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Effects of 2,5-di-*t*-butyl-1,4-benzohydroquinone (BHQ) on rat aorta smooth muscle

Fabio Fusi *, Beatrice Gorelli, Massimo Valoti, Katia Marazova [1], Gian P. Sgaragli

Istituto di Scienze Farmacologiche, Università di Siena, via E.S. Piccolomini 170, 53100 Siena, Italy

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Abstract

To characterise the pharmacological activity of 2,5-di-*t*-butyl-1,4-benzohydroquinone (BHQ) on vascular smooth muscle, the different effects of BHQ on rat aorta were investigated under several experimental conditions. In aortic rings at rest or depolarised with 80 mM K^+ in the presence of 1 μM nifedipine, BHQ evoked a slow tonic contraction which was antagonised by 1 mM Ni^{2+}. Depolarised rings contracted in response to addition of 1 mM Ca^{2+}, with an EC_{50} value of 32.4 ± 1.0 mM for K^+. At 20 mM K^+, Ca^{2+}-induced contraction was enhanced by BHQ. This effect was antagonised by 1 mM Ni^{2+}, but not by 1 μM nifedipine. By contrast, at 40, 80 and 128 mM K^+, BHQ antagonised Ca^{2+}-induced contraction. This effect was partially reversed by 1 μM methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay K 8644) or by increasing extracellular Ca^{2+} concentration. In the presence of nifedipine and Ni^{2+}, depolarised rings (80 mM K^+) contracted in response to addition of 1 μM phenylephrine; this response was fast and then slowly decreased. When the preparations were preincubated with BHQ, the phenylephrine-induced contraction was transient and antagonised in a concentration-dependent manner by BHQ. These results indicate that the myotonic effect of BHQ on rat aortic rings depends on activation of Ca^{2+} influx via a Ni^{2+}-sensitive pathway, whereas its myolytic activity is due either to antagonism of Ca^{2+} entry via L-type Ca^{2+} channels or depletion of intracellular Ca^{2+} stores. © 1998 Elsevier Science B.V.

Keywords: BHQ (2,5-di-*t*-butyl-1,4-benzohydroquinone); Ca^{2+} channel, Ni^{2+}-sensitive; Ca^{2+} channel, L-type; Endoplasmic reticulum; Aorta, rat

1. Introduction

Molecules possessing a phenol moiety are generally antioxidants. Previous studies from this laboratory (Gorelli et al., 1995; Sgaragli et al., 1993; Fusi et al., 1994) showed that a series of phenol derivatives related to 2-*t*-butyl-4-methoxyphenol (BHA), besides their well-known antioxidant activity, relaxed smooth muscle cells by affecting Ca^{2+} homeostasis and that the two properties were linearly correlated. These compounds are characterised by at least one hydroxyl group on the aromatic ring and a highly lipophilic area. The most active of them, 2,6-di-*t*-butyl-4-methoxyphenol, was proposed as a lead compound for the synthesis of drugs useful for preventing tissue damage caused by ischemia-reperfusion (Sgaragli et al., 1993). However, the phenol derivatives studied showed much lower Ca^{2+} antagonistic activity ($IC_{50} > 10$ μM) than the Ca^{2+} antagonists currently used in clinical practice. In the search for more active compounds, our attention was attracted by 2,5-di-*t*-butyl-1,4-benzohydroquinone (BHQ). The structural requirements for the bifunctional activity described above occur twice in BHQ; moreover, the plane of symmetry separating the two moieties suggests that this molecule has a high probability of acting as a powerful smooth muscle-relaxing agent as well as an antioxidant.

BHQ was initially studied in the sixties as an antioxidant (Wilson and Poley, 1960; Ershoff, 1963, 1969) and has been described as a selective inhibitor of endoplasmic reticulum Ca^{2+} ATPase (Moore et al., 1987). It was recently found to inhibit Ca^{2+} ATPases bound to inositol 1,4,5-trisphosphate (IP_3)-sensitive and IP_3-insensitive Ca^{2+} stores (Oldershaw and Taylor, 1990; Nakamura et al., 1992) without affecting mitochondrial Ca^{2+} fluxes and plasma membrane Ca^{2+}-ATPase activity (Moore et al., 1987) or the sensitivity of the myofibrillar proteins to Ca^{2+} (Westerblad and Allen, 1994). BHQ was also shown

* Corresponding author. Tel.: +39-577-221255/48724; fax: +39-577-281928; e-mail: fusif@unisi.it

[1] Present address: Department of Pharmacology and Toxicology, Faculty of Medicine, 1431 Sofia, Bulgaria.

to reduce passive Ca^{2+} leakage from internal stores of permeabilized A7r5 vascular smooth muscle cells (Missiaen et al., 1992) and to inhibit plasma membrane Ca^{2+} influx in parotid acinar cells (Foskett and Wong, 1992).

