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Relaxant effects of some benzothiazolinone derivatives on isolated rabbit corpus cavernosum

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Abstract In the present study, two 6-(fluorobenzoyl)-3-piperazinomethyl-2-benzothiazolinone derivatives were synthesized and their relaxant effects on isolated rabbit corpus cavernosum investigated. Compounds Y-16 and Y-21 can alter the ability of corpus cavernosum smooth muscle to contract. Strips of rabbit corpus cavernosum smooth muscle were mounted in isolated tissue baths for measurement of isometric contractile force. Compounds (10^{-6}–10^{-3} M) did not cause contraction but induced relaxation in precontracted corpus cavernosum smooth muscle. Neither *N*-nitro-L-arginine methylester (L-NAME) nor indomethacin affected the relaxant effect of these compounds. Glibenclamide and tetraethylammonium chloride (TEA) also did not influence the relaxation induced by the compounds. In conclusion, in isolated rabbit corpus cavenosum, Y16 and Y21 have a relaxant potency equal or superior to known vasoactive agents. Further investigations are needed to show the importance of these effects for the diagnosis and treatment of erectile dysfunction.

Key words Benzothiazolinone · Smooth muscle relaxation · Corpus cavernosum · Erectile dysfunction

Introduction

Normal male sexual function depends on a complex interplay of psychological, neurological, vascular and endocrine factors [7,12]. Penile erection is due to the relaxation of smooth muscle fibers in the penile arteries, leading to an increase in blood flow to the penis, and to the relaxation of smooth muscle fibers in the two erectile tissues, the corpora cavernosa, resulting in the engorgement of the penis with blood [3]. The balance between the contractile and the relaxant factors controls the smooth muscle of the corpus cavernosum and determines the functional state of the penis (detumescence and flaccidity versus tumescence and erection) [4]. Changes in penile hemodynamics are the prerequisite for the induction and maintenance of penile erection through the intracavernous administration of vasoactive substances. The commonly used substances for the treatment of erectile dysfunction are papaverine, combination of papaverine and phentolamine [18], prostaglandin E_1 (alprostadil) [5], and sildenafil which is a selective inhibitor of the cGMP-specific phosphodiesterase [6]. In addition to these established substances, several other regimens such as linsidomine (SIN-1), calcitonin gene-related peptide (CGRP), moxisylyte, and various triple or quadruple drug mixtures have been described [16]. Benzothizolinone derivatives have been reported to have a wide range of biological activities, including anticholinergic, analgesic, antiinflammatory, antiplatelet and calcium antagonist [1, 8, 9, 10, 13].

In this study our aim was to investigate the relaxant effects of Y-16 and Y-21 on the rabbit corpus cavernosum in an effort to find a more desirable agent for the diagnosis and treatment of erectile impotence.

Materials and methods

Chemistry

All chemicals used in this study were supplied by Aldrich (Stenheim, Germany). Melting points were determined with a Thomas Hoover melting point apparatus (Philadelphia, Penn., USA) and the values were uncorrected. UV Spectra were recorded with a 10^{-5} M Shimadzu UV-160A UV-Visible Spectrophotometer. IR Spectra were recorded with a Perkin Elmer FT-IR Spectrophotometer 1720 × (KBr disc). ^{1}H-NMR spectra were measured on a Bruker AC 80 MHz ($CDCl_3$) and FT NMR and Bruker DPX 400 MHz spectrophotometer; tetramethylsilane was used as the

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internal standard. Elemental analysis of the compounds was performed on a Perkin Elmer 240 °C and a Leco CHNS-932 at the Instrumental Analysis Laboratory of TUBITAK (Scientific and Technical Research Council of Turkey), Ankara-Turkey.

6-benzoyl and 6-(2-fluorobenzoyl)-2-benzothiazolinones

Both 0.01 M 2-benzothiazolinone and 0.01 M benzoic acid were heated in 30 ml polyphosphoric acid (PPA) at 130 °C for 1 h, then poured into 200 ml ice water and stirred for 6 h. The precipitate formed was washed with water and crystallized from meOH.

3-[4-(piperonyl)piperazino]methyl-6-benzoyl (2-fluorobenzoyl)-2-benzothiazolinones

A mixture of 0.01 M 6-benzoyl-2-benzothiazolinone and/ or 6-(2-fluorobenzoyl)-2-benzothia-zolinone, 0.01 M 4-piperonyl-piperazine and 0.02 M formaldehyde solution (35%) was heated in methanol. Then the solution was concentrated under diminished pressure. The precipitate was filtered, washed with water, and crystallized from alcohol.

