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Mechanisms underlying the vasorelaxing effects of butylidenephthalide, an active constituent of Ligusticum chuanxiong, in rat isolated aorta

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Abstract

Butylidenephthalide (BDPH) is one of the most potent vasorelaxants isolated from *Ligusticum chuanxiong* Hort. The objective of the current study is to investigate the underlying vasorelaxation mechanisms in rat aorta. In 9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2\alpha}$ (U46619) precontracted preparations, endothelium removal, the nitric oxide (NO) synthase inhibitor *N*ω-nitro-L-arginine methyl ester (L-NAME) and the soluble guanylate cyclase inhibitor 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) partially inhibited the BDPH relaxation response to a similar extent. The cyclooxygenase inhibitor indomethacin, β-adrenoceptor antagonist propranolol, adenylate cyclase inhibitors 9-(tetrahydro-2-furanyl)-9H-purin-6-amine (SQ 22536) and 2′,5′-dideoxyadenosine, and K^+ channel blocker tetraethylammonium had no effect. BDPH produced full relaxation against contractions induced by KCl and U46619 in the presence of the L-type voltage-operated Ca^{2+} channel (Ca_v 1.2) blocker nifedipine. In a receptor-operated Ca^{2+} channel protocol where contraction was mediated by Ca^{2+} re-addition in the presence of U46619 and nifedipine, BDPH produced relaxation. In the absence of extracellular Ca^{2+}, BDPH inhibited contractions induced by phorbol-12,13-dibutyrate and U46619.

Our results suggest that BDPH-mediated vasorelaxation comprises both endothelium-dependent (NO) and independent components. It is suggested that BDPH acting through an inhibitory mechanism downstream to L-type voltage-operated and prostanoid TP receptor-operated Ca^{2+} channels operating late in the contractile pathway.
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Keywords: Butylidenephthalide; *Ligusticum chuanxiong*; Smooth muscle relaxation; Calcium; Endothelium

1. Introduction

Rhizoma Chuanxiong, the rhizome of *Ligusticum chuanxiong* Hort., is a traditional Chinese medicine widely used for the treatment of cardiovascular disorders such as angina pectoris and stroke in China. According to traditional Chinese medicine theory, the beneficial effects of the herb are related to its action on "facilitation of blood circulation" and "removal of blood stasis" (Zhu, 1998). Nevertheless, the scientific basis of the therapeutic value of the herb remains to be established.

Our laboratory has previously quantified 10 main constituents in *Ligusticum chuanxiong*, of which 80% (w/w) are phthalides (1(3H)-isobenzofuranones) (Li et al., 2003; Yan et al., 2004). Among the phthalide constituents, butylidenephthalide (BDPH, Fig. 1) is one of the most potent smooth muscle relaxants (Ko, 1980 and our research team unpublished observation). There are several reports suggesting that BDPH-mediated smooth muscle relaxation involves the inhibition of extracellular Ca^{2+} entry and is independent of endothelium (Ko, 1980; Ko et al., 1997, 1998, 2002). However, our research team has previously reported that BDPH may modulate the vasorelaxing effects of endothelium-derived nitric oxide (NO) (Chan et al., 2004). The pathway by which BDPH inhibits Ca^{2+} mobilization is also not resolved. The objective of the current study is to investigate the involvement of endothelium and Ca^{2+} mobilization in BDPH-mediated vasorelaxation.

2. Materials and methods

2.1. Animals and tissue preparation

Male Sprague–Dawley rats (250–300 g) were obtained from the Laboratory Animal Service Centre, Chinese University of

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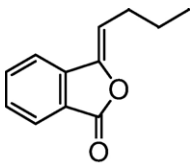

Fig. 1. Structure of butylidenephthalide.

