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Investigation of smooth muscle contraction by organ bath

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Organ bath:
What is it?

- **approach for measuring and exploring the contraction of smooth muscle**
- **„myography“**
- **intact, fresh tissues, in vitro**

Organ bath device,
Urological Research Lab

Smooth muscle:
No skeletal muscle, unconscious motoric actions

- blood vessels (vascular smooth muscle)
- Airways
- Lower urinary tract: urinary bladder, prostate
- Gastrointestinal system
- Kidney, corpus cavernosum: vascular smooth muscle

Smooth muscle:
Functions, diseases

organ	function	disease	drugs
Blood vessel (peripheral) [1]	Blood pressure, blood flow (systemic, regional)	Arterial hypertension [1]	AT_1R antagonists (sartans), ACE inhibitor, Ca^{2+} antagonist
Airway	Respiration	COPD, asthma	β-agonist
Prostate	Reproduction	Voiding symptoms, BPH	α_1-blockers, PDE5 inhibitor
Bladder	Micturition	Storage symptoms, incontinence	Anticholinergics, β_3-agonist
Kidney [1]	Renal function	diabetes	
Corpus cavernosum [1]	Reproduction	Erectile dysfunction	PDE5 inhibitors

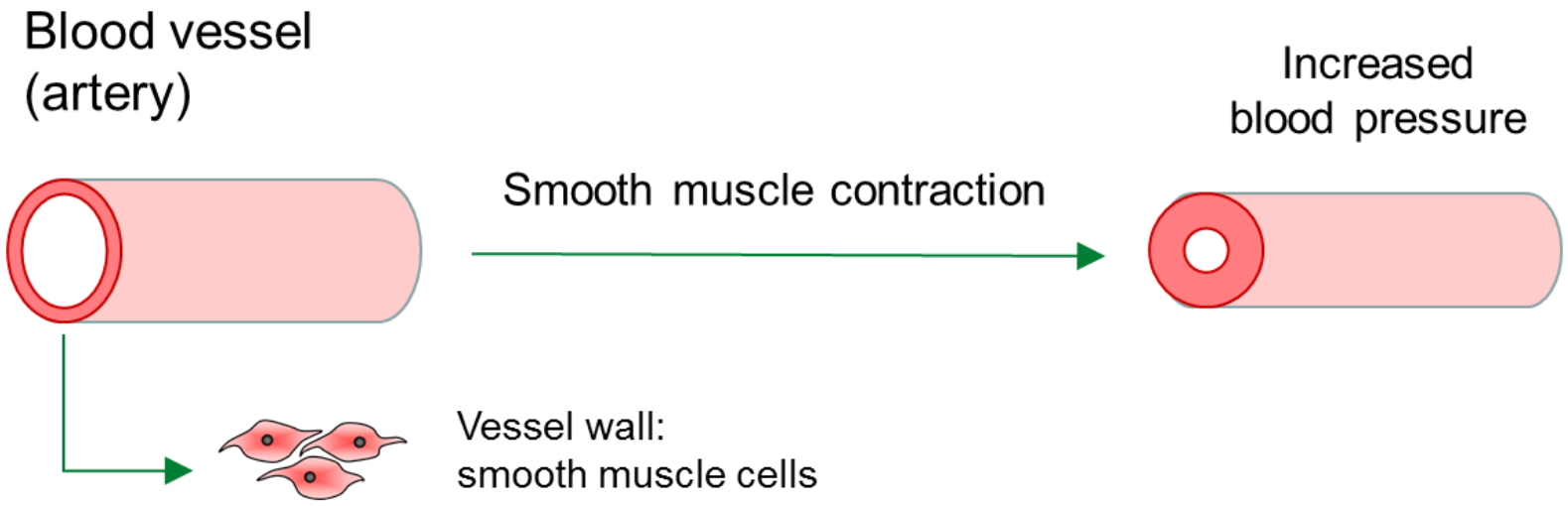
[1] vascular smooth muscle, [2] plus pulmonary hypertension, portal hypertension (liver cirrhosis)

Smooth muscle-based diseases:
Prevalence, clinical & socioeconomical relevance, costs

Organ system	disease	Number of patients	costs
Cardiovascular system	• Arterial hypertension • „cardiovasuclar disease“	• 3.5 Mrd (ww) >110-115 mmHg • 874 Mio (ww) >140 mmHg • 9.4 Mio death/a	1 Trillion USD ww/a
	Liver cirrhosis: portal hypertension	100 Mio ww	
Lower urinary tract	LUTS: • prostate (BPH) • bladder (OAB)	1 Mrd (ww, 2018) (=OAB+BPH)	• BPH, medications: 4.8 Mrd $, ww/2009 • OAB, only USA: 65.9 Mrd $ (medical + non-medical)
Airways	Asthma, COPD	358 Mio asthma 174 Mio COPD (ww)	19.3 Mrd €/a (only asthma in Europe)

ww, worldwide

Vascular smooth muscle contraction:
Increase of blood pressure, target of medical treatment

