THE JOURNAL OF BIOLOGICAL CHEMISTRY
Vol. 266, No. 19, Issue of July 5, pp. 12612-12617, 1991
Printed in U.S.A.

Regional and Mechanistic Differences in Platelet-derived Growth Factor-Isoform-induced Alterations in Cytosolic Free Calcium in Porcine Vascular Smooth Muscle Cells*

(Received for publication, February 19, 1991)

Pamela A. Diliberto‡, Gerry Gordon‡, and Brian Herman‡§¶

From the ‡Laboratories for Cell Biology, Department of Cell Biology and Anatomy and the §Cancer Cell Biology Program, Lineberger Cancer Research Center, School of Medicine, University of North Carolina, Chapel Hill, North Carolina 27599

Three specific platelet-derived growth factor (PDGF) isoforms are thought to bind with differing affinities to two distinct PDGF receptors which undergo activation following dimerization. Recent evidence has been presented that marked differences exist between the ability of PDGF-AA *versus* PDGF-AB and PDGF-BB to stimulate alterations in second messengers in cultures of vascular smooth muscle cells (VSMC), a result which was thought to be due to low numbers of the A-type receptor in this cell type (Sachinidis, A., Locker, R., Vetter, W., Tatje, D., and Hoppe, J. (1990) *J. Biol. Chem.* 265, 10238–10243, 1990). In particular, PDGF-BB and PDGF-AB *but not* PDGF-AA could elicit alterations in cytosolic free calcium (Ca^{2+}_i). However, because these studies were performed on large cell populations using biochemical assays of PDGF activity, a minor PDGF-AA-Ca^{2+}-responsive population of cells might go undetected. To test this possibility, VSMC were isolated from either thoracic or abdominal pig aorta, and alterations in Ca^{2+}_i were monitored using Multiparameter Digitized Video Microscopy following stimulation with PDGF isoforms alone, or either before or after exposure of VSMC to 5 mM EGTA. PDGF-AA-responsive cells were found to exist only in cultures of thoracic VSMC, caused oscillations in Ca^{2+}_i, represented 20% of the PDGF-BB-responsive cells, and were subsequently responsive to PDGF-BB. PDGF-BB elicited monophasic alterations in Ca^{2+}_i in both thoracic and abdominal VSMC. Prior addition of EGTA inhibited PDGF-AA *but not* PDGF-BB-induced alterations in Ca^{2+}_i. Addition of EGTA during PDGF-AA-induced Ca^{2+}_i oscillations inhibited subsequent oscillations in Ca^{2+}_i, while addition of EGTA at the peak of the PDGF-BB Ca^{2+} response resulted in a more rapid return of Ca^{2+}_i to prestimulation levels. These data suggest that regional differences in the distribution of PDGF-A- and B-type receptor exists *in vivo*, and that activation of the A- and B-type PDGF receptors results in distinct alterations in Ca^{2+}_i.

PDGF[1] elicits a variety of biological activities in VSMC (mitogenesis, chemotaxis, and vasoconstriction) through the existence of distinct PDGF isoforms and receptors (1–4). Human PDGF consists of two disulfide-bonded homologous peptide chains, A and B (PDGF-AB). Each chain is the product of a distinct cellular gene, their expression being independently regulated and the two chains sharing approximately 50% homology (5–7). Porcine PDGF appears to be a BB homodimer as is PDGF isolated from most animal species (except human) (8). Skeletal and smooth muscle, growth factor-stimulated fibroblasts and a number of tumor cell lines produce exclusively PDGF-AA (9–11).

These different types of PDGF molecules are thought to bind to at least two distinct types of PDGF receptors. The originally described receptor (B-type, 180 kDa, similar to the CSF-1 receptor) was found to bind the B chain of PDGF, is located on chromosome 5 and possesses tyrosine kinase activity (12). A second type of PDGF receptor (A-type, 170 kDa, similar to the c-kit gene product) is thought to bind both PDGF A and B chains, is located on chromosome 4, and also contains tyrosine kinase activity (13). Since the receptors display some selectivity in terms of which PDGF chains they can bind, and because active PDGF molecules are thought to exist as dimers, a number of models have recently been proposed where PDGF binding to its receptors requires dimerization of the A- and B-type receptor subunits to elicit functional activity (14–16). These models predict that dimerization of two A-type receptors could lead to binding of all three types of PDGF isoforms (AA, BB, AB), while dimerization of two B-type receptors could only lead to binding of PDGF-BB (although some data suggests that PDGF-AB can bind to cells containing only the B-type receptor albeit at 10-fold lower affinity than PDGF-BB). A key aspect of these models is that in cells lacking the A-type PDGF receptor, there should be no response to either PDGF-AA or PDGF-AB.

The mechanisms by which PDGF binding to its receptor is transduced into biological activity are still unclear. Our laboratory has been studying the role of PDGF-induced alteration in Ca^{2+}_i in PDGF-stimulated mitogenesis (17–21). PDGF addition to a variety of cells causes a rapid transient increase in Ca^{2+}_i within seconds of addition (22), and results from our laboratory as well as others (17–21, 23–31) demonstrate that inhibition of PDGF-stimulated increases in Ca^{2+}_i also inhibit

* This work was supported by Grants AG07218, AG10104, 2-P01-CA29589, and 2-P30-CA16086 from the National Institutes of Health, the Gustavus and Louise Pfeiffer Research Foundation, and Grant FRA-383 from the American Cancer Society. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¶ To whom correspondence should be addressed: Dept. of Cell Biology and Anatomy, CB 7090, 232 Taylor Bldg., University of North Carolina, Chapel Hill, NC 27599.

[1] The abbreviations used are: PDGF, platelet-derived growth factor; Ca^{2+}_i, cytosolic free calcium; VSMC, vascular smooth muscle cells; MDVM, multiparameter digitized video microscopy; DMEH-H, Dulbecco's modified Eagle's medium with high glucose; EGTA, [ethylenebis(oxyetheylenenitrilo)]tetraacetic acid; IP_3, inositol 1,4,5-trisphosphate.

