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The Action of Ginkgo Bibloba Extract in the Isolated Rabbit Corpus Cavernosum

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=ABSTRACT=

The extract of Ginkgo bibloba (EGb) is a complex mixture of natural products from the Ginkgo leaves and clinically used for the treatment of cerebral and peripheral circulatory disturbances due to its combined activity of several vasoactive principles. In this study we investigated the action of EGb and its mechanism in isolated rabbit corporal smooth muscle to evaluate the possibility of using this material as a pharmacoerecting agent. Strips of rabbit corpus cavernosum were mounted in organ chambers to measure isometric tension. EGb began to exert an relaxing effect at 1 mg/ml in the submaximally precontracted muscle strips with phenylephrine (PHE, 5×10^{-6} M); causing concentration-dependent relaxation with maximal effect at 3~5 mg/ml. That relaxation was partially inhibited by removal of the smooth muscle endothelium or by pretreatment with a NO scavenger, pyrogallol (10^{-4} M) or the guanylate cyclase inhibitor, methylene blue (10^{-4} M). Pretreatment with EGb (3 mg/ml) inhibited PHE- (5×10^{-6} M) or KCl- (20 and 40 mM) induced contraction of muscle strip. In calcium-free high potassium solution EGb depressed the basal tone of the depolarized muscle strip and inhibited calcium-induced contraction when $CaCl_2$ (10^{-4} M) was added. These results suggest that EGb relaxes rabbit corpus cavernosal smooth muscle through multiple action mechanisms that include increasing the release of nitric oxide from the corporal sinusoidal endothelium, sequestration of intracytosolic calcium, and maybe a hyperpolarizing action.

Key Words: Extract of Ginkgo bibloba, Corporal smooth muscle

INTRODUCTION

The widespread use of intracavernous injections of vasoactive agents has revolutionized the treatment of erectile dysfunction. Several vasoactive agents such as papaverine, phentolamine and prostaglandin E1 have proven effective in relaxing cavernosal smooth muscle and are useful in the pharmacotherapy of erectile dysfunction (Barada & McKimmy, 1994). However, patient dissatisfaction with these agents has been associated with a high incidence of prolonged erection, significant pain in the penis or corporal fibrosis (Levine et al, 1989; Chiang et al, 1990; Lakin et al, 1990).

The extract of Ginkgo bibloba (EGb), a complex mixture containing flavonoid glycosides and several other natural products is known to occur in

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Ginkgo leaves (Nasr et al, 1986), The major clinical indications of EGb treatment are, briefly, insufficiency states of the cerebral and peripheral circulation, parenchyma and neurosensory organs (Chatterjee & Gabard, 1982). The combined activity of several action principles of the extract is responsible for its beneficial effects. The present study was undertaken to investigate the effects of EGb on isolated rabbit corporal smooth muscle for evaluation of the possibility of developing this material as an pharmacoerecting agent.

MATERIAL AND METHODS

Animal preparation

The New Zealand White rabbit (2.5 to 3.5 kg.) was chosen as the animal model based on the close similarities that have been reported in the reactivity in vitro of human and rabbit corpus cavernosum (Azadzoi et al, 1988). The animals were sacrificed by exsanguination following anesthesia by intracavernous injection of pentobarbital sodium (50 mg/kg). The corpora were removed en bloc to maintain the integrity of the tunica. After a ventral incision was made on the right and left corpora, the tunica was dissected and the corpus cavernosum tissue was exposed. The right and left corporal tissue were dissected and subsequently studied in the organ chambers. Each rabbit provided two to four strips of corpus cavernosum tissue that were studied in separate chambers.

Drugs

EGb was provided as a vial (50 mg/vial) from Sunkyong Pharm. LTD. Phenylephrine hydrochloride, acetylcholine chloride, pyrogallol and methylene blue were obtained from Sigma Chemical Co, (St. Louis, MO, USA).

