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A tyrosine kinase regulates α-adrenoceptor-stimulated contraction and phospholipase D activation in the rat aorta

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Abstract

Since previous studies had indicated a role for tyrosine kinases in α_2-adrenoceptor-induced contractile responses in other blood vessels, as well as in the activation of phospholipase D, we examined the sensitivity of these responses in rat aorta to the tyrosine kinase inhibitor genistein. Contractions induced by both noradrenaline and the α_2-adrenoceptor-selective agonist UK14304 (5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline) were fully inhibited by genistein, with the latter responses being more sensitive. Contractions induced by high K^+ buffer were also inhibited, but to a lesser extent. Both agonists caused a stimulation of phospholipase D activity, which could be blocked by pretreatment with pertussis toxin, indicating involvement of either G_i or G_o. Genistein completely inhibited the agonist-induced phospholipase D activity and also substantially reduced the basal level of phospholipase D activity. Pretreatment with either the α_1-adrenoceptor antagonist prazosin or the α_2-adrenoceptor antagonist rauwolscine was also effective in eliminating the agonist-induced increase of phospholipase D. These results indicate that a tyrosine kinase-regulated phospholipase D plays a critical role in α-adrenoceptor-induced contractions of the rat aorta and that stimulation of both α_1- and α_2-adrenoceptors is essential to allow phospholipase activation.

Keywords: α_2-Adrenoceptor; Phospholipase D; Smooth muscle, vascular; Tyrosine kinase; $pp60^{v-src}$; Hypertension; Protein kinase C

1. Introduction

α-Adrenoceptors can elicit contraction of vascular smooth muscle by multiple mechanisms. In some tissues, α_1-adrenoceptors have been shown to stimulate phosphoinositide hydrolysis leading to intracellular Ca^{2+} release and protein kinase C activation (Campbell et al., 1986), while in other tissues, contraction occurs in the absence of an increase of intracellular Ca^{2+} (Jiang and Morgan, 1987), implying an alternative mechanism. Contractions induced by α_2-adrenoceptor stimulation are known to be highly dependent upon extracellular calcium, but the precise events in the coupling pathway are not fully understood.

Previous studies on rat aorta have noted characteristics suggesting the presence of both α_1- and α_2-adrenoceptors. Thus, yohimbine, a selective antagonist of α_2-adrenoceptors, exhibits a K_D of 20 nM (Ruffolo et al., 1982), similar to its affinity for the cloned α_{2D}-adrenoceptor (Lanier et al., 1991), while prazosin, the selective α_1-adrenoceptor antagonist exhibits a K_D of 0.22 nM (Tian et al., 1990). More recently, the α_1-adrenoceptor subtype has been suggested to be of the α_{1D} subtype (Buckner et al., 1995) The contractile response to noradrenaline has been shown to involve two components: (1) A phasic response which can only be elicited once after removal of extracellular Ca^{2+}. (2) A sustained response which can be repeatedly generated in the absence of extracellular Ca^{2+} (Heaslip and Rahwan, 1982). Two different contractile mechanisms may, therefore, contribute to the overall response.

Stimulation of a number of receptors, including the α_{2A}-adrenoceptor (MacNulty et al., 1992), as well as receptors for endothelin-1 (Liu et al., 1992) and angiotensin II (Lassegue et al., 1991), increases the activity of phospholipase D, resulting in the formation of phosphatidic acid, which in turn can be converted to diacylglycerol by the action of the enzyme phosphatidate phosphohydrolase. In an earlier study, noradrenaline was shown to activate phospholipase D in rat aorta (Jones et al., 1993), although the receptor responsible for the increase was not specifically identified. Such activation could, therefore,

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lead to increased protein kinase C activity and contraction. In studies with rat tail artery, noradrenaline was also found to activate phospholipase D, and since the response was inhibited by the α_1-adrenoceptor antagonist prazosin, α_1-adrenoceptors were implicated as the source of activation (Gu et al., 1992).

The ability of receptors to stimulate phospholipase D has been shown to be dependent upon tyrosine kinase activity while stimulation by protein kinase C is not similarly dependent (Uings et al., 1992; Dubyak et al., 1993). While, there is uncertainty about the nature of the tyrosine kinase and the mechanism of its involvement, it is known that phospholipase D activity is regulated by low molecular weight G-proteins, such as ARF (Cockcroft et al., 1994) and Rho (Bowman et al., 1993), which introduces a potential role for tyrosine kinase involvement.

