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The mechanism of action of α_2-adrenoceptors in human isolated subcutaneous resistance arteries

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1 The effect of noradrenaline and the selective α_2-adrenoceptor agonist, azepexole, on tone and intracellular Ca^{2+} ($[Ca^{2+}]_i$) was examined in human isolated subcutaneous resistance arteries. Isolated arteries were mounted on an isometric myograph and loaded with the Ca^{2+} indicator, fura-2, for simultaneous measurement of force and $[Ca^{2+}]_i$.

2 High potassium solution (KPSS), noradrenaline and azepexole increased $[Ca^{2+}]_i$ and contracted subcutaneous arteries in physiological saline. When extracellular Ca^{2+} was removed and the calcium chelator, BAPTA, added to the physiological saline (PSS_o), responses to noradrenaline were transient and reduced, and responses to azepexole were markedly inhibited.

3 Ryanodine, an agent which interferes with Ca^{2+} release from intracellular stores, had little effect on contractile responses to KPSS, noradrenaline or azepexole in physiological saline. The response to caffeine in physiological saline was inhibited by ryanodine. In PSS_o, ryanodine partially inhibited contractile responses to noradrenaline and azepexole, and completely abolished the response to caffeine.

4 Noradrenaline and azepexole both significantly increased maximum force achieved by cumulative addition of Ca^{2+} to a Ca^{2+}-free depolarizing solution and shifted the calculated relationship between $[Ca^{2+}]_i$ and force to the left, suggesting these agents increase the sensitivity of the contractile apparatus to $[Ca^{2+}]_i$.

5 (–)-202 791, a dihydropyridine antagonist of voltage-operated calcium channels partially inhibited both the contractile response and the rise in $[Ca^{2+}]_i$ induced by azepexole. Pre-treatment of arteries with pertussis toxin inhibited responses to azepexole, but had no significant effect on tone induced by KPSS or noradrenaline. ETYA, an inhibitor of phospholipase A_2, lipoxygenase and cyclo-oxygenase, had no effect on azepexole-induced contraction in the presence of N^{ω} nitro-L-arginine methyl ester.

6 Azepexole, a selective α_2-adrenoceptor agonist, contracts human subcutaneous resistance arteries by a mechanism largely dependent on the influx of extracellular Ca^{2+}, probably through voltage-operated calcium channels. This action involves a pertussis toxin-sensitive G protein, possibly G_i.

Keywords: α-Adrenoceptor; α_2-adrenoceptor; intracellular Ca^{2+}; fura-2; G protein; ryanodine; pertussis toxin; phospholipase A_2

Introduction

The contractile effects of adrenaline and noradrenaline may be mediated by both postjunctional α_1- and α_2-adrenoceptors on vascular smooth muscle (McGrath, 1982; Faber, 1988). In man, postjunctional α_2-adrenoceptors have been demonstrated *in vivo* (Elliot & Reid, 1983), and in isolated resistance arteries from a variety of sites (Nielsen *et al.*, 1989; 1991). Resistance arteries are defined as small muscular arteries with internal diameters between 100–500 μm (Mulvany & Aalkjaer, 1990). These small arteries make a significant contribution to peripheral vascular resistance (Mulvany & Aalkjaer, 1990), and exert an important regulatory influence on local blood flow. Previous *in vitro* studies suggest that postjunctional α_2-adrenoceptors contribute to the response to both exogenous catecholamines (Nielsen *et al.*, 1989; 1990) and to sympathetic nerve stimulation in human resistance arteries (Parkinson *et al.*, 1992).

