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European Journal of Pharmacology 517 (2005) 224 – 231

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Effect of sildenafil citrate and a nitric oxide donating sildenafil derivative, NCX 911, on cavernosal relaxation and superoxide formation in hypercholesterolaemic rabbits

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Received 3 March 2005; received in revised form 10 May 2005; accepted 19 May 2005
Available online 15 June 2005

Abstract

Hypercholesterolaemia promotes erectile dysfunction through increased superoxide formation and negation of nitric oxide (NO) bioactivity in cavernosal tissue. The source of superoxide has not been clearly defined, however. Sildenafil (Viagra™), the standard therapy for erectile dysfunction, may also be rendered more effective by the presence of an NO donor. One drug that intrinsically fulfils this criterion is sildenafil nitrate (NCX 911), an NO donating derivative of sildenafil. The objective of this study, therefore, was to determine the source of superoxide and its effect on erectile function in corpus cavernosum from hypercholesterolaemic rabbits and to determine whether NCX 911 confers an improvement over sildenafil citrate in this model. Hypercholesterolaemia elicited an increase in superoxide formation by rabbit cavernosal tissue and a reduction of carbachol-stimulated relaxation both of which were reversed by diphenylene iodonium chloride and apocynin (NADPH oxidase inhibitors). In response to sodium nitroprusside, hypercholesterolaemia also caused an attenuation of cavernosal relaxation which was not reversed with NADPH oxidase inhibitors. Both sildenafil citrate and NCX 911 significantly reversed impaired carbachol-stimulated relaxation and inhibited superoxide formation by cavernosal tissue from hypercholesterolaemic rabbits, NCX 911 being more potent. NCX 911 also augmented cavernosal cGMP levels, an effect blocked by the guanylyl cyclase inhibitor, 1H-{1,2,4}oxadiazolo {4,3-*a*}quinoxalin-1-one (ODQ). These data demonstrate that hypercholesterolaemia promotes erectile dysfunction through an augmentation of superoxide derived from NADPH oxidase in cavernosal tissue. It also indicates that NO donating sildenafil may be therapeutically more beneficial than conventional sildenafil in treating erectile dysfunction with an oxidative stress-related aetiology.

Keywords: Erectile dysfunction; Cholesterol; Superoxide; NADPH oxidase

1. Introduction

There is a strong epidemiological association between vasculogenic erectile dysfunction and hypercholesterolaemia (Feldman et al., 1994; Kim, 2000; Martin-Morales et al., 2001; Roumeguere et al., 2003; Wei et al., 1994), the converse being that vasculogenic erectile dysfunction is a predictor for cardiovascular disease (Barrett-Connor, 2004; Bocchio et al., 2004; Roumeguere et al., 2003). Aetiologically, vasculogenic erectile dysfunction has been ascribed mainly to a reduction of nitric oxide (NO) derived from non-cholinergic nonadrenergic nerves and the penile endothelium (Cashen et al., 2002; Gonzalez-Cadavid et al., 1999). NO relaxes the corpus cavernosum and associated penile arteries, through inhibition of calcium mobilisation, an effect that is mediated by the cyclic GMP-protein kinase G system (Gonzalez-Cadavid et al.,

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doi:10.1016/j.ejphar.2005.05.012

1999). Thus, a reduction of NO bioavailability results in impaired relaxation of penile corpus cavernosum and therefore vasculogenic erectile dysfunction.

A principal mechanism underlying vasculogenic erectile dysfunction is the over-production of vascular superoxide (Jeremy et al., 2000; Jones et al., 2002). Superoxide reacts with NO to form reactive nitrogen species, effectively reducing NO bioavailability (Jeremy et al., 2004; Kojda and Harrison, 1999; Li and Shah, 2004). In turn, it is well established that hypercholesterolaemia promotes increased vascular superoxide formation (O'Hara et al., 1993; Mugge et al., 1994; Seo et al., 1999), an effect mediated by an increase in NADPH oxidase activity (Itoh et al., 2002; Warnholtz et al., 1999). Although it has been shown that hypercholesterolaemia augments cavernosal superoxide formation (Kim et al., 1997), the enzymic sources of cavernosal superoxide in hypercholesterolaemia have hitherto not been determined.

The first objective of this study, therefore, was to investigate the role of superoxide in mediating vasculogenic erectile dysfunction in hypercholesterolaemia, with particular emphasis on the enzymic source of superoxide using a cholesterol-fed rabbit model.