PDGF-mediated mitogenesis. Recently, we have provided evidence that alterations in Ca^{2+}_i which immediately follow PDGF binding are necessary for PDGF-induced mitogenesis (32). Collectively, these data support the contention that alterations in Ca^{2+}_i are a necessary component in the signal transduction cascade of PDGF. However, two recent reports have indicated that PDGF isoforms in VSMC differ in their ability to stimulate components of the second messenger pathways; in particular, PDGF-AA was unable to cause an increase in Ca^{2+}_i (33, 34). Both PDGF-AA and PDGF-BB in these and other studies stimulated mitogenesis (35–37), although PDGF-AA was a less effective mitogen, a result explained by the lower number of A-type relative to B-type PDGF receptors. If correct, these results would suggest that Ca^{2+}_i is not a necessary component of PDGF mitogenesis activity. However, we report here that PDGF-AA does stimulate increases in Ca^{2+}_i in VSMC but that PDGF-AA-induced increases in Ca^{2+}_i occur only in a small percentage of cells whose distribution is not uniform throughout the aorta. In addition, the mechanisms that PDGF-AA and PDGF-BB employ to elicit alterations in Ca^{2+}_i in VSMC are distinct.

MATERIALS AND METHODS

Cell Culture—Secondary cultures of porcine VSMC were derived from explants of thoracic or abdominal aorta and cultured as previously described (18–19). Cells were grown in DMEM-H supplemented with 10% fetal bovine serum, 5.6 mM glutamine, 100 units/ml penicillin, and 10 μg/ml streptomycin. VSMC used for experiments were between passages 3–12 and either serum starved for 5–7 days before use or placed in serum-free medium (DMEM/F-12 (1:1) supplemented with insulin/transferrin/selenium) for 48 h before use. PDGF responsiveness was compared in thoracic and abdominal regions from the same or different animals.

Source of PDGF Isoforms—Recombinant PDGF-AA and PDGF-BB were obtained from either Upstate Biotechnology Inc., or Biosource Inc. All PDGF isoforms were aliquoted and stored frozen in 10 μg/ml in aliquots of 0.1 N acetic acid. Before addition to cells, the isoforms were diluted to 500 ng/ml in DMEM-H containing 0.1% bovine serum albumin and neutralized with 5 mM Na_2CO_3.

Ca^{2+}_i Measurements—Ca^{2+}_i was measured in individual living cells using MDVM and ratio imaging. The method and the design of the instrumentation for measuring Ca^{2+}_i in VSMC has been previously described (17–21). Briefly, VSMC grown on glass coverslips were chilled for 10 min at 4 °C then loaded with the Ca^{2+}-sensitive fluorophore Fura-2-AM (5 μM Fura-2-AM, 20 min at 37 °C). It has been previously demonstrated that the Fura-2 in VSMC loaded in this manner is in the cytosolic compartment and does not become sequestered in the mitochondria or other organelles (38). Coverslips with the loaded cells were then sealed in a specially designed chamber which holds 0.5 ml of medium, and mounted on the microscope stage. Prior to stimulation of the cells with PDGF, a background image of an area of the coverslip with no cells and two base-line images of the cells to be stimulated were collected. An image typically contained two to five cells. After stimulation with PDGF, images were collected every 10 s for the first 3 min, and every 20 s for an additional 15–18 min for a total of 20 min. Images were obtained at two different wavelengths: 340 nm (Ca^{2+}-bound Fura-2) and 380 nm (Ca^{2+}-free Fura-2) with the emission recorded at >450 nm. Following subtraction of the background image at each excitation wavelength, the 340-nm image was divided by the 380-nm image (ratioed) to give a two-dimensional representation of Ca^{2+} within the cells. The ratio approach corrects for problems due to differences in accessible volume and pathlength in the cell. Standard curves were generated through the microscope optics using Ca^{2+}-EGTA buffers as previously described (17–21). Both average Ca^{2+}_i levels in all cells in the image and in each individual cell were calculated.

RESULTS

Differential Response of Thoracic and Abdominal VSMC to PDGF Isoforms—Previous studies in a variety of cell types have reported considerable variability in the ability of PDGF-AA to stimulate mitogenesis (35–37). When PDGF-AA was able to stimulate mitogenesis, it was much less effective than either PDGF-AB or PDGF-BB, with PDGF-BB slightly more effective than PDGF-AB at similar doses (12, 15, 34). Two explanations have been put forth to account for these findings. 1) The ratio of A to B-type receptor is very low such that cells respond weakly to PDGF-AA but strongly to PDGF-BB and PDGF-AB, the latter potentially acting via both dimerization of A- and B-type receptors or through just the B-type receptor alone. 2) Differences in the signal transduction pathways stimulated by the different PDGF isoforms, with the B-type receptor (but not the A-type receptor) efficiently coupled to the mitogenic pathway. Either of these hypotheses, alone or in combination, could account for the previously reported data. However, all of these experiments were performed in cell culture where population heterogeneity may obscure individual cell responses, especially when the events under study occur rapidly or suddenly. In addition, responses of distinct but minor cell populations may also be obscured. For example, the lack of a Ca^{2+}_i response to PDGF-AA in VSMC could be due to low A-type receptor levels on *all* VSMC in the population or to the presence of a small, nondetectable but fully functional subpopulation of PDGF-AA-responsive VSMC. In an attempt to determine if this latter possibility might explain previously reported data in VSMC (33, 34), we employed MDVM and the Ca^{2+}-sensitive fluorophore Fura-2, to monitor the ability of PDGF-AA and PDGF-BB to elicit increases in Ca^{2+}_i in VSMC obtained from either thoracic or abdominal aorta. A particular advantage of the MDVM approach is that it allows examination of the behavior of individual cells within a population, permitting observance of very low frequency events that might otherwise be obscured in studies which employ whole cultures or suspensions of cells making similar measurements.