In studies comparing rabbit saphenous vein and aorta contractile responses, we found that several tyrosine kinase inhibitors were able to completely inhibit α_2-adrenoceptor responses in the venous preparation, while α_1-adrenoceptor responses of the aorta were unaffected (Jinsi and Deth, 1995b). We, therefore, undertook the current experiments on rat aorta to determine whether the α-adrenoceptor-induced phospholipase D activation and associated contractile response were sensitive to tyrosine kinase inhibition.

2. Materials and methods

2.1. Tissue preparation

Male rats (300–400 g) were decapitated and their thoracic aorta excised and placed in normal Krebs–Henseleit bicarbonate buffer of the following composition (mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25, KH_2PO_4 1.2, glucose 11.1, equilibrated with 95% O_2/5% CO_2 to a final pH of 7.4. Tissues were cleaned, cut into transverse strips and the endothelium removed by briefly rubbing them on filter paper.

2.2. Contraction studies

Aortic strips were mounted in an organ bath containing buffer maintained at 37°C under 2 g passive tension and isometric tension was recorded. After an initial equilibration period of 60 min, contractions were elicited with a 1 μM dose of noradrenaline until successive responses were within 10% of each other. A first dose–response curve was then obtained by the cumulative addition of either noradrenaline or the α_2-adrenoceptor-selective agonist UK14304 (5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline). After washout and relaxation, tissues were incubated with antagonists or other drugs prior to obtaining a second dose–response curve. Responses were calculated as a percentage of the maximum response obtained during the first dose–response protocol. In some studies, tissues were contracted by substitution of a buffer with elevated K^+ (60 mM), in which case NaCl was lowered by an equimolar amount, or by addition of the Ca^{2+} channel activator BAYK8644 (1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridine carboxylic acid methyl ester. In some instances, drugs were added in solutions containing up to 0.1% DMSO, a concentration which had no significant effect on the contractility of tissues.

2.3. Phospholipase D assay

The assay is based upon the ability of phospholipase D to form phosphatidylbutanol in the presence of the added alcohol. Aortic strips were incubated in buffer containing [^{3}H]myristic acid (2–5 μCi/ml) for 6 h and then treated with agonists and/or various inhibitors in the presence of 0.4% butanol for the indicated time. Reaction was stopped by removing the tissue from the buffer and placing it in ice-cold $CH_3OH/CHCl_3$ (2 : 1). After 1 h, the tissue samples were homogenized in additional $CH_3OH/CHCl_3$ containing 0.1% HCl and centrifuged at 2000 rpm for 20 min. The pellet was further extracted with $CH_3OH/CHCl_3$/HCl and the supernatants combined. Phases were resolved by addition of water and $CHCl_3$ and the lower $CHCl_3$ layer containing phospholipids was removed and dried under nitrogen. Samples were redissolved in CH_3OH/CH_3OH for spotting on silica gel G-plates and were separated chromatographically using the following solvent system: benzene/chloroform/pyridine/formic acid (45 : 38 : 4 : 2). Commercial standards of phosphatidylbutanol were separated in adjacent lanes and were visualized by iodine vapor staining and the corresponding [^{3}H]phosphatidylbutanol bands ($R_f = 0.45$) were scraped from the plate and counted. This migration was also confirmed by autoradiography in some samples. Data was normalized to the total radioactivity in the initial $CH_3OH/CHCl_3$ extract. [^{3}H]Phosphatidylbutanol counts were typically 0.5–1.0% of the total radioactivity in the phospholipid extract.

2.4. Drugs

UK14304 was provided by Research Biochemicals International (Natick, MA) as part of the Chemical Synthesis Program of the National Institute of Mental Health, Contract 278–90–007. BAYK8644 was kindly provided by Miles Pharmaceuticals (West Haven, CT) and prazosin by Pfizer Pharmaceuticals (Groton, CT). Other sources: genistein and methyl 2,5-dihydroxycinnamate (LC Laboratories, Woburn, MA), pertussis toxin (Calbiochem, LaJolla, CA), noradrenaline (Sigma, St. Louis, MO), rauwolscine (Research Biochemicals International, Natick, MA).

