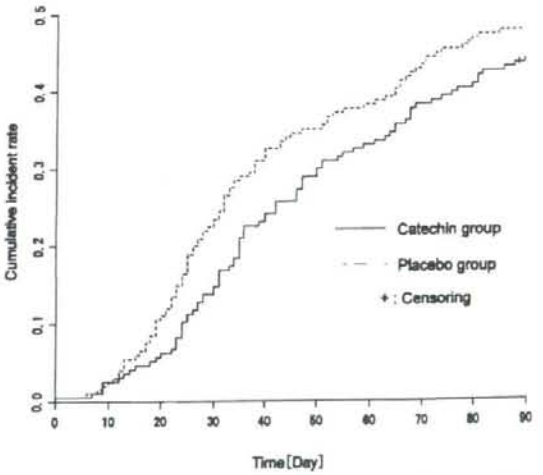
Table 1 Baseline characteristics of the participants in the group gargling with tea catechin extracts solution (catechin group) and the placebo (control) group*

	Catechin group (N=200)	Control group (N=204)	p value
Age (years)	39.6±11.4	40.2±11.5	0.5849
Men/Women	36/164	52/152	0.0718
Body mass index†	21.1±2.77	21.5±2.77	0.1476
Smoking (+/past/−)	39/12/149	47/14/143	0.6147
Alcohol (+/past/−)	117/4/79	114/1/89	0.3080
Tea drinking (mL/day)	607±333	591±341	0.6471
Taking health foods (+/−)	33/167	41/159	0.5820

*Plus-minus values are means±SD. There were no significant differences between the groups.
†The body mass index is the weight in kilograms divided by the square of the height in meters.



Kaplan-Meier estimate [95% confidence interval] of cumulative incident rate

	Catechin group	Placebo group
Cumulative incident rate at 30 day	0.1341[0.0851-0.1820]	0.2350[0.1763-0.2938]
Cumulative incident rate at 60 day	0.3270[0.2607-0.3933]	0.3820[0.3144-0.4495]
Cumulative incident rate at 90 day	0.4374[0.3662-0.4923]	0.4790[0.4094-0.5486]

p-value: 0.1720[Generalized Wilcoxon test]

Fig. 2 Kaplan-Meier incidence curves of influenza or upper respiratory tract infections according to intervention

The incidence rate of influenza infection in the catechin group (1.0%, 2 participants) was half that in the placebo group (2.0%, 4 participants), but no significant difference was observed between the two groups. The incidence rate of upper respiratory infections was also not significantly different between the two groups (48.2%, 94 participants in the catechin group vs. 51.5%, 103 participants in the placebo group). Figure 2 shows the cumulative incidence-free time curves of influenza or upper respiratory infections in each group. The incidence-free time was not significantly different (p value, 0.1720). As determined by the Kaplan-Meier method, the cumulative incidence rate at 30, 60, and

Table 2 The comparison of the incidence rates of influenza and respiratory tract infection, severity and duration of the respiratory tract infection between the group gargling with tea catechin extract solution (catechin group) and the placebo (control) group

	Catechin group (N=195)	Control group (N=200)	p value
Influenza illness	2 (1.0%)	4 (2.0%)	0.8423
Influenza-like illness	1	1	
Upper respiratory infections	94 (48.2%)	103 (51.5%)	0.4515
(1 time)	64	76	
(2 times)	26	20	
(3 times)	4	7	
Severity (total Jackson scores)			0.8934
(1-5)	31	37	
(6-10)	44	46	
(10<)	19	20	
Duration			
Median (Quartile)	7 (11)	9 (10)	0.1886
(Min.- Max.)	(2-55)	(2-41)	

90 days was 23.5%, 38.2%, and 47.9%, respectively, in the placebo group. On the other hand, this value was 13.4%, 32.7%, and 43.7% respectively, in the catechin group. The severity of the symptoms and duration of the cold among incident cases did not differ significantly between the two groups (Table 2).

In each group, there were no severe complications such as bronchopneumonia or encephalitis and no cases that required hospital admission. No significant differences in job absence were observed between the groups. The interventions were well tolerated by the participants. Only 2 participants experienced an adverse event, i.e., mild throat irritation. The frequency of throat irritation was similar in the 2 groups (0.5% in each group), and the symptoms disappeared after discontinuation of the gargling despite the continued consumption of tea. No serious adverse events such as respiratory tract irritation and obstruction or allergic bronchial spasm were observed during the study.

Discussion

The present study is the first randomized, double-blind, placebo-controlled study to investigate the effects of gargling with tea catechin extracts on the prevention of influenza infection in healthy adults. Contrary to a previous report on elderly nursing home residents[19], we could not confirm that the positive effects of catechin extracts on the prevention of influenza infection in healthy adults inoculated with the influenza vaccine. However, the discrepancy in the results of the two studies might be due to the low incidence rate of influenza infection in our study (1.5%) that was insufficient to obtain the required statistical power. In a typical epidemic season, approximately 5%-15% of adults and children develop symptomatic influenza[21]. The 2005-2006 season that we studied was interpandemic in the United States as well as in Japan, and no remarkable pandemic outbreak occurred also in the study areas[22,23]. Further, the selection of healthy adults inoculated with influenza vaccine lowered the frequency of influenza infection. The rate of influenza infection is higher in people not vaccinated against the influenza virus and in children, elderly, or immunosuppressed people[24,25]. Therefore, it can be speculated that the incidence of influenza infection is higher if the study population comprised people who are highly vulnerable to influenza, and hence, the results would vary.

In experimental studies, catechins bind to the hemagglutinin of the influenza virus, and they inhibit viral adsorption to Madin-Darby canine kidney (MDCK) cells ; these results provide an insight into the mechanisms by which tea catechin extracts inhibit the influenza virus[9-14]. Although evidence from basic experiments is accumulating, data from randomized, controlled clinical trials

that are linked to the basic experimental results are not yet established. Further studies are required to clarify the effects of tea catechins in humans in order to recommend their use against influenza infection.

As shown in the results, we did not observe any significant effects of catechin extracts on the prevention of upper respiratory tract infections, although the cumulative incidence rate in the catechin group was slightly lower than that in the control group. In Japan, gargling is generally recommended as a preventive modality for upper respiratory tract infections[26]. Recently, Satomura et al reported that mere gargling with water was effective in preventing upper respiratory tract infections compared to the usual hygienic care[27]. Therefore, it should be considered that gargling itself has a placebo effect which is similar to that of gargling with tea catechin extracts.

Tea catechins are reported to be well tolerated, except in tea factory workers with occupational asthma induced by the inhalation of green tea dust[28-30]. During the three months of gargling, no serious side effects were observed in the participants, except for throat irritation in 0.5% of the participants in each group. The symptoms disappeared after discontinuation of gargling despite the continued consumption of tea. Therefore, the adverse effects were believed to be related to the gargling itself, not to catechins.

In summary, we could not find significant effects of gargling with tea catechin on the prevention of influenza or upper respiratory tract infections in the healthy adults who had been inoculated with the influenza vaccine. However, the effect on more susceptible groups, i.e., those not vaccinated against influenza, children, elderly, or immunosuppressed people remain inconclusive.

Acknowledgments

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EFFECTS OF SAW PALMETTO EXTRACT ON MICTURITION REFLEX OF RATS AND ITS AUTONOMIC RECEPTOR BINDING ACTIVITY

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ABSTRACT

Purpose: We examined the effects of saw palmetto extract (SPE) on the rat micturition reflex and on autonomic receptors in the lower urinary tract.

Materials and Methods: The effect of SPE was examined on cystometrograms of anesthetized rats induced by intravesical infusion of saline or 0.1% acetic acid. SHR/NDmc-cp (cp/cp) rats received repeat oral administration of SPE and nighttime urodynamic function was determined. The autonomic receptor binding activity of SPE in the rat bladder and prostate was examined by radioligand binding assay.

Results: Intraduodenal administration of SPE (60 mg/kg) in anesthetized rat cystometry caused a significant increase in the micturition interval, micturition volume and bladder capacity during intravesical saline infusion. Also, similar administration of SPE at doses of 12 and 20 mg/kg significantly reversed the shortened micturition interval as well as the decreased micturition volume and bladder capacity due to 0.1% acetic acid infusion in a dose dependent manner. In conscious SHR/NDmc-cp (cp/cp) rats repeat oral administration of SPE (6 mg/kg daily) constantly increased the micturition interval and concomitantly decreased voiding frequency. SPE inhibited specific binding of [^{3}H]NMS ([N-methyl-^{3}H]scopolamine methyl chloride) (bladder) and [^{3}H]prazosin (prostate) with IC_{50} values of 46.1 and 183 μg/ml, respectively.

Conclusions: SPE significantly alleviates urodynamic symptoms in hyperactive rat bladders by increasing bladder capacity and subsequently prolonging the micturition interval. Our data may support the clinical efficacy of SPE for the treatment of lower urinary tract symptoms.

KEY WORDS: bladder; urodynamics; Permixon; rats, Sprague-Dawley

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS) are common disorders in aging men. The 2 main forms of internationally accepted medical treatment are inhibitors of 5α-reductase, such as finasteride or α_1-adrenoceptor antagonists, with the latter being more effective.[1] Nevertheless, plant extracts are widely used for the treatment of BPH and related LUTS. In fact, phytotherapeutic agents, including saw palmetto extract (SPE), are popular in many European countries, where herbal remedies represent up to 80% of all drugs prescribed for these disorders.[2] Debruyne et al reported that Permixon (Pierre Fabre Médicament, Castres, France), a lipid-sterolic extract of SPE, and α_1-blocker were equivalent for the medical treatment of LUTS in men with BPH during 12 months.[3]

Numerous mechanisms of SPE have been proposed, including 5α-reductase inhibition, and antiandrogenic, antiproliferative, anti-inflammatory and anti-edema effects.[4] However, most pharmacological effects were observed at relatively high doses of SPE.[5,6] Thus, none of these mechanisms for SPE has ever convincingly been demonstrated to be therapeutically relevant in vivo.[7,8] We noted the relatively potent smooth muscle relaxant activity by SPE observed in isolated tissues such as the bladder of guinea pigs and rats.[9] However, to our knowledge the smooth muscle relaxation of this extract in the lower urinary tract function has not been previously verified by in vivo pharmacology. Therefore, in this study we clarified the effect of SPE on urodynamic functions in anesthetized rat cystometry, and in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats. Our data demonstrate that SPE is effective for improving the hyperactive bladder response of rats, suggesting the clinical efficacy of this extract for LUTS in patients with overactive bladder. Also, this extract has been shown to bind to autonomic receptors in the rat lower urinary tract.