The cellular mechanism underlying the contractile action of α_2-adrenoceptor activation is unclear. α_2-Adrenoceptor activation is linked to inhibition of adenylate cyclase activity in a number of cell types (Limbird, 1988; MacNulty *et al.*, 1992). α_2-Adrenoceptor-induced inhibition of adenylate cyclase is believed to be mediated in many cells through a class of heterotrimeric GTP-binding proteins (G proteins), known as G_i (Limbird, 1988). G_i and some other classes of G proteins are ADP-ribosylated and consequently inactivated by pertussis toxin (Kaslow & Burns, 1992). However, despite the association between α_2-adrenoceptor activation and inhibition of adenyl cyclase, there is little evidence to suggest that inhibition of basal generation of adenosine 3′:5′-cyclic monophosphate (cyclic AMP) will cause an increase in vascular smooth muscle tone (Limbird, 1988; Wright *et al.*, 1995), and it seems likely that some other mechanism is involved in α_2-adrenoceptor-induced tone. α_2-Adrenoceptor activation has been linked to stimulation of phospholipase A_2 (PLA_2) in some cell types (Fraser, 1991). PLA_2 acts on phospholipids to release arachidonic acid, a substrate for eicosanoid synthesis; this leads to production of a variety of lipid mediators such as prostaglandins, leukotrienes, lipoxins and platelet-activating factor (Piomelli, 1993). Arachidonic acid has been reported to induce contraction in vascular smooth muscle (Jancar *et al.*, 1987; Toda *et al.*, 1988), and has also been proposed as a modulator of K channels (Ordway *et al.*, 1989), and the Ca^{2+}-sensitivity of the contractile apparatus of vascular smooth muscle (Gong *et al.*, 1992). Nevertheless the role of arachidonic acid generation in the contractile action of α_2-adrenoceptors in vascular smooth muscle is not known.

Most evidence suggests that intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is a major determinant of vascular smooth muscle tone (Somlyo & Himpens, 1989). Contractile agonists may increase $[Ca^{2+}]_i$ by causing release of stored intracellular Ca^{2+} and/or influx of extracellular Ca^{2+} through calcium channels. In vascular smooth muscle, inositol 1,4,5-trisphosphate (IP_3) appears to be an important signal which causes release of Ca^{2+} from intracellular stores associated with the

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endoplasmic reticulum (Hashimoto *et al.*, 1986; Ehrlich & Watras, 1988). However in contrast to α_1-adrenoceptors (Gu *et al.*, 1992; Lepetre *et al.*, 1994), most studies indicate that α_2-adrenoceptors are not coupled to phospholipase C and do not cause an elevation in inositol 1,4,5-trisphosphate (Schoepp *et al.*, 1984; Limbird, 1988).

The purpose of this study was to examine the effect of α_2-adrenoceptor activation on force and $[Ca^{2+}]_i$ in human isolated resistance arteries by use of the selective α_2-adrenoceptor agonist, azepexole (previously known as BHT 933; Pichler *et al.*, 1980). Previous studies (Nielson *et al.*, 1989) have demonstrated that azepexole induces tone in human resistance arteries by a selective action on postjunctional α_2-adrenoceptors. The object of these studies was to define the respective contribution of intracellular release and influx of extracellular Ca^{2+} to these responses. An additional purpose was to investigate the possible role of a pertussis toxin-sensitive G protein and activation of PLA_2 with release of arachidonic acid in α_2-adrenoceptor-induced tone.

Methods

Resistance arteries (normalized internal diameter = 108–474 μm, $n=31$) were dissected from subcutaneous fat removed during coronary artery bypass graft surgery (26 patients; age 45–70). The use of this tissue conformed with local Ethics Committee guidelines. Arteries were mounted as intact rings in a myograph in physiological saline (PSS) comprising (mM): NaCl 118, KCl 4.7, $CaCl_2$. $6H_2O$ 2.5, $MgSO_4$. $7H_2O$ 1.17, $NaHCO_3$ 25.0, $NaH_2PO_4.2H_2O$ 1.0, Na_2EDTA 0.03 and glucose 5.5, maintained at 37°C and bubbled with 95% O_2: 5% CO_2 as previously described (Aalkjaer *et al.*, 1986). Vessels were allowed to equilibrate for 30 min during which time they were normalised to $0.9L_{100}$ (Mulvany & Halpern, 1976). Subsequently vessel viability was checked by exposing the arteries to a high potassium solution (PSS containing equimolar substitution of K^+ for Na^+; KPSS), then washed with PSS, and subsequently exposed to 10 μM noradrenaline, and again washed out with PSS. Arteries which failed to produce an active tension equal to, or greater than that equivalent to an effective active pressure of 100 mmHg in response to these activators were discarded. No effort was made to remove the endothelium from the vessels.