Fig. 1 demonstrates PDGF-AA- and PDGF-BB-induced alterations in Ca^{2+}_i in thoracic and abdominal VSMC. Fig. 1, *a-f*, represent thoracic VSMC and *g-i* abdominal VSMC. Basal Ca^{2+}_i levels were 41.3 nm ± 5.6 (S.E.) for the cells shown in Fig. 1, *a*, *d*, *g*; this value is representative of that seen in the many cells we have examined (see below). In thoracic VSMC, PDGF-AA caused a small percentage (approximately 20%) of cells to alter their Ca^{2+}_i (Fig. 1, *a-c*; see Fig. 2), while PDGF-BB elicited an increase in Ca^{2+}_i in almost all (147) thoracic VSMC examined (n = 155) (Fig.1, *d-f*; Fig. 2). In contrast, in abdominal VSMC (Fig. 1*h*), PDGF-AA never caused an increase in Ca^{2+}_i, yet these cells were universally responsive to PDGF-BB (Fig. 1*i*). Interestingly, on average, the maximal increase (percent basal) in Ca^{2+}_i observed in PDGF-AA-responsive thoracic VSMC was essentially equivalent to that seen in PDGF-BB-responsive thoracic VSMC (393 ± 73 (S.E.; n = 9) for PDGF-AA *versus* 415 ± 68 (S.E.; n = 8) for PDGF-BB).

Fig. 2 presents a compilation of our data to date. In VSMC isolated from two different pigs, we examined a total of 432 cells from different cell passages and regions of the aorta. PDGF-AA-responsive cells were found only in VSMC isolated from the thoracic segment of the aorta and represented 17–20% of the total PDGF-BB responding population. The majority (>95%) of cultured VSMC obtained for either thoracic or abdominal aorta responded to PDGF-BB.

PDGF Isoforms Elicit Different Types of Ca^{2+}_i Responses in Thoracic VSMC—In porcine VSMC, we previously reported that PDGF-AB-induced increases in Ca^{2+}_i could be blocked by prior treatment of cells with EGTA or voltage-sensitive Ca^{2+} channel antagonists (19). We also found that PDGF-AB induced increases in phosphatidylinositol 4,5-bisphosphate hydrolysis, but at concentrations that were one log order

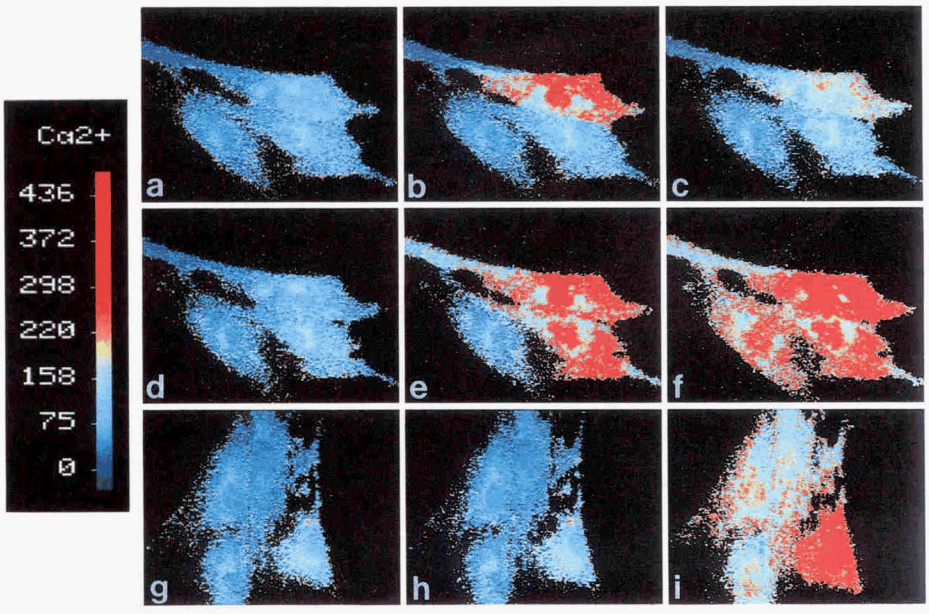

FIG. 1. **Alterations in Ca^{2+}_i in thoracic (*a–f*) and abdominal (*g–i*) VSMC exposed to PDGF-AA (*b*, *c*, and *h*) or PDGF-BB (*e*, *f*, and *i*).** Cultured VSMC were labeled with Fura-2, and Ca^{2+}_i in single cells was measured as described under "Materials and Methods." Thoracic VSMC were exposed to PDGF-AA (40 ng/ml) (*b–d*), and the same cells were then exposed to PDGF-BB (40 ng/ml) 10 min later (*e* and *f*). Abdominal VSMC (*g*) were first exposed to PDGF-AA (40 ng/ml) (*h*) and then 10 min later to PDGF-BB (40 ng/ml) (*i*). The time elapsed from PDGF addition was as follows: *a*, zero; *b*, 30 s; *c*, 490 s; *d*, 570 s; *e*, 620 s; *f*, 640 s; *g*, zero; *h*, 30 s; *i*, 650 s.

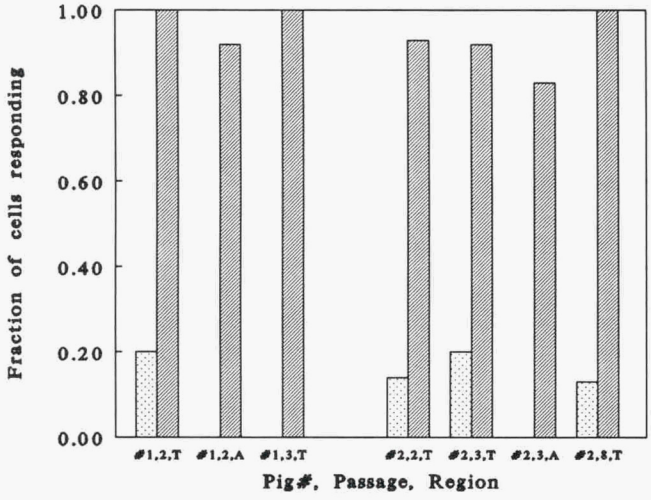

FIG. 2. **Alterations in Ca^{2+}_i in VSMC cultures obtained from thoracic and abdominal aorta in response to PDGF-AA and PDGF-BB.** VSMC isolated from either thoracic or abdominal segments of porcine aorta from two pigs were loaded with Fura-2 and Ca^{2+}_i monitored following addition of PDGF-AA (40 ng/ml) or PDGF-BB (20 ng/ml). The fraction of cells responding to either PDGF-AA or PDGF-BB as a function of pig number, cell passage number, and region form which VSMC were obtained was then calculated from a total of 432 cells examined.

