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# Stimulation of calcium uptake by norepinephrine or high external potassium in human calyces and renal pelvis

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**Summary.** The effects of stimulation with either 10 μmol/l norepinephrine or 85 mmol/l extracellular potassium concentration on calcium uptake were studied in muscle strips from human renal calyces and from the renal pelvis. The apparent uptake of calcium under control conditions was essentially complete after 30 min. Stimulation of the muscle strips with norephinephrine or high external potassium significantly ($P<0.05$) increased the calcium uptake over the control values at 30 and 100 min, whereas $^{45}Ca$ efflux was virtually not affected. It is concluded that the mechanical responses of the muscle strips to norepinephrine or high external potassium correspond with an increased uptake of calcium suggesting that both types of activation are mediated by an increase of intracellular calcium concentration similar to that described in other smooth muscles.

**Key words:** $^{45}Ca$ – Calcium fluxes – Human upper urinary tract

## Introduction

The role of calcium as an essential link in smooth muscle contraction is well established. One of the next steps in improving our understanding of excitation-contraction coupling in smooth muscle of the urinary tract is to investigate the characteristics of calcium mobilization processes stimulated by different modes of activation.

Norepinephrine and high external potassium depolarization represent the two most prominent means of activation of smooth muscles; the former acting by binding to the specific adrenoreceptors on the smooth muscle membrane and the latter by depolarization of the cell membrane which is not related to any specific receptor activation. We used norepinephrine and high potassium induced contractions to study the role of calcium in the activation of the smooth muscle of the upper urinary tract [6, 7]. A concept emerging from these studies was that different types of mechanical activity in the upper urinary tract are mediated by different pools of activator calcium [7].

In principle, various procedures can be used to describe calcium mobilization processes. The four main approaches are: 1. removal of calcium from the extracellular fluid, 2. blockade of transmembrane calcium transport with selective antagonists, 3. measurement of calcium fluxes with radioactive labeled calcium, and 4. electrophysiological studies. Each procedure has its own limitations and especially the intracellular registration of membrane potentials in human ureter because of the extremely small size of the cells.

In this paper we present data, showing that measurements of calcium fluxes may be useful tools in the investigation of calcium mobilization processes in the human upper urinary tract.

## Material and methods

### *General*

Human tissue was obtained from kidneys removed at operation for renal carcinoma. The age of the patients ranged between 55 and 62 years. The collecting system showed no signs of infection or obstruction. Immediately after excision of the kidney the renal pelvis was dissected free and removed for further preparation. In a dissection chamber containing warm, oxygenated Tyrode solution the renal pelvis was opened and muscle strips approximately 10 by 3 to 4 mm weighing 50 to 120 mg were excised. In order to obtain calyceal segments, that part of the kidney unaffected by tumor was dissected to display minor and major calyces and strips of calyceal tissue were excised.

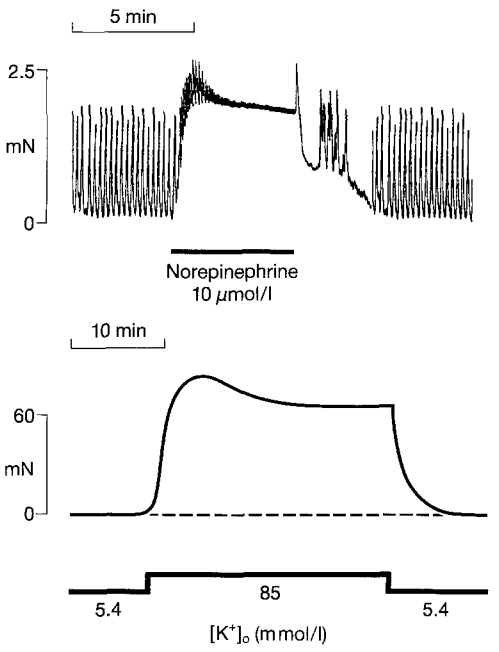


**Fig. 1.** Original tracings of effects of norepinephrine and high external potassium on mechanical actitity. Both modes of activation produced stable and reproducible contractions

### *Contractions*

Immediately after excision the preparations were suspended in 10 ml organ baths containing Tyrode solution at 37°C gassed with 95% $O_2$ and 5% $CO_2$. The tension was measured under isometric conditions with inductive force displacement transducers and recorded on paper. The preload tension was adjusted to 10 mN. An interval of at least an hour was allowed for equilibration, after which experiments were performded. The treatment schedules chosen were based on our previous studies [6, 7].

