British Journal of Pharmacology (1998) 125, 1677–1684
http://www.stockton-press.co.uk/bjp

Relaxation of corpus cavernosum and raised intracavernous pressure by berberine in rabbit

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1 In the present study, we have investigated the effect of berberine in rabbit isolated corpus cavernosum and measured the intracavernous pressure (ICP) change after intracavernosal injection of berberine in rabbit.

2 Berberine alone suppressed the basal tone and induced a concentration (0.1–100 μM)-dependent relaxation in phenylephrine (PE)-precontracted corpus cavernosum.

3 Tetrodotoxin (0.1 and 1 μM) treatment had no significant effect on the berberine-induced relaxation. Phentolamine (1 and 10 μM), propranolol (1 and 3 μM) and atropine (1 and 3 μM) were also without effect. These results suggest that berberine might cause relaxation of the cavernosal strip by direct action on the corpus cavernosum, not by a neuronal effect. Furthermore, muscarinic- and β-adrenoceptors were not involved.

4 Berberine-induced relaxations were significantly reduced by endothelium removal and by exposure to L-N^G-nitro arginine methyl ester (0.1 and 0.3 mM), but not indomethacin (30 μM).

5 In endothelium-deprived corpus cavernosal tissues, berberine-induced relaxations were significantly reduced in high K^+ medium (KCl = 60 mM), by charybdotoxin (ChTX) and 4-aminopyridine (4-AP) but not by glibenclamide and apamin.

6 After intracavernous injection of berberine (1, 2, 3 and 5 mg kg^{-1}), the ICP rose from 12.7 ± 3.6 to 13.2 ± 5.4, 25.3 ± 6.1, 46.5 ± 8.2, and 63.4 ± 10.2 mmHg, respectively. The duration of tumescence ranged from 11.5–43.7 min.

7 The results show that berberine possesses a relaxant effect on rabbit corpus cavernosal tissues which is attributable to both endothelium-dependent and-independent properties. While the former component is apparently due to the release of NO from sinusoidal endothelium, the endothelium-independent mechanism involved in berberine relaxation is probably linked to ChTX- and 4-AP-sensitive K^+ channel activation in the cavernosal vasculature.

Keywords: Berberine; rabbit corpus cavernosum; nitric oxide; K^+ channels; intracavernous pressure

Abbreviations: 4-AP, 4-aminopyridine; ChTX, charbdotoxin; ICP, intracavernous pressure; Kca, Ca^{2+}-activated K^+ channel; L-NAME, L-N^G-nitro arginine methyl ester; PE, phenylephrine; SNP, sodium nitroprusside

Introduction

For erection to take place, the penile arteries and sinusoids have to dilate, thereby increasing the blood flow into the penis. There is increasing evidence that release of L-arginine-derived nitric oxide (NO) from nonadrenergic-noncholinergic (NANC) nerves and from the sinusoidal endothelium, is a major event in penile smooth muscle relaxation (Holmquist *et al*., 1991; Kim *et al*., 1991; Rajfer *et al*., 1992). Since the drugs most often used, papaverine, phentolamine, and prostaglandin (PG)E_1, are associated with various side effects such as priapism, local fibrosis and pain (Junemann & Alken, 1989), there has been an increasing interest in finding effective and safe alternatives. K^+ channel openers and drugs acting by liberation of NO have been shown to effectively relax human isolated corpus cavernosum (Kim *et al*., 1991; Giraldi & Wagner, 1990; Holmquist *et al*., 1990a,b; Hedlund *et al*., 1994) and produce erection when injected intracorporeally into animals and man. Therapeutically, there are several drug types that, through enhancing the NO-cyclic GMP axis of cyclic AMP signal transduction, may prove beneficial in treating erectile dysfunction (Jeremy *et al*., 1997). One such class of drugs is the phosphodiesterase (PDE) inhibitors that prevent the hydrolysis of cyclic GMP and/or cyclic AMP, thereby evaluating levels of these cyclic nucleotides. Sildenafil (Viagra), an inhibitor of cyclic GMP-specific PDE, is currently undergoing evaluation as an oral therapy for the treatment of male erectile dysfunction (Moreland *et al*., 1998; Goldstein *et al*., 1998). Sildenafil also enhances NO-mediated relaxation of rabbit corpus cavernosum *in vitro* (Ballard *et al*., 1996; Tang *et al*., 1996).

One potential source for novel impotence therapy is the diverse area of natural products. Berberine is a benzodiozoloquinolizine alkaloid widely distributed in nature, particularly in the various species of berberis plants (such as *Berberis aristata* and *Berberis vulgaris*) and *Hydrastis canadensis* (Jan, 1941). In a previous study, it was found that berberine induces a potent relaxing effect on rat isolated mesenteric arteries (Chiou *et al*., 1991). Thus, we speculated that berberine might also have a relaxing effect on corpus cavernosal smooth muscle.

Before any drug is used in the treatment of erectile dysfunction, it is desirable to have information on its effects on penile erectile tissue. Many experimental animals have been employed in *in vivo* studies of penile erection, such as dogs, monkeys, cats, rats and rabbits (Chen *et al*., 1992; Domer *et al*., 1978; Lin *et al*., 1985; Lue *et al*., 1983; Stacle *et al*., 1988). Lin & Lin (1996) reported that the effects of vasoactive drugs

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on the rabbit corpus cavernosum are similar to those in humans; thus the rabbit model is a suitable alternative for further physiological and pharmacological studies of penile erection.

In this study, we have characterized the effect of berberine on rabbit corpus cavernosum, and investigated its mechanism(s) of action, using organ bath experiments and measuring the effect on intracavernous pressure (ICP) in anaesthetized rabbits.

Methods

Tissue procurement

Male New Zealand White rabbits (2–3 kg) were anaesthetized with sodium pentobarbital and exsanguinated. Rabbit penises were surgically removed *en bloc*, with care being taken to keep the tunica albuginea intact. The corpus spongiosum and urethra were excised. The corpus cavernosum tissue was carefully dissected free from the surrounding tunica albuginea and mounted in organ baths (see below).

