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SB203580 reverses adrenomedullin's effect on proliferation and apoptosis in cultured mesangial cells

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Abstract

Adrenomedullin is a potent vasodilatory peptide that has a variety of effects in a number of different systems including kidney. In cultured rat glomerular mesangial cells adrenomedullin increases cAMP, decreases proliferation and increases apoptosis. Associated with the anti-proliferative and apoptotic effects, adrenomedullin also causes a decrease in extracellular signal-regulated kinase2 (ERK2) and an increase in cJun N-terminal kinase1 (JNK1) and P38 mitogen-activated protein kinase (P38 MAPK) activities. The purpose of the present study was to examine the role of P38 MAPK on adrenomedullin-mediated inhibition of [^{3}H]thymidine incorporation (an index of proliferation) and on adrenomedullin-stimulated nucleosome-associated cytoplasmic DNA fragmentation (an index of apoptosis) in mesangial cells, using a selective inhibitor of P38 MAPK, SB203580 {[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole}, and also to characterize the proximal signal transduction pathways of the three MAPKs in relation to [^{3}H]thymidine incorporation and cytoplasmic DNA fragmentation using a phosphotidyl inositol-3-kinase inhibitor, wortmannin {[1*S*-(1α,6*b*α,9*a*β, 11α,11*b*β)]-11-(acetyloxy)-1,6*b*,7,8,9*a*,10,11,11*b*-octahydro-1-(methoxymethyl)-9*a*,11*b*-dimethyl-3*H*-furo[4,3,2-de]indeno[4,5-*h*]-2-benzopyran-3,6,9-trione}. SB203580 significantly reversed the effects of adrenomedullin on [^{3}H]thymidine incorporation and cytoplasmic DNA fragmentation, and inhibited only P38 MAPK activity. It had no effect on ERK2 and JNK1 activities. Wortmannin, on the other hand, inhibited only adrenomedullin-stimulated cytoplasmic DNA fragmentation and did not affect adrenomedullin-mediated inhibition of [^{3}H]thymidine incorporation. Wortmannin also inhibited adrenomedullin-stimulated P38 MAPK activity without affecting ERK2 and JNK1 activities. These results indicate that: (a) In rat mesangial cells adrenomedullin-mediated inhibition of [^{3}H]thymidine incorporation and stimulation of nucleosome-associated cytoplasmic DNA fragmentation are sensitive to SB203580, and (b) adrenomedullin activates a P38 MAPK through a wortmannin-sensitive kinase. The data using SB203580 suggest an important physiological role for P38 MAPK in rat mesangial cell proliferation and apoptosis.

Keywords: Proliferation; Mesangial cell; Apoptosis; P38 MAPK (P38 mitogen-activated protein kinase); ERK (extracellular signal-regulated kinase); JNK (cJun N-terminal kinase)

1. Introduction

Adrenomedullin, a derivative of proadrenomedullin, is a 52 amino acid peptide and is a potent vasodilator and natriuretic factor. Discovered in 1993, it is thought to belong to the calcitonin gene-related peptide (CGRP) superfamily (Kitamura et al., 1993; Sakata et al., 1993). Since its initial discovery, a number of reports have appeared describing the physiological and pharmacological actions of adrenomedullin, both in animal and cell culture models (Chini et al., 1997; Ebara et al., 1994; Gardiner et al., 1995; Haynes and Cooper, 1995). In all systems studied so far, adrenomedullin has been shown to activate adenylate cyclase resulting in the accumulation of cAMP (Kohno et al., 1995; Osajima et al., 1996; Chini et al., 1995, 1997). In some cellular systems, adrenomedullin receptor is also coupled to phosphotidyl inositol hydrolysis (Shimekake et al., 1995). In glomerular mesangial cells adrenomedullin activates adenylate cyclase without any increase in phosphotidyl inositol hydrolysis (Osajima et al., 1996).

Mesangial cell proliferation is a common feature in chronic renal diseases such as glomerulonephritis (Klahr et

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al., 1988; Floege et al., 1993; El Nahas et al., 1997) and, therefore, understanding the mechanism of mesangial cell turnover is critical for our understanding of the role of mesangial cells in the development of chronic renal disease. Although a number of agents have been shown to have proliferative and antiproliferative effects, the mechanisms of action of these agents, however, have not been completely characterized. Adrenomedullin is one such peptide that has been shown recently to have an anti-proliferative effect in mesangial cells (Chini et al., 1997). We have also reported an apoptotic effect of adrenomedullin in rat mesangial cells (Parameswaran et al., 1999). The purpose of the present study was to delineate the mechanisms of these two responses.