Although the biochemical effects of BHQ, presumed to underlie its pharmacological properties, have been well documented, full characterisation of its pharmacological activity on vascular smooth muscle has not yet been undertaken. In the present study the contraction–relaxation cycle and force production of rat aorta rings were monitored to investigate the effects of BHQ on either the intracellular or extracellular Ca^{2+} handling.

In this model system BHQ exerted either tonic or lytic effects. The results obtained with different concentrations of extracellular K^+ ($[K^+]_o$) suggest that its myotonic effect depends on activation of Ca^{2+} influx via a Ni^{2+}-sensitive channel, whereas the myolytic effect seems due to depletion of intracellular Ca^{2+} stores or blockade of L-type Ca^{2+} channels.

2. Materials and methods

2.1. Aortic ring preparation and equilibration period

Male Wistar rats (250–350 g) were anaesthetised with a mixture of Ketavet® and Rompum®, decapitated and exsanguinated. The aorta was immediately removed, cleaned of connective tissue and the thoracic or abdominal portion of the vessel cut into 1.5-mm rings. The endothelium was removed by rubbing the lumen of the artery with a forceps. Each arterial ring was mounted over two rigid parallel stainless-steel tubes, one fixed in place and the other attached to an isometric transducer (Basile, Varese, Italy). The preparation was immersed in a water-jacketed organ bath (37°C), containing 5 ml of a modified Krebs–Henseleit physiological salt solution (PSS) (composition, mM: NaCl, 124; KCl, 4; $CaCl_2$, 1.8; $MgCl_2$, 1.1; KH_2PO_4, 0.4; $NaHCO_3$, 25; and glucose, 5.5) bubbled with a 95% O_2–5% CO_2 gas mixture to give a pH of 7.4. The vessel segments were allowed to equilibrate for 1 h at a resting tension of 1 g. Under these conditions maximal plateau levels of active tension of about 380 mg were obtained following full depolarization with 80 mM KCl. During the equilibration period, PSS was changed every 15 min. The K^+-rich PSS containing 20 (K20), 40 (K40), 80 (K80) and 128 mM KCl (K128) respectively, was prepared by replacing NaCl with equimolar KCl. The nominally Ca^{2+}-free PSS was prepared by omitting Ca^{2+}; EGTA was deliberately omitted since prolonged exposure of rat aorta to Ca^{2+}-free PSS may lead to increased permeability to subsequently added Ca^{2+} (Guan et al., 1988). After the equilibration period, the aortic rings were stimulated with K^+-rich PSS, until a constant response was obtained, to test for the integrity of the voltage-dependent Ca^{2+} channels. The absence of relaxation to 12 μM acetylcholine of blood vessels precontracted with 0.1–1 μM phenylephrine was used to confirm that the endothelium has been removed. The rings were then washed and equilibrated for another 30 min before testing the different experimental settings (see below).

2.2. Effects of BHQ on basal tone

The effects of BHQ on aortic rings were examined under resting conditions and in K80 PSS containing 1 μM nifedipine. BHQ (50 μM) was added to the organ bath; when the contraction provoked by BHQ reached a maximum, 1 mM Ni^{2+} was added.

2.3. Effects of BHQ on contraction promoted by extracellular Ca^{2+} influx at different $[K^+]_o$

The following experimental protocol was used to examine the effects of BHQ on Ca^{2+} influx via L-type Ca^{2+} channels. The preparations were contracted by adding 1 mM Ca^{2+} to Ca^{2+}-free PSS containing various concentrations of K^+ (20–128 mM). The vessels were then washed with the same Ca^{2+}-free, K^+-rich PSS, until baseline was reached, incubated for 15 min with dimethyl sulphoxide (DMSO) (control) or 50 μM BHQ and stimulated again with 1 mM Ca^{2+}. 1 mM Ni^{2+}, 1 μM nifedipine or 1 μM methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate (Bay K 8644) was added 5 min before DMSO or BHQ.