higher than those required to stimulate EGTA-inhibitable alterations in Ca^{2+}_i. We postulated that PDGF-AB activates both the A- and B-type PDGF receptors, which are coupled to distinct second messenger pathways. Subsequently, evidence has been presented in support of this hypothesis (34). To further clarify whether A- and B-type PDGF receptors are coupled to distinct second messenger pathways, we monitored alterations in Ca^{2+}_i induced by the different PDGF isoforms in thoracic VSMC. VSMC exposed to a wide range of doses of PDGF-BB (1–40 ng/ml) displayed a monophasic increase in Ca^{2+}_i (Fig. 3*A*), while PDGF-AA (20–80 ng/ml) elicited oscillations in Ca^{2+}_i (Fig. 3*B*). PDGF-AA-induced oscillations in Ca^{2+}_i had a frequency of one oscillation every 3.4 ± 0.38 min. These distinct PDGF isoform-specific alterations in Ca^{2+}_i could also occur in the same cell. VSMC were first exposed to PDGF-AA (40 ng/ml), and a responsive cell was identified by the typical oscillatory pattern of alterations in Ca^{2+}_i. PDGF-BB (20 ng/ml) was then added, and the oscillatory alteration in Ca^{2+}_i was converted into the characteristic PDGF-BB monophasic increase in Ca^{2+}_i. Subsequent addition of 10% fetal bovine serum resulted in a rapid spike in Ca^{2+}_i followed by smaller oscillations in Ca^{2+}_i. Thus, a single VSMC has the capacity to sequentially respond to PDGF-AA, PDGF-BB, and fetal bovine serum. This finding coupled with the distinct character of the alterations in Ca^{2+}_i elicited by PDGF-AA and PDGF-BB supports the hypothesis that these agents are altering Ca^{2+}_i by different mechanisms.

PDGF-AA but Not PDGF BB-induced Alterations in Ca^{2+}_i Are Sensitive to EGTA—Ligand-induced alterations in Ca^{2+}_i are thought to occur through a combination of release of Ca^{2+} from intracellular stores and influx of Ca^{2+} from the extracellular environment (39). Monophasic alterations in Ca^{2+}_i are thought to be initially due to IP_3-mediated release of Ca^{2+} from intracelluar stores and subsequent influx of extracellular Ca^{2+} (40). Characteristically, the initial rise in Ca^{2+}_i in this type of response is insensitive to chelation or removal of extracellular Ca^{2+}, but the duration of the Ca^{2+}_i response is shortened. Oscillations in Ca^{2+}_i are also thought to be due to an initial IP_3-mediated Ca^{2+} release followed by some type of feedback response involving replenishment of intracellular Ca^{2+} stores from influx of Ca^{2+} from the extracellular medium (41–44). Here, the frequency of oscillations is diminished or inhibited by removal or chelation of extracellular Ca^{2+}, but an initial oscillation always occurs. Cells may also contain voltage-sensitive or receptor-operated plasma membrane Ca^{2+} channels (45). Ligand-induced changes in plasma membrane potential may lead to opening of voltage-dependent Ca^{2+} channels. Ligand binding could also lead to alterations in plasma membrane potential by causing an increase in Ca^{2+}_i and stimulating Ca^{2+}-activated K^+ channels. Lastly, ligand binding could also open receptor-operated Ca^{2+} channels. The net effect is the influx of extracellular Ca^{2+}. Here, removal or chelation of extracellular Ca^{2+} would prevent any ligand-induced alterations in Ca^{2+}_i. In order to determine the mechanisms by which PDGF-AA and PDGF-BB cause alterations in Ca^{2+}_i in VSMC, buffered EGTA (5 mM) was added to cells before or after exposure to the PDGF isoforms (Fig. 4). EGTA addition both prior to as well as after PDGF-AA rapidly inhibited PDGF-AA-induced oscillations in Ca^{2+}_i (Fig. 4, *A*