Preparation of tissue in organ chambers

Strips of rabbit corpus cavernosum tissue measuring approximately $2 \times 2 \times 6$ mm. were submerged in 10 ml organ chmbers containing Tyrode buffer solution. The strips were suspended with a silk ties to a force transducer on one end, and fixed to a metallic support on the opposite end. Composition of Tyrode solution (mEq/L) was Na^+ 153.6, K^+ 5.3, Ca^{2+} 3.0, Mg^{2+} 1.2, Cl^- 157.2, $H_2PO_4^-$ 0.6, SO_4^{2-} 1.2, HCO_3^- 7.1 and glucose 5.0. The solution was gassed with 95% air and 5% CO_2. The pH of the solution was 7.4 and the temperature was maintained at 37C. Isometric tension was measured with a force transducer (Narco F-60, USA) and monitored with a physiograph (Narco Physiograph MK-IV, USA). The corpus cavernosum tissue was stretched incrementally for a period of 2 hours and the optimal resting isometric tension for contraction was determined. After every three stretches (0.5 gm. tension/stretch), the tissue was contracted with phenylephrine (5×10^{-6} M). When the amplitude of the contraction was within 10% of the previous contraction, that tension was considered optimal for isometric contraction. Relaxations were studied following contraction with phenylephrine (5×10^{-6} M). Disruption of the endothelium was achieved by rubbing cavernosal tissue strips between the thumb and index finger for about 20 sec (Saenz de Tejada et al, 1988). After rinsing in-chilled Tyrode solution, tissue strips were gently rolled across a dry paper towel to generate shear forces across the endothelial surfaces of the lacunar spaces. Removal of the endothelium was confirmed by the absence of the relaxation response of the strip to acetylcholine (10^{-5} M) or relaxed within 10% range in the control state.

Data and statistical analysis

All relaxant responses were expressed as percentage of maximal relaxation which was calculated from the perpendicular vertical distance between phenylephrine-induced maximal contraction point and the largest downward deflection in

the tracing at any given experiment. Inhibitory actions on contractile responses were also expressed as a percentage of the contraction in the control state. The statistical analysis was performed using student's t test. N denotes the number of cavernosal strips tested and results are expressed as mean ± standard deviation of the mean. $P<0.05$ was taken as being of statistical significance.

RESULTS

Responses to EGb

Rabbit corpus cavernosum strips submaximally precontracted with phenylephrine (5×10^{-6} M) began to exert a relaxing effect at 1 mg/ml of EGb (approximately 20% relaxation) and caused concentration-dependent relaxation with maximal effect (up to approximately 100% relaxation) at 3~5 mg/ml of EGb (Fig. 1 and Fig. 2). The EC50 value calculated from the linear regression curve of best fit by the method of least squares was 1.86 mg/ml EGb.

Effects of treatments with inhibitors of endothelium derived relaxing factor (EDRF) or nitric oxide (NO) action

Relaxations to EGb were significantly reduced ($p<0.01$), but not completely abolished, by removing the endothelium (Fig. 3). Also, pretreatment with a guanylate cyclase inhibitor, methylene blue (10^{-4} M) or a NO scavenger, pyrogallol (10^{-4} M) inhibited the relaxation of muscle strips significantly ($p<0.05$) at lower doses of EGb (≤3 mg/ml) (Fig. 3).

Effects of EGb pretreatment on phenylephrine-, Ca^{2+}-and K^{+}- induced contraction

Pretreatment with EGb inhibited phenylephrine-(5×10^{-6} M) induced contraction up to 10.1% of the control in a dose-dependent fashion (Fig. 4). In Ca^{2+} free high potassium depolarizing solution,

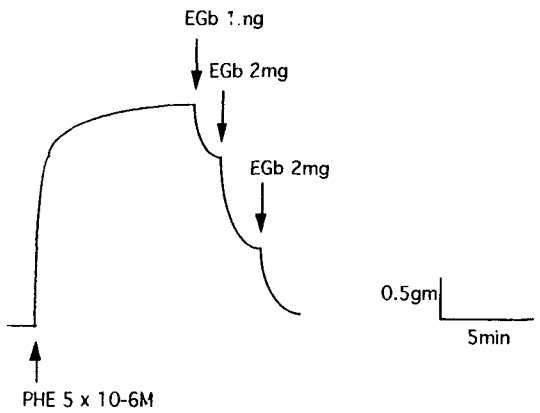

Fig. 1. Representative tracing of EGb effects on the isolated rabbit corpus cavernosum precontracted by phenylephrine. EGb relaxed submaximally precontracted muscle strip in a rapid fashion.

