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Saw Palmetto Extracts Potently and Noncompetitively Inhibit Human α_1-Adrenoceptors In Vitro

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BACKGROUND. We wanted to test whether phytotherapeutic agents used in the treatment of lower urinary tract symptoms have α_1-adrenoceptor antagonistic properties in vitro.
METHODS. Preparations of β-sitosterol and extracts of stinging nettle, medicinal pumpkin, and saw palmetto were obtained from several pharmaceutical companies. They were tested for their ability to inhibit [^{3}H]tamsulosin binding to human prostatic α_1-adrenoceptors and [^{3}H]prazosin binding to cloned human α_{1A}- and α_{1B}-adrenoceptors. Inhibition of phenylephrine-stimulated [^{3}H]inositol phosphate formation by cloned receptors was also investigated.
RESULTS. Up to the highest concentration which could be tested, preparations of β-sitosterol, stinging nettle, and medicinal pumpkin were without consistent inhibitory effect in all assays. In contrast, all tested saw palmetto extracts inhibited radioligand binding to human α_1-adrenoceptors and agonist-induced [^{3}H]inositol phosphate formation. Saturation binding experiments in the presence of a single saw palmetto extract concentration indicated a noncompetitive antagonism. The relationship between active concentrations in vitro and recommended therapeutic doses for the saw palmetto extracts was slightly lower than that for several chemically defined α_1-adrenoceptor antagonists.
CONCLUSIONS. Saw palmetto extracts have α_1-adrenoceptor-inhibitory properties. If bioavailability and other pharmacokinetic properties of these ingredients are similar to those of the chemically defined α_1-adrenoceptor antagonists, α_1-adrenoceptor antagonism might be involved in the therapeutic effects of these extracts in patients with lower urinary tract symptoms suggestive of benign prostatic obstruction. *Prostate 38:208–215, 1999.*

KEY WORDS: **saw palmetto; stinging nettle; medicinal pumpkin; β-sitosterol; α_1-adrenoceptor**

INTRODUCTION

Plant extracts are widely used in the treatment of lower urinary symptoms (LUTS) suggestive of benign prostatic obstruction (BPO) in various countries. While the clinical efficacy for the majority of plant extracts remains to be proven, randomized, controlled clinical trials are emerging for some preparations. For example, improvements of symptom scores and peak urinary flow rates have been shown in a double-blind, placebo-controlled, 6-month trial with β-sitosterol involving 200 patients with LUTS suggestive of BPO [1]. Other double-blind studies have compared the effects of saw palmetto (*Serenoa repens*, a.k.a. *Sabal serrulata*) extracts with those of finasteride and found similar efficacy during a 6-month period in 1,098 patients with LUTS suggestive of BPO [2] or a 12-month period in 543 patients [3].

The use of phytotherapy in the rational medical treatment of patients with LUTS has also been limited

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by the lack of knowledge regarding their possible mechanism of action. A variety of mechanisms have been proposed for various phytotherapies, including inhibition of 5α-reductase, cylcooxygenase, lipoxygenase, and sex hormone-binding globuline [4]. However, none of these mechanisms has ever convincingly been demonstrated to be operative in vivo at therapeutic doses. For example, several studies have shown inhibition of 5-reductase activity by high concentrations of phytotherapeutic agents in vitro, but the in vivo treatment of patients with these preparations did not result in a lowering of dihydrotestosterone levels [5,6].

Among medical treatments for patients with LUTS suggestive of BPO which have a proven efficacy relative to placebo, the α_1-adrenoceptor antagonists are now the most widely used [7]. In recent years, Teng et al. [8–10] have systematically investigated plant extracts which are used in traditional Chinese medicine. They have identified a number of active ingredients which are high-affinity α_1-adrenoceptor antagonists. Thus, dicentrine is an apomorphine derivative isolated from *Lindera megaphylla* [8]. N-methylactinodaphine is also an apomorphine derivative which has been isolated from *Illigera luzonensis* and has some selectivity for α_{1B}- over α_{1A}-adrenoceptors [9]. (-)-discretamine is an α_1-adrenoceptor antagonist which has been isolated from *Fissistima glaucescens* and may be somewhat selective for α_{1D}-adrenoceptors [10].

Therefore, we performed a systematic screening of several preparations of β-sitosterol and extracts from saw palmetto, stinging nettle (*Urtica dioica*), and medicinal pumpkin (*Cucurbita pepo*) for their ability to bind to and inhibit α_1-adrenoceptors. Human prostatic and cloned human α_{1A}- and α_{1B}-adrenoceptors were used for this purpose in radioligand binding and [^{3}H]inositol phosphate accumulation studies.