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MATERIALS AND METHODS

Materials. SPE (Serenoa repens purified extract) was suspended in 0.5% methyl cellulose. [^{3}H]NMS ([N-methyl-^{3}H]scopolamine methyl chloride) (3.03 TBq/mmol) and [^{3}H]prazosin (2.98 TBq/mmol) were purchased from PerkinElmer Life Sciences, Inc., Boston, Massachusetts. All other chemicals were purchased from commercial sources.

Animals. Male Sprague-Dawley rats (SLC Co., Ltd., Hamamatsu, Japan) weighing about 250 to 350 gm were used. Also, the male subline of spontaneously hypertensive/National Institutes of Health corpulent rats [SHR/NDmc-cp (cp/cp)] (SLC Co., Ltd.) was also used. They were housed in the laboratory with free access to food and water, and maintained on a 12-hour dark/light cycle in a room with controlled temperature (about 20C to 26C) and humidity (about 30% to 70%).

Cystometry. The procedure of cystometry in anesthetized rats was performed as previously described.[10] Rats were anesthetized by subcutaneous injection of urethane (1.0 gm/

kg) and the duodenum was cannulated for the administration of SPE. The bladder was exposed through a short midline incision. It was cannulated with polyethylene tubing (PE-50, Clay-Adams, Parsippany, New Jersey) that was heat flared at the end, inserted into the bladder dome and ligated. Using a T connector the bladder catheter was connected to a pressure transducer (DX-360, Nihon Kohden, Tokyo, Japan) and to an infusion pump (STC-521, Terumo Corp., Tokyo, Japan). Saline or 0.1% acetic acid maintained at 37C at a rate of 4.0 ml per hour was instilled into the bladder of rats and urodynamic parameters were monitored by cystometry recording. Voided urine was cumulatively collected into a urine cup containing water that was placed on a microbalance (HF-200, A and D Co., Ltd., Tokyo, Japan). Analog voltage from the microbalance was received by a PowerLab/8sp (AD Instruments Pty Ltd., Castle Hill, New South Wales, Australia) and processed by MacLab8s proprietary client software, version 3.3 (Apple, Sunnyvale, California). After a stable cystometrogram was obtained urodynamic parameters were measured, including maximum micturition pressure, baseline pressure, threshold pressure, micturition interval, mean micturition volume and bladder capacity (infusion rate × micturition interval). The pretreatment period was considered the 20 minutes of saline or 0.1% acetic acid infusion before SPE administration. The effects of SPE (6 to 60 mg/kg) were analyzed 20 to 40 minutes after intraduodenal administration.

Frequency-volume charts. SHR/NDmc-cp (cp/cp) rats were administered SPE (6 mg/kg) orally from 3:00 to 5:00 p.m. for 14 days. The frequency-volume chart was continuously monitored from 6:00 p.m. to 6:00 a.m. on the next day. Voided urine was cumulatively collected into a urine cup containing liquid paraffin that was placed on a microbalance. Analog voltage from the microbalance was received by a PowerLab/8sp and processed by MacLabs8s. Voiding frequency and mean voided volume for 12 hours was calculated by proprietary client software. Water intake was measured before and during the oral administration of SPE.

Binding assay of [^{3}H]NMS and [^{3}H]prazosin in rat tissues. Binding assays of muscarinic and α_1-adrenoceptor receptors were performed using [^{3}H]NMS[11] and [^{3}H]prazosin, respectively. Rats were exsanguinated by taking the blood from the descending aorta under temporary anesthesia with diethyl ether and the tissues were then perfused with cold saline from the aorta. The bladder and prostate were then dissected and the tissues were minced with scissors. For the [^{3}H]NMS binding assay the bladder was homogenized using a Polytron homogenizer (Kinematica AG, Lucerne, Switzerland) in 19 volumes of ice-cold 30 mM Na^+/HEPES buffer (pH 7.5). The homogenate was then centrifuged at 40,000 × gravity for 20 minutes at 4C. The resulting pellet was finally resuspended in ice-cold buffer. For the [^{3}H]prazosin binding assay the prostate was homogenized in 30 volumes of ice-cold 50 mM tris-HCl buffer (pH 7.5). The homogenate was then centrifuged at 40,000 × gravity for 20 minutes at 4C. After suspension in cold buffer the pellet was centrifuged further at 40,000 × gravity for 20 minutes at 4C and the resulting pellet was finally resuspended in cold buffer.

In the presence of various concentrations (10 to 300 μg/ml) of SPE rat bladder and prostate homogenates were incubated with [^{3}H]NMS (456 pM) and [^{3}H]prazosin (516 pM) in 30 mM Na^+/HEPES buffer (pH 7.5) and 50 mM tris-HCl buffer (pH 7.5), respectively. Incubation was done for 60 ([^{3}H]NMS) and 30 ([^{3}H]prazosin) minutes at 25C. The reaction was terminated by rapid filtration (Cell Harvester, Brandel Co., Gaithersburg, Maryland) through GF/B glass fiber filters (Whatman, Brentford, United Kingdom) and the filters were rinsed 3 times with 3 ml ice-cold buffer. Tissue bound radioactivity was extracted from the filters by placing them overnight by immersion in scintillation fluid and radioactivity was then determined. Specific binding of [^{3}H]NMS and [^{3}H]prazosin was determined experimentally from the difference between counts in the absence and presence of 1 μM atropine and 10 μM phentolamine, respectively.

Data analysis. Cystometry and frequency-volume chart data are expressed as the mean ± SEM and analyzed by Student's paired t test. The binding activities of SPE to muscarinic and α_1-adrenocetors were estimated by IC_{50} values, which are the concentrations of SPE necessary to displace 50% of the specific binding of [^{3}H]NMS and [^{3}H]prazosin, as determined by log probit analysis. Statistical significance was considered at $p < 0.05$.

RESULTS

Effects of single administration on cystometric parameters. Figure 1 shows that reproducible micturition patterns were obtained throughout the cystometry period in anesthetized rats. In saline treated rats the intraduodenal administration of vehicle had little effect on urodynamic parameters (see table, fig. 1, *A*). These urodynamic parameters in saline infused rats were unaffected by the intraduodenal administration of SPE at the 6 mg/kg dose but the higher 60 mg/kg dose of SPE exerted a significant increase (40.5% to 42.6%) in micturition interval, mean micturition volume and bladder capacity as well as a significant decrease (8.1%) in maximum micturition pressure (see table, fig. 1, *B*). SPE (6 and 60 mg/kg) had little effect on baseline and threshold bladder pressure.

Following the intravesical infusion of saline containing 0.1% acetic acid in anesthetized rats compared with saline infusion alone the micturition interval (6.22 ± 0.57 vs 8.64 ± 0.73 minutes in 10 and 12, respectively) was significantly shortened (28.0%, $p < 0.05$). Mean micturition volume (0.60 ± 0.05 vs 0.42 ± 0.04 ml) and bladder capacity (0.57 ± 0.05 vs 0.42 ± 0.04 ml) were also significantly decreased (30.0% and 26.3%, respectively, $p < 0.05$).

Following the intraduodenal administration of SPE at doses of 12 and 20 mg/kg compared to results in 0.1% acetic acid treated rats there were significant and dose dependent increases in the micturition interval (15.1% vs 47.7%), mean micturition volume (15.9% vs 65.0%) and bladder capacity (14.0% vs 47.5%) (see table, fig. 1, *C*). In extract administered rats baseline pressure and maximum micturition pressure were slightly but significantly decreased but the effect was not dose related. Threshold pressure in rats was unaffected by SPE administration.

Effects of repeat administration on urodynamic parameters. The effect of repeat oral administration of SPE on urodynamic parameters in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats was examined by recording nighttime micturition frequency-volume charts. Following the oral administration of a relatively low dose of SPE (6 mg/kg) in SHR/NDmc-cp (cp/cp) rats the micturition interval was continuously increased during repeat treatment for 3 to 8 days after the initial transient decrease compared with pretreatment values (46.0 ± 2.3 minutes in 6, fig. 2, *A*). Concomitantly voiding frequency in these rats constantly decreased after the initial transient increase following SPE administration (fig. 2, *C*). On the other hand, the 2 urodynamic parameters during treatment for 9 to 14 days were comparable with pretreatment values. Mean micturition volume tended to constantly increase during oral SPE administration (fig. 2, *B*). Also, total micturition volume was significantly increased for 9 to 14 days after the start of SPE administration (fig. 2, *D*). Water intake was little changed by repeat SPE administration (data not shown).

Effects on bladder muscarinic and prostatic α_1-adrenergic receptors. SPE at concentrations of 10 to 300 μg/ml competed with [^{3}H]NMS and [^{3}H]prazosin for binding sites in the rat bladder and prostate, respectively, in a concentration dependent manner (fig. 3). SPE IC_{50} values of [^{3}H]NMS and [^{3}H]prazosin binding were 46.1 ± 5.6 μg/ml in 7 preparations

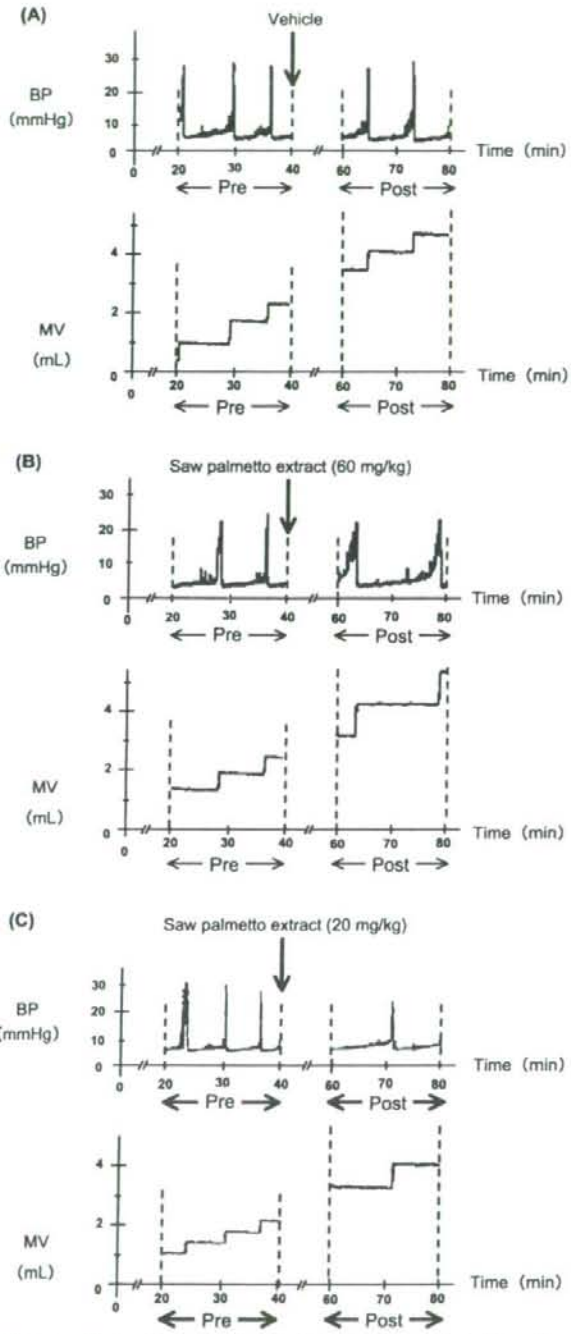

FIG. 1. Effects of intraduodenal administration of 6 to 60 mg/kg SPE on bladder pressure (*BP*) and micturition volume (*MV*) on cystometrograms of saline (*A* and *B*) and or 0.1% acetic acid (*C*) infused anesthetized rats. There were spontaneous contractions during bladder filling. Arrow indicates intraduodenal administration of 0.5% methyl cellulose vehicle (*A*), and 60 mg/kg (*B*) and 20 mg/kg (*C*) SPE. *Pre*, pretreatment. *Post*, posttreatment.