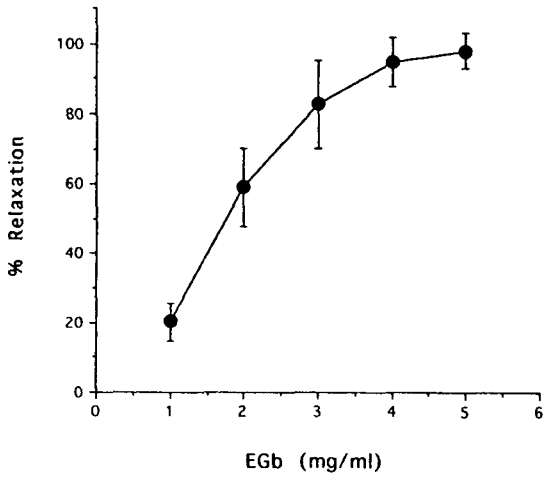

Fig. 2. Effects of EGb on the submaximally contracted muscle strips by phenylephrine (5×10^{-6}M) (n=18). Values represents mean ± SD. Note the concentration dependent relaxation up to 98.2±5.0% at 5 mg of EGb.

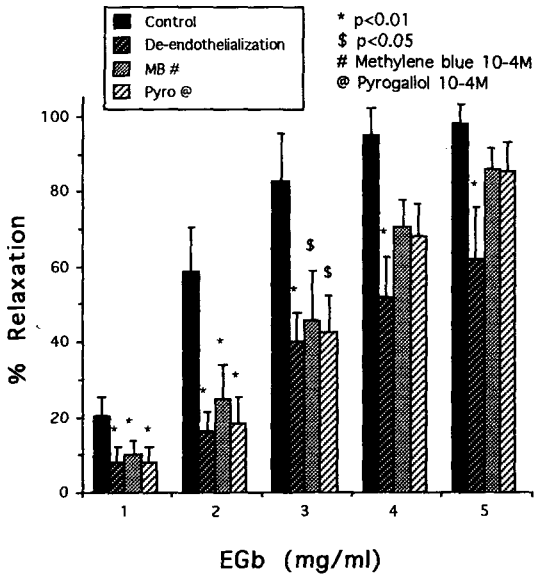

Fig. 3. Effects of various treatments on the relaxation respose of musxle strips to EGb. Values represent mean ± SD (n=8). Deendothelialization, treatments by methylene blue or pyrogallol significantly reduced the relaxation of muscle strips at lower doses of EGb.

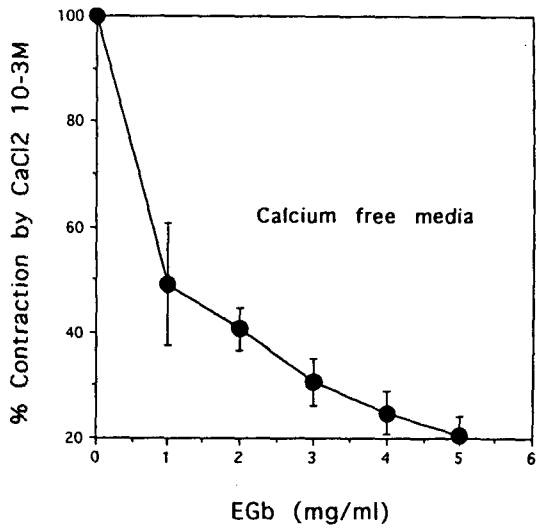

Fig. 5. Effect EGb pretreatment on calcium chloride induced contraction of muscle strips in calcium free, high potassium depolarizing media. Values represent mean ± SD (n=6). Pretreatment of EGb causes concentration dependent inhition of $CaCl_2$ (10^{-6}M) induced contraction of muscle strips.