Stimulation of a receptor leading to the activation of second messenger systems has been shown to be coupled to divergent intracellular signaling pathways including the MAPK pathway. Three parallel MAPK pathways are currently known. They are the extracellular signal-regulated kinase (ERK), cJun N-terminal kinase (JNK) and P38 mitogen-activated protein kinase (P38 MAPK) pathways. Although these MAPKs are stimulated through a kinase cascade, the exact nature of the proximal signaling event depends on the cell system as well as the ligand (Denhardt, 1996; Gutkind, 1998). In mesangial cells, adrenomedullin receptor is coupled to all the three pathways differently, that is, adrenomedullin causes a decrease in ERK and an increase in JNK and P38 MAPK activities. Only the decrease in ERK and the increase in P38 MAPK are protein kinase-A mediated (Parameswaran et al., 1999). The aims of the present study were (1) to study the involvement of the MAPK pathways modulated by adrenomedullin specifically, P38 MAPK, in adrenomedullin-mediated inhibition of proliferation (using [^{3}H]thymidine incorporation as an index) and stimulation of apoptosis (using nucleosome-associated cytoplasmic DNA fragmentation as an index) and (2) to characterize the proximal signaling pathways, using pharmacological inhibitors of specific signaling molecules. In order to understand the role of MAPKs as well as phosphotidyl inositol-3-kinase in adrenomedullin-mediated apoptosis and inhibition of proliferation, we used two inhibitors of signaling molecules, SB203580 {[4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1*H*-imidazole}, a P38 MAPK inhibitor (Lee et al., 1994) and wortmannin {[1*S*-(1α,6*b*α,9*a*β,11α,11*b*β)]-11-(acetyloxy)-1,6*b*,7,8,9*a*,10,11,11*b*-octahydro-1-(methoxymethyl)-9*a*,11*b*-dimethyl-3*H*-furo[4,3,2-de]indeno[4,5-*h*]-2-benzopyran-3,6,9-trione}, a phosphotidyl inositol-3-kinase inhibitor (Ui et al., 1995). SB203580 preferentially inhibits P38 MAPK without affecting other kinases significantly (Lee et al., 1994; Young et al., 1997). Wortmannin is a potent, selective, cell permeable and irreversible inhibitor of phosphotidyl inositol-3-kinase (IC_{50} 1–10 nM). At 100 fold higher concentrations, it has also been shown to inhibit phosphotidyl inositol-4-kinase, myosin light chain kinase (MLCK) and phospholipase-D (Bonser et al., 1991; Nakanishi et al., 1992; Ui et al., 1995). Wortmannin was also shown to inhibit a novel isoform of phosphotidyl inositol-3-kinase with a lower potency (IC_{50} of around 200–300 nM) (Stephens et al., 1994).

The results of this study indicate a role for P38 MAPK in adrenomedullin-mediated mesangial cell turnover. We demonstrate for the first time that the inhibition of P38 MAPK but not JNK or ERK, by SB203580 reverses the effect of adrenomedullin on [^{3}H]thymidine incorporation and nucleosome-associated cytoplasmic DNA fragmentation. Furthermore we also show the possible presence of a wortmannin-sensitive kinase upstream of a P38 MAPK but not of ERK or JNK. Wortmannin affects only adrenomedullin-stimulated nucleosome-associated cytoplasmic DNA fragmentation but does not alter adrenomedullin-mediated inhibition of proliferation. These data indicate differential regulatory mechanisms controlling adrenomedullin-mediated changes on mesangial cell proliferation and apoptosis.

2. Materials and methods

2.1. Materials

Adrenomedullin was purchased from phoenix pharmaceuticals (Belmont, CA), myelin basic protein, from sigma (St. Louis). Polyclonal anti-ERK2, anti-P38 MAPK and anti-JNK1 antibodies were purchased from Santa Cruz laboratories (Santa Cruz, CA). GST-cJun was purchased from Alexis Biochemicals (San Diego, CA). RPMI-1640, fetal bovine serum, penicillin and streptomycin were from Gibco (Grand Island, NY). SB203580 was a kind gift from

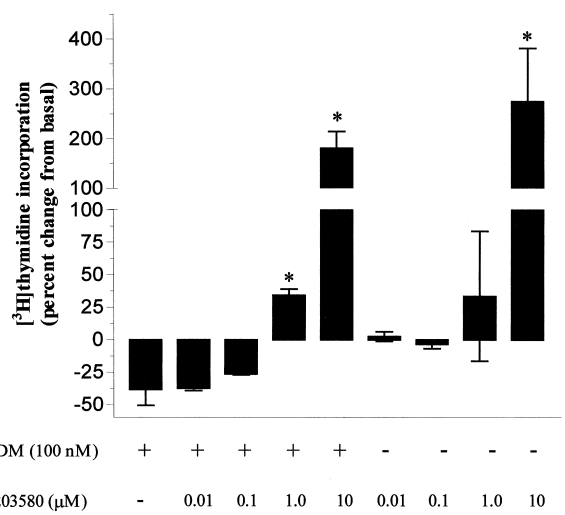

Fig. 1. Effect of adrenomedullin (ADM) and SB203580 (P38 MAPK inhibitor) on [^{3}H]thymidine incorporation in rat mesangial cells. Experiment was done as described in Section 2. Cells were pretreated with the inhibitor for a period of 30 min before the addition of adrenomedullin. ADM caused a significant decrease in [^{3}H]thymidine incorporation (n = 3). * $P < 0.01$ compared to ADM.