Hong Kong. The use of animals as detailed below was approved by the Animal Experimental Ethics Committee, Chinese University of Hong Kong. Animals were sacrificed by cervical dislocation and exsanguination. Thoracic aortic rings of 3–4 mm in length were mounted in an organ bath apparatus at an initial force of 1 g tension and bathed with Krebs–Henseleit buffer (in mM: NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, D-glucose 10.6) aerated with carbogen (95% O_2/5% CO_2) at 37 °C. Isometric tension was measured with a Grass FT03 force transducer (Grass Instrument, West Warwick, RI, USA) and relayed via a PowerLab data acquisition system (ADInstruments, Castle Hill, Australia) connected to a computer. In some preparations, endothelium was mechanically removed by a wooden rod and its absence was verified by a lack of acetylcholine (1 μM) relaxation response (less than 10% relaxation) against phenylephrine (1 μM)-induced contraction. The endothelium was considered intact when such an acetylcholine response caused more than 80% relaxation. After examining the integrity of functional endothelium, the aortic rings were subjected to one of the following protocols.

2.2. Protoco1 1: general characteristic

9,11-dideoxy-9α,11α-methanoepoxyprostaglandin $F_{2\alpha}$ (U46619, 30 nM), a prostanoid TP receptor agonist, was employed as the contractile agent. After a stable contraction was established, BDPH 1–300 μM) was cumulatively administered. Various inhibitors were administered 30 min prior to the contractile agent. Papaverine (100 μM), a cyclic nucleotide phosphodiesterase inhibitor, was added at the end of each experiment to determine the maximal relaxation response.

2.3. Protocol 2: L-type voltage-operated calcium channels

KCl (60 mM) was used to induce contraction following by the cumulative addition of BDPH (1–300 μM). In another set of experiments, after a U46619 (30 nM)-mediated contraction was established, the L-type voltage-operated Ca^{2+} channel (VOCC, Ca_v 1.2) inhibitor nifedipine (1 μM) was administered for 30 min. BDPH (1–300 μM) and another L-type VOCC inhibitor verapamil (0.1–30 μM) were then cumulatively added.

2.4. Protocol 3: prostanoid TP receptor-operated calcium channels

U46619 (30 nM) was first administered to produce a reference contraction in normal Krebs–Henseleit buffer. After washout with Ca^{2+}-free buffer containing 200 μM EGTA, U46619 (30 μM) was used to induce contraction, following by the administration of nifedipine (1 μM) for 15 min. BDPH (200 μM) was then administered for 20 min prior to the cumulative addition of $CaCl_2$ (0.25–7.5 mM). In another set of experiments, $CaCl_2$ (2.5 mM) was administered after U46619 and nifedipine to cause further contraction, following by the application of increasing concentrations of BDPH (1–300 μM).

2.5. Protocol 4: store-operated calcium channels

In the absence of extracellular Ca^{2+} (with 200 μM EGTA), thapsigargin (3 μM, a sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase inhibitor) was added for 90 min. $CaCl_2$ (2.5 mM) was administered to induce contraction following by nifedipine (1 μM) for 15 min. Cumulative concentrations of BDPH (1–300 μM), or the combination of the non-selective cation channel inhibitor 3,4-dihydro-6,7-dimethoxyisoquinoline-1-yl-2-phenyl-*N*,*N*-di-[2-(2,3,4-trimethoxyphenyl)ethyl]acetamide (LOE 908, 30 μM) and the non-selective cation channel and store-operated Ca^{2+} channel inhibitor 1-[β-[3-(4-methoxyphenyl)propoxy]-4-methoxyphenethyl]-1H-imidazole (SKF 96365, 30 μM) were then added.

2.6. Protocol 5: contractions induced by internal calcium stores sensitive to phenylephrine

In the absence of extracellular Ca^{2+} (with 200 μM EGTA), phenylephrine (10 μM) was used to induce a transient contraction. After a washout period of 30 min, BDPH (300 μM) or the NO donor sodium nitroprusside (100 nM) was administered for 10 min, followed by the second challenge of phenylephrine (10 μM). Contractions induced by the first and second challenges of phenylephrine were compared.