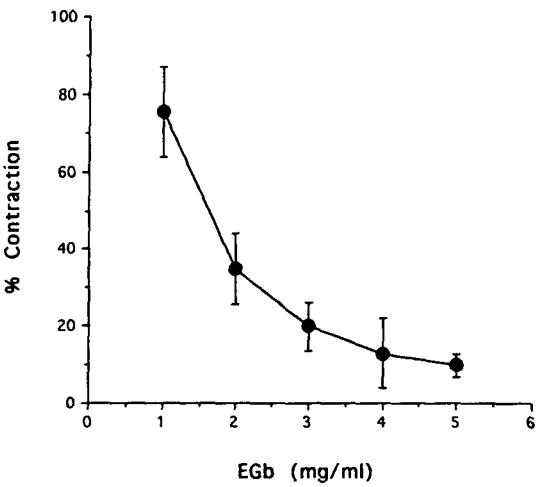

Fig. 4. Effect EGb pretreatment on phenylephrine induced contraction of muscle strip. Values represent mean ± SD (n=6). Pretreatment of EGb causes concentration dependent inhibition of phenylephrine- (5×10^{-6}M) induced contraction of muscle strips.

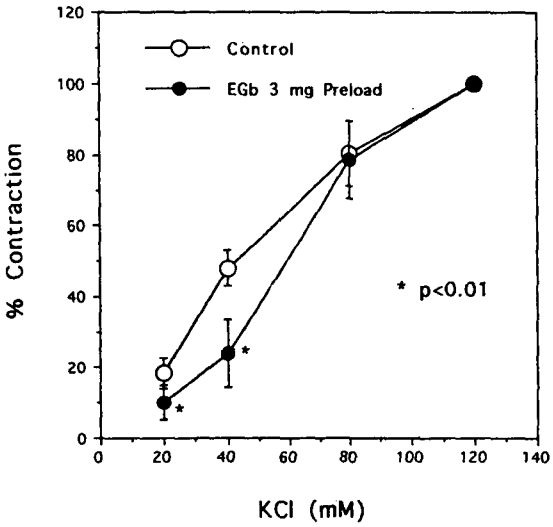

Fig. 6. Effect of EGb pretreatment on potassium chloride-induced contraction of muscle strips. Values represent mean ± SD (n=6). Pretreatment of EGb (3mg/ml) inhibits the contraction of muscle strips at lower doses of KCl (20 and 40 mM) only.

adding EGb depressed the basal tone of muscle strip to some degree (data not shown), and then inhibited contraction induced by adding $CaCl_2$ (10^{-4} M) dose-dependently up to 20% of the control with the pretreatment with 5 mg/ml EGb (Fig. 5). Pretreatment with 3 mg/ml EGb also inhibited the contraction induced by depolarization with 20 and 40 mM of KCl ($p<0.01$), however, in high concentrations of KCl of 80 and 120 mM, this inhibitory effect did not occur (Fig. 6).

DISCUSSION

Penile erection follows the relaxation of penile smooth muscle (Saenz de Tejada et al, 1985). Dilatation of the cavernosal and helicine arteries increases blood flow to the lacunar spaces. Relaxation of the trabecular smooth muscle dilates the lacunar spaces, causing engorgement of penis. The relaxed trabecular walls by trapping the blood against tunica albugenia compresses the plexus of subtunical venules, reduces venous outflow in the lacunar space, and elevates lacunar space pressure, making the penis rigid (Lue & Tanagho, 1987). Therefore, corporal smooth muscle relaxation plays a critical role in erection, which is largely nerve-mediated by a nonadrenergic, noncholinergic(NANC) mechanism, however, endothelium- dependent cholinergic neurotransmission may also mediate penile erection (Trigo-Rocha et al, 1993). Recent studies have shown that nitric oxide (NO) is the major neuronal mediator of erection (Rajfer et al, 1992).