Dr. John Lee, Smithkline Beecham Pharmaceuticals. Wortmannin was from Calbiochem. All other reagents were of high quality available.

2.2. Cell culture

Rat mesangial cells were obtained from the glomeruli of kidney cortex isolated from Sprague Dawley rats as described before (Albrightson et al., 1992), and were grown in RPMI-1640 with 15% fetal bovine serum. Passages between 15 and 30 were used for the experiments.

2.3. [^{3}H]-Thymidine incorporation

Cells were plated in 24 well plates at 30 000 cells/well and grown for 2 days, after which they were serum starved for 48 h. Cells were then treated with the test compounds for a period of 16 h and pulsed with [^{3}H]thymidine (1 μCi/ml) for 4 h. The radioactivity was counted in Beckman LS counter, after washing the cells and stopping the reaction with 5% trichloro acetic acid and solubilising the cells in 0.5 ml of 0.25 N sodium hydroxide. Each experiment was done in quadruplicates and was repeated at least 3 times.

2.4. Kinase assays

Cells were plated in p100 plates and were serum-starved overnight on reaching confluency. The agonist solutions were prepared in the growth media without serum. Cells were treated with the agonists for 30 min. In experiments where inhibitors were used, the cells were pretreated with the inhibitor for a period of 30 min before adrenomedullin treatment. The time points and experimental protocols

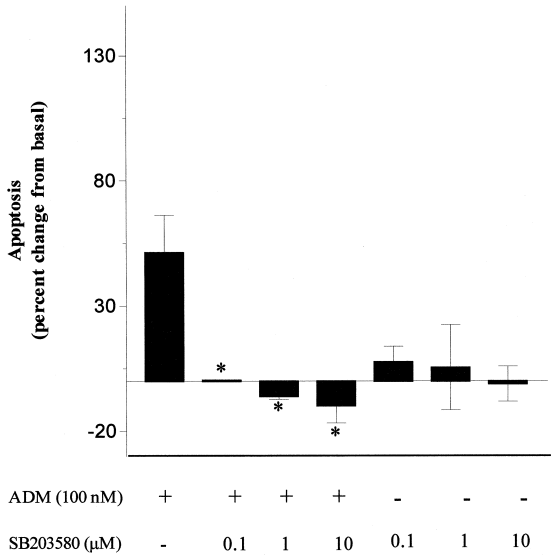

Fig. 3. Effect of adrenomedullin (ADM) and SB203580 (P38 MAPK inhibitor) on cytoplasmic nucleosome-associated DNA fragmentation (an index of apoptosis) in rat mesangial cells. Experiment was done as described in Section 2. Cells were pretreated with the inhibitor for a period of 30 min before the addition of adrenomedullin. ADM caused a significant increase in DNA fragmentation. SB203580 by itself did not affect DNA fragmentation at any of the concentrations tested ($n = 3$). $^*P < 0.01$ compared to ADM.

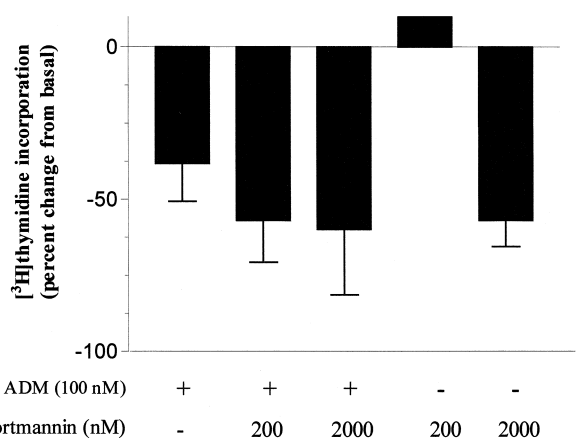

Fig. 2. Effect of adrenomedullin (ADM) and wortmannin (PI-3 kinase inhibitor) on [^{3}H]thymidine incorporation in rat mesangial cells. Experiment was done as described in Section 2. Cells were pretreated with the inhibitor for a period of 30 min before the addition of adrenomedullin. (ADM caused a significant decrease in [^{3}H]thymidine incorporation.) Effect of wortmannin was not significantly different from ADM treatment ($n = 3$).