2.4. Effects of BHQ on contraction induced by Ca^{2+} mobilisation from the endoplasmic reticulum

To determine the contribution of intracellular Ca^{2+} stores to muscle contraction, the aortic rings were stimulated with 1 μM phenylephrine (the tension attained at the peak was taken to be 100%) and then washed with PSS. Then 1 μM nifedipine was added to the bath. After 15 min, PSS was drained and quickly replaced with K80 PSS containing 1 μM nifedipine. Under these conditions K80 PSS failed to contract the rings, demonstrating that extracellular Ca^{2+} influx via L-type Ca^{2+} channels has been blocked. After 5 min, the substances being studied or the vehicle were added for a 15-min incubation period. The rings were then contracted with 1 μM phenylephrine and the tension expressed as a percentage of the first response to phenylephrine. The response to phenylephrine in the presence of nifedipine was taken to represent the contribution of intracellular Ca^{2+} stores to the contractile response, being the resultant of the amount flowing out of endoplasmic reticulum store sites and that pumped in. Obviously, this model system—endoplasmic reticulum store sites/cy-

toplasm Ca^{2+} bidirectional exchange and tension reflecting intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) —may be influenced by any extracellular Ca^{2+} influx via non L-type Ca^{2+} channels.

2.5. Drugs: commercial sources and solutions

BHQ, $NiCl_2$, nifedipine, Bay K 8644, phenylephrine, cyclopiazonic acid and thapsigargin were purchased from Sigma Chimica (Milan, Italy). All other compounds were of analytical grade and used without further purification.

Stock solutions of BHQ in 100% DMSO, and nifedipine and Bay K 8644 in absolute ethanol were shielded from light with aluminium foil, stored at −20°C and used within one week.

Water for solutions was first distilled and then passed through a NANOpure II deionization system (Barnstead-Sybron, Boston, MA, USA), to obtain Type I Reagent Grade water (resistivity 18 MΩ). DMSO and ethanol at the highest concentration used (both 0.1%) did not affect the response of the aorta rings. The concentrations given are the final concentrations in the bath chambers.

2.6. Statistical analysis

All values were expressed as mean ± S.E.M.; n is the number of animals (indicated in parentheses). The statistical significance of differences was analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test for multiple comparisons; P values < 0.05 were considered significant.

3. Results

3.1. Effects of BHQ on basal tone

In rings at rest, 5 or 50 μM BHQ evoked slow and moderate tonic contractions which were 33.1 ± 13.6% ($n = 7$) and 55.9 ± 7.8% ($n = 10$) of the contractions produced by 0.1 μM phenylephrine (276.9 ± 35.6 mg, $n = 8$; 100%). 50 μM BHQ-evoked contraction was not modified by 1 μM nifedipine (Fig. 1), whereas 1 mM Ni^{2+} antagonised BHQ effects in a highly significant manner (6.8 ± 2.4%, $n = 8$, $P < 0.01$).

To further analyse this effect, we added 50 μM BHQ to rings in K80 PSS containing 1 μM nifedipine and observed a slow tonic contraction (165.3 ± 36.1 mg, $n = 5$) which plateaued in about 45 min (Fig. 2a) and was reversed by 1 mM Ni^{2+}. The addition of 10 μM cyclopiazonic acid (Fig. 2b) or 1 μM thapsigargin (Fig. 2c) also gave rise to a slow tonic contraction (66.2 ± 39.2 and 72.6 ± 40.1 mg, respectively; $n = 4$) which was reversed by the further addition of Ni^{2+}.

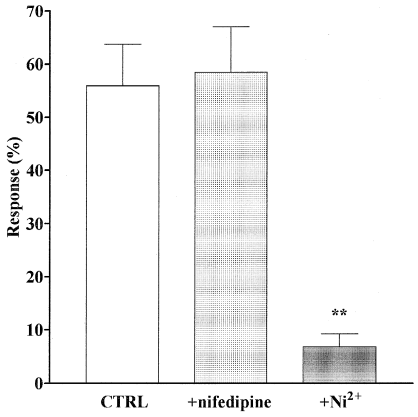

Fig. 1. Contraction evoked by BHQ in rat aorta rings at rest. Responses are given as a percentage of the contraction induced by 0.1 μM phenylephrine (276.9 ± 35.6 mg, $n = 8$). Columns represent mean values ± S.E.M. 50 μM BHQ (CTRL, $n = 10$) was added to rings incubated in PSS; when the contraction provoked by BHQ reached a maximum, 1 mM Ni^{2+} ($n = 8$) or 1 μM nifedipine ($n = 6$) was added to the organ bath. ** $P < 0.01$ represents significant difference with respect to CTRL, Dunnett's test.