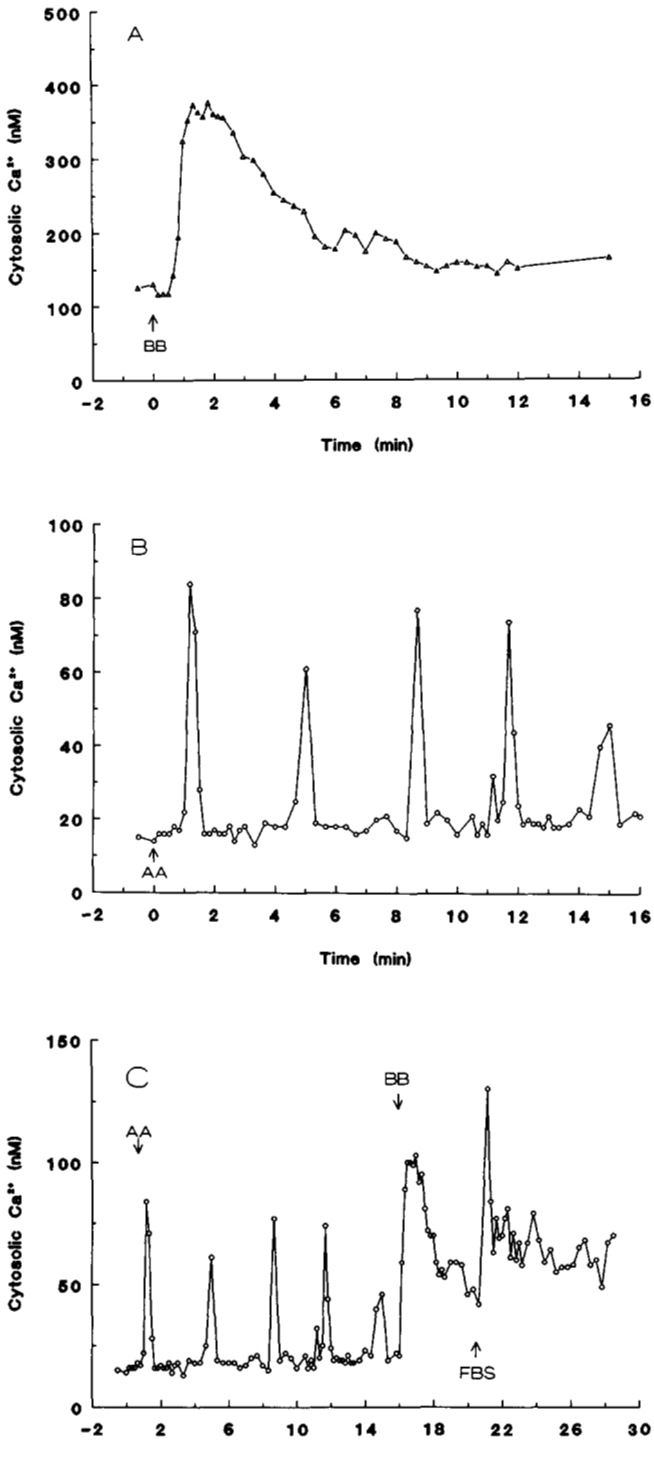

FIG. 3. **Characteristics of changes in $Ca^{2+}{}_i$ in VSMC cell cultures obtained from thoracic aorta following exposure to PDGF isoforms.** Fura-2-loaded VSMC were exposed to PDGF-BB (*A*, 20 ng/ml), PDGF-AA (*B*, 40 ng/ml) or sequentially to PDGF-AA (40 ng/ml), PDGF-BB (20 ng/ml), and 10% fetal bovine serum (*C*). *Arrows* and *labels* indicate point of addition of the various reagents. These traces are representatives of 32 independent experiments comprising 134 cells examined.

and *B*). Conversely, addition of EGTA to VSMC after PDGF-BB shortened the duration of the monophasic $Ca^{2+}{}_i$ response (Fig. 4*C*), while prior addition of EGTA did not inhibit PDGF-BB-induced alterations in $Ca^{2+}{}_i$ (Fig. 4*D*).

DISCUSSION

The present communication presents evidence that both PDGF-AA and PDGF-BB, acting through the A- and B-type PDGF receptors, cause increases in $Ca^{2+}{}_i$, but the characteristics of the $Ca^{2+}{}_i$ response as well as the source of $Ca^{2+}{}_i$ are distinct. Our findings also indicate that a nonuniform distribution of PDGF-AA- and PDGF-BB-responsive porcine aortic VSMC exists, with PDGF-BB-responsive cells predominating, being found both in thoracic and abdominal segments, while PDGF-AA-responsive cells are localized to the thoracic portion of the aorta and make up only a small portion of the total PDGF-responsive cell population. Lastly, we demonstrate that the same VSMC has the capability to sequentially respond to PDGF-AA, PDGF-BB, and fetal bovine seum, and that PDGF-BB Ca^{2+} mobilization mechanisms predominate over those of PDGF-AA when cells are challenged sequentially with both PDGF molecules.

A number of previous reports have provided conflicting data regarding the mitogenic activity of PDGF-AA (35–37). Much of these data have been reconciled by suggesting that PDGF-AA needs to act in concert with other growth factors to promote optimal mitogenesis (35), or that the number of A-type receptors is very low in the less or nonresponsive cell types (1–3, 12). Another potential explanation that has been put forth is that the A- and B-type PDGF receptors are coupled to distinct second messenger pathways (33, 34), one which may be more effective in stimulating mitogenesis. In rat thoracic VSMC, PDGF-BB stimulated alterations in IP_3 levels, $Ca^{2+}{}_i$, intracellular pH, diacylglycerol production, and [^{3}H]thymidine uptake, whereas PDGF-AA stimulated only diacylglycerol production, and did not alter IP_3 levels, $Ca^{2+}{}_i$, or intracellular pH. In these cells, 10–20-fold higher concentrations of PDGF-AA were required to stimulate mitogenesis as compared to PDGF-BB or PDGF-AB (34).

Common to these previous studies examining the differences in the biological activity and potency of different PDGF molecules is the reliance on measurements from large cell populations, either in suspension or culture. This situation has the danger that rare events occurring in a minor population of the cells may be missed as a given response may be buried in the noise of the system. Our data indicate that PDGF-AA-responsive cells (in terms of $Ca^{2+}{}_i$) do exist, but in small number and only at particular sites relative to PDGF-BB-responsive cells. These findings may explain the low number of A-type PDGF receptors and little or no PDGF-AA biological activity observed in previous studies in VSMC; PDGF-AA responses were so small relative to PDGF-BB as to be considered noise in the measurement system. The technique of MDVM has allowed us to detect this low number of PDGF-AA-responsive VSMC in thoracic aorta.