MATERIALS AND METHODS

Chemicals

Individual plant extracts are identified by Roman numerals which were assigned in consecutive order. β-sitosterol powders I, II, and III were obtained from AZU Pharma (Gerlingen, Germany), Hoyer (Monheim, Germany), and TAD Pharma (Cuxhaven, Germany), respectively. They were dissolved in ethanol at 1 mM, diluted 1:20 in binding buffer (see below), and used at a maximum assay concentration of 10 μM. Pumpkin seed extract powder IV was obtained from Hoyer. Stinging nettle extract powders VIII, IX, X, and XI were obtained from AZU Pharma, Hoyer, Kanoldt (Ismaning, Germany), and TAD Pharma, respectively; stinging nettle extract granules XII were from Dr. Willmar Schwabe (Karlsruhe, Germany). These powders and granules were dissolved at 10 mg/ml in dimethylsulfoxide under sonication, diluted 1:20 in binding buffer, and used at a maximum assay concentration of 100 μg/ml. Pumpkin seed extract oils V, VI, and VII were from Smith Kline Beecham (Herrenberg, Germany). They were diluted with 20% Tween-80, and then diluted 1:400 with binding buffer under sonication to reach a maximum assay concentration of 0.1%. Saw palmetto extract powders XIII and XIV were provided by Smith Kline Beecham. They were dissolved at 40 mg/ml in dimethylsulfoxide under sonication, diluted 1:20 in binding buffer, and used at a maximum assay concentration of 400 μg/ml. Saw palmetto extracts oils XV, XVI, XVII, and XVIII were obtained from Hoyer, Sanofi Winthrop (Munich, Germany), Dr. Willmar Schwabe, and TAD Pharma, respectively. They were diluted with 20% Tween-80, and then diluted 1:400 with binding buffer to reach a maximum assay concentration of 0.1%.

[^{3}H]prazosin (specific activity 74 Ci/mmol) and [^{3}H]tamsulosin (also known as [^{3}H]YM 617, specific activity 44 Ci/mmol) were obtained from New England Nuclear (Dreieich, Germany); [^{3}H]myo-inositol (specific activity 74.0 Ci/mmol) was from Amersham (Braunschweig, Germany). Phenylephrine HCl was purchased from Sigma-Aldrich Chemie (Munich, Germany), and phentolamine HCl was a gift from Novartis (Basel, Switzerland).

Radioligand Binding

Rat-1 cells, which had been stably transfected with the cloned human α_{1A}- or α_{1B}-adrenoceptor [11], were kindly provided by Pfizer Central Research (Sandwich, Kent, UK). Human prostate was obtained from patients undergoing cystectomy due to bladder cancer or transurethral prostate resection due to benign prostatic enlargement. Tissue preparation and radioligand binding techniques have previously been described in detail [12]. Briefly, 100 μl of membrane suspension were incubated in a total volume of 1,000 μl binding buffer (50 mM TRIS, 0.5 mM EDTA, pH 7.4) for 45 min at 25°C. The incubations were terminated by rapid vacuum filtration of Whatman GF/C filters (HJ-Bioanalytik, Mönchengladbach, Germany). Nonspecific binding was defined as binding in the presence of 10 μM phentolamine. Protein content was determined by the method of Bradford [13], using bovine IgG as the standard. [^{3}H]tamsulosin was used as the radioligand instead of [^{3}H]prazosin in human prostate, since it labels a similar number of sites but has less nonspecific binding and thus a more favorable signal/noise ratio in this tissue [12,14]. In saturation binding experiments, six concentrations of [^{3}H]prazosin and [^{3}H]tamsulosin ranging between 50–1,800 pM and 10–1,200 pM, respectively, were used. In competition

binding experiments the concentration of [^{3}H]prazosin and [^{3}H]tamsulosin was ≈350 pM and ≈1,000 pM, respectively.

