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Mechanisms underlying postjunctional synergism between responses of the vas deferens to noradrenaline and ATP

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Abstract

Mechanisms of postjunctional synergism between adenosine 5′-triphosphate (ATP) and noradrenaline were studied in isolated guinea pig vas deferens. Whereas prior exposure to ATP had no significant effect on noradrenaline-mediated contractions, noradrenaline concentration-dependently enhanced ATP-induced contractions. Similarly to noradrenaline, histamine, which also acts via phospholipase-coupled receptors, induced contractions of the vas deferens and enhanced subsequent responses to ATP. Although phorbol-12, 13-dibutyrate (PDBu), a stimulant of protein kinase C (PKC), failed to induce contractions, it significantly potentiated ATP-induced contractions. The PKC inhibitor, Calphostin C, prevented this effect and the noradrenaline-mediated enhancement of ATP-induced contractions. The phosphatase inhibitor cantharidin induced a time- and concentration-dependent tonic contraction and markedly increased subsequent contractions to ATP. It is suggested that noradrenaline potentiates the contractile response of the vas deferens to ATP via a PKC-mediated mechanism. This may involve the inhibition of myosin light chain phosphatase (MLCP) and subsequent calcium sensitisation.

Keywords: Noradrenaline; ATP; Vas deferens; Synergism; PKC

1. Introduction

Although the sympathetic cotransmitters adenosine 5′-triphosphate (ATP) and noradrenaline both stimulate the contraction of isolated guinea pig vas deferens (Wadsworth, 1973, 1974; Sneddon and Burnstock, 1984; Sneddon and Westfall, 1984), the nature of the respective contractions are markedly different. ATP elicits a rapid phasic contraction, whereas noradrenaline induces a phasic, then well-maintained tonic contraction (Sneddon et al., 1984; Burt et al., 1998). Such differences are most likely a reflection of the distinct signal transduction pathways activated by the two neurotransmitters. The ATP induced contraction is predominantly mediated by $P2X_1$ ligand-gated ion channels (Mulryan et al., 2000), whilst noradrenaline acts via its phospholipase C (PLC)-coupled α_1-adrenoceptors (Summers and McMartin, 1993; Burt et al., 1998). Phospholipase C produces inositol (1,4,5)-triphosphate (IP_3) and diacylglycerol (DAG) from membrane phospholipids (Berridge and Irvine, 1984). Stimulation of both $P2X_1$-receptors and α_1-adrenoceptors results in an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$) concentration. This increase in $[Ca^{2+}]_i$ concentration is essential for myosin light chain kinase (MLCK) activation and subsequent phosphorylation of myosin and, thus, smooth muscle cell contraction (Somlyo and Somlyo, 1994). Not only do the different signal transduction pathways activated by noradrenaline and ATP produce dissimilar contractile responses, they also permit diverse postjunctional interactions between the two cotransmitters. Indeed, it has been demonstrated that synergism exists between noradrenaline and ATP at various smooth muscle preparations, including the portal vein, mesenteric arterial bed and vas deferens (Holck and Marks, 1978; Kennedy and

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Burnstock, 1986; Huidobro-Toro and Parada, 1988; Kishi et al., 1990; Ralevic and Burnstock, 1990; Witt et al., 1991; Fujita et al., 1996). That is, noradrenaline and ATP in combination induce a greater contraction of smooth muscle than that of the sum of the individual responses. The underlying mechanisms of this postjunctional interaction have yet to be elucidated. Since a rise in $[Ca^{2+}]_i$ triggers smooth muscle cell contraction, it is possible that the rise in $[Ca^{2+}]_i$ induced by noradrenaline and ATP in combination exhibits synergism. However, it has been shown that, despite potentiating the rapid ATP-mediated contraction of guinea pig vas deferens, noradrenaline and ATP used in combination do not induce a concomitant increase in smooth muscle cell $[Ca^{2+}]_i$ concentration (Fujita et al., 1996). This strongly suggests that an alternative mechanism(s), not affecting the rise in $[Ca^{2+}]_i$ is responsible for synergism between noradrenaline and ATP. Such a finding points towards the phenomenon of 'Ca^{2+} sensitisation'. Ca^{2+} sensitisation of a cell simply means that a greater response (in this case contraction) will occur for a given rise in $[Ca^{2+}]_i$ concentration. Several reports have implicated the reversible inhibition of myosin light chain phosphatase (MLCP) as a contributory factor to Ca^{2+} sensitisation (Kitazawa et al., 1991; Kubuta et al., 1992; Somlyo and Somlyo, 1994; Kitazawa et al., 2000). MLCP dephosphorylates myosin and thus opposes contraction by terminating the interaction between myosin and actin. Inhibition of MLCP in addition to activation of MLCK contributes to the development of contraction triggered by various agonists (Kitazawa et al., 2000). Furthermore, an endogenous 17-kDa inhibitor of MLCP, CPI-17 has been identified in smooth muscle, which is activated upon phosphorylation by kinases such as protein kinase C (PKC) and Rho kinase (Kitazawa et al., 2000). As would be expected, inhibition of MLCP by CPI-17 leads to an increase in phosphorylated myosin regulatory light chain and a concomitant increase in smooth muscle cell contraction (Kitazawa et al., 2000). It has also been demonstrated that contractile agonists such as histamine and phenylepherine trigger the phosphorylation and activation of CPI-17 (via PKC and Rho kinase; Kitazawa et al., 2000). Since PLC produces the PKC stimulant, 1,6-diacylglycerol from phosphatidylinositol bisphosphate (a membrane phospholipid), it is plausible that noradrenaline sensitises smooth muscle cells to increases in $[Ca^{2+}]_i$ triggered by ATP, via a PKC-mediated inhibition of MLCP. In the present study we have assessed the contribution of PKC and MLCP to this hypothesis, in the isolated guinea pig vas deferens.