Therapeutically, sildenafil citrate (Viagra™) has revolutionised the treatment of erectile dysfunction (Palumbo et al., 2001). Sildenafil, a type 5 phosphodiesterase (PDE5) inhibitor enhances the NO–cGMP pathway through inhibition of the hydrolysis of cGMP to inactive GMP by PDE5). Despite its undoubted clinical benefits, patients with vasculogenic erectile dysfunction show a diminished clinical response to sildenafil citrate compared to patients with a non-vascular aetiology (Rosen and Kostis, 2003). A possible limitation of sildenafil citrate is that its effects rely on endogenous NO formation (drive), which in turn activates guanylate cyclase which generates cyclic GMP (Jeremy et al., 1997). Sildenafil effectively acts by amplifying intravascular levels of cGMP by preventing its hydrolysis (Jeremy et al., 1997), but does not itself augment the formation of NO. However, the greater the degree of depression of NO formation, the less effective sildenafil may be, therapeutically. It has therefore been suggested that the administration of an NO donor with sildenafil may compensate for reduced 'NO drive' in vasculopathic states (Jeremy et al., 1997, 2000; Seidler et al., 2002; Kalsi et al., 2004). In this context, sildenafil nitrate (NCX 911) is a novel NO-releasing PDE5 inhibitor that has been shown to be at least as potent as sildenafil citrate in relaxing isolated human corpus cavernosum and more potent in promoting cGMP formation (Seidler et al., 2002; Kalsi et al., 2004). However, the effects of NCX 911 in cavernosal tissue from patients or animals with endothelial dysfunction or risk factors thereof have not been reported. The second objective, therefore, was to compare the effects of NCX 911 with sildenafil citrate on cavernosal relaxation from hypercholesterolaemic rabbits using an organ bath. Since we have recently demonstrated that NO–cGMP axis inhibits the formation of superoxide through inhibition of NADPH oxidase activity and expression (Muzaffar et al., 2004), the effect of NCX 911 on superoxide formation by cavernosal tissue was also studied. Since NO is released from NCX 911, comparative effects of the drugs and a guanylyl cyclase inhibitor, 1H-{1,2,4}oxadiazolo{4,3-*a*}quinoxalin-1-one (ODQ), on cavernosal GMP formation were also studied.

2. Methods

2.1. Drugs

The following drugs were purchased from Sigma Chemical Co (Poole, Dorset, UK): allopurinol, apocynin, carbachol, catalase, diphenyleneiodonium chloride, L-nitroarginine methyl ester (L-NAME), phenylephrine, sodium nitroprusside, 1H-{1,2,4}oxadiazolo{4,3-*a*}quinoxalin-1-one (ODQ), isobutylmethylxanthine, copper–zinc superoxide dismutase (SOD). Sildenafil (5-[2-ethoxy-5-(4-methylpiperazin-1-ylsulfonyl)phenyl]-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-*d*]pyrimidin-7-one) citrate and nitrate (NCX 911) were kind gifts of Nicox SA, Nice, France.

2.2. Rabbit model

Adult New Zealand White male rabbits (3 kg) were fed a 1% cholesterol diet (Kim et al., 1997), while control animals were fed standard chow. Hypercholesterolaemia was confirmed in the cholesterol fed rabbits by serum total cholesterol after 8 weeks: mean total cholesterol was 42.8±4 mM; $n=16$ for cholesterol-fed rabbits and 5.52±0.3 mM; $n=16$ for controls.