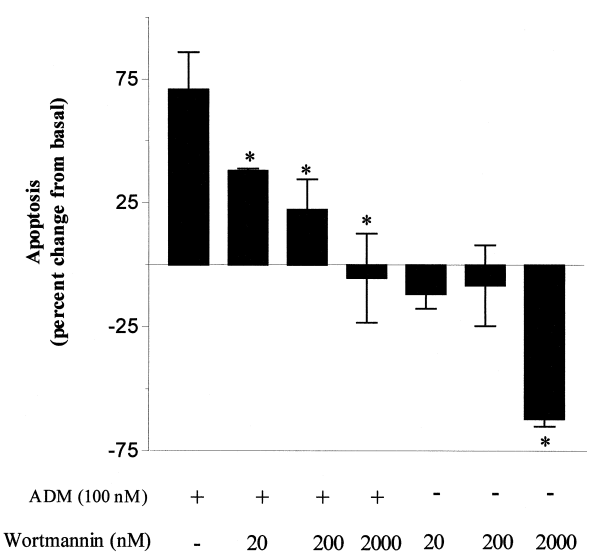

Fig. 4. Effect of adrenomedullin (ADM) and wortmannin (PI3 kinase inhibitor) on cytoplasmic nucleosome-associated DNA fragmentation (an index of apoptosis) in rat mesangial cells. Experiment was done as described in Section 2. Cells were pretreated with the inhibitor for a period of 30 min before the addition of adrenomedullin. ADM caused a significant increase in DNA fragmentation ($n = 4$). $^*P < 0.01$ compared to ADM.

were similar to that described before (Parameswaran et al., 1999). The cell lysates were prepared as described (Bogoyevitch et al., 1995; Li et al., 1995). In the meantime specific antibodies (10 μg/reaction) were incubated with protein A agarose (Gibco) for 30 min at room temperature. After normalizing for protein concentration, the cell lysates were incubated with the specific antibody agarose conjugate for 2 h at 4°C with constant shaking. The kinase assays were done after washing the immunoprecipitate three times with HNTG (20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol) buffer and two times with kinase buffer (50 mM Tris–HCl, 100 mM NaCl, 10 mM $MnCl_2$ and 0.1 mM sodium *ortho*-vanadate). The functional assay was done in the presence of 50 μM ATP, 5 μCi γ [^{32}P]ATP, 10 μg of specific substrate (myelin basic protein (MBP) for ERK2 and P38 MAPK, and glutathione-*S*-transferase–c-Jun (GST-cJun) for JNK1), and the immunoprecipitate. The reactions were performed at 30°C for 15 min and then stopped with sodium dodecyl sulphate buffer. The samples were electrophoresed on 12% polyacrylamide gel with appropriate molecular weight standards. The gels were dried and subjected to phosphoimager plates. The intensity of the bands was visualized and quantitated using imagequant program.

