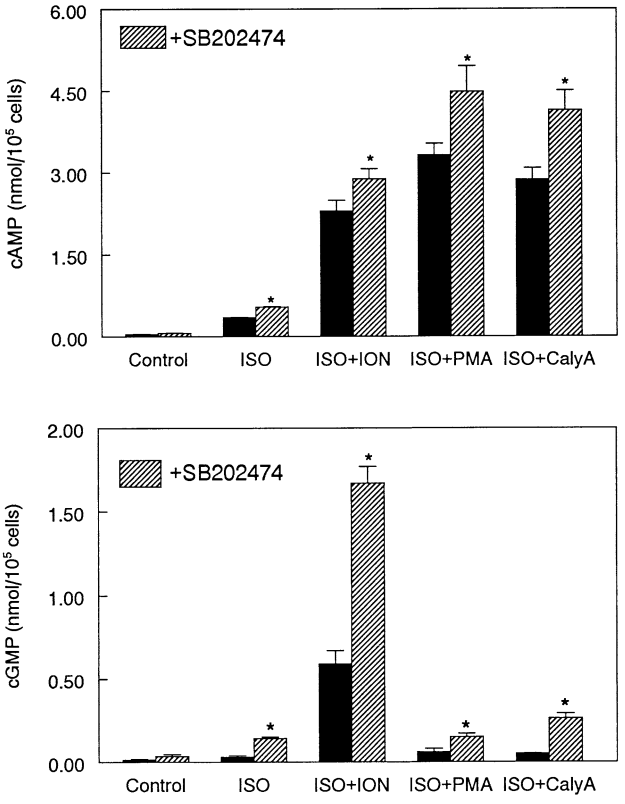

Fig. 5. Effect of SB202474 on cAMP and cGMP accumulation stimulated by the combination of ISO and established potentiating agents. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with ISO (1 μM) in the presence or absence of ionomycin (ION, 1 μM), PMA (0.1 μM), or calyculin A (CalyA, 0.1 μM) for 15 min in the presence or absence of SB202474 (1 μM). Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. Key: (*) significantly different from the corresponding treatment without SB202474, $P < 0.05$.

NE-stimulated cAMP and cGMP accumulation were smaller (Fig. 4).

3.2. Effects of p38MAPK inhibitors on the potentiation of the ISO-stimulated cAMP and cGMP accumulation by PMA, ionomycin, and calyculin A

Previous studies have shown that the ISO-stimulated cyclic nucleotide responses can be potentiated by elevation of intracellular Ca^{2+}, activation of PKC, or inhibition of serine/threonine phosphatase activity [7–10]. As shown in Fig. 5, elevation of Ca^{2+} by ionomycin (1 μM), activation of PKC by PMA (0.1 μM), or inhibition of phosphatase by calyculin A (0.1 μM) was effective in enhancing the ISO-stimulated cAMP and cGMP accumulation as reported previously [7–9]. When the three known potentiating pathways were maximally activated by pharmacological agents, SB202474 remained effective in enhancing the cyclic nucleotide responses (Fig. 5). These results suggest that these three potentiating pathways are unlikely to be involved in the enhancing effects of SB202474 on the cyclic nucleotide responses.

3.3. Effects of IL-1β on basal and ISO-stimulated cAMP and cGMP accumulation

IL-1β has been shown to activate the p38MAPK signaling pathway in other tissues [3], and there is an abundant expression of IL-1β and its specific receptor in the rat pineal gland [17]. In rat pinealocytes, we have shown previously that IL-1β has an inhibitory effect on the L-type Ca^{2+} channel current [18]. Therefore, we examined whether IL-1β had an effect on basal or ISO-stimulated cAMP and cGMP accumulation. It was found that IL-1β had no effect on basal or ISO-stimulated cAMP and cGMP accumulation (Table 1), suggesting that activation of the p38MAPK pathway has no effect on stimulated cyclic nucleotide responses.