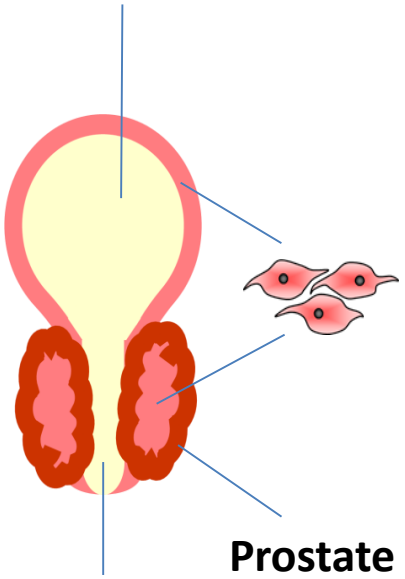


Antihypertensive medications:

- Inhibition of vascular smooth muscle contraction → decrease of blood pressure
- Sartans (Ang-II receptor antagonists), ACE inhibitors, Ca^{2+} channel blockers

Bladder/prostate smooth muscle contraction:
Lower urinary tract symptoms, target of medical treatment

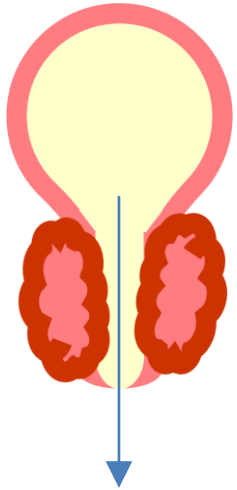
Urinary bladder



Prostate

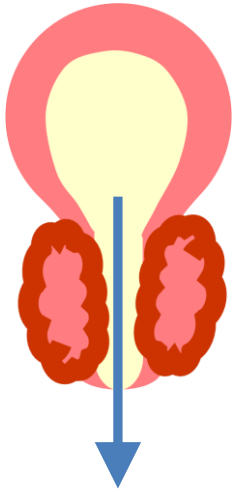
Urethra

Normal micturition:
Bladder smooth muscle contraction



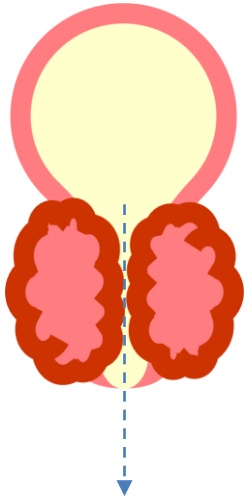
Urinary flow,
bladder emptying

Overactive bladder:
Increased bladder smooth muscle contraction



Urgency,
incontinence

BPH:
Increased prostate smooth muscle tone



Urethral obstruction,
impaired voiding

Medications: inhibition of bladder/prostate smooth muscle contraction

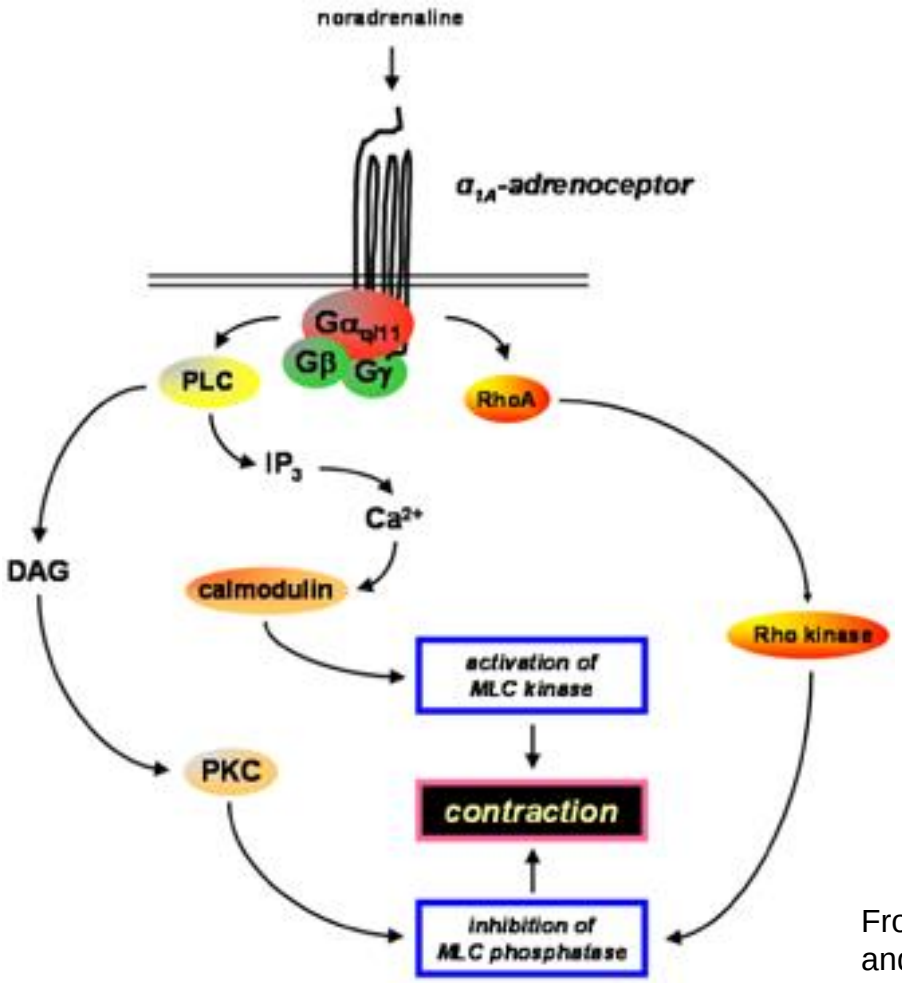
Investigation of smooth muscle contraction: purposes

