Effects of Stinging Nettle Root Extracts and Their Steroidal Components on the Na^+, K^+-ATPase of the Benign Prostatic Hyperplasia[1]

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[1] Part of this work was presented at the IVth Clinical Experimental Conference Concerning Questions to the Benign Prostatic Hyperplasia, Nov. 12–15, 1992, Eze

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Received: February 1, 1993; Revision accepted: April 20, 1993

Abstract

The effects of organic-solvent extracts of *Urtica dioica* (Urticaceae) on the Na^+,K^+-ATPase of the tissue of benign prostatic hyperplasia (BPH) were investigated. The membrane Na^+,K^+-ATPase fraction was prepared from a patient with BPH by a differential centrifugation of the tissue homogenate. The enzyme activity was inhibited by 10^{-4}–10^{-5} M of ouabain. The hexane extract, the ether extract, the ethyl acetate extract, and the butanol extract of the roots caused 27.6–81.5 % inhibition of the enzyme activity at 0.1 mg/ml. In addition, a column extraction of stinging nettle roots using benzene as an eluent afforded efficient enzyme inhibiting activity. Steroidal components in stinging nettle roots, such as stigmast-4-en-3-one, stigmasterol, and campesterol inhibited the enzyme activity by 23.0–67.0 % at concentrations ranging from 10^{-3}–10^{-6} M. These results suggest that some hydrophobic constituents such as steroids in the stinging nettle roots inhibited the membrane Na^+,K^+-ATPase activity of the prostate, which may subsequently suppress prostate-cell metabolism and growth.

Key words

Urtica dioica, Urticaceae, stinging nettle roots, organic-solvent extract, membrane Na^+,K^+-ATPase, stigmast-4-en-3-one, benign prostatic hyperplasia.

Introduction

An aqueous methanolic extract from the roots of *Urtica dioica* (Urticaceae) is used clinically for treatment of benign prostatic hyperplasia (BPH) in Europe. Some biological (1) and histological (2) data demonstrated the effect of the roots on the androgen dependent growth of prostatic cells. Recent studies have suggested that *Urtica* extracts inhibit growth factor-receptor interaction in the prostate. The precise mechanism of these effects or the active components of the remedy, however, are still unclear.

Stinging nettle roots have been known to contain several steroidal and phenolic components (3). On the other hand, some kinds of synthetic and naturally occurring steroids and phenolic compounds are reported to inhibit Na^+,K^+-ATPase activity of heart or kidney origin (4–6). Recent conceptions, as those reported by Farnsworth (7), are that effects of androgens on proliferation, metabolism, biosynthesis, and secretion of the prostate are mediated not simply through binding of the steroid-receptor complex to nuclei, but also through binding to plasma membrane receptors. Several investigations proved that one of the androgen binding sites is membrane Na^+,K^+-ATPase (7–10). Taking these observations into consideration, in the present study, we examined the influence of stinging nettle root extracts and their known components on BPH Na^+,K^+-ATPase activity.

Materials and Methods

Preparation of membrane ATPase from BPH tissue

The plasma membrane ATPase was prepared according to the methods we described previously (4, 5). In brief, five grams of BPH tissue obtained from a BPH patient were cut into pieces in 100 ml of 5 mM EDTA solution containing 0.32 M sucrose. These were homogenized and centrifuged at 6,000 × g for 40 min at 4 °C to remove the cell debris. The supernatant was centrifuged at 85,000 × g for 60 min at 4 °C to give a microsomal pellet which was subsequently treated with 2 M NaI in the presence of 50 mM cysteine, 5 mM $MgCl_2$, 3 mM ATP, and 5 mM EDTA. The mixture was centrifuged at 20,000 × g for 90 min and the resulting pellet was resuspended in 5 ml of an assay buffer. This preparation was used as an enzyme fraction.

Stepwise extraction from stinging nettle roots

Extraction of stinging nettle roots was performed with cyclohexane, diethyl ether, ethyl acetate, and *n*-butanol, one after another. 600 g of stinging nettle roots were cut into pieces and extracted with 3 × 4 l methanol for 24 h at room temperature with occasional stirring. The solvent was removed in vacuo and the residue was suspended in 1 l water. The mixture was thoroughly extracted with 4 × 500 ml cyclohexane. The combined cyclohexane layers were washed with 200 ml water, dried over Na_2SO_4, and evaporated to dryness in vacuo. The water which was used to wash the organic layer was combined with the original water suspension. Following the same procedure the water suspension was extracted successively with 4 × 500 ml diethyl ether,

4 × 500 ml ethyl acetate, and 4 × 500 ml *n*-butanol. Extraction yields of methanol-raw extract, cyclohexane extract, ether extract, ethyl acetate extract, *n*-butanol extract, and the residual water soluble materials were 11.9, 0.38, 0.25, 0.17, 1.0, and 10.1 %, respectively. The extracts thus obtained were dried and redissolved in ethanol to an appropriate concentration for use in the ATPase assay.