2.5. Statistical analysis

Groups of data were analysed by Student's t-test. A probability of $P < 0.05$ was selected as the criterion for statistical significance.

3. Results

3.1. Contraction studies

In order to determine whether a tyrosine kinase played a role in the contractile response of rat aorta to α-adrenoceptor stimulation, dose–response curves for the nonselective agonist noradrenaline were compared before and after pretreatment with various concentrations of the tyrosine kinase inhibitor genistein. As shown in Fig. 1A, genistein produced a graded noncompetitive inhibition at doses from 10 to 100 μM, with complete inhibition occurring at the latter dose. Maximal contractions induced by the α_2-adrenoceptor-selective agonist UK14304 were significantly smaller (0.26 ± 0.04 g) than contractions induced by noradrenaline (0.68 ± 0.12 g), and the rate of tension development in response to UK14304 was distinctly slower than for noradrenaline. Dose–response curves for UK14304 were also steeper than those for noradrenaline. These differences raise the possibility that UK14304 may be activating only a portion of the noradrenaline-activated contractile mechanisms. UK14304 responses were also sensitive to genistein (Fig. 1B), and in fact appeared to be more sensitive, since complete inhibition was obtained at 25 μM. Similar inhibitory effects were also observed for both agonists following incubation with methyl 2,5-dihydroxycinnamate (25 μM), another inhibitor of tyrosine kinase activity, which reduced noradrenaline and UK14304 responses by 65 ± 10 and 98%, respectively. However, in confirmation of the earier finding of Sauro and Thomas (1993), the tyrosine kinase inhibitor tyrphostin did not inhibit α-adrenoceptor responses of the rat aorta.

These results suggest an important role for a tyrosine kinase in the α-adrenoceptor-mediated contractile mechanism, especially for the α_2-adrenoceptor pathway. However, the particular tyrosine kinase involved is apparently not sensitive to all inhibitors.

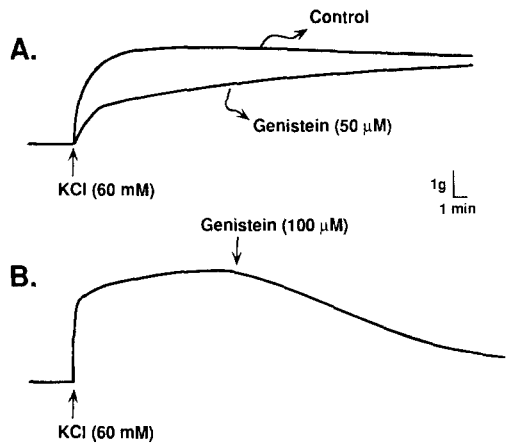

Fig. 2. Genistein inhibition of contractile responses to high K^+ in rat aorta. Genistein was added either 30 min prior to (A) or 10 min after (B) the addition of high K^+ (60 mM) buffer. Result shown is a tracing of a single experiment which was replicated three times.

Genistein also had an inhibitory effect on contractions induced by high K^+ buffer, although less so than for α-adrenoceptor response. Pretreatment with genistein (50 μM) caused a clear slowing of the rate of tension development induced by a 60 mM K^+ buffer, although eventually the contraction reached a height similar to the pretreatment level (Fig. 2A). Addition of genistein (100 μM) to a tissue stably contracted by high K^+ resulted in a prompt reduction to ~ 50% of the initial level of tension (Fig. 2B). Contractions induced by the L-type Ca^{2+} channel opener BAYK8644 (0.1 μM) were almost completely inhibited by pretreatment with 100 μM genistein (data not shown).

Since both high K^+ and BAYK8644 produce contractions via augmenting cytoplasmic levels of Ca^{2+}, normal tyrosine kinase activity may contribute to the degree of tension produced by an increase in Ca^{2+}. However, this result contrasts with the inability of tyrosine kinase inhibitors to affect high K^+-induced contractions in other