- compounds for inhibition of smooth muscle contraction: new medications
- target identification, validation (knockouts)
- pathophysiology
- basic sciences: mechanisms of contraction, relaxation

Investigation of smooth muscle contraction:
Who? Where?

- **Internal medicine**
- **Urology**
- **Preclinical medicine: Pharmacology, Physiology**
- **Non-academic: pharmaceutic industry**

Smooth muscle contraction:
Receptor-induced, intracellular pathways



Contractile receptors:

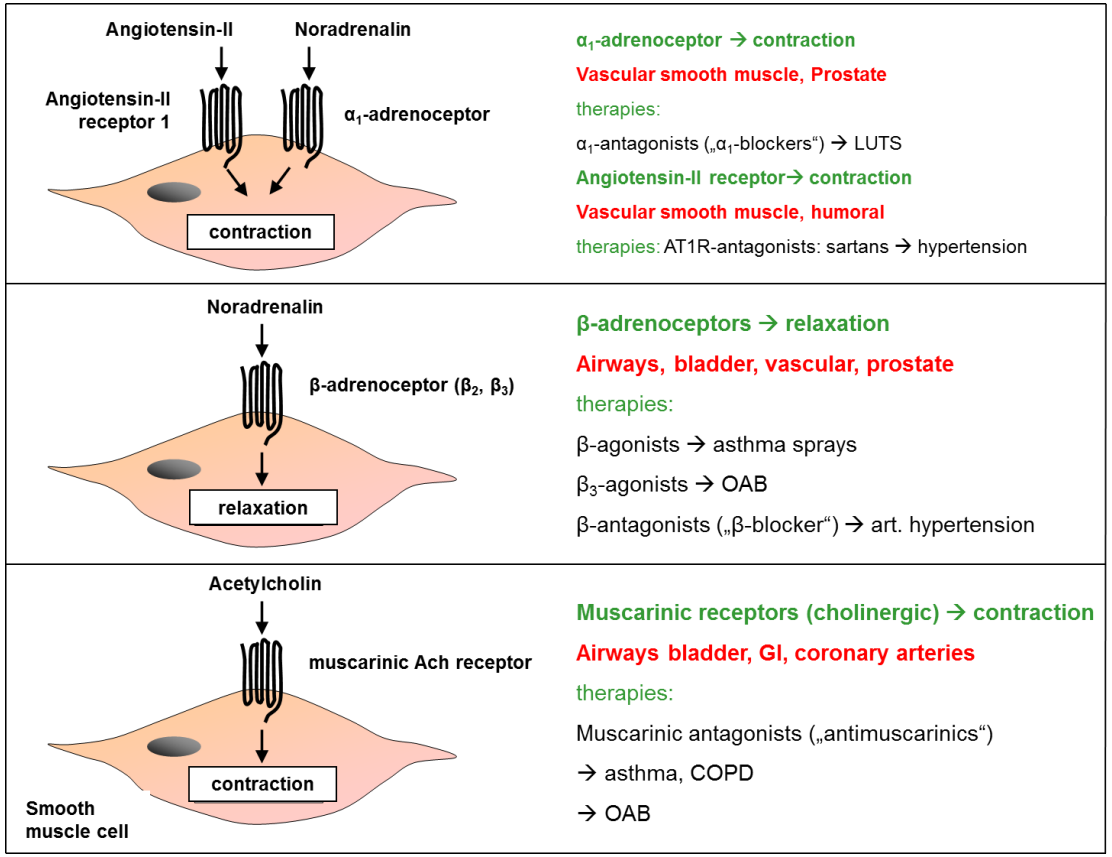
- α_1-adrenoceptor
- Muscarinic/cholinergic
- Angiotensin-II
- Thromboxane A2
- Endothelin

Intracellular pathways:

