Regulation of glucose transport in aortic smooth muscle cells by cAMP and cGMP

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We have studied the ability of cGMP and cAMP to modulate platelet-derived growth factor (PDGF)-stimulated 2-deoxy-D-glucose (deGlc) transport in primary cultures of vascular smooth muscle cells (VSMC) from rat aorta. PDGF stimulated deGlc transport in a time- and concentration-dependent manner. 8-Bromo-cGMP and atrial natriuretic peptide(1–28) [ANP(1–28)] were found to reduce PDGF-stimulated deGlc transport without affecting basal (unstimulated) transport activity. In contrast, 8-bromo-cAMP and dibutyryl-cAMP stimulated basal deGlc transport 2-fold and were without effect on PDGF-stimulated deGlc transport. 8-Bromo-cGMP also inhibited 8-bromo-cAMP-stimulated deGlc transport. The stimulation of deGlc transport by PDGF was sensitive to the mitogen-activated protein kinase (MAPK)/extracellular-signal-regulated kinase (ERK) kinase (MEK) inhibitor PD98059, and we show that

ERK1/2 was activated by PDGF. Neither 8-bromo-cGMP nor ANP(1–28) inhibited PDGF-stimulated ERK activation, suggesting that the effects of cGMP and ANP(1–28) were not mediated by inhibition of this kinase. Our data also argue against a role for cGMP-dependent protein kinase in mediating the effects of cGMP or ANP(1–28). Collectively, our data suggest that in VSMC: (i) cGMP and cAMP have opposing effects on deGlc transport; (ii) PDGF and cAMP have common elements in the pathways by which they activate deGlc transport; and (iii) a common element may be the target of the cGMP-mediated inhibition of deGlc transport.

Key words: atrial natriuretic peptide, extracellular-signal-regulated kinase, vascular smooth muscle cells.

INTRODUCTION

Atherosclerosis is a form of arterial disease that accounts for approx. 50 % of all mortality in the U.S.A., Europe and Japan. Atherosclerotic plaques form in the lumen of the blood vessel in response to an insult or injury to the endothelium and can lead to myocardial infarction, stroke and peripheral vascular disease [1]. The development of a plaque is dependent on abnormal smooth muscle cell proliferation and platelet aggregation; platelet-derived growth factor (PDGF) secreted from the platelets at the site of an injury is one of the factors which stimulates the proliferation and migration of the smooth muscle cells [1]. Glucose transport is probably an important factor in the development of atherosclerotic plaques, since one of the early cellular responses to growth factors, such as PDGF, is an increase in the rate of glucose transport, a fundamental requirement for growth [2]. Despite the clinical importance of atherosclerosis, its dependence on smooth muscle cell proliferation, and its association with diabetes, little is known about the mechanism and regulation of growth-factor-stimulated glucose transport in vascular smooth muscle cells (VSMC).

As well as important roles for growth factors, such as PDGF, in VSMC physiology, cAMP and cGMP have been shown to have fundamental roles in these cells as mediators of smooth-muscle relaxation [3,4]. Abnormal proliferation of VSMC is central to the aetiology of vascular disease, and it is known that NO released from the endothelium participates in the regulation

of VSMC growth via cGMP-dependent events [5]. Indeed, recent studies have shown that elevated cGMP inhibits mitogenesis/proliferation in VSMC by interfering with the activation of the Ras/Raf/extracellular-signal-regulated kinase (ERK) signalling cascades [6,7] and also by inactivating ERK1/2 through the action of mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1) [8]. As well as an important role for cGMP in VSMC, it is now well established that cAMP levels play a fundamental role in the vasodilatory action of these cells, for example cAMP is known to mediate vasodilation in response to β -adrenoceptor agonists [9]. Although their respective cyclic-nucleotide-dependent kinases are believed to mediate the effects of cAMP and cGMP, the issue is complicated by the ability of these cyclic nucleotides to activate each others kinase [10–13]. Although cAMP has been shown to regulate glucose transport in other cells (see [14–20]) its role in the control of glucose transport in VSMC remains poorly understood.

