Effect of platelet-derived growth factor on voltage-operated calcium channels in rabbit isolated ear artery cells

¹S. Wijetunge & A.D. Hughes

Department of Clinical Pharmacology, St. Mary's Hospital Medical School, Imperial College of Science Technology & Medicine, London W2 1NY

1 Platelet derived growth factor (PDGF), AB and BB isoforms (100 pM) increased calcium channel currents measured by whole cell voltage clamp technique in single vascular smooth muscle cells isolated from rabbit ear arteries.

2 Tyrphostin-23 (100 μ M) a selective inhibitor of protein tyrosine kinases, reduced calcium channel currents. Pre-incubation with tyrphostin-23 prevented PDGF-AB induced increase in calcium channel currents. However, in these same cells 10 nM (+)-202791, a dihydropyridine calcium channel agonist, did increase calcium channel currents.

3 Bistyrphostin (10 μ M), a selective inhibitor of epidermal growth factor (EGF)-kinase also reduced calcium channel currents and inhibited PDGF-AB-induced increases in calcium channel currents.

4 Genistein (100 μ M) a selective inhibitor of tyrosine kinases, structurally unrelated to the tryphostins, also inhibited calcium channel currents and pre-incubation with genistein prevented the PDGF-AB-induced rise in calcium channel currents.

5 These results indicate that PDGF increases calcium channel currents in vascular smooth muscle. This action of PDGF probably involves a tyrosine kinase.

Keywords: Platelet-derived growth factor; calcium channels; vascular smooth muscle; tyrosine kinase; tyrphostin; bistyrphostin; genistein

Introduction

Platelet derived growth factor (PDGF) is a 28–32 kDa peptide which acts as a potent mitogen and chemotactic factor for many cells of mesenchymal origin including vascular smooth muscle cells (Ross, 1989; Bobik & Campbell, 1993). It is a dimeric glycoprotein formed of two distinct but related peptide chains; peptide A of molecular weight 14–18 kDa and peptide B (16 kDa). The two chains are linked by disulphide bonds to form two homologous (PDGF-AA and PDGF-BB) isoforms and a heterologous (PDGF-AB) isoform, all of which have been isolated from natural sources (Johnsson *et al.*, 1982; Stroobant & Waterfield, 1984; Heldin *et al.*, 1986). In the vasculature, PDGF is produced by several types of cells including platelets, endothelial cells, macrophages and vascular smooth muscle cells although each cell may produce different amounts of each isoform (Bobik & Campbell, 1993).

Membrane receptors for PDGF are 170–180 kDa, transmembrane spanning glycoproteins formed of two subunits, α and β (Hart *et al.*, 1988). Binding of the PDGF isoforms to the extracellular domain of the receptors promotes formation of dimers; $\alpha\alpha$, $\beta\beta$ and $\alpha\beta$, the dimer formed being dependent on the isomer of PDGF (Seifert *et al.*, 1989). The consequence of binding and dimerization of receptors is to activate a tyrosine kinase which is intrinsic to the receptor (Heldin *et al.*, 1989), leading to autophosphorylation of the receptor at multiple tyrosine residues of the intracellular domain (Pazin & Williams, 1992). The autophosphorylated receptors act as specific binding sites for a variety of cytoplasmic proteins containing src homologous (SH2) domains, which link the receptor to a variety of cytoplasmic and nuclear events (Pazin & Williams, 1992). The tyrosine kinase activity of PDGF receptors is known to be critical for the signal transduction pathway required for PDGF-induced mitogenesis (Ullrich & Schlessinger, 1990).

It has been found that PDGF elicits vascular smooth muscle contraction (Berk *et al.*, 1986; Berk & Alexander, 1989; Hughes, 1995). However, the mechanism by which PDGF

causes contraction of vascular smooth muscle or whether there is a link between contraction and cell proliferation is not known. PDGF-induced vasoconstriction is reported to be unaffected by inhibition of 5-hydroxytryptamine (5-HT) receptors, α -adrenoceptors or cyclo-oxygenase (Berk *et al.*, 1986) and therefore probably involves a direct action of the PDGF receptor. The role of protein tyrosine kinase (PTK) in regulating calcium channels is also not clear, although we have previously shown that selective inhibitors of PTK such as tyrphostin-23 and genistein, which inhibit tyrosine kinase by different mechanisms, inhibit voltage-operated calcium channels in rabbit ear artery cells (Wijetunge *et al.*, 1992).