2. Materials and methods

2.1. Animals

Sexually mature, male Hartley guinea pigs weighing 250–350 g were used for the study.

2.2. Tissue preparation

The guinea pigs were killed by carbon dioxide asphyxiation, according to UK Home Office regulations covering Schedule 1 procedures and approved by the institutional ethics committee. The vasa deferentia were then dissected out and stripped of adhering fat and connective tissue. Each vas deferens, of approximately 15 mm, was ligated with silk thread, one end attached to a rigid support and the other to a FTO3C force displacement transducer. The tissues were suspended in 10 ml organ baths containing gassed (95% O_2/5% CO_2) Krebs solution of the following composition (mM): NaCl, 133; KCl, 4.7; $NaHCO_3$, 16.4; $MgSO_4$, 0.6; NaH_2PO_4, 1.4; glucose, 7.7 and $CaCl_2$, 2.5; pH 7.3. Experiments were carried out at 37±1 °C.

2.3. Concentration–response curves

Concentration–response curves were constructed using noradrenaline and ATP, both in a non-cumulative fashion using single doses. The time interval between applications of noradrenaline and ATP was 15–20 min to avoid desensitisation. In experiments to determine whether noradrenaline enhances responses to ATP, ATP was given approximately 1.5 min after noradrenaline. This coincided with the development of the noradrenaline-induced tonic contraction. In the converse experiments, noradrenaline was given after the rapid phasic contraction of ATP returned to baseline.

Tissues were incubated with phorbol-12, 13-dibutyrate (PDBu) for 3 min and calphostin C for 1 h in bright light (standard bench lamp), which is essential for the activation of this PKC inhibitor. In experiments designed to investigate the contribution of MLCP inhibition to synergism, tissues were incubated with the MLCP inhibitor cantharidin for 80 min.

The concentrations of inhibitors chosen for this study were taken from previous work investigating the selective effects of PKC, rho kinase and phosphatase inhibitors on contractions in various smooth muscle preparations, (Burt et al., 1998; Knapp et al., 1998; Sward et al., 2000).

Frequency–response curves to electrical field stimulation were also constructed (100 V, 0.3 ms, 0.5–16 Hz, 15 s stimulation every 5 min). The effect of noradrenaline, PDBu and cantharidin on responses to 2 Hz electrical field stimulation was assessed.

2.4. Drugs used

ATP, histamine, noradrenaline (bitartrate salt) and prazosin were obtained from Sigma (Poole, UK). Pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS; tetrasodium salt) was supplied by Tocris Cookson (Avonmouth, UK). Calphostin C, cantharidin, 1-(5-isoquinoline-

sulfonyl)-homopiperazine (HA1077) and PDBu were obtained from Calbiochem (Beeston, UK).

Noradrenaline was dissolved in 100 μM ascorbic acid. Cantharidin, calphostin C and PDBu were dissolved in dimethylsulfoxide (DMSO), all other drugs were prepared in distilled water. The volume added to the organ bath to produce the final bath concentration was not in excess of 100 μl. At the concentrations used, ascorbic acid and DMSO had no effect on basal tone or agonist-stimulated contractions.