### *Calcium uptake*

Parallel experiments were carried out to study the effects of potassium- or norepinephrine-induced activation on net $^{45}Ca$ uptake in muscle strips of the renal pelvis and of the calyces. Initially, the tissues were allowed to equilibrate for 60 min in an oxygenated Tyrode solution at 37°C. The muscle strips were then exposed for different times (0 to 100 min) to normal Tyrode solution or Tyrode solutions containing 10 µmol/l norepinephrine or 85 mmol/l KCl, which were labeled with $^{45}Ca$ (0.1 ml per 100 ml solution). At the end of the exposure the tissues were blotted, weighed and incubated in 1 ml of tissue solubilizer (TS-1, Zinsser) at 65°C for 3 h. Finally, 1 ml of 1 N HCl and 10 ml of scintillation cocktail containing Triton X-100 (Unisolve, Zinsser) were added and vials analyzed for $^{45}Ca$ in a liquid scintillation counter (Packard, Model 3380). $^{45}Ca$ space was calculated from the ratio of radioactivity in the tissue to radioactivity in incubation medium. The uptake of calcium was calculated from the ratio of radioactivity in the tissue and in the incubation medium and the concentration of calcium in the Tyrode solution. The data were expressed as calcium uptake in µmol. per gram tissue wet weight.

### *Calcium efflux*

For measurements of calcium efflux, tissues of the renal pelvis and calyces were exposed to $^{45}Ca$ labeled Tyrode solution for 60 min in order to label all the exchangeable cellular calcium. At the end of the exposure, the tissues were bathed three times in inactive Tyrode solution in order to remove extracellular $^{45}Ca$. For determination of the time course of $^{45}Ca$ efflux, the Tyrode solution in the organ baths was changed every 5 min and collected in vials. 10 ml of scintillation cocktail (Minisolve I, Zinsser) was added. In the first 30 min the organ baths were rinsed with inactive Tyrode solution (control phase). Afterwards normal Tyrode solution or Tyrode solutions containing 10 µmol/l norepinephrine or 85 mmol/l KCl were used alternatively to rinse the organ baths (test phase). The radioactivity in the collected solutions as well as the residual activity in the muscle strips at the end of the experiments was determined in a liquid scintillation counter as described above. From the measured values the $^{45}Ca$ content in the tissues at the rinsing moments was calculated by cumulative addition. The data were expressed as percentages of initial activity.

### *Solutions*

The Tyrode solution used was prepared with distilled deionized water and had the following composition (mmol/l): NaCl 136.9, KCl 5.4, $MgCl_2$ 1.05, $NaH_2PO_4$ 0.42, $NaHCO_3$ 11.9, $CaCl_2$ 1.8 and glucose 5.5. The pH of the solution was 7.2 to 7.4. The Tyrode solution used for potassium-induced contractions contained 85 mmol/l KCl and 57.3 mmol/l NaCl; other ingredients remained the same as above. The stock solution of norepinephrine was prepared in distilled water. From this stock solution, desired concentration of norepinephrine was prepared in Tyrode solution, and pH was maintained at $7.2 \pm 0.1$.

### *Drugs*

L-norepinephrine bitartrate (Serva), $^{45}Ca$ (specific radioactivity: 29.84 mCi/mg) was obtained from New England Nuclear, Boston.

Results are expressed as means ± standard error of the means (SEM). An overall statistical comparison of independent mean values was based on the analysis of variance, Documenta Geigy, equations 623–625 (3); this procedure was followed by standard t statistics. A p-value of less than 0.05 was considered significant.

## Results

Stimulation of muscle strips from the calyces and from the renal pelvis with high external potassium or norepinephrine produced sustained and reproducible contractions. The concentrations chosen were based on our previous studies. [6, 7]; they represent submaximal concentrations according to concentration-response relationships obtained in these tissues. In the experiments with high external potassium, phentolamine $10^{-5}$ mol/l was added to the Tyrode solution to prevent effects of norepinephrine at depolarization. The original tracings of Fig. 1 show the effects of

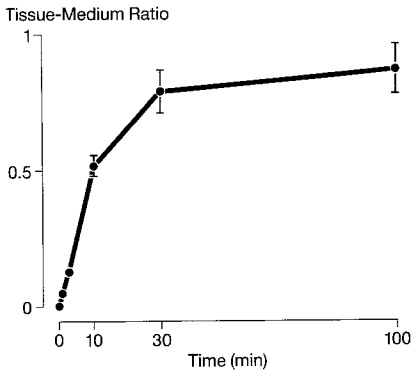


**Fig. 2.** Time dependent changes of $^{45}Ca$ space in unstimulated calyceal muscle strips (Means ± SEM, $N=6$). $^{45}Ca$ space after 100 min: 0.87 ± 0.09 ml/g tissue, indicating that overall calcium content of isolated preparation is about equal to that of Tyrode solution