Inositol Phosphate Accumulation

Phospholipase C activation was assessed as [^{3}H]inositol phosphate formation, as previously described [15]. Rat-1 cells expressing the human α_{1A}-adrenoceptor were grown in 12 well plates. During the last 24 hr prior to the experiment, cells were labeled with myo-[^{3}H]inositol (3 μCi/ml) in inositol-free medium. Thereafter, the subconfluent cells were washed twice with medium containing 20 mM HEPES and 50 mM LiCl at pH 7.4 and 37°C. The cells were incubated with or without phenylephrine at 37°C for 30 min. Reactions were stopped by addition of 1 ml ice-cold methanol. The cells were removed by a cell scraper into vials, and 1.5 ml chloroform and 0.5 ml water were added. The mixture was vigorously vortexed twice for 30 sec, and thereafter the phases were separated by centrifugation at 820*g* for 10 min at 4°C. Aliquots (450 μl) of the upper phase were placed on Dowex AG 1-X8 columns (230 mg/column; Bio-Rad, München, Germany). Free inositol was eluted with 10 ml water and 10 ml of 60 mM ammonium formate. Total inositol phosphates were eluted by addition of 2 ml of 1 M ammonium formate dissolved in 100 mM formic acid. All measurements were done in triplicate in each experiment.

Data Analysis

Saturation and competition binding experiments were analyzed by fitting rectangular hyperbolic and sigmoidal functions, respectively, to the experimental data. Where possible, IC_{50} values from the competition experiments were converted into K_i values by the equation $K_i = IC_{50}/(1 + (L/K_d))$, where L and K_d are the concentration and affinity, respectively, of the radioligand. Data are mean ± SEM of n experiments. The statistical significance of difference was determined by Student's *t*-test or by one-way analysis of variance, as indicated.

RESULTS

Radioligand Binding Studies With Human Prostate

[^{3}H]tamsulosin bound to human prostatic α_1-adrenoceptors with a K_d of 38 ± 7 pM and a B_{max} of 16 ± 3 fmol/mg protein (n = 6). Ethanol (1%) and dimethylsulfoxide (1%) did not affect [^{3}H]tamsulosin binding (−6.9 ± 5.9% and −1.0 ± 2.3% inhibition, respectively; n = 3 each), while Tween-80 (0.02%) caused 40.3 ± 3.3% inhibition (n = 3).

At the highest tested concentration, the β-sitosterol preparations and the stinging nettle and pumpkin extracts did not inhibit [^{3}H]tamsulosin binding to human prostatic membranes (Table I). While the saw palmetto extract powders had little effect on [^{3}H]tamsulosin binding, the saw palmetto extract oils caused concentration-dependent inhibition (Fig. 1). However, the interpretation of these inhibition curves was complicated by a concomitant concentration-dependent inhibition of [^{3}H]tamsulosin binding by the vehicle, Tween-80 (Fig. 1). Moreover, the inhibition curves of the saw palmetto extract oils were bell-shaped in some cases; thus, maximum inhibition was seen at concentrations of approximately 0.03%, and higher concentrations caused less inhibition of [^{3}H]tamsulosin binding due to interference with the filtration process. When only the descending part of the concentration-inhibition curve was analyzed, half-maximal inhibition occurred between 0.005–0.01%, i.e., at concentrations where the Tween-80 alone inhibited [^{3}H]tamsulosin binding only by up to 13% (Fig. 1).

Radioligand Binding Studies With Cloned Human α_1-Adrenoceptors

Specific [^{3}H]prazosin to cloned human α_{1A}-adrenoceptors had a K_d of 168 ± 17 pM and a B_{max} of 991 ± 34 fmol/mg protein (Table II). Ethanol (1%) did not affect [^{3}H]prazosin binding (0.2 ± 2.2% inhibition, n = 6), while dimethylsulfoxide (1%) caused 8.5 ± 0.9% inhibition (n = 20), and Tween-80 (0.02%) caused 46.0 ± 1.2% inhibition (n = 3).

β-sitosterol from three different sources in concentrations up to 10 μM did not cause significant inhibition of [^{3}H]prazosin binding to cloned human α_{1A}-adrenoceptors (Table I). The inhibition by five different stinging nettle extracts was also very weak and did not exceed 14% at the highest tested concentration (Table I). The pumpkin seed powder in concentrations up to 100 μg/ml and the pumpkin seed extract oils in concentrations up to 0.1% did not significantly inhibit [^{3}H]prazosin binding (Table I).