Disruption of endothelium

The endothelium lining the lacunar spaces of rabbit corpus cavernosum was disrupted and/or removed by detergent treatment using a modification of a protocol for blood vessels, described elsewhere (Tesfamariam *et al*., 1985; Kim *et al*., 1991). The intact, isolated penis was placed in a tray containing chilled physiological salt solution (PSS). A 21-gauge minicatheter was inserted into each (left and right) corporal body at the proximal end of the crus of the penis. A third minicatheter was inserted into the distal end, below the glans penis, where the right and left corpora communicate. While the distal and one proximal minicatheter were clamped, 3 ml of 0.3% CHAPS (wt/vol.) in a solution of normal saline was infused into the remaining proximal catheter. Leakage of the CHAPS solution through venous drainage was minimal. After a short interval ($\sim$20 s), the clamped minicatheters were opened and the preparation was extensively washed by infusion of PSS. This procedure was repeated for the proximal catheter on the opposite side. The corpora cavernosa were then removed and tested for endothelial integrity. Of the tissues treated with CHAPS, 73% did not relax or relaxed poorly ($<$10% of maximal relaxation) to acetylcholine (ACh, 1 μM) and were considered to be functionally denuded of endothelium. These tissues were used for this study.

Organ bath experiments

Strips of rabbit corpus cavernosum were mounted with surgical suture to a fixed metal loop from below and a metal wire from above, connected to a force transducer (Model FT03; Grass Instruments, Quincy, MA, U.S.A.). The preparation was then immersed in 12-ml baths maintained at 37°C and containing Krebs' solution (aerated with 5% CO_2, 95% O_2 to attain pH 7.4). As previously described (Saenz de Tejada *et al*., 1989), optimal isometric tension was achieved by gradual, incremental stretching. The tissue was periodically tested by contracting with 3 μM phenylephrine (PE). Tissues were considered to have reached optimal isometric tension when two successive contractions were within 10% of each other. After this determination, tissues were relaxed maximally with 10 μM sodium nitroprusside (SNP) to determine baseline. After thorough washout, the tissues were contracted with either PE or 60 mM KCl and subjected to cumulative additions of acetylcholine (1 nM–10 μM), SNP (10 nM–0.1 mM) or berberine (0.1–100 μM). Tetrodotoxin, atropine and propranolol were added 20 min prior to PE contraction. In some experiments, NO or PGI_2 synthesis in intact corpus cavernosum tissues was inhibited by addition of the NO synthase inhibitor L-N^G-nitro arginine methyl ester (L-NAME) or cyclo-oxygenase inhibitor indomethacin (Palmer *et al*., 1988; Azadzoi *et al*., 1992) before the addition of vasoactive agents and then throughout the rest of the experiment. Responses to berberine were also examined in the absence or presence of various K^+ channel blockers added 45 min before the contraction with PE. The relaxant responses were calculated as per cent relaxations of active muscle tone induced by PE or KCl (running from 0–100%).

Animal preparation for recording intracavernous pressure

Male New Zealand White rabbits weighing 2–3 kg were used for the investigation. After sedation with an intramuscular injection of ketamine 10 mg kg^{-1}, the rabbits were anaesthetized with intraperitoneal pentobarbital sodium (30 mg kg^{-1}). Anaesthesia was maintained with 10 mg kg^{-1} as needed. The animals breathed spontaneously. The rabbits were then placed in the supine position, and the body temperature was maintained at 37°C using a heating pad and lamp. The femoral artery on one side was cannulated for continuous monitoring of systemic arterial pressure (SAP), mean systemic arterial pressure (MSAP) and heart rate (HR) *via* a Gould 23 ID pressure transducer on Gould RS3400 polygraph. Under sterile conditions, the skin overlying the penis was incised and the corpora cavernosum was exposed at the root of the penis. A 25-gauge needle was inserted into the corpus cavernosum for pressure recording (Gould Polygraph RS3400). The needle was connected to a three-way stopcock, thus permitting the intracavernous injection of the drugs. The tube was filled with heparinized saline (50 IU/2–3 h) to prevent clotting.

Intracavernous injection of vasocative drugs and normal saline

In eight rabbits, increasing concentrations of berberine (1, 2, 3 and 5 mg kg^{-1}) were injected intracavernously in a volume of less than 0.15 ml. Normal saline in increasing volumes (0.06, 0.09 and 0.15 ml) was injected in four rabbits as a control group. The effects of berberine and normal saline on the intracavernous pressure (ICP) and on the duration of action were evaluated. In order to minimize the effect of the previous drug, the cavernous body was flushed with 0.15 ml normal saline before each injection and the time interval between each injection was at least 1 h.

Drugs and solutions

Acetylcholine hydrochloride, berberine chloride, atropine sulphate, propranolol, phentolamine, indomethacin, L-N^G-nitro arginine methyl ester (L-NAME), phenylephrine hydrochloride, KCl, sodium nitroprusside (SNP), tetrodotoxin (TTX) and glibenclamide were obtained from Sigma Chemical Company (St. Louis, MO, U.S.A.). Apamin, 4-aminopyridine (4-AP) and charybdotoxin (ChTX) were purchased from Research Biochemicals International (Natick, MA, U.S.A.). Phenylephrine HCl was dissolved in 0.9% saline containing 0.1% ascorbic acid and stored frozen at -20°C. On the day of

the experiments, final dilutions of PE were made with Krebs' solution. Indomethacin, ChTX and glibenclamide were first dissolved in 0.1% $NaHCO_3$, ethanol and DMSO, respectively, and then further diluted with water. Unless stated otherwise, drugs were prepared by using triple-distilled water. Stock solutions were prepared and stored at $-20^{\circ}C$ and further fresh dilutions were prepared daily. Stock solution (100 mM) of berberine chloride was dissolved in warm triple-distilled water and further diluted. The amount of vehicle added did not produce significant effects on the responsiveness of the tissues to drugs. The Krebs' solution used had the following composition (mM): NaCl 119, KCl 4.7, $CaCl_2$ 1.5, $MgCl_2$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 24, and glucose 11 (pH 7.3–7.4). When a high potassium solution (60 mM) was used, an equimolar reduction in NaCl was performed.

Analysis of data

The relaxations induced by each concentration of ACh, SNP and berberine chloride were expressed as a percentage of active muscle tone induced by PE (3 μM) running from 0–100% and used in the construction of the concentration-response curves. The EC_{50} (the molar concentration required to cause half-maximal relaxation) was determined by linear interpolation for each concentration-response curve.