Thus VSMC physiology is greatly influenced both by growth factors, such as PDGF, and by cyclic nucleotides. In the present study we have examined the effects of cGMP and cAMP on glucose transport in VSMC with the aim of characterizing how these fundamental signalling molecules influence VSMC physiology. The impact of these cyclic nucleotides on growth-factor action was also examined. The data presented show that cGMP and cAMP produce opposing effects on glucose transport in VSMC: cGMP inhibits growth-factor-stimulated glucose transport and cAMP analogues elevate glucose transport. Our data

Abbreviations used: ANP(1–28), atrial natriuretic peptide(1–28); deGlc, 2-deoxy-D-glucose; DMEM, Dulbecco's modified Eagle's medium; ERK, extracellular-signal-regulated kinase; KRP, Krebs's Ringer Phosphate medium; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK kinase; MKP-1, MAPK phosphatase-1; PDGF, platelet-derived growth factor; PKA, cAMP-dependent protein kinase; PKG, cGMP-dependent protein kinase; VSMC, vascular smooth muscle cells.

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also show that both PDGF and cAMP analogues stimulate glucose transport via distinct intracellular signalling cascades. Furthermore, we show that the inhibition of glucose transport by cGMP is not mediated by inhibition of the MAPK/ERK kinase (MEK)/ERK cascade. Rather cGMP inhibition acts at a step common to both PDGF- and cAMP-stimulated glucose transport cascades, and this is unlikely to be cGMP-dependent protein kinase (PKG).

MATERIALS AND METHODS

Materials

The standard laboratory chemicals were AnalaR grade and were obtained from BDH-Merck Ltd. (Poole, Dorset, U.K.). Dulbecco's modified Eagle's medium (DMEM; 4.5 g/l D-glucose, 4 mM L-glutamine and 0.37 % NaHCO₃), L-glutamine, penicillin and streptomycin were purchased from Sigma (Poole, Dorset, U.K.). Heat-inactivated fetal bovine serum was purchased from Gibco BRL (Paisley, Renfrewshire, Scotland, U.K.). ERK1/2 antiserum was obtained from New England Biolabs (Hitchin, Herts., U.K.), anti-GLUT1 was purchased from Chemicon Inc. (Harrow, Middx., U.K.), GLUT3 and GLUT4 antisera were raised in-house [21], and the anti-(PKG I α / β) antibody was purchased from Calbiochem (Nottingham, U.K.). Rat recombinant PDGF- $\beta\beta$, 8-bromo-cAMP, dibutyl-cAMP, 8-bromo-cGMP, H-89, rat atrial natriuretic peptide(1–28) [ANP(1–28)] and SB-203580 were all obtained from Calbiochem-Novabiochem (Nottingham, U.K.). R0 20-1724 was obtained from RBI (Boston, MA, U.S.A.).

Cell culture

VSMC were prepared from the thoracic aortas of 2–3-month-old Wistar-Kyoto rats by enzymic digestion [22]. The cells were cultured in DMEM supplemented with 10 % (v/v) fetal bovine serum, 100 units of penicillin/ml, 100 μ g of streptomycin/ml and 2 mM L-glutamine, at 37 °C in a humidified 5 % CO₂ atmosphere. The cells were grown in 75 cm² flasks and were fed twice weekly. Prior to assay, when the cells were approx. 80 % confluent, the cells were quiesced by a 48 h incubation in serum-free medium. Cells were used between passages 4 and 14. The identity of the smooth muscle cell cultures was confirmed periodically by staining with a monoclonal antibody against smooth muscle α -actin.