2.3. Organ bath experiments

After 8 weeks, rabbits were killed by intravenous injection of pentobarbitone (Euthatal 100 mg/kg) via the lateral ear vein (Jones et al., 2005). The penis was removed and corpus cavernosum dissected from the tunica vaginalis, placed in cold Dulbecco's Modified Eagle Medium (DMEM, GIBCO BRL Life Technologies Ltd., Paisley, Scotland, UK), and used within 6 h. Strips of tissue (8 by 2 mm) were mounted in organ baths for isometric tension studies. The size and weight of the cavernosal tissues were the same in both the control and cholesterol-fed rabbits (Jones et al., 2005). The strips were mounted vertically in 20 ml chambers, equipped with two parallel platinum electrodes, containing Kreb's Ringer bicarbonate buffer with the following composition (millimole per liter in distilled water): 119NaCl, 4.7KCl, 1.17$MgSO_4 \cdot 7H_2O$, 1.18KH_2PO_4, 2.5$NaHCO_3$, 2.5$CaCl_2$, 5 glucose, maintained at 37 °C by a thermoregulated circuit. Tissues were suspended between 2 tissue bearers, one in fixed position and the other attached to a force-displacement transducer, and data recorded on disc (MacLab®) (Jones et al., 2005). The Kreb's Ringer bicarbonate buffer was gassed with a mixture of 95% O_2/5% CO_2 maintained at pH 7.4. An initial tension of 2 g was applied to the suspended tissue strips. All strips were equilibrated for 1 h with frequent changes of Kreb's Ringer bicarbonate buffer. Following equilibration, tissues were pre-contracted with phenylephrine (100 μM) and then relaxed with cumulative doses of the acetylcholine analogue, carbachol (0.01–

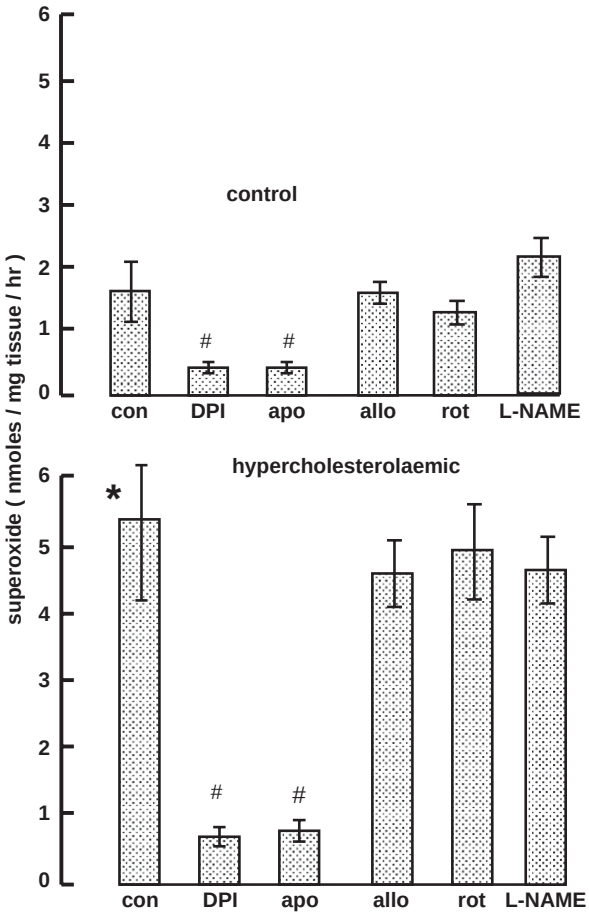

Fig. 1. Superoxide formation by isolated corpus cavernosum from hypercholesterolaemic and control rabbits and effect of: apocynin [Apo] and diphenyleneiodonium chloride [DPI] (NADPH oxidase inhibitors), allopurinol [allo] (xanthine oxidase inhibitor), rotenone (inhibitor of mitochondrial electron transport chain) and L-NAME (NOS inhibitor). $^{*}p<0.001$ comparing control to hypercholesterolaemic (without inhibitors) and $^{\#}p<0.01$ comparing control to effect of inhibitors. Each point=mean± S.E.M., $n=6$ animals.

10 μM) or the endothelium-independent vasodilator, sodium nitroprusside (SNP; 0.01–10 μM) (Jones et al., 2005). Relaxation responses were expressed as percentage inhibition of phenylepherine-induced contraction.

To investigate the involvement of NADPH oxidase in mediating the impairment of endothelium-dependent cavernosal relaxation, tissues were incubated for 30 min with 10 μM diphyleneiodonium chloride or 10 μM apocynin (inhibitors of NADPH oxidase) or 100 μM allopurinol (an inhibitor of xanthine oxidase). At these concentrations we have established that these inhibitors all inhibit superoxide formation (Muzaffar et al., 2005). Relaxation with carbachol was then repeated as described above.