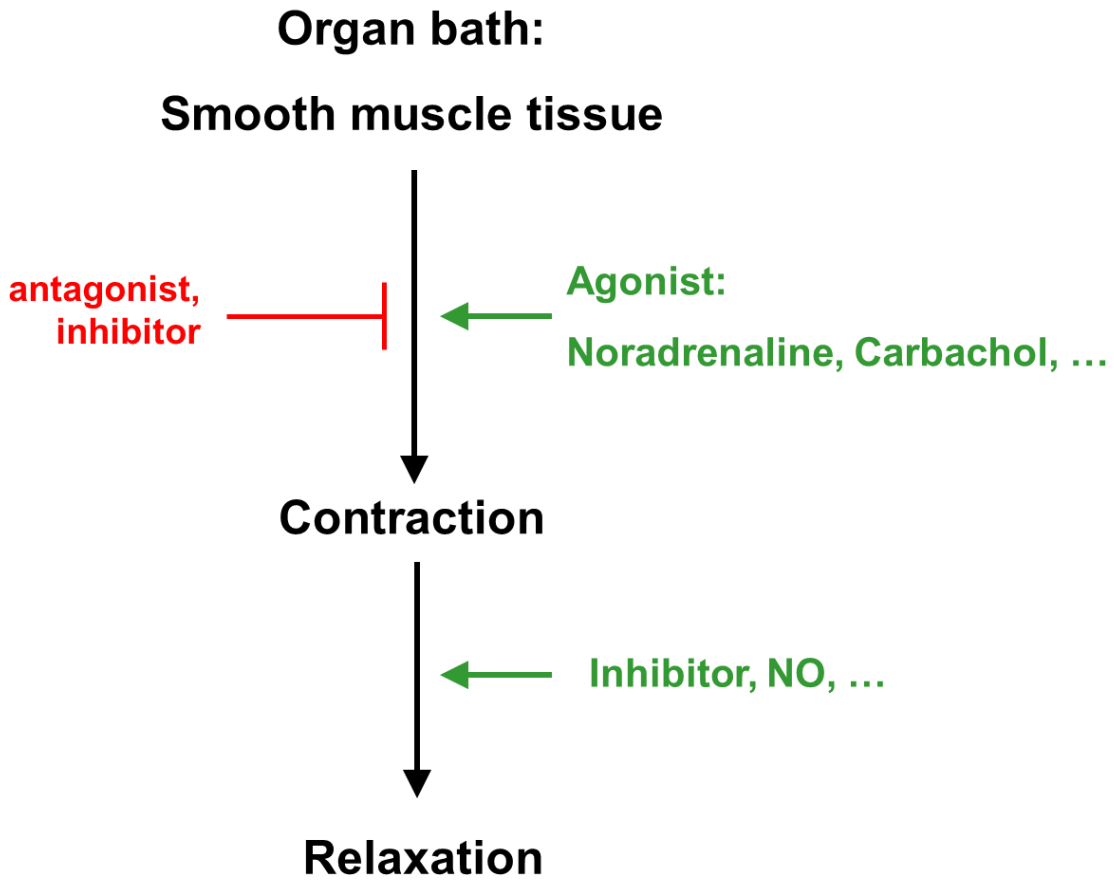
- Calcium
- PKC
- RhoA/Rho kinase
- others

From: Hennenberg et al.: Prostatic α1-adrenoceptors: new concepts of function, regulation, and intracellular signaling. Neurourol Urodyn 2014;33:1074-85.

Bladder/prostate smooth muscle contraction:
Lower urinary tract symptoms, target of medical treatment



Organ bath experiments:
Principle strategy



Organ bath experiments:
Which tissues?

- **Mice, rats**
  - **knockout models**
  - **disease models**
  - **Without disease, without knockout**
- **Human**
  - **high translational value**
  - **non-diseased controls not always available**
  - **cheap**

Tissue samples for organ bath experiments:
Organ-specific considerations

Lower urinary tract:

- Prostate, bladder: rPx, rCx
- Prostate: resection flakes from ablative approaches (TURP): traumatized tissues, bad results
- rodents: very small samples, limited translational value, permissions, very bad reputation in Europe (not: USA, China)

Cardiovascular system:

- Aorta, mesenteric vessels: rat, mouse; very common, cheap
- pig: similarity to human, slaughterhouse, butcher, easy
- Human material: umbilical veine
- other human vessels: organ transplantation (hepatic artery), cancer surgery (renal artery)

Airway smooth muscle:

- bovine: slaughterhouse, large, much, cheap
- guinea pig: experimentally-induced COPD, allergic models

Organ bath device:
Myograph 720M

4 chambers

Gas supply (carbogen)