and 183 ± 30 μg/ml in 4, respectively. The SPE IC_{50} value of bladder [^{3}H]NMS binding was significantly lower than that of prostatic [^{3}H]prazosin binding (¼, $p < 0.001$).

DISCUSSION

The intraduodenal administration of SPE at the 60 mg/kg dose significantly increased bladder capacity in anesthetized rats, resulting in a prolonged micturition interval and enhanced mean micturition volume. It is well known that hyperreflexia manifested by enhanced voiding frequency is evoked by stimulating sensory afferents with dilute acetic acid solution in the cystometry infusate.[10,11] Also, in the current study continuous intravesical infusion of 0.1% acetic acid in anesthetized rats produced a hyperactive bladder response, which was characterized by a decrease in bladder capacity and mean micturition volume with a concomitant shortening of the micturition interval. It was evident that such a hyperactive bladder response due to acetic acid infusion was significantly attenuated by relatively low doses (12 and 20 mg/kg) of SPE given intraduodenally in a dose dependent manner. In fact, the values of these urodynamic parameters on rat cystometry after SPE administration at the 20 mg/kg dose were almost identical to control values in saline infused rats without acetic acid.

It should be noted that the intensity of the enhanced micturition interval, mean micturition volume and bladder capacity by SPE at the intraduodenal dose of 20 mg/kg in acetic acid infused rats was almost equivalent to that at the 60 mg/kg dose in saline infused rats (see table). The interpretation of such results is that the hyperactive bladder may be 3-fold more susceptible to SPE than the normal bladder. Thus, it is notable that hyperactive rather than normal bladder appears to be more sensitive to SPE. At any rate the current study may provide the first in vivo functional evidence to suggest that SPE significantly improves bladder instability.

Although the precise mechanism by which SPE attenuates a hyperactive bladder response in acetic acid infused rats remains to be clarified, there are several indications that significantly suppress smooth muscle tone. Previous pharmacological studies have demonstrated the spasmolytic effects of SPE on different isolated smooth muscle organs, such as the ileum, bladder, aorta and uterus in guinea pigs and rats.[9] These investigators speculated that the inhibitory effects of SPE on agonist or KCl induced smooth muscle contractions are mediated at least in part via the antagonistic effects on α-adrenoceptors, muscarinic cholinoceptors and calcium channels with the possible involvement of other activities. In fact, Goepel et al noted that SPE displaced α_1-adrenoceptor radioligands to bind to human prostatic and cloned human α_1-adrenoceptors in a noncompetitive manner and concomitantly suppressed agonist induced formation of [^{3}H]-inositol phosphate.[12] Furthermore, Noronha-Blob et al observed in in vitro and in vivo functional studies that anticholinergic activity rather than spasmolytic or local anesthetic activity of smooth muscle relaxants may have an important role in prevailing therapies for the suppression of bladder hyperactivity.[13] Also, it is worth noting that a relatively low concentration of SPE competed with bladder [^{3}H]NMS binding sites as well as prostatic [^{3}H]prazosin binding sites in a concentration dependent manner and binding activity was significantly (4 times) greater to muscarinic receptors than to α_1-adrenoceptors (fig. 3). Thus, it seems likely that SPE increases bladder capacity, possibly via antimuscarinic and/or spasmolytic effects on bladder smooth muscle, resulting in increased micturition volume and a prolonged micturition interval. Nevertheless, it cannot be ruled out that SPE may have some additional peripheral or central neuronal effects because bladder instability may possibly arise not only from a myogenic, but also from a neurogenic mechanism.[14]

We further examined the urodynamic effect of repeat oral administration of SPE in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats. The SHR/NDmc-cp (cp/cp) strain is a strain of spontaneously hypertensive/National Institutes of

Effects of intraduodenal SPE administration (6 to 60 mg/kg) on urodynamic parameters in cystometrograms of saline and 0.1% acetic acid infused anesthetized rats

	Mean Saline ± SE (mg/kg SPE)		Mean Acetic Acid ± SE (mg/kg SPE)	
	6	60	12	20
Micturition interval (mins):				
Pretreatment	9.93 ± 1.07	7.71 ± 0.90	6.44 ± 1.10	5.99 ± 0.47
Posttreatment	9.07 ± 1.09	10.83 ± 1.77*	7.41 ± 1.25*	8.85 ± 1.07*
Mean micturition vol (ml):				
Pretreatment	0.69 ± 0.07	0.54 ± 0.06	0.44 ± 0.08	0.40 ± 0.04
Posttreatment	0.66 ± 0.09	0.77 ± 0.13*	0.51 ± 0.08*	0.66 ± 0.08*
Bladder capacity (ml):				
Pretreatment	0.65 ± 0.06	0.51 ± 0.06	0.43 ± 0.07	0.40 ± 0.03
Posttreatment	0.60 ± 0.07	0.72 ± 0.12*	0.49 ± 0.08*	0.59 ± 0.07*
Baseline pressure (mm Hg):				
Pretreatment	3.35 ± 0.19	3.17 ± 0.10	3.99 ± 0.26	3.54 ± 0.16
Posttreatment	3.20 ± 0.22	2.93 ± 0.21	3.69 ± 0.23	3.37 ± 0.11*
Threshold pressure (mm Hg):				
Pretreatment	5.02 ± 0.13	4.74 ± 0.16	5.06 ± 0.31	4.44 ± 0.19
Posttreatment	4.78 ± 0.13	4.81 ± 0.15	5.13 ± 0.27	4.62 ± 0.25
Max micturition pressure (mm Hg):				
Pretreatment	26.1 ± 2.2	28.3 ± 0.8	29.8 ± 1.7	25.6 ± 1.4
Posttreatment	23.9 ± 2.0	26.0 ± 0.7*	25.7 ± 2.0†	23.0 ± 0.5

Values in 5 to 7 rats.
* Significantly different vs pretreatment (Student's paired t test $p < 0.05$).
† Significantly different vs pretreatment (Student's paired t test $p < 0.01$).

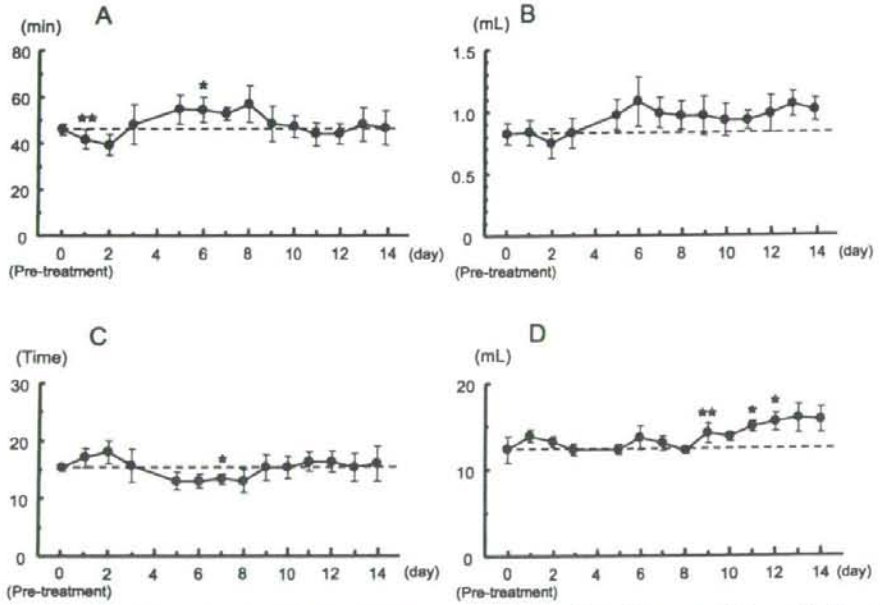

FIG. 2. Effects of repeat SPE oral administration on nighttime urodynamic function from 6:00 p.m. to 6:00 a.m., that is micturition interval (*A*), mean micturition volume (*B*), voiding frequency (*C*) and total micturition volume (*D*), in conscious and unrestrained SHR/NDmc-cp (cp/cp) rats that received 6 mg/kg SPE orally daily for 14 days. Point represent mean ± SE of 3 SHR/NDmc-cp (cp/cp) rats. Dotted lines indicate pretreatment values. Single asterisk indicates significantly different vs pretreatment (Student's paired t test $p < 0.05$). Double asterisks indicate significantly different vs pretreatment (Student's paired t test $p < 0.01$).