For the evaluation of direct effects of NCX 911 and sildenafil citrate, cavernosal strips from cholesterol fed rabbits were precontracted with phenylephrine then exposed to cumulative increasing doses of NCX 911 or sildenafil citrate (0.1–100 μM). In other experiments, cavernosal strips from HCh rabbits were incubated for 30 min with NCX 911 or sildenafil citrate prior to contraction and relaxation by acetylcholine. Eight cavernosal strips were used for each arm of the study for organ bath experiments.

2.4. Superoxide formation

The measurement of superoxide release by cavernosal segments was performed by detection of ferricytochrome *c* reduction (Muzaffar et al., 2003). Cavernosal segments of approximately 50 mg were equilibrated in DMEM without phenol red for 10 min at 37 °C in a 95% air–5% CO_2 incubator (Heraeus, Hera Cell, Kandro Laboratory Products, Germany). Twenty micromolar cytochrome *c* (Sigma Chemical Co.) with or without 500 U/ml copper–zinc superoxide dismutase (SOD; Sigma Chemical Co.) was added to the segments and incubated at 37 °C in a 95% air–5% CO_2 incubator for 1 h. The final volume of the reaction mixture was 0.5 ml per well. The reaction medium was then removed and optical density measured by spectrophotometry at 550 nm and converted to nanomoles of cytochrome *c* reduced superoxide using the molar extinction $\Delta E_{550\ nm}=21.1\ mM^{-1}\ cm^{-1}$. The reduction of cytochrome *c* that was inhibitable with SOD reflected actual superoxide release. Segments were blotted, dried and weighed, data being expressed as nanomoles of superoxide per milligram tissue per hour.

2.5. Cyclic GMP formation

In order to determine whether NCX 911 promotes cyclic GMP formation, as one would expect if it is an NO donor, cyclic GMP was measured using enzyme-linked immunoassay (R&D Systems, UK) following incubation of cavernosal tissue with drugs. Segments of cavernosal tissue (50 mg) were incubated with sildenafil citrate or NCX 911 (± the guanylyl

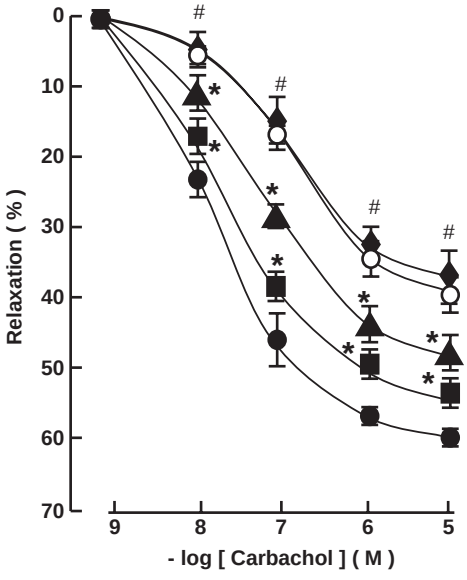

Fig. 2. Concentration–response curves for carbachol-mediated relaxation of rabbit corpus cavernosum: controls (●); hypercholesterolaemic (♦); hypercholesterolaemic+100 μM allopurinol (○); hypercholesterolaemic+10 μM apocynin (▲); hypercholesterolaemic+10 μM diphenyleneiodonium chloride (■). Each point=mean±S.E.M., $n=6$ animals $^{*}p<0.001$ controls vs. hypercholesterolaemic. $^{\#}p<0.001$ hypercholesterolaemic vs. hypercholesterolaemic+drug.

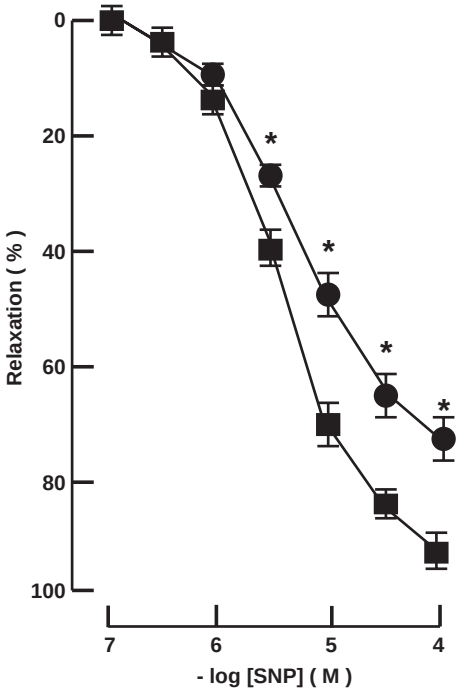

Fig. 3. Concentration–response curves for sodium nitroprusside-stimulated relaxation of rabbit corpus cavernosum: controls (●); hypercholesterolaemic (■); Each point=mean±S.E.M., $n=6$ animals; *$p<0.001$ when comparing controls to hypercholesterolaemics.