The role of extracellular Ca^{2+} in responses to α-adrenoceptor activation was examined by comparing responses to single supramaximal concentrations of noradrenaline (10 μM) and azepexole (10 μM) in PSS with responses in Ca-free PSS (PSS_o) from which Ca^{2+} was omitted and 1 mM 1,2-*bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), a Ca^{2+} chelator, was added. Vessels were exposed to PSS_o for 3 min before addition of agonist. In some experiments 3 min after addition of agonist, PSS_o was washed out and replaced with PSS and α-adrenoceptor agonist to restore the extracellular Ca^{2+} concentration. The role of the ryanodine-sensitive intracellular Ca^{2+} store in the contractile response of these vessels was examined by comparing responses to noradrenaline (10 μM) and azepexole (10 μM) and caffeine (20 mM) in PSS and PSS_o, before and after 30 min preincubation with 10 μM ryanodine. After incubation with ryanodine all subsequent exposure to stimulants was in the continued presence of ryanodine (10 μM).

The effect of pertussis toxin treatment on contractile responses was examined by exposing vessels to 1 μg ml^{-1} pertussis toxin for 2 h in PSS. This treatment had no effect on resting tone. Pertussis toxin was then washed out and the vessels were re-exposed to KPSS, noradrenaline and azepexole. Preliminary studies indicated that this procedure in the absence of pertussis toxin had no effect on contractile response to any stimulant (data not shown).

Some vessels were used to examine the effect of α-adrenoceptor activation on force and $[Ca^{2+}]_i$ measured concurrently. These vessels were incubated with 6 μM fura-2/AM for 2 h at 37°C. After washing, the artery was allowed to recover in PSS for 30 min and then the myograph was placed on the stage of an Axiovert-35 inverted fluorescence microscope (Carl Zeiss, Germany). During measurement of $[Ca^{2+}]_i$ the vessel was illuminated at 340 nm and 380 nm via a ×10 Ultrafluor objective (N.A. 0.2; Carl Zeiss, Germany), a dichroic mirror (FT 395 nm, Carl Zeiss, Germany) and fibre optic light guides connected to a Deltascan D101 (Photon Technology International Inc., U.S.A.) high intensity dual wavelength light source. When $[Ca^{2+}]_i$ was not being measured the vessel was not illuminated to minimize photobleaching of dye during the experiment. During the experimental period (up to 3 h) there was minimal leakage of dye out of the vessel (data not shown). Epifluorescence was collected via the objective, passed through the dichroic filter and filtered by a band pass filter (BP500–530 nm, Carl Zeiss, Germany) before passing to a photomultiplier tube. Fluorescence at 500–530 nm, and force were acquired on-line using an A/D interface (PTI Inc.) into an IBM AT personal computer using commercially available software (Delta, PTI Inc.) at a sampling rate of 4–10 Hz.

$[Ca^{2+}]_i$ was estimated on the basis of the ratio of fluorescence emission measured at 510±5 nm which was evoked by excitation at 340 nm and 380 nm. Experiments were not calibrated using the technique outlined by Grynkiewicz *et al.* (1985), since ionomycin calibration was found to be inconsistent in these vessels and was often associated with leakage of dye. Consequently changes in $[Ca^{2+}]_i$ were normalised by expressing them as % change in peak ratio of 340/380 nm signal induced by depolarization with KPSS as previously described (Garcha & Hughes, 1994).

The effect of removal of extracellular Ca^{2+} on simultaneous measurements of force and $[Ca^{2+}]_i$ was examined using the protocols described above. In addition the effect of noradrenaline and azepexole on the Ca^{2+}-sensitivity of contraction was examined by comparing responses to the cumulative addition of Ca^{2+} to a Ca^{2+}-free depolarizing solution containing 80 mM potassium ($KPSS_o$) in the presence and absence of agonist. Concentrations of Ca^{2+} referred to are those added to the nominally Ca^{2+}-free $KPSS_o$ and extracellular $[Ca^{2+}]$ is likely to differ from these. Vessels were bathed in $KPSS_o$ for 3 min before cumulative addition of Ca^{2+} (1 nM–3 mM) and Ca^{2+} concentration-response curves were constructed.