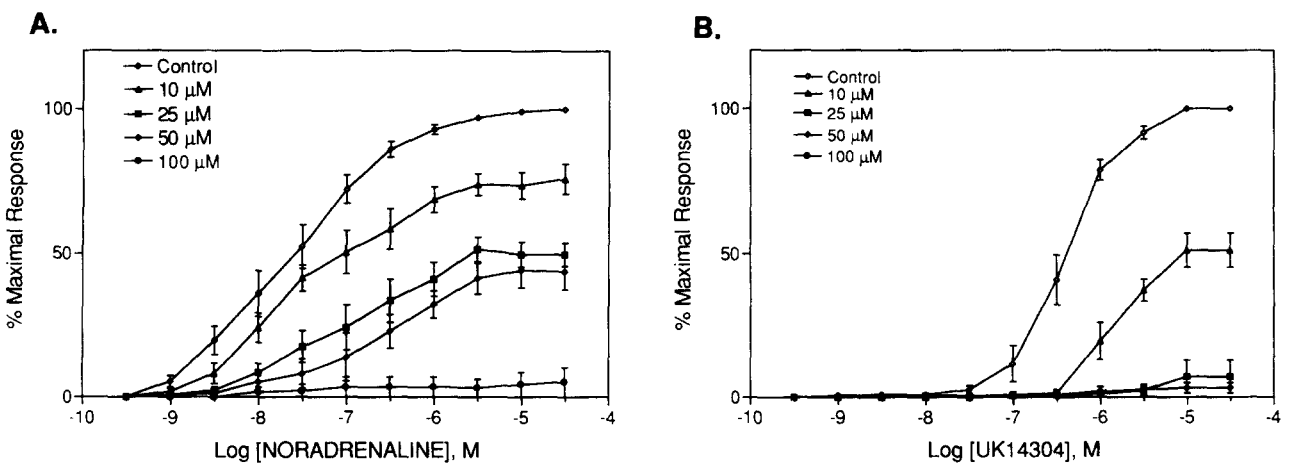

Fig. 1. Genistein inhibition of contractile responses of rat aorta induced by noradrenaline (A) or UK14304 (B). After obtaining an initial dose–response curve (Control), tissues were exposed to genistein at the indicated concentrations for 45 min prior to obtaining a second dose–response curve. Each data point is the mean ± S.E.M. of at least four determinations.

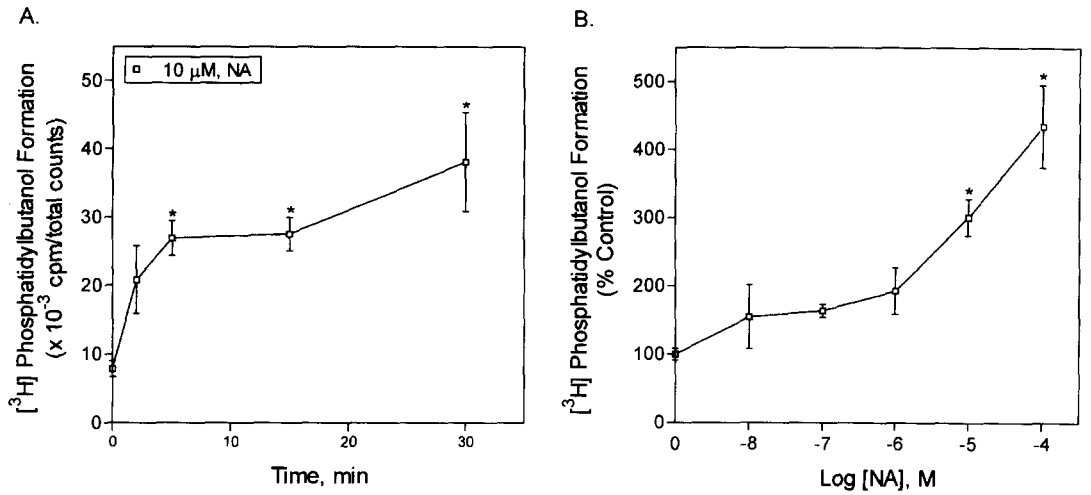

Fig. 3. Stimulation of phospholipase D activity in rat aorta segments by noradrenaline. Tissues were labeled with [^{3}H]myristic acid for 6 h and were treated with noradrenaline (10μM) in the presence of 0.4% butanol for the indicated times (Fig. 3A). Alternatively, tissues were treated with different concentrations of noradrenaline for 15 min in the presence of 0.4% butanol (Fig. 3B). [^{3}H]Phosphatidylbutanol formation was measured as described in Materials and methods. Each point is the mean ± S.E.M. of four or more determinations. * denotes statistical significance from the butanol-only group ($P \leq 0.05$).

vascular tissues (Di Salvo et al., 1993; Jinsi and Deth, 1995b), suggesting that Ca^{2+} effectiveness in the rat aorta is uniquely dependent upon the activity of a tyrosine kinase-regulated process.