3.2. Effects of BHQ on contraction promoted by extracellular Ca^{2+} influx at different $[K^+]_o$

In this set of experiments, muscle tension developing at different $[K^+]_o$ was taken as an index of Ca^{2+} influx from the extracellular space promoted by membrane depolarisation. Full tension was attained over a 20–80 mM range of

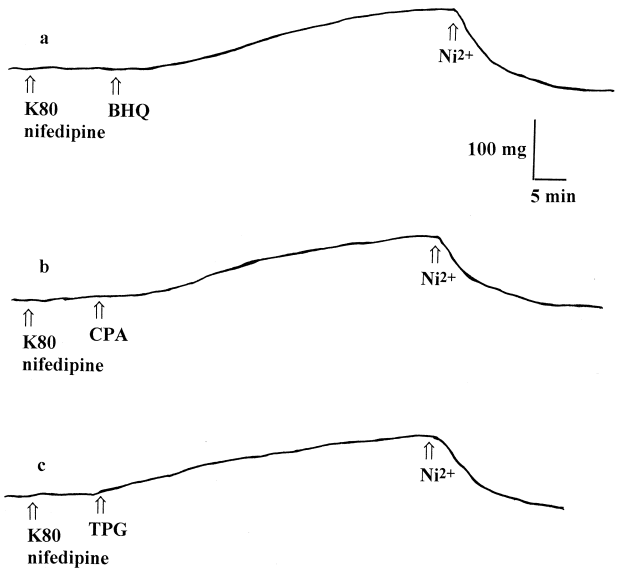

Fig. 2. Contraction evoked by BHQ (a), cyclopiazonic acid (CPA) (b) and thapsigargin (TPG) (c) in rat aorta rings in K80 PSS containing 1 μM nifedipine. 50 μM BHQ, 10 μM cyclopiazonic acid or 1 μM thapsigargin was added to the K80 PSS containing 1 μM nifedipine in the organ bath; when the contraction provoked by BHQ, cyclopiazonic acid or thapsigargin reached a maximum, 1 mM Ni^{2+} was added. Traces are representative of at least four different animals.

$[K^+]_o$ in the presence of 1 mM Ca^{2+} (Fig. 3a–d) showing an EC_{50} value of 32.4 ± 1.0 mM ($n = 8$). The effect of 50 μM BHQ in relation to $[K^+]_o$ was biphasic: contractions increased 10-fold in K20 PSS (Fig. 3a) or were increasingly antagonised in K40, K80 and K128 PSS (Fig. 3b to 3d). At K30 PSS (not reported in Fig. 3), pretreatment with BHQ led to an increase (117.6 ± 9.6 mg, $n = 4$) or a decrease (70.3 ± 19.9 mg, $n = 3$) in the contractile response to Ca^{2+} with respect to control conditions (90.0 ± 16.7 mg, $n = 7$). The average, however, (97.3 ± 13.2 mg, $n = 7$) was not significantly different with respect to control conditions. When the concentration of extracellular Ca^{2+} ($[Ca^{2+}]_o$) was increased to 1.8 mM in K20 PSS, stimulation of muscle contraction by BHQ was higher than that observed at 1 mM Ca^{2+}; on the contrary, BHQ antagonism was fully reversed for K40 and K80, but only partially for K128 PSS.

In order to clarify the mechanism underlying this biphasic effect, the response to Ca^{2+} was studied in rings preincubated with BHQ in the presence of nifedipine, Bay K 8644 or Ni^{2+}. As shown in Fig. 3a, the increase in response to Ca^{2+} caused by BHQ in K20 PSS was abolished by 1 mM Ni^{2+}, but only partially reduced by 1 μM nifedipine. When K40, K80 and K128 PSS contained 1 μM Bay K 8644, BHQ antagonism of Ca^{2+}-induced contraction was partially reversed, as compared to the contraction observed in the presence of Bay K 8644 alone, but the response was not significantly different from that observed in control conditions (Fig. 3b–d).