Our data demonstrate EGb relaxes isolated rabbit corporal smooth muscle strip. EGb is a well defined and complex product prepared from green leaves of Ginkgo bibloba and has a vascular relaxant effect via several mechanisms. Experiments with rabbit aortic strips showed that the vasorelaxant effect of EGb depends upon an intact endothelium (DeFeudis, 1985). Our study also revealed that removal of the endothelium of muscle strips inhibited relaxation significantly by EGb. This result indicates that the vasorelaxant effect of EGb is mediated by the release or augmentation of the spontaneous release of endothelium derived relaxing factor (EDRF).

Nitric oxide was first described in 1979 as a potent relaxant of peripheral vascular smooth muscle, with an action mediated by cyclic GMP (Gruetter et al, 1979). Acetylcholine was postulated to stimulate the formation of an EDRF (Furchgott & Zawadzki, 1980), which was subsequently identified as being either nitric oxide or a chemically unstable nitroso precursor (Ignarro et al, 1978, Palmer et al, 1987). Nitric oxide is synthesized from endogenous L-arginine by the nitric oxide synthase system, located in the vascular endothelium (Palmer et al, 1988). The present study shows that both guanylate cyclase inhibitor, methylene blue and nitric oxide scavenger, pyrogallol can inhibit the relaxation effect of EGb on cavernous muscle strip. This indicates that the relaxing action of EGb in the isolated rabbit corporal smooth muscle is mediated by nitric oxide and/or cyclic GMP. It seems possible that certain flavonoid constituents of EGb could mediate a papaverine-like action; ie efficient inhibition of cyclic GMP phosphodiesterase (Ruckstuhl et al, 1979; Middleton, 1984), and that papaverine and other cyclic GMP phosphodiesterase inhibitors induce endothelium dependent relaxation by potentiating the effects of spontaneously released EDRF (Martin et al, 1986). The vasorelaxation induced by EDRF could involve sequestration of intracellular Ca^{2+} (DeFeudis, 1988). Auguete and Clostre (1983) reported that EGb, like papaverine, has an action related to inhibition of the influx of extracellular Ca^{2+}. It seems that this action explains the results of our study that EGb inhibited $CaCl_2$-induced contraction of muscle strip in the Ca^{2+} free media.

However, this inhibited relaxant response of muscle strips to EGb by removal of the endo-

thelium or addtion of methylene blue and pyrogallol ocurred partially, but not completely. Furthermore, at a higher dose of 4 or 5 mg/ml EGb, there was no significant inhibitory action of relaxation by pretreatment with methylene blue or pyrogallol (Fig. 3). This indicates that the relaxing action of EGb includes not only an endothelium mediated mechanism but also other mechanisms. Norepinephrine or phenylephrine induces concentration-dependent contraction of isolated strip preparations of corpus cavernosum tissue and this contraction is attenuated or blocked by alpha adrenoreceptor blockers (Adaikan & Karim, 1981; Hedlund & Anderson, 1985). The results obtained in the present study of inhibitory action of EGb pretreatment in strips with phenylephrine induced contracion suggest that EGb might have an alpha adrenergic blocking effect. EGb pretreatment of muscle strips also inhibited K^+-induced contraction at the concentration range of 20~40 mM. Hamilton et al (1986) reported that a potassium channel opener prevented activation of the voltage operated calcium channel by potassium induced depolarization at the same range of potassium concentration of 20~40 mM, with little efecct above 80 mM. Therefore, EGb also has a hyperpolarizing effect of like a potassium channel opener in the isolated corporal tissue.

Collectively, present study indicates that at least part of the relaxing effect of EGb on the corporal smooth muscle is mediated by the release (or augmentation of the spontaneous release) of EDRF or nitric oxide as well as inhibition of calcium mobilization into the cytosol from the intracellular sarcoplasmic reticulum or extracellular fluid. Additionally, a hyperpolarizing effect via potassium channel opening might be also related to this relaxing effect. These results indicate that there are many potentioally potentially active constituents in EGb that could influence its final action. Therefore, further sudies are required in this area to clarify the individual mechanism of each component of EGb and purify and select the proper components for achieving better relaxation of corporal smooth muscle.

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