The objective of this study was to investigate the effect of PDGF on the regulation of voltage-operated calcium channels in vascular smooth muscle cells and to study the role of tyrosine phosphorylation in the action of PDGF by use of selective PTK inhibitors, tyrphostin-23, bistyrphostin and genistein.

Methods

Single smooth muscle cells were freshly dispersed from rabbit ear arteries by a method previously described (Benham & Bolton, 1986). Short (2–3 mm) segments of artery were incubated for 50 min in a modified physiological salt solution (PSS) containing (mM): NaCl 130, KCl 6, CaCl₂ 0.01, MgCl₂ 1.2, glucose 14, and HEPES 10.7 buffered to pH 7.2 with NaOH, 2 mg ml⁻¹ bovine serum albumin, 1 mg ml⁻¹ collagenase (130 u mg⁻¹), 0.5 mg ml⁻¹ papain (15 u mg⁻¹) and 5 mM dithiothreitol. Cells were dispersed by mild agitation and resuspended after centrifugation in normal PSS containing 1.7 mM CaCl₂. Cells were stored on cover slips at 4°C and used within 4–6 h. Internal pipette solution contained (mM): NaCl 126, MgCl₂ 1.2, EGTA 2, MgATP 2, TEA 10, and HEPES 11 buffered to pH 7.2 with NaOH. Patch pipettes were made from borosilicate glass and had resistances of 3–5 M Ω .

Calcium channel currents were measured by the whole cell configuration of the patch clamp technique (Hamill *et al.*,

¹ Author for correspondence.

Figure 1 The effects of platelet-derived growth factor (PDGF) and inhibitors of tyrosine kinase on calcium channel currents in rabbit ear artery cells. Figure shows % increase in peak calcium current induced by PDGF, evoked by 200 ms depolarization to +10 mV from a holding potential of -60 mV. Columns represent means $\pm$ s.e. means of (n) observations. Bistyr, bistyrphostin; Tyr, tyrphostin.

1981) using a List EPC-7 amplifier and a Labmaster A/D interface board with commercially available software (Pclamp 5.5, Axon Instruments CA, U.S.A.) on an IBM compatible PC. The experiments were carried out in a 'high barium' solution (BaCl_2 110 mM, HEPES 10 mM and buffered to pH 7.2 with TEA-OH) to increase the size of the inward current and to minimize calcium-dependent inactivation of currents (Aaronson *et al.*, 1988). Data were recorded on-line, or on digital audio tape with a DAT recorder (Biologic, France), and analysed off-line after analogue-to-digital conversion. The currents were digitally filtered at 2 kHz and leak currents were subtracted digitally, using average values of steady leakage currents elicited by a 10 mV hyperpolarizing pulse (Aaronson *et al.*, 1988). Drugs were applied by bath perfusion and exchange of bath perfusate was complete in approximately 1 s. All recordings were made at room temperature (22–25°C). A standard concentration of PDGF (100 pM) which was found to be a near maximal effective dose in previous studies (Hughes, 1995) was used. For the inhibitors, concentrations in excess of the IC_{50} from previous studies (Levitzki & Gilon, 1991; Wijetunge *et al.*, 1992) were used.

Drugs and chemicals

The following (source in parentheses) were used: (+)-202791 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridine-carboxylate) (gift from Sandoz Switzerland), bistyrphostin (Calbiochem, Nottingham), bovine serum albumin (essentially fatty acid free) (Sigma, Dorset), collagenase (Worthington, Reading), genistein (Gibco BRL, Scotland), PDGF-AB and PDGF-BB (Calbiochem, Nottingham), papain (Sigma, Dorset), tyrphostin-23 (Calbiochem, Nottingham).