The results are expressed as means ± s.e.mean. *N* designates the number of rabbits and *n* the number of corpus cavernosal strips examined in each experiment. Student's *t*-test (two-tailed) for paired or unpaired observations where appropriate. A value of $P<0.05$ was considered to be statistically significant.

Results

Responses to berberine

Berberine alone produced a concentration (1–30 μM)-dependent inhibition of the stretch-evoked passive tension in rabbit corpus cavernosum in the absence of constrictors (Figure 1a). When corporeal smooth muscle strips were pre-contracted by phenylephrine (PE), berberine applied to the plateau phase caused a concentration-dependent relaxation (Figures 1b and 2). The induction of relaxation was immediate upon application of berberine. To investigate whether the relaxant response of the cavernosal smooth muscle to berberine is due to direct action of berberine or due to the neuronal effect on the cavernosal smooth muscle, tetrodotoxin (0.1 and 1 μM), phentolamine (1 and 10 μM), propranolol (1 and 30 μM) and atropine (1 and 30 μM) were employed. When pretreated with tetrodotoxin to block neuronal transmission, relaxation was still induced by berberine (Figure 2). Propranolol and atropine treatment also failed to affect the berberine-induced relaxation (Table 1).

Role of endothelium on berberine-induced relaxation

To evaluate endothelium-dependency, isolated corpus cavernosal strips with and without endothelium were contracted with PE to similar magnitude and then exposed to increasing concentrations of berberine (0.1–100 μM). The amplitude of the contractile response to PE was not significantly changed by previous endothelium deprivation. However, the relaxant effect of berberine was reduced significantly in endothelium-deprived preparations (Figure 3), thus implicating that a partially endothelium-dependent mechanism was contributing to its effect. Consequently, L-NAME and indomethacin were employed in the following experiment to examine the possible involvement of NO and PGI_2. In endothelium-intact cavernosal tissue, L-NAME (0.1 and 0.3 mM) induced an increase in basal tension. The contraction to PE was also increased in the presence of L-NAME and the concentration of PE was reduced to 0.5 μM to obtain contractions comparable to those in

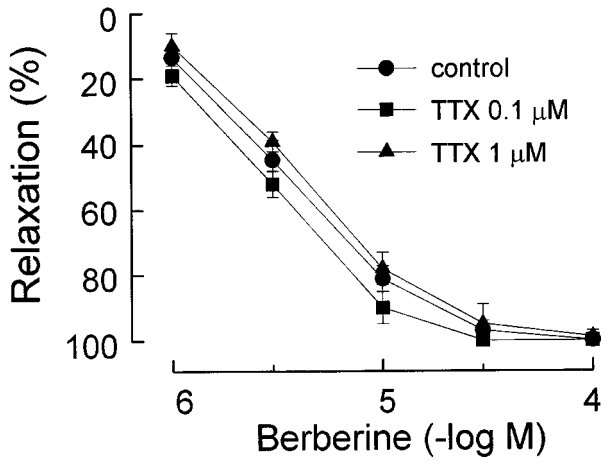

Figure 2 Relaxation effects of berberine on phenylephrine-precontracted endothelium-intact rabbit corpus cavernosum in the absence (control) and presence of TTX (0.1 and 1 μM). Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means ± s.e.mean ($n=6–9$).

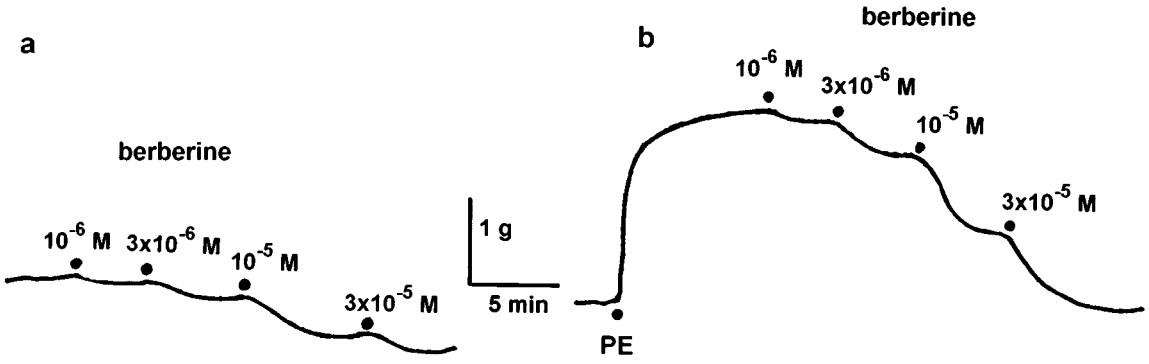

Figure 1 Relaxation effects of berberine (1–30 μM) against (a) stretch-evoked passive tension and (b) phenylephrine (PE, 1 μM)-evoked contraction in endothelium-intact rabbit corpus cavernosum.

Table 1 Effects of phentolamine, propranolol, and atropine treatment on berberine-induced relaxation in rabbit corpus cavernosum

	EC_{50} (μM)	E_{max} (%)
Control	3.26±0.17	100
Phentolamine (10 μM)	2.84±0.26	100
Propranolol (3 μM)	3.52±0.23	100
Atropine (3 μM)	3.45±0.19	100

Results are expressed as means±s.e.mean (n=6–9).

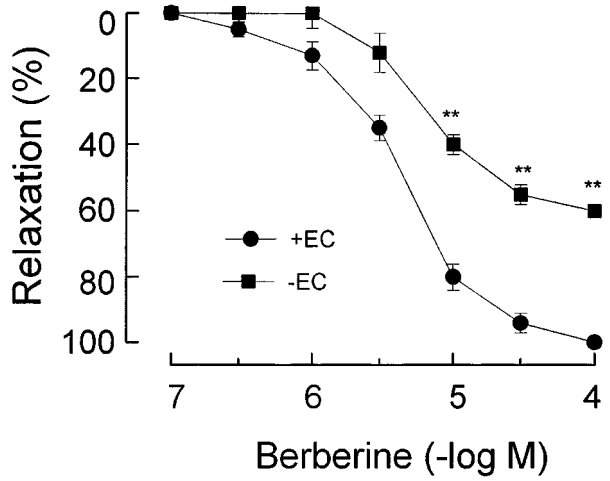

Figure 3 Relaxation effects of berberine in endothelium-intact (+EC) and -denuded (−EC) rabbit corpus cavernosum. Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means±s.e.mean (n=5–7). (**P<0.01 as compared with control).