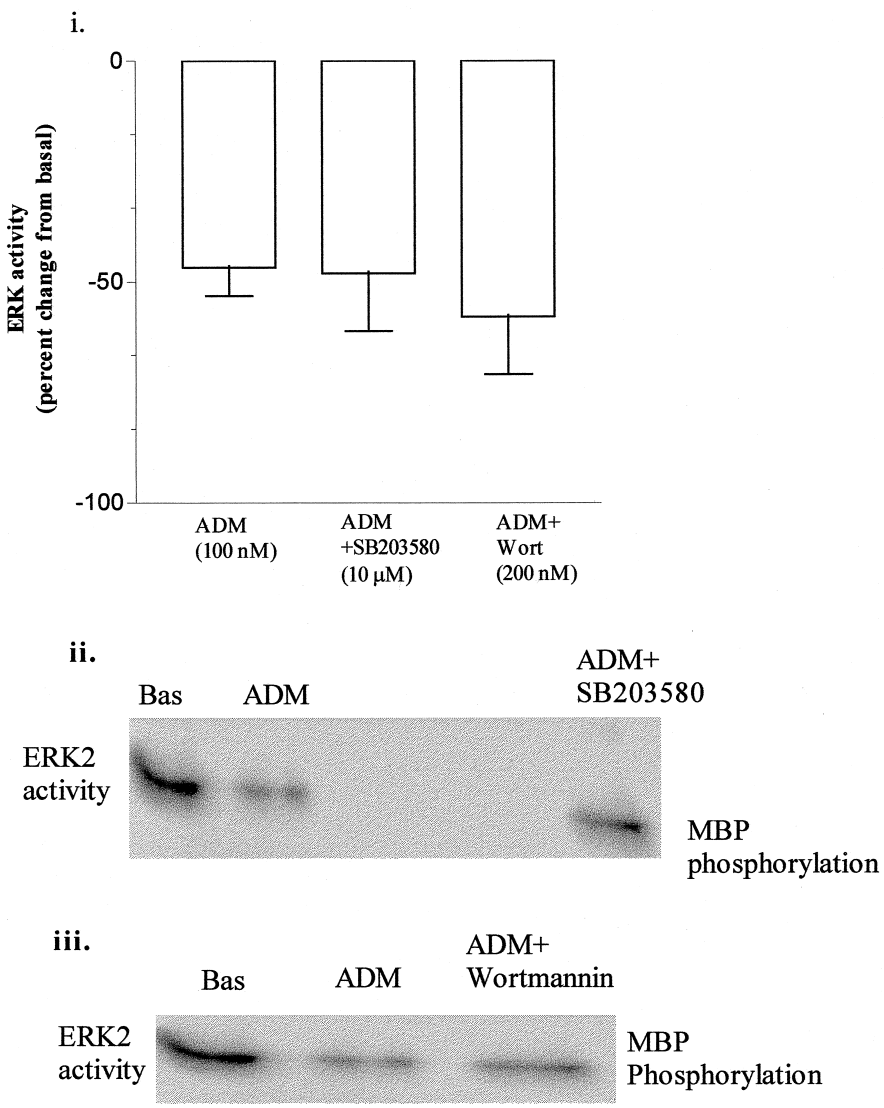

Fig. 5. The kinase assays were done after 30 min treatment with adrenomedullin (ADM). In experiments where inhibitors were used, the cells were pretreated for a period of 30 min before adrenomedullin treatment. (i) Effect of ADM, SB203580 and wortmannin on ERK2 activity in rat mesangial cells ($n = 3$). SB203580 or wortmannin alone did not affect ERK2 activity. Adrenomedullin decreased ERK2 activity significantly below basal levels as measured by immuno-complex assay with specific anti-ERK2 antibody using MBP as substrate. (ii) A representative autoradiogram showing the effect of ADM and SB203580 on ERK2 activity in rat mesangial cells. (iii) A representative autoradiogram showing the effect of ADM and wortmannin on ERK2 activity in rat mesangial cells.

2.5. Enzyme linked immunosorbant assay (ELISA) for apoptosis

The ELISA kit was obtained from Boehringer Mannheim (Indianapolis, IN), which specifically detects the cytoplasmic nucleosomal DNA. For that, cells were plated in 48 well plates and after 24 h were serum starved for 24 h. Different agonists (prepared in the media) were added to the cells and incubated for another 20 h. The cells were lysed with the lysis buffer and centrifuged to separate cytoplasmic and nuclear fractions. The cytoplasmic fraction was then tested for DNA still attached to nucleosomes using the ELISA protocol from Boehringer Mannheim, Indianapolis, IN. The assay was done in triplicates or quadruplicates and repeated 3–5 times.

3. Results

3.1. [^{3}H]Thymidine incorporation

Exposure of rat mesangial cells to adrenomedullin resulted in a significant decrease in basal [^{3}H]thymidine incorporation ($-38.13 \pm 12.5\%$) (Fig. 1). SB203580 completely reversed adrenomedullin-mediated decrease in proliferation (Fig. 1). At higher concentrations (10 μM), SB203580 by itself caused a significant increase ($275 \pm 106\%$) in [^{3}H]thymidine incorporation (Fig. 1). However at 1 μM, SB203580 by itself did not increase proliferation consistently ($33 \pm 50\%$); but it completely reversed adrenomedullin-mediated decrease in [^{3}H]thymidine incorporation ($34 \pm 4.7\%$).