In contrast, all tested saw palmetto extract powders and oils concentration-dependently inhibited [^{3}H]prazosin binding to cloned human $_{1A}$-adrenoceptors (Table III, Fig. 2). For the two extract powders, maximum inhibition was seen with 125 and 160 μg/ml and was 74 ± 4% and 59 ± 17%, respectively. Higher concentrations of these extracts caused less inhibition of [^{3}H]prazosin binding due to interference with the filtration process. Half-maximal inhibition was observed with approximately 60 μg/ml for

TABLE I. Inhibition of Radioligand Binding to Human Prostatic α_1-Adrenoceptors and Cloned Human α_{1A}-Adrenoceptors*

Extract	Form	Highest concentration tested	Percent inhibition	
			Human prostate α_1-adrenoceptor	Cloned human α_{1A}-adrenoceptor
β-sitosterol				
I	Powder	10 μM	6 ± 8 (3)	−5 ± 8 (3)
II	Powder	10 μM	3 ± 7 (3)	−3 ± 4 (3)
III	Powder	10 μM	12 ± 8 (3)	−1 ± 2 (3)
Medicinal pumpkin (*Cucurbita pepo*) seeds				
IV	Powder	100 μg/ml	−4 ± 4 (3)	4 ± 4 (3)
V	Oil	0.1%	−7 ± 6 (3)	−17 ± 3 (3)
VI	Oil	0.1%	−6 ± 3 (3)	−16 ± 3 (3)
VII	Oil	0.1%	0 ± 4 (3)	−13 ± 4 (3)
Stinging nettle (*Urtica dioica*) roots				
VIII	Powder	100 μg/ml	−5 ± 3 (4)	1 ± 5 (3)
IX	Powder	100 μg/ml	−1 ± 3 (3)	1 ± 5 (3)
X	Powder	100 μg/ml	−12 ± 2 (3)	14 ± 2 (3)
XI	Powder	100 μg/ml	−24 ± 3 (3)	4 ± 1 (3)
XII	Granules	100 μg/ml	−9 ± 2 (4)	9 ± 2 (3)

*Cloned human α_{1A}-adrenoceptors were labeled with [^{3}H]prazosin, and human prostatic α_1-adrenoceptors with [^{3}H]tamsulosin. Final concentrations of the solvents ethanol (β-sitosterol) and dimethylsulfoxide (all other powders and granules) in the assays were 1%. Date are mean ± SEM of the number of experiments given in parentheses.

both extracts, but due to the bell-shaped inhibition curve this could not be calculated reliably. The saw palmetto extract oils behaved slightly different. They inhibited [^{3}H]prazosin binding almost completely (Fig. 2). The slopes of the inhibition curves for the saw palmetto extract oils were very steep, with Hill coefficients of approximately 5–8. This was not explained by the inhibition caused by the vehicle Tween-80 alone (Fig. 2). Half-maximal inhibition of [^{3}H]prazosin binding was seen at 0.001–0.002% (Table III). Similar data were obtained with cloned human α_{1B}-adrenoceptors (Table III).

Due to the steepness of the inhibition curves, [^{3}H]prazosin saturation binding experiments were performed with cloned α_{1A}-adrenoceptors in the presence of 0.001% of the saw palmetto extract oils to test for competitive inhibition. In these experiments, the vehicle Tween-80 (0.0002%) did not have significant effects on the K_d or B_{max} of [^{3}H]prazosin saturation binding (Table II). In contrast, all four saw palmetto extract oils significantly reduced the detectable number of binding sites by ≈30–40% (Table II). The saw palmetto extracts significantly ($P < 0.05$) lowered K_d in some cases (XV, 97 ± 21 pM; XVII, 83 ± 6 pM; XVIII, 92 ± 6 pM) but not in others (XVI, 128 ± 25 pM) relative to values observed in the presence of Tween-80 (158 ± 9 pM).

For comparative purposes, we also performed competition binding experiments with clinically-used α_1-adrenoceptor antagonists. They inhibited [^{3}H]prazosin binding with monophasic curves and an order of potency tamsulosin > terazosin ≈ alfuzosin ≈ doxazosin (Table IV). All α_1-adrenoceptor antagonist inhibition curves had Hill coefficients close to unity (Table IV), and the calculated K_i values were in good agreement with those obtained at the bovine α_{1A}-adrenoceptor under identical conditions [16].

Inositol Phosphate Accumulation Studies With Cloned α_{1A}-Adrenoceptors

Phenylephrine stimulated [^{3}H]inositol phosphate formation, with an EC_{50} of 3.4 ± 0.4 μM; maximal stimulation was 2,213 ± 676% above basal values (n = 5). In all further experiments, phenylephrine was used at a concentration of 25 μM. Ethanol (1%) and Tween-80 (0.02%) did not significantly affect phenylephrine-stimulated inositol phosphate formation (0 ± 2% inhibition, n = 3, and 5.6 ± 7.2% inhibition, n = 5, respectively). Dimethylsulfoxide (1%) inhibited phenylephrine-induced [^{3}H]inositol phosphate formation by 14.6 ± 7.6% (n = 7). Inhibitory effects of all agents are expressed relative to the values obtained in the presence of vehicle.