3-[4-(piperonyl)piperazino]methyl-6-benzoyl-2-benzothiazolinone (Y-16)

m.p.: 191 °C. IR (KBr): ν (cm^{-1}) = 1690 C = O ring, 1650 C = O ketone, ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.15–2.70 (4H; m; piperazine H^2, H^6), 2.80–3.30 (4H; m; piperazine H^3, H^5), 3.40 (2H; s; -N-CH_2-Ar), 4.80 (2H; s; –N-CH_2-N-), 5.80 (2H; s; –O-CH_2-O–), 7.10–8.00 (m; 11H ; aromatic protons), Analysis for $C_{27}H_{25}N_3O_4S$ (M.W.: 487.57), calculated: C: 66.51, H: 5.17, N: 8.62, found C: 66.14, H: 5.29, N: 8.83.

3-[4-(piperonyl)piperazino]methyl-6-(2-fluorobenzoyl)-2-benzothiazolinone (Y-21)

m.p.: 216 °C. IR (KBr): ν (cm^{-1}) = 1700 C = O ring, 1660 C = O ketone, ^{1}H-NMR (DMSO-d_6): δ (ppm) 2.10–2.70 (4H; m; piperazine H^2,H^6), 2.80–3.35 (4H; m; piperazine H^3, H^5), 3.40 (2H; s; –N-CH_2-Ar), 4.85 (2H; s; –N-CH_2-N-), 5.85 (2H; s; –O-CH_2-O-), 7.05–8.00 (m; 10H; aromatic protons), Analysis for $C_{27}H_{24}FN_3O_4S$ (M.W.: 505.56) calculated: C: 64.15, H: 4.78, N: 8.31, found C: 64.01, H: 5.02, N: 8.44.

The compounds were synthesized by the Mannich reaction of 6-benzoyl and/or 6-(2-fluoro- benzoil)-2-benzothiazolinone. 6-Aroyl-2-benzothiazolinones were synthesized by the reaction of 2-nezothiazolinone, the appropriate benzoic acid derivative and polyphosphoric acid.

The structure of the compounds was elucidated by IR, ^{1}H-NMR, and elemental analyses and all spectral data are in accordance with assigned structures. In IR spectra, no absorption bands were detected in the range of 3200–3300 cm^{-1} indicating the absence of an NH group. This is evidence for the Mannich reaction. In addition elemental analyses of the compounds confirmed their structures.

Preparation of tissue in organ chambers

Mature male albino rabbits weighing 2.5–3 kg were used. At the time of study, rabbits were sacrificed with a subcutaneous injection of ketamine and xylazine followed by exsanguination. Penises were surgically removed at the level of the crural attachments to the pubo-ischal bones and 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 containing Krebs-bicarbonate solution maintained at 37 °C. Each rabbit provided four strips of corpus cavernosum tissue which were studied in separate chambers.

Strips of corpus cavernosum tissue measuring approximately 3 × 3 × 15 mm were studied in 20-ml organ chambers for isometric tension measurement. The strips were tied with silk to a force transducer (Grass FT 03. Quincy MA) at one end and fixed with silk ties to a glass support at the other. The organ chambers contained Krebs-bicarbonate solution composed of: NaCl: 118 g; KCl: 4.7 g; $CaCl_2$: 2.5 g; $NaHCO_3$: 25 g; Mg SO_4: 12 g; $KHPO_4$: 1.2 g; and glucose: 11 g, and the temperature was maintained at 37 °C. The solution was aerated continuously with 5% CO_2, 95% O_2. The pH of the saturated solution was 7.4.

After mounting, the preparations were allowed to equlibrate for 2 h. During this time the resting tension was adjusted to 2 g, a value which was previously found to be optimal for the measurement of changes in tension, and the solution was renewed every 15 min. After a stabilization period, the strips were precontracted submaximally using phenylephrine (10^{-5} M) until the contraction reached a plateau.

Concentration-response relaxation for Y-16 and Y-21 were obtained by adding these compounds into the bath in a cumulative manner. In another set of experiments, L-arginine methyl ester (L-NAME, the nitric oxide synthase inhibitor) (3.10^{-5} M), indomethacin (PGOx inhibitor) (10^{-5} M), tetraethylammonium (TEA, a nonspecific calcium-activated potassium channel inhibitor) (5.10^{-4} M), and glibenclamide (ATP- sensitive potassium channel inhibitor) (10^{-6} M) were added into the organ bath 15 min before the precontraction in order to test the effects of nitric oxide, prostaglandins and K^+ channel opening activity, all of which could have contributed to the corporal smooth muscle relaxation induced by Y-16 and Y-21.