cyclase inhibitor, 1H-{1,2,4}oxadiazolo{4,3-*a*}quinoxalin-1-one [ODQ]) for 1 h. All incubations were carried out in the presence of the broad spectrum PDE-inhibitor, isobutylmethylxanthine (250 μM), to inhibit the hydrolysis of cGMP (Jeremy et al., 1997). After incubation, media was removed and 200 μl of 0.1 N HCl was added to the cells and incubated for 20 min

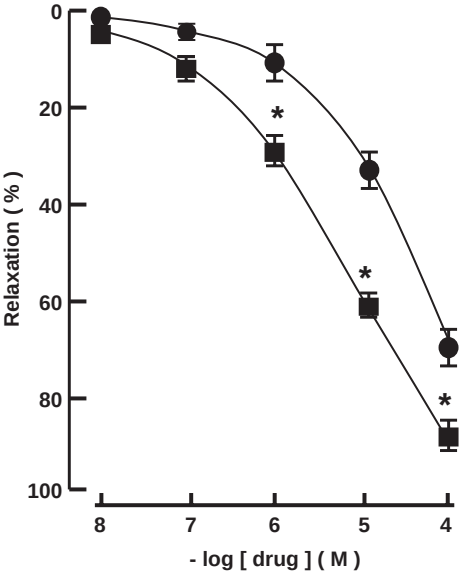

Fig. 4. Direct effect of sildenafil citrate (●) and NCX 911 (■) on relaxation of the corpus cavernosum (precontracted with phenylephrine) from hypercholesterolaemic rabbits. Each point=mean±S.E.M., $n=6$ animals; *$p<0.001$ when comparing effect of NCX 911 with that of sildenafil citrate.

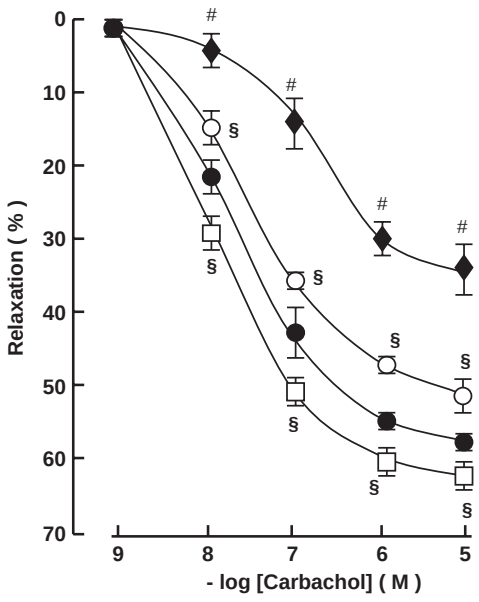

Fig. 5. Effect of 1 μM sildenafil citrate (○) and 1 μM NCX 911 (□) on carbachol-stimulated relaxation of the corpus cavernosum from hypercholesterolaemic rabbits compared to untreated controls (●) and untreated tissues from hypercholesterolaemic rabbits (♦). Each point=mean±S.E.M., $n=6$ animals; $^{\#}p<0.001$ when comparing response of sildenafil citrate with that of NCX 911 and $^{\S}p<0.001$ when comparing response of sildenafil citrate with that of NCX 911.

to extract cGMP. cGMP concentrations were then measured according to manufacturer's instructions. The protein content was determined by the BCA-protein assay kit (R&D Systems, UK) and cGMP levels expressed as femtomole per milligram tissue per hour.