2-Deoxy-D-glucose (deGlc) transport

Cells grown on 6-well plates were incubated in serum-free medium for 48 h prior to assay. Cells were then washed in Krebs' Ringer Phosphate medium (KRP; 136 mM NaCl, 4.7 mM KCl, 1.25 mM MgSO₄, 1.25 mM CaCl₂ and 5 mM NaH₂PO₄, pH 7.4) and were incubated in 1 ml of KRP containing 0.1 % (w/v) BSA, for the period of the assay, at 37 °C. Transport was initiated by the addition of an aliquot of tritiated deGlc (50 μ M final concentration; 0.5 μ Ci/ml) and uptake was measured for a period of 5 min [23]. Under these conditions the uptake of glucose was linear for at least 20 min (results not shown). Uptake was stopped by rapid removal of the medium, followed by three washes in ice-cold PBS. Cells were solubilized in 1 % (v/v) Triton X-100, and the radioactivity associated with the cells was determined by scintillation counting. Uptake in the presence of 10 μ M cytochalasin B (a potent inhibitor of facilitated glucose transport) was also measured, in order to determine the level of non-specific association of deGlc with the cells. This value was typically less than 10 % of the total uptake, and was subtracted

from the total to obtain a measurement of specific deGlc transport.

Thymidine incorporation

DNA synthesis was assayed by measuring the incorporation of [6-³H]thymidine. The cells were grown on 12-well plates and quiesced in serum-free medium for 24 h at 40 % confluency. Cells were incubated with agonist and 1 μ Ci/ml [6-³H]thymidine for 24 h. Following incubation the medium was aspirated and the cells were washed in ice-cold PBS (pH 7.4), followed by three washes in 5 % (w/v) trichloroacetic acid and three washes in ethanol. The cells were solubilized in 0.3 M NaOH, and the incorporated radioactivity was measured using scintillation counting.

Immunoblot analysis

After a 48 h quiescing period in serum-free medium the cells were washed in KRP, and were incubated in KRP containing 0.1 % BSA. The cells were treated with inhibitor and/or agonist for the required period, the medium was then aspirated and the cell layer was washed in ice-cold PBS (pH 7.4). The cell layer was solubilized in 200 μ l of lysis buffer [20 mM Hepes (pH 7.4), 1 % (v/v) Triton X-100, 0.05 % SDS, 1 mM EDTA, 1 μ g/ml aprotinin, 1 μ g/ml leupeptin, 10 μ g/ml pepstatin A, 1 μ g/ml PMSF, 50 μ g/ml soya bean trypsin inhibitor, 1 mM NaF and 1 mM Na₃VO₄] per well of a 6-well plate or 1 ml of lysis buffer per 75 cm² flask. The lysate was snap-frozen and stored at –80 °C prior to SDS/PAGE and immunoblotting. Total VSMC protein (5–40 μ g) was separated on 10 % (w/v) polyacrylamide gels and transferred on to nitrocellulose membranes as described previously [23]. The membranes were blocked in 5 % (w/v) non-fat milk/0.1 % Tween-20 in TBST-1 (20 mM Tris, 150 mM NaCl and 0.02 % Tween-20), and were probed with antibodies specific for ERK1/2, phospho-ERK1/2, PKG I α / β , GLUT1, GLUT3 or GLUT4. The blots were developed using ECL[®] (Amersham). A range of exposures of X-ray films were produced and quantified using densitometry, such that signals were measured in the linear range.

RESULTS

PDGF stimulates glucose transport in VSMC

PDGF-stimulation of VSMC results in a biphasic stimulation of deGlc uptake. In our hands, an approx. 5-fold increase in deGlc uptake was evident at 1 h after exposure to PDGF, which after a brief plateau phase continued to rise over the following 6–8 h (Figure 1, top panel). In agreement with studies in many other cell types, we have found that the chronic phase of PDGF-stimulated deGlc uptake is sensitive to inhibitors of protein synthesis (results not shown; see [2] for review). In the present study we will discuss aspects of the acute-phase stimulation of deGlc uptake in VSMC. Figure 1 (bottom panel) illustrates that PDGF stimulates acute phase deGlc transport with an EC₅₀ consistent with the action of PDGF through its cognate receptor. The VSMC used in this study express predominantly the GLUT1 isoform, with no GLUT4 immunoreactivity observed (C. J. MacKenzie and G. W. Gould, unpublished work). It is presently unclear whether the increases observed in response to acute stimulation with PDGF arise as a consequence of the cellular redistribution of glucose transporters or their intrinsic activation in this cell type.