3.3. Effects of BHQ on contraction promoted by Ca^{2+} mobilisation from the endoplasmic reticulum

The following set of experiments was designed to evaluate the effects of BHQ on intracellular Ca^{2+} mobilisation. Fig. 4 shows the typical traces of 1 μM phenylephrine-induced contraction in rings depolarised with K80 PSS in the presence of nifedipine with or without Ni^{2+} and/or BHQ. Phenylephrine evoked a fast response ($60.4 \pm 3.2\%$ of the first phenylephrine-induced contraction, $n = 9$)

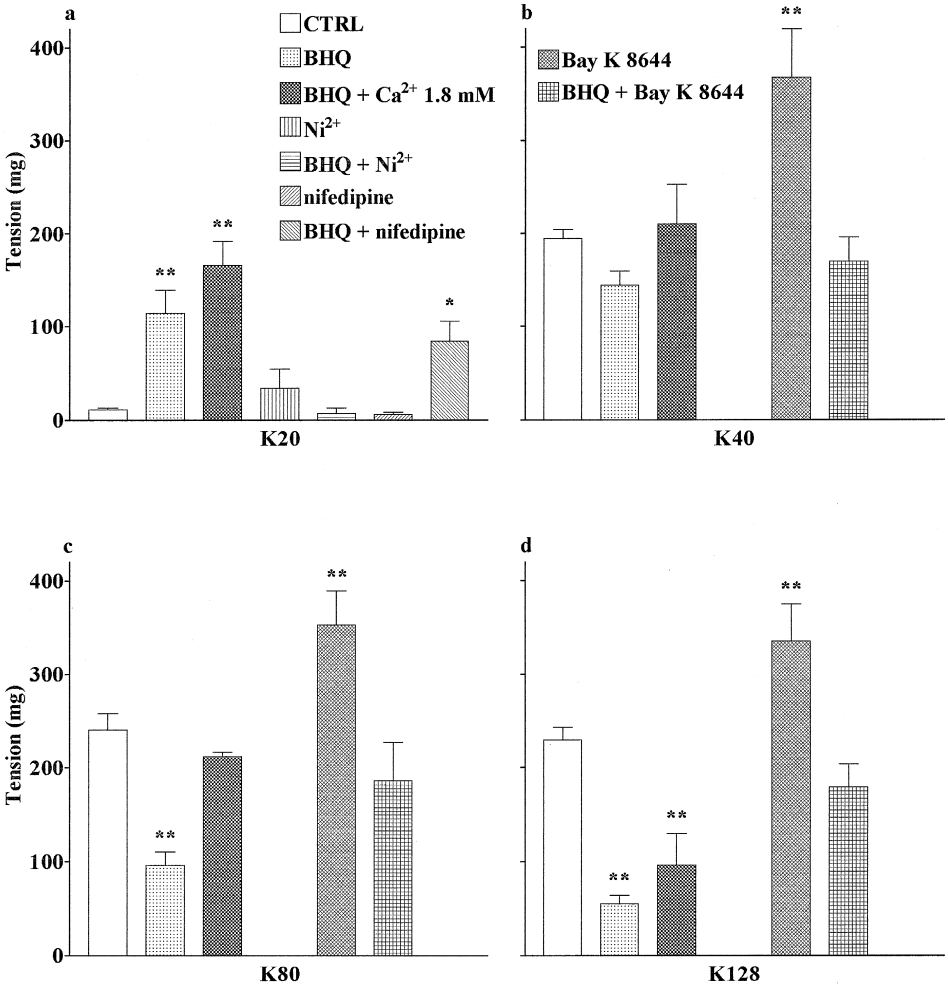

Fig. 3. Effects of BHQ on Ca^{2+}-dependent contraction at different $[K^+]_o$ (mM): (a) 20; (b) 40; (c) 80; (d) 128. Ca^{2+}-induced contractions were obtained in Ca^{2+}-free PSS containing various concentrations of K^+ (20–128 mM). 50 μM BHQ (or DMSO) was added 15 min before Ca^{2+} (1 mM). 1 mM Ni^{2+}, 1 μM nifedipine or 1 μM Bay K 8644 additions were made 5 min before DMSO or BHQ. Data represent mean values of tension (mg) $\pm$ S.E.M. ($n = 4$–10). $* P < 0.05$, $** P < 0.01$ represent significant differences with respect to CTRL, Dunnett's test.

