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Energetics of smooth muscle taenia caecum of guinea-pig: a ^{31}P-NMR study

Niek A. Vermuë and Klaas Nicolay*

Department of Pharmacology, University of Groningen, Bloemsingel 1, 9713 BZ Groningen and **Department of Physical Chemistry, University of Groningen, Nijenborgh 16, 9747 AG Groningen, The Netherlands*

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Smooth muscle cell energetics of taenia caeci during relaxation, spontaneous activity and maximal contraction were investigated using ^{31}P-NMR. In relaxed muscle obtained in calcium-free medium, [ATP], [phosphocreatine] and [sugar phosphate] were 4.4 mM, 7.7 mM and 2.8 mM, respectively. There was only a small difference in the energetics of spontaneously active and maximally contracted muscles, but under both conditions substantial changes occurred as compared with relaxed muscles. The internal pH in relaxed muscle was found to be 7.05, which acidified to 6.5 during contraction. The level of sugar phosphates was found to be not a limiting factor in energetics.

^{31}P-NMR *Internal pH* *Smooth muscle* *Taenia caecum* *Spontaneous contraction* *Energetics*

1. INTRODUCTION

A remarkable feature of smooth muscle is the spontaneous contractions that are generally exhibited by these preparations [1,2]. It is important to study energetics of smooth muscle under well-defined conditions. So, we have studied the smooth muscle taenia caecum during spontaneous activity, maximal contraction and complete relaxation using ^{31}P-NRM. With this technique it is possible to measure the concentrations of phosphorylated metabolites and the internal pH in intact tissue under physiological conditions. Because NMR is non-invasive, it does not require destruction of the tissue for chemical analysis [3–6]. This method has been widely employed to study the bio-energetics of striated muscle [7], but has hardly been applied to the study of smooth muscle. In this study on guinea-pig taenia caecum it was investigated how the energetics develop on spontaneous activity and maximal contraction.

* Present address: Department of Biochemistry, State University of Utrecht, Padualaan 8, 3508 TB Utrecht, The Netherlands

Abbreviations: PCr, phosphocreatine; SP, sugar phosphates; NMR, nuclear magnetic resonance; pH_i, internal pH; P_i, orthophosphate

2. MATERIALS AND METHODS

2.1. *Muscle preparation*

The guinea-pigs had unlimited access to food and drink. Animals of either sex, which weighed about 500 g, were used for the experiments. They were killed by cervical dislocation and the taenia caeci discerted out. After being prepared free from adhesive tissue, they were equilibrated in well-aerated Krebs solution for 30 min at 23°C.

2.2. *Composition of solutions*

The Krebs solution for the experiments at 23°C contained 2.8 mM KCl, 122 mM NaCl, 1.2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 12 mM glucose, 23 mM $NaHCO_3$ and set at pH 7.35 by gassing with 95% O_2 and 5% CO_2. In the Krebs solution for the experiments at 37°C [KCl] and [$NaHCO_3$] were in-

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creased to 5.8 mM and 25 mM, respectively; [glucose] was decreased to 11.4 mM (pH 7.35).

To obtain maximal contraction of the preparations a 30 mM K^+-Krebs solution was used, in which the [KCl] was enhanced to 30 mM by substitution of NaCl. Complete relaxation of the muscles was obtained in Ca-free solution in which the $CaCl_2$ was omitted and 0.4 mM ethyleneglycol–bis-tetraacetic acid (EGTA) was added. The [$MgCl_2$] was enhanced to 5.6 mM to compensate for the $CaCl_2$ [8].

All solutions were 10% (v/v) in ^2H_2O in order to allow magnetic field-optimization in the NMR experiments; ^2H_2O did not effect the contraction pattern.

2.3. *Contraction measurements*

The contractions were measured isotonically. A minimum load was applied to balance the weight of the muscle.

2.4. *^{31}P-NMR measurements*

Except for the load applied to the muscles, the conditions in the contraction and NMR experiments were the same. For each experiment 6–8 taenia caeci of about 50 mg were used. Unless otherwise stated, experiments were performed at 23 ± 1°C.

