Erectile dysfunction and the biochemical/molecular/ regulation of human penile arteries

Ph.D. Thesis

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Aims of the present study

Erection is a hemodynamic event where vasodilatation of penile arteries is followed by increased arterial blood inflow to the corpora cavernosa. In this process endothelium is an important contributor, since it plays a key role in the regulation of vascular tone via synthesis / secretion of vasorelaxant mediators, like NO and prostanoids, and it is involved in the endothelium derived hyperpolarization (EDH) - type relaxation. It is accepted that there is a correlation between endothelial and erectile dysfunction. In the present thesis we were investigated the mechanisms involved in the endothelium dependent relaxation.

Our studies were focused on the following:

1. Improve surgical technique of male-to female transsexual surgery required to obtain human penis with intact vasculature for in vitro experiments.

2. *In vivo* investigation of flow mediated dilation in patients suffering from erectile dysfunction.

3. *In vitro* investigation of endothelium-dependent vasorelaxation in human isolated intracavernous arteries.

4. *In vitro* investigation of the role of C-type natriuretic peptide in penile vascular relaxation.

5. *In vitro* investigation of BK_{Ca} channels in endothelium-dependent and -independent relaxation of penile arteries

METHODS

1.1.INDIRECT CLINICAL INVESTIGATION OF HUMAN PENILE ARTERIES

1.1.1. Measurement of flow-mediated dilation

1.1.1.1.Patients

Correlation between common risk factors of ED and cardiologic diseases was examined on 51 patients suffering from ED, with the lack of cardiac symptoms at the University of Szeged in the Urological and Internal Department between 01.01. 2006 – 31.12. 2007. Patients were selected randomly and their average age is 51,3 years.

The patients suffering from ED for six months were divided based on International index of erectile function-5 (IIEF-5) into the following groups: under 10 scores into severe one, 11-15 into moderate one, 16-20 mild one. Due to the low number of cases only two groups were used: a severe group, and the second group from the moderate and mild ones.

After that a detailed anamnesis was made, extended with the function of partnership. We were striving for excluding psychogenic ED patients. Systematic diseases, pelvic and genital previous surgeries and traumas and medications were asked. Besides a general physical examination and following an observation of secondary genital characteristic, testicles, penis and rectal digital examinations were made.

After measuring BMI, blood pressure, ECG, exercise ECG and heart US were made. The following rates were measured from blood: Htc, Hgb, liver and kidney functions, ions, LDL, HDL, triglyceride, Hgb A1C%, testosterone, prolactin, LH, FSH.

To exclude or identify vascular origin of the ED flow mediated vasodilation /FMD/ technique was applied. During the technique the condition of endothelium of artery brachialis was examined which is a similar vessel to intracavernosus artery so its condition is well represented.

Endothel function was validated with coronarography, PET examination, plethysmography and arteria brachialis ultrasound was applied by us.

1.1.1.2 Experimental procedure

Diameter of arteria brachialis was measured with the patient relaxing for 10 minutes. The examination is considered to be informative between 3-6 mm brachial diameter. The cuff of the blood pressure set was blown by 250 Hgmm, brachial diameter was measured after release

mode. It was shown that the highest vasodilatation happened in the first two minutes /Fig. 1/ always that rate was measured. Endothelium was intact if FMD rate was 10% or above, or damaged if it was under 10%. No further groups were created due to the small number of cases, although a 5-10% is considered to be a threshold limit. 200microgramm sublingual nitrate was given for control, of which effect was checked with several measures in two minutes Reperfusion following ischaemia and NO from endothelium cells causes vasodilatation. If endothelium is damaged, vasodilatation will be smaller.

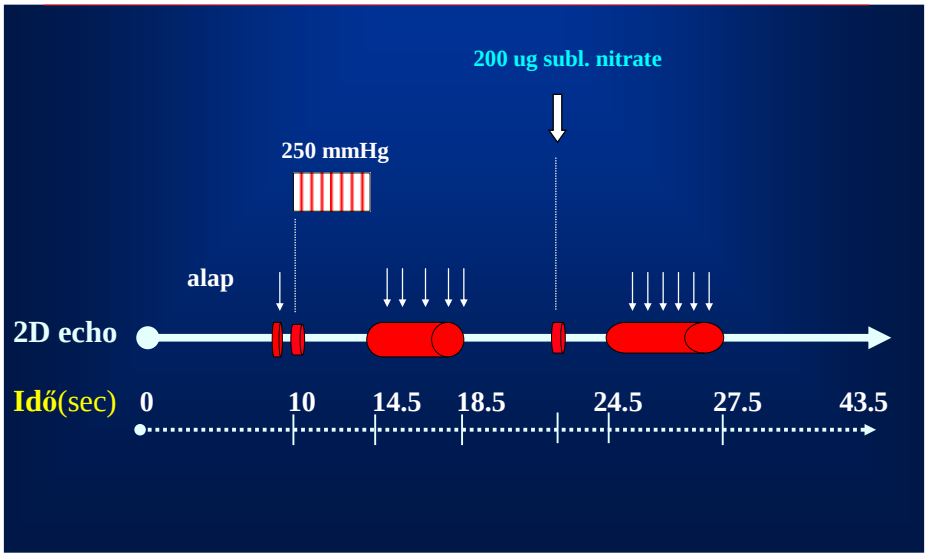


Figure 1: The protocol of the experimental procedure

1.2. Measurement of isometric tension in human intracavernous arteries

2.2.1. Tissue preparation and mounting

Penile erectile tissue was obtained in connection with transsexual operations. The penis was excised and submerged immediately in ice-cold (4ºC) physiological salt solution (PSS). The penile artery was carefully dissected and cleaned from the adherent connective tissue. Ring segments (ca. 2 mm long) were mounted on two 40-µm wires of isometric double myograph (Danish Myotechnology, Aarhus, Denmark). One wire was fixed to a force-transducer, the other to a length displacement device (Mulvany & Halpern 1977; Fig. 2). Before starting the experiment, preparations were allowed to equilibrate in PSS solution, at 37 °C and pH 7.4, for 30 min.