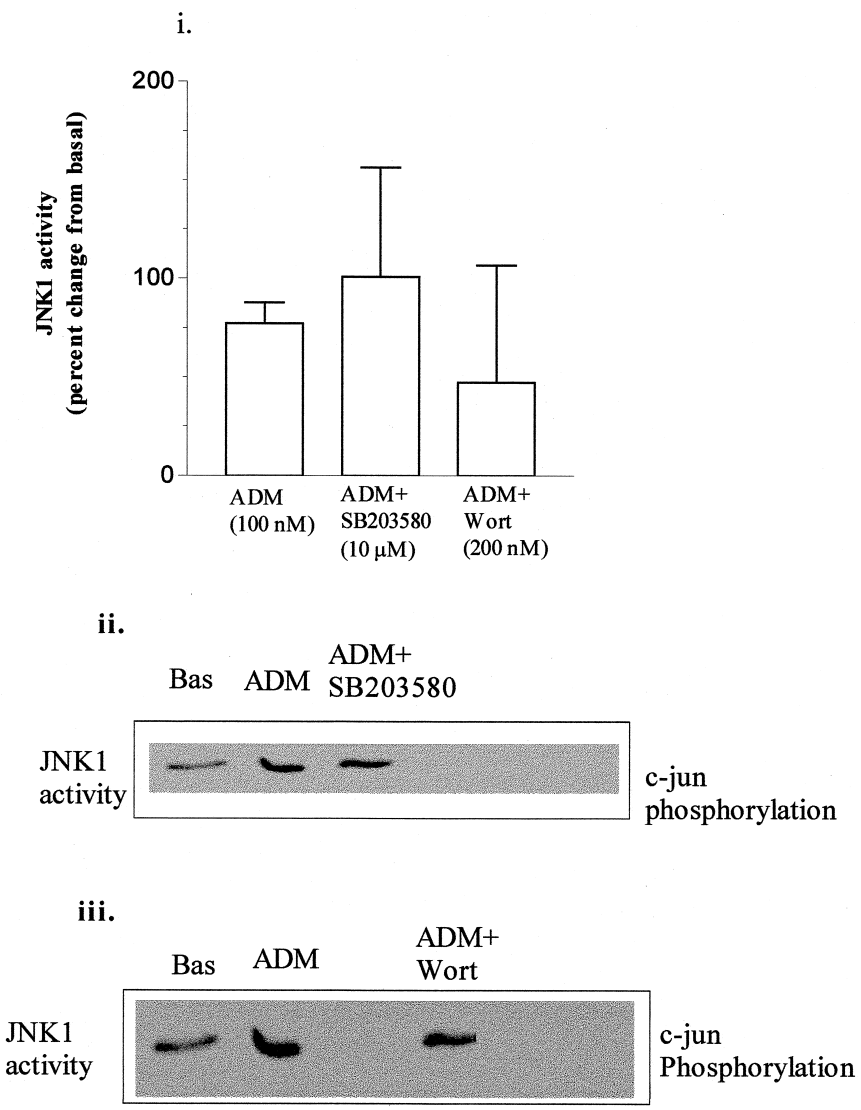

Fig. 6. The kinase assays were done after 30 min treatment with adrenomedullin (ADM). In experiments where inhibitors were used, the cells were pretreated for a period of 30 min before adrenomedullin treatment. (i) Effect of ADM, SB203580 and wortmannin on JNK1 activity in rat mesangial cells ($n = 3$). SB203580 or wortmannin alone did not affect JNK1 activity. Adrenomedullin increased JNK activity significantly above basal levels as measured by immuno-complex assay using specific anti-JNK1 antibody and GST-cJun as substrate. (ii) A representative autoradiogram showing the effect of ADM and SB203580 on JNK1 activity in rat mesangial cells. (iii) A representative autoradiogram showing the effect of ADM and wortmannin on JNK1 activity in rat mesangial cells.

Wortmannin, at concentrations known to be effective for inhibition of phosphotidyl inositol-3-kinase activity (100–200 nM), did not affect adrenomedullin-mediated inhibition of [^{3}H]thymidine incorporation (Fig. 2). At 200 nM, wortmannin by itself had no consistent effect on thymidine incorporation, but at higher concentrations (2 μM), it inhibited basal [^{3}H]thymidine incorporation in mesangial cells significantly ($-57 \pm 8.5\%$) (Fig. 2). In addition, at these concentrations, wortmannin also inhibited nucleosome-associated cytoplasmic DNA fragmentation (see below).

3.2. Cytoplasmic nucleosome-associated DNA fragmentation

Adrenomedullin caused a significant increase in nucleosome-associated cytoplasmic DNA fragmentation. Unlike [^{3}H]thymidine incorporation, adrenomedullin-mediated increase in cytoplasmic DNA fragmentation was inhibited by both inhibitors (SB203580 as well as wortmannin). SB203580 inhibited adrenomedullin-mediated increase in cytoplasmic DNA fragmentation significantly (even at 0.1 μM) with no significant effect on its own (Fig. 3). Wortmannin also inhibited adrenomedullin-mediated increase in cytoplasmic DNA fragmentation (Fig. 4). Although wortmannin by itself had no effect on basal apoptosis at 200 nM, it had a significant effect on basal apoptosis at 2 μM ($-62 \pm 3\%$) (Fig. 4).

3.3. MAPK pathway

Because of the differential effects observed with SB203580 and wortmannin on adrenomedullin-mediated proliferation and apoptosis, it was of interest to test the activities of various mitogen-activated protein kinases. The kinase assays were done after 30 min treatment with adrenomedullin. In experiments where inhibitors were used, the cells were pretreated for a period of 30 min before adrenomedullin treatment.

3.3.1. ERK2

Adrenomedullin decreased ERK2 activity significantly below basal levels as measured by immuno-complex assay with specific anti-ERK2 antibody using MBP as substrate (Fig. 5). The effect of adrenomedullin on ERK2 activity was not affected by both SB203580 and wortmannin (Fig. 5).