which then slowly decreased (see Fig. 4a as an example). In the presence of 50 μM BHQ, the phenylephrine-induced contraction was similar in amplitude but sustained (71.6 ± 5.6%, $n = 7$); the subsequent addition of Ni^{2+} resulted in a reversal of this sustained contraction towards basal tone levels (see Fig. 4b as an example). The addition of 1 mM Ni^{2+} did not affect the response to phenylephrine (52.9 ± 6.1%, $n = 5$; see Fig. 4c as an example). Furthermore, when tissues were preincubated with Ni^{2+}, the response to phenylephrine in the presence of BHQ was phasic and significantly reduced (24.9 ± 5.0%, $n = 4$, $P < 0.01$; see Fig. 4d as an example).

The phasic contraction in response to phenylephrine in the presence of nifedipine was investigated at various BHQ concentrations. BHQ only caused inhibition at the lowest concentration tested (0.5 μM) (42.9 ± 1.3%, $n = 5$, $P < 0.05$) and was ineffective at the other concentrations (up to 50 μM). However, in the presence of 1 mM Ni^{2+}, BHQ antagonised the phenylephrine-induced contraction in a concentration-dependent manner. In fact, when plotting the ratio of the phasic contractions to phenylephrine

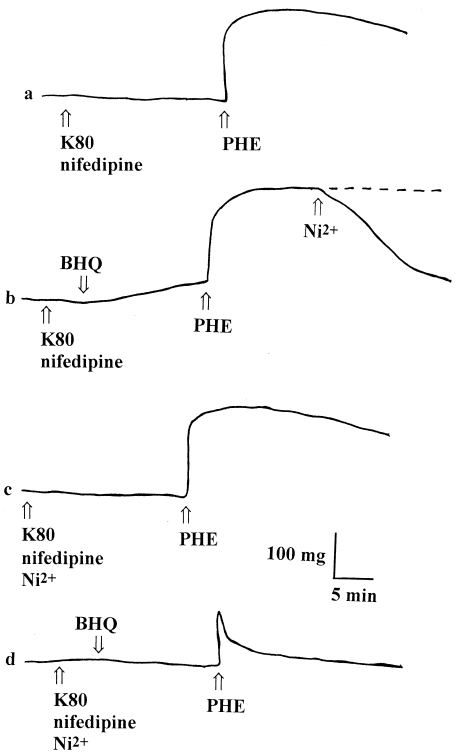

Fig. 4. Effects of BHQ on Ca^{2+} mobilisation from intracellular stores. All traces show 1 μM phenylephrine (PHE)-induced contraction of rat aorta rings incubated in K80 PSS containing 1 μM nifedipine. This response was assumed to represent the contribution of intracellular Ca^{2+} sources to the contractile response. (a) Control; (b) phenylephrine-induced response after 15 min incubation with 50 μM BHQ; the dotted line indicates tension when no Ni^{2+} was added; (c) phenylephrine-induced response after 20 min incubation with 1 mM Ni^{2+}; (d) phenylephrine-induced response after 15 min incubation with 50 μM BHQ in the presence of Ni^{2+}. Traces are representative of at least four different animals.

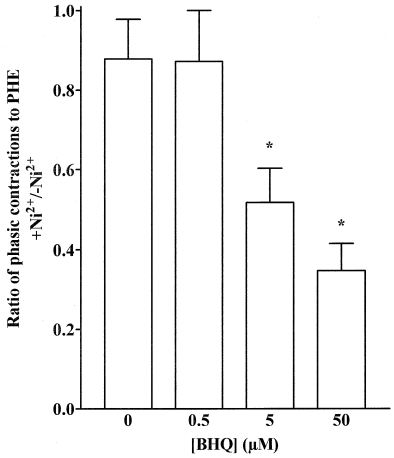

Fig. 5. Concentration–response relationship of BHQ on phenylephrine (PHE)-induced contraction of rat aorta rings in the presence of nifedipine and Ni^{2+}. The rings were stimulated with 1 μM phenylephrine in K80 PSS containing 1 μM nifedipine. Columns represent mean values ± S.E.M. ($n = 5$–9) of the ratio of phasic contractions in response to phenylephrine in the presence and absence of 1 mM Ni^{2+} obtained at various BHQ concentrations. Contractions (%) were calculated with respect to the contraction induced by 1 μM phenylephrine in PSS. $* P < 0.05$ represents significant difference with respect to control (no BHQ), Dunnett's test.

with and without Ni^{2+}, concentration-dependent antagonism by BHQ was observed (Fig. 5).