2.7. Protocol 6: phorbol ester- and U46619-induced contractions in the absence of extracellular calcium

Phorbol 12,13-dibutyrate (10 μM) and U46619 (100 nM) were used to induce contraction in the absence of extracellular Ca^{2+} (with 2 mM EGTA). BDPH (1-300 μM), the protein kinase C inhibitor 2-[8-[(dimethylamino)methyl-]-6,7,8,9-tetrahydropyrido[1,2-a]indol-3-yl]-3-(1-methyl-1H-indol-3-yl)maleimide (Ro-32-0432, 0.1–3 μM) and the Rho kinase inhibitor (R)-(+)-*trans*-*N*-(4-pyridyl)-4-(1-aminoethyl)-cyclohexanecarboxamide (Y27632, 0.1–3 μM) were then cumulatively administered.

2.8. Data analysis

Results are expressed as mean±S.E.M. unless specified otherwise. Concentration-response curves were fitted into a sigmoidal equation:

$$\text{Response} = \text{Lower asymptote} + \frac{\text{Upper asymptote} - \text{Lower asymptote}}{1 + 10^{(\text{Hillslope} \times (\log EC_{50} - \log[\text{Drug}]))}}$$

Table 1
Effects of endothelium removal, L-NAME, ODQ, indomethacin, SQ 22536 and 2′,5′-dideoxyadenosine on BDPH-mediated relaxation in rat aorta pre-contracted with 30 nM U46619 (n=4–7)

Treatment	pEC_{50}	E_{max} (% relaxation)
Control	4.20±0.07	107±2
Endothelium removal	4.02±0.08*	95±4
L-NAME (100 μM)	3.88±0.05*	96±6
ODQ (10 μM)	3.89±0.04**	98±5
Indomethacin (1 μM)	4.19±0.05	108±7
SQ 22536 (100 μM)	4.22±0.17	95±2
2′,5′-dideoxyadenosine (10 μM)	4.20±0.07	96±4

*P<0.05, **P<0.01 vs. Control (one-way analysis of variance, Bonferroni post hoc test).
Data are expressed as mean±S.E.M.

"Response" is expressed as a percentage of the vascular tone induced by the contractile agent; "Lower asymptote" is the maximal relaxing response of the test compound in percentage (E_{max}); "Upper asymptote" is the initial vascular tone induced by the contractile agent in percentage (i.e., 100% in most cases); "EC_{50}" is the effective concentration of the test compound to cause 50% of its maximal response.

Concentration-response data were analyzed by repeated-measures two-way analysis of variance to determine the statistical significance of treatments (main effects) and also to compare data pairs at a particular relaxant concentration. Relaxation potency was expressed as the negative logarithm of EC_{50} (pEC_{50}). One-way analysis of variance with Bonferroni post hoc test (or unpaired Student t-test for comparing two treatment groups) was used to determine the statistical significance among pEC_{50} and E_{max} values. Statistical significance was set as P<0.05 (two tailed; *: P<0.05, **: P<0.01, ***: P<0.001).

2.9. Materials

BDPH was purchased from Aldrich (St Louis, MO, USA). U46619 was purchased from Sapphire Bioscience (Redfern, Australia). 2′,5′-dideoxyadenosine, phorbol-12,13-dibutyrate, Ro-32-0432, thapsigargin and Y27632 were purchased from Calbiochem (San Diego, CA, USA). All other chemicals were purchased from Sigma (St Louis, MO, USA). BDPH was dissolved in dimethyl sulfoxide (DMSO) and the highest concentration of DMSO used (0.3%) did not produce any significant effect in various protocols as described above.