Column fractionation of extracts A, B, and C

A water extract or water/alcohol extract of the stinging nettle roots was prepared. Extract A contained 50 % water/methanol extract from the roots and 50 % lactose. Extract B contained water extract from the roots, and extract C, 70 % ethanol/water-extract from the roots. Extracts A and B are known to mainly contain hydrophilic components such as lectin *Urtica dioica* agglutinin (UDA) and polysaccharides, while extract C is known to contain steroidal and phenolic compounds (3).

Portions of 100 mg of extracts A–C were dissolved in 1 ml water, turbidities removed by centrifugation, and 300 µl of the resulting solution were extracted using a rapid-flow fractionation procedure RFF (11) with 7 ml of ethanol/dichloromethane (1/9, v/v) as eluent. The extract was dried and redissolved in 20 µl of ethanol. The preparations from extract A– C had a yellow color, which seriously disturbed our colorimetric assay. The ethanol solutions, therefore, were further applied to a silica gel column (gel volume 100 µl), and then extracted with a three-step benzene (3 ml) elution, ethanol/benzene (1/9 v/v) (3 ml), and ethanol (3 ml), respectively. The final ethanol fraction still had color, and therefore we did not examine its activity. The former two fractions were dried, redissolved in ethanol, and subsequently used for the ATPase assay.

ATPase assay

The ATPase assay sample was dissolved in assay buffer or ethanol. The assay buffer contained 100 mM NaCl, 50 mM Tris-HCl (pH 7.4), 6 mM $MgCl_2$, and 20 mM KCl. When ethanol was used, the final % of alcohol in the assay mixture was 2%. The control tubes contained the same concentration of ethanol. 20 µl of sample solution were added to 160 µl of assay buffer, and then 20 µl of the enzyme fraction, prepared as described above were subsequently added to this mixture. After incubation of this mixture at 37 °C for 10 min, 20 µl of 30 mM ATP were added, and the mixture was incubated for 10 min more. At the end of this incubation, 200 µl of ice-cold 10 % trichloroacetic acid were added, and the liberated orthophosphate was determined by a colorimetric assay procedure as described elsewhere (4–6). Then Na^+,K^+-ATPase activity was calculated by the amount of phosphate (µmol)/h/mg protein.

Materials

Ouabain, campesterol, and stigmasterol were obtained from Sigma Chemical Co. (USA). Stigmast-4-en-3-one and hecogenin were the gifts of Boots Pharma GmbH (Germany). All other reagents were of the best grade available.

Results

Ouabain, which has been known as a potent inhibitor of Na^+,K^+-ATPase from various tissues (12), inhibited BPH enzyme activity at concentrations ranging from 10–100 µM (Fig. **1**), which is almost equivalent to Farnsworth's results (7).

Then, we examined the effects of the organic-solvent extracts of stinging nettle roots on BPH Na^+,K^+-ATPase activity. The hexane extract, the ether extract, the ethyl acetate extract, and the butanol extract of the roots caused 27.6–85.1 % inhibition of the enzyme activity at a concentration of 0.1 mg/ml (Fig. **2**). The hexane extract caused the strongest inhibition (85.1 % inhibition) at 0.1 mg/ml. At a final concentration of 0.01 mg/ml of the extract, each sample inhibited the enzyme activity by 0.5–44.3 %. Addition of more than 0.1 mg/ml of the extract decreased the accuracy of the assay, since the color of the extract seriously increased background OD.

Fig. **3** shows the effects of the benzene extract and ethanol/benzene extract of extracts A–C on the enzyme activity. These fractions showed 10–85 % inhibition of enzyme activity, and significant effects ($p < 0.05$) were obtained by use of benzene extract and ethanol/benzene extract of extract C. These results support that some organic solvent-soluble constituents showed inhibitory activity against BPH Na^+,K^+-ATPase.

Stinging nettle roots are known to contain several steroidal and phenolic components (3). Among those, in the present study, stigmast-4-en-3-one, stigmasterol (stigmasta-5,22-dien-3β-ol), and campesterol (24*R*-ergost-5-en-3β-ol) were examined for their effects on BPH enzyme activity. These compounds are easily soluble in organic solvents. Stigmast-4-en-3-one showed an inhibitory effect at concentrations less than 10^{-5} M (Fig. **4**). This steroid at 10^{-5} M inhibited more than 50 % of the enzyme activity, suggesting that the steroid effect is equal to or even stronger than that of ouabain. Stigmasterol also inhibited enzyme activity, but its potency was less than that of stigmast-4-en-3-one (Fig. **4**). Campesterol also showed inhibitory activity (4–23 %) against the enzyme at concentrations ranging from 10^{-7}–10^{-4} M (Fig. **5**). We also examined the effect of hecogenin (3-hydroxy-5-spirostan-12-one) on BPH Na^+,K^+-ATPase activity. Hecogenin is not an ingredient of stinging nettle roots, but its structure prompted us to examine its activity. The steroid showed considerable suppressive activity against the enzyme (Fig. **5**).