controls: the contractions to PE were 2.2±0.3 g and 2.5±0.2 g in the absence and the presence of L-NAME, respectively. We initially confirmed the previous observation that ACh-mediated relaxation in rabbit cavernosal tissue is mediated through an NO pathway (Kim *et al*., 1991). ACh (1 nM–10 μM) produced a relaxation of the contracted cavernosal tissue. Cavernosal tissue was first exposed to L-NAME (0.1 mM) for 20 min, followed by PE and then ACh. The addition of L-NAME at optimal isometric tension blocked fully the ACh-induced relaxation (<98%, n=4, P<0.01). However, the same concentration of L-NAME (0.1 mM) only moderately attenuated berberine-induced relaxation (Figure 4, ■) when compared with control groups (Figure 4). A higher concentration of L-NAME (0.3 mM) still failed to block completely the berberine-evoked response (Figure 4). The concentration-relaxant effect curve to berberine obtained in the presence of 0.3 mM L-NAME was almost completely superimposable on those observed in endothelium-deprived preparations. Indomethacin, a cyclo-oxygenase inhibitor, influenced neither the basal tension nor the PE-induced contraction, and had no effect on berberine (Figure 4).

Effects of external K^+ concentration on berberine-induced relaxation

The observation that a consistent part of the berberine relaxant effect was still present in endothelium-deprived corpus cavernosum, suggested that berberine has a direct effect on corpus cavernosal smooth musculature. The following experiments were conducted to evaluate the endothelium-resistant

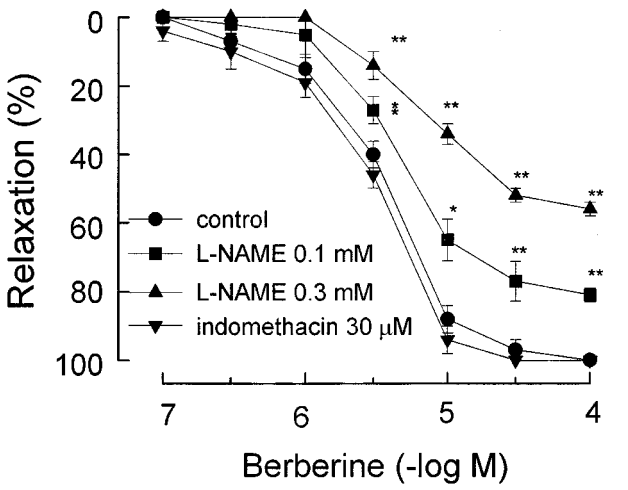

Figure 4 Relaxation effects of berberine on phenylephrine-precontracted endothelium-intact rabbit corpus cavernosum in the absence (control) and presence of L-NAME (0.1 and 0.3 mM) or indomethacin (30 μM). Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means±s.e.mean (n=8–11). (*P<0.05, **P<0.01 as compared with control).

response in endothelium deprived corpus cavernosum. First, the role of K^+ permeability in the action of berberine was studied in high K^+ medium (60 mM). The amplitude of contractile response to KCl (60 mM) was not significantly different from that induced by PE (2.4±0.5 vs 2.6±0.3 g contractile force, n=8–11). However, the relaxant activity of berberine was greatly reduced (Figure 5a, ■). The degree of relaxation observed with the higher concentration of berberine (100 μM) was reduced from 61.1±3.8 to 6.8±4.5%. In contrast to berberine, the relaxant response to SNP was not affected in high K^+ medium (Figure 5b).

Effects of K^+ channel blockers

In order to ascertain further the contribution of membrane K^+ channels to berberine-induced relaxation, we test the effect of several K^+-channel blockers (glibenclamide, apamin, charybdotoxin, and 4-AP) on the residual relaxation to berberine. In endothelium-deprived corpus cavernosum precontracted by PE, berberine (0.1–100 μM)-evoked relaxation was significantly reduced both by charybdotoxin (ChTX, 0.1 μM) and 4-AP (0.3 mM) treatment for 45 min, with the maximum responses being reduced from 60.2±4.0 to 32.6±3.1% and 44.5±2.7%, respectively (Figure 6, n=5–7; P<0.05). However, exposure to glibenclamide (0.1 μM) and apamin (1 μM) did not attenuate significantly the berbering-evoked relaxation (Figure 6). Concentration-response curves for berberine-induced relaxation in the absence and presence of different concentration of ChTX (50 nM and 0.1 μM) and 4-AP (0.1 and 0.5 mM) are shown in Figure 7. The degree of relaxation observed with the highest concentration of berberine was reduced to 48.6±3.7 and 32.1±2.6% by 50 nM and by 0.1 μM ChTX treatment, respectively (Figure 7a). Likewise, the degree of relaxation observed with the higher concentration of berberine was reduced to 55.6±4.1 and 39.7±2.8% by 0.1 and 0.3 mM 4-AP treatment, respectively (Figure 7b).

Effect of berberine on ICP

The baseline ICP recorded was 12.7±3.6 mmHg. Although transient rises or an unstable ICP following needle insertion