145.78 MHz ^{31}P-NMR experiments were performed on a Bruker HX-360 spectrometer operating in the Fourier transform mode. Experiments were carried out without locking the instrument and without proton-decoupling. Spectra (500 or 2000 scans) were accumulated in 4096 time–domain addresses with a recycle time of 1 s and a spectral width of 6000 Hz. 60° radiofrequency pulses were employed. NMR peak positions are given in ppm from endogenous phosphocreatine at 0 ppm. The sample tube (10 mm diam.) contained 6 ml medium which was vigorously aerated with a gas mixture (95% O_2:5% CO_2) through a capillary.

2.5. *Measurement of internal pH*

The internal pH was determined from the chemical shift of internal P_i. The chemical shift was converted to pH, using a calibration curve with pK_2 6.7 and chemical shifts at low and high pH of 3.27 and 5.69 ppm, respectively [9].

2.6. *Determination of concentrations*

The concentration of PCr was determined as in [10] on a muscle preparation bathed in calcium-free solution, supplemented with 10 mM P_i which was included for calibration. It was assured that no significant penetration of P_i occurred. [P_i] and [ATP] were determined by calibration of their peak areas against the above [PCr]. In order to determine real concentrations, peak intensities were corrected for partial saturation due to rapid pulsing by comparison with spectra acquired with a recycle time of 20 s. Uncertainties in the concentrations were estimated to be ± 0.2 mM.

3. RESULTS

3.1. *Contraction*

After being placed in the set-up for the isotonic contraction measurements, the muscles almost immediately became spontaneously active in Krebs solution at 23°C (fig.1). The spontaneous activity consists of phasic contractions, which do not com-

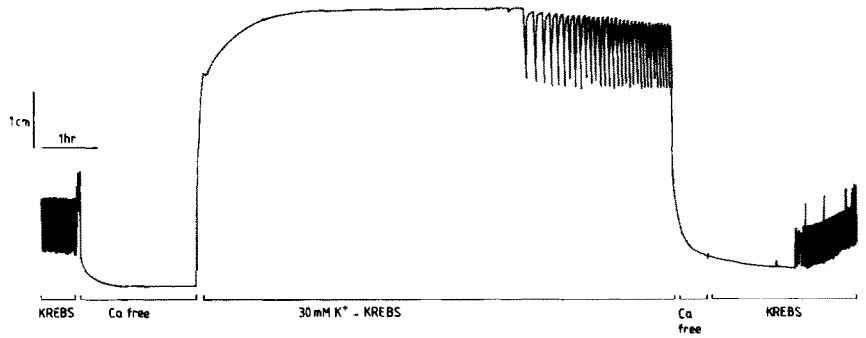

Fig.1. The contractile state of taenia caecum measured isotonically at 23°C in the presence of Krebs solution, high-potassium (30 mM) and calcium-free medium. This procedure was also followed in the ^{31}P-NMR experiment.

pletely relax at the end of a single contraction due to the high repetition frequency. Complete relaxation occurred in Ca^{2+}-free solution. When the muscles were subsequently exposed to the 30 mM K^+-Krebs, the maximal contraction was reached in 12 min and was maintained for nearly 2 h. At that time the contraction was partially released. The frequency of these contraction releases progressively increased, while the maximal contraction simultaneously decreased. After this series of manipulations the muscle will always become spontaneously active again in normal Krebs solution (fig.1). Analogous experiments carried out at 37°C only revealed a small increase in spontaneous activity and the duration of the maximal contraction increased to 3 h.

3.2. *High-energy phosphates*

In the ^{31}P-NMR spectrum of relaxed muscles (fig.2A), resonances of sugar phosphates (SP), phosphocreatine (PCr) and ATP are present. P_i and ADP were not detected in relaxed muscles, indicating that the preparation was well oxygenated [11]. [ATP], [PCr] and [SP] were determined to be 4.