Figure 1 PDGF-stimulated deGlc transport in rat VSMC

Top panel: quiescent VSMC were stimulated with 10 ng/ml PDGF- $\beta\beta$ for various periods of time, and deGlc transport was assayed as described in the Materials and methods section. Data are shown from a typical experiment, with each point representing the mean $\pm$ S.E.M. of triplicate determinations. This graph is representative of two experiments. Bottom panel: VSMC were stimulated with PDGF at the concentrations shown for 1 h prior to assay of deGlc transport. Data are shown from a typical experiment, with each point representing the mean $\pm$ S.E.M. of triplicate determinations. This graph is representative of two experiments. In both panels, the data were corrected by subtracting the non-specific association of deGlc with the cells, determined from parallel assays performed in the presence of 10 μ M cytochalasin B. These values typically represented less than 10% of the total counts in each assay. In the experiments shown, basal deGlc transport was 49 ± 8 pmol/min per mg protein (each well of a 6-well plate contained 98 ± 7 μ g of protein).

cGMP can modulate glucose transport in VSMC

We examined the effects of 8-bromo-cGMP on basal and PDGF-stimulated deGlc uptake in VSMC (Figure 2, top panel). Although without effect on basal deGlc transport, 8-bromo-cGMP was observed to potently inhibit PDGF-stimulated deGlc transport. Such data suggested to us that cGMP may play a

Figure 2 Effects of 8-bromo-cGMP and ANP(1-28) on basal and PDGF-stimulated deGlc transport in rat VSMC

Top panel: quiescent VSMC were preincubated with various concentrations of 8-bromo-cGMP for 15 min. PDGF (10 ng/ml) was added for 30 min, and deGlc transport was assayed as described in the Materials and methods section. Data are expressed as percentages of the basal (unstimulated) rate. The data from a typical experiment are shown, in which triplicate determinations are presented as means $\pm$ S.E.M. The experiment was repeated three times with similar results. *Statistically significant inhibition ($P < 0.05$). Bottom panel: the experiment shown in the top panel was repeated using various concentrations of ANP(1-28). Cells were exposed to ANP(1-28) for 15 min prior to addition of PDGF for 30 min and assay of deGlc transport. The data are expressed as means $\pm$ S.E.M. of triplicate observations. This graph is representative of four experiments. *Statistically significant inhibition in response to ANP(1-28) ($P < 0.05$).

fundamental role in the control of glucose transport into VSMC. To test this hypothesis, in a physiologically relevant context, we examined the effect of ANP(1-28) on deGlc uptake. ANP(1-28) stimulates the production of cGMP in VSMC through activation

Figure 3 8-Bromo-cAMP- and dibutyryl-cAMP-stimulated deGlc transport in rat VSMC

Quiescent VSMC were stimulated with various concentrations of 8-bromo-cAMP (●) or dibutyryl-cAMP (○) for 1 h prior to assay of deGlc transport. The data from a typical experiment are presented, and are expressed as mean values that have been corrected for non-specific association of deGlc with the cells. This graph is representative of three experiments. Error bars are omitted for clarity, but were usually within 10% of the mean. *Statistically significant increase compared with basal cells ($P < 0.05$).

Figure 4 Effect of the PKA inhibitor H-89 on dibutyryl-cAMP-stimulated deGlc transport in rat VSMC

Quiescent VSMC were preincubated with 10 or 100 nM H-89 for 30 min, and were then stimulated with 1 mM dibutyryl-cAMP for 60 min, prior to assay of deGlc transport. The data from a typical experiment are presented, and are expressed as means $\pm$ S.E.M. of triplicate observations that have been corrected for non-specific association of deGlc with the cells. This graph is representative of three experiments. *Statistically significant inhibition ($P < 0.05$).

of an endogenous receptor with intrinsic guanylate cyclase activity [24]. ANP(1–28) also inhibited PDGF-stimulated deGlc uptake in these cells (Figure 2, bottom panel). Similar effects of 8-bromo-cGMP and ANP(1–28) were observed on 3-O-methyl-D-glucose transport (results not shown).