Health corpulent rat as a model of type 2 diabetic nephropathy. SHR/NDmc-cp (cp/cp) rats showing significant hypertension retain a common genetic background with nondiabetic SHR rats.[15] Adrenergic innervation is generally increased in the SHR strain but SHR bladders are hyperactive relative to Wistar-Kyoto rat bladders.[16] Clemow et al reported that with an increase in adrenergic efferent innervation there is an increase in sensory afferent innervation in SHR bladders.[17] Their data suggest that the increase in sensory afferent nerve density may underlie an enhanced voiding reflex. It was shown that repeat oral administration of SPE (6 mg/kg daily) in conscious SHR/NDmc-cp (cp/cp) rats for 8 days produced a transient shortening and subsequent sustained prolongation of the micturition interval and concomitantly SPE administration decreased voiding frequency. Thus, it is likely that repeat SPE administration alleviates hyperactivity of the SHR bladder, as observed by a single intraduodenal administration of this extract on anesthetized rat cystometry. However, such urodynamic improvement of SPE in SHR/NDmc-cp (cp/cp) rats apparently disappeared after longer repeat administration more than 9 days in duration. The clear explanation for this result is lacking but it may be in part attributable to the significant increase in total micturition volume associated with extract administration for

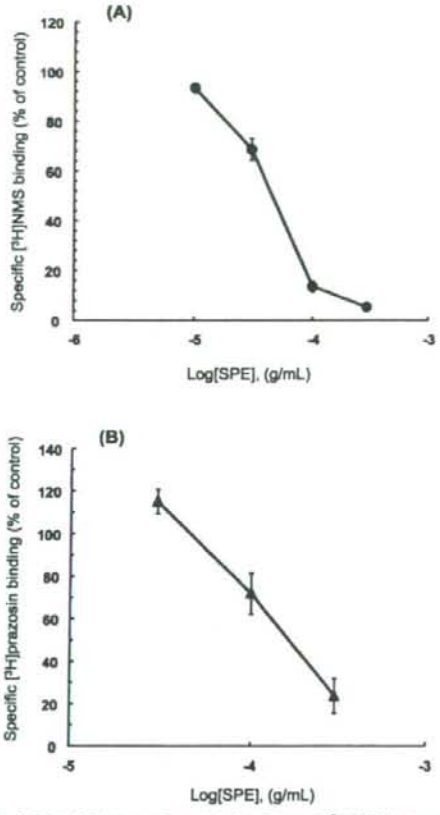

FIG. 3. SPE inhibition of specific binding of [^{3}H]NMS in rat bladder (*A*) and [^{3}H]prazosin in prostate (*B*). Specific binding of [^{3}H]NMS (456 pM) and [^{3}H]prazosin (516 pM) in homogenates of rat bladder and prostate, respectively, was measured in absence and presence of 3 or 4 concentrations (10 to 300 μg/ml) of SPE. Points represents mean ± SE in 4 ([^{3}H]prazosin) or 7 ([^{3}H]NMS) rats.

more than 9 days. To our knowledge basic pharmacological evidence for the diuretic effect of SPE has not been reported previously but a mild diuretic effect in humans has been described.[18] However, until the pharmacological effects of SPE on lower urinary tract function are definitely known we should continue to interpret variable urodynamic effects of this extract in conscious SHR/NDmc-cp (cp/cp) rats with caution.

In the majority of men with BPH and LUTS the major treatment goal is the relief of irritative and obstructive symptoms. These symptoms have been shown to be significantly alleviated by phytotherapeutic agents, including SPE.[3,19] Our data support this clinical usefulness.

CONCLUSIONS

SPE may improve urodynamic symptoms in hyperactive rat bladders by increasing bladder capacity and prolonging the micturition interval. In addition, SPE contains the constituent(s) exerting the binding activities of muscarinic and α_1-adrenergic receptors in the lower urinary tract. Thus, our data may support the clinical efficacy of SPE for the treatment of LUTS accompanying overactive bladder.

SHR/NDmc-cp (cp/cp) rats were established at Disease Model Cooperative Research Association, Kyoto, Japan. Serenoa repens purified extract was provided by Indena Japan Co., Ltd., Tokyo, Japan. K. Sakakura provided technical assistance.

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Muscarinic and Alpha 1-Adrenergic Receptor Binding Characteristics of Saw Palmetto Extract in Rat Lower Urinary Tract

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OBJECTIVES To elucidate the in vitro and ex vivo effects of saw palmetto extract (SPE) on autonomic receptors in the rat lower urinary tract.

METHODS The in vitro binding affinities for alpha 1-adrenergic, muscarinic, and purinergic receptors in the rat prostate and bladder were measured by radioligand binding assays. Rats received vehicle or SPE (0.6 to 60 mg/kg/day) orally for 4 weeks, and alpha 1-adrenergic and muscarinic receptor binding in tissues of these rats were measured.

RESULTS Saw palmetto extract inhibited specific binding of [^{3}H]prazosin and [N-methyl-^{3}H]scopolamine methyl chloride (NMS) but not alpha, beta-methylene adenosine triphosphate [2,8-^{3}H]tetrasodium salt in the rat prostate and bladder. The binding activity of SPE for muscarinic receptors was four times greater than that for alpha 1-adrenergic receptors. Scatchard analysis revealed that SPE significantly reduced the maximal number of binding sites (B_{max}) for each radioligand in the prostate and bladder under in vitro condition. Repeated oral administration of SPE to rats brought about significant alteration in B_{max} for prostatic [^{3}H]prazosin binding and for bladder [^{3}H]NMS binding. Such alteration by SPE was selective to the receptors in the lower urinary tract.

CONCLUSIONS Saw palmetto extract exerts significant binding activity on autonomic receptors in the lower urinary tract under in vitro and in vivo conditions. UROLOGY 69: 1216–1220, 2007. © 2007 Elsevier Inc.

In many European countries phytotherapeutic agents, including saw palmetto extract (SPE), are widely used for the treatment of benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS), and these herbal remedies represent up to 80% of all drugs prescribed for these disorders.[1,2] Some clinical trials support the efficacy of SPE in improving symptoms associated with BPH and LUTS.[3–5]

Numerous mechanisms of SPE have been proposed, including inhibition of 5 alpha-reductase, antiandrogenic effects, antiproliferative effects, anti-inflammatory effects (inhibition of cyclo-oxygenase and lipoxygenase), and anti-edema effects.[6] However, these pharmacologic actions of SPE were observed by using relatively high concentrations or large dosages.[7,8] Therefore, it is uncertain whether the reported modes of action of SPE are therapeutically relevant.[3,4] Gutierrez *et al.*[9] showed relatively potent smooth muscle relaxant activity by SPE in isolated tissues, such as the urinary bladder of guinea pigs and rats. Furthermore, our recent study revealed that intraduodenally administered SPE significantly improves urodynamic symptoms in hyperactive bladder of rats by increasing bladder capacity and prolonging micturition interval.[10] Such improvement of urodynamic symptoms by SPE has been suggested to arise partly from its significant antagonism of autonomic receptors in the lower urinary tract.[10,11] The aim of this study was to clarify the in vitro and ex vivo effects of SPE on autonomic (alpha 1-adrenergic, muscarinic, and purinergic) receptors involved in lower urinary function.

MATERIAL AND METHODS

Materials

Saw palmetto extract (*Serenoa repens* purified extract) was provided by Indena Japan (Tokyo, Japan). Saw palmetto extract was obtained with hypercritical CO_2 (SABAL SELECT, Indena S.p.A., Milan, Italy). This extract contained high-molecular-weight compounds, which are esters of long chain alcohol with fatty acid. Saw palmetto extract was suspended in 0.5% methylcellulose. [N-methyl-^{3}H]scopolamine methyl chloride ([^{3}H]NMS, 2.997 TBq/mmol), [7-methoxy-^{3}H]prazosin (2.979

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TBq/mmol), (+)-[^{3}H]PN 200-110 (3.180 TBq/mmol), and alpha, beta-methylene adenosine triphosphate [2,8-^{3}H]tetrasodium salt ([^{3}H]alpha, beta-MeATP, 580.9 GBq/mmol) were purchased from PerkinElmer Life Sciences (Boston, Mass). All other chemicals were purchased from commercial sources.

Animals and Administration of SPE

Male Sprague-Dawley rats weighing 250 to 350 g were purchased from SLC (Hamamatsu, Japan). Rats were housed with a 12-hour light–dark cycle and fed laboratory food and water ad libitum. Rats received SPE (0.6, 6, and 60 mg/kg/day) by gavage once per day for 4 weeks. The control animals received vehicle alone. The repeated SPE administration had little effect on the eating and drinking behaviors and on the body weight of rats. This study was done according to guidelines approved by the experimental animal ethics committee of the University of Shizuoka.

Binding Assay of [^{3}H]Prazosin, [^{3}H]NMS, and [^{3}H]Alpha, Beta-MeATP

The radioligand binding assays for alpha 1-adrenergic, muscarinic, and purinergic receptors were performed using [^{3}H]prazosin,[12] [^{3}H]NMS,[13] and [^{3}H]alpha, beta-MeATP,[14] respectively. The bladder, prostate, submaxillary gland, heart, and spleen were dissected from rats under temporary anesthesia, and the tissues were minced with scissors. In the case of binding assays of [^{3}H]prazosin, each tissue was homogenized by a Kinematica Polytron homogenizer (Kinematica, PT 10-35) in ice-cold 50 mmol/L Tris-HCl buffer (pH 7.5). The homogenates were then centrifuged at 40,000*g* for 20 minutes at 4°C. The pellet, after suspension in the cold buffer, was centrifuged further at 40,000*g* for 20 minutes at 4°C, and the resulting pellet was finally resuspended in the cold buffer to use in the radioligand binding assay. In the case of [^{3}H]NMS binding assay, the bladder and submaxillary gland were similarly homogenized in 30 mmol/L Na^+/N-(2-hydroxyethyl)-piperazine-N'-2-ethanesulfonic acid (pH 7.5). The homogenate was then centrifuged at 40,000*g* for 20 minutes at 4°C. The suspension of the resulting pellet was used. In the case of [^{3}H]alpha, beta-MeATP binding assay, the bladder homogenate in 50 mmol/L Tris/HCl buffer was centrifuged at 2000*g* for 10 minutes at 4°C. The pellet suspension was further centrifuged at 2000*g* for 10 minutes at 4°C, and the supernatant was combined with the original supernatant. The supernatant was centrifuged at 48,000*g* for 20 minutes at 4°C, and the suspension of the resulting pellet was used.

In in vitro displacement experiments, the rat tissue homogenates were incubated with [^{3}H]prazosin (500 pmol/L, prostate and spleen), [^{3}H]NMS (460 pmol/L, bladder and submaxillary gland), and [^{3}H]alpha, beta-MeATP (4.5 nmol/L, bladder) in the presence of various concentrations (10 to 1000 μg/mL) of SPE. In in vitro and ex vivo saturation experiments, the rat tissue homogenates were incubated with [^{3}H]prazosin (0.03 to 0.5 nmol/L, prostate, submaxillary gland, heart, and spleen) and [^{3}H]NMS (0.06 to 1.0 nmol/L, bladder and submaxillary gland). Thus, Scatchard analysis with a full range of radioligands was conducted to estimate apparent dissociation constant (K_d) and maximal number of binding sites (B_{max}) values.