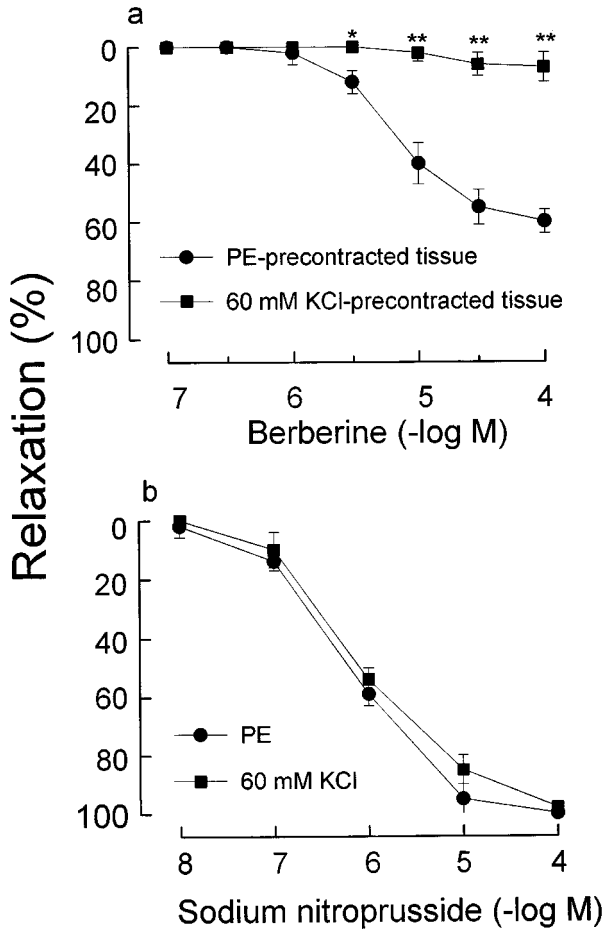

Figure 5 Relaxation effects of (a) berberine and (b) sodium nitroprusside against phenylephrine- and 60 mM KCl-evoked contraction in endothelium-deprived rabbit corpus cavernosum. Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means ± s.e.mean ($n = 7-10$). (*$P<0.05$, **$P<0.01$ as compared with PE-precontracted tissues).

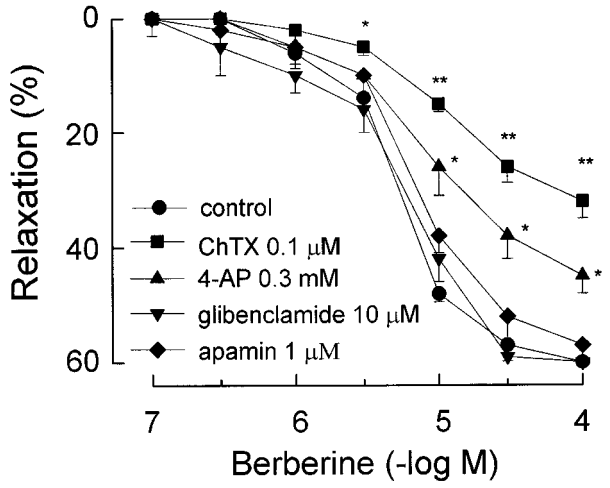

Figure 6 Relaxation effects of berberine on phenylephrine-precontracted endothelium-deprived rabbit corpus cavernosum in the absence (control) and presence of ChTX (0.1 μM), 4-AP (0.3 mM), glibenclamide (10 μM) or apamin (1 μM). Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means ± s.e.mean ($n = 5-7$). (*$P<0.05$, **$P<0.01$ as compared with control).

were found occasionally, the ICP restabilized within 10–20 min. In all rabbits studied, intrapenile injection of berberine induced tumescence as documented by a sustained increase in ICP. Figure 8a shows the time-course changes in ICP after intracavernous injection of berberine (3 mg kg^{-1}). During the injection periods, the SAP, MSAP and HR were unchanged. Intracavernous injection of normal saline induced a transient rise in ICP in a volume-dependent manner (Figure 8b). Nevertheless, the pressure rises often returned to the resting level within 1 min and the spike-like pressure tracing curves were different from those of berberine. We believe that the transient rise in ICP was due to the volume effect of injection of normal saline. Administration of berberine in increasing dose (1, 2, 3, and 5 mg kg^{-1}) induced a dose-dependent elevation in ICP (Figure 9). The ICP was increased respectively from basal to 13.2 ± 5.4, 25.3 ± 6.1, 46.5 ± 8.2, and 63.4 ± 10.2 mmHg, with a duration of 11.5–43.7 min. We also found in some cases tumescence of the penile shaft, but not full erection. Intracavernous injection of the highest dose of berberine (5 mg kg^{-1}) induced a slight and transient reduction of SAP (data not shown).

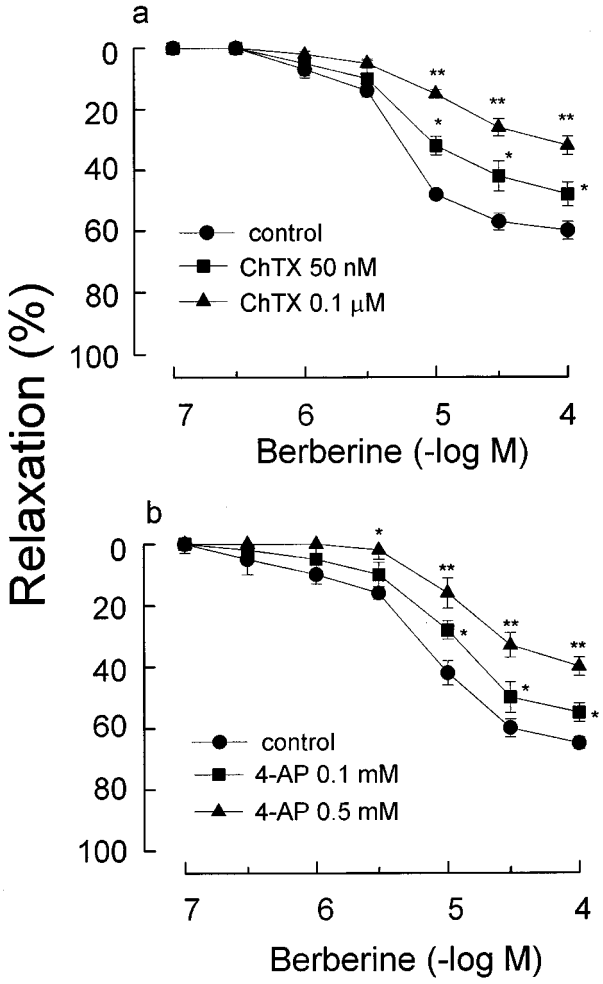

Figure 7 Relaxation effects of berberine on phenylephrine-precontracted endothelium-deprived rabbit corpus cavernosum in the absence (control) and presence of (a) ChTX (50 nM) or (b) 4-AP (0.1 and 0.5 mM). Results are expressed as per cent relaxation according to concentration in logarithmic scale and given as means ± s.e.mean ($n = 6-10$). (*$P<0.05$, **$P<0.01$ as compared with control).