Drugs

Drugs were obtained from the following sources: (–)-202791 (isopropyl-4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridine-carboxylate) (a gift from Sandoz, Switzerland), arachidonic acid (Sigma, Poole, Dorset), azepexole (BHT 933) (a gift from Boehringer Ingelheim), 1,2-*bis*(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (Sigma, Poole, Dorset), caffeine (Sigma, Poole, Dorset), cremophor EL (Sigma, Poole, Dorset), 5,8,11,14-eicosatetraynoic acid (ETYA; Calbiochem, CA, U.S.A.), fura-2/AM (Molecular Probes, Oregon, U.S.A.), N^{ω}-nitro-L-arginine methyl ester HCl (L-NAME; Sigma, Poole, Dorset), noradrenaline bitartrate (Sigma, Poole, Dorset), pertussis toxin (Sigma, Poole, Dorset), pluronic F-127 (Calbiochem, CA, U.S.A.), ryanodine (Calbiochem, CA, U.S.A.). Prior to use, fura-2/AM was dissolved in dimethylsulphoxide (DMSO) containing cremophor EL (0.1% v/v final concentration) and pluronic F-127 (0.1 mg ml^{-1} v/v final concentration) by sonication, the final concentration of DMSO (0.5% v/v), pluronic F-127 and cremophor EL had no effect on vessel reactivity.

Statistics and calculations

Data are presented as means±s.e.means. Single statistical comparisons were made by the non-parametric Wilcoxon signed ranks test. Multiple comparisons were made by non-parametic analysis of variance by the Freidman test, followed by Conover's technique (Conover, 1980), if analysis of variance proved significant. A value of $P<0.05$ was considered significant.

Results

KPSS, noradrenaline, caffeine and azepexole in PSS all increased $[Ca^{2+}]_i$ and induced contraction in human isolated subcutaneous arteries (Figures 1 and 2). In PSS_o, resting tone and $[Ca^{2+}]_i$ were not significantly altered, but peak force and $[Ca^{2+}]_i$ in response to noradrenaline were transient and were reduced to $21 \pm 9\%$ and $45 \pm 11\%$ of control in PSS ($n=6$; $P<0.05$). In the absence of extracellular Ca^{2+}, responses to azepexole were markedly reduced to $1 \pm 1\%$ (force) and $5 \pm 5\%$ ($[Ca^{2+}]_i$) respectively of the response in PSS ($n=5$; $P<0.05$) (Figures 1 and 2). In both cases re-exposure to PSS and agonist restored force and $[Ca^{2+}]_i$ to levels similar to responses in PSS without prior exposure to PSS_o.

Ryanodine (10 μM) had no significant effect on resting tone nor did it significantly affect the contraction induced by noradrenaline and azepexole in PSS (Figure 3) or KPSS (control $= 3.3 \pm 0.4$ Nm^{-1}; ryanodine $= 3.3 \pm 0.4$ Nm^{-1}; $n=13$). In contrast, the transient contraction in response to 20 mM caffeine in PSS was significantly reduced to $26\% \pm 4$ of control values by ryanodine ($P<0.05$) (Figure 3). In PSS_o contractions induced by caffeine were totally abolished by 10 μM ryanodine. In the absence of extracellular Ca^{2+}, noradrenaline-induced contraction was reduced to 13% and azepexole to 30% of the prior control response in PSS_o by ryanodine, although this latter effect was not statistically significant.

Under depolarizing conditions noradrenaline and azepexole both increased the sensitivity of contraction to $[Ca^{2+}]_i$, as judged from the calculated relationship between force and $[Ca^{2+}]_i$ (Figure 4). Neither noradrenaline nor azepexole had a marked effect on the relationship between extracellular Ca^{2+} and $[Ca^{2+}]_i$ although both agonists tended to increase the maximum force achived by addition of 3 mM Ca^{2+}; however, this effect was not statistically significant by analysis of variance.

Preincubation with the dihydropyridine calcium channel antagonist (–)-202 791 (5 μM) (Hering *et al.*, 1993), a concentration sufficient to inhibit completely responses to KPSS, partially inhibited responses to azepexole (control response to azepexole = $57 \pm 12\%$ KPSS (force) $55 \pm 8\%$ KPSS ($[Ca^{2+}]_i$; response to azepexole in the presence of (–)-202 791 $= 17 \pm 9\%$ KPSS (force) $16 \pm 9\%$ KPSS ($[Ca^{2+}]_i$; $n=3$).