4. Discussion

$[Ca^{2+}]_i$ has a crucial role in regulating the contractile activity of smooth muscles. In this study we did not measure $[Ca^{2+}]_i$ directly, but a relationship is known to exist between changes in $[Ca^{2+}]_i$ and muscle tone (Morgan, 1990). Changes in tension were therefore assumed to be the consequence of changes in $[Ca^{2+}]_i$. The control of $[Ca^{2+}]_i$ involves two integrated membrane systems: (i) the plasmalemma, which is controlled by membrane potential, agonists and endoplasmic reticulum (Irvine, 1992), and (ii) the endoplasmic reticulum, which is under the control of intracellular second messengers (Van Breemen and Saida, 1989). The effects of BHQ on contraction can be explained by discussing the role of these two mechanisms.

At plasmalemmal level, L-type Ca^{2+} channels open in response to depolarisation of the membrane, and extracellular Ca^{2+} follows the electrochemical gradient into the cell. The present study shows that the effect of BHQ on depolarised rings contracting in response to extracellular Ca^{2+} addition was biphasic and dependent on $[K^+]_o$, 30 mM K^+ being the threshold concentration below which BHQ activated, and above which it inhibited Ca^{2+} influx. At higher $[K^+]_o$, inhibition of L-type Ca^{2+}-channel activity by BHQ increased by increasing the degree of depolarisation, thus mimicking the effect of dihydropyridine Ca^{2+} antagonists (Bean et al., 1986). Opening of the K^+ chan-

nels does not seem to be involved in the myorelaxant effect of BHQ, because at high $[K^+]_o$, K^+ channel openers fail to relax vascular smooth muscle (Gurney, 1994). If Ca^{2+}-antagonism exerted by BHQ at high $[K^+]_o$ is regulated by a nifedipine-sensitive Ca^{2+} channel, one would expect this antagonism to be reversed by the Ca^{2+} channel activator Bay K 8644 or by increasing $[Ca^{2+}]_o$. Our results showed, in fact, that both experimental conditions reversed BHQ antagonism on Ca^{2+}-induced contraction, suggesting that the blockade of a nifedipine-sensitive Ca^{2+} channel is responsible for the antispasmodic property of BHQ. However, at low $[K^+]_o$ (< 30 mM), the Ca^{2+} antagonistic effect of BHQ vanished and a marked stimulation of Ca^{2+}-induced contraction occurred. This stimulation paralleled the increase in $[Ca^{2+}]_o$ up to 1.8 mM, was partly inhibited by nifedipine and was blocked by Ni^{2+}. This suggests that it depends on the influx of extracellular Ca^{2+} which mainly takes place through Ni^{2+}-sensitive Ca^{2+} channels. Also, the slowly developing and sustained contraction caused by BHQ in rings exposed to PSS alone or K80 PSS containing nifedipine was due to extensive Ca^{2+} influx across the plasma membrane through a Ni^{2+}-sensitive Ca^{2+} channel. Though the mechanism by which BHQ promotes Ca^{2+} influx is unknown, cyclopiazonic acid (an indoletetramic acid) and thapsigargin (a sesquiterpene lactone), two inhibitors of endoplasmic reticulum Ca^{2+}-ATPase (Seidler et al., 1989; Takemura et al., 1989) structurally unrelated to BHQ, produced the same effect under similar experimental conditions. Since these two Ca^{2+} ATPase inhibitors have been shown to mobilise endoplasmic reticulum Ca^{2+} slowly by preventing its reuptake, the depletion of intracellular Ca^{2+} stores brought about by these three agents might be the signal which activates Ca^{2+} influx from the extracellular space *via* a channel sensitive to divalent or trivalent cations (Fasolato et al., 1994). This hypothesis is sustained by the slow rate of development of the tonic contraction induced by these agents which conceivably reflects the kinetic of mobilisation of intracellular Ca^{2+} stores, as established in non-excitable cells for cyclopiazonic acid, thapsigargin and BHQ (Foskett and Wong, 1992; Demaurex et al., 1992). Activation of Ca^{2+} influx by BHQ was observed in PSS alone, K20 PSS and K80 PSS containing nifedipine, indicating that this mechanism is potential-independent. These findings confirm a previous observation that depletion of endoplasmic reticulum Ca^{2+} stores by vasopressin, IP_3 or A23187 in rat aortic smooth muscle cells stimulates $^{45}Ca^{2+}$ uptake, which, however, does not occur through voltage-operated Ca^{2+} channels (Missiaen et al., 1990).