3. Results

3.1. Experiment 1: general characteristics

3.1.1. Endothelium, soluble guanylate cyclase and adenylate cyclase

As illustrated in Table 1, endothelium removal, the NO synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME, 100 μM) and the soluble guanylate cyclase inhibitor 1H-[1,2,4] oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM) all slightly inhibited the BDPH vasorelaxation response to a similar extent (pEC_{50}: 4.02±0.08, 3.88±0.05 and 3.89±0.04 respectively, vs. control 4.20±0.07, P<0.05, n=4–7). The cyclooxygenase inhibitor indomethacin (1 μM), and the two adenylate cyclase inhibitors 9-(tetrahydro-2-furanyl)-9Hpurin-6-amine (SQ 22536, 100 μM) and 2′,5′-dideoxyadenosine (10 μM) were without effect (pEC_{50}: 4.19±0.05, 4.22±0.17 and 4.20±0.07 respectively, vs. control 4.20±0.07, n=4–7). Endothelium removal and the inhibitors did not significantly alter the basal and U46619-induced tone (P>0.05, data not shown).

3.1.2. β-adrenoceptor and potassium channels

In endothelium-removed preparations, the β-adrenoceptor antagonist propranolol (1 μM) did not affect the BDPH vasorelaxation response (pEC_{50}: 3.93±0.03 vs. control 4.02 ±0.08, n=4–7) against U46619 (30 nM)-induced contraction, nor did the non-selective K^+ channel blocker tetraethylammonium (10 mM) (pEC_{50}: 4.17±0.02 vs. control 4.09±0.07, n=6).

3.2. Experiment 2: L-type voltage-operated calcium channels

In endothelium-removed preparations, BDPH had a slightly higher relaxation potency against contractions induced by 60 mM KCl (pEC_{50}: 4.00±0.03, n=5) than that by 30 nM U46619 (pEC_{50}: 3.76±0.03, P<0.01, n=5, Fig. 2). Contractions induced by 60 mM KCl and 30 nM U46619 were not significantly different (1.75±0.07 and 1.96±0.16 g wt respectively, P>0.05).

As illustrated in Fig. 3, post-treatment of nifedipine at 1 μM (a concentration which totally abolished 60 mM KCl-induced contraction, data not shown) reduced the U46619 tone by 36%. BDPH completely inhibited the remaining nifedipine-insensitive tone (pEC_{50}: 4.78±0.09, n=5) while verapamil induced only weak relaxation. Maximal relaxation was achieved by papaverine (100 μM) in verapamil-treated preparations.

3.3. Experiment 3: prostanoid TP receptor-operated calcium channels

A typical tracing of the effect of BDPH in a prostanoid TP-receptor-operated Ca^{2+} channel (TP-ROCC) protocol in endothelium-removed preparations is illustrated in Fig. 4. Contraction induced by U46619 (30 nM) in the absence of extracellular Ca^{2+} was reduced to 28% of its response in normal Ca^{2+}-containing buffer, and nifedipine (1 μM) had no

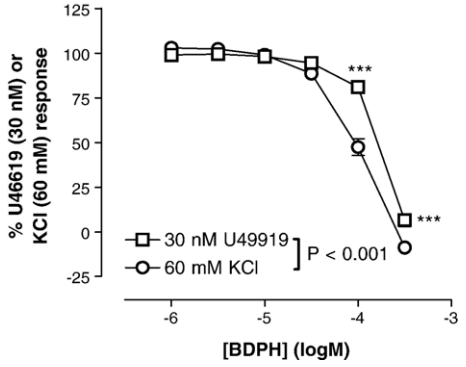

Fig. 2. The relaxing response of BDPH against contractions induced by U46619 (30 nM) and KCl (60 mM) in endothelium-removed rat aorta (n=5).