The relaxant effects of the compounds were expressed as percentages of the precontraction using phenylephrine. To evaluate the effects of agonists, the maximum response (E_m) and pD_2 values [the negative logarithm of the concentration for the half-maximal response, (EC_{50})] were calculated, as predicted from the Scatchard equation for drug–receptor interaction. Agonist pD_2 values (apparent agonist affinity constants) were calculated from each agonist concentration–response curve by linear regression of the linear part of the curve and taken as a measure of the sensitivity of the tissues to each agonist. All data were expressed as mean ± standard error. Statistical comparisons between groups were performed using general linear models by Scheffe's F test and *P* values of less than 0.05 were considered to be statistically significant (Table 1).

Drugs

Phenylephrine hydrochoride, L-NAME, indomethacin, TEA, and glibenclamide were supplied by Sigma. All drugs were dissolved in distilled water except for glibenclamide (in dimethyl-sulfoxide) and indomethacin (in 1% sodium carbonate). The solvents had no effect on the tone of the strips. L-NAME was initially dissolved in distilled water and stored frozen. On the day of use it was thawed and diluted in distilled water. All other drugs were prepared daily.

Results

Compounds Y-16 and Y-21 did not induce contraction but exerted concentration-dependent relaxation

Table 1 The relaxant effect of compounds Y-16 and Y-21 as well as various drugs on isolated strips of rabbit corporal smooth muscle. Relaxation is expressed as a percentage of the precontraction induced by phenylephrine (3 μM). E_{max} and pD_2 values of the drugs, except Y-16 and Y-21, were previously calculated in our laboratory. The maximal effect (E_{max}, %) and pD_2 values represent mean values ± SE for 9 muscle strips from 3 to 4 different preparations

	Maximum relaxation (E_{max} %)	pD_2
Compound Y-16	96.7 ± 3.3	4.12 ± 0.064
Compound Y-21	97.3 ± 2.7	4.94 ± 0.069
Papaverine hydrochloride	98.4 ± 1.4	6.57 ± 0.059
Sodium nitroprusside	97.6 ± 2.3	5.35 ± 0.063
Zaprinast	96.3 ± 3.2	4.528 ± 0.038
Carbachol chloride	78.3 ± 5.2	6.79 ± 0.053
Prostaglandin E_1	67.2 ± 4.01	5.26 ± 0.096
Adenosine	61.2 ± 4.3	4.26 ± 0.012
Isosorbide dinitrate	60.2 ± 6.1	5.07 ± 0.813
ATP	28.6 ± 5.2	4.42 ± 0.021

responses in the cavernosal strips precontracted with phenylephrine (Fig. 1). The smooth muscle endothelium is known to modulate corpus cavernosum responsiveness to a variety of contractile and relaxant stimuli [4].

To investigate whether relaxation induced by the test compounds was due to an interaction with the cyclooxygenase or nitric oxide pathways, tissues were pretreated with indomethacin (10^{-5} M) or L-NAME (3×10^{-5} M), respectively. Treatment of cavernosal tissue strips with these inhibitors did not significantly alter the relaxant activity of compoundsY-16 or Y-21. Investigate whether relaxation induced by compounds Y-16 and Y-21 involved the opening of ATP-sensitive K^+ channels and calcium-activated K^+ channels, the effects of glibenclamide and TEA were investigated. We tested the responses to compounds Y-16 and Y-21 before and after a 30 min application of 10^{-6} M glibenclamide and 5.10^{-4} M TEA. Neither TEA nor glibenclamide affected the relaxant effects or the pD_2 or E_{max} values of the compounds. Compound Y-16 exerted 96.7% maximal relaxation with pD_2 value of 4.12 and compound Y-21 exerted 97.3% maximal relaxation with pD_2 value of 4.94.

Our results showed that compounds Y-16 and Y-21 had a potency for relaxing isolated rabbit cavernous smooth muscle similar to those of papaverine and SNP and a greater efficiency than carbachol, adenosine, ATP, and PGE_1.