Figure 5 cGMP inhibits 8-bromo-cAMP-stimulated deGlc transport in rat VSMC

Quiescent VSMC were preincubated with various concentrations of 8-bromo-cGMP for 15 min, prior to addition of 1 mM 8-bromo-cAMP for 1 h, and deGlc transport assay. Data are expressed as a percentage of basal transport rates. The data shown are the means of triplicate experiments (each point performed in triplicate). *Statistically significant inhibition ($P < 0.05$).

cAMP stimulates deGlc transport in VSMC

In marked contrast with the effects of cGMP, long-lasting cAMP analogues (dibutyryl-cAMP and 8-bromo-cAMP) were both found to stimulate deGlc transport (Figure 3). Neither of these cAMP analogues had any effect on PDGF-stimulated deGlc uptake (results not shown). These experiments clearly demonstrate that the effects of cGMP (Figure 2) are unlikely to be the consequence of cross-activation of cAMP-dependent processes.

A specific inhibitor of cAMP-dependent protein kinase (PKA) was employed to determine whether the effects of cAMP on deGlc uptake were mediated by the action of this kinase. H-89 inhibited both 8-bromo-cAMP- and dibutyryl-cAMP-stimulated deGlc transport in VSMC at a concentration consistent with the known IC_{50} of this compound (Figure 4, and results not shown). The extent of inhibition was sufficient to suggest that the effect of cAMP analogues on glucose transport is mediated entirely by the action of PKA. In contrast, PDGF-stimulated glucose transport was resistant to H-89, with no inhibition of PDGF-stimulated deGlc transport observed at concentrations of H-89 up to 1 μ M ($99 \pm 12\%$ of the control PDGF-stimulated response; $n = 4$). Such data suggests that cAMP production/PKA activation does not play a key role in PDGF-stimulated glucose transport in VSMC.

In common with PDGF, 8-bromo-cAMP-stimulated deGlc transport was inhibited by 8-bromo-cGMP (Figure 5). This result suggests that cAMP- and PDGF-stimulated signalling pathways converge on common effector molecule(s), and this point of convergence is the probable source of regulation of transport by cGMP.

Role of ERK1/2 in the regulation of glucose transport

The MAPKs ERK1/2 have been shown in a range of cells to modulate growth-factor-stimulated glucose transport [2,22]. In

Figure 6 PD98059 inhibits PDGF-stimulated deGlc transport in VSMC

Quiescent VSMC were preincubated with PD98059 (0–50 μ M) for 60 min, prior to stimulation with 10 ng/ml PDGF. DeGlc transport was determined as described in the Materials and methods section, and the data are presented as the percentage inhibition of transport rate (each point is the mean of triplicate determinations at each condition). The maximal dose of PD98059 tested (50 μ M) had no significant effect on basal transport ($110 \pm 3\%$ of the basal control value). The graph is representative of three separate experiments. *Statistically significant inhibition ($P < 0.05$).

order to address the role of these kinases in PDGF-stimulated glucose transport, we employed the MAPK/ERK kinase (MEK) inhibitor PD98059 to inhibit the activation of the ERK cascade. PD98059 was found to inhibit PDGF-stimulated deGlc uptake over the concentration range expected for this inhibitor (Figure 6), implicating the Raf/MEK/ERK cascade as a fundamental component of PDGF-stimulated deGlc uptake in these cells.