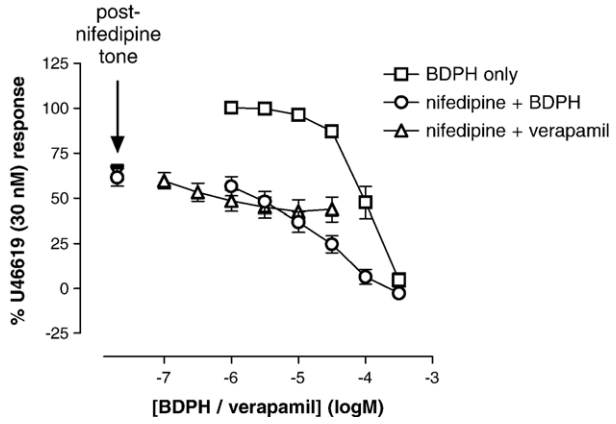

Fig. 3. Effect of nifedipine (1 μM) on BDPH-mediated relaxation in endothelium-removed rat aorta ($n=5$). Tone was induced by U46619 (30 nM).

effect on this response. Subsequent Ca^{2+} re-addition produced further contraction to 80% of the U46619 response in normal buffer. BDPH now induced relaxation, which was complete at 300 μM (pEC_{50}: 4.29±0.03, $n=5$, Fig. 5A). On control preparations, cumulative addition of DMSO did not alter the contractile tone; application of EGTA (2 mM) reduced the tension back to the initial U46619 response (Figs. 4 and 5A). Pre-treatment with BDPH (200 μM) significantly inhibited the Ca^{2+} re-addition-mediated contraction (Fig. 5B).

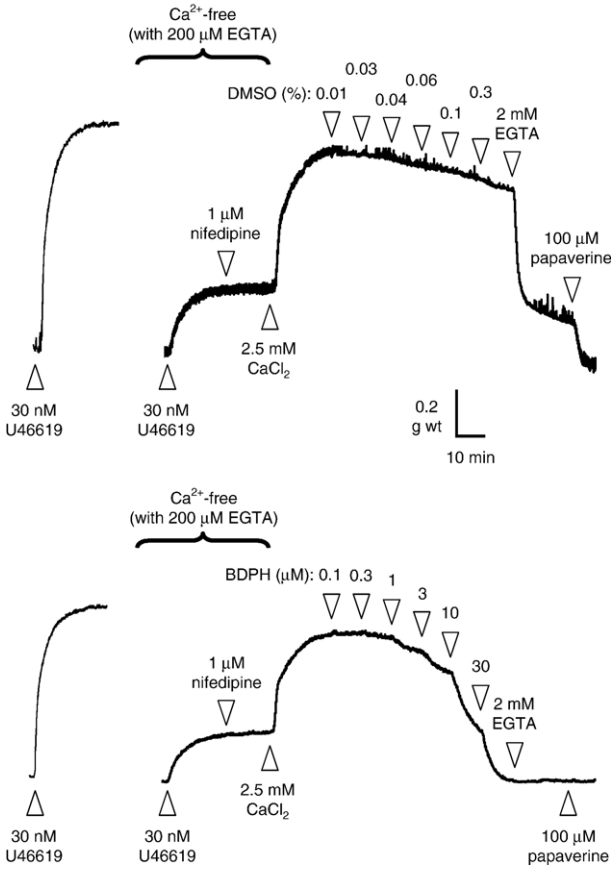

Fig. 4. Typical tracings of the effects of vehicle (upper panel) and BDPH (lower panel) in a TP-ROCC protocol in endothelium-removed rat aorta.

3.4. Experiment 4: store-operated calcium channels

In the absence of extracellular Ca^{2+}, thapsigargin (3 μM) did not cause significant increase in tension (0.05±0.02 g, $n=6$) in endothelium-removed preparations. Subsequent Ca^{2+} re-addition produced a tonic contraction which was not inhibited by 1 μM nifedipine (0.11±0.01 g both before and after nifedipine, $n=6$). BDPH inhibited the remaining nifedipine-insensitive tone (pEC_{50}: 4.57±0.12, $n=6$) while DMSO did not.