Statistics and data analysis

Data are presented as means $\pm$ s.e. means of (n) observations. Comparisons of data were made with a Wilcoxon signed ranks

Figure 2 Onset of platelet-derived growth factor (PDGF)-AB induced increase of calcium currents. Currents were evoked every 1 s using a 20 ms pulse to +10 mV from a holding potential of -60 mV. (a) Currents recorded in a single cell at 1 s, 5 s, 15 s, 30 s and 60 s following addition of PDGF-AB are shown. (b) Each dot represents the average current recorded over a 5 s period from a single cell before and after addition of PDGF-AB. Period of exposure to PDGF-AB (100 pM) is indicated by the bar. Similar results were observed in four other cells.

test for single paired comparisons. Multiple comparisons were made using non-parametric two-way analysis of variance by the Friedman test followed by the multiple comparison method described by Conover (1980). $P < 0.05$ was considered statistically significant.

Results

In cells voltage-clamped at a holding potential of -60 mV, 100 pM PDGF-AB increased the peak calcium channel currents evoked every 1 s by a 200 ms step to +10 mV from 37.2 ± 3 to 49.6 ± 4 pA which corresponds to a $31.6 \pm 2\%$ ($P < 0.05$, $n = 5$) increase in current (Figure 1). To examine the onset of the effect of PDGF-AB, currents were evoked every 1 s by a 20 ms depolarizing step to +10 mV from a holding potential of -60 mV. The onset of this effect was rapid and observed within a minute of application of PDGF (Figure 2). Following washout of PDGF-AB, currents slowly recovered to levels similar to those seen prior to application of PDGF ($n = 2$). PDGF increased currents across the range of depolarizing test potentials from -50 mV to +60 mV (Figure 3 and Figure 4a). PDGF-BB (100 pM) increased the peak calcium channel currents evoked every 1 s by a 200 ms step to +10 mV from 45.1 ± 2.0 to 58.0 ± 6.4 pA which corresponds to a $28.5 \pm 6.0\%$ increase in currents ($P < 0.05$, $n = 3$), (Figure 1).

Addition of 100 μ M tyrphostin-23, a selective inhibitor of PTK, decreased peak calcium current evoked by a 200 ms

Figure 3 The effect of platelet-derived growth factor (PDGF)-AB on calcium channel currents in a rabbit ear artery cell. (a) Traces of control and PDGF-AB-induced currents evoked by a 200 ms depolarizing step to +10 mV from a holding potential of -60 mV. Control = current prior to application of PDGF-AB; PDGF-AB = current in the presence of PDGF-AB (100 pM). (b) The effect of 100 pM PDGF-AB on the peak current-voltage relationship. Currents were evoked by 200 ms depolarizing steps from -50 mV to +70 mV from the holding potential, -60 mV. (○) Control; (●) PDGF-AB 100 pM. Similar results were observed in four other cells.

depolarization to +10 mV from a holding potential of -60 mV from 44.1 ± 2 to 29.6 ± 2 pA ($P < 0.05$, $n = 6$). Application of PDGF-AB (100 pM) in the presence of tyrphostin-23 had no statistically significant effect on calcium channel currents (Figure 1 and Figure 4b). However, addition of 10 nM (+)-202791 a dihydropyridine calcium channel agonist (Williams *et al.*, 1985) which acts independently of tyrosine kinase did increase calcium currents significantly in the same cells, despite the presence of tyrphostin-23 ($P < 0.01$, $n = 3$), (Figure 4b).

Bistyrphostin (10 μ M), a selective inhibitor of epidermal growth factor (EGF)-kinase, reduced calcium current evoked by a 200 ms depolarization to +10 mV from a holding potential of -60 mV from 52.3 ± 2 to 41.5 ± 4 pA ($P < 0.05$, $n = 3$). Pre-incubation with bistyrphostin (10 μ M) abolished the PDGF-AB induced increase in calcium current (Figure 1).

Genistein (100 μ M) a selective tyrosine kinase inhibitor reduced calcium currents evoked by a 200 ms depolarization to +10 mV from a holding potential of -60 mV from 38.0 ± 5.0

Figure 4 The effect of tyrphostin-23 on responses to platelet-derived growth factor (PDGF)-AB and (+)-202791. Plots show current-voltage relationships in a single rabbit ear artery cell. Current was evoked by a 200 ms depolarization steps from a holding potential of -60 mV. (a) (○) Control; (●) following PDGF-AB 100 pM. Similar results were shown in four other cells. (b) (●) Control; (□) tyrphostin-23 100 μ M; (■) 100 pM PDGF-AB in the presence of 100 μ M tyrphostin-23; (△) (+)-202791 10 nM in the presence of 100 μ M tyrphostin-23. Similar results were observed in two more cells.

to 16.5 ± 0.8 pA ($P < 0.05$, $n = 3$). PDGF-AB (100 pM) in the presence of genistein did not cause a significant rise in calcium channel currents (Figure 1).