Several studies have shown that cGMP and ANP(1–28) exhibit anti-proliferative action in VSMC by antagonizing the ability of growth factors to activate this cascade [6–8]. We therefore examined the activity of ERK1/2 in these cells following cGMP or ANP(1–28) treatment (Figure 7). PDGF stimulation resulted in the rapid activation of ERKs 1 and 2 in VSMC. Strikingly, we were unable to demonstrate any effect of ANP(1–28) on ERK activation in response to PDGF (Figure 7). Thus we suggest that the ability of ANP(1–28) to inhibit PDGF-stimulated glucose transport is unlikely to be mediated by inhibition of ERK activity.

Moreover, the data presented in Figure 7 clearly show that 8-bromo-cAMP does not stimulate ERK1/2 activity in these cells. Such data reinforce the distinction between PDGF- and cAMP-stimulated deGlc uptake and suggest that the pathways of PDGF- and cAMP-stimulated glucose are, at least in part, distinct.

Role of PKG in cGMP inhibition of stimulated deGlc transport

We subsequently investigated whether the effects of cGMP on deGlc transport are mediated by PKG. The PKG inhibitor KT-

Figure 7 Effects of PDGF, 8-bromo-cAMP and ANP(1–28) on ERK1/2 activation

Quiescent VSMC were preincubated for 15 min with or without 200 nM ANP(1–28), and were stimulated for the indicated times with 20 ng/ml PDGF- $\beta\beta$ or 1 mM 8-bromo-cAMP. ERK activity was determined using band-shift assays with anti-ERK1/2 (a) or using a phospho-specific ERK antibody (b). This immunoblot is typical of three separate experiments using three separate batches of cells.

Figure 8 Immunoblot analysis of PKG levels in aorta and cultured VSMC

Protein (50 μ g) from different samples was analysed by SDS/PAGE and immunoblotting for PKG I α / β . A representative immunoblot is shown. Lane 1, rat brain homogenate (negative control); lane 2, homogenate of aorta from a stroke-prone spontaneously hypertensive rat; lane 3, homogenate of aorta from a Wistar-Kyoto rat; lane 4, passage 2 Wistar-Kyoto cultured VSMC (high confluence); lane 5, passage 11 Wistar-Kyoto cultured VSMC (low confluence); lane 6, passage 11 Wistar-Kyoto cultured VSMC (high confluence). The approximate position of the nearest molecular-mass standard is shown.

5823, at doses up to 1 μ M, did not block the effects of 8-bromo-cGMP or ANP(1–28) on PDGF-stimulated deGlc transport (results not shown). In addition, PKG I α / β protein could not be detected by immunoblotting whole-cell lysates from cultured VSMC at high or low passage (Figure 8). These data strongly suggest that the effects of cGMP are unlikely to be mediated by PKG, at least in this cell population.

DISCUSSION

cAMP and cGMP occupy central positions in the control of VSMC physiology: analysis of the mechanisms by which these cyclic nucleotides exert their action must take into consideration the well documented ability of these molecules to activate each others kinases (see [10–13]). However, in the present study we have shown that in cultured VSMC the effects of cAMP and cGMP on deGlc transport can be clearly distinguished. A cGMP analogue and an agonist that elevates cGMP levels inhibited PDGF-stimulated deGlc transport in VSMC (Figure 2), whereas cAMP analogues did not. Moreover, cAMP analogues, but not cGMP analogues, stimulated basal deGlc transport (Figure 3).

Activators of guanylate cyclase and cGMP analogues have been found to inhibit VSMC proliferation [25,26]. Consistent with this, we have shown that 8-bromo-cGMP and ANP(1–28) inhibited PDGF-stimulated deGlc transport, a key early event in mitogenesis. In previous studies, cGMP-mediated inhibition of VSMC proliferation was found to occur at the level of Ras activation of Raf-1, preventing the activation of the MEK/ERK cascade that is essential for cell proliferation [6]. Sugimoto et al. [8] also reported the induction of MKP-1 by ANP(1–28) in VSMC as an alternative mechanism for cGMP-mediated inhibition of ERK1/2. Consistent with an important role for this cascade in the control of mitogen-stimulated glucose transport in other cells [23], we have shown that the MEK inhibitor, PD98059, also inhibited PDGF-stimulated deGlc transport in VSMC (Figure 6).