Pertussis toxin pretreatment reduced contractile responses to azepexole to $19 \pm 5\%$ ($n=5$; $P<0.05$) of control responses to azepexole, though responses to noradrenaline and KPSS were unaffected (Figure 5). In the presence of L-NAME, an inhibitor of NO synthase (Rees *et al.*, 1990; Vila *et al.*, 1991), 30 min pre-exposure to ETYA (10 μM), an inhibitor of PLA_2 and several arachidonic acid metabolizing enzymes, such as lipoxygenase and cyclo-oxygenase (Lanni & Becker, 1985; Knepel & Meyer, 1986), had no significant effect on resting tone or contraction induced by azepexole compared with the effect of azepexole in the presence of L-NAME alone (control responses to azepexole $= 63 \pm 10\%$ KPSS; response to azepexole in the presence of ETYA = $57 \pm 13\%$ KPSS; $n=13$). However, this concentration of ETYA completely inhibited contractile responses evoked by 10 μM arachidonic acid (data not shown).

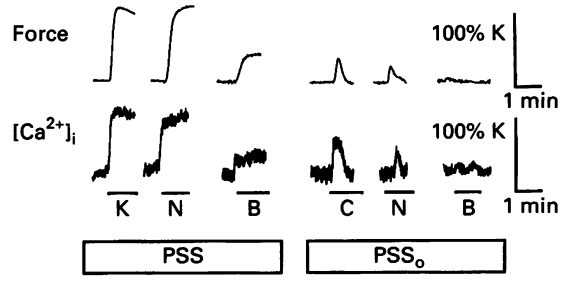

Figure 1 Representative traces showing simultaneous measurements of force and $[Ca^{2+}]_i$ in human isolated subcutaneous arteries mounted in a myograph and loaded with fura-2 as described in Methods. Drugs (K = KPSS, N = noradrenaline (10 μM), B = azepexole (10 μM), C = caffeine (20 mM)) were applied for the period indicated by the bars. Experiments were conducted in physiological saline or in Ca^{2+}-free physiological saline (PSS_o) as indicated by the bars. A scale bar is also shown. Traces are representative of 4–7 separate experiments.

Discussion

These results indicate that the contractile response to the α_2-adrenoceptor selective agonist, azepexole, is associated with a rise in $[Ca^{2+}]_i$ in human isolated subcutaneous resistance arteries. Recently a study using the Ca^{2+}-sensitive protein photo-aequorin reported that UK 14,304 a selective α_2-adre-

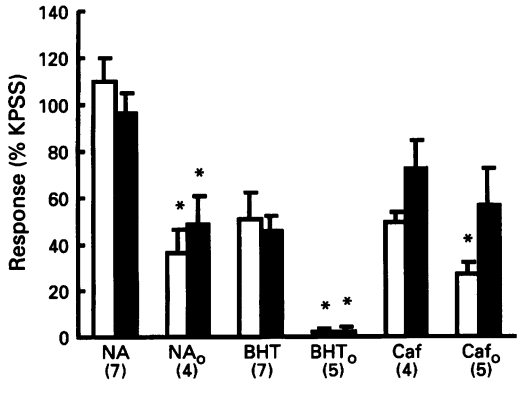

Figure 2 Effect of agonists on force (open columns) and $[Ca^{2+}]_i$ (solid columns) in human isolated resistance arteries. (NA = noradrenaline (10 μM) in physiological saline, NA_o = noradrenaline (10 μM) in Ca^{2+}-free physiological saline, BHT = azepexole (10 μM) in physiological saline, BHT_o = azepexole (10 μM) in Ca^{2+}-free physiological saline, Caf = caffeine (20 mM) in physiological saline, Caf_o = caffeine (20 mM) in Ca^{2+}-free physiological saline). Columns represent means ± s.e.means of 4–7 observations, (*n*) for each observation is shown below the label. $^*P<0.05$ compared with response to stimulant in physiological saline.