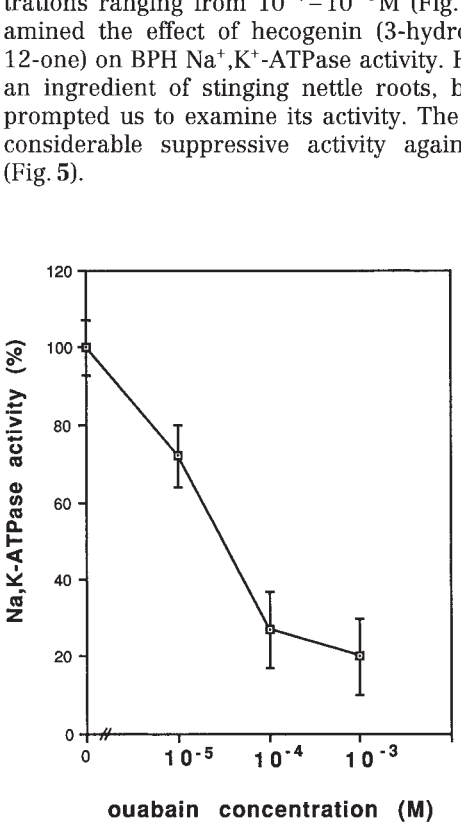

Fig. 1 Effect of ouabain on BPH Na^+,K^+-ATPase activity.

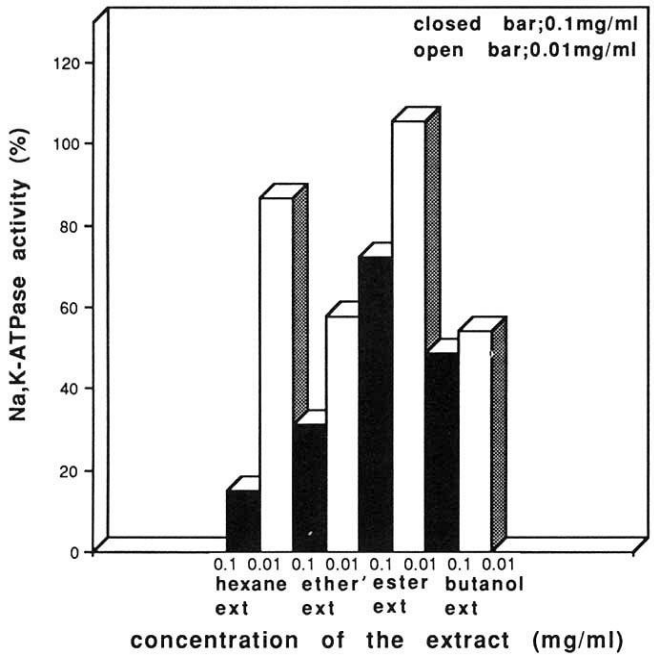

Fig. 2 Effect of organic-solvent extracts of stinging nettle roots on BPH Na^+,K^+-ATPase activity. The roots were extracted with cyclohexane, diethyl ether, ethyl acetate, and *n*-butanol, one after another. The extracts were dried and redissolved in ethanol to an appropriate concentration. The enzyme assay was carried out in the presence of 0.1 or 0.01 mg/ml of each extract.