3.2. Phospholipase D activation studies

Phospholipase D activity in aortic segments was measured as the formation of [^{3}H]phosphatidylbutanol in the transphosphatidylation reaction catalyzed by phospholipase D in the presence of 0.4% butanol. As shown in Fig. 3A, noradrenaline (10 μM) caused a time-dependent increase in [^{3}H]phosphatidylbutanol formation, reaching an increase of ~ 240% during the 5–10-min interval. Since the rate of initial tension development in response to noradrenaline is extremely fast in rat aorta ($t_{1/2}$ = 5 s), the time course of phospholipase D activation is more consistent with participation in the second stage of sustained tension. The dose–response relationship for phospholipase D activation by noradrenaline did not show saturation up to the highest concentration studied (100 μM), and an EC_{50} of at least 5 μM could be estimated (Fig. 3B). Similar to the findings of Jones et al. (1993), the latter value is almost 1000-fold higher than the EC_{50} for noradrenaline-induced contractile response. This suggests the possibility that several processes with differing agonist sensitivities may contribute to

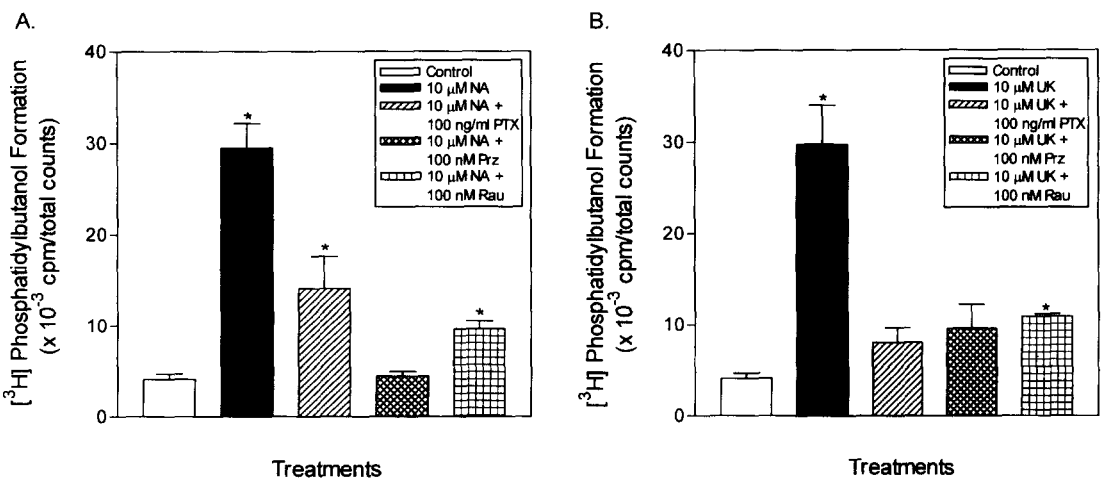

Fig. 4. Influence of pertussis toxin (PTX), prazosin or rauwolscine on noradrenaline-(A) or UK14304-stimulated (B) phospholipase D activity in rat aorta. Aortic segments were labeled with [^{3}H]myristic acid for 6 h and exposed to either agonist (10μM) for 15 min in the presence of 0.4% butanol. Groups of tissues were either treated with pertussis toxin (100 ng/ml) during the 6 h labeling period (PTX), or with prazosin (100 nM; PRZ) or with rauwolscine (100 nM; RAU) for 15 min prior to and during noradrenaline exposure. Each point is the mean ± S.E.M. of at least four determinations. * denotes statistical significance ($P \leq 0.05$) from the butanol-only group.

the net contractile response, with phospholipase D activation being a low sensitivity component.