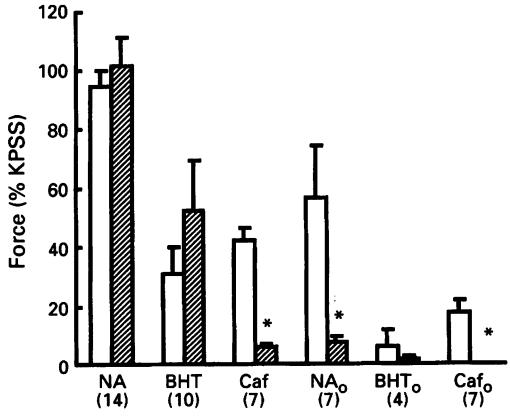

Figure 3 Effect of ryanodine (10 μM) on contractile response to stimulants: NA = noradrenaline (10 μM) in physiological saline, NA_o = noradrenaline (10 μM) in Ca^{2+}-free physiological saline, BHT = azepexole (10 μM) in physiological saline, BHT_o = azepexole (10 μM) in Ca^{2+}-free physiological saline, Caf = caffeine (20 mM) in physiological saline, Caf_o = caffeine (20 mM) in Ca^{2+}-free physiological saline. Open columns = control data; hatched columns = responses following ryanodine, columns represent means ± s.e.means of *n* observations, (*n*) for each observation is shown below the label. $^*P<0.05$ compared with control response in the absence of ryanodine.

noceptor agonist, increased $[Ca^{2+}]_i$ and myosin light chain phosphorylation in rabbit saphenous vein (Aburto *et al.*, 1993). In our studies both the contraction and the rise in $[Ca^{2+}]_i$ are largely dependent on the influx of extracellular Ca^{2+}. A dihydropyridine calcium channel antagonist, (–)-202 791, inhibited responses to azepexole. Other dihydropyridines have previously been reported to inhibit partially α_2-adrenoceptor-induced tone in human subcutaneous resistance arteries (Garcha *et al.*, 1991; Nielsen *et al.*, 1992) and these observations suggest that α_2-adrenoceptor activation causes Ca^{2+} influx, at least in part, through voltage-operated calcium channels. α_2-Adrenoceptor stimulation has been reported to cause a slow depolarization in rat saphenous vein (Cheung, 1985). The ionic basis for this depolarization is unknown, but changes in cation, chloride and potassium conductance have all been suggested as possible mediators of agonist-induced depolarization in smooth muscle (Byrne & Large, 1988; Cole & Sanders, 1989). Recently α_2-adrenoceptor activation in voltage-clamped rat portal vein cells has also been reported to increase directly voltage-activated calcium channel currents by a G protein-dependent mechanism linked to activation of protein kinase C (Leprêtre *et al.*, 1994).

In the presence of extracellular Ca^{2+}, ryanodine had little effect on the responses to either noradrenaline or azepexole, despite effectively inhibiting responses to caffeine. These findings suggest that when extracellular Ca^{2+} is present, influx of Ca^{2+} is sufficient to supply the Ca^{2+} necessary for contraction induced by noradrenaline and azepexole despite depletion of a caffeine/ryanodine-sensitive intracellular Ca^{2+} store. Recently we have reported similar findings with respect to α_1-adrenoceptors activation in rat isolated small arteries (Garcha & Hughes, 1995). It has been suggested that α_2-adrenoceptor activation may mobilize intracellular Ca^{2+} from a ryanodine-sensitive Ca^{2+} store in human subcutaneous arteries (Nielsen *et al.*, 1992), as it does in rabbit ear vein (Daly *et al.*, 1990). These findings contrast with the failure of α_2-adrenoceptor agonists to release Ca^{2+} stores in rabbit saphenous vein (Jim & Matthews, 1985; Daly *et al.*, 1990). The mechanism by which α_2-adrenoceptor activation releases intracellular Ca^{2+} stores in vascular smooth muscle is unclear. Unlike α_1-adrenoceptors (Gu *et al.*, 1992; Leprêtre *et al.*, 1994), IP_3 production is not generally thought to be involved in α_2-adrenoceptor mediated responses (Schoepp *et al.*, 1984; Limbird, 1988; MacNulty *et al.*, 1992), though one report in human digital arteries has described α_2-adrenoceptor mediated rise in inositol phosphates (Stevens & Moulds, 1990). Further studies will be necessary to determine whether the small degree of release of intracellular Ca^{2+} stores by azepexole is due to a weak stimulation of IP_3 production or if some other second messenger is involved as