None of the tested β-sitosterol preparations and most of the pumpkin, stinging nettle, or saw palmetto

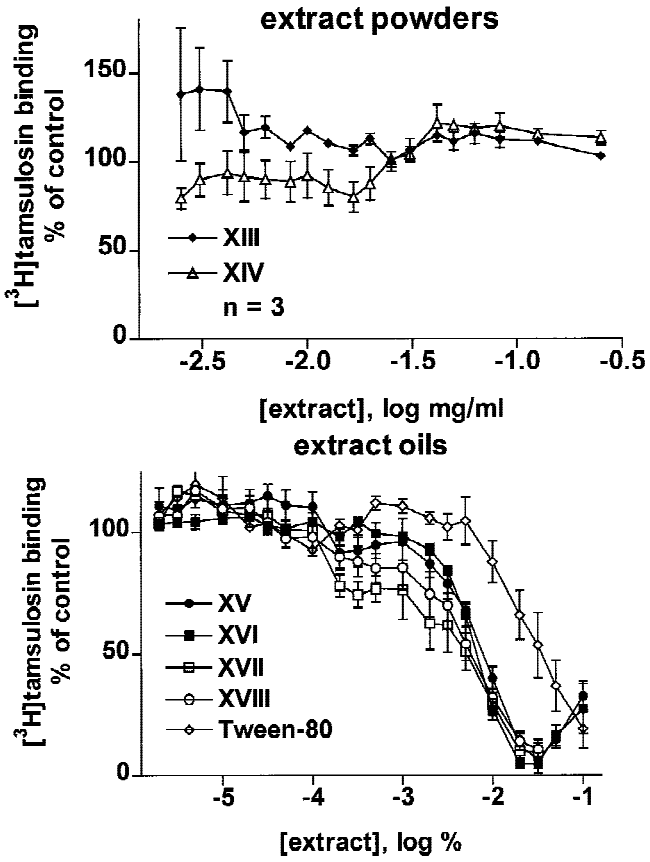

Fig. 1. Inhibition of [^{3}H]tamsulosin binding to human prostatic membranes by saw palmetto extracts. Data are mean ± SEM of three experiments. Roman numerals identify individual extracts (see Materials and Methods).

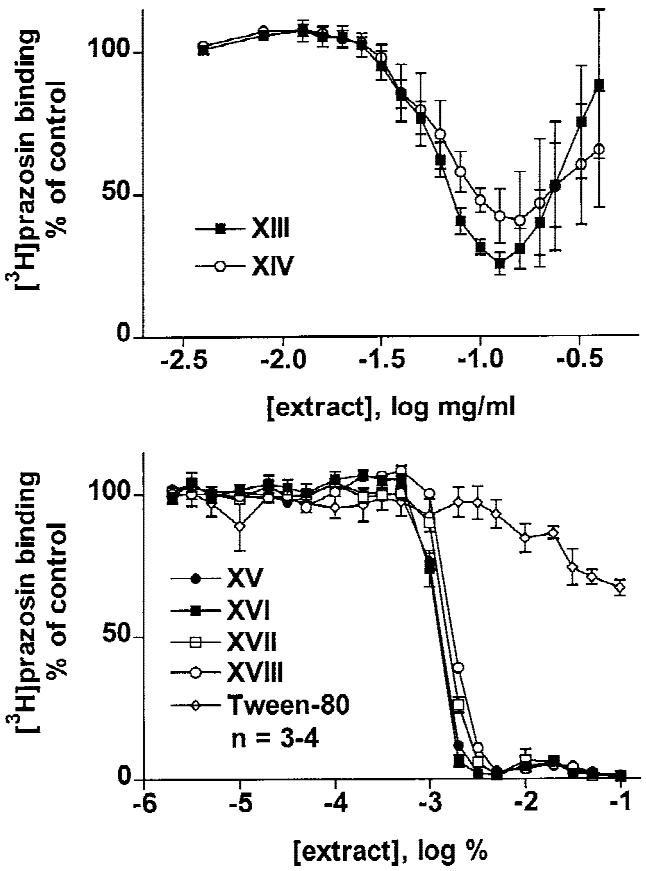

Fig. 2. Inhibition of [^{3}H]prazosin binding to cloned human α_{1A}-adrenoceptors by saw palmetto extracts. Data are mean ± SEM of 3–4 experiments. Roman numerals identify individual extracts (see Materials and Methods).