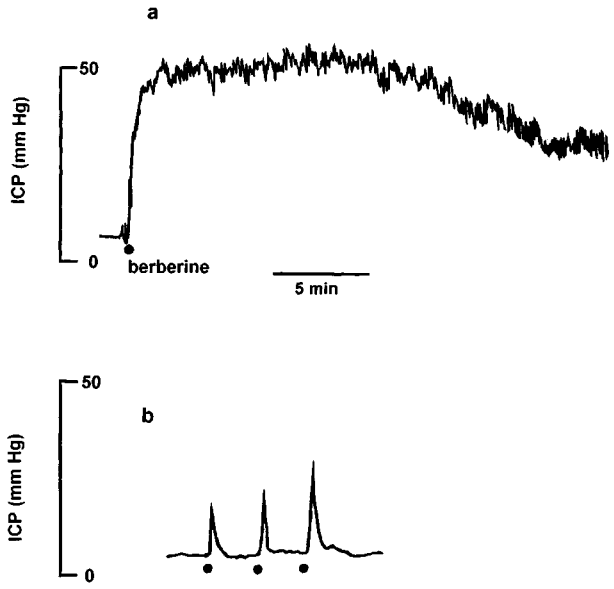

Figure 8 Representative intracavernosal pressure (ICP) change after intracavernous injections of (a) berberine (3 mg kg^{-1}) and (b) normal saline in different doses (0.06, 0.09, and 0.15 ml).

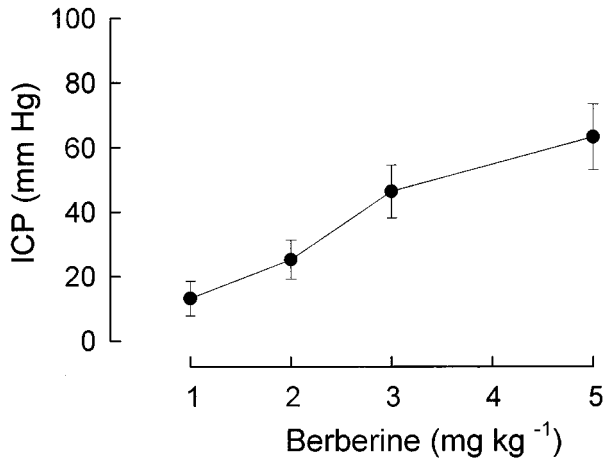

Figure 9 Administration of berberine (1, 2, 3, and 5 mg kg^{-1}) induced a dose-dependent elevation in intracavernosal pressure (ICP). Results are expressed as means ± s.e.mean. ($N = 5-8$).

Discussion

The present study, which represents the first attempt to describe the corpus cavernosum vascular effects of berberine, demonstrates that the drug has concentration-dependent relaxant activity in the isolated rabbit corpus cavernosum. In corpus cavernosum precontracted by phenylephrine (PE), the relaxant effect of berberine is attributable to both an endothelium-dependent and -independent mechanism of action. The conclusion is based chiefly upon results showing that the relaxant effect of berberine was significantly reduced by removing endothelium from the preparations. The endothelium-dependent component of the effect of berberine seems to be completely attributable to the production of NO-like substances by the endothelial cells. In fact, berberine-induced relaxation was concentration-dependently inhibited by nitric oxide synthase inhibitor, L-NAME (Palmer *et al*., 1988). The degree of relaxation induced by berberine in the presence of L-NAME was almost completely superimposable on that observed in endothelium-deprived corpus cavernosal preparations. Although cyclo-oxygenase products (especially PGI_2), as well as NO, play important roles in modulating corpus cavernosum smooth muscle tone (Azadzoi *et al*., 1992), indomethacin treatment did not influence the berberine-induced relaxing effect in intact preparation, indicating that the PGI_2-pathway is not implicated in the endothelium-dependent component.

However, the observation that a consistent part of the berberine relaxant effect was still present in endothelium deprived preparations and in those treated with a nitric oxide synthase inhibitor, suggests that berberine has a direct effect on vascular smooth muscle cells. This finding prompted us to study the response to berberine of endothelium-deprived preparations exposed to molecules able to interfere with smooth muscle relaxation. In corpus cavernosum the relaxant activity of berberine is strongly reduced in a high K^+ medium. Since the increase in tonic tension obtained in this experimental condition is due to the opening of calcium channels (Spedding & Cavero, 1984), the reduced effectiveness of berberine in relaxing corpus cavernosum demonstrates that the drug does not block voltage operated calcium channels. This was consistent with our previous finding that berberine had only a slight effect on high K^+-induced contraction in rat mesenteric artery when compared with PE-induced contraction (Chiou *et al*., 1991). However, the present result was opposed to the finding in guinea-pig ventricular myocytes that berberine antagonizes the L- and T-type calcium channels (Xu *et al*., 1997). It is possible that the interaction between berberine and calcium channel may vary from tissue to tissue.

Christ *et al*. (1993) examined the role of K^+ channels in regulating corporeal smooth muscle cell tone, and demonstrated that K^+ channels play a significant role in corporeal smooth muscle tone. These authors further suggested that impairment in K^+ channel activity may contribute to erectile dysfunction. Thus, the possibility of K^+ channel activation by berberine was further investigated. The first evidence of the importance of K^+ channel-mediated hyperpolarization in the actions of berberine was provided by the differential potency of berberine in relaxing PE-induced contraction versus 60 mM KCl-induced contraction. Vasodilators dependent on the K^+ channel mechanism lose their effects when exposed to high K^+ solutions because an increase in extracellular K^+ attenuates the K^+ gradient across the plasma membrane, thus rendering the K^+ channel-activating mechanism ineffective (Adeagbo & Triggle, 1993; Amedee *et al*., 1990; Khan *et al*., 1998). Berberine is believed to produce relaxation in this way since its effect was almost completely blunted in high K^+ (60 mM) condition. At 60 mM KCl, inhibition was so pronounced that even a 30 fold increase in the berberine concentration could not restore maximal relaxations (data not shown).