Effects of PDGF-AB in the presence of tyrphostin-23, bistyrphostin and genistein were tested in batches of cells that responded to PDGF-AB in the absence of these inhibitors.

Discussion

Voltage-operated calcium channels are a major route of calcium entry into vascular smooth muscle cells (Van Breeman & Saida, 1989) and play an important role in excitation-contraction coupling in response to most contractile agonists (Bolton, 1979). PDGF is known to contract vascular smooth muscle and increase intracellular Ca^{2+} by a mechanism involving voltage-operated calcium channels in rabbit isolated ear artery (Hughes, 1995). A rise in intracellular Ca^{2+} is also believed to be an important, though not an essential, mitogenic

signal evoked by several growth factors including PDGF (Magni *et al.*, 1991; Kobayashi *et al.*, 1994). The role of voltage-operated calcium channels in mediating this rise in intracellular Ca^{2+} in dividing cells is unclear, although calcium channel antagonists such as nifedipine and verapamil have been reported to inhibit smooth muscle proliferation (Nilsson *et al.*, 1985; Stein *et al.*, 1987). Protein phosphorylation on tyrosine residues is known to occur during both normal and malignant cell growth and in particular tyrosine phosphorylation has been shown to be critical in the mitogenic response of vascular smooth muscle cells (Tsuda *et al.*, 1991). Several other growth factors such as EGF, fibroblast growth factor (FGF) and insulin like growth factor-1 (IGF-1) also appear to act by binding to receptors which are themselves tyrosine kinases (Ullrich & Schlessinger, 1990). The possibility that tyrosine phosphorylation plays a critical role in other processes such as calcium entry and force development by smooth muscle is now also receiving attention (Hollenberg, 1994a). A recent report by Hollenberg showed that EGF causes contraction of gastric smooth muscle and that this was inhibited by selective PTK inhibitors tyrphostin and genistein (Hollenberg, 1994b). Intracellular Ca^{2+} ($[\text{Ca}^{2+}]_i$) was not measured in these studies, but $[\text{Ca}^{2+}]_i$ is known to regulate the tone of smooth muscle and EGF has been reported to cause Ca^{2+} influx into fibroblasts (Magni *et al.*, 1991). Recently it was reported by Selinfreund & Blair (1994) that IGF-1, which is also linked to a PTK caused a rapid increase in voltage-operated calcium channel currents in clonal pituitary cells. This action of IGF-1 was inhibited by genistein a selective inhibitor of tyrosine kinases. Similarly we found that two tyrphostins, tyrphostin-23 and bistyrphostin, also inhibited the stimulatory effect of PDGF on voltage-operated calcium channel currents in vascular smooth muscle cells.

Tyrphostins are selective inhibitors of tyrosine kinase which bind to the substrate binding site of the PTK (Gazit *et al.*, 1989). They are low molecular weight compounds which have been shown to inhibit the effects of PTK in a number of systems (Gazit *et al.*, 1989). Tyrphostins have been shown to possess no significant effects on several serine or threonine protein kinases such as protein kinase C (PKC), cyclic AMP-dependent kinase or cyclic GMP-dependent kinase (Levitzki & Gilon, 1991). However some tyrphostins have been reported to interfere with other cellular processes and enzymes such as fatty acid synthesis, pyruvate oxidation and aldehyde dehydrogenase (Young *et al.*, 1993), therefore some caution with regard to their specificity towards PTK is needed. Nevertheless, tyrphostins have been shown to inhibit tyrosine autophosphorylation of the PDGF-receptor accompanying PDGF-induced proliferation in vascular smooth muscle cells (Bilder *et al.*, 1991). Tyrphostins have also been reported to inhibit rises in $[\text{Ca}^{2+}]_i$ and contraction in vascular smooth muscle at concentrations consistent with a selective action on tyrosine kinases (Sauro & Thomas, 1993; Hughes, 1995). In this study tyrphostin-23, reduced calcium currents as we have previously reported (Wijetunge *et al.*, 1992) at a concentration which inhibits PTKs (Gazit *et al.*, 1989). At present it is un-