The relation between resting wall tension and internal circumference of the vessels was

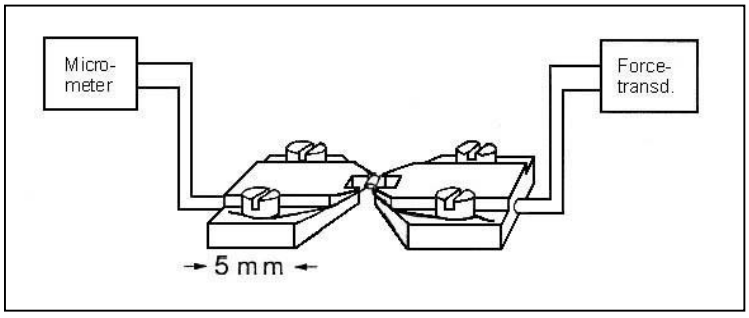


Figure 2. Schematic figure of myograph

determined, and the internal circumference, $L_{100,}$ corresponding to a transmural pressure of 100 mmHg in a relaxed vessel was calculated. Subsequently, the internal circumference of the vessels were set to L_1, where $L_1 = 0.9 \times L_{100}$. The effective internal lumen diameter was determined as $l_1 = L_1 / \pi$.

1.2.2. Experimental procedure

To test contractility of the preparations, they were exposed twice to phenylephrine (PE, 10 μM). The presence of intact endothelium was evaluated by inducing a stable contraction with phenylephrine (10 μM) followed by addition of acetylcholine (10 μM). Relaxation greater than 50% was taken as evidence of endothelial integrity.

Arteries were incubated with N^G-nitro-L-arginine methyl ester (L-NAME, 300 μM) and indomethacin (10 μM) for 20 min before being contracted with phenylephrine (3 μM) and cumulative concentration-response curves were constructed for the endothelium-dependent vasodilator acetylcholine (ACh, 1 nM -10 μM), the endothelium-independent vasodilator NO-donor sodium nitroprusside (SNP, 1 nM -10 μM), CNP (0.01–1 mM) and cANF4-23 (0.01–1 mM). the BK_{Ca} channel activator NS11021 (0,1 μM – 100 μM) and for the biologically inactive analogue of NS11021, NS13558 (0,1 μM – 100 μM). When the effects of inhibition of K+ channels or Na+-K+-ATPase were tested on vasodilations, the arteries were subsequently incubated for 20 minutes with either the combination of charybdotoxin (0.1 mM) plus apamin (0.5 mM), barium (Ba^{2+}, 30 mM) plus ouabain (5 mM), charybdotoxin (0.1 mM), or iberiotoxin (0.1 mM), and a second concentration–response curve was obtained for the agonist.

2. RESULTS

2.1. MEASUREMENT OF CHANGE IN FLOW-MEDIATED DILATION IN PATIENTS SUFFERING FROM ERECTILE DYSFUNCTION

2.1.1. Patients characteristics

23 patients from the 51 ones with ED were healthy and the other 28 patients considered themselves to be healthy but the examinations showed high blood pressure and laboratory deviation. FMD rate was below 10% with 13 patients from the 23 ‘healthy‘ ones and in the other group 22 from the 28 ones were pathological. 4 patients had psychogenic ED from the normal FMD level ones and their average age was lower and they did not smoke .

If cases of psychogenic origin are excluded, pathological FMD was found with 35 patients from 47 ED ones, and that means a high 76% rate.

2.1.2. Effect of age and smoking on flow-mediated dilation

A negative correlation between FMD and smoking, FMD and age. Significance level between FMD and age is (p=0,001)

Following the exclusion of ED patients of psychological origin, 20 patients from the 32 mild moderate ED ones had pathological FMD level (62%) while 14 patients from the 15 severe ED ones had pathological FMD (93%). Although the difference is striking if FMD rates are coordinated to IIEF scores, no linear correlation can be determined.

2.1.3. Effect of body-mass index, cholesterol and triglyceride level on flow-mediated dilation

Due to high scattering no correlation was found between BMI and FMD scores. No correlation could be shown between FMD and triglyceride level either and between FMD and cholesterol. It can be explained that only one blood sample was made and the increased rate of blood lipids and the existence period of their pathological rate was not examined. We assume that the high blood lipid level existing for decades causes endothelium dysfunction.

2.2. INVESTIGATION OF ENDOTHELIUM-DEPENDENT RELAXATION IN HUMAN INTRACAVERNOUS ARTERIES

Human penile small arteries with normalized internal lumen diameter of 557 ± 20 mm were contracted with phenylephrine (10 μM) (n = 74 arteries from 21 patients). Average contraction was 6.5 ± 0.2 N/m (n = 74).