TABLE II. Effects of Saw Palmetto Extract Oils on B_{max} of [^{3}H]Prazosin Saturation Binding†

Extract	fmol/ml protein	Percent of control
Control	991 ± 34	
Tween-80	971 ± 50	98 ± 2
XV	623 ± 73*	63 ± 7*
XVI	614 ± 114*	61 ± 11*
XVII	682 ± 74*	69 ± 6*
XVIII	702 ± 72*	71 ± 6*

†[^{3}H]prazosin saturation binding experiments (≈50–1,800 pM) were performed in the absence (control) and presence of 0.0002% Tween-80 or 0.001% of the indicated saw palmetto extract oils. Data are mean ± SEM of four experiments.
*$P < 0.001$ vs. Tween-80 in a repeated measures one-way analysis of variance followed by a Dunnett multiple comparison test.

extracts lacked major direct effects on [^{3}H]inositol phosphate formation (data not shown); however, stinging nettle extract IX and pumpkin extract IV (both provided by the same supplier) stimulated [^{3}H]inositol phosphate formation in the absence of phenylephrine (13.8 ± 3.6% and 35.4 ± 3.0%, respectively, of 25 μM phenylephrine values; n = 4 each). However, none of the tested β-sitosterol preparations or stinging nettle or pumpkin extracts caused relevant inhibition of phenylephrine-stimulated [^{3}H]inositol phosphate formation (Table V). In contrast, the saw palmetto extract powders and oils inhibited 25 μM phenylephrine-induced [^{3}H]inositol phosphate formation by ≈66% and ≈38%, respectively (Table V).

DISCUSSION

It has been reported that saw palmetto extracts can inhibit noradrenaline-induced smooth muscle contraction in vitro, but contractions by acetylcholine and receptor-independently by KCl were also inhibited in the same concentration range; therefore, it could not be determined from these data whether α_1-adrenoceptor antagonism was involved [17,18]. In contrast to that indirect approach, we directly investigated whether plant extracts used in the treatment of LUTS suggestive of BPO contain α_1-adrenoceptor antagonistic ingredients.

Studies on plant extracts require the use of organic

TABLE III. Inhibition of Radioligand Binding to Cloned Human α_1-Adrenoceptors by Saw Palmetto Extracts*

Extract	Form	Cloned human α_{1A}-adrenoceptor		Cloned human α_{1B}-adrenoceptor	
		Hill slope	–log IC_{50}	Hill slope	–log IC_{50}
XIII	Powder		1.13 ± 0.03		
XIV	Powder		1.08 ± 0.08		
XV	Oil	4.8 ± 0.4	2.90 ± 0.01	2.9 ± 0.6	2.98 ± 0.10
XVI	Oil	8.5 ± 2.4	2.94 ± 0.03	4.3 ± 0.4	2.98 ± 0.12
XVII	Oil	4.9 ± 0.5	2.81 ± 0.01	3.7 ± 0.3	3.18 ± 0.02
XVIII	Oil	5.5 ± 0.1	2.74 ± 0.01	2.9 ± 0.3	3.13 ± 0.02

*Data are mean ± SEM of three experiments. Affinity estimates are shown as negative logarithms of the concentration of extract which inhibited [^{3}H]prazosin binding by 50% (–log IC_{50}), calculated as mg/ml for powder forms and as % for oils.

TABLE IV. Competition of Clinically Used Antagonists for [^{3}H]Prazosin Binding to Cloned Human α_{1A}-Adrenoceptors*

Antagonist	Hill slope	–log IC_{50} (mol/l)	–log K_1 (mol/l)
Alfuzosin	0.85 ± 0.07	7.66 ± 0.01	8.14 ± 0.01
Doxazosin	0.98 ± 0.11	7.61 ± 0.07	8.09 ± 0.07
Tamsulosin	1.02 ± 0.10	9.83 ± 0.07	10.30 ± 0.07
Terazosin	0.93 ± 0.06	7.75 ± 0.05	8.22 ± 0.04