Incubation was carried out for 60 minutes at 25°C (4°C in [^{3}H]alpha, beta-MeATP). The reaction was terminated by rapid filtration through Whatman GF/B glass fiber filters, and the filters were rinsed with ice-cold buffer. Tissue-bound radioactivity was extracted from the filters by immersion in scintillation fluid, and radioactivity was determined by a liquid scintillation counter. Specific binding of [^{3}H]prazosin, [^{3}H]NMS, and [^{3}H]alpha, beta-MeATP was determined experimentally from the difference between counts in the absence and presence of 10 μmol/L phentolamine, 1 μmol/L atropine, and 3 μmol/L alpha, beta-MeATP, respectively.

Data Analysis

The K_d and B_{max} for radioligands were estimated by Scatchard analysis of the saturation data.[15] The binding activities of SPE to each receptor were estimated by IC_{50} values, which are the concentrations of SPE necessary to displace 50% of specific binding of each radioligand. Statistical analysis of the data was performed by a one-way analysis of variance followed by Dunnett's test for multiple comparisons.

RESULTS

In Vitro Effects on Alpha 1-Adrenergic, Muscarinic, and Purinergic Receptors

Saw palmetto extract (50 to 200 μg/mL) inhibited specific [^{3}H]prazosin binding in the prostate and spleen (Fig. 1A), and their IC_{50} values were 169 ± 24 and 188 ± 47 μg/mL, respectively. Similarly, SPE (10 to 1000 μg/mL) inhibited specific [^{3}H]NMS binding in the bladder and submaxillary gland of rats in a concentration-dependent manner (Fig. 1B), and the IC_{50} values were 40.0 ± 4.1 and 52.3 ± 4.4 μg/mL, respectively. Thus, the inhibitory effect by SPE of bladder [^{3}H]NMS binding was significantly (4.2 times, $P < 0.001$) more potent than that of prostatic [^{3}H]prazosin binding. It had little effect on bladder [^{3}H]alpha, beta-MeATP binding (Fig. 1C).

Scatchard analysis revealed that SPE (150 μg/mL) significantly reduced B_{max} values for specific [^{3}H]prazosin binding in the rat prostate (55.3%), compared with the corresponding control value (Table 1). Similarly, in the presence of SPE (50 μg/mL), there was a significant decrease of B_{max} values for specific [^{3}H]NMS binding in the bladder (55.2%). There was little change in K_d values for specific binding of each radioligand in rat tissues, except for a significant (32.0%) decrease of the K_d value for prostatic [^{3}H]prazosin binding.

Effects of Repeated Oral Administration of SPE on Autonomic Receptors

Repeated oral administration of SPE brought about a significant increase in B_{max} values for specific [^{3}H]prazosin binding in the prostate and spleen of rats, compared with the corresponding control values (Table 2). The enhancement in B_{max} values was significant in the prostate (23.6% and 36.7%, respectively) at the dose of 6 and 60 mg/kg/day and in the spleen (26.1%) at the dose of 60 mg/kg/day. Thus, the enhancement in B_{max} values in the prostate was exerted by a relatively low dose (6 mg/kg/day) of SPE. In the submaxillary gland and heart, there was little change in [^{3}H]prazosin binding parameters, except a significant (30.5%) increase in the heart at the dose of 60 mg/kg.

After repeated oral administration of SPE (0.6, 6, and 60 mg/kg/day), there was a significant (31.8% to 41.2%)

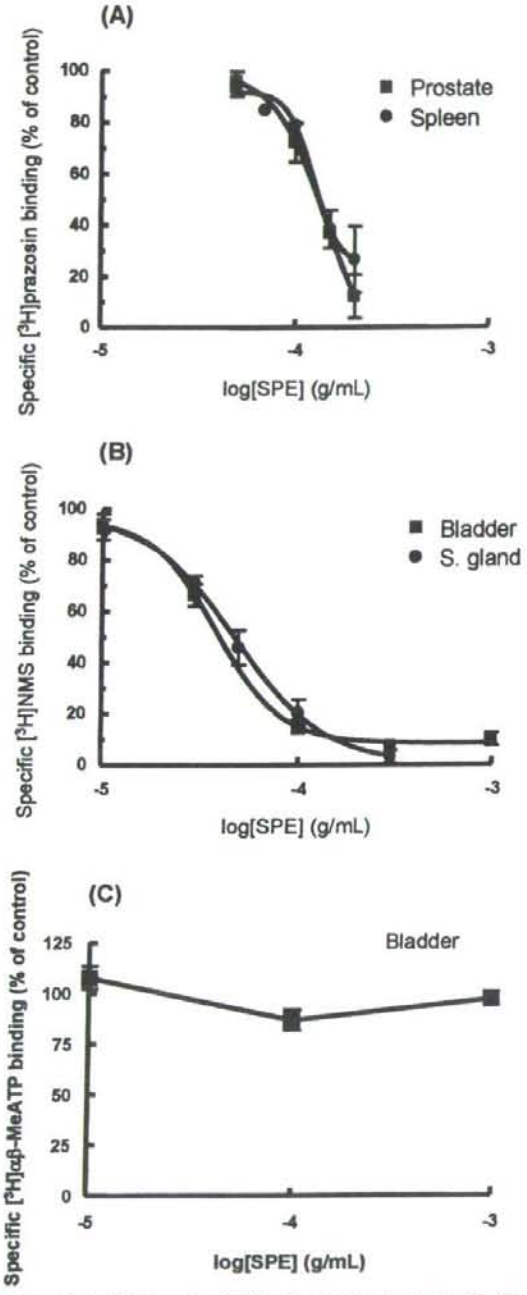

Figure 1. Inhibition by SPE of specific binding of **(A)** [^{3}H]prazosin, **(B)** [^{3}H]NMS, and **(C)** [^{3}H]alpha, beta-MeATP in rat tissues. Specific binding of [^{3}H]prazosin (500 pmol/L), [^{3}H]NMS (460 pmol/L), and [^{3}H]alpha, beta-MeATP (4.5 nmol/L) in rat tissues was measured in the absence and presence of four or five different concentrations (10 to 1000 μg/mL) of SPE. Each point represents the mean ± standard error of 5 to 8 ([^{3}H]prazosin), 8 to 9 ([^{3}H]NMS), and 3 ([^{3}H]alpha, beta-MeATP) determinations.

decrease of B_{max} values for specific [^{3}H]NMS binding in the rat bladder compared with the corresponding control value (Table 2). Similarly, the B_{max} value was significantly (17.9%) reduced in the submaxillary gland by SPE only at the high dose (60 mg/kg/day). Thus, the decrease of B_{max} values in the bladder compared with the submaxillary gland was seen by relatively low doses (0.6 and 6 mg/kg/day) of SPE. There was little change in K_d values for [^{3}H]NMS, except a slight (13.8%) increase in the submaxillary gland at the dose of 0.6 mg/kg.

COMMENT

In the majority of men with BPH and LUTS, the major goal of pharmacologic treatment is the relief of irritative and obstructive symptoms. These symptoms are effectively alleviated by alpha 1-adrenergic and muscarinic receptor antagonists. The in vitro and in vivo binding activities of SPE on autonomic receptors expressing in the rat lower urinary tract were examined. The administered oral doses of SPE were determined on the basis of in vivo pharmacologic doses in reducing detrusor contractility and number of micturition in rats.[10] In fact, it may be of pharmacologic significance that oral doses (0.6 to 60 mg/kg/day) of SPE were considerably lower than those previously reported for pharmacologic effects such as inhibition (100 to 640 mg/kg/day) of rat prostatic hyperplasia induced by hyperprolactinemia[16] and reduction (50, 100 mg/kg/day) of mast cell accumulation in the rat prostate.[17]

The in vitro experiment has shown that SPE inhibited specific binding of [^{3}H]prasozin and [^{3}H]NMS but not [^{3}H]alpha, beta-MeATP in the prostate, bladder, and other tissues of rats, in a concentration-dependent manner. According to IC_{50} values, the binding activity of SPE for muscarinic receptors was shown to be four times greater than that for alpha 1-adrenergic receptors. These receptor-binding affinities of SPE for autonomic receptors were nearly comparable to or greater than in vitro pharmacologic potencies of this extract (eg, inhibition of 5 alpha-reductase [IC_{50}: 71 μg/mL], anti-inflammatory effect [inhibition of cyclo-oxygenase and 5-lipoxygenase, IC_{50}: 28.1 and 18.0 μg/mL, respectively], and antiandrogenic effect [IC_{50}: 1004 μg/mL]) previously reported.[18,19] Furthermore, Scatchard analysis revealed that SPE caused a significant decrease of B_{max} values for specific binding of [^{3}H]prazosin and [^{3}H]NMS in the prostate and bladder of rats. Therefore, it could be presumed that SPE binds noncompetitively to alpha 1-adrenergic and muscarinic receptors in rat tissues.

To clarify whether SPE has some effects on neurotransmitter receptors under in vivo conditions, we investigated the effect of oral administration of SPE on autonomic receptors in the lower urinary tract of rats. Repeated oral administration of SPE produced a significant decrease of B_{max} values for specific [^{3}H]NMS binding in the rat bladder and submaxillary gland. Notably, such reduction in the number of [^{3}H]NMS binding sites was observed with relatively lower doses (0.6 and 6

Table 1. Effects of SPE on K_d and B_{max} for specific binding of [^{3}H]prazosin and [^{3}H]NMS in the rat prostate and bladder

Radioligands	Tissues	K_d (pmol/L)	B_{max} (fmol/mg protein)
[^{3}H]prazosin	Prostate		
	Control	59.2 ± 7.1	22.6 ± 1.8
	SPE (150 μg/mL)	40.3 ± 1.6*	10.1 ± 0.6†
[^{3}H]NMS	Bladder		
	Control	256 ± 77	192 ± 27
	SPE (50 μg/mL)	190 ± 14	86.1 ± 11.7*

SPE = saw palmetto extract; K_d = dissociation constant; B_{max} = maximal number of binding sites; [^{3}H]NMS = [N-methyl-^{3}H]scopolamine methyl chloride.
Values are mean ± standard error of 4 to 7 rats.
* $P < 0.01$ vs. control.
† $P < 0.05$ vs. control.