2.2.1. Determination of the role of EDHF in ACh-evoked relaxation

In penile arteries contracted with phenylephrine (3 μM), ACh (1 nM – 10 μM) evoked concentration-dependent relaxation in endothelium-intact but not in endothelium-denuded segments with maximal relaxation and pD_2 value of 87.1 ± 2.6 % and 7.5 ± 0.2 (n=14), respectively. Incubation with an inhibitor of NO synthase, L-NOARG (100 mM), and cyclooxygenase, indomethacin (10 μM), significantly reduced the ACh relaxation (Figure 3). The pD_2 value for ACh in the presence of L-NOARG + indomethacin was 6.8 ± 0.1 (n=14).

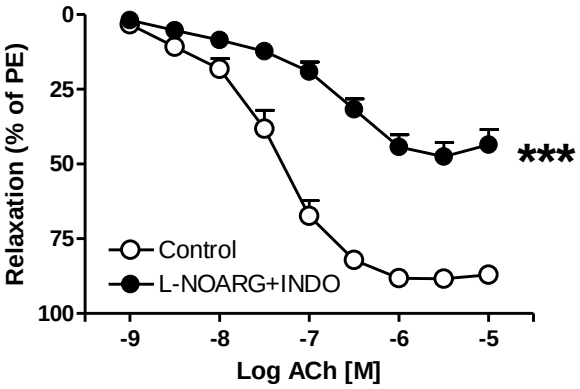


Figure 3. Concentration–response curves for ACh in the absence (control) and presence of NG-nitro-L-arginine (L-NOARG), and indomethacin (INDO), inhibitors of nitric oxide synthase and cyclooxygenase, respectively. *** p<0.001 by two-way ANOVA.

2.2.2. Effect of blockers of the EDHF pathway on ACh relaxation

In the presence of L-NOARG and indomethacin, the residual ACh relaxation was inhibited by treating the arteries with charybdotoxin (ChTX, 0.1 μM), a blocker of intermediate-conductance Ca^{2+}-activated K^+-channel (Control: 61.2 ± 5.8 % vs ChTX: 33.2 ± 6.1 %, n = 5, Figure 4a). Combination of charybdotoxin and apamin, blockers of intermediate- and small-conductance Ca^{2+}-activated K^+-channels, respectively, abolished the ACh-induced vasodilation (Control: 55.1 ± 9.0 % vs ChTX (0.1 μM) +Apamin (0.5 μM): 14.6 ± 2.5 %, n = 5, Figure 4b).

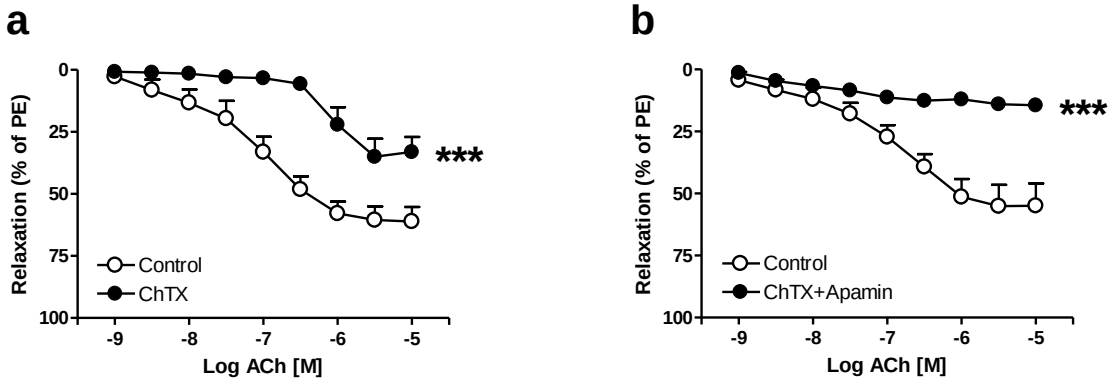


Figure 4. Concentration–response curves for ACh in the absence (control) and presence of (**a**) charybdotoxin (ChTX) and (**b**) charybdotoxin plus apamin. *** p<0.001 by two-way ANOVA.

2.2.3. Effect of IbTX on ACh relaxation

To investigate the role of BK_{Ca} channels in endothelium-dependent and -independent relaxation, iberiotoxin (IbTX, 0.1 μM), a selective blocker of BK_{Ca} channels, was used. Iberiotoxin inhibited the endothelium-dependent, acetylcholine-evoked vasodilatations both in the absence (control: 89.9 ± 1.7 % vs IbTX: 53.0 ± 9.4 %, n=7, $p < 0.001$, Figure 5) and presence of L-NAME plus indomethacin (L-NAME+INDO: 35.5 ± 4.1 % vs. L-NAME+INDO+IbTX: 14.8 ± 8.6 %, n=7, $p < 0.001$, Figure 10).