2.5. Statistical analysis

All concentration–response curves are expressed as mean percentage contraction of the KCl contraction (120 mM)±standard error (S.E.M.) of n animals. Statistical differences between groups were determined by analysis of variance followed by post hoc analysis by Bonferroni's test or by Student's t test, using the GraphPad InStat software (GraphPad Software, San Diego, USA). $P<0.05$ was considered significant.

3. Results

3.1. ATP and noradrenaline-induced contractions of isolated guinea pig vas deferens

ATP produced a rapid phasic contraction (Fig. 1), which was inhibited by the $P2X_1$-receptor antagonist, pyridoxalphosphate-6-azophenyl-2′,4′-disulfonic acid (30 μM; data not shown). Unlike ATP, the noradrenaline-induced contraction consisted of two components. An initial slow (with respect to ATP) phasic contraction, then a well-maintained tonic contraction (Fig. 1). Both components of the contraction were blocked by the α_1-adrenoceptor antagonist, prazosin (1 μM; data not shown). The contractions induced by ATP and noradrenaline were concentration-dependent (Fig. 2A and B).

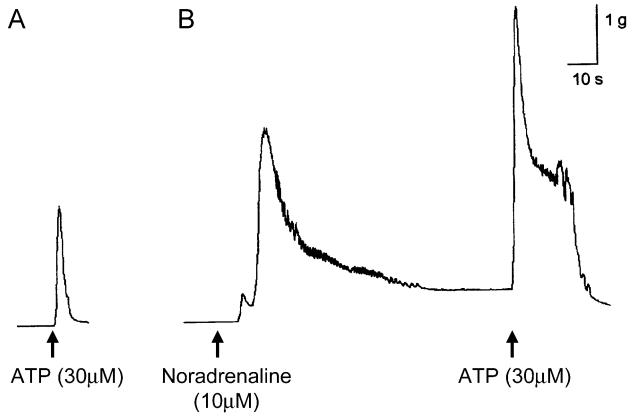

Fig. 1. Typical tracings from isolated guinea pig vas deferens of the contractile responses induced by (A) noradrenaline (10 μM) and (B) ATP (30 μM). The effect of noradrenaline on subsequent exposure to ATP is also shown in (B).

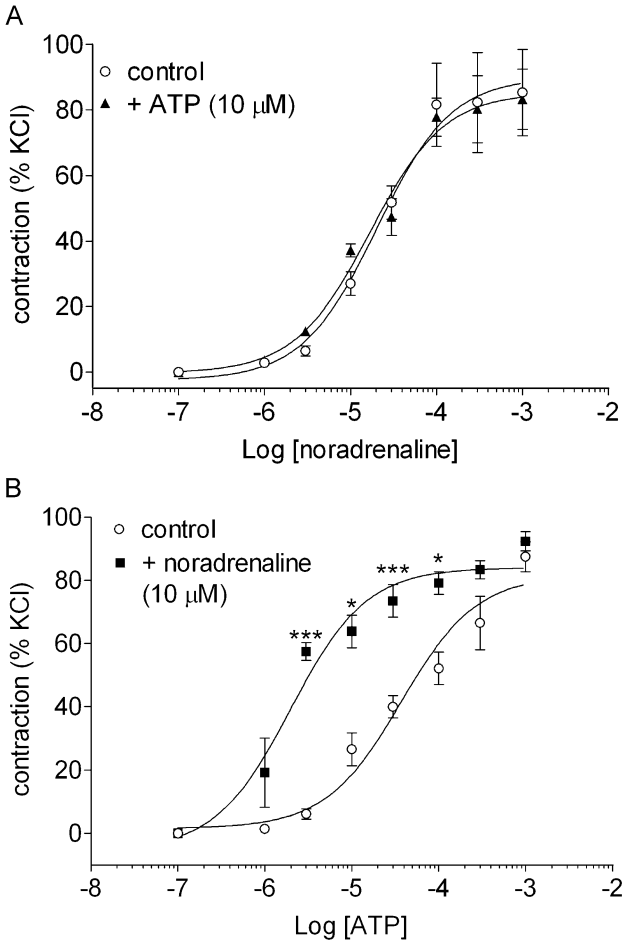

Fig. 2. The effect of (A) ATP (10 μM) and (B) noradrenaline (10 μM) on non-cumulative concentration–response curves to (A) noradrenaline and (B) ATP in isolated guinea pig vas deferens. Points are means±S.E.M. values of contraction (percent of KCl response) at the concentration of (A) noradrenaline or (B) ATP indicated by the abscissa (n=4). *$P<0.05$ and ***$P<0.001$ from control by two-way ANOVA followed by post hoc Bonferroni's test.