Table 2. K_d and B_{max} for specific binding of [^{3}H]prazosin and [^{3}H]NMS in rat tissues after repeated oral administration of SPE at doses of 0.6, 6, and 60 mg/kg

Organs	Dose (mg/kg)	K_d (pM)	B_{max} (fmol/mg protein)
Specific [^{3}H]prazosin binding			
Prostate	Control	49.7 ± 2.4	26.7 ± 0.5
	0.6	43.1 ± 2.0	30.1 ± 1.6
	6	51.9 ± 4.8	33.0 ± 1.3*
	60	54.6 ± 2.8	36.5 ± 1.2†
Submaxillary gland	Control	53.7 ± 3.0	165 ± 8
	0.6	50.7 ± 2.5	160 ± 5
	6	54.8 ± 2.2	162 ± 7
	60	60.6 ± 2.1	164 ± 8
Spleen	Control	26.4 ± 2.8	68.3 ± 4.0
	0.6	28.8 ± 2.2	75.1 ± 3.8
	6	27.5 ± 1.5	65.7 ± 5.1
	60	30.1 ± 1.2	86.1 ± 4.3†
Heart	Control	26.6 ± 1.6	68.7 ± 4.4
	0.6	27.1 ± 1.4	72.4 ± 6.9
	6	26.0 ± 1.5	77.2 ± 4.8
	60	34.7 ± 1.4*	77.3 ± 1.9
Specific [^{3}H]NMS binding			
Bladder	Control	157 ± 10	144 ± 12
	0.6	127 ± 13	84.7 ± 8.1†
	6	135 ± 11	98.2 ± 3.8†
	60	137 ± 5	97.5 ± 2.7†
Submaxillary gland	Control	98.4 ± 3.8	145 ± 3
	0.6	112 ± 3†	149 ± 10
	6	101 ± 2	149 ± 8
	60	105 ± 2	119 ± 2*

K_d = dissociation constant; B_{max} = maximal number of binding sites; [^{3}H]NMS = [N-methyl-^{3}H]scopolamine methyl chloride; SPE = saw palmetto extract.
Values are mean ± standard error of 4 to 7 rats.
* $P < 0.01$ vs. control.
† $P < 0.05$ vs. control.

mg/kg/day) of SPE in the bladder and only with a high dose (60 mg/kg/day) in the submaxillary gland. Similarly, a significant enhancement of B_{max} values for specific [^{3}H]prazosin binding was observed in the rat prostate by repeated treatment with the low dose (6 mg/kg/day) of SPE, but not in the submaxillary gland, spleen, and heart. On the other hand, in vitro experiment has shown that SPE exhibits little tissue selectivity in binding activities of each receptor. We have no clear explanation for such receptor binding selectivity by oral administration of SPE in the rat lower urinary tract. The most plausible reason may be preferential distribution of receptor binding constituents in the lower urinary tract after systemic administration of SPE. It is reported that SPE contains a complex mixture of free fatty acids and their esters, small quantities of phytosterols (eg, beta-sitosterol), aliphatic alcohols, and various polyprenic compounds.[20] A systemic distribution study in rats administered [^{14}C]oleic acid or [^{14}C]sitosterol-supplemented SPE has shown that these components are accumulated in the prostate to a greater extent compared with other tissues.[21] Because the prostate is particularly rich in free fatty acids, it would be expected that greater amounts of lipophilic substances accumulate in the prostate than in other tissues.

It is well known that chronic treatment with agonists and antagonists induces compensatory alteration of au-

tonomic receptor expression.[15,22,23] Saw palmetto extract has been shown to exert inhibitory effects against phenylephrine-induced formation of [^{3}H]inositol phosphate formation, which is suggestive of an alpha 1-adrenoceptor antagonistic effect.[11] Thus, it is plausible that a significant enhancement of [^{3}H]prazosin binding sites in the rat prostate after repeated administration of SPE reflects alpha 1-adrenoceptor upregulation due to the sustained receptor blockade. In fact, Kersting *et al.*[24] have shown that chronic treatment with prazosin may cause upregulation of alpha 1-adrenoceptors in patients with congestive heart failure. Furthermore, it is considered that a significant decrease of bladder [^{3}H]NMS binding sites in SPE-treated rats may represent downregulation of muscarinic receptors, resulting from the continuous receptor stimulation. Alternatively, there is a possibility that SPE contains slowly dissociating muscarinic antagonists, as previously suggested in the underlying mechanism for unsurmountable receptor antagonists.[25] It may be conceivable that apparent inverse compensation of autonomic receptors occurs, because SPE may be present as a mixture in agonists and antagonists for autonomic receptors.

CONCLUSIONS

The present study has revealed that SPE exerts significant binding activities on alpha 1-adrenergic and muscarinic receptors in the rat lower urinary tract. Thus, our data may contribute significantly to the further understanding of the pharmacologic effects of SPE in the treatment of patients with BPH and LUTS.

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【総　説】

ノコギリヤシ果実抽出液の排尿機能及び下部尿路受容体に対する作用

Effects of Saw Palmetto Extract on Urodynamic Function and Receptors in the Lower Urinary Tract

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【要　旨】
ノコギリヤシ果実抽出液 (SPE) は，ヨーロッパでは前立腺肥大症 (BPH) に対する治療薬として用いられ本邦でも健康食品として汎用されている．SPE の薬理作用には抗アンドロゲン作用などがある．臨床的には，SPE (320 mg/day) の 6 ヶ月投与により BPH やそれに伴う頻尿に有効との報告がある．排尿機能及び下部尿路受容体に対する SPE の作用を調べたところ，酢酸誘発頻尿ラットシストメトリーにおいて，SPE は排尿間隔及び一回排尿量を有意に増加させ，頻尿改善作用を示した．さらに SPE は前立腺 α_1 受容体，膀胱ムスカリン性及び 1,4-ジヒドロピリジン系 Ca 拮抗薬受容体に対し結合活性を示した．SPE は反復経口投与により，テストステロン誘発肥大前立腺における α_1 受容体数の増加を抑制した．また，SPE の反復投与はラットの血液臨床検査値，肝機能及び肝薬物代謝酵素活性に影響しなかった．以上，SPE は下部尿路受容体への直接作用による BPH の機能的閉塞の解除や頻尿の抑制などの薬理作用を示すことが示唆された．

【キーワード】
ノコギリヤシ果実抽出液，排尿パラメータ，前立腺α_1受容体，膀胱ムスカリン性受容体

1. はじめに

近年，わが国においては，代替医療の普及と高齢者人口の増加に伴い，健康増進や疾患の予防・治療を目的として健康食品への関心が高まっている．欧米では，民間薬として伝承されてきたメディカルハーブを医療の現場において積極的に活用しており，本邦でも健康食品として容易に入手可能である．特に高齢者では，医薬品とともに健康食品の摂取率が高く，この傾向は今後益々増加すると予想される．一方において健康食品の使用頻度が増大するに従い，その過剰摂取による健康被害や医薬品と併用した場合の有害事象が報告されている．しかしながら，メディカルハーブを含めいわゆる健康食品に関しては医薬品の場合と比較して，有効性についてそのメカニズムを含めた科学的検証は未だ十分とは言えず，健康食品それ自体の有害事象や医薬品との相互作用に関して信頼できる情報の提供も行われていない．従って，健康食品の適正な使用を確保するためには，有効性及び安全性に関する科学的検証が急務といえる．ここでは，近年特に注目されている健康食品のうち，前立腺肥大による排尿症状の改善目的で使用されるノコギリヤシ果実を取り上げ，それらの有効性及び安全性について，我々の最近の知見も交えて紹介する．

2. 前立腺肥大症とノコギリヤシ果実抽出液

食生活の欧米化や高齢者人口の増加に伴い，前立腺肥大症 (BPH) 患者が急増している．BPH は，前立腺組織の肥大による機械的閉塞や，前立腺平滑筋の α_1 受容体緊張亢進による機能的閉塞により排尿困難や残尿を呈する疾患である．この薬物治療には，現在 α_1 遮断薬やホルモン剤が汎用されるが，これらの医薬品では起立性低血圧や性機能障害などの副作用が問題となっており，また BPH 患者の約半数においては頻尿症状も出現することから，抗コリン薬も併用される場合も多い．カンザス大学の泌

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尿器外来に来院する男性患者を対象に代替医療食品の使用率を調査したところ，302 例中約半数の患者が前立腺の健康を目的にノコギリヤシを含めた何らかの代替医療食品を使用していた．これより，泌尿器系疾患の男性において，代替医療食品が普及していること，また年齢が上がるにつれその使用率も高まると報告している[1]．

ヤシ科シュロ属のノコギリヤシ果実抽出液（saw palmetto extract: SPE）は，北米原産の低灌木で，南東部の海岸地帯の砂丘の松林に生息している．超臨界抽出法により精製されたイタリアインデナ社の SPE（SABALSELECT）は，飽和・不飽和脂肪酸が 90%以上を占め，その他，高級アルコール及びステロール等で構成されている．現在報告されている SPE の薬理作用としては，5α-reductase 阻害作用[2]，アンドロゲン受容体遮断作用[3]，抗炎症作用[4]，細胞増殖抑制作用[5]，α_1 受容体遮断作用[6]，鎮痙作用[7]などが知られている．しかし，SPE の有効成分が明らかになっていないこと，また本邦においてはいわゆる健康食品として扱われていることから，様々な規格の SPE が販売されている．そこで，SPE の全体像を明らかにする目的で，今まで報告されている SPE の臨床試験，また筆者らが実施した SPE の排尿機能に対する作用や排尿障害の薬物治療において標的部位となる下部尿路受容体（α_1 受容体やムスカリン性受容体など）に対する作用について紹介する．

3. ノコギリヤシ果実抽出液の臨床試験

SPE を用いた臨床試験を表 1 に示した[8-10]．これまでに 10 を超えるプラセボ対照試験[11-21]と 4 つの実薬対照試験[22-25]などが実施されている．

(1) プラセボ対照試験

プラセボ対照試験の SPE 投与量はいずれの試験においても 320 mg/日（160 mg/回，1 日 2 回）であった．このうち 1980 年代に報告された試験は，被験者数が限られており，また試験期間も短いものが多かった．一方，2000 年以降に発表された試験は，比較的被験者数も多く，試験期間も 6 ヶ月以上と長い．

多くの試験において，SPE の最大尿流量率及び夜間頻尿の改善が認められた．また，最近の試験で評価項目として用いられている IPSS（International Prostate Symptom Score：国際前立腺症状スコア）あるいは AUASI（American Urological Association Symptom Index：米国泌尿器科学会症状指標）では，2 つの試験で有効性が認められたものの，これらの指標が測定された他の 2 つの試験では認められなかった．結果のばらつきについては，被験者数，試験期間が各試験で異なっていること，さらに使用された SPE の規格が統一されていないこと，プラセボの識別不能性，対象患者の特性など，様々な要因が挙げられる[11]．今後，これらの点を考慮した試験の実施と結果の集積が望まれる．