clear whether the effect of tyrphostin-23 on calcium currents involves a tyrosine kinase but it is unlikely to represent a non-specific block of calcium channels because the dihydropyridine calcium channel agonist (+)-202791 (Williams *et al.*, 1985; Hughes *et al.*, 1990), which activates calcium channels by a mechanism independent of PTK increased currents in the presence of tyrphostin-23.

Bistyrphostin is a more selective and potent inhibitor of EGF and PDGF receptor-linked tyrosine kinases. Bistyrphostin alone, at a concentration in excess of that necessary to inhibit EGF-kinase had reduced calcium currents, although less potently than tyrphostin-23, and also blocked PDGF-AB-induced increases in calcium currents.

Genistein is an inhibitor of PTK which competitively binds to the ATP binding site on the PTK. It is an isoflavonoid compound and has been shown to inhibit specifically tyrosine kinases in a variety of systems without inhibiting the activities of serine and threonine-specific protein kinases (Akiyama *et al.*, 1987). Like the tyrphostins, genistein also prevented the PDGF-induced increase in calcium channel currents.

Together these results are consistent with tyrosine phosphorylation being an obligate signal in PDGF-induced increase in calcium channel currents.

The mechanism by which tyrosine phosphorylation may modulate calcium channel activity is not clear. It is possible that the tyrosine kinases may phosphorylate the voltage-operated calcium channel itself. At present, there is no evidence of tyrosine phosphorylation of the L-type calcium channel, though tyrosine phosphorylation of the nicotinic acetylcholine receptor channel and of a delayed rectifier potassium channel has been reported (Siegelbaum, 1994). Alternatively PDGF acting through a tyrosine kinase may modulate channel activity by activation of a second messenger system such as stimulation of PKC via phospholipase C- γ (PLC γ). Dimerization and autophosphorylation of growth factor receptors leads to activation of several cytoplasmic and nuclear events including activation of ras p21 (Schlessinger, 1993), mitogen-activated protein kinases (Cadena & Gill, 1992), PLC γ (Rhee, 1991), GTPase activating protein (GAP) for ras (Cadena & Gill, 1992) and phosphatidylinositol-3-kinase (Schlessinger & Ullrich, 1992). Each of these is believed to possess important second messenger roles, though in many cases their precise functions remain to be defined.

In conclusion PDGF, a potent mitogen for vascular smooth muscle, increases voltage-operated calcium channel currents. This effect is probably mediated by a process involving tyrosine phosphorylation. This action of PDGF may be important for Ca^{2+} entry into vascular smooth muscle cells in response to PDGF and therefore play an important role in the contractile and mitogenic action of this growth factor.

This work was supported by a grant from the Medical Research Council.

References

- AARONSON, P.I., BOLTON, T.B., LANG, R.J. & MACKENZIE, I. (1988). Calcium currents in single isolated smooth muscle cells from the rabbit ear artery in normal calcium and high barium solutions. *J. Physiol.*, **405**, 57–75.
- AKIYAMA, T., ISHIDA, J., NAKAGAWA, S., OGAWARA, H., WATANABE, S., ITOH, N., SHIBUYA, M. & FUKAMI, Y. (1987). Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J. Biol. Chem.*, **262**, 5592–5595.
- BENHAM, C.D. & BOLTON, T.B. (1986). Spontaneous transient outward currents in single visceral and vascular smooth muscle cells of the rabbit. *J. Physiol.*, **381**, 385–406.
- BERK, B.C. & ALEXANDER, R.W. (1989). Vasoactive effects of growth factors. *Biochem. Pharmacol.*, **38**, 219–225.
- BERK, B.C., ALEXANDER, R.W., BROCK, T.A., GIMBRONE, M.A. Jr., & WEBB, R.C. (1986). Vasoconstriction: a new activity for PDGF. *Science*, **232**, 87–90.
- BILDER, G.E., KRAWIEC, J.A., MCVETY, K., GAZIT, A., GILON, C., LYALL, R., ZILBERSTEIN, A., LEVITZKY, A., PERRONE, M.H. & SCHREIBER, A.B. (1991). Tyrphostins inhibit PDGF-induced DNA synthesis and associated early events in smooth muscle cells. *Am. J. Physiol.*, **260**, C721–C730.
- BOBIK, A. & CAMPBELL, J.H. (1993). Vascular derived growth factors: Cell biology, pathophysiology and pharmacology. *Pharmacol. Rev.*, **45**, 1–42.
- BOLTON, T.B. (1979). Mechanisms of action of transmitters and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