Further proof of the existence of $Ca^{2+}{}_i$-responsive PDGF-AA VSMC, in agreement with previous findings that distinct PDGF receptors can be coupled to different signal transduction pathways, is our detection of distinct types of alterations in $Ca^{2+}{}_i$ elicited by PDGF-AA and PDGF-BB. PDGF-AA caused oscillations in $Ca^{2+}{}_i$ which were blocked by chelation of extracellular Ca^{2+}, while PDGF-BB-induced increases in $Ca^{2+}{}_i$ were only partially sensitive to EGTA treatment. The source of Ca^{2+} in response to exposure of cells to PDGF-BB is likely to be a combination of release from intracelluar stores and influx of extracellular Ca^{2+}. Data from our laboratory as

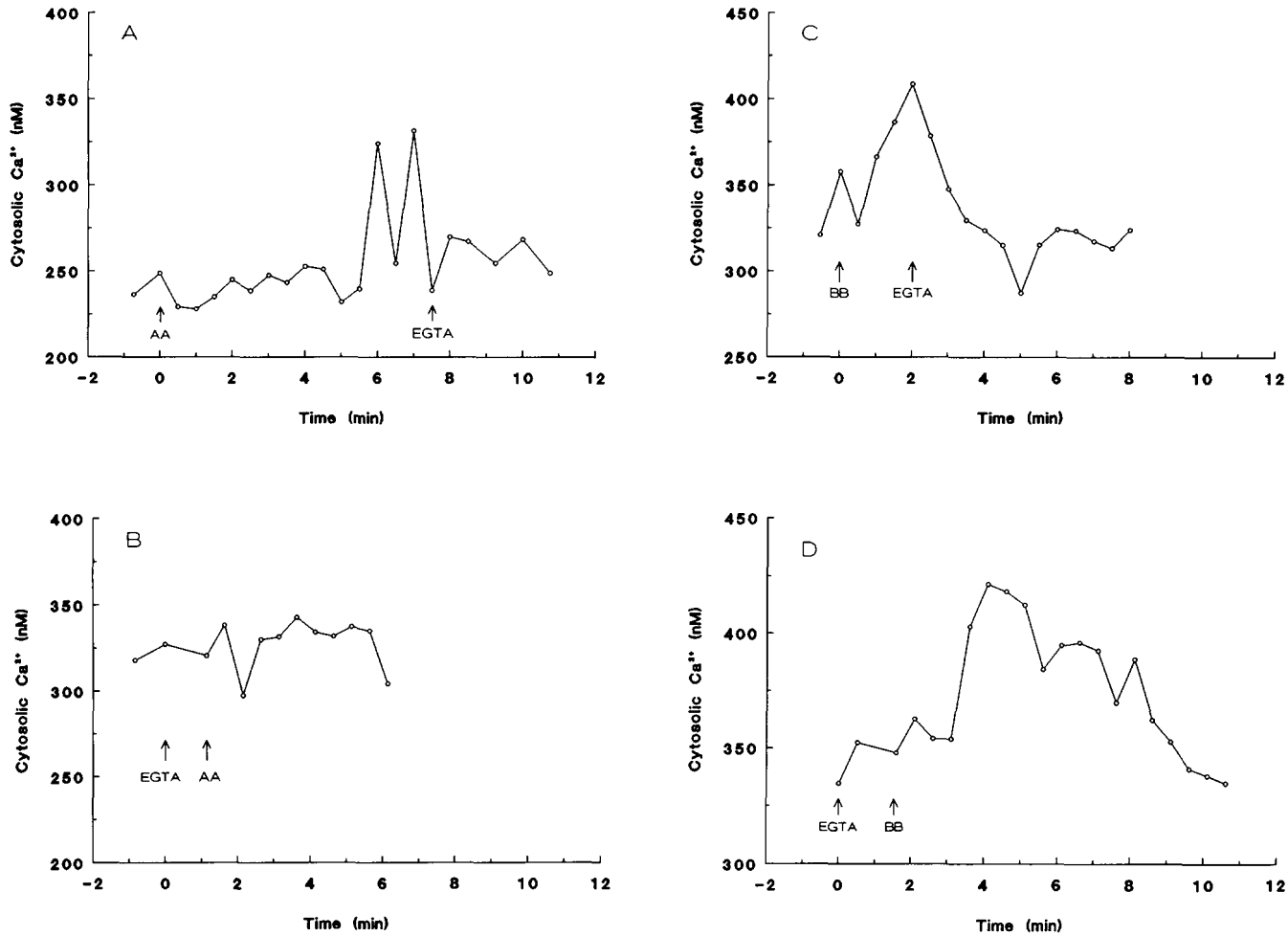

FIG. 4. **Effect of EGTA on PDGF-AA and PDGF-BB-induced alterations in $Ca^{2+}{}_i$ in VSMC.** VSMC cell cultures obtained from the thoracic portion of the aorta were loaded with Fura-2 and exposed to PDGF-AA (40 ng/ml, *A* and *B*) or PDGF-BB (20 ng/ml, *C* and *D*). 5 mM EGTA was added either after (*A* and *C*) or before (*B* and *D*) PDGF. These results are representative of 29 independent experiments comprising 147 cells examined.

well as others have shown that both PDGF-BB and PDGF-AB (which can bind to the B-type PDGF receptor) stimulates IP_3 production which would lead to release of intracellularly stored Ca^{2+} (19, 33, 34). The fact that the $Ca^{2+}{}_i$ response of VSMC exposed to PDGF-BB was not blocked by EGTA treatment but was shortened, is consistent with an IP_3-mediated Ca^{2+} release mechanism (40). In contrast to PDGF-BB, PDGF-AA-responsive VSMC displayed oscillatory changes in $Ca^{2+}{}_i$. Based on the binding properties of the two PDGF receptors (14–16), and the differential type of Ca^{2+} responses elicited by PDGF-AA and PDGF-BB reported in this study (purportedly through the A- and B-type receptor, respectively), and the ability of PDGF-AA-responsive cells to sequentially alter their $Ca^{2+}{}_i$ in response to PDGF-BB, our data would indicate that, minimally, the thoracic aorta contains two cell populations, one with both the A- and B-type receptor and the other with just the B-type receptor, whereas abdominal VSMC seem only to contain the B-type PDGF receptor.