(2) 実薬対照試験及び open 試験

SPE の実薬対照試験として，4 試験[22-25]が報告されている．

Grasso ら[24]は，SPE と Alfuzosin の比較試験の結果，Boyarsky's total score 及び排尿障害スコアにおいて Alfuzosin は SPE に比較して統計的に有意に優れていたことを報告している．一方，これ以外の実薬対照試験 3 試験[22,23,25]において SPE は既存の臨床薬とほぼ同様の効果を示した（表 1）．Alfuzosin を対照とした試験は，評価期間が 3 週間と短く，一方その他の試験は 3 ヶ月から 12 ヶ月と比較的長い．これまでに動物試験の結果から SPE の効果発現までには 2–3 ヶ月の期間が必要であることが示唆されており[32]，Alfuzosin を対照とした試験と他の 3 試験の結果が異なった一因として，試験期間の長短が関与しているものと考察される．

Debruyne ら[33]は，2002 年に報告した試験[22]をさらに層別解析し，重症（IPSS>21）BPH 患者における刺激症状及び閉塞症状に対する SPE 及び Tamsulosin の効果を検討した結果，SPE は Tamsulosin に比較して同等以上の効果が認められたことを示した．しかしながらこの解析はレトロスペクティブなものであり，既存薬に対する SPE の効果についても更なる検証が必要である．現在，米国において National Institute of Diabetes and Digestive and Kidney Diseases（国立糖尿病・消化器・腎疾病研究所），National Center for Complementary and Alternative Medicine（国立補完代替医療センター），及び Office of Dietary Supplements をスポンサーとした前立腺肥大症に対する SPE，Pygeum africanum（アフリカ原産常緑樹であるピジウムの樹皮エキス），及び Tamsulosin を比較する大規模な第 III 相臨床試験が実施されている[34]．

4. ノコギリヤシ果実抽出液の排尿機能に対する作用

SPE の排尿機能に対する作用を検討するため，ウレタン麻酔下ラットを用いたシストメトリーにより膀胱内圧及び排尿量の測定を行った[35,36]．まず，生理食塩水を膀胱内に注入した正常ラットにおいて，SPE（6 mg/kg）を十二指腸内投与したところ，排尿間隔，一回排尿量，膀胱容量，静止時膀胱圧，排尿閾値圧及び最大膀胱収縮圧は投与前後で有意な差異がなく，排尿パラメータに殆ど影

表1 前立腺肥大症患者を対象とした臨床試験における SPE の IPSS，最大尿流量率及び夜間頻尿に及ぼす効果

Study	治療群	用量	試験期間	IPSS		最大尿流量率		夜間頻尿	
				n	change	n	change	n	change
プラセボ対照試験									
Bent et al., 2006	SPE	160*2	12 m	112	−0.68#	112	0.42		
	Placebo	Placebo		113	−0.72#	113	−0.01		
Willetts et al., 2003	SPE	160*2	12 m			46	1.5		
	Placebo	Placebo				47	4.4		
Gerber et al., 2001	SPE	160*2	6 m	41	−4.4	41	1.0		
	Placebo	Placebo		44	−2.2	44	1.4		
Marks et al., 2000	SPE (blend)	106*3	6 m	21	−2.24	21	1.27		
	Placebo	Placebo		23	−1.39	23	0.09		
Descotes et al., 1995	SPE	160*2	1 m			82	3.42	82	−0.67
	Placebo	Placebo				94	1.06	94	−0.32
Reece et al., 1986	SPE	160*2	3 m			33	2.35	33	−1.0
	Placebo	Placebo				37	2.3	37	−1.0
Cukier et al., 1985	SPE	2*80*2	2–3 m					43	−1.1
	Placebo	Placebo						47	−0.5
Tasca et al., 1985	SPE	160*2	3 m			14	3.3	14	−2.6
	Placebo	Placebo				13	0.6	13	−1.2
Champault et al., 1984	SPE	2*80*2	1 m			46	2.7	47	−1.4
	Placebo	Placebo				39	0.25	41	−0.5
Boccafoschi et al., 1983	SPE	160*2	2 m			11	4.13	11	−2.2
	Placebo	Placebo				11	1.96	11	−1.0
Emili et al., 1983	SPE	160*2	1 m			15	3.37	15	−1.6
	Placebo	Placebo				15	0.2	15	−0.4
実薬対照試験									
Debruyne et al., 2002	SPE	320*1	12 m	350	−4.4		1.79		
	Tamsulosin	0.4*1		354	−4.4		1.89		
Carraro et al., 1996	SPE	160*2	6 m	464	−5.8		2.68	464	−0.74
	Finasteride	5		477	−6.2		3.26	477	−0.69
Grasso et al., 1995	SPE	160*2	0.75 m			31	2.8	32	−1.0
	Alfuzosin	7.5				32	4.7	31	−0.9
Adriazola et al., 1992	SPE	160*2	3 m			20	1.5	20	−0.2
	Prazosin					22	0.47	22	−0.4
オープン試験									
Aliaev et al., 2002	SPE	160*2	60 m	26	−8.8		4.13		
Pytel et al., 2002	SPE	160*2	24 m	155	−5.33		1.13		
Stepanov et al., 1999	SPE	160*2	3 m	100	−6.5		1.4		
	SPE	320*1			−6.4		1.8		
Gerber et al., 1998	SPE	160*2	6 m	46	−7.0	46	−0.7		
Foroutan, 1997	SPE	2*80*2	3 m	592	−6.5	592	2.93	592	−1.0
Authie et al., 1987	SPE	2*80*2	3 m					500	−2.2

#: AUASI: American Urological Association Symptom Index

響しなかった（表2）．次に，高用量の SPE (60 mg/kg) 投与において，排尿間隔，一回排尿量及び膀胱容量はいずれも有意に（約40%）増加した（図1A, 表2）．次に，0.1%酢酸溶液をラット膀胱内に注入することにより，排尿間隔，一回排尿量及び膀胱容量は正常ラットの場合に比べ有意に減少し，頻尿状態が観察された．この頻尿モデルラットに SPE (12, 20 mg/kg) を投与することにより，排尿間隔，一回排尿量及び膀胱容量はいずれも投与量に依存して有意に増加し，頻尿改善作用を示すことが明らかになった（図1B, 表2）．この SPE の作用は正常ラットに比べ酢酸誘発頻尿ラットにおいて低用量で発現したことから，病態特異的である可能性が示された．ところで，

表2 麻酔下ラットシストメトリーにおけるSPE (6–60 mg/kg) 投与前後の排尿パラメータ

投与量 (mg/kg)	排尿間隔 (min)		一回排尿量 (mL)		膀胱容量 (mL)	
	投与前	投与後	投与前	投与後	投与前	投与後
正常ラット（生理食塩水注入）						
6	9.93±1.07	9.07±1.09	0.69±0.07	0.66±0.09	0.65±0.06	0.60±0.07
60	7.71±0.90	10.8±1.77*	0.54±0.06	0.77±0.13*	0.51±0.06	0.72±0.12*
頻尿ラット（0.1%酢酸溶液注入）						
12	6.44±1.10	7.41±1.25*	0.44±0.08	0.51±0.08*	0.43±0.07	0.49±0.08*
20	5.99±0.47	8.85±1.07*	0.40±0.04	0.66±0.08*	0.40±0.03	0.59±0.07*
投与量 (mg/kg)	静止時膀胱圧 (mmHg)		排尿閾値圧 (mmHg)		最大膀胱収縮圧 (mmHg)	
	投与前	投与後	投与前	投与後	投与前	投与後
正常ラット（生理食塩水注入）						
6	3.35±0.19	3.20±0.22	5.02±0.13	4.78±0.13	26.1±2.2	23.9±2.0
60	3.17±0.10	2.93±0.21	4.74±0.16	4.81±0.15	28.3±0.8	26.0±0.7*
頻尿ラット（0.1%酢酸溶液注入）						
12	3.99±0.26	3.69±0.23	5.05±0.31	5.13±0.27	29.3±1.7	25.7±2.0**
20	3.54±0.16	3.37±0.11*	4.44±0.19	4.62±0.25	25.6±1.4	23.0±0.5

*P<0.05, **P<0.01 vs. 投与前

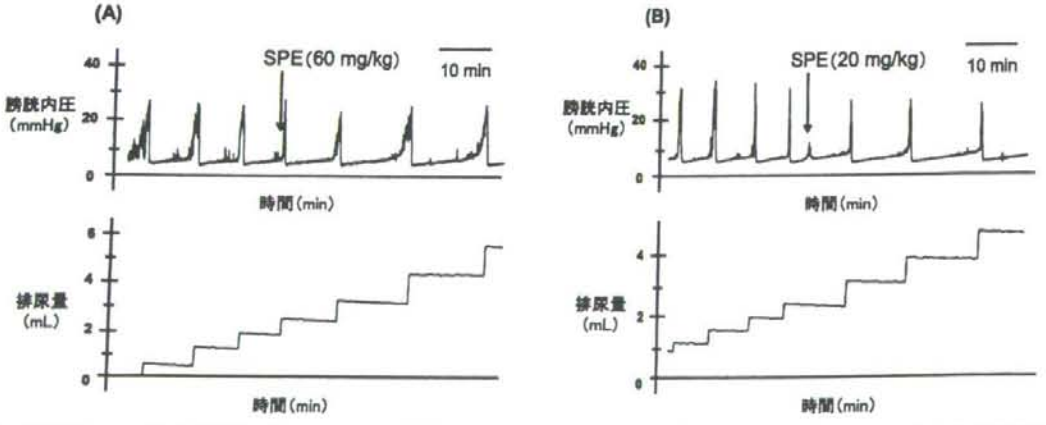

図1 麻酔下ラット（A: 生理食塩水注入, B: 0.1%酢酸溶液注入）シストメトリーにおけるSPE (A: 60 mg/kg, B: 20 mg/kg) 十二指腸内投与時のシストメトログラム.