3.3.2. JNK1

Adrenomedullin increased JNK activity significantly above basal levels as measured by immuno-complex assay using specific anti-JNK1 antibody and GST-cJun as substrate (Fig. 6). Neither SB203580 nor wortmannin had any consistently significant effect on adrenomedullin-stimulated JNK1 activity (Fig. 6).

3.3.3. P38 MAPK

Adrenomedullin increased P38 MAPK activity significantly above basal levels as measured by immuno-complex assay using specific anti-P38 MAPK antibody and MBP as substrate (Fig. 7A). Only P38-MAPK activity was sensitive to SB203580. Similar to SB203580, wortmannin inhibited adrenomedullin-stimulated P38 MAPK activity (Fig. 7B).

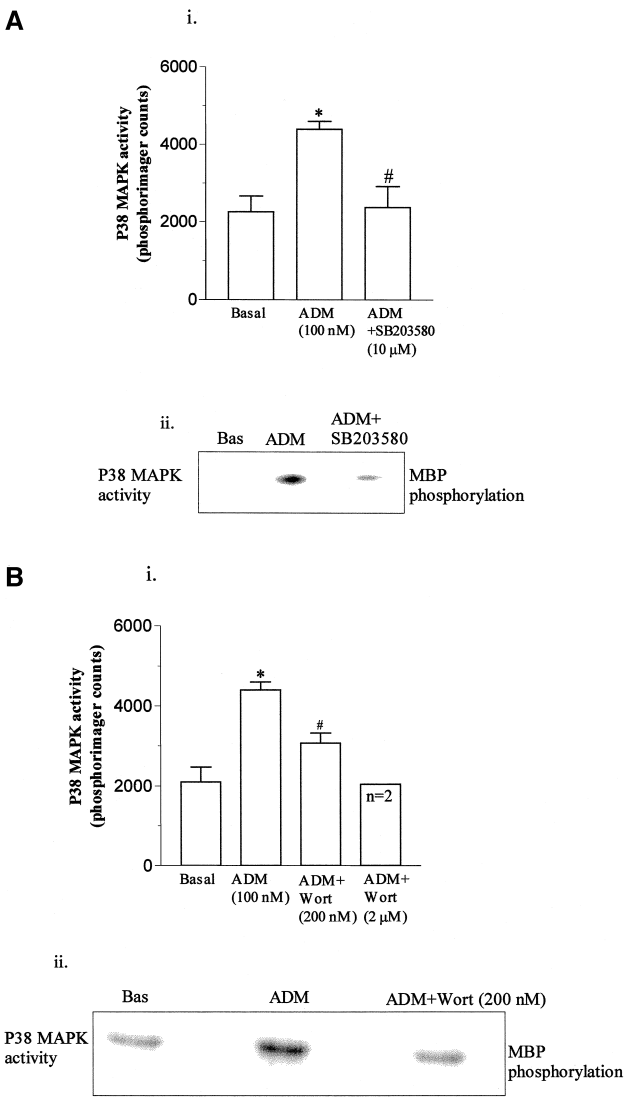

Fig. 7. The kinase assays were done after 30 min treatment with adrenomedullin (ADM). In experiments where inhibitors were used, the cells were pretreated for a period of 30 min before adrenomedullin treatment. (A.i) Effect of ADM and SB203580 on P38 MAPK activity in rat mesangial cells ($n = 4$). $^*P < 0.01$ compared to basal and $^{\#}P < 0.01$ compared to ADM. Adrenomedullin increased P38 MAPK activity significantly above basal levels as measured by immuno-complex assay using specific anti-P38 MAPK antibody and MBP as substrate. (A.ii) A representative autoradiogram showing the effect of ADM and SB203580 on P38 MAPK activity in rat mesangial cells. (B.i) Effect of ADM and wortmannin on P38 MAPK activity in rat mesangial cells ($n = 3$). $^*P < 0.01$ compared to basal and $^{\#}P < 0.01$ compared to ADM. (B.ii) A representative autoradiogram showing the effect of ADM and wortmannin (200 nM) on P38 MAPK activity in rat mesangial cells.

The inhibitors by themselves did not cause any significant change in the activities of these kinases within the time frame tested (data not shown).