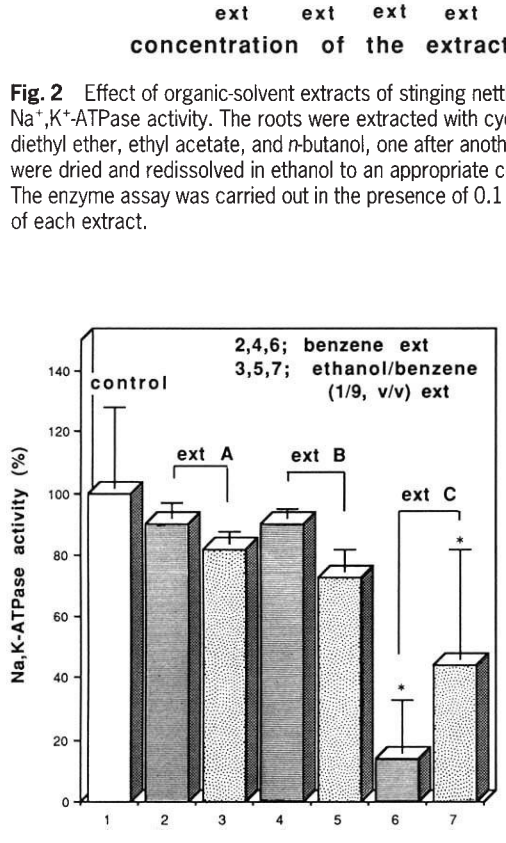

Fig. 3 Effect of benzene extract or ethanol/benzene extract of extracts A–C (see Materials and Methods) on BPH Na^+,K^+-ATPase activity. 300 μl of 100 mg/ml extract were finally fractionated through a silica gel column with a stepwise elution using benzene and ethanol/benzene (1/9, v/v) as eluents. Each extract was dried, redissolved in ethanol, and subsequently used for the ATPase assay. Statistical analysis was carried out by Student's t-test. p values less than 0.05 are considered to be significant (*).

Discussion

The data as described above suggest suppressive effects of some organic-solvent soluble components in stinging nettle roots, such as stigmast-4-en-3-one, stigmasterol, and campesterol, on BPH Na^+,K^+-ATPase activity.

The plasma membrane ATPase we obtained from the prostate of the untreated patient was proven to be an ouabain-sensitive Na^+,K^+-ATPase, which is relevant to Farnsworth's observations (7). The enzyme, however, when compared with those of the other tissues (6, 12), appears to be relatively resistant to ouabain, since 70% or more of the enzyme activity remained in the presence of 10^{-5} M ouabain. In the case of Na^+,K^+-ATPase from dog kidney cortex, in contrast, the enzyme was almost completely inhibited in the presence of 10^{-5} M ouabain (6).

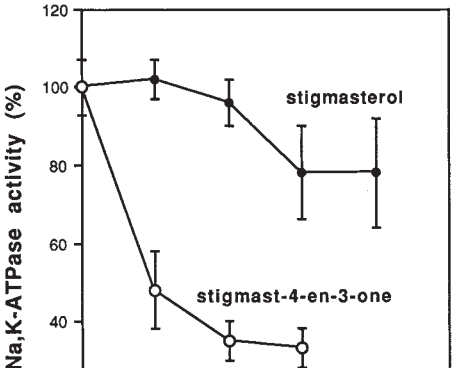

Fig. 4 Effects of stigmasterol and stigmast-4-en-3-one on BPH Na^+,K^+-ATPase activity.

Fig. 5 Effects of campesterol and hecogenin (3-hydroxy-5-spirostan-12-one) on BPH Na^+,K^+-ATPase activity.

The extracts of the stinging nettle roots significantly inhibited BPH Na^+,K^+-ATPase activity. Our stepwise extraction from the roots with 4 different organic solvents suggests that the inhibitory activity was extracted efficiently with solvents of higher hydrophobicity. It should be noticed that the benzene extract of extract C has a considerable effect on the Na^+,K^+-ATPase of the prostate. These results suggest that some organic-solvent soluble components possess strong inhibitory activity against the BPH Na^+,K^+-ATPase.

It has been known that stinging nettle roots contain several steroidal and phenolic components (3). Among those, stigmast-4-en-3-one showed strong inhibitory effects on BPH enzyme activity, which appears to be superior to that of ouabain. The binding site of this steroid on the enzyme is a problem that needs to be clarified, since androgens are considered to accelerate enzyme activity via binding to the Na^+,K^+-ATPase at a site other than that of ouabain (7, 10). Stigmasterol, campesterol, and hecogenin also showed inhibitory activities against the enzyme, however, the potencies of these steroids were not as high as that of stigmast-4-en-3-one. The difference of these potencies could possibly be due to a structural difference between the steroids, i.e., the 3-oxo-4-ene structure may be critical. The enzyme inhibition of these steroids, except for stigmast-4-en-3-one, appears to be unspecific, since the inhibition curves do not correspond with that of ouabain. In our preliminary study, the steroids were less effective against Na^+,K^+-ATPase from canine kidney cortex (data not shown), suggesting that the steroids are relatively selective to the enzyme of prostate origin.

An aqueous methanolic extract of stinging nettle roots is clinically used for the treatment of BPH. Light microscopic evaluation of BPH tissues from patients treated with the remedy revealed some morphological effects of the drug on the prostate (2). The precise mechanisms for the efficacy or the active components of the remedy, however, are still vague. Our present data suggest that at least some of the organic-solvent soluble components of stinging nettle roots, such as steroids, strongly suppress BPH membrane Na^+,K^+-ATPase activity. The steroid block of the ATPase may subsequently suppress the accelerated proliferation and function of the prostate.

Acknowledgements

Boots Pharma GmbH, Höchstadt, Germany are gratefully acknowledged for providing the extracts and the prostate tissues, for helpful discussions, and for financial support of this work. We also thank T. Yamamoto and C. Yajima for their technical assistance.

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