Because the high K^+ condition can produce multiple effects, a more direct pharmacological approach was taken by the use of Ca^{2+}-activated K^+ channel (K_{Ca} channel) blockers. ChTX is a highly selective blocker that inhibit high-conductance K_{Ca} in smooth muscle and neuroendocrine tissues (Jones *et al*., 1990; Hamaguchi *et al*., 1992). In a concentration range of berberine (10–100 μM), a significant relaxant component appears to be highly sensitive to blockade by the K_{Ca} channel blocker. On the other hand, the relaxant effect of berberine in endothelium-deprived preparations was effectively antagonized by 4-AP (an inhibitor of voltage-dependent K^+ channels) (Okabe *et al*., 1987; Beech & Bolton, 1989; Robertson & Nelson, 1994). However, berberine induced relaxation was not attenuated by a K_{ATP} channel blocker, glibenclamide (Standen *et al*., 1989), or by a small conductance

Ca^{2+}-activated K^+ channel blocker, apamin (Kolb, 1990). The lack of an effect of glibenclamide or apamin on berberine-induced relaxations also demonstrates the pharmacological selectivity of the former K^+ channel blockers. These data collectively identify ChTX- and 4-AP-sensitive K^+ channels as the possible mechanisms involved in the direct effect exerted by berberine on corpus cavernosal vasculature.

In the present study, intracavernous injection of berberine resulted in a rise of ICP without significant change in blood pressure, although a transient hypotensive action of berberine has been noticed (Fukuda *et al*., 1969). Ko & Lim (1980) reported that intravenous administration of berberine to anaesthetized rabbits at doses of 0.5, 1.5 and 5.0 mg kg^{-1}, resulted in dose-related decrements in blood pressure, ranging from 6.6 ± 0.4 to 23.4 ± 0.9 mmHg. However, the observed duration was transient and blood pressure returned to the original level within 3–10 min. At the dose used (1, 2 and 3 mg kg^{-1}) in our study, intrapenile administration of berberine did not cause any significant hypotensive effects. It is therefore reasonable to assume that the local penile effect of berberine observed in the present study was not influenced by any systemic haemodynamic changes.

In its structure, berberine shows some resemblance to papaverine (Ko & Lim, 1980). Lin & Lin (1996) reported that intracavernous injection of papaverine (0.25–1 mg kg^{-1}) induced a increase in ICP, with a maximal ICP ranging from 24–56 mmHg and a duration of tumescence from 5.3–15 min. Based on the doses used, their report showed that papaverine seemed to be more effective than berberine in inducing penile erection. However, the duration of tumescence caused by berberine (11.5–43.7 min) is considerably longer than papaverine.

In the present study, we have demonstrated that berberine has potent relaxant properties on the rabbit corpus cavernosum due to both endothelium-dependent and -independent mechanisms, and has the potential to be used as a drug for intracavernous injection therapy.

This investigation was supported by grants NSC87-2314-B-077-015 to Prof Chieh-Fu Chen and NSC88-2314-B-077-002 to Dr Wen-Fei Chiou from the National Science Council of Republic of China and in part by National Research Institute of Chinese Medicine, Taiwan, ROC.

References

ADEAGBO, A. & TRIGGLE, C. (1993). Varying extracellular [K^+]: a functional approach to separating EDHF- and EDNO-related mechanisms in perfused rat mesenteric arterial bed. *J. Cardiovasc. Pharmacol.,* **21,** 423–429.

AMEDEE, T., BENHAM, C., BOLTON, T., BYRNE, N. & LARGE, W. (1990). Potassium, chloride, and non-selective cation conductances opened by noradrenaline in rabbit ear artery cells. *J. Physiol.,* **423,** 551–568.

AZADZOI, K.M., KIM, N., BROWN, M.L., GOLDSTEIN, I., COHEN, R.A. & TEJADA, I.S. (1992). Endothelium-derived nitric oxide and cyclooxygenase products modulate corpus cavernosum smooth muscle tone. *J. Urol.,* **147,** 220–225.

BALLARD, S.A., TURNER, L.A. & NAYLOR, A.M. (1996). Sildenafil, a potent selective inhibitor of type 5 phosphodiesterase enhances nitric oxide-dependent relaxation of rabbit corpus cavernosum. *Br. J. Pharmacol.,* **118,** 153P.

BEECH, D.J. & BOLTON, T.B. (1989). Two components of potassium current activated by depolarization of single smooth muscle cells from the rabbit portal vein. *J. Physiol.,* **418,** 293–309.

CHEN, K.K., CHAN, Y.J., CHANG, L.S., CHEN, M.J. & CHAN, H.H. (1992). Intracavernous pressure as an experimental index in a rat model for the evaluation of penile erection. *J. Urol.,* **147,** 1124–1128.

CHIOU, W.F., YEN, M.H. & CHEN, C.F. (1991). Mechanism of vasodilatory effect of berberine in rat mesenteric artery. *Eur. J. Pharmacol.,* **204,** 35–40.

CHRIST, G.J., SPRAY, D.C. & BRINK, P.R. (1993). Characterization of K currents in cultured human corporal smooth muscle cells. *J. Androl.,* **14,** 319–328.

DOMER, F.R., WESSLER, G., BROWN, R.L. & CHARLES, H.C. (1978). Involvement of the sympathetic nervous system in the urinary bladder internal sphincter and in penile erection in anesthetized cat. *Invest. Urol.,* **15,** 404–407.

FUKUDA, H., WATANABE, J. & KUDO, Y. (1969). Some observations on the cardiovascular effects of 9-substituted berberine. *Chem. Pharmaceu. Bull.,* **18,** 1299–1304.

GIRALDI, A. & WAGNER, G. (1990). Effect of pinacidil upon penile erectile tissue, in vitro and in vivo. *Pharmacol. Toxicol.,* **67,** 235–238.

GOLDSTEIN, I., LUE, T.F., PADMA-NATHAN, H., ROSEN, R.C., STEERS, W.D. & WICKER, P.A. (1998). Oral sildenafil in the treatment of erectile dysfunction. *N. Engl. J. Med.,* **338,** 1397–1404.

HAMAGUCHI, M., ISHIBASHI, T. & IMAI, S. (1992). Involvement of charbdotoxin-sensitive K^+ channel in the relaxation of bovine tracheal smooth muscle by glyceryl trinitrate and sodium nitroprusside. *J. Pharmacol. Exp. Ther.,* **262,** 263–270.

HEDLUND, P., HOLMQUIST, F., HEDLUND, H. & ANDERSSON, K.E. (1994). Effects of nicorandil on human isolated corpus cavernosum and cavernous artery. *J. Urol.,* **151,** 1107–1113.