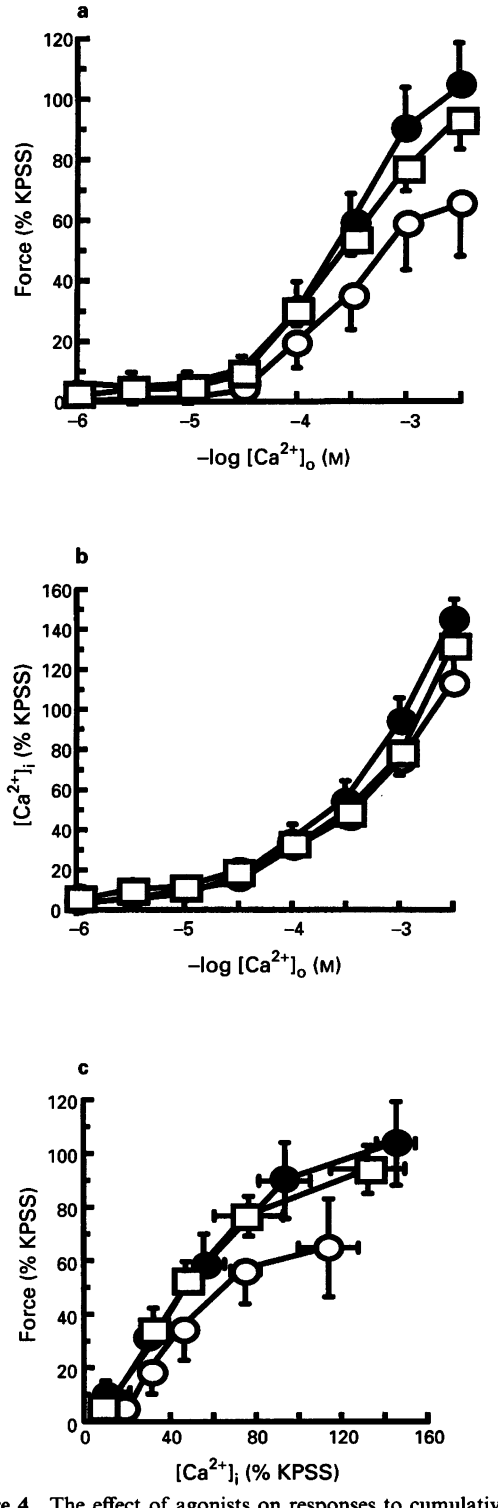

Figure 4 The effect of agonists on responses to cumulative addition of Ca^{2+} to nominally Ca^{2+}-free depolarized conditions ($KPSS_o$). (a) Concentration-responses relationship between added extracellular Ca^{2+} and force in $KPSS_o$ (○), noradrenaline 10 μM (●), or azepexole 10 μM (□). Agonist was added immediately prior to cumulative addition of Ca^{2+}. Increase in force is expressed as % contraction to KPSS in the same vessel. (b) Concentration-responses relationship between added extracellular Ca^{2+} and $[Ca^{2+}]_i$ in $KPSS_o$ (○), noradrenaline 10 μM (●), or azepexole 10 μM (□). Change in $[Ca^{2+}]_i$ is expressed as % change in $[Ca^{2+}]_i$ in response to KPSS in the same vessel. (c) Relationship between $[Ca^{2+}]_i$ and force derived from data in (a) and (b). Each value represents the mean ± s.e.means of data from 4 separate experiments.

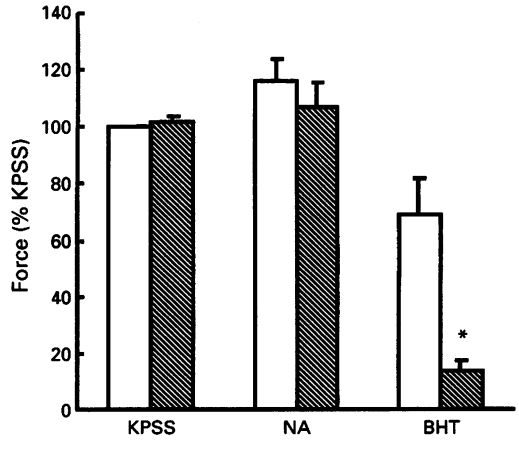

Figure 5 The effect of pertussis toxin (1 μg ml^{-1}) pretreatment on responses to high potassium (KPSS), noradrenaline (10 μM; NA), and azepexole (10 μM; BHT). Force in response to stimulants prior to pertussis toxin treatment are shown as open columns; cross-hatched columns indicate responses following pertussis toxin exposure. Data are means ± s.e.means of 5 observations in each case. *$P < 0.05$ compared with control response before pertussis toxin treatment.