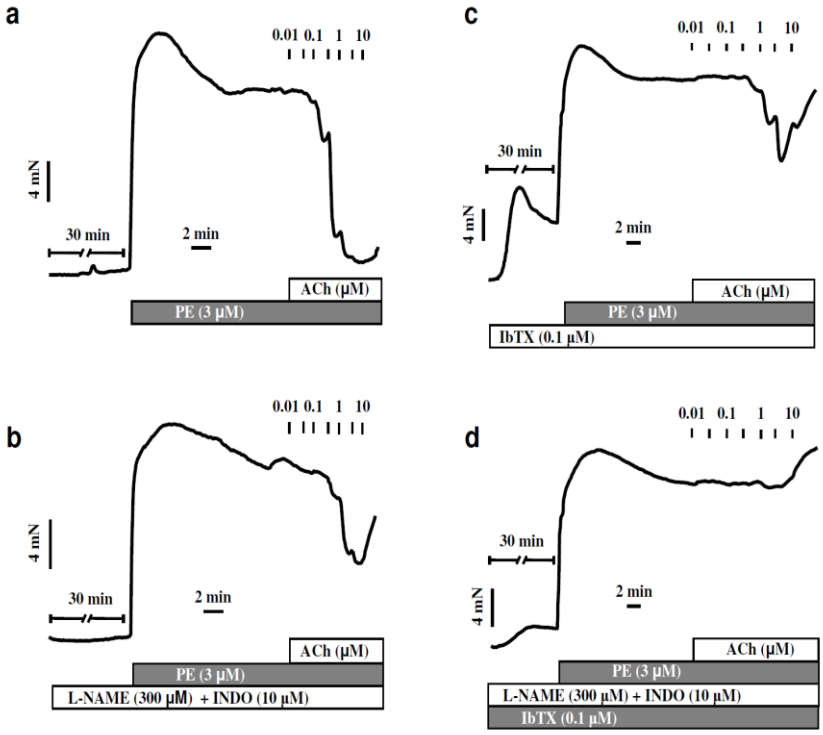


Figure 5. Original traces showing the relaxing effect of ACh in the absence (**a, b**) and presence (**c, d**) of iberiotoxin (IbTX). ACh-evoked relaxations were recorded in the absence (**a, c**) and presence (**b, d**) of L-NAME plus indomethacin. The horizontal bar indicates time, and the vertical bar indicates increase in force (mN).

Iberiotoxin also induced rightward shift in dose-response curve (pD_2 values for control: 7.7 ± 0.3 vs IbTX: 6.7 ± 0.1, n=7, $p < 0.05$; pD_2 values for L-NAME+INDO: 6.7 ± 0.1 vs. L-NAME+INDO+IbTX: 6.3 ± 0.3, n=7, $p > 0.05$).

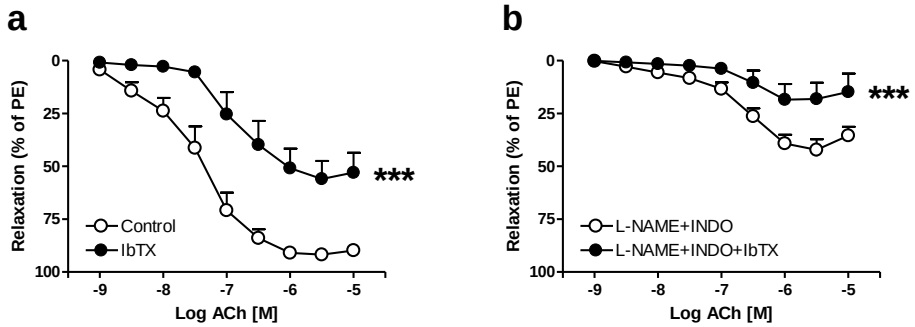


Figure 6. Concentration –response curves for ACh in the absence and presence of (**a**) iberiotoxin (IbTX) and (**b**) L-NAME plus INDO. *** $p<0.001$ by two-way ANOVA.

2.3. INVESTIGATION OF ENDOTHELIUM-INDEPENDENT RELAXATION IN HUMAN INTRACAVERNOUS ARTERIES

2.3.1. Characterization of CNP-evoked relaxation

In arteries with endothelium (Figure 7) and contracted with phenylephrine in the presence of L-NOARG and indomethacin, CNP (0.01–1 mM) evoked concentration-dependent relaxations (Figures 7 and 8). The concentration–response curves for CNP were reproducible up to three times and were unaltered in preparations without endothelium. In the presence of L-NOARG and indomethacin and contracted with high extracellular K^+ (80 mM), CNP relaxation was markedly blunted (Figures 7 and 8).

In the presence of L-NOARG and indomethacin, incubation of the arteries with charybdotoxin alone (Figure 8) or a combination of charybdotoxin plus apamin (Figure 8), markedly reduced CNP relaxation.