3.2. Postjunctional synergism between ATP and noradrenaline

When the isolated guinea pig vas deferens were exposed to ATP and noradrenaline in combination, the response to ATP occurred more rapidly than noradrenaline alone making it difficult to ascertain synergism. Therefore, experiments were performed in which ATP was given prior to noradrenaline and vice versa (see Materials and Methods). ATP (10 μM) had no effect on subsequent contractions induced by noradrenaline (1 μM to 1 mM; Fig. 2A). Furthermore, higher concentrations of ATP (30 μM to 1 mM) also failed to enhance subsequent contractions to noradrenaline (data not shown). Unlike ATP, noradrenaline (10 μM) caused an increase in subsequent ATP-induced contractions (Figs. 1 and

2B). Although noradrenaline did not increase maximal contractions to ATP, there was a marked leftward shift to the concentration–response curve (Fig. 2B). The effect of noradrenaline on ATP was concentration-dependent (Fig. 3A) and was abolished by prazosin (1 μM; Fig. 3B).

3.3. The potentiation of ATP-induced contractions by histamine and phorbol-12, 13-dibutyrate

Similarly to noradrenaline, histamine (10 μM) induced contractions of the guinea pig vas deferens and markedly potentiated ATP-induced contractions (from 23±5.6% to 51±12.2% of the KCl response for 30 μM ATP). Since both α_1-adrenoceptors and histamine H_1-receptors couple to PLC and thus stimulate PKC, the effect of the PKC stimulant PDBu was assessed. PDBu (10 μM) did not induce a contraction of the isolated guinea pig vas deferens (data not shown), however, it markedly augmented contractions induced by ATP (Fig. 4A).

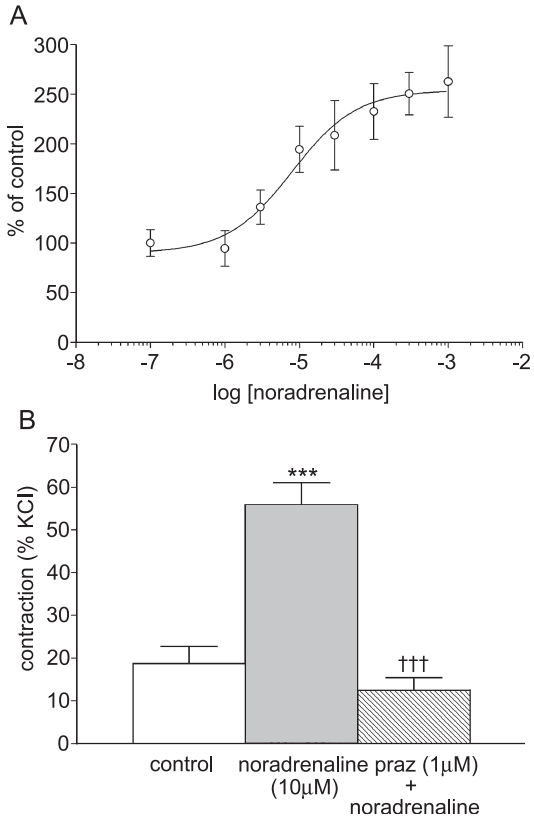

Fig. 3. (A) The dose-dependent effect of noradrenaline on ATP (30 μM)-induced contractions of isolated guinea pig vas deferens. Points are means±S.E.M. values of contraction (percent of 30 μM ATP response) in the presence of the concentration of NA indicated by the abscissa (n=4). (B) The effect of the α_1-adrenoceptor antagonist prazosin (praz; 1 μM) on the NA-induced potentiation of ATP (30 μM) mediated contractions. Bars are means±S.E.M. values of the ATP-induced contraction (percent of KCl response) in the absence or presence of NA±prazosin (n=4). ***P<0.001 from control; $^{\dagger\dagger\dagger}P$<0.001 from noradrenaline by ANOVA followed by post hoc Bonferroni's test.