Oscillations in Ca^{2+} can occur in both excitatable and nonexcitatable cells and be of two types, amplitude-dependent or frequency-dependent (46). In amplitude-dependent oscillations, the size of each oscillation is dependent on agonist concentration, while in frequency-dependent oscillations, the frequency of the oscillations is agonist dependent but the amplitude of the oscillations is agonist concentration-independent. Such oscillations might be part of a frequency-encoded signaling system (47), whereby, certain cells can convey information by varying the frequency of the oscillations, which act as a digital signal. Such a signaling system obviates the noise problems of an amplitude-dependent system (where the biological response is proportional to the amplitude of the signal). Small amplitudes are hard to detect over noise, whereas constant large amplitudes with varying frequency are easy to discriminate even in a noisy system.

Oscillators are usually classed as either membrane oscillators, where a change in plasma membrane potential or the repetitive opening and closing of a receptor-operated Ca^{2+} channel regulates Ca^{2+} influx into cells, or cytosolic oscillators, where the source of Ca^{2+} for the oscillations is intracellular. Cytosolic oscillators can generate Ca^{2+} responses in the absence of extracellular Ca^{2+} (although the frequency of the oscillations is dependent on extracellular Ca^{2+}), while membrane oscillators are totally dependent on extracellular Ca^{2+}. Our data indicate that PDGF-AA-induced oscillations require extracellular Ca^{2+}; EGTA addition before or during oscillations inhibits any subsequent change in $Ca^{2+}{}_i$. This would suggest that PDGF-AA-induced $Ca^{2+}{}_i$ oscillations are due to influx of Ca^{2+} from extracellular sources (membrane oscillator) and not from intracellular release, (cytosolic oscillator).

While the exact type of membrane oscillator involved in PDGF-AA-induced alterations in $Ca^{2+}{}_i$ is not yet known, VSMC plasma membranes contain potential dependent (voltage regulated) Ca^{2+} channels (48), and PDGF-AB has been shown to cause a depolarization of the plasma membrane potential, apparently opening an L-type (based on inhibitor specificity) voltage-sensitive Ca^{2+} channel in a G protein-dependent fashion (19). Earlier studies have also demonstrated changes in plasma membrane potential and a dependence on extracellular Ca^{2+} for the increase in $Ca^{2+}{}_i$ that follows growth factor (*i.e.* PDGF) binding (49–53). Further work monitoring PDGF-induced alterations in VSMC plasma membrane potential relative to alterations in $Ca^{2+}{}_i$ and the effects of antagonists of putative voltage-sensitive Ca^{2+}-channels and Ca^{2+}-activated K^+ channels following exposure to the different PDGF isoforms should clarify the regulation of PDGF-AA induced Ca^{2+} oscillations in VSMC.

A key question that remains is whether both PDGF-AA and PDGF-BB are mitogenic and if so, whether the type of Ca^{2+} response we have observed differentiates the two PDGF molecules in terms of mitogenesis or other PDGF-dependent functions. Answers to these questions will have to wait until studies of sequential observation of alterations in $Ca^{2+}{}_i$ and mitogenesis can be performed at the single cell level. Such studies are now underway in our laboratories. However, at this time, preliminary data[2] indicate that between 20–30% of thoracic VSMC, but no abdominal VSMC, respond mitogenically to PDGF-AA (using a BrdU single cell incorporation assay), and this mitogenic response can be inhibited by pretreatment of cells with the Ca^{2+} chelator BAPTA. This is the same percentage of thoracic VSMC which display alterations in Ca^{2+} to PDGF-AA. 70–80% of both thoracic and abdominal VSMC respond to PDGF-BB and PDGF-AB. Thus, these data are consistent with the number of cells which display alterations in $Ca^{2+}{}_i$ when exposed to the different PDGF molecules and support the contention that alterations in $Ca^{2+}{}_i$ are associated with PDGF-stimulated mitogenesis.

[2] P. A. Diliberto and B. Herman, unpublished results.