3.5. Experiment 5: contractions induced by internal calcium stores sensitive to phenylephrine

In the absence of extracellular Ca^{2+}, phenylephrine (10 μM) produced a small, transient and repeatable contraction (0.46 ±0.08 g and 0.43±0.05 g for the first and second challenges of phenylephrine respectively, $n=4$) in endothelium-removed preparations. BDPH (300 μM) and sodium nitroprusside (100 nM) decreased contraction induced by the second phenylephrine challenge by 45% and 79% respectively while DMSO did not (Fig. 6).

3.6. Experiment 6: phorbol ester- and U46619-induced contractions in the absence of extracellular calcium

In endothelium-removed preparations, the protein kinase C activator phorbol-12,13-dibutyrate (10 μM) in the absence of

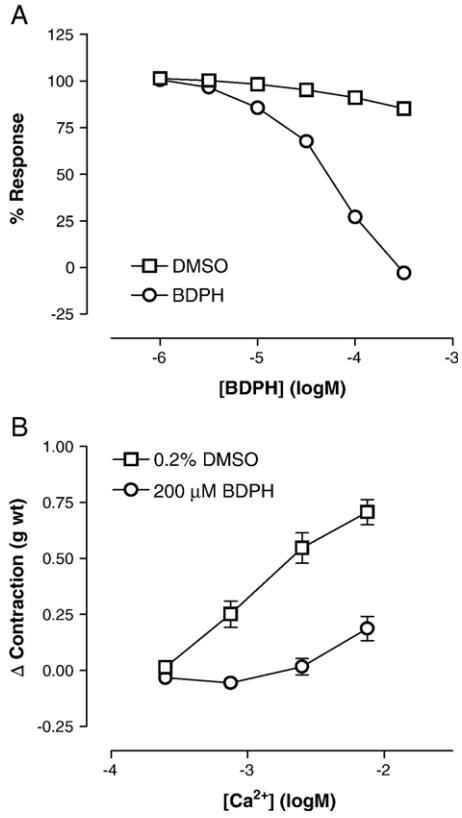

Fig. 5. The relaxing response of BDPH (A) pre-treatment ($n=4$) and (B) post-treatment ($n=5$) in a TP-ROCC protocol in endothelium-removed rat aorta.

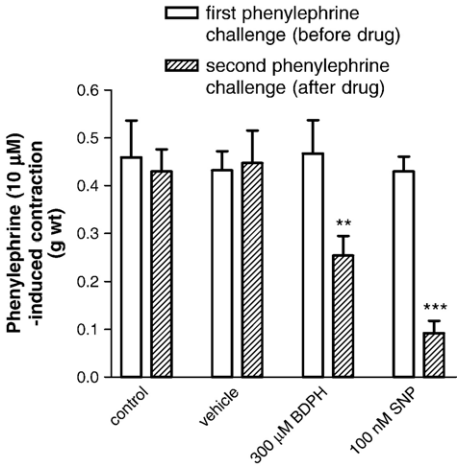

Fig. 6. Effect of BDPH on contraction induced by Ca^{2+} release from phenylephrine-sensitive internal stores in endothelium-removed rat aorta ($n=4$). BDPH and sodium nitroprusside (SNP) were administered 30 min after the first and 10 min before the second challenges of phenylephrine (10 μM) in the absence of extracellular Ca^{2+}(with 200 μM EGTA). Statistical comparison of first and second phenylephrine challenges was performed by Student *t*-test.

extracellular Ca^{2+} induced a slow-onset tonic contraction (1.06±0.03 g). BDPH (300 μM) and Ro-32-0432 (3 μM) abolished this tone (97%±5% and 100%±3% relaxation respectively, $n=5$). Y27632 (3 μM) was without any significant effect while subsequent addition of papaverine (100 μM) caused maximal relaxation (data not shown).