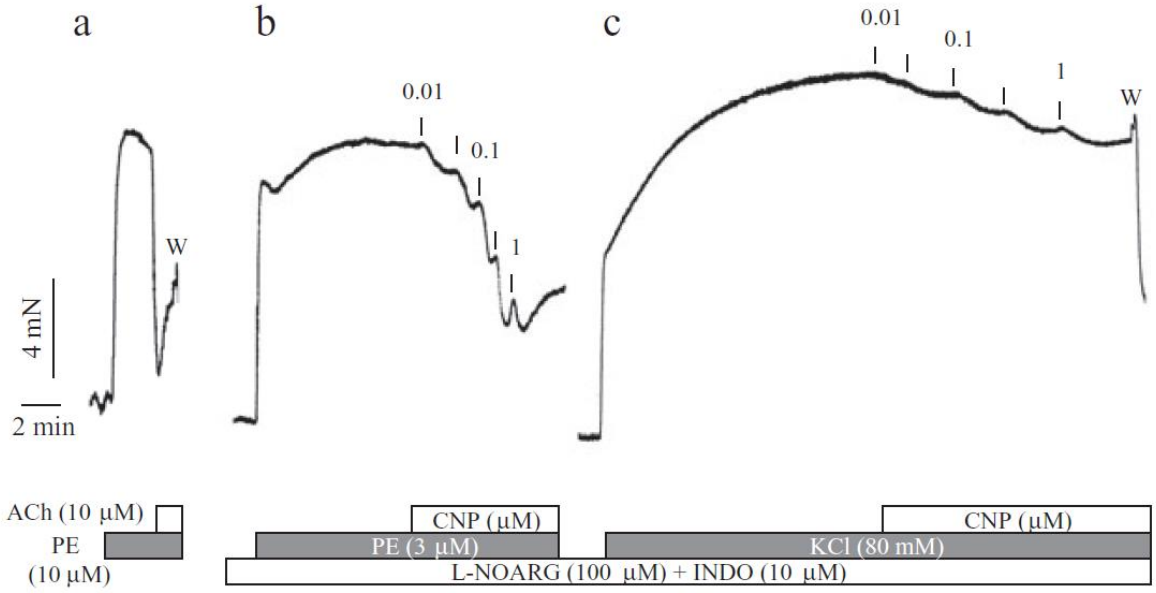


Figure 7. Original traces showing the relaxing effect of C-type natriuretic peptide (CNP) in human penile small artery. (**a**) Contraction induced by phenylephrine (PE, 10 μM) followed by acetylcholine (ACh, 10 μM). (**b**) In the arterial segment contracted by PE, CNP induced concentration-dependent relaxation. (**c**) In the same arterial segment contracted with 80-mM K^+-rich physiological salt solution, CNP relaxation was markedly reduced. The horizontal bar indicates time, and the vertical bar indicates increase in force (mN). W = washout.

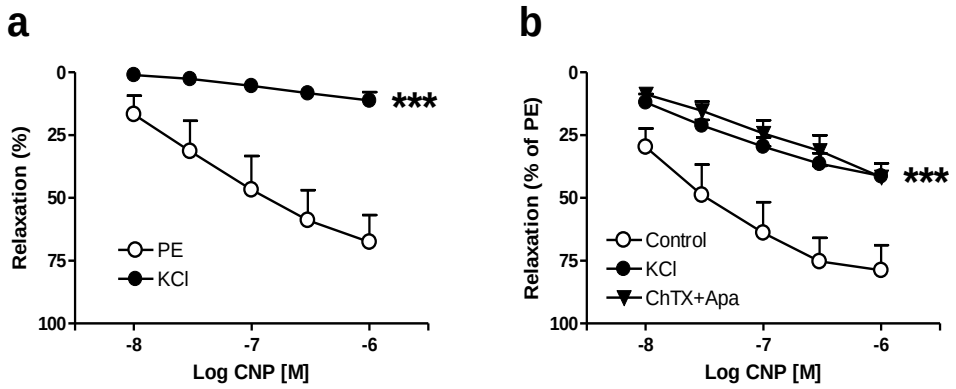


Fig.: **8.** (**a**) Average concentration–response curves for CNP in preparations contracted with phenylephrine (PE) or with 80 mM K^+ (n = 5). (**b**) Average concentration–response curves for CNP alone or in combination with charybdotoxin (ChTX) (n = 4) or ChTX plus apamin (ChTX + Apa) (n = 4) in PE-contracted penile arteries. Results are means ± standard error of the mean. *** p<0.001 by two-way ANOVA.

2.3.2. Effect of IbTX on CNP-evoked relaxation

To test whether BKCa channels were involved in CNP-evoked relaxation, the blood vessels were incubated with iberiotoxin (0.1 μM). In the presence of L-NOARG and indomethacin, iberiotoxin markedly reduced the concentration–response curves for CNP (Figure 9) compared to parallel control experiments (Figure 9)

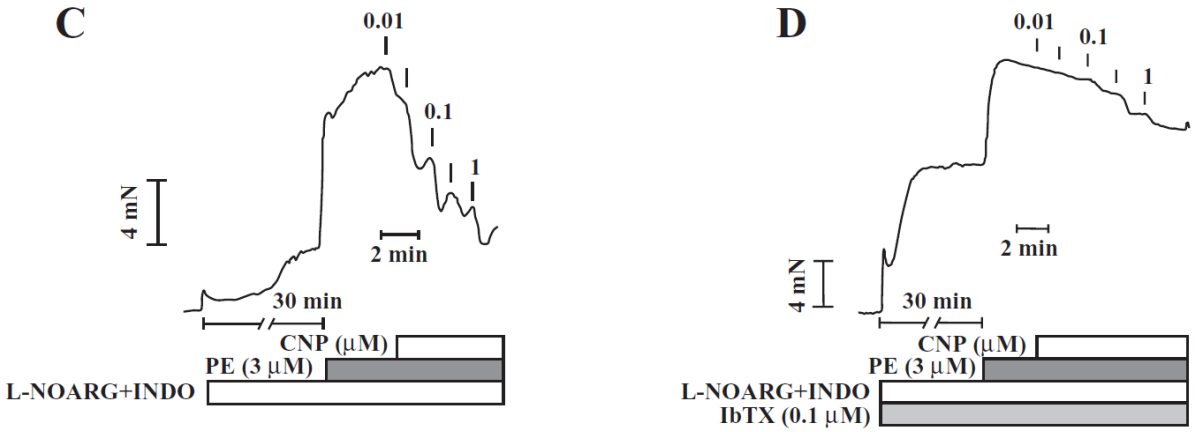


Figure 9. BK_{Ca} channels involved in C-type natriuretic peptide (CNP)-evoked relaxation. Original traces showing the relaxing effect of CNP in human penile small arteries in the (**C**) absence and (**D**) presence of a blocker of BK_{Ca} channels, iberiotoxin (IbTX). The traces are representative for two experiments obtained from two patients. The horizontal bar indicates time, and the vertical bar indicates increase in force (mN).