HOLMQUIST, F., ANDERSSON, K.E., FOVAEUS, M. & HEDLUND, H. (1990b). K^+-channel openers for relaxation of isolated penile erectile tissue from rabbit. *J. Urol.,* **144,** 146–151.

HOLMQUIST, F., ANDERSSON, K.E. & HEDLUND, H. (1990). Effects of pinacidil on isolated human corpus cavernosum penis. *Acta Physiol. Scand.,* **138,** 463–469.

HOLMQUIST, F., HEDLUND, H. & ANDERSSON, K.E. (1991). L-N^G-nitro-arginine inhibits non-adrenergic, non-cholinergic relaxation of human isolated corpus cavernosum. *Acta Physiol. Scand.,* **141,** 441–442.

JANG, C.S. (1941). The action of berberine on mammalian hearts. *J. Pharmacol., Exp. Ther.,* **1,** 178–186.

JEREMY, J.Y., BALLARD, S.A., NAYLOR, A.M., MILLER, M.A.W. & ANGELINI, G.D. (1997). Effects of sildenafil, a type-5 cGMP phosphodiesterase inhibitor, and papaverine on cyclic GMP and cyclic AMP levels in the rabbit corpus cavernosum in vitro. *Br. J. Urol.,* **79,** 958–963.

JONES, T.R., CHARETTE, L., GARCIA, M.L. & KACZOROWSKI, G.J. (1990). Selective inhibition of relaxation of guinea-pig trachea by charybdotoxin, a potent Ca^{2+}-activated K^+ channel inhibitor. *J. Pharmacol. Exp. Ther.,* **255,** 697–706.

JUNEMANN, K.P. & ALKEN, P. (1989). Pharmacotherapy of erectile dysfunction: a review. *Int. J. Impotence Res.,* **1,** 71–93.

KHAN, S.A., HIGDON, N.R. & MEISHERI, K.D. (1998). Coronary vasorelaxation by nitroglycerin: involvement of plasmalemmal calcium-activated K^+ channels and intracellular Ca^{2+} stores. *J. Pharmacol. Exp. Ther.,* **284,** 838–846.

KIM, N., AZADZOI, K.M., GOLDSTEIN, I. & SAENZ DE TEJADA, I. (1991). A nitric oxide-like factor mediates noradrenergic-noncholinergic neurogenic relaxation of penile corpus cavernosum smooth muscle. *J. Clin. Invest.,* **88,** 112–118.

KO, S.T. & LIM, D.Y. (1980). Influence of berberine on the blood pressure of rabbits. *Arch. Pharm. Res.,* **3,** 23–30.

KOLB, H.A. (1990). Potassium channels in excitable and non-excitable cells. *Rev. Physiol. Biochem. Pharmacol.,* **115,** 51–91.

LIN, J.S.N., WANG, J.M. & MA, C.P. (1985). Hemodynamic study of penile erection in dogs. *Eur. Urol.,* **11,** 401–406.

LIN, Y.M. & LIN, S.N. (1996). The rabbit as an intracavernous injection study model. *Urol. Res.,* **24,** 27–32.

LUE, T.F., TAKAMURA, T., SCHMIDT, R.A., PAULBINSKAS, A. & TANAGHO, E.A. (1983). Hemodynamics of erection in the monkey. *J. Urol.,* **130,** 1237–1241.

MORELAND, R.B., GOLDSTEIN, I. & TRAISH, A. (1998). Sildenafil, a novel inhibitor of phosphodiesterase type 5 in human corpus cavernosum smooth muscle cells. *Life Sci.,* **62,** 309 – 318.

OKABE, K., KIKAMURA, K. & KURIYAMA, H. (1987). Features of 4-aminopyridine sensitive outward current observed in single smooth muscle cells from the rabbit pulmonary artery. *Pflugers Arch.,* **409,** 561 – 568.

PALMER, R.M.J., ASHTON, D.S. & MONCADA, S. (1988). Vascular endothelial cell synthesize nitric oxide from L-arginine. *Nature,* **333,** 664 – 666.

RAJFER, J., ARONSON, W.J., BUSH, P.A., DOREY, F.J. & IGNARRO, C.J. (1992). Nitric oxide as a mediator of relaxation of the corpus cavernosum in response to noradrenergic, noncholinergic neurotransmission. *N. Engl. J. Med.,* **326,** 90 – 94.

ROBERTSON, B.E. & NELSON, M.T. (1994). Aminopyridine inhibition and voltage dependence of K^+ currents in smooth muscle cells from cerebral arteries. *Am. J. Physiol.,* **267,** C1589 – C1597.

SAENZ DE TEJADA, I., KIM, N., LAGAN, I., KRANE, R.J. & GOLDSTEIN, I. (1989). Regulation of adrenergic activity in penile corpus cavernosum. *J. Urol.,* **142,** 1117 – 1121.

SPEDDING, M. & CAVERO, I. (1984). 'Calcium antagonists' a class of drugs with a bright future. Part II. Determination of basic pharmacological properties. *Life Sci.,* **35,** 575 – 587.

STACLE, W., LOUPAL, G. & HOLZMANN, A. (1988). Intracavernous injection of vasocative drugs in the rabbit. *Urol. Res.,* **16,** 455 – 458.

STANDEN, N.B., QUAYLE, J.M., DAVIS, N.W., BRAYDEN, J.E., HUANG, Y. & NELSON, T.N. (1989). Hyperpolarizing vasodilators activate ATP-sensitive potassium channels in arterial smooth muscle. *Science,* **245,** 177 – 180.

TANG, K., TURNER, L.A., BALLARD, S.A. & NAYLOR, A.M. (1996). Effects of a novel phosphodiesterase type 5 inhibitor, sildenafil, on methacholine induced relaxation of isolated rabbit corpus cavernosum. *Br. J. Pharmacol.,* **118,** 154P.

TESFAMARIAM, B., HALPERN, W. & OSOL, G. (1985). Effects of perfusion and endothelium on the reactivity of isolated resistance arteries. *Blood Vessels,* **22,** 301 – 305.

XU, S.Z., ZHANG, Y., REN, J.Y. & ZHOU, Z.N. (1995). Effects of berberine on L- and T- type calcium channels in guinea-pig ventricular myocytes. *Acta Pharmacologica Sinica,* **18,** 515 – 518.

(Received July 14, 1998
Revised September 23, 1998
Accepted September 25, 1998)