3.4. Effect of PDE inhibition on SB202474-mediated potentiation of cyclic nucleotide production in rat pinealocytes

To determine whether the effects of p38MAPK inhibitors were due to inhibition of metabolism of cyclic nucleotides, the effects of these inhibitors on NE- and ISO-stimulated cyclic nucleotide accumulation were determined in the presence of a PDE inhibitor, IBMX. IBMX (1 mM) significantly ($P < 0.05$) enhanced basal and NE-stimulated cAMP and cGMP accumulation (Fig. 6). In the presence of IBMX, SB202474 (10 μM) did not have an additional effect

Table 1
Effect of IL-1β on basal and ISO-stimulated cAMP and cGMP accumulation

Treatment	cAMP accumulation (pmol/100,000 cells)	cGMP accumulation (pmol/100,000 cells)
Control	12 ± 1.5	3.5 ± 0.5
IL-1β (10 ng/mL)	13 ± 1.8	4.1 ± 0.6
ISO (1 μM)	315 ± 11.5*	20.5 ± 1.5*
ISO (1 μM) + IL-1β (10 ng/mL)	330 ± 12.9*	22.9 ± 1.5*

Pinealocytes were incubated with different treatments for 15 min; cells were pelleted, and cAMP and cGMP were determined by RIA. Each value is the mean ± SEM of the amount of cAMP and cGMP in quadruplicate samples.

* Significantly different from control, $P < 0.05$.

on NE-stimulated cAMP or cGMP accumulation (Fig. 6), suggesting that the effect of SB202474 on cyclic nucleotide accumulation is likely mediated through inhibition of PDE activity.

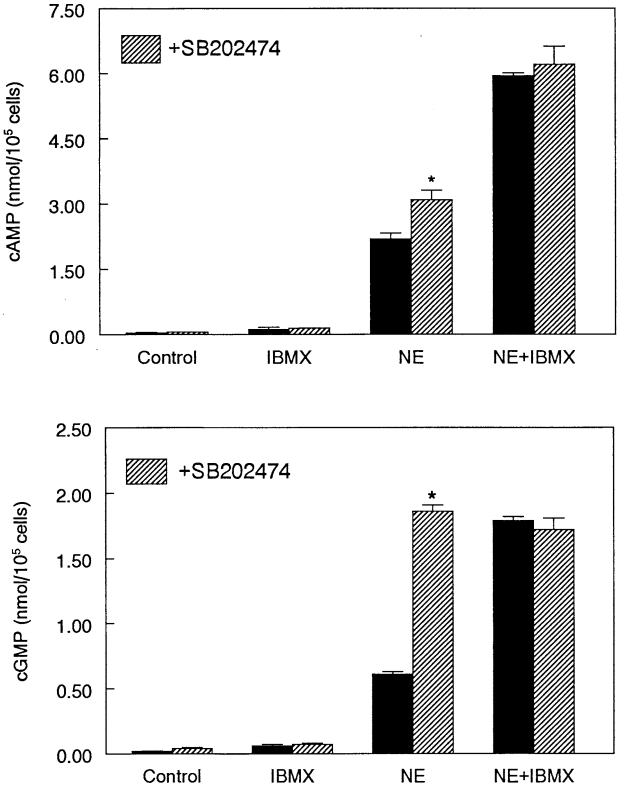

Fig. 6. Effect of SB202474 on cAMP and cGMP accumulation stimulated by NE in the presence of maximal phosphodiesterase inhibition. Pinealocytes (2×10^4 cells/0.4 mL) were incubated in DMEM and stimulated with IBMX (1 mM), NE (10 μM), or NE (10 μM) + IBMX (1 mM) in the presence or absence of SB202474 (1 μM) for 15 min. Cellular levels of cAMP and cGMP were determined by RIA, and each value is the mean ± SEM from four samples of cells. Key: (*) significantly different from the corresponding treatment without SB202474, $P < 0.05$.