In endothelium-removed preparations, U46619 (100 nM) in the absence of extracellular Ca^{2+} induced a small tonic contraction (0.28±0.01 g), which was only 12% of its tone in the presence of extracellular Ca^{2+}. The extracellular Ca^{2+}-insensitive U46619 tone was significantly attenuated by BDPH (300 μM) and Y27632 (3 μM) (93%±10% and 68%±6% relaxation respectively, $n=5$–6). Ro-32-0432 (3 μM) was without any significant effect while subsequent addition of papaverine (100 μM) caused maximal relaxation (data not shown).

4. Discussion

In the current investigation, several mechanisms were probed for their contribution to BDPH-mediated vasorelaxation. Endothelium removal and L-NAME caused small inhibition of the BDPH vasorelaxing response (Table 1), indicating that BDPH-mediated vasorelaxation contained both endothelium-dependent and independent components. NO is likely to be the endothelium-dependent component, since inhibition of the BDPH response by L-NAME was comparable to that by endothelium removal. It remains uncertain whether BDPH released NO or basal NO augmented the BDPH response. Direct measurement of NO level is required. ODQ also inhibited the BDPH response to a similar extent to endothelium removal and L-NAME (Table 1). This observation implied that the activation of soluble guanylate cyclase was more likely due to NO binding than a direct effect of BDPH. On the other hand, indomethacin, propranolol and tetraethylammonium did not affect the BDPH response, implying that the activation of cyclooxygenase, β-adrenoceptor and K^+ channels was not involved. Adenylate cyclase was also not involved in the BDPH vasorelaxing effect, as the two adenylate cyclase inhibitors SQ 22536 and 2′,5′-dideoxyadenosine failed to modify the BDPH response. Although direct measurement of intracellular cyclic AMP levels was not performed in the present study, both inhibitors have been reported to significantly inhibit intracellular cyclic AMP levels and adenylate cyclase-mediated vasorelaxation at comparable potency (Dubey et al., 2000; Kageyama et al., 2003; Uchida et al., 2005).

Ko et al. (1998, 2002) previously reported that BDPH-mediated vasorelaxation was independent of endothelium, BK_{Ca} channels (K_{Ca} 1.1), K_{ATP} (K_{ir} 6.1) channels, soluble guanylate cyclase, adenylate cyclase and β-adrenoceptor. While the current study supports the non-involvement of K^+ channels, adenylate cyclase and β-adrenoceptors in BDPH's activity, endothelium removal and soluble guanylate cyclase inhibition were found to slightly inhibit the BDPH response. The discrepancy in the case of soluble guanylate cyclase may be due to other group's use of methylene blue in inhibiting soluble guanylate cyclase. Methylene blue is a weak soluble guanylate cyclase inhibitor (our lab unpublished observation) and may not have completely inhibited the enzyme. The disagreement on endothelium dependency was not known. In the current study, endothelium was considered as intact if 1 μM acetylcholine caused more than 80% relaxation and removed if the same concentration of acetylcholine produced less than 10% relaxation (see Materials and methods). However, the definitions of endothelium-intact and removal were not properly described (not mentioned) in the previous report (Ko et al., 1998). Hence, it is difficult to compare the results of both studies.

In the current study, several extracellular Ca^{2+}-related protocols were utilized to investigate the interaction between BDPH-mediated vasorelaxation and extracellular Ca^{2+}. KCl is a membrane depolarizing agent which is generally accepted to induce smooth muscle contraction chiefly by L-type VOCC opening. Hence, the observation that BDPH relaxed against KCl-induced contraction implies that BDPH inhibits a pathway involving L-type VOCC. In previous studies using similar protocols in several isolated smooth muscle preparations, other investigators suggested that BDPH inhibited L-type VOCC (Ko, 1980; Ko et al., 1997, 1998, 2002). However, direct evidence supporting this conjecture by means of ion channel recording and intracellular Ca^{2+} imaging has not yet been reported.