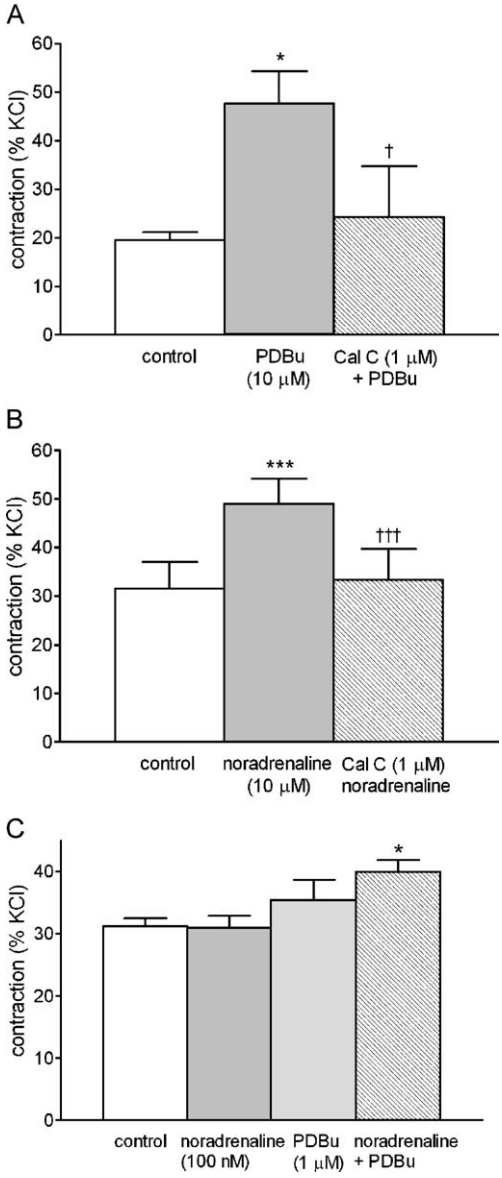

Fig. 4. The effect of the PKC inhibitor, calphostin C (Cal C; 1 μM) on the (A) phorbol-12, 13-dibutyrate (PDBu; 10 μM)- and (B) noradrenaline (10 μM)-induced potentiation of ATP (30 μM)-mediated contractions of isolated guinea pig vas deferens. (C) The effect of subthreshold concentrations of (100 nM) and PDBu (1 μM) in combination on subsequent ATP (30 μM) responses. Bars are means± S.E.M. values of the ATP-induced contraction (percent of KCl response) in the absence or presence of (A) PDBu±Cal C (n=5), (B) noradrenaline±Cal C (n=5) or (C) noradrenaline±PDBu (n=4). *P<0.05 from control; ***P<0.001 from control; $^{\dagger}P$<0.001 from noradrenaline; $^{\dagger\dagger\dagger}P$<0.001 from noradrenaline by ANOVA followed by post hoc Bonferroni's test.

3.4. The effect of PKC inhibition on postjunctional synergism between noradrenaline and ATP

The PKC inhibitor Calphostin C (1 μM) abolished the enhancing action of both PDBu and NA, on subsequent ATP-induced contractions (Fig. 4A and B). Furthermore, subthreshold concentrations of noradrenaline and PDBu, in

combination, potentiated subsequent ATP-induced contractions (Fig. 4C).

3.5. The effect of rho kinase inhibition on postjunctional synergism between noradrenaline and ATP

The rho kinase inhibitor 1-(5-isoquinolinesulfonyl)-homopiperazine (HA1077; 30 μM) significantly attenuated the noradrenaline-induced potentiation of ATP stimulated contractions (Fig. 5A).

3.6. The effect of phosphatase inhibition on ATP-induced contractions

The phosphatase inhibitor cantharidin induced a gradual time- and concentration-dependent increase in tonic contraction of isolated guinea pig vas deferens (data not shown). More significantly, preincubation of the vas deferens with cantharidin (10 μM, 80 min), markedly enhanced subsequent responses to ATP (Fig. 5B).

3.7. The effect of noradrenaline, PDBu and cantharidin on electrical field stimulation-induced contractions

Similar to the effect on exogenous ATP, the compounds noradrenaline, PDBu and cantharidin all enhanced contractions induced by electrical field stimulation (Fig. 6).