2.6. Data analysis and statistics

All measurements represent the mean of means. Quadruplicate measurements were made for each parameter per animal and the mean calculated. In turn, for each parameter, measurements were made in tissue from 12 rabbits (6 controls and 6

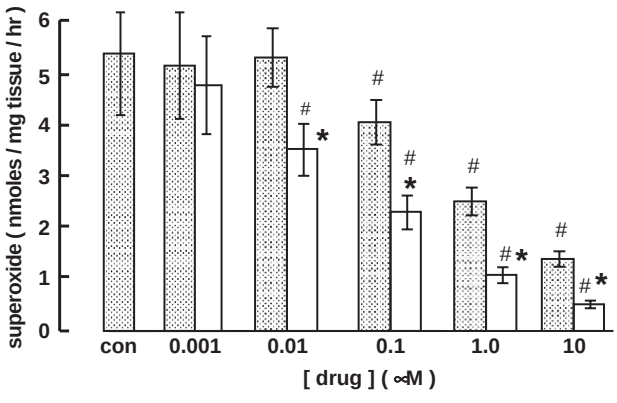

Fig. 6. Effect of sildenafil citrate (shaded bars) and NCX 911 (open bars) on superoxide formation by the corpus cavernosum from hypercholesterolaemic rabbits. Each point=mean±S.E.M., $n=6$ animals; $^{\#}p<0.001$ when comparing effect drugs with zero values *$p<0.001$ when comparing response of sildenafil citrate with that of NCX 911 at each drug concentration.

hypercholesterolaemics). Where applicable, the dose–response data for sildenafil citrate and NCX 911 were best fit by nonlinear regression (sigmoidal dose response, variable slope) to the following equation: $Y=\text{bottom}+(\text{top}-\text{bottom})/(1+10\text{^}((\log ED_{50}-X)*H))$, where ED_{50} is the concentration of sildenafil citrate or NCX 911 resulting in 50% inhibition, X is the log of sildenafil citrate or NCX 911 concentration, Y is the response and H is the Hill coefficient. The difference between the NCX 911 and sildenafil ED_{50} values with 95% confidence intervals was analysed using the F-test ($P<0.05$). Data analysis was done using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego, CA, USA). Other statistical analyses were carried out using one-way analysis of variance (ANOVA) followed by Bonferroni's t test. Differences among means were considered significant at $P<0.05$. Data were analysed using Graphpad (San Diego, Ca, USA).

3. Results

Hypercholesterolaemia elicited a statistically significant increase in superoxide formation by rabbit cavernosal tissue compared with controls (Fig. 1). Superoxide formation was inhibited by diphenyleneiodonium chloride (10 μM) and apocynin (10 μM), but not allupurinol (100 μM), rotenone (10 μM), or L-nitroarginine methyl ester (L-NAME; 500 μM) by cavernosal tissue from hypercholesterolaemic rabbits (Fig. 1).

Hypercholesterolaemia elicited a marked attenuation of cavernosal relaxation in response to carbachol, consistent with impaired cavernosal NO-dependent relaxation (Fig. 2). Diphenyleneiodonium chloride (10 μM) and apocynin (10 μM), but not allupurinol (100 μM), significantly reversed the inhibitory effect of hypercholesterolaemia on relaxation of cavernosum in response to carbachol (Fig. 2).

In response to the NO donor, sodium nitroprusside, hypercholesterolaemia also caused a statistically significant attenuation of cavernosal relaxation (Fig. 3). Neither diphenyleneiodonium chloride nor apocynin, at concentrations that reversed carbachol-stimulated relaxation, had any effect on relaxation elicited with nitrosprusside, however (data not shown).

In pre-contracted cavernosal strips from hypercholesterolaemic rabbits, both sildenafil citrate and NCX 911 induced relaxation in a concentration-dependent manner (Fig. 4). NCX 911 was significantly ($p<0.001$) more potent (ED_{50}; 7.24 μM) than sildenafil citrate (ED_{50}; 53 μM) in inducing relaxation (Fig. 4). Both 1 μM sildenafil citrate and 1 μM NCX 911 significantly reversed impaired carbachol-stimulated relaxation by cavernosal tissue from hypercholesterolaemic rabbits, NCX 911 being more potent than sildenafil citrate (Fig. 5). Both sildenafil citrate and NCX 911 also inhibited superoxide formation, NCX 911 being significantly ($p<0.001$) more potent (ED_{50}; 0.09 μM) than sildenafil citrate (ED_{50}; 0.98 μM) (Fig. 6). NCX 911 stimulated the formation of cGMP in a dose-dependent manner, an effect blocked by ODQ (Fig. 7).