In order to further characterize the origin of the phospholipase D activation, aortic strips were incubated in buffer containing pertussis toxin (100 ng/ml) for 6 h prior to noradrenaline stimulation (10 μM), and the level of increased phospholipase D activity compared to untreated controls. Pertussis toxin significantly reduced the level of noradrenaline-induced phospholipase D activation by 60%, although the remaining activity was still higher than that for unstimulated tissues (Fig. 4A). UK14304 (10 μM) was able to stimulate phospholipase D activity to the same extent as noradrenaline and pertussis toxin pretreatment reduced this stimulation to a level not significantly different from the untreated control group (Fig. 4B). In contraction studies, this concentration of UK14304 marked the onset of its maximal response, suggesting that for this agonist, unlike noradrenaline, phospholipase D activation was the primary coupling event for contraction. Thus, while UK14304 clearly behaved as a slow-onset partial agonist for contractile response, it was a full agonist with regard to phospholipase D activation. Pertussis toxin sensitivity indicates that G_i or G_o most likely mediate this activation.

Pretreatment of tissues with either the α_1-adrenoceptor antagonist prazosin or the α_2-adrenoceptor antagonist rauwolscine at 100 nM resulted in a large decrease in both noradrenaline- and UK14304-stimulated phospholipase D activity (Fig. 4A,B), indicating that activation of both receptor types is essential for its stimulation. This is consistent with the earlier observation that the noradrenaline-induced α-adrenoceptor contractile response of rat aorta exhibits the properties of both α_1- and α_2-adrenoceptors (Ruffolo et al., 1982). We confirmed this dual sensitivity for the UK14304 response by determination of K_B values for each antagonist (0.29 and 63 nM, respectively, for prazosin and rauwolscine). These values are in close agreement with the previously reported affinities of these antagonists for the α_{1D}- and α_{2D}-adrenoceptor subtypes, respectively (Lanier et al., 1991; Perez et al., 1991). UK14304 is relatively selective for α_2-adrenoceptors over α_1-adrenoceptors with pA_2 values of 8.1 and 5.3, respectively (Cambridge, 1981), but may provide some critical α_1-adrenoceptor activation at the concentrations required for contraction and phospholipase D activation.

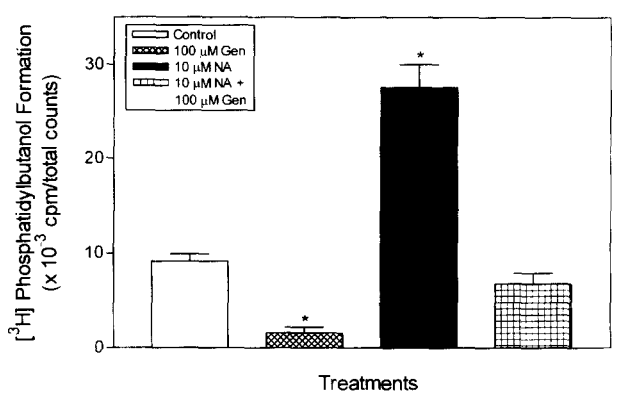

Fig. 5. Genistein inhibition of noradrenaline-stimulated phospholipase D activity in rat aorta. Tissues were labeled with [^{3}H]myristic acid for 6 h prior to a 15 min incubation in 0.4% butanol with or without noradrenaline (10 μM). Groups of tissues were treated with genistein (100 μM) for 30 min prior to the butanol or butanol and noradrenaline exposure. Each data point is the mean ± S.E.M. of at least four determinations. * denotes statistical significance ($P < 0.05$) from the butanol-only group.

Similar to contractile responses, the stimulation of phospholipase D activity by noradrenaline was almost completely eliminated by pretreatment with genistein (100 μM, 30 min) (Fig. 5). Additionally, genistein was observed to cause a significant inhibition of basal phospholipase D activity, to the extent of ~ 80%. Tyrosine kinase activity, therefore, appears to be important for both agonist-dependent and agonist-independent phospholipase D activity in the rat aorta.

4. Discussion

The current study examines the coupling of α-adrenoceptors in rat aorta to phospholipase D activation, a pathway whose significance has only more recently been recognized. Our results provide the first demonstration that the ability of α-adrenoceptors to activate phospholipase D requires concomitant activity of a tyrosine kinase.