Connection to AD converter

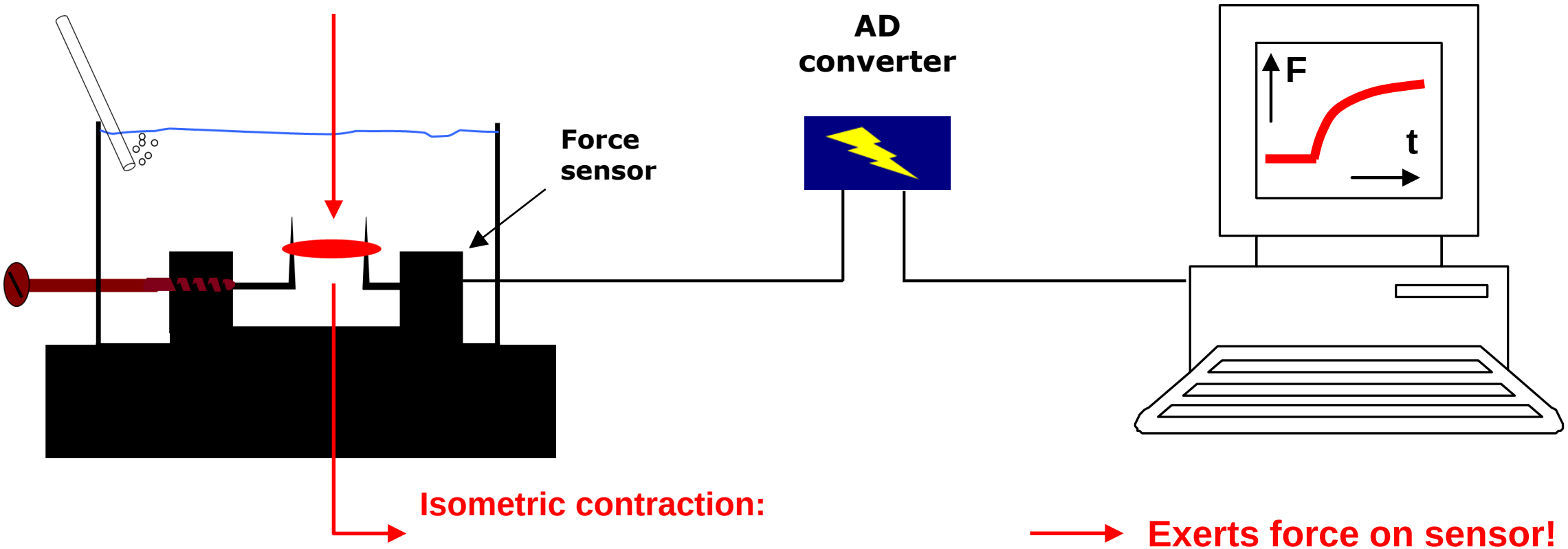
Force sensor, transducer

Needles, hooks: attachment of tissue

Screw: stretching hooked tissue, pretension

Complete setting:
Myograph, AD converter, computer, software

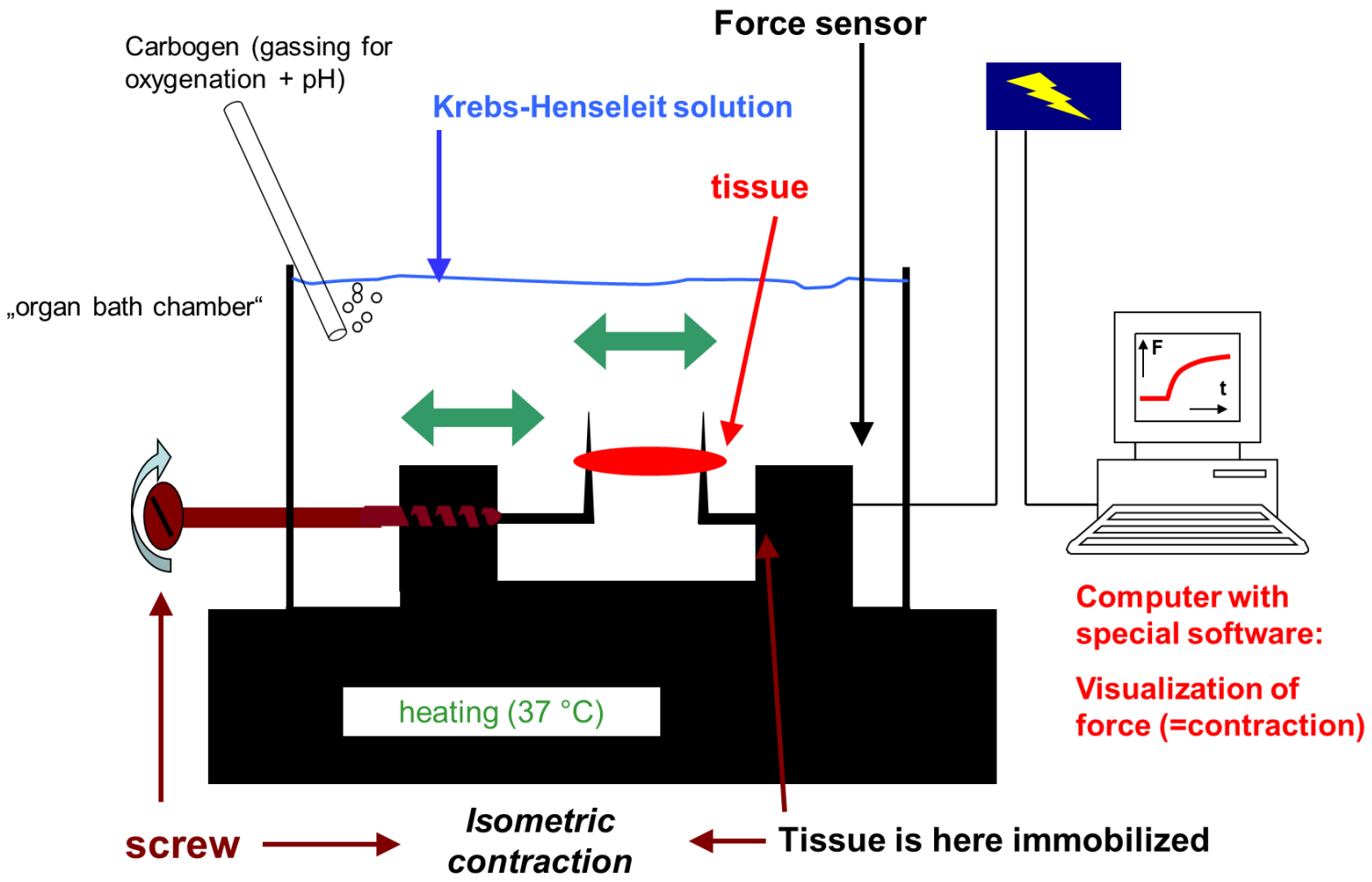
Agonist: e. g. noradrenaline



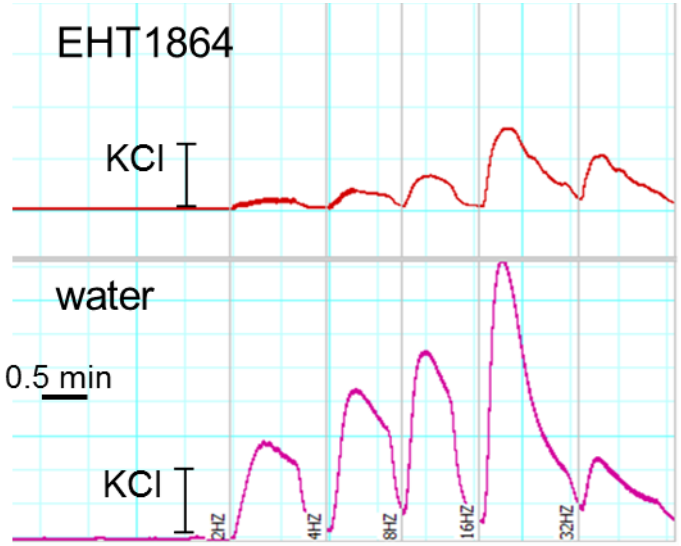
Isometric contraction:

contraction without changing length → **Exerts force on sensor!**

Isometric contraction:
Contraction withouth change in length



Induction of contractions:
Concentraction response curves



Example #1:
- Human prostate tissue
- Noradrenaline: cumulative concentrations
- With and without α_1-blocker

Example #2:
- Human bladder tissue
- Electric field stimulation: neurogenic contractions
- frequence response curves
- With and without inhibitor