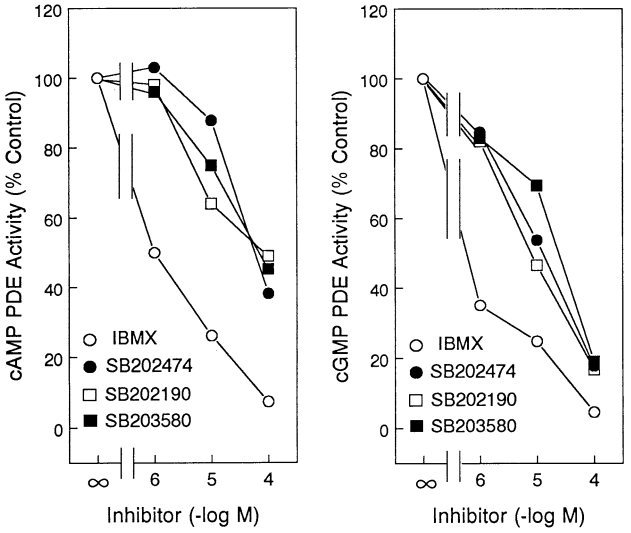

Fig. 7. Effects of p38MAPK inhibitors on cAMP- and cGMP-PDE activities. Cytosolic cAMP- and cGMP-PDE activities from pineal glands were determined in the presence or absence of various concentrations of SB202474, SB202190, and SB203580 as indicated. The results are expressed as a percentage of PDE activities determined in the presence of DMSO but without any p38MAPK inhibitors. One hundred percent cAMP-PDE activity = 73 ± 5 nmol/min/mg protein, and 100% cGMP-PDE activity = 132 ± 10 nmol/min/mg protein. Each data point is the mean from four separate determinations, and the SEM was less than 2% in all cases.

3.5. Effects of p38MAPK inhibitors on cAMP- and cGMP-PDE activities

All three compounds, SB202474, SB202190, and SB203580, were effective in inhibiting *in vitro* pineal cAMP- and cGMP-PDE activities (Fig. 7). At 100 μM, all three compounds inhibited cAMP-PDE activity by about 50% and cGMP-PDE activity by about 80%, respectively (Fig. 7), suggesting that the cGMP-PDE in the rat pineal gland is more sensitive to inhibition by p38MAPK inhibitors than the cAMP-PDE. In the presence of Ca^{2+} and CM, pineal cAMP- and cGMP-PDE activities were about 30% higher (Table 2). Under this condition, SB202474, SB202190, and SB203580 inhibited cAMP-PDE activity by about 55% and cGMP-PDE activity by about 80% (Table 2), suggesting that the three pyridinyl imidazole compounds likely act as a general PDE inhibitor.

4. Discussion

Since the discovery of p38MAPK as a new member of the MAPK superfamily, significant advances have been made in respect to its mechanism of activation and the role it plays in apoptosis, cytokine production, and cell differentiation [1,2]. These investigations have been facilitated by the discovery of specific p38MAPK inhibitors [3,4]. The two pyridinyl imidazole compounds, SB202190 and

Table 2
Effects of p38MAPK inhibitors on cAMP- and cGMP-PDE activities in the absence and presence of Ca^{2+}/CM

Treatment	cAMP-PDE activity (nmol/min/mg protein)		cGMP-PDE activity (nmol/min/mg protein)	
	$-Ca^{2+}$/CM	$+Ca^{2+}$/CM	$-Ca^{2+}$/CM	$+Ca^{+2}$/CM
Control	70.1 ± 5.3	91.5 ± 8.2	141.5 ± 10.3	192.5 ± 14.3
SB202474 (100 μM)	28.3 ± 3.9*	37.1 ± 4.8*	30.5 ± 3.6*	41.2 ± 3.4*
SB202190 (100 μM)	31.8 ± 4.5*	39.1 ± 5.8*	34.4 ± 2.9*	45.3 ± 2.5*
SB203580 (100 μM)	32.5 ± 5.4*	40.1 ± 3.8*	35.0 ± 3.7*	42.3 ± 3.6*

cAMP- and cGMP-PDE activities of the cytosolic fractions of pinealocytes were determined in the presence of p38MAPK inhibitors with or without Ca^{2+} (1 mM) and CM (10 μM). Each value is the mean ± SEM of determinations done in duplicate from at least three separate experiments. For further details see "Materials and methods."

* Significantly different from control, $P < 0.05$.

SB203580, were originally prepared as inhibitors of inflammatory cytokine synthesis [3]. They were subsequently found to be selective inhibitors of p38MAPK and had no effect on other members of the MAPK superfamily [4]. SB202474, a structurally related compound that does not inhibit p38MAPK, has been used as a negative control in investigating and confirming the function of p38MAPK [3].

In this study, we found that all three pyridinyl imidazole compounds, SB202190, SB203580, and the putative inactive compound, SB202474, were effective in potentiating adrenergic-stimulated cAMP and cGMP accumulation in rat pinealocytes. Of the three compounds, SB202474, the inactive p38MAPK inhibitor, was found to be more potent than the two active p38MAPK inhibitors in potentiating the effect. These findings suggest that the potentiating effects of the pyridinyl imidazole compounds are likely due to pharmacological actions of these compounds that are unrelated to their inhibition of p38MAPK activity.