Discussion

The results of this study indicate that compounds Y-16 and Y-21 induce the relaxation of rabbit corpus cavernosum tissue in vitro. This relaxation was not mediated by cyclooxygenase products and nitric oxide which is synthesized and released either from nonadrenergic and noncholinergic nerves or from the endothelium of the corpus cavernosum. To investigate whether relaxation induced by the test compounds was due to an interaction with the cyclooxygenase or nitric oxide pathways, tissues were pretreated with indomethacin or L-NAME, respectively. Treatment of cavernosal tissue strips with these inhibitors did not significantly alter the relaxant activity of compounds Y-16 or Y-21. In our study, the relaxation induced by the compounds was not impaired by glibenclamide or TEA. K^+ channel openers such as pinacidil, nicorandil, and kromakalim which relax various types of smooth muscle cells by opening the rubidium permeable K^+ channels leading to hyperpolarization of the cells. To investigate whether relaxation induced by compounds Y-16 and Y-21 involved the opening of ATP-sensitive K^+ channels and calcium-activated K^+ channels, the effects of glibenclamide and TEA were investigated. Neither TEA nor glibenclamide affected the relaxant effects or the pD_2 or E_{max} values of the compounds. These results indicate that K^+ channel opening does not play an important role in the relaxation responses of these compounds. Thus, the mechanism underlying the relaxation induced by Y-16 and Y-21 is not known at present; however, it is likely to be due to a direct effect on corpus cavernosum smooth muscle. To test the relaxant effect of the compounds on other vascular structures, rat aorta, with and without endothelium, was precontracted with phenylephrine and then treated with the compounds in an organ bath. A dose–relaxation response curve similar to those of the corpus cavernosum was obtained, indicating that the relaxant effect of these compounds also occurred in rat aorta. (unpublished data).

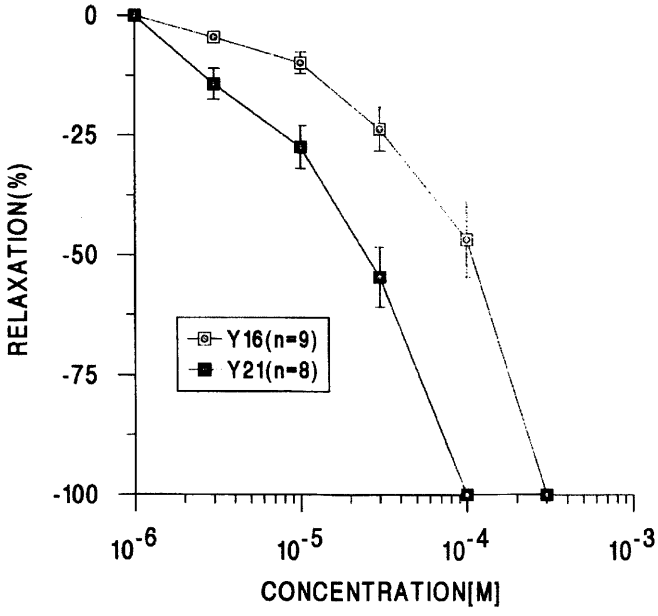

Fig. 1 Concentration–response curves of relaxation induced by compounds Y-16 and Y-21 in isolated strips of rabbit corpus cavernosum. Each *point* is expressed as a percentage of the contraction induced by 3 μM phenylephrine and shows the mean ± SE. *Numbers in parentheses* indicate the number of preparations used

The discovery that penile erection can be induced by the intracavernous injection of pharmacological agents dramatically changed the diagnosis and treatment of impotence. In 1982 Virag reported the attainment of erection after intracavernous injection of papaverine in men [17]. Intracavernous injection of papaverine, with or without phentolamine, has since gained widespread acceptance. However, side effects, such as priapism, fibrosis formation, hypotension and dizziness, have also been reported [11]. Intracavernous injection of prostaglandin E_1 has proven superior to injection of papaverine [14]. PGE_1 more closely mimics a physiological erection, produces less complications, and is possibly metabolized in the penis. The main disadvantages of PGE_1 are its high cost relative to papaverine and its chemical instability. The L-arginine/NO/guanylate cyclase/cGMP pathway seems to be the most important for penile erection in some species and recent results obtained with sildenafil, a selective inhibitor of the cGMP-specific phosphodiesterase (PDE 5) in the human corpus cavernosum [6,15], further support the suggestion that this may alsobe the case in humans [16]. Our present results show that the compounds Y-16 and Y-21

have a potency at least equal to those of papaverine, sodium nitroprusside (SNP), and zaprinast and a stronger efficiency than PGE1, carbachol, and ATP in the relaxation of isolated rabbit corporal smooth muscle.

To detect whether the compounds Y-16 and Y-21, alone or together with other vasoactive agents, can be used for the diagnosis and treatment of erectile dysfunction, further investigations are required. The next step is to investigate the effectiveness of these compounds by administering them intracavernously in the rabbits.

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