Presentation of results:
Curves, EC_{50}, E_{max} values, analyses

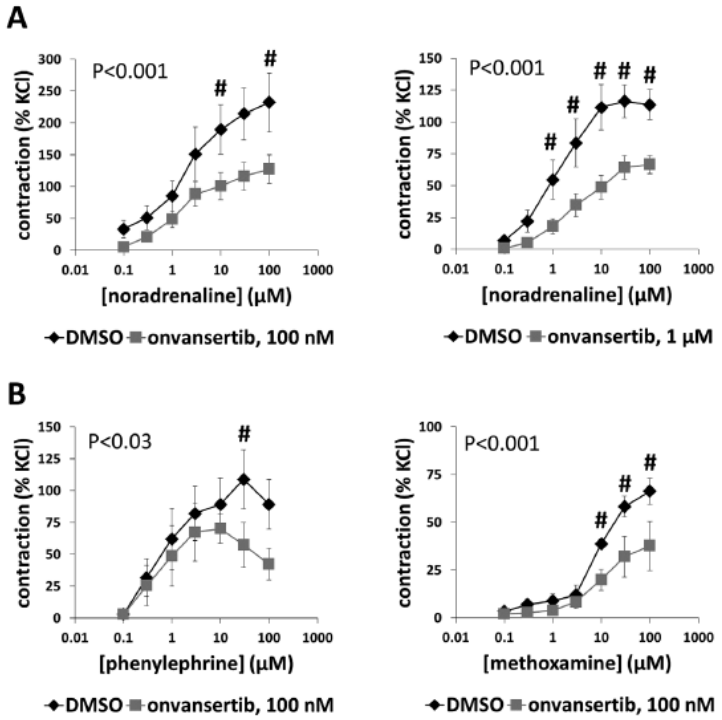


Fig. 1. Effects of onvansertib on adrenergic contractions of human prostate strips. Contractions of human prostate strips were induced by noradrenaline **(A)**, or by the α_1-adrenoceptor agonists phenylephrine and methoxamine **(B)** in an organ bath, after addition of onvansertib in a concentration of 100 nM or 1 µM, or of DMSO (controls), or induced by noradrenaline **(C)** following washout of DMSO and onvansertib (100 nM) for 30 min. In each experiment, DMSO and onvansertib were applied to separate samples, which were obtained from the same prostates. To eliminate heterogeneities due to individual variations, different degree of BPH or other varying smooth muscle content, tensions have been expressed as % of contraction by highmolar KCl, being assessed before application of onvansertib or DMSO. Data are means ± S.E.M. from series with tissues from n = 5 (noradrenaline with 100 nM onvansertib without washout), n = 6 (noradrenaline with 1 µM onvansertib), n = 5 (phenylephrine), n = 5 (methoxamine), and n = 5 (noradrenaline after washout) patients, in which samples from each patient were allocated to both the control and inhibitor groups (#P < 0.05 after multivariate analysis at indicated concentration; P value for whole groups after two-way ANOVA as indicated in inserts).

From: Wang et al, Eur J Pharmacol 2020;872:172985.

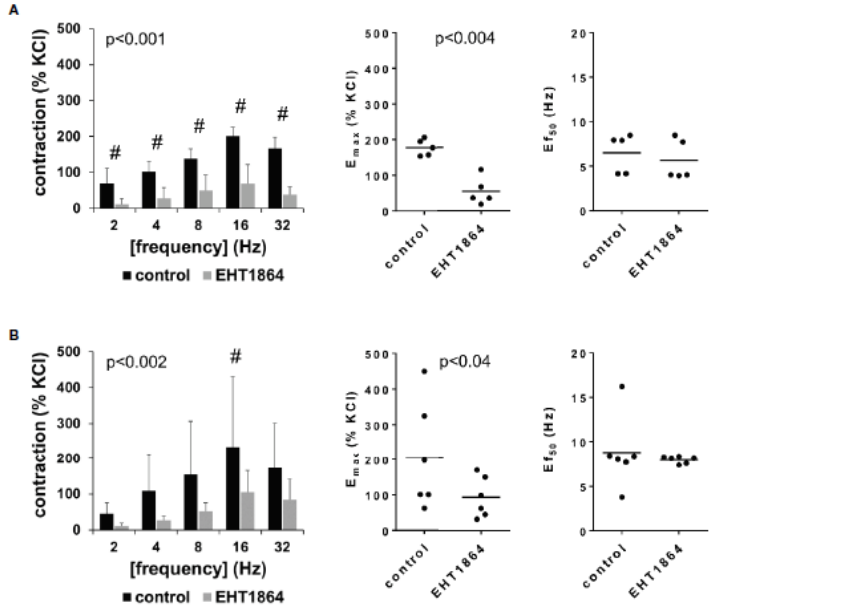
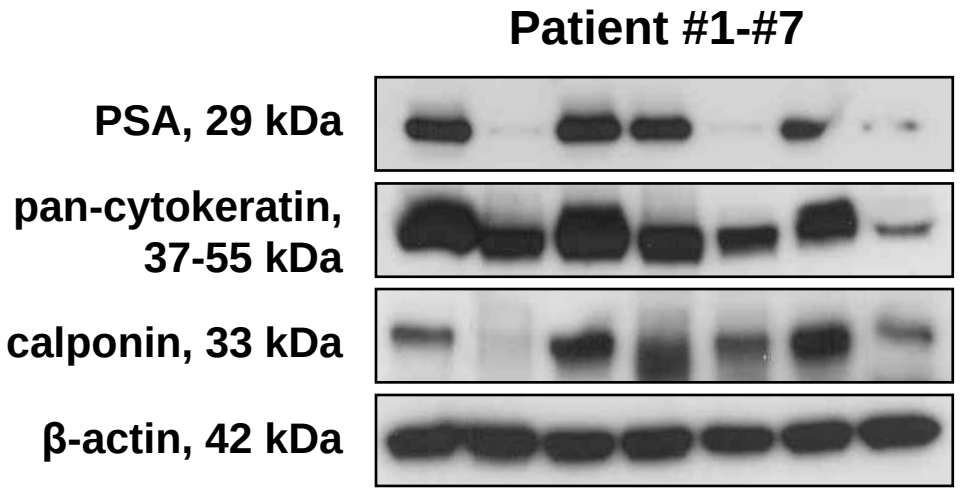


FIGURE 2 | Effects of EHT1864 on electric field stimulation (EFS)–induced contractions of human detrusor tissues. Contractions of female **(A)** and male **(B)** tissues from the lateral urinary bladder wall were induced by EFS in an organ bath, 30 min after addition of EHT1864 (100 µM) or an equivalent amount of water (controls), which was used as solvent for EHT1864. The stock solution of EHT1864 had a concentration of 10 mM, so that 100 µl of water was added to organ bath chambers. Each chamber contained 10 ml Krebs-Henseleit solution, resulting in a concentration of 0.99% for solvent-related water. Shown are data from n=5 female and n=6 male patients, which are means ± SD in frequency response curves $^{\#}p < 0.05$ for control vs. EHT1864 by two-way ANOVA, and p values for whole groups in inserts from 1-way ANOVA), and E_{max} values and frequencies inducing 50% of the maximum EFS-induced contraction (Ef_{50}) for single experiments (calculated by curve fitting) in scatter plots (p value from paired Student's t-test).