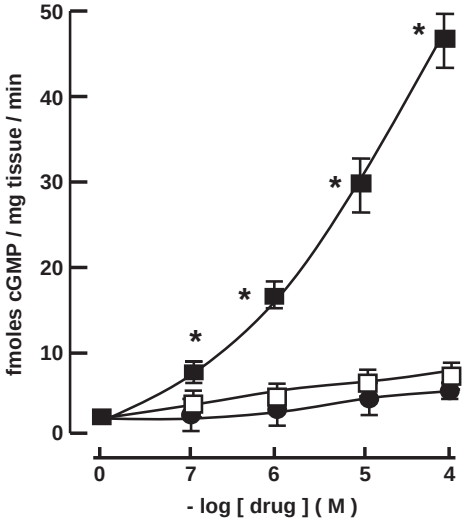

Fig. 7. Effect of sildenafil citrate (●) and NCX 911 (■) and NCX 911+ODQ (□) on cyclic GMP formation by the corpus cavernosum from hypercholesterolaemic rabbits. Each point=mean±S.E.M., $n=6$ animals; *$p<0.001$ when comparing response of sildenafil citrate with that of NCX 911.

4. Discussion

The present study firstly confirms previous reports that hypercholesterolaemia leads to an impairment of endothelium-dependent (i.e. carbachol-stimulated) relaxation and an increase in superoxide formation in isolated cavernosal tissue. Nitroprusside-stimulated relaxation was also reduced indicating that hypercholesterolaemia also promotes an impairment of cyclic GMP formation or subsequent signal transduction systems, possibly protein kinase G. Cavernosal relaxation to exogenous NO donors has been reported previously to be significantly impaired in hypercholesterolaemic rabbits (Kim et al., 1997).

Superoxide formation by cavernosal tissue from hypercholesterolaemic rabbits was three-fold greater than controls. Since this increase was inhibitable with diphenylene iodonium and apocynin these data indicate that this superoxide formation is mediated by an increase in endogenous NADPH oxidase expression or activity. These results are consistent with a previous report that superoxide formation is enhanced in aortae from hypercholesterolaemic rabbits was also inhibitable with diphenyleneiodonium chloride (Itoh et al., 2002). Increased superoxide formation is associated with impaired relaxation since the free radical reacts with NO to form reactive nitrogen species, effectively lowering NO bioavailability and therefore reducing cavernosal relaxation and erection (Jones et al., 2002). In the present study, diphenyleneiodonium chloride and apocynin reversed the inhibitory impact of hypercholesterolaemia on cavernosal relaxation. It is reasonable to conclude, therefore, that superoxide derived from NADPH oxidase plays a central role in mediating erectile dysfunction in the hypercholesterolaemic rabbit.

One point of interest is that we did not achieve complete reversal with these NADPH oxidase inhibitors. As noted above, the response to sodium nitrosprusside was also impaired indicating a defect down stream of NO formation,

namely the guanylyl cyclase–protein kinase G axis. Apocynin and diphenylene iodonium had no effect on impaired nitroprusside-stimulated relaxation indicating that superoxide derived from NADPH oxidase has no direct effect on this pathway. It is also possible that hypercholesterolaemia may augment the expression of PDE5 itself and as such warrants further study.

In the present study, sildenafil citrate inhibited the formation of superoxide and reversed the inhibitory effect of hypercholesterolaemia on carabachol-stimulated relaxation of cavernosal strips at 1 μM. Sildenafil citrate also had a direct relaxing effect on corpus cavernosum from hypercholesterolaemic rabbits, albeit at concentrations greater than those required to inhibit PDE5 (K_D approximately 5.4. nM; Chuang et al., 1998). However, circulating levels of sildenafil following oral administration of the drug in man are in the range of approximately 0.5–10 μM (Jetter et al., 2002; Rosen and Kostis, 2003), indicating that the effects we observed on superoxide formation with an ED_{50} of 0.98 μM may be of therapeutic relevance. While it was initially reported that sildenafil citrate induces cavernosal relaxation only in the presence of exogenous or stimulated release of NO (Ballard et al., 1998), sildenafil citrate has been shown to have a direct relaxant effect on cavernosal tone at concentrations of 100 nM or greater (McAuley et al., 2001). This effect was independent of the NO–cGMP pathway, as the effect was not inhibitable with L-NAME (a nitric oxide synthase inhibitor) and only partially prevented by oxadiazolo quinoxaline, a guanylyl cyclase inhibitor (McAuley et al., 2001). It was concluded that a higher tissue concentration of sildenafil relaxes cavernosal smooth muscle through a hitherto undefined mechanism. It is suggested from our study that this mechanism may entail, in part, the supression of superoxide formation.