Activation of a number of G-protein-coupled receptors has been shown to lead to phospholipase D stimulation, including both angiotensin II (Lassegue et al., 1991) and α_2-adrenoceptors (MacNulty et al., 1992). In the latter case, this has been demonstrated for the α_{2A}-adrenoceptor subtype, whose species homologue in the rat is designated as the α_{2D}-subtype because of its anomalous low affinity for yohimbine and rauwolscine (Lanier et al., 1991). In studies with the cloned α_{2D}-adrenoceptor expressed in PC12 cells, we have confirmed its ability to activate phospholipase D (Jinsi and Deth, 1995a), but notably, this could only be detected in the presence of simultaneous protein kinase C activation. A critical role for tyrosine kinase activity in allowing phospholipase D activation by a G-protein-coupled receptor has been described for the neutrophil chemotactic peptide receptor (Dubyak et al., 1993). This is distinct from phospholipase D activation by phorbol esters or by increased levels of Ca^{2+}, which do not require tyrosine kinase activity. Since the ability of α_2-adrenoceptors to activate G-proteins is not altered after tyrosine kinase inhibition (A. Jinsi and R.C. Deth, unpublished observation), tyrosine kinase involvement would appear to be at the level of G-protein–effector coupling.

The recognition that low molecular weight G-proteins regulate phospholipase D activity (Bowman et al., 1993; Malcolm et al., 1994) provides a potential link between tyrosine kinases and heterotrimeric G-protein-coupled signaling pathways. Thus, it is well-established that GTP

cycling at low molecular weight G-proteins is regulated by accessory proteins, such as GTPase-activating or GDP dissociation-inhibiting proteins, which are in turn regulated by tyrosine phosphorylation. Although other possibilities exist, the requirement of tyrosine kinase activity for both the contractile (Fig. 1) and phospholipase D (Fig. 5) responses to α-adrenoceptor stimulation may reflect a role for low molecular weight G-proteins in allowing coupling of a pertussis toxin-sensitive protein (i.e. G_i/G_o) to phospholipase D.

Involvement of tyrosine kinases in vascular contractile responses to α-adrenoceptor stimulation has been reported by Di Salvo et al. (1993) who found that genistein and several other kinase inhibitors inhibited the receptor-induced contraction of guinea pig mesenteric microvessels, but, unlike our findings for rat aorta, had little effect on KCl-induced contractions. They also showed that genistein, at a concentration of 15 μM, had no inhibitory effect on artery-derived myosin light chain kinase activity, so that its inhibitory effects on α-adrenoceptor and KCl-induced response in the current study cannot be attributed to a nonspecific inhibition of myosin phosphorylation. Sauro and Thomas (1993) reported that another tyrosine kinase inhibitor, tyrphostin, failed to inhibit phenylephrine contractions of rat aorta, although contractions induced by platelet-derived growth factor were inhibited, implying that genistein and tyrphostin may have different selectivity. These authors proposed that tyrphostin-induced inhibition could be due to a reduction in the tyrosine phosphorylation of myosin light chains, while the ability of genistein to inhibit receptor activation of phospholipase D is clearly a separate phenomenon.

In an earlier study, we demonstrated the ability of genistein to selectively inhibit UK14304-induced contractions of the rabbit saphenous vein (an α_2-adrenoceptor response) with little effect on phenylephrine-induced contractions of the rabbit aorta (an α_1-adrenoceptor response) (Jinsi and Deth, 1995b). Several additional observations in the current study suggest that tyrosine kinase-dependent stimulation of phospholipase D is mediated primarily by α_2-adrenoceptor activation: (1) UK14304-induced contractions, while smaller and slower in time course than those induced by noradrenaline, were more sensitive to genistein inhibition. (2) Noradrenaline and UK14304 produced similar increases in phospholipase D. (3) The slower time course of phospholipase D activation by noradrenaline was similar to the slower rate of tension development during α_2-adrenoceptor activation induced by UK14304. (4) Rauwolscine inhibited the UK14304 contractile response with a K_B similar to its reported affinity for the α_{2D}-adrenoceptor and also effectively inhibited stimulation of phospholipase D.

The above features notwithstanding, the α_1-adrenoceptor antagonist prazosin also potently inhibited UK14304-induced contractile response and phospholipase D activation. However, several previous studies have noted that the efficacy of α_2-adrenoceptors in vascular smooth muscle is highly dependent upon co-stimulation of α_1-adrenoceptors (Daly et al., 1988). As illustrated in Fig. 6, we propose that activation of α_1-adrenoceptors in responsible for the initial norepinephrine-induced contractile response while at the same time it serves to enable α_2-adrenoceptor coupling to phospholipase D which is more important for sustained contraction. Insofar as the initial α_1-adrenoceptor response could provide the dominant source of activation in dose–response studies, this relationship could account for the lower EC_{50} for noradrenaline stimulation of phospholipase D observed in Fig. 3.