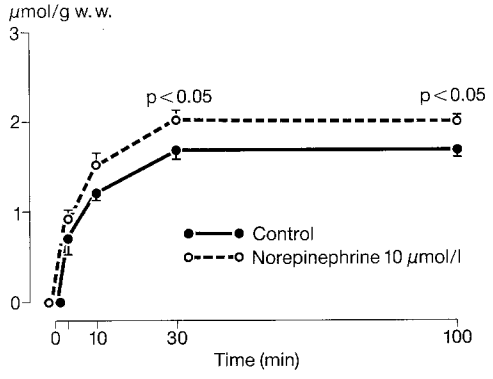


**Fig. 3.** Time course of calcium uptake in muscle strips from calyces and renal pelvis (Means ± SEM, $N=8$). Calcium uptake under control conditions was essentially complete after 30 min with no significant further increase occuring between 30 and 100 min. Stimulation with norepinephrine significantly ($P<0.05$) increased the calcium uptake over the control values at 30 and 100 min (w.w. ≙ wet weight)

norepinephrine and high external potassium on mechanical activity. Addition of norepinephrine ($10^{-5}$ mol/l) to the organ bath caused an increase in tension to a sustained plateau together with an increase in frequency and a marked reduction of the amplitude of the phasic, spontaneous contractions. After wash-out of the agonist the isolated minor calyx segment immediately returned to its original activity. Muscle strips exposed to a Tyrode solution containing 85 mmol/l potassium showed a rapid increase in tension which, following a transient peak, remained stable for several hours. After wash-out of the high potassium Tyrode solution the muscle strip immediately returned to its original resting tension (lower tracing in Fig. 1). In norepinephrine-induced contractions the maximal tension development over baseline was noted and the mean ± SD value was 11.98 ± 2.7 mN per 100 mg tissue

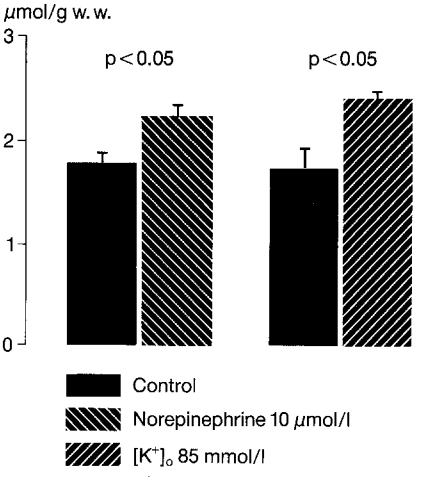


**Fig. 4.** Stimulation of muscle strips from the calyces and renal pelvis with norepinephrine or high external potassium ([K+]0) significantly ($P<0.05$) increased calcium uptake (Means ± SEM, $N=8$) over control values (w.w. ≙ wet weight)

wet weight ($N=12$). The steady state tension development of muscle strips of the renal pelvis after activation with 85 mmol/l extracellular potassium amounted to 30.98 ± 4.93 mN per 100 mg tissue wet weight (mean ± 1 SD, $N=12$).

Apparent calcium uptake was defined as the quantity of calcium taken up the tissue based upon the number of counts found in the tissue as compared with the number of counts per unit concentration in the medium. Figure 2 shows the time dependent changes of $^{45}Ca$ space in unstimulated calyceal muscle strips. The $^{45}Ca$ space after 100 min amounted to 0.87 ± 0.09 ml/g tissue (mean ± SEM of 6 preparations), indicating that the overall calcium content of the isolated preparation is about equal to that of the Tyrode solution. Figure 3 shows the time course of calcium uptake in unstimulated (control) and in muscle strips stimulated with norepinephrine $10^{-5}$ mol/l. The uptake of calcium under control conditions was essentially complete after 30 min with no significant further increase occuring between 30 and 100 min and amounted to 1.82 ± 0.03 µmol/g tissue wet weight ($N=12$). Stimulation of the muscle strips with norepinephrine significantly ($P<0.05$) increased the calcium uptake over the control values at 30 and 100 min. Figure 4 shows the effects of stimulation with either $10^{-5}$ mol/l norepinephrine or 85 mmol/l extracellular potassium concentration on calcium uptake in muscle strips of the calyces and the renal pelvis at 100 min after exposure to $^{45}Ca$ labeled test solutions. Both modes of activation significantly ($P<0.05$) increased the calcium uptake over control values. The values were 2.25 ± 0.11 µmol/g tissue wet weight ($N=8$) for norepinephrine and