- CADENA, D.L. & GILL, G.N. (1992). Receptor tyrosine kinases. *FASEB J.*, **6**, 2332–2337.
- CONOVER, C.W. (1980). *Practical Non-Parametric Statistics*, 2nd Edition, New York: John Wiley.
- GAZIT, A., YAISH, P., GILON, C. & LEVITZKI, A. (1989). Tyrphostins I: synthesis and biological activity of protein tyrosine kinase inhibitors. *J. Med. Chem.*, **32**, 2344–2352.
- HAMILL, O.P., MARTY, A., NEHER, E., SAKMANN, B. & SIGWORTH, F.J. (1981). Improved patch clamp techniques for high resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.*, **391**, 85–100.
- HART, C.E., FORSTROM, J.W., KELLY, J.D., SEIFERT, R.A., SMITH, R.A., ROSS, R., MURRAY, M.J. & BOWEN-POPE, D.F. (1988). Two classes of PDGF receptor recognize different isoforms of PDGF. *Science*, **240**, 1529–1531.
- HELDIN, C.H., ERNLUND, A., ROSSMAN, C. & RONNSTRAND, L. (1989). Dimerization of B-type platelet derived growth factor receptors occurs after ligand binding and is closely associated with the receptor tyrosine kinase activation. *J. Biol. Chem.*, **264**, 8905–8912.
- HELDIN, C.H., JOHNSSON, A., WENNERGREEN, S., WERNSTEDT, C., BETSHOLTZ, C. & WESTERMARK, B. (1986). A human osteosarcoma cell line secretes a growth factor structurally related to a homodimer of PDGF-A chains. *Nature*, **319**, 511–514.
- HOLLENBERG, M.D. (1994a). The acute actions of growth factors in smooth muscle systems. *Life Sci.*, **54**, 223–235.
- HOLLENBERG, M.D. (1994b). Tyrosine kinase pathways and the regulation of smooth muscle contractility. *Trends Pharmacol. Sci.*, **15**, 108–114.
- HUGHES, A.D., HERING, S. & BOLTON, T.B. (1990). Evidence that agonist and antagonist enantiomers of the dihydropyridine PN 202791 act at different sites on the voltage-dependent calcium channel of vascular muscle. *Br. J. Pharmacol.*, **101**, 3–5.
- HUGHES, A.D. (1995). Increase in tone and intracellular Ca^{2+} in rabbit isolated ear artery by platelet derived growth factor. *Br. J. Pharmacol.*, **114**, 138–142.
- JOHNSSON, A., HELDIN, C.H., WESTERMARK, B. & WASTESON, A. (1982). Platelet-derived growth factor: identification of constituent polypeptide chains. *Biochem. Biophys. Res. Commun.*, **104**, 66–74.
- KOBAYASHI, S., NISHIMURA, J. & KANAIDE, H. (1994). Cytosolic Ca^{2+} transients are not required for platelet derived growth factor to induce cell cycle progression of vascular smooth muscle cells in primary culture. *J. Biol. Chem.*, **269**, 9011–9018.
- LEVITZKI, A. & GILON, C. (1991). Tyrphostins as molecular tools and potential antiproliferative drugs. *Trends Pharmacol. Sci.*, **12**, 171–174.
- MAGNI, M., MELDOLESI, J. & PANDIELLA, A. (1991). Ionic events induced by epidermal growth factor. *J. Biol. Chem.*, **266**, 6329–6335.
- NILSSON, J., SJOLUND, M., PALMBERG, L., VON EULER, A.M., JONZON, B. & THYBERG, J. (1985). The calcium antagonist nifedipine inhibits arterial smooth muscle proliferation. *Atherosclerosis*, **58**, 109–122.