*Data are mean ± SEM of three experiments each. Affinity estimates are given as logarithms of the concentration inhibiting [^{3}H]prazosin by 50% (–log IC_{50}) or occupying 50% of the receptors (–log K_i).

solvents. Since these solvents can cause assay perturbations, the perturbing effects of the solvents can become limiting for the biological analysis of plant extracts. Our data demonstrate that the employed concentrations of ethanol and dimethylsulfoxide caused only minor if any perturbation of the radioligand binding or [^{3}H]inositol phosphate accumulation assays. While Tween-80 had very little effect on the [^{3}H]inositol phosphate assay, it caused marked concentration-dependent inhibition of radioligand binding to α_1-adrenoceptors. This was more pronounced with prostatic membranes than with membranes from rat-1 cells transfected with cloned α_1-adrenoceptors, since more membrane (and thus more lipid) was required in the assay for human prostate due to a lower receptor expression density. Therefore, we analyzed all data on plant extracts relative to the effects of the respective vehicles alone. Moreover, all key findings were observed at extract dilutions, where the corresponding solvent concentrations had minimal effects.

[^{3}H]prazosin is the standard radioligand for the detection of α_1-adrenoceptors. While [^{3}H]prazosin and [^{3}H]tamsulosin label similar numbers of α_1-adrenoceptors in human prostate, [^{3}H]tamsulosin exhibits less nonspecific binding and thus a more favorable signal/noise ratio in this tissue [12,14]. Therefore, we used [^{3}H]tamsulosin as the radioligand for our present experiments on human prostate, and [^{3}H]prazosin with the cloned receptors. Both of these radioligands are α_1-adrenoceptor antagonists, and hence our experiments tested for a possible interaction between the plant extracts and both the α_1-adrenoceptors and the chemically defined α_1-adrenoceptor antagonists.

β-sitosterol preparations and extracts from stinging nettle or pumpkin in the highest concentrations which could be tested did not cause relevant inhibition of radioligand binding to prostatic or cloned human α_1-adrenoceptors or of [^{3}H]inositol phosphate accumulation. These findings were obtained consistently with preparations obtained from several different suppliers. Therefore, our data suggest that α_1-adrenoceptor antagonism does not play a role in the possible beneficial therapeutic effects of such drugs.

In contrast, the saw palmetto extracts concentration-dependently inhibited radioligand binding to human α_1-adrenoceptors. Qualitatively this was detected in the prostate and with cloned human α_{1A}- and α_{1B}-adrenoceptors. Quantitatively the data in the prostate were more difficult to interpret, since the extract powders did not cause detectable inhibition and since the inhibition curves for the extract oils were biphasic. Both observations were likely due to technical difficulties with the filtration process, which was hindered by the combination of a high membrane content in the assay and high concentrations of extracts. Therefore, most further experiments were performed with the cloned α_{1A}-adrenoceptors. Their use was justified because α_{1A}-adrenoceptors predominate in the human prostate at the mRNA [19] and protein [16] levels. Since our transfected rat-1 cells contained a much greater density of α_1-adrenoceptors than human pros-

TABLE V. Effects of Phytotherapeutic Drugs on Phenylephrine-Stimulated [^{3}H]Inositol Phosphate Formation†

Extract	Tested concentration	Percent inhibition
β-sitosterol		
I	10 μM	5 ± 2 (3)
II	10 μM	2 ± 2 (3)
III	10 μM	6 ± 2 (3)
Medicinal pumpkin (*Cucurbita pepo*) seeds		
IV	100 μg/ml	5 ± 12 (3)
V	0.1%	2 ± 23 (3)
VI	0.1%	−12 ± 17 (3)
VII	0.1%	−6 ± 11 (3)
Stinging nettle (*Urtica dioica*) roots		
VIII	100 μg/ml	8 ± 7 (5)
IX	100 μg/ml	−3 ± 18 (3)
X	100 μg/ml	−11 ± 9 (5)
XI	100 μg/ml	−17 ± 7 (5)
XII	100 μg/ml	−1 ± 6 (5)
Saw palmetto (*Sabal serrulata*) fruits		
XIII	400 μg/ml	69 ± 16* (3)
XIV	400 μg/ml	63 ± 15* (3)
XV	0.08%	39 ± 8** (4)
XVI	0.08%	46 ± 11** (4)
XVII	0.08%	33 ± 11** (4)
XVIII	0.08%	35 ± 12* (4)

†[^{3}H]Inositol phosphate formation was stimulated by 25 μM phenylephrine. Inhibition by the plant extracts was assessed at the indicated concentrations. Data are expressed as percent inhibition relative to the effects of the corresponding vehicle. Data are mean ± SEM of the number of experiments indicated in parentheses.
*Statistically significant from 0 at the $P < 0.05$ level in a two-tailed, one-sample *t*-test.
**Statistically significant from 0 at the $P < 0.01$ level in a two-tailed, one-sample *t*-test.

tate, the transfected cells yielded a better signal/noise ratio than human prostate membranes. Moreover, the resulting use of lower membrane concentrations in the assay also resulted in less assay interference by the solvents.

Radioligand binding to cloned human α_{1A}- and α_{1B}-adrenoceptors was potently inhibited by all saw palmetto extract powders and oils. These data clearly demonstrate that saw palmetto extracts contain ingredients which bind to human α_1-adrenoceptors. Our inositol phosphate formation experiments clearly showed that these ingredients are antagonists of the α_1-adrenoceptors. The effects of the extract powders and oils are not easy to compare directly, since the concentrations of active ingredients in the two types of extract preparations are unknown. Observed quantitative differences between the extract powders and oils could relate to differential extraction processes which were used by the respective manufacturers, which may have resulted in differential enrichment in the α_1-adrenoceptor-blocking ingredients. However, within each extract form, the inhibitory effects of preparations from several suppliers were quantitatively similar, indicating consistency in their content of active ingredient despite possible differences in plant sources and extraction procedures.

The competition curves of the saw palmetto extracts were much steeper than those of the chemically defined, competitive α_1-adrenoceptor antagonists. Therefore, saturation binding experiments were performed with cloned α_{1A}-adrenoceptors in the presence of a single concentration of extract oils. The presence of extract oils did not increase the apparent K_d values for the radioligand, but in some cases even reduced them. On the other hand, the number of detectable receptors was significantly reduced. These data demonstrate that the inhibition of α_1-adrenoceptors by saw palmetto extracts is noncompetitive.

Previous studies demonstrated that some plant extracts used in the treatment of LUTS suggestive of BPO inhibit 5α-reductase in vitro [5,6]. However, upon administration of therapeutic doses in man, no reductions of dihydrotestosterone plasma concentrations were observed in vivo [5]. The present data cannot definitively show whether the in vitro α_1-adrenoceptor antagonism by saw palmetto extracts observed in our study results in in vivo antagonism in man upon administration of therapeutically relevant doses. To obtain circumstantial evidence, we calculated a ratio between the dose of extract causing a 50% inhibition of radioligand binding to cloned α_{1A}-adrenoceptors in vitro and the recommended daily dose for the treatment of LUTS suggestive of BPH. Our calculations show that this ratio ranges from 98,000–1,100,00 for the chemically defined α_1-adrenoceptor antagonists, while it ranges from 20,000–32,000 for the saw palmetto oils. Thus, the difference between the two groups for this ratio is smaller than the differences within the classical α_1-adrenoceptor antagonists. Thus, it can be envisioned that saw palmetto extracts may yield α_1-adrenoceptor inhibition in vivo if the pharmacokinetic properties (e.g., bioavailability, volume of distribution, terminal half-life) of their active ingredients are similar to those of the chemically defined α_1-adrenoceptor antagonists.

While our data clearly demonstrate α_1-adrenoceptor antagonism of saw palmetto extracts in vitro, they do not allow us to conclude whether this also occurs in vivo and whether this yields a clinically relevant therapeutic effect. Thus, a possible α_1-adreno-

ceptor antagonism of saw palmetto extracts in vivo can only be tested by clinical studies. In the absence of such data it is interesting to look at direct comparative studies of saw palmetto extracts and chemically defined α_1-adrenoceptor antagonists. Unfortunately, such studies are available only on a very limited scale. Thus, in one study with 45 patients with LUTS suggestive of BPO being treated for 12 weeks, the α_1-adrenoceptor antagonist prazosin was significantly more efficacious than a saw palmetto extract [20]. Another study with 63 patients being treated for 3 weeks found that the α_1-adrenoceptor antagonist alfuzosin reduced irritative and obstructive symptoms in the Boyarski score to a greater extent than a saw palmetto extract [21]. However, these studies do not provide definitive evidence due to the limited number of patients and/or short study duration. The present observation of potent α_1-adrenoceptor antagonism by saw palmetto extracts from several manufacturers indicates that additional clinical studies on possible α_1-adrenoceptor antagonism by these extracts are warranted.

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