The ability of genistein to impair Ca^{2+}-induced contractions of the rat aorta is an unexpected finding, since this does not occur in other tissues (Di Salvo et al., 1993; Jinsi and Deth, 1995a). Genistein does inhibit basal phospholipase D activity, however, and the associated loss of basal protein kinase C kinase activity may account for the

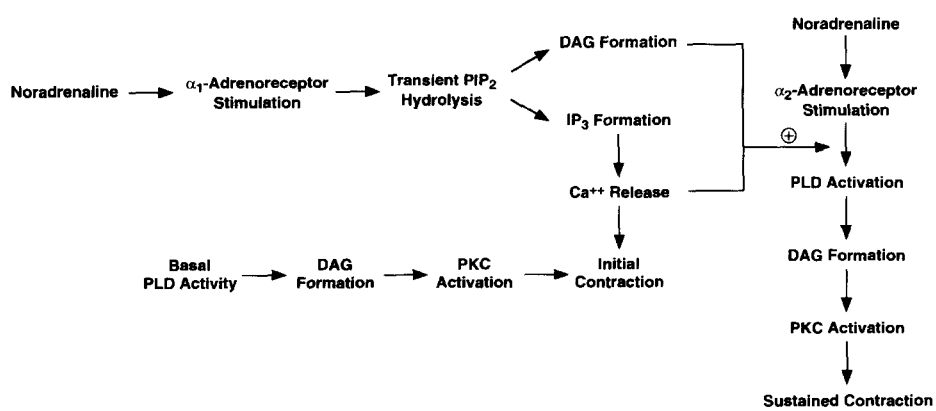

Fig. 6. Outline of a possible mechanism by which both α_1- and α_2-adrenoceptor stimulation may combine to produce the transient and sustained contractile components of noradrenaline response in rat aorta. Stimulation of α_1-adrenoceptors may initiate a transient hydrolysis of phosphatidylinositol diphosphate (PIP_2) providing for inositol triphosphate (IP_3)-induced release of Ca^{2+} whose effectiveness is dependent upon basal phospholipase D (PLD) activity. These initial α_1-adrenoceptor-induced events are necessary for the effective coupling of α_2-adrenoceptors to phospholipase D activation, which provides for sustained contraction via a diacylglycerol (DAG) and protein kinase C (PKC)-dependent mechanism.

impaired effectiveness of Ca^{2+}, whether it is provided by influx or by intracellular release. Indeed, it is well-recognized that the effectiveness of Ca^{2+} is 'sensitized' during sustained responses to receptor stimulation, and protein kinase C activation has been proposed to account for such sensitization (Nishimura et al., 1989). Inhibition of basal phospholipase D activity may account for the fact that the noradrenaline contractile response was completely inhibited by genistein since the usual effectiveness of Ca^{2+} provided by α_1-adrenoceptor stimulation could be greatly reduced when phospholipase D activity is inhibited. In this regard, we previously noted that rat aorta exhibits an 8–10-fold higher rate of basal phospholipid hydrolysis than does rabbit aorta, with phosphatidylcholine showing the greatest difference (Campbell et al., 1986). The current results suggest that phospholipase D may be a significant component of the higher basal activity.

The in vivo significance of tyrosine kinase regulation of α-adrenoceptor-induced vascular tone remains to be clarified, however, genetic analysis of spontaneously hypertensive rats vs. normotensive controls found that segregation of alleles of the cytoplasmic tyrosine kinase $pp60^{src}$ in cross-bred hybrids predicted the occurrence of hypertension (Postnov, 1990). Since increased vascular resistance is an important feature of the hypertensive state in this model, an increase in arterial phospholipase D activity consequent to overactivity of $pp60^{src}$ provides a potential link between genetic factors and vascular reactivity. In support of such a relationship, cells expressing the constituitively active v-Src form of $pp60^{src}$ have been shown to exhibit an elevated phospholipase D activity which is G-protein-dependent (Jiang et al., 1994).

In summary, activation of phospholipase D by noradrenaline in rat aorta appears to require the co-stimulation of α_1- and α_2-adrenoceptors and is dependent upon the activity of a tyrosine kinase.

Acknowledgements

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