The mechanism that mediates the potentiating effect of the pyridinyl imidazole compounds appears to be independent from the established mechanisms known to potentiate adrenergic-stimulated cAMP and cGMP accumulation. This is based on the observations that SB202474 remains effective in enhancing the cyclic nucleotide responses even when the three potentiating pathways are maximally activated by pharmacological agents including ionomycin (elevation of intracellular calcium), PMA (activation of PKC), and calyculin A (inhibition of phosphatase). In contrast, the effect of SB202474 was abolished in the presence of IBMX, a PDE inhibitor, suggesting that PDE is a probable site of action of the pyridinyl imidazole compounds. This is supported by *in vitro* studies that showed inhibition of cAMP- and cGMP-PDE activities by all three compounds. Moreover, there was a greater inhibition of the cGMP-PDE than the cAMP-PDE activity by the three compounds, thus providing an explanation for their greater potentiation of the cGMP than the cAMP response. These results are also consistent with our previous observations that the cGMP response is more sensitive than the cAMP response to PDE inhibition [11,12]. Furthermore, the inhibitory effects of the three compounds on cAMP- and cGMP-PDE activities are not influenced by the presence of Ca^{2+} and CM, suggesting that these compounds likely act as a general PDE inhibitor.

The specificity of pyridinyl imidazole compounds, in particular SB203580, towards a wide range of protein kinases has been examined. At micromolar concentrations, SB203580 is specific for p38MAPK, but at 100- to 500-fold higher concentrations, SB203580 also inhibits other kinases such as lymphocyte kinase, glycogen synthase kinase 3β, and protein kinase B-α [19], indicating that among different kinases, these compounds are highly specific for p38MAPK. However, our current study indicates that these compounds may have an additional effect towards enzymes other than kinases. Considering that these compounds inhibit the catalytic activity of p38MAPK by competitive binding in the ATP pocket of the enzyme [20], it is not surprising that they also interact with other enzymes that act on nucleotides such as PDE. Although a direct inhibition of cAMP- and cGMP-PDE activities could account for the potentiating effects of these compounds on the cyclic nucleotide responses, it fails to explain the greater potentiation caused by SB202474 than the other two pyridinyl imidazole compounds. The *in vitro* inhibitory potencies of the three compounds against cAMP- and cGMP-PDE activities were similar.

At present, it is not possible to fully explain why SB202474 is more potent than SB203580 or SB202190 in enhancing the cyclic nucleotide responses in intact cells. One possible explanation is that because of the structural differences, SB202474 may attain a higher cellular concentration. Alternatively, SB202474 may be more effective than the other compounds in inhibiting PDE activities under *in vivo* conditions, and this difference could not be detected in broken cell preparations in which the conditions for the enzymatic reaction have been optimized.

Both SB202190 and SB203580 were originally prepared as inhibitors of inflammatory cytokine synthesis [3]. An earlier study indicated that these compounds had no effect on cAMP levels in human monocytes alone or in the presence of agents known to increase cytokine production such as lipopolysaccharide, indicating that they likely act through pathways that are independent of cAMP elevating mecha-

nisms [21]. In contrast, in this study, we found that these compounds were effective in potentiating adrenergic-stimulated cyclic nucleotide production in rat pinealocytes, an effect likely related to inhibition of cAMP- and cGMP-PDE activities. This difference in the effects of pyridinyl imidazole compounds on cyclic nucleotide levels could be related to different cell types or alternatively to whether cyclic nucleotide has been stimulated. Although these compounds are effective in potentiating adrenergic-stimulated cyclic nucleotide production in rat pinealocytes, IL-1β, a cytokine highly expressed in rat pinealocytes [17], had no effect on basal or ISO-stimulated cAMP and cGMP production. However, IL-1β is effective in activating p38MAPK in rat pinealocytes (our unpublished observation), suggesting that the potentiating effects of these compounds on cyclic nucleotide production are unlikely to be related to inhibition of p38MAPK.

In summary, all three pyridinyl imidazole compounds were effective inhibitors of cAMP- and cGMP-PDE activities in rat pinealocytes. Since only SB203580 and SB202190 but not SB202474 are selective inhibitors of p38MAPK, their effects on the adrenergic-stimulated cyclic nucleotide are unlikely to be related to inhibition of p38MAPK. Therefore, our results underscore the importance of considering p38MAPK-independent action when these compounds are used as pharmacological tools to establish the involvement of p38MAPK.

Acknowledgments

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