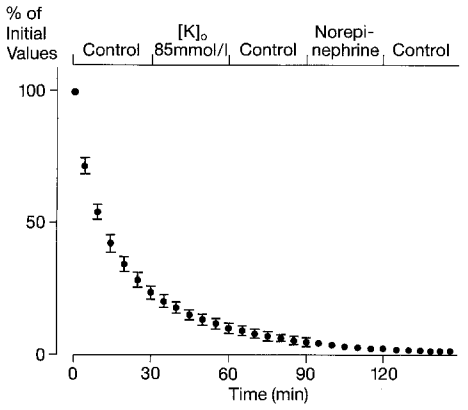


**Fig. 5.** Time course of $^{45}Ca$ efflux from muscle strips of the renal pelvis. $^{45}Ca$ activity in the tissues rapidly decreased over time after exposure to inactive Tyrode solutions and was virtually not influenced by stimulation with either high extracellular potassium or norepinephrine

$2.42 \pm 0.05$ µmol/g tissue wet weight ($N=8$) for high external potassium.

The efflux of $^{45}Ca$ from preparations of the renal pelvis was defined as the quantity of calcium delivered by the tissues based upon the number of counts found in the bath solutions per time unit. Figure 5 shows the time course of $^{45}Ca$ efflux from muscle strips of the renal pelvis. This curve represents the $^{45}Ca$ activity in the tissues in per cent of the initial values. The figure clearly shows that $^{45}Ca$ activity in the tissues rapidly decreased over time after exposure to inactive Tyrode solutions and was virtually not influenced by stimulation with either 85 mmol/l KCl or $10^{-5}$ mol/l norepinephrine.

## Discussion

An essential role for calcium in induction and maintenance of smooth muscle contractility has been delineated in numerous studies (for review see [4]). Generally, the qualitative and quantitative response of the smooth muscle system to a specific agent is a function of the intracellular free calcium concentration, and this in turn is directly related to the amounts of calcium mobilized into the cytoplasm. Much of the research in smooth muscle physiology has centred on the identification of sources and sinks for calcium operating during excitation and relaxation. It is now widely accepted that the source of calcium leading to an activation of the contractile proteins can be either the external medium or the intracellular store or both. From our earlier studies on smooth muscles of the upper urinary tract we hypothesized that contractions induced by norepinephrine and contractions induced by depolarizing potassium solutions may use different pools of calcium [7]. In addition we found marked differences between ureteral muscle strips and muscle strips from the renal pelvis and calyx with respect to their response to α-adrenergic stimulation suggesting differences in the underlying calcium mobilization processes [7]. In the present work we therefore tried to obtain more information about the calcium movements during stimulation of muscle strips of the upper tract. Two approaches are useful to study some details of ionic movements during excitation and contraction: 1. Electrophysiological studies allow for a registration of membrane potentials during rest and excitation and give some information on the time course of ionic movements through the cell membrane; however, these studies are not ion specific and interpretation of data is difficult without extensive variation of experimental parameters. 2. Radioisotope studies allow the direct measurement of ionic fluxes but are hampered by large ion compartments capable of masking the physiologically important ion fluxes.

Among the cations involved in smooth muscle contractility, calcium is clearly the most complex from a kinetic viewpoint [8]. Therefore, many unsuccessful attempts have been made to measure calcium fluxes across the cell membranes of smooth muscles. A probably reason for this failure is that the very large degree of extracellular calium binding has obscured the relatively small membrane calcium fluxes. Techniques were developed later to quench the bound extracellular calcium with little depletion of the cellular calcium content. Pioneering work was done by Godfraind [5], van Breemen et al. [10], Weiss [11] and Deth [2] in vascular smooth muscle and tenia coli. In the present work we adopted methods from Deth and van Breemen [1] and Meisheri et al. [9] to measure $^{45}Ca$ fluxes in muscle strips from calyces and the renal pelvis. Parallel contraction experiments were performed. The data from the present study clearly show that contractions induced by norepinephrine or high external potassium are accompanied by an increased uptake of calcium. This is in agreement with previous observations on a variety of smooth muscle tissues.

In our experiments, the time course of $^{45}Ca$ efflux was virtually not affected by stimulation of the muscle strips with either norepinephrine or high external potassium. However, small changes in $^{45}Ca$ efflux may be masked by larger movements of calcium unrelated to the effects of norepinephrine or high external potassium. We cannot decide from our experiments, whether or not also small changes of $^{45}Ca$ efflux can indeed be detected under more special conditions.

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