From: Li et al, Front Pharmacol 2020;11:409.

Normalization of contractions:
Compensaton of tissue heterogeneities

Heterogeneity of tissues:
- May affect contractions!
- In particular: human tissues

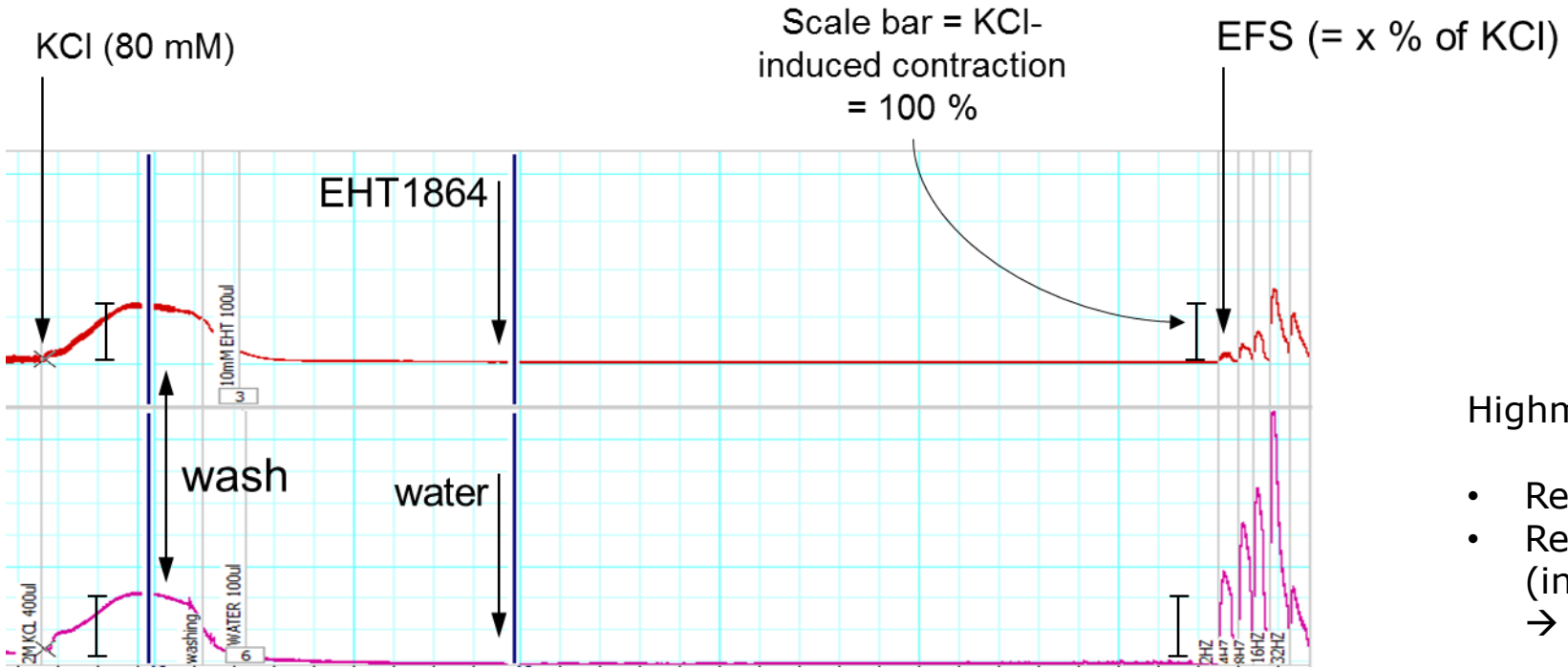


Sources of heterogeneity:
- Degree of disease
- Individual variation
- Inflammation
- Tissue size
- Composition of tissue

Normalization to:
- KCl (80-120 mM)
- Sample wet weight
- Sample length

Without normalization:
- Force (mN)
- Weight (g)

Normalization:
Highmolar KCl-induced contraction = 100%



Highmolar KCl:

- Receptor-independent contraction
- Reversal of [K^+] gradient (intra/extracellular) → depolarization → increase of intracellular Ca^{2+}

Advantages/Disadvantages of organ bath research:
Considerations for young researchers

advantages	disadvantage
Running costs: low	Prime costs for device: high (720M ca. 20,000 €)
Required lab space: small (15 m^2)	
Required accessories: rather few	
Level of difficulty: very low	
Tissues, samples: cheap (except rodents)	
Quick!! 1 Experiment: 3 h Series with n=5 → few days	

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Thanks for Your attention!

Questions, comments to
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