As mentioned above, BHQ inhibits endoplasmic reticulum Ca^{2+}-ATPase and therefore causes the depletion of the Ca^{2+} content of the endoplasmic reticulum (Nakamura et al., 1992). However, when studying the response to phenylephrine in the presence of nifedipine (taken as the contribution of intracellular Ca^{2+} stores to the contractile response) BHQ only inhibited the phasic response to phenylephrine at the lowest concentration tested (0.5 μM) and was ineffective at the other concentrations (up to 50 μM). Obviously, this model system—endoplasmic reticulum store sites/cytoplasm Ca^{2+} bidirectional exchange and muscle tension reflecting $[Ca^{2+}]_i$—may be influenced by any Ca^{2+} influx from the extracellular space via non L-type Ca^{2+} channels. Since high concentrations of BHQ activate an influx of Ca^{2+} via Ni^{2+}-sensitive Ca^{2+} channels, endoplasmic reticulum Ca^{2+}-ATPase inhibition was studied in the presence of Ni^{2+} and nifedipine to block any influx of Ca^{2+} from the extracellular space. It was possible to determine the consequence of the inhibition of endoplasmic reticulum Ca^{2+}-ATPase under these experimental conditions. In fact, the response to phenylephrine in the presence of BHQ was significantly reduced in a concentration-dependent manner, indicating that BHQ was progressively exhausting endoplasmic reticulum Ca^{2+} stores. Furthermore, when considering the effects of BHQ on the response to phenylephrine, we can not rule out that this might be due to a decrease of the sensitivity to Ca^{2+} of contractile elements (Somlyo and Somlyo, 1994; Karaki, 1989) and not only to the inhibition of the endoplasmic reticulum Ca^{2+}-ATPase. However, this hypothesis needs to be examined through the means of measurement of $[Ca^{2+}]_i$ and/or using permeabilized smooth muscle preparations, which is a matter for further investigation in this laboratory.

Ni^{2+} has been shown to block agonist-induced Ca^{2+} entry which is dependent on the emptying of the intracellular stores (Kwan and Putney, 1990)—the so called 'capacitative model' (Putney, 1990). Activation of this Ca^{2+} influx has been demonstrated in several cell types, if not ubiquitously and is insensitive to classical inhibitors of voltage gated Ca^{2+} channels (Fasolato et al., 1994). Hoth and Penner (1992) demonstrated that depletion of intracellular stores leads to activation of a Ca^{2+} current named I_{CRAC} (Ca^{2+}-release-activated Ca^{2+} current), that is blocked by Ni^{2+} (Zweifach and Lewis, 1996). BHQ therefore may activate I_{CRAC} (as a consequence of the emptying of intracellular Ca^{2+} stores), allowing Ca^{2+} influx to bypass nifedipine blockade of L-type Ca^{2+} channels. Ni^{2+} has been shown to exercise some selectivity in blocking T-type Ca^{2+} channels as well (Spedding and Paoletti, 1992). These channels have been identified in a variety of cells, including vascular smooth muscle cells (Bolton et al., 1988). However, T-type Ca^{2+} channels are known to activate and inactivate at very low membrane potentials (-70 to -60 mV) (Janssen, 1997) and they should be mainly in the inactive state under the condition used in the present study. Furthermore, nifedipine, at 1 μM, affects also T-type channels to some extent (Hirakawa et al., 1994). It is therefore unlikely that T-type channels contribute significantly to the relaxing effects of Ni^{2+} on BHQ stimulated contraction.

In conclusion, the pleiotypic effects of BHQ in rat aortic rings can be ascribed to interaction with specific

cellular targets: the myotonic effect depends on activation of Ca^{2+} influx via a Ni^{2+}-sensitive pathway, while myolytic activity is due to antagonism of Ca^{2+} entry via L-type Ca^{2+} channels or depletion of intracellular Ca^{2+} stores.

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