In the present study, the NO donating version of sildenafil citrate, NCX 911, elicited a more potent effect on cavernosal tissue from hypercholesterolaemic rabbits. The enhanced relaxation with NCX 911 may reflect the NO donating capacity of this drug in addition to amplification of the effects of the NO–cGMP axis through inhibition of PDE5 (Seidler et al., 2002). Indeed, in the present study we demonstrated that NCX 911 stimulates the formation of cyclic GMP to a greater degree than sildenafil citrate. This effect was reversed by oxadiazoloquinoxaline, a guanylyl cyclase inhibitor, demonstrating the effect was mediated by guanylyl cyclase and that NCX 911 releases NO. It is reasonable to conclude, therefore, that NCX 911 promotes relaxation of corpus cavernosum from hypercholesterolaemic rabbits through a dualistic effect of PDE5 inhibition and donation of NO. However, NCX 911 also inhibited the formation of superoxide at an ED_{50} of 0.09 μM by cavernosal tissue from hypercholesterolaemic rabbits, indicating that NCX 911 exerts an inhibitory effect on NADPH oxidase directly, as well as effects on the NO–cGMP axis. In support of this proposal, it has been shown that other NO donors inhibit the formation of superoxide and inhibit NADPH oxidase activity in isolated pulmonary artery endothelial cells (Muzaffar et al., 2004). It is also possible that the NO derived from NCX 911 reacts with superoxide to form reactive nitrogen species, effectively "quenching" and removing superoxide from the system by direct chemical reaction. However, such a reaction may actually prove to be deleterious since reactive nitrogen species such as peroxynitrite are toxic to tissues (Jeremy et al., 2004).

These data also indicate that NCX 911 may prove effective in patients who do not respond to sildenafil citrate therapy. However, the concomitant use of sildenafil and NO donors are associated with hypotension (Webb et al., 2000) and fatal cardiac events (Morales et al., 1998). For this reason, the current or recent use of NO donors remains the only contraindication to sildenafil prescription in current practice (Cheitlin et al., 1999). However, NCX 911 appears to be a relatively weak NO donor and effects on blood pressure may therefore be minimal. The influence of NCX 911 on blood pressure in humans is currently unknown. Furthermore, recent data indicate that sildenafil may not elicit as much cardiopathic side effects as has been purported (Corbin et al., 2003).

It is also of interest to note that sildenafil citrate also reversed the inhibitory effect of diabetes mellitus on impaired relaxation of the corpus cavernosum, again in a rabbit model (Thompson et al., 2001). Diabetes mellitus is another major risk factor for erectile dysfunction (Sullivan et al., 1999) and is also associated with increased vascular NADPH oxidase activity and increased superoxide formation (Jones et al., 2002). It is reasonable to suggest, therefore, that a similar oxidative aetiology is involved in mediating both diabetic and hypercholesterolaemic erectile dysfunction and that NCX 911 may be therapeutically active through similar mechanisms in both conditions.

To summarise, hypercholesterolaemia in rabbits augments the formation of superoxide in cavernosal tissue, principally through an increase in NADPH oxidase activity. In turn, superoxide generated by NADPH oxidase impairs NO-mediated relaxation. Hypercholesterolaemia also appears to significantly impair relaxation (i.e. at the cyclic GMP–PKG axis level) through a superoxide-independent mechanism. Sildenafil citrate normalises cavernosal relaxation from hypercholesterolaemic rabbits, in part through suppression of NADPH oxidase activity. NCX 911 was even more potent, exerting an acute stimulatory effect on both guanyl cyclase, inhibition of PDE5 and inhibition of superoxide formation by upregulated NADPH oxidase. The concentrations at which these drugs elicit these effects are within the known therapeutic range of circulating sildenafil citrate after ingestion, suggesting that these novel mechanisms, at least in part, may contribute to the therapeutic impact of the drugs in treating erectile dysfunction. By virtue of its capacity to donate NO, NCX 911 may be useful in treating erectile dysfunction in

patients who do not respond to sildenafil citrate due to an excessive reduction of NO drive elicited by excess superoxide formation.

Acknowledgment

This study was funded by a grant from the Ralph Shackman Trust, UK.

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