has been proposed for α_2-adrenoceptor-induced release of intracellular Ca^{2+} in human erythroleukaemia cells (Michel *et al.*, 1989).

Noradrenaline and azepexole caused a leftward shift and an increase in maximum in the concentration-response relationship between $[Ca^{2+}]_i$ and tone in depolarized subcutaneous arteries. This suggests that in addition to causing influx and to a lesser extent release of Ca^{2+} from intracellular stores, noradrenaline and azepexole increase the sensitivity of the contractile machinery of the cell to $[Ca^{2+}]_i$. Similar findings have been reported in other tissues (Aburto *et al.*, 1993), and for other agonists (Nishimura *et al.*, 1988; Himpens *et al.*, 1990; Jensen *et al.*, 1992), and this action may represent an important mechanism involved in the generation of arterial tone which does not specifically involve the elevation of $[Ca^{2+}]_i$. The mechanism by which agonists increase the sensitivity of the contractile machinery to $[Ca^{2+}]_i$ is unknown. Activation of protein kinase C (Nishimura *et al.*, 1990), mitogen-activated protein (MAP) kinase (Khalil & Morgan, 1993) and inhibition of protein phosphatase (Somlyo & Somlyo, 1994) have all been proposed to be involved in this process.

The inhibitory effect of pertussis toxin on contractile responses to azepexole is consistent with the participation of a pertussis toxin-sensitive G protein in the action of the α_2-adrenoceptor. Pertussis toxin has previously been reported to inhibit α_2-adrenoceptor-mediated responses in both vascular endothelium and smooth muscle in dog (Miller *et al.*, 1991). The most likely subtype of G protein mediating this effect is G_i as linkage between α_2-adrenoceptors and this G protein has been described in other cell types (Limbird, 1988; Nieuwland *et al.*, 1994). However, the possibility that the action of α_2-adrenoceptors is mediated by some other pertussis toxin-sensitive G protein cannot be excluded. The inhibitory effect of pertussis toxin is unlikely to represent a non-specific effect on vascular smooth muscle cells since it failed to inhibit the contractile response to a high potassium solution. The action of α_1-adrenoceptors has been widely reported to be insensitive to pertussis toxin treatment (Ruffolo *et al.*, 1991; Lepêtre *et al.*, 1994). The failure of pertussis toxin to inhibit responses to the non-selective α-adrenoceptor agonist, noradrenaline, in this tissue may therefore indicate that there is a receptor reserve of α_1-adrenoceptors which is adequate to compensate for the inhibition of α_2-adrenoceptor-mediated effects by pertussis toxin.

A possible role for arachidonic acid in excitation-contraction coupling was suggested recently (Gong *et al.*, 1992). Interestingly both α_2-adrenoceptor activation (Nebigil & Malik, 1992; 1993) and vasopressin receptor activation (Ito *et al.*, 1993) have been reported to release arachidonic acid in vascular smooth muscle. Although ETYA, a selective inhibitor of PLA_2, inhibited arachidonic acid-induced contraction in these studies it failed to affect azepexole-induced contraction. This finding does not support activation of PLA_2 as a primary mechanism of action of α_2-adrenoceptor in human subcutaneous arteries. However given that more than one isoform of PLA_2 exists (Piomelli, 1993) and that ETYA blocks several enzymes metabolizing archidonic acid as well as PLA_2 (Lanni & Becker, 1985; Knepel & Meyen, 1986), a possible role of arachidonic acid, perhaps within the cell, cannot be excluded by these observations. At present the link between α_2-adrenoceptor induced activation of a pertussis toxin-sensitive G protein and vascular contraction is unexplained and requires further investigation.

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