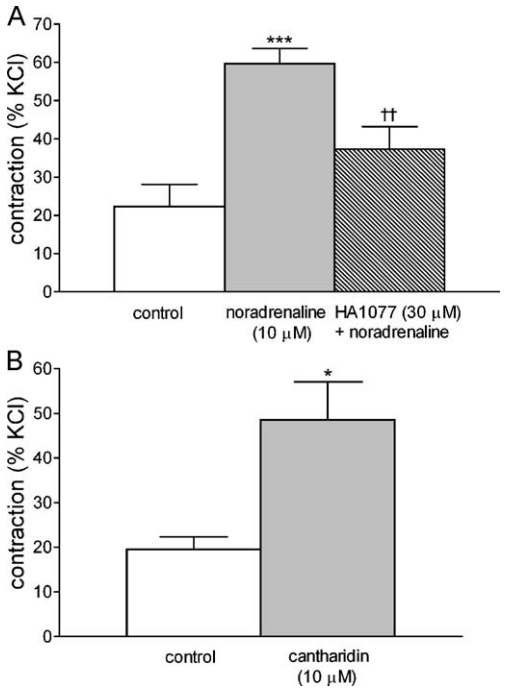

Fig. 5. The effect of the (A) rho kinase inhibitor HA1077 (30 μM) on the noradrenaline (NA; 10 μM)-induced potentiation of ATP (30 μM)-mediated contractions of isolated guinea pig vas deferens. (B) The effect of the myosin light chain phosphatase inhibitor cantharidin (10 μM) on ATP (30 μM)-induced contractions of isolated guinea pig vas deferens. Bars are means± S.E.M. values of the ATP-induced contraction (percent of KCl response) in the absence or presence of (A) noradrenaline±HA1077 (n=4), (B) cantharidin (n=4). *P<0.05 from control; ***P<0.001 from control; ††P<0.01 from noradrenaline by Student's t test.

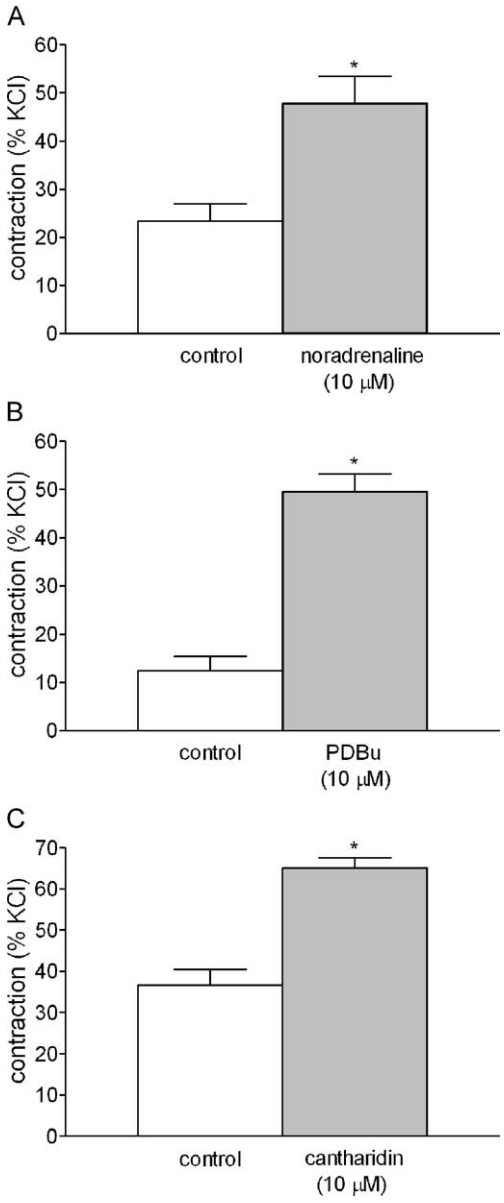

Fig. 6. The effect of (A) noradrenaline (10 μM), (B) phorbol-12, 13-dibutyrate (PDBu; 10 μM) and (C) cantharidin (10 μM) on electrical field stimulation (2 Hz)-induced contractions. Bars are means±S.E.M. values of the electrical field stimulation-induced contraction (percent of KCl response) in the absence or presence of (A) noradrenaline (n=4), (B) PDBu (n=4) or (C) cantharidin (n=4). *P<0.05 from control by Student's t test.

4. Discussion

We studied the potential mechanisms of postjunctional interaction between noradrenaline and ATP in isolated guinea pig vas deferens. Both noradrenaline and ATP stimulated the contraction of isolated guinea pig vas deferens via α_1- and $P2X_1$-receptors, respectively. Similar to previous studies, we found that noradrenaline potentiated

contractions induced by ATP, and as would be expected, this effect was abolished by the α_1-adrenoceptor antagonist, prazosin. However, ATP had no significant effect on noradrenaline-induced contractions. As was found for noradrenaline, histamine also potentiated ATP-induced contractions of isolated guinea pig vas deferens. Since both noradrenaline and histamine operate via PLC-coupled receptors, we ascertained the effect of the PKC stimulant PDBu on ATP-induced contractions. PDBu did not induce contractions but potentiated ATP-induced contractions. In addition, calphostin C attenuated the effect of PDBu and noradrenaline on ATP-induced contractions. This suggests that PKC activation contributes to postjunctional synergism between noradrenaline and ATP. Furthermore, inhibition of MLCP with cantharidin, also potentiated ATP-induced contractions.