4. Discussion

In mesangial cells, adrenomedullin caused a significant decrease in [^{3}H]thymidine incorporation (an index of proliferation) and an increase in cytoplasmic nucleosome-associated DNA fragmentation (an index of apoptosis). In addition, pretreatment of mesangial cells with adrenomedullin resulted in a decrease in ERK2 and an increase in JNK1 and P38 MAPK activities. SB203580, a P38 MAPK inhibitor completely reversed the effect of adrenomedullin on both proliferation and apoptosis, suggesting that adrenomedullin-stimulated P38 MAPK may be involved in adrenomedullin-mediated decrease in proliferation and increase in apoptosis. The fact that SB203580 did not affect either ERK2 or JNK1 activity, further indicates that the effect of this inhibitor is likely through inhibition of P38 MAPK. Recent reports using other cell lines have indicated a role for P38 MAPK in apoptosis (Wang et al., 1998). Although P38 MAPK has been shown to be involved in other biological responses such as cardiac myocyte hypertrophy, and cytokine secretion (Lee et al., 1994; Wang et al., 1998), this is the first demonstration of the involvement of P38 MAPK in adrenomedullin-mediated effects on mesangial cell proliferation. The fact that SB203580 by itself at high concentrations stimulated mesangial cell proliferation, suggests that a P38 MAPK is active basally that controls the normal turnover. This may have important implications in progressive renal diseases where aberrant proliferation of mesangial cells is a characteristic feature. It remains to be seen whether P38 MAPK activity is altered in the progression of such diseases.

Wortmannin, on the other hand, did not affect [^{3}H]thymidine incorporation that was inhibited by adrenomedullin but inhibited adrenomedullin-stimulated apoptosis at concentrations that are known to be selective for phosphotidyl inositol-3-kinase inhibition. The rationale for testing wortmannin was to identify the proximal signaling mechanisms of JNK and P38 MAPK activation. A number of reports have suggested recently that both these kinase pathways could be regulated by phosphotidyl inositol-3-kinase especially through the $\beta\gamma$ subunit of G-protein involved in receptor activation (Yamauchi et al., 1997; Gutkind, 1998; Lopez-Ilasaca et al., 1998). In HL60-granulocytes, wortmannin was found to inhibit ERK and P38 MAPK activities but not JNK activity stimulated by formyl peptide receptor (Rane et al., 1997). Moreover, we have also found that adrenomedullin-stimulated hyaluronic acid secretion can be inhibited significantly by wortmannin (Parameswaran et al., 1999; Manuscript submitted for publication, Eur. J. Pharmacol.). Our present results indicate that adrenomedullin-stimulated P38 MAPK may be dependent on a wortmannin-sensitive kinase, but JNK activation by adrenomedullin is not. We have found that adrenomedullin-stimulated JNK activation is not consistently sensitive to any of the inhibitors that we tested including a protein kinase-A inhibitor.

Wortmannin inhibited adrenomedullin-stimulated P38 MAPK activity. This suggests that the kinase sensitive to wortmannin is present upstream of P38 MAPK. Wortmannin affected only adrenomedullin-induced apoptosis and not the proliferation response while SB203580, a P38 MAPK inhibitor affected both. These findings lead us to suggest that, there are probably two different pathways that are P38/SB203580 sensitive and only one is possibly sensitive to wortmannin. This might indicate that the effect of SB203580 could be a combination of P38 MAPK-dependent and -independent mechanisms or alternatively, that the wortmannin-sensitive pathway might be activating an isoform of P38 MAPK that affects apoptosis but not proliferation. The fact that SB203580 did not affect either ERK or JNK activity (the most closely related kinases to P38 MAPK), possibly argues against the former. Different isoforms of P38 have been shown to cause different effects in myocardial cells (Wang et al., 1998). The fact that 10 μM SB203580 caused mesangial cell proliferation while, did not affect basal apoptosis suggests that the enzymes regulating these two processes are active differently, in quiescent cells.

The differential effects of wortmannin could also be unrelated to P38. That is, wortmannin has been shown to decrease receptor-dependent regulation of calcium-entry in human platelets, without affecting intracellular Ca^{2+} stores (Von Appen et al., 1997). Since late apoptosis requires an increase in Ca^{2+}, it is possible that wortmannin might be inhibiting apoptosis by inhibiting the calcium entry. Obviously extensive studies are necessary to test this hypothesis.

Other factors have been shown to induce mesangial cell apoptosis. Of particular interest are the fas and ceramide-mediated apoptosis (Coroneos et al., 1996; Gonzalez-Cuadrado et al., 1997). It is not quite clear what MAPKs are required for the apoptosis induced by these agents. But Fas-induced apoptosis in T cells was shown to be independent of P38, even though Fas increased P38 activity (Salmon et al., 1997). Further studies examining the regulation of apoptosis by these factors and adrenomedullin will be quite useful in understanding the pathophysiology of proliferative glomerulonephritis.

Our results demonstrate for the first time that the adrenomedullin-mediated decrease in [^{3}H]thymidine incorporation and increase in nucleosome-associated cytoplasmic DNA fragmentation are sensitive to SB203580 and hence possibly mediated through P38 MAPK pathway. Our data also suggests the presence of wortmannin-sensitive and -insensitive p38 MAPK/SB203580 sensitive pathways, the former possibly regulating apoptosis and the latter proliferation. Taken together, these results indicate

that P38 MAPK may play an important role in mesangial cell turnover mediated by adrenomedullin.

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