2.3.3. Effect of IbTX on SNP-induced relaxation

The NO-donor, endothelium-independent vasodilator, sodium nitroprusside-induced relaxations were inhibited by iberiotoxin (Control: 88.0 ± 8.4% vs. IbTX: 66.0 ± 4.6 %, n=6, $p < 0.001$, Figure 10). The corresponding pD_2 values for SNP were 7.9 ± 0.2 and 7.2 ± 0.1 in the absence and presence of IbTX, respectively (n=6, $p < 0.05$).

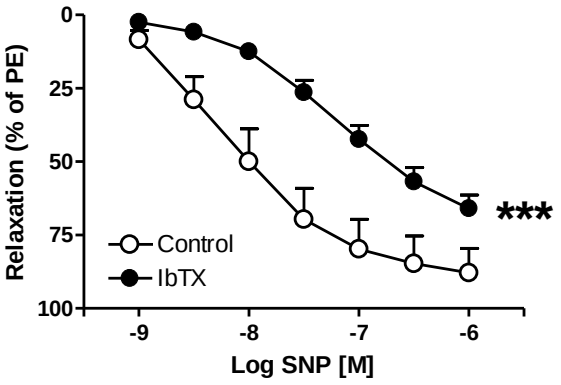


Figure 10. Concentration–response curves for SNP in the absence and presence of iberiotoxin (IbTX). *** p<0.001 by two-way ANOVA.

2.3.4. EFFECT OF NS11021, A PUTATIVE BKCA OPENER, IN HUMAN INTRACAVERNOUS ARTERIES

NS11021, a selective opener of BK_{Ca} channels at low concentrations, evoked powerful relaxation, which was inhibited and right-shifted in the presence of the BK_{Ca} channel blocker iberiotoxin (Figure11). NS11021 pD_2 values were 5.8 ± 0.1 and 5.0 ± 0.1 in the absence and presence of IbTX, respectively (n=5, $p < 0.01$). NS13558, the biologically inactive analogue of NS11021, did not induce significant relaxation in human penile small arteries, compare to NS11021 (38.1 ± 5.8 % vs. 95.6 ± 2.0 %, respectively n=4-5, $p < 0.001$, Figure 11).

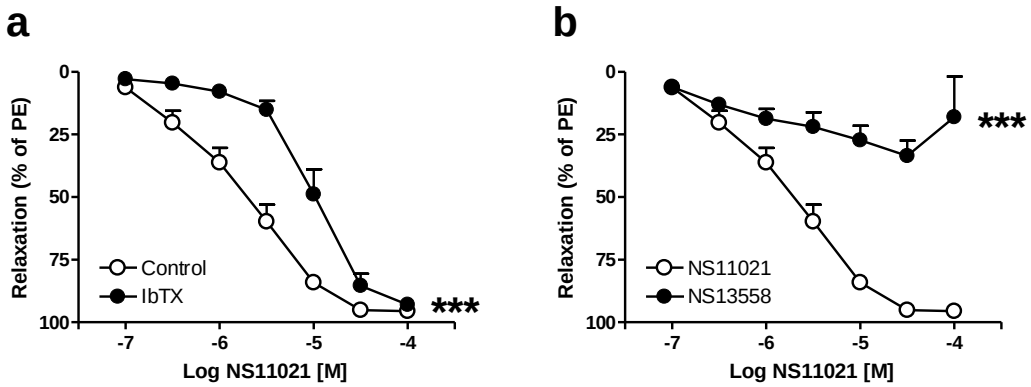


Figure 11. Concentration–response curves for NS11021, a BK_{Ca} channel opener, (**a**) in the absence and presence of iberiotoxin (IbTX) and (**b**) comparison of NS11021 with NS13558, the biologically inactive analogue of NS11021. *** p<0.001 by two-way ANOVA.

3. TRANSSEXUAL OPERATION

3.1. CHANGES IS SURGICAL TECHNIQUE IN TRANSSEXUAL OPERATION

The examination of erection research of human penis was made possible male by female sexual reassignment surgery at the University of Szeged in the Urological Department. Transformation of sex is a multiphase operation, its essential part is creating a new female genital organ which operates both esthetically and functionally well. The current operational technique was has been developed by continuous changes based on 14 year experience.

The operation is made in a lithotomy position. The earlier turned down u-shape perineal incision, which resulted a threshold building at the neovagina, was exchanged by median sagittal incision. (Figure 12).

Degloving of penis is made after castration. When the patient does not circumcise and its individual sizes make it possible to save a part of the inner layer of the foreskin, the inner lips are created from that one. The next step is to separate the urethra from corpus cavernosum penis (Figure 13a).

Division of penis into parts is continued. Saving the penile dorsal neurovascular bundle is essential to the function of newly created clitoris. The neurovascular bundle used to be separated from the corpus cavernosum along with tunica albuginea, so that the possibility of neurovascular injury was minimized. (Figure 13.b,c).