However, in our hands, neither 8-bromo-cGMP nor ANP(1–28) had any observable effect on PDGF-stimulated ERK1/2 activation (Figure 7), and we were unable to observe induction of MKP-1 in the time-frame over which cGMP or ANP(1–28) inhibit deGlc transport (results not shown). Although these mechanisms clearly play a role in the inhibition of cell proliferation/DNA synthesis, these effects are manifest over longer time periods (> 12 h) [6,8]. Thus the effects of cGMP and ANP(1–28) observed in the present study on the early stages of PDGF-stimulation of deGlc transport (approx. 1 h) appear to be mediated via a distinct mechanism.

The effects of NO and cGMP in the cell are often mediated by PKG [27]. In the majority of tissues PKG is either absent or

present at negligible levels, however, in smooth muscle [28] and platelets [29] PKG levels are high. The amount of PKG in cultured VSMC decreases with subculturing of the cells [3]. Freshly isolated VSMC express high levels of PKG I, but expression appears to decline with passage [3]. We have observed PKG I protein expression in the whole aorta but not in our cultures of VSMC (Figure 8). Thus the effects of cGMP and ANP(1–28) are unlikely to be mediated through the action of PKG. It is interesting that the loss of PKG is associated with VSMC in the de-differentiated state [30]. VSMC in this phenotypic state are central to the development of atherosclerotic plaques [1], and the loss of PKG may therefore be important for the regulation of this process.

We have shown that cAMP analogues can stimulate deGlc transport by a PKA-dependent mechanism. This mechanism is, at least in part, distinct to the mechanism employed by PDGF, as PDGF-stimulated deGlc transport was insensitive to H-89 (results not shown) over the same concentration range. Thus these data imply that multiple intracellular signalling systems are capable of stimulating glucose transport in VSMC. One caveat with these studies is that the elevations in cAMP observed using non-hydrolysable analogues are unlikely to mirror physiological situations in which agonists trigger elevated cAMP levels. Indeed, the β -adrenergic receptor agonist, isoprenaline, which causes an elevation of cAMP levels in VSMC (a 30 min stimulation with 300 nM isoprenaline elevated cAMP levels to 7.6 ± 2.2 -fold of basal control) had no effect on deGlc transport (results not shown). However, the possibility that highly localized cAMP pools may act to modulate glucose transport in VSMC should not be ignored, as the action of many signalling systems is highly spatially organized. Moreover, it is well documented that elevated levels of cAMP accumulate in cells from diabetic subjects; such accumulations may imply that cAMP could significantly modulate glucose transport in VSMC cells. Further studies will be required to delineate the importance of cAMP in the control of glucose transport in this cell type.

These caveats notwithstanding, the fact that both PDGF- and cAMP-stimulated deGlc transport were inhibited by 8-bromo-cGMP or ANP(1–28) argues that the pathways by which these distinct agonists stimulate glucose transport converge at a point that is sensitive to regulation by cGMP. Studies are currently in progress to identify this molecular target.

SUMMARY

The present study has demonstrated opposing actions of cAMP and cGMP on glucose transport in VSMC. We have shown that cGMP or ANP(1–28) inhibit growth-factor-stimulated deGlc transport by a mechanism that appears independent of inhibition of the ERK cascade and probably does not involve PKG. The identification of the target of cGMP in these cells represents an important objective. Previous studies suggest that VSMC in culture adopt a phenotype similar to that of the VSMC involved in the development of atherosclerotic plaques [1,30], a view supported by the demonstrated absence of PKG from the cultured cells. The presence of PKG would profoundly alter the responses of the cell to cyclic nucleotides. The regulation of glucose transport by cyclic nucleotides, as we have described it, is therefore of particular relevance to the de-differentiated VSMC associated with atherosclerotic-plaque development.

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