The first finding in this study is that whilst noradrenaline markedly potentiated ATP-induced contractions, the converse was not true. A similar finding in guinea pig vas deferens has been reported before (Kishi et al., 1990). It is very probable that differences in the signal transduction pathways and thus the second messengers activated by noradrenaline and ATP account for this. Whereas α_1-adrenoceptors are coupled to PLC activation, $P2X_1$-receptors are ligand-gated ion channels. Although subtypes of PKC may be activated by the ATP-induced rise in $[Ca^{2+}]_i$, the extent of PKC activation will probably be less than that achieved by α_1-adrenoceptors, which couple directly to PLC and thus produce the PKC stimulant 1,6-DAG. Indeed, it has been shown that noradrenaline stimulates a well-maintained, PKC-dependent tonic contraction in addition to an initial phasic contraction (Burt et al., 1998). Such a tonic contraction is not seen with ATP, suggesting that the extent of PKC activation is not enough to produce and maintain a tonic contraction in the isolated guinea pig vas deferens.

Since particular subtypes of P2X-receptors (e.g., $P2X_2$) are affected by extracellular pH and catecholamines have been shown to potentiate responses to ATP by decreasing the extracellular pH (Wildman et al., 1997), it was important to ensure that the present findings with noradrenaline were mediated by an adrenoceptor. Indeed, the noradrenaline-induced potentiations of ATP-stimulated contractions were abolished by prazosin, an α_1-adrenoceptor antagonist, clearly implicating the specific activation of α_1-adrenoceptors, rather than a change in extracellular pH, as the trigger for this contractile enhancement.

If PLC activation contributes to the potentiation of ATP-induced contractions, we reasoned that alternative PLC-coupled receptors should also enhance responses to ATP. Guinea pig vasa deferentia are endowed with histamine H_1-receptors (Vohra, 1981), which couple to PLC. We found that histamine, similarly to noradrenaline, induced the contraction of isolated guinea pig vas deferens with a phasic and tonic component. More significantly, contractions induced by ATP were markedly potentiated. Angiotensin II has also been shown to potentiate responses to ATP in isolated rat vas deferens (Sum et al., 1996). Moreover, this effect of angiotensin II was mediated by angiotensin AT_1 receptors, which also couple to PLC. This and our findings suggest that PLC activation could contribute to the enhancement of ATP-mediated contractions by noradrenaline and histamine. Since previous studies have implicated PKC in the development of smooth muscle contraction, we tested whether the PKC stimulant, PDBu, triggered contraction and/or potentiation of ATP-induced responses. Although PDBu (10 μM) did not induce contraction of isolated vas deferens, it potentiated subsequent contractions to ATP. A previous study reported that PDBu induced a contraction of isolated rat vas deferens (Burt et al., 1998). In addition to the possibility of species variation, the experiments performed with PDBu in that study were at 25 °C, rather than 37 °C. Such differences in temperature were shown (in the same study) to markedly enhance contractions induced by noradrenaline; this could also apply to responses produced by PDBu.

To ascertain whether noradrenaline and PDBu potentiate contractions produced by endogenous ATP, electrical field stimulation experiments were performed. Although the contribution of presynaptic receptors was not assessed or accounted for in this study, similarly to that found for exogenous ATP, both noradrenaline and PDBu markedly augmented subsequent responses to field stimulation. A relatively low frequency (2 Hz) was used for these experiments since it has previously been demonstrated that there is a greater ATP component to the contraction during low frequency stimulation (Kennedy et al., 1986). At higher frequencies, the noradrenaline component is increased.

Since PKC activation potentiated responses to ATP, the contribution of PKC to postjunctional interactions between noradrenaline and ATP was assessed. Calphostin C, a PKC inhibitor, attenuated the effect of noradrenaline on subsequent responses to ATP, clearly suggesting a role for PKC activation and synergism between noradrenaline and ATP. Furthermore, we found that subthreshold concentrations of noradrenaline and PDBu in combination potentiated the ATP-induced contraction. The above finding and the fact that there is a significant concentration-dependent relationship for noradrenaline on the enhancement of subsequent responses to ATP suggest that increased production of DAG (a stimulant of PKC) contributes to synergism between noradrenaline and ATP. Since rho kinase has also been found to contribute to the contraction of smooth muscle induced by various agonists including noradrenaline (Kitazawa et al., 2000) we also assessed the ability of a rho kinase inhibitor to attenuate the potentiating effect of noradrenaline on ATP-induced contractions. HA1077 significantly reduced the enhancing effect of noradrenaline on ATP-induced contractions, however, the incomplete inhibition seen suggests that rho kinase activation may not contribute to synergism as much