Whether BDPH has an L-type VOCC-insensitive relaxation component was studied by the use of the L-type VOCC inhibitor nifedipine. In the presence of a saturating concentration of nifedipine (1 μM), BDPH produced full relaxation of the remaining U46619 response while another L-type VOCC inhibitor verapamil did not (Fig. 4). This observation indicated that BDPH could induce vasorelaxation by a mechanism other than direct L-type VOCC blockade.

In the absence of extracellular Ca^{2+}, U46619-induced contraction was mediated by internal Ca^{2+} mobilization and Ca^{2+} sensitization (Huang et al., 2004). Subsequent Ca^{2+} re-

addition caused further contraction which was chiefly mediated by TP-ROCC but not by L-type VOCC, since this contractile response was abolished by the Ca^{2+} chelator EGTA but not by nifedipine (Fig. 4). BDPH also relaxed against this contraction (Figs. 4 and 5), suggesting that BDPH inhibits a pathway involving TP-ROCC.

In the store-operated Ca^{2+} channel protocol, thapsigargin was employed to deplete Ca^{2+} in the sarcoplasmic reticulum, thereby opening store-operated Ca^{2+} channels (Iwamuro et al., 1998, 1999). Subsequent Ca^{2+} re-addition induced contraction which was insensitive to nifedipine. The combination of the non-selective cation channel inhibitor LOE 908 (30 μM) and the non-selective cation channel and store-operated Ca^{2+} channel inhibitor SKF 96365 (30 μM) produced full relaxation (data not shown), indicating that the contraction was mediated by the store-operated Ca^{2+} channel. BDPH also relaxed against this contractile tone, suggesting BDPH to inhibit a pathway involving the store-operated Ca^{2+} channel.

In the absence of extracellular Ca^{2+}, phenylephrine produced a fast-onset, non-sustainable contraction, which has been shown to be mediated by Ca^{2+} release from phenylephrine-sensitive stores in the sarcoplasmic reticulum (Hisayama et al., 1990). BDPH at 300 μM reduced phenylephrine-induced transient contraction by 45% (Fig. 6), indicating an inhibitory action on this mechanism. However, Ca^{2+} release from internal stores is not a crucial mechanism in inducing contraction since it is suggested mainly to regulate Ca^{2+}-utilizing events in the plasmalemmal space (Karaki et al., 1997; van Breeman et al., 1995).

Phorbol ester such as phorbol-12,13-dibutyrate induced contraction by activating protein kinase C (Nunes, 2002). Both the protein kinase C inhibitor Ro-32-0432 and BDPH inhibited the phorbol-12,13-dibutyrate response, suggesting that BDPH inhibits a pathway involving protein kinase C. U46619 has been suggested to activate Rho kinase to induce contraction in the absence of extracellular Ca^{2+} (Wareing et al., 2005). The observation that the Rho kinase inhibitor Y27632 inhibited U46619-induced contraction in the absence of extracellular Ca^{2+} further supported this conjecture. BDPH was also found to inhibit this contraction, suggesting a possibility of BDPH to inhibit a pathway involving Rho kinase. Further investigations using molecular biology approaches are required to verify these speculations.

As discussed above, BDPH appeared to inhibit one or more contractile pathways involving L-type VOCC, TP-ROCC, store-operated Ca^{2+} channels, phenylephrine-sensitive internal Ca^{2+} store, PKC and Rho kinase. Although there remains a distant possibility that BDPH directly inhibits all of the above-mentioned targets, the results from the current study can neither support nor reject this conjecture. A more plausible explanation is that BDPH interacts with an inhibitory mechanism operating downstream of these targets (e.g., the contractile apparatus). Other investigational approaches such as experiments with skinned fibers are much needed to elucidate this issue.

In the present study, BDPH was found to cause relaxation in isolated rat aorta. An inhibitory mechanism downstream to L-type VOCC and TP-ROCC appears to be involved. BDPH was also found to have an endothelium- and NO-dependent relaxation component. The specific pathways involved in BDPH-mediated vasorelaxation await further investigation.

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