As the patients are genetically male, the pelvis of male to female of transsexual patients is tight, the neurovascular bundle separated together with tunica albuginea takes a big place, so the entry of the neovagina is critically tightening, with the increased experience we have returned to the isolation of neurovascular bundle itself because it takes a smaller place and the number of tissue lost of the newly created clitoris has not increased.

Corpus cavernosum is exstirpated from the inferior wing of symphisis. A greater part of the glans is used at the creation of clitoris, one part of it is deepithelialized and it is sank under the skin (Fig. 14.). The inner lips are created in the inner layer of foreskin left on glans and the fixing sutures of the neo clitoris is placed into foreskin to avoid circular damage.

After the separation of penis the preparation of the space of the neovagina is followed. The bulbar urethra is separated from the rectum the centrum tendineum is cut. When the place is too tight, the spongiosus tissue of bulbar urethra may be resected. The prostate is reached and partly mobilized as in the case of perineal prostatectomy. The muscle of pelvic floor is perforated and it is incised ceating a fairly wide cave for the neo vagina. The parameter of

vagina is determined by the individual size of the penis. The patient expects a proper diameter neovagina. The solution of the problem was provided by the non used pendular urethra, which is separated from the neomeatus in dorsal two third, but the ventral one third of the urethra is preserved to keep blood supply and that part of the urethra is detubularised and the anterior wall of the neovagina is created from that part. (Figure 15). The result of that is the diameter growth of the vagina and the spongiosus tissue participating in vagina creation increases the pleasure of sexual intercourse. The distal part of the penile skin which is the end of neovagina closes with absorbable running sutures(Figure 16 a).

The next step is to fit the neovagina into its place and to appoint the place of clitoris and the meatus (Figure 16 b.). If it is necessary, the skin of the abdomen is mobilized and occasionally the umbilicus is replaced. The clitoris and urethra are sutured in the earlier demonstrated way, then the neovagina is fixed with tissue glue. The inner lips are created from the inner layer of the foreskin and the outer lips are created from the resectioned scrotum skin. The upper part of the outer lips are approximated to each other after a transverse incision and sagittal suturing of the skin above the neoclitoris(Figure 17. a). Urethra catheter and perianal drain are left behind. The vagina is kept fixed with bandage filled condom. The drain and the condom are removed on the second postoperative day while the urethra catheter is removed on the seventh postoperative day. A hyperol and betadine lavage is applied daily. The operation is made LMWH protection. Antibiotic effecting on anaerob bacteria are given for two days parenterally and five more days enterally. The success of the operation is greatly influenced by the postoperative care. A Betadine soap treatment of the operative field is necessary at home. A regular vagina widening should immediately be started after wound healing which is made by the patient itself.

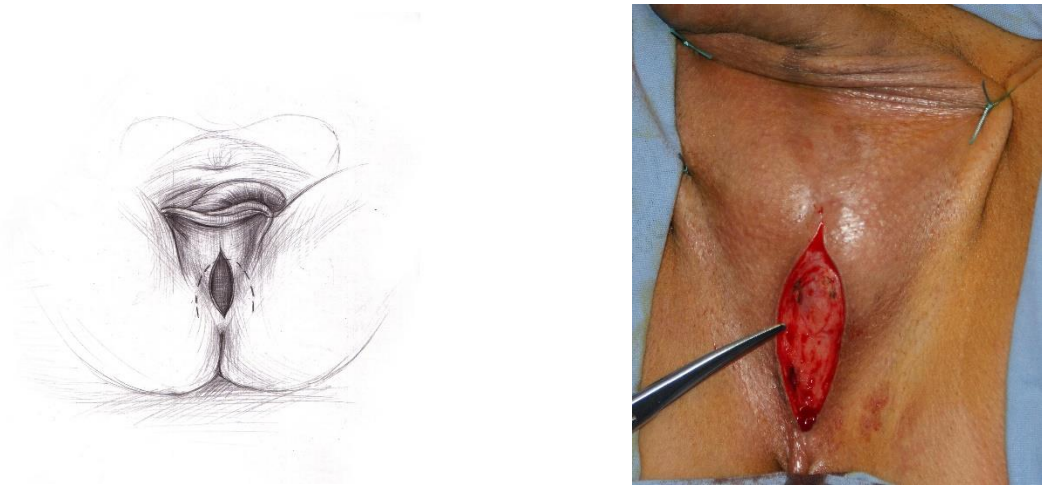


Figure 12: The earlier turned down u-shape perineal incision was exchanged by median saggital incision.