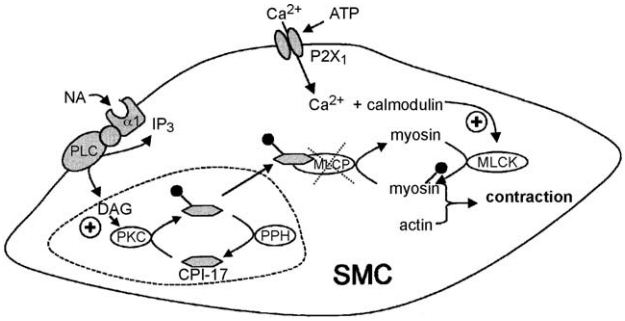

Fig. 7. Schematic illustrating a possible mechanism of postjunctional synergism between noradrenaline and ATP in smooth muscle cells (SMC) of the guinea pig vas deferens. ATP, acting predominantly at $P2X_1$ ligand-gated ion channels, triggers the influx of Ca^{2+}, which in combination with calmodulin stimulates myosin light chain kinase (MLCK). MLCK phosphorylates myosin, allowing interaction with actin and thus contraction. Noradrenaline stimulates α_1-adrenoceptors, triggering phospholipase C (PLC) activity, which produces inositol (1,4,5)-triphosphate (IP_3) and diacylglycerol (DAG) from membrane phospholipids. DAG stimulates protein kinase C (PKC), which in turn phosphorylates and activates the myosin light chain phosphatase (MLCP) inhibitor CPI-17. Since MLCP dephosphorylates myosin and therefore opposes contraction, inhibition of MLCP sensitises smooth muscle to Ca^{2+}. Hence, the contractile response to ATP is enhanced with prior activation of α_1-adrenoceptors.

as PKC in guinea pig vas deferens. It should be noted that a relatively high concentration of HA1077 was used in these experiments (30 μM) so the incomplete inhibition of synergism is unlikely to reflect insufficient attenuation of rho kinase (Sward et al., 2000).

Next, we attempted to uncover the mechanism of PKC-mediated potentiation of ATP-induced contractions. Although PKC-mediated phosphorylation of Ca^{2+} channels can trigger the influx of Ca^{2+} (Gleason and Flaim, 1986; Chiu et al., 1988; Murthy et al., 2000b), it has been reported that there is no concomitant increase in $[Ca^{2+}]_i$ during contractile synergism between noradrenaline and ATP (Fujita et al., 1996). Many studies have demonstrated that the regulation of MLCP activity is important for the development of smooth muscle cell contraction (Kitazawa et al., 1991; Kubuta et al., 1992; Somlyo and Somlyo, 1994; Kitazawa et al., 2000). Furthermore, in intestinal smooth muscle cells, it was shown that the tonic contraction produced by G-protein-coupled receptors was a result of PKC-mediated MLCP inhibition (Murthy et al., 2000a). Therefore, we investigated the effect of MLCP inhibition on basal tone and ATP-induced contractions of isolated guinea pig vas deferens. Cantharidin has been shown to have a vasoconstrictor effect that is probably solely due to phosphatase inhibition (Knapp et al., 1998). In the present study, similar to the findings in bovine coronary artery rings (Knapp et al., 1998; Waurick et al., 1999), cantharidin caused a slow developing, well-maintained contraction of the isolated guinea pig vas deferens. The contraction produced by cantharidin was not dissimilar from the tonic contraction produced by noradrenaline and histamine. More significantly, the marked enhancing effect of cantharidin on ATP- and electrical field stimulation-induced contractions clearly demonstrates that inhibition of MLCP is a potential mechanism for the synergism seen between noradrenaline and ATP. In fact, an endogenous 17-kDa inhibitor of MLCP, CPI-17, has been identified in smooth muscle, which is activated upon phosphorylation by kinases such as protein kinase C (Kitazawa et al., 2000). Once MLCP is inhibited by CPI-17, MLCK is unopposed; hence, there is an increase in phosphorylated myosin regulatory light chain and a concomitant increase in smooth muscle cell contraction (Kitazawa et al., 2000). We therefore propose that noradrenaline enhances ATP-induced contractions of vas deferens by sensitising smooth muscle cells to Ca^{2+} via a PKC-mediated inhibition of MLCP, which probably involves activation of CPI-17 (see Fig. 7).

Acknowledgements

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