- PAZIN, M.J. & WILLIAMS, L.T. (1992). Triggering signalling cascades by receptor tyrosine kinases. *Trends Biochem. Sci.*, **17**, 374–378.
- RHEE, S.G. (1991). Inositol phospholipid specific phospholipase C: interaction of the γ_1 isoform with tyrosine kinase. *Trends Biochem. Sci.*, **16**, 297–300.
- ROSS, R. (1989). Platelet derived growth factor. *Lancet*, **i**, 1179–1182.
- SAURO, M.D. & THOMAS, B. (1993). Tyrphostin attenuates platelet derived growth factor induced contraction in aortic smooth muscle through inhibition of protein tyrosine kinases. *J. Pharmacol. Exp. Ther.*, **267**, 1119–1125.
- SCHLESSINGER, J. (1993). How receptor tyrosine kinases activate Ras. *Trends Biochem. Sci.*, **18**, 273–275.
- SCHLESSINGER, J. & ULLRICH, A. (1992). Growth factor signalling by tyrosine kinases. *Neuron*, **9**, 383–391.
- SEIFERT, R.A., HART, C.E., PHILLIPS, P.E., FORSTROM, J.W., ROSS, R., MURRAY, M.J. & BOWEN-POPE, D.F. (1989). Two different subunits associate to create isoform-specific platelet derived growth factor receptors. *J. Biol. Chem.*, **264**, 8771–8778.
- SELINFREUND, R.H. & BLAIR, L.A.C. (1994). Insulin-like growth factor-1 induces a rapid increase in calcium currents and spontaneous membrane activity in clonal pituitary cells. *Mol. Pharmacol.*, **45**, 1215–1220.
- SIEGELBAUM, S.A. (1994). Ion channel control by tyrosine phosphorylation. *Current Biol.*, **4**, 242–245.
- STEIN, O., HALPERIN, G. & STEIN, Y. (1987). Long term effects of verapamil on aortic smooth muscle cells cultured in the presence of hypercholesterolaemic serum. *Arteriosclerosis*, **7**, 585–592.
- STROOBANT, P. & WATERFIELD, M.D. (1984). Purification and properties of porcine platelet derived growth factor. *EMBO J.*, **3**, 2963–2967.
- TSUDA, T., KAHAWARA, Y., SHII, K., KOIDE, M., ISHIDA, Y. & YOKOYAMA, M. (1991). Vasoconstrictor induced protein-tyrosine phosphorylation in cultured vascular smooth muscle cells. *FEBS Lett.*, **285**, 44–48.
- ULLRICH, A. & SCHLESSINGER, J. (1990). Signal transduction by receptor tyrosine kinase activity. *Cell*, **61**, 203–212.
- VAN BREEMAN, C. & SAIDA, K. (1989). Cellular mechanisms regulating Ca^{2+} in smooth muscle. *Annu. Rev. Physiol.*, **51**, 315–329.
- WIJETUNGE, S., AALKJAER, C., SCHACTER, M. & HUGHES, A.D. (1992). Tyrosine kinase inhibitors block calcium channel currents in vascular smooth muscle cells. *Biochem. Biophys. Res. Commun.*, **189**, 1620–1623.
- WILLIAMS, J.S., GRUPP, I.L., DUMONT, L., SCHWARTZ, A., YATANI, A., HAMILTON, S. & BROWN, A.M. (1985). Profile of the oppositely acting enantiomers of the dihydropyridine 202791 in cardiac preparations: receptor binding, electrophysiological and pharmacological studies. *Biochem. Biophys. Res. Commun.*, **131**, 13–21.
- YOUNG, S.W., POOLE, R.C., HUDSON, A.T., HALESTRAP, A.P., DENTON, R.M. & TAVARE, J.M. (1993). Effects of tyrosine kinase inhibitors on protein-kinase independent systems. *FEBS Lett.*, **316**, 278–282.

(Received November 30, 1994

Revised February 10, 1995

Accepted February 17, 1995)