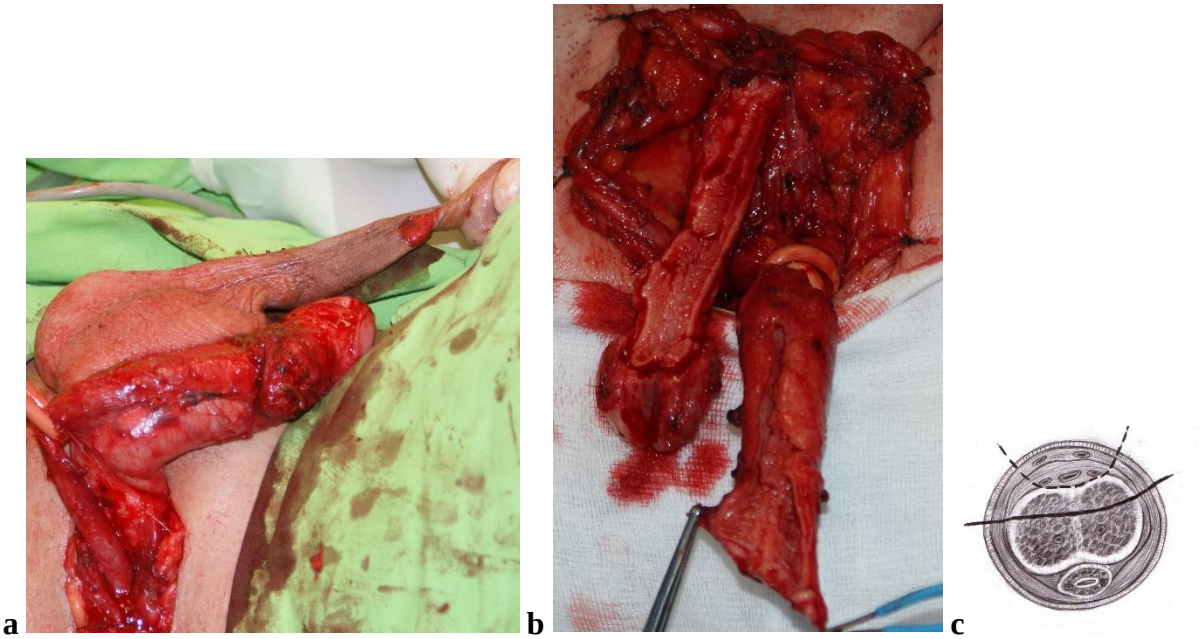


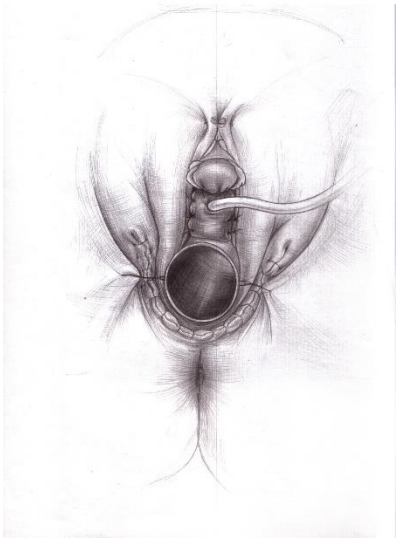
Figure 13: Separate the urethra from the corpus cavernosum/a/.The neurovascular bandle used to be separated from the corpus cavernosum along with tunica albuginea/b,c/

Figure 14: The greater part of the clitoris is used at the creation of the clitoris, one part of it deepithelialized and it is sank under the skin

Figure 15: The pendular urethra is detubularised and the anterior wall of the vagina is created from the part

a  b 

Figure 16: The neovagina is created from the penile skin/a/.Fit the neovagina into its place and marking out the proper place of the meatus and clitoris/b/.

a  b 

a  b 

Figure 17: The outer lips are created from the resectioned scrotal skin/a/. Six months after the operation/b/

Conclusion

1. Flow mediated vasodilation as a new, non-invasive diagnostic procedure in the examination of vascular type erectile dysfunction.
2. CNP has a strong vasodilatation effect on human resistance vessels in the presence of NO synthase and cyclooxygenase enzyme inhibitors
3. That relaxation demonstrates a similar characteristics as an EDHF released by Ach
4. CNP has a smooth muscle cells hyperpolarization effect similar to Ach
5. Our examinations prove CNP is an EDHF in human penile resistance vessels which plays an essential role in the reduction of vessel tone supporting erection.
6. CNP or its similar compounds may be successful in the treatment of erectile dysfunction.
7. NO and EDHF take part in the endothelium dependence relaxation human penile intracavernosus arteries.
8. BKC channels play an essential role in the establishment of the effect enduced by both of NO and EDHF
9. During our examination the opening compounds of BKC channels are assumed to be effective in the treatment of disregulation of penile arteries.
10. The Hungarian implementation of female to male sexual reassignment operation

Development of the surgical technique in our Department of Urology has lead to better functional and esthetical results. These modifications are the following

-The turned down u-shape perineal incision was exchanged by median sagittal incision

- The expert preparation of neurovascular bundle

- Reaching clitoral orgasm a greater part of the glans is used at the creation of clitoris, one part of it is deepithelialized and it is sunk under the skin. The fixing sutures of the neo clitoris is placed into foreskin to avoid circular damage.

6. Acknowledgments

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List of publication related to the thesis

I. **Kiraly I**, Pataricza J, Bajory Z, Simonsen U, Varro A, Papp JG, Pajor L, Kun A
Involvement Of Large-Conductance Ca(2+) -Activated K(+) Channels In Both Nitric Oxide And Endothelium-Derived Hyperpolarization-Type Relaxation In Human Penile Small Arteries
BASIC & CLINICAL PHARMACOLOGY & TOXICOLOGY 113:(1) pp. 19-24. (2013)

II. **Király I**, Kun A, Pajor L
A merevedés kialakulásának sejtszintű mechanizmusa, különös tekintettel a magas konduktanciájú Ca2+ aktiválta K+ csatornák ebben játszott szerepére.
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III. **Király I**, Pajor L.
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IV. Kun A, **Király I**, Varró A, Simonsen U, Pataricza J, Papp JGy, Pajor L
Endothelium in health and disease involvement of large-conductance Ca2+ - activated K+ channels in acetylcholine-evoked vasodiation of human penile small arteries.
BASIC & CLINICAL PHARMACOLOGY & TOXICOLOGY 107:(Suppl.1) p. 396. (2010)

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