REFERENCES

1. Heldin, C.-H., Backstrom, G., Östman, A., Hammacher, A., Ronnstrand, L., Rubin, K., Nister, M., and Westermark, B. (1988) *EMBO J.* **7,** 1387–1393
2. Heldin, C.-H., and Westermark, B. (1990) *J. Cell Sci.* **96,** 193–196
3. Claesson-Welsh, L., and Heldin, C.-H. (1989) *Acta Oncol.* **28,** 331–334
4. Ross, R., Raines, E. W., and Bowen-Pope, D. F. (1986) *Cell* **46,** 155–169
5. Hammacher, A., Hellman, U., Johnsson, A., Östman, A., Gunnarsson, K., Westermark, B., Wasteson, A., and Heldin, C.-H. (1988) *J. Biol. Chem.* **263,** 16493–16498
6. Heldin, C.-H., and Westermark, B. (1989) *Trends Genet.* **5,** 108–111
7. Paulsson, Y., Hammacher, A., Heldin, C.-H., and Westermark, B. (1987) *Nature* **328,** 715–717
8. Bowen-Pope, D. F., Hart, C. E., and Seifert, R. A. (1989) *J. Biol. Chem.* **264,** 2502–2508
9. Heldin, C.-H., Johnson, A., Wennergren, S., Wernstedt, C., Beltsholtz, C., Westermark, B. (1986) *Nature* **319,** 511–514
10. Westermark, B. Johnsson, A., Paulsson, Y., Betsholtz, C., Heldin, C.-H., Herlyn, M., Rodeck, U., and Koprowski, H. (1986) *Proc. Natl Acad. Sci. U. S. A.* **83,** 7197–7200
11. Terracio, L., Rnnstrand, L., Tingstrom, A., Rubin, K., Claesson-Welsh, L., Funa, K., and Heldin, C.-H. (1988) *J. Cell Biol.* **107,** 1947–1958
12. Hart, C. E., Forstrom, J. W., Kelly, J. D., Seifert, R. A., Smith, R. A., Ross, R., Murray, M. J., and Bowen-Pope, D. F. (1988) *Science* **240,** 1529–1531
13. Claesson-Welsh, L., Ericksson, A., Westermark, B., and Heldin, C.-H. (1989) *Proc. Natl Acad. Sci. U. S. A.* **86,** 4917–4921
14. Hoppe, J., Weich, H. A., Eichner, W., and Tatje, D. (1990) *Eur. J. Biochem.* **187,** 207–214
15. Seifert, R. A., Hart, C. E., Phillips, P. E., Fostrom, J. W., Ross, R., Murray, M. J., and Bowen-Pope, D. F. (1989) *J. Biol. Chem.* **264,** 8771–8778
16. Heldin, C.-H., Ernlund, A., Rorsman, C., and Rnnstrand, C. (1989) *J. Biol. Chem.* **264,** 8905–8912
17. Bernacki, S. H., Wray, B. E., Kusmik, W., and Herman, B. (1990) *in Optical Microscopy for Biology* (Herman, B., and Jacobson, K., eds) pp. 307–322, Wiley/Liss, New York
18. Herman, B., Roe, M., Harris, C., Wray, B., and Clemmons, D. (1987) *Cell Motil. Cytosk.* **8,** 91–105
19. Roe, M., Hepler, J. R., Harris, C., and Herman, B. (1989) *J. Cell Physiol.* **139,** 100–108
20. Zagari, M., Hepler, J. R., Harris, C., and Herman, B. (1988) *Biochem. Biophys. Res. Commun.* **150,** 1207–1212
21. Zagari, M., Stephens, M., Earp, H. S., and Herman, B. (1989) *J. Cell Physiol.* **139,** 167–174
22. Sperti, G., and Colucci, W. S. (1987) *Fed. Proc.* **46,** 974
23. Frantz, C. N. (1985) *Exp. Cell Res.* **158,** 287–300
24. Tucker, R. W., and Fay, F. S. (1990) *Eur. J. Cell Biol.* **51,** 120–127
25. Tucker, R. W., Chang, D. T., and Meade-Cobun, K. (1989) *J. Cell. Biochem.* **39,** 139–151
26. Hesketh, T. R., Smith, G. A., Moore, J. P., Taylor, M. V., and Metcalf, J. C. (1983) *J. Biol. Chem.* **258,** 4876–4882
27. McNeil, P. L., McKenna, M. P., and Taylor, D. L. (1985) *J. Cell Biol.* **101,** 372–379
28. Mix, L. L., Dinerstein, R. J., and Villereal, M. L. (1984) *Biochem. Biophys. Res. Commun.* **119,** 69–75
29. Moolenear, W. H., Tertoolen, L. G. J., and de Laat, S. W. (1984) *J. Biol. Chem.* **259,** 8066–8069
30. Morris, J. D. H., Metcalte, J. C., Smith, G. A., Hesketh, T. R., and Taylor, M. V. (1984) *FEBS Lett.* **169,** 189–193
31. Tacher, R. W., Snowdowne, K. W., and Borte, A. B. (1986) *Eur. J. Cell Biol.* **41,** 347–351
32. Diliberto, P. A., and Herman, B. (1990) *FASEB J.* **4,** A489
33. Block, L. H., Emmons, L. R., Vogt, E., Sachinidio, A., Vetter, W., and Hoppe, J. (1989) *Proc. Natl Acad. Sci. U. S. A.* **86,** 2388–2392
34. Sachinidis, A., Locher, R., Vetter, W., Tatje, D., and Hoppe, J. (1990) *J. Biol. Chem.* **265,** 10238–10243
35. Majack, R. A., Majesky, M. W., and Goodman, L. V. (1990) *J. Cell Biol.* **111,** 239–247
36. Raines, E. W., Dower, S. K., and Ross, R. (1989) *Science* **243,** 393–396
37. Sjohund, M., Hedin, U., Sejersen, T., Heldin, C.-H., and Thyberg, J. (1988) *J. Cell. Biol.* **106,** 403–413
38. Roe, M. W., Lemasters, J. J., and Herman, B. (1990) *Cell Calcium* **11,** 63–73
39. Hepler, J. R., Nakahata, N., Lovenberg, T. W., DiGuiseppi, J., Herman, B., Earp, H. S., and Harden, T. K. (1987) *J. Biol. Chem.* **262,** 2951–2956
40. Rasmussen, H. (1986) *N. Engl. J. Med.* **314,** 1094–1110, 1164–1170
41. Ambler, S. K., Poenie, M., Tsien, R. Y., and Taylor, P. (1988) *J. Biol. Chem.* **263,** 1952–1959
42. Jacob, R., Merritt, J. E., Halbum, T. J., and Rink, T. J. (1988) *Nature* **335,** 40–45
43. Sage, S. O., Adams, D. J., and van Breeman, C. (1989) *J. Biol. Chem.* **264,** 6–9
44. Woods, N. M., Cuthbertson, K. S. R., and Cobbold, D. H. (1987) *Cell Calcium* **8,** 79–100
45. Khalil, R., Lodge, N., Sarda, K., and van Breeman, C. (1987) *J. Hypertension* **5,** (Supl. 4) s5–s15
46. Berridge, M. J., and Galione, A. (1988) *FASEB J.* **2,** 3074–3082
47. Berridge, M. J. (1990) *J. Biol. Chem.* **265,** 9583–9586
48. Sadoshima, J., Alkaike, N., Kanaide, H., and Nakamura, M. (1988) *Am. J. Physiol.* **255,** H754–H759
49. Frace, A. M., and Gargus, J. J. (1989) *Proc. Natl. Acad. Sci. U. S. A.* **86,** 2511–2515
50. Moolenaar, W. H., DeFrize, L. H. K., and DeLaat, S. W. (1986) *J. Exp. Biol.* **124,** 359–373
51. Moolenaar, W. H., DeLaat, S. W., aVan der Saag, D. T. (1979) *Nature* **279,** 721–723
52. Rothenberg, P., Reuss, L., and Galser, L. (1982) *Proc. Natl. Acad. Sci. U. S. A.* **79,** 7783–7787
53. Moolenaar, W. H., Tertoolen, L. G. J., and DeLaat, S. W. (1984) *J. Biol. Chem.* **259,** 8066–8069