SPEは高カリウム，ノルエピネフリン及びアセチルコリンによるラット摘出平滑筋組織の収縮を抑制する[7,37]ことから，*in vivo*において膀胱平滑筋の弛緩により頻尿改善作用を示すことが考えられた．また，SPEの主な含有成分である遊離脂肪酸は，K^+, Na^+, Ca^{2+}などのイオンチャネル透過性や神経伝達などの生理機能に対して影響を及ぼすこと[38]，並びに酢酸誘発頻尿は膀胱知覚神経の活性化に基づく[39]ことから，SPEは下部尿路神経に作用することも推定された．

5. *In vitro*におけるノコギリヤシ果実抽出液の各種受容体に対する作用

次に，排尿障害治療薬の作用部位となる下部尿路自律神経受容体（α_1 受容体，ムスカリン性受容体，1,4-ジヒドロピリジン (DHP) 系 Ca 拮抗薬受容体，ATP (P2X) 受容体）に対するSPEの結合活性を，各受容体の選択的標識リガンドを用いる受容体結合測定法により検討した[35,36]．その結果，SPEはラット前立腺及び脾臓の α_1 受容体（IC_{50} 値：それぞれ 169, 188 μg/mL），ラット膀胱及び顎下腺のムスカリン性受容体（IC_{50} 値：それぞれ 40.4,

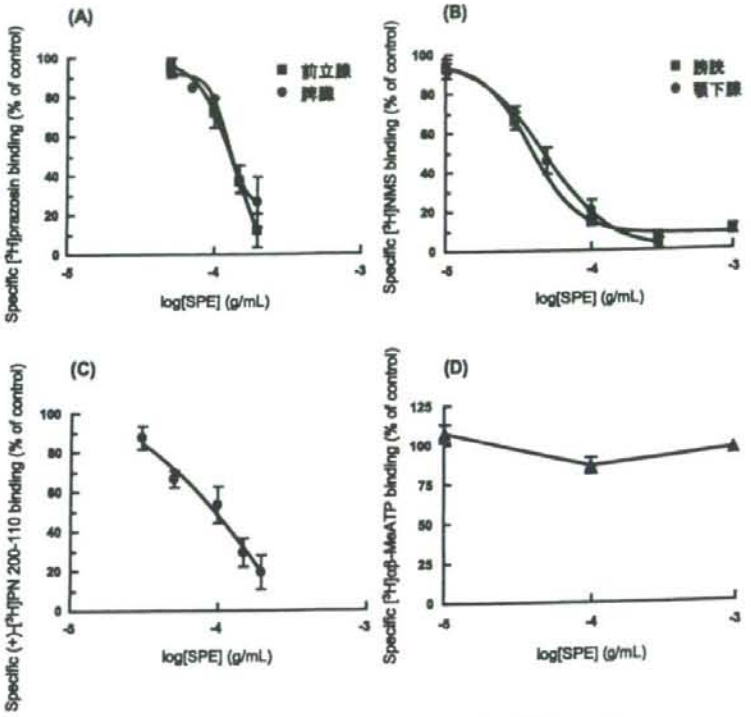

図 2 (A) ラット前立腺及び脾臓における [^{3}H]prazosin 特異的結合に対する SPE の抑制作用 (n=5–8)
(B) ラット膀胱及び顎下腺における [^{3}H]NMS 特異的結合に対する SPE の抑制作用 (n=8–9)
(C) ラット膀胱における (+)-[^{3}H]PN 200-110 特異的結合に対する SPE の抑制作用 (n=5)
(D) ラット膀胱における [^{3}H]αβ-MeATP 特異的結合に対する SPE の抑制作用 (n=2)
(Mean±S.E.)

52.3 μg/mL)，ラット膀胱の 1,4-DHP 系 Ca 拮抗薬受容体 (IC_{50} 値：97.3 μg/mL) に対し結合活性を有すること（図 2），またその結合様式は非競合的（受容体結合後の解離が遅い）であることが明らかとなった．一方，SPE はラット膀胱の P2X 受容体に対し結合活性を示さなかった．SPE が α_1 受容体結合活性を有することは既に Goepel ら[6]もヒトクローン発現細胞系を用いて認めているが，本研究結果は α_1 受容体サブタイプの分布密度が異なるラット前立腺（α_{1A} 受容体が優位）と脾臓（α_{1B} 受容体が優位）において SPE の α_1 受容体結合活性を明らかにし，さらに両臓器での SPE の結合親和性には差異がないことを示した．また，SPE はムスカリン性受容体及び 1,4-DHP 系 Ca 拮抗薬受容体に結合活性を示し，両受容体結合活性は IC_{50} 値の比較より，α_1 受容体に比べ 2–4 倍高いことが示された．この SPE によるムスカリン性受容体と 1,4-DHP 系 Ca 拮抗薬受容体の結合活性についてはこれまで報告がなく，本研究で初めて得られた知見である．これより，SPE のこれら各受容体に対する結合活性は，ラット平滑筋弛緩作用[7,37]及びラット頻尿改善作用（図 1，表 2）の発現に寄与することも考えられた．また，これら受容体結合活性を示す SPE の活性成分は，主に n-ヘキサン及びジエチルエーテル可溶性画分に含まれることが明らかとなり，脂肪酸類である可能性の高いことが考えられた．今後受容体への結合活性成分の分離同定は興味深い課題となる．さらに，SPE はヒト前立腺 α_1 受容体及び膀胱ムスカリン性受容体に対しても結合活性（IC_{50} 値：それぞれ 53.2, 71.2 μg/mL）が認められたことから，ヒトにおいても下部尿路受容体への作用を介して排尿障害を改善することが示唆された．

6. ラットの各臓器受容体に対するノコギリヤシ果実抽出液の反復投与の影響

SPE の *in vivo* における下部尿路自律神経受容体に対する作用を検討した[36,40]．正常ラットに SPE (0.6, 6, 60 mg/kg) を 4 週間反復経口投与することにより，前立腺 α_1 受

容体数の有意（24–37%）な増加，並びに膀胱ムスカリン性受容体数の有意（32–41%）な減少が認められた（図3）ことから，SPE は経口投与により下部尿路臓器の各受容体に作用することが示された．また，前立腺及び膀胱におけるこの両受容体数の変動は，脾臓や顎下腺などの他の臓器の受容体に比べ SPE の低用量(0.6, 6 mg/kg)で発現したことから，SPE は生体内において下部尿路受容体に対し選択性を示すことが考えられた．

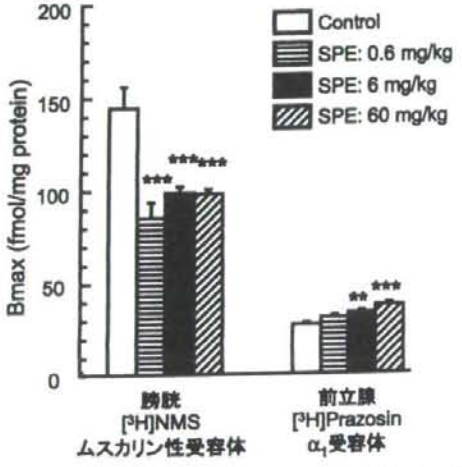

図3 ラット前立腺 α_1 受容体数（α_1 受容体の標識リガンド：[3H]prazosin 特異的結合の最大結合部位数：Bmax）及び膀胱ムスカリン性受容体数（ムスカリン性受容体の標識リガンド：[3H]NMS 特異的結合の Bmax）に対する SPE 反復（4 週間）経口投与の影響．
Mean±S.E., (n=3–7). **P<0.01, ***P<0.001 vs. Control.

次に，前立腺肥大モデルラットに対する SPE 反復投与の影響を検討した．ラットにテストステロン (3 mg/kg) を4週間皮下投与することにより，sham 群に比べ前立腺重量は有意（約2倍）に増加し，さらに前立腺 α_1 受容体数も有意（62%）に増加した（図4）．次に，6 及び 60 mg/kg の SPE をテストステロンとともに4週間経口投与した場合，前立腺重量は control（テストステロン単独投与）群の場合と比べ有意な差は認められなかったが，前立腺 α_1 受容体数はテストステロン投与で認めた増加を抑制した．前立腺肥大に対する SPE の作用については，ラットへの SPE (100, 320 mg/kg) 30 日間投与によるスルピリド誘発前立腺肥大抑制[41]，SPE (50 mg/kg) 60 日間投与によるテストステロン誘発前立腺肥大抑制[42] を示した報告がある．本研究ではこれらの結果を確認できなかった理由として，SPE の投与量が低く且つ投与期間が短かったことが考えられる．実際に，抗アンドロゲン剤における前立腺肥大抑制効果発現には 2–3 ヶ月の連続投与を要することが知られている[32]．しかしながら，Rhodes ら[43] はテストステロン誘発ラット前立腺肥大が高用量 (180, 1800 mg/day) SPE の反復投与によっても全く影響されないことを示している．本実験で用いた 6 ないし 60 mg/kg という SPE の用量は，ヒトでの内服量 (320 mg/day) と同等もしくはその 10 倍量であったことから，SPE の臨床での薬効発現量に等しいと考えられる．これまでの研究から，SPE は 5α-reductase 阻害作用や抗アンドロゲン作用などに基づく前立腺肥大の抑制により，BPH の機械的閉塞を解除し排尿障害を改善することが主たる薬理作用と

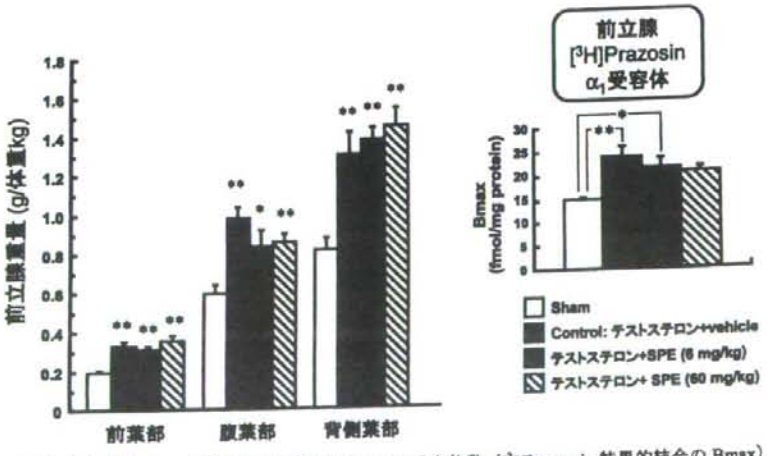

図4 テストステロン処置（4 週間）ラット前立腺の組織重量及び α_1 受容体数（[3H]prazosin 特異的結合の Bmax）に対する SPE 反復（4 週間）経口投与の影響．
Mean±S.E., (n=5). *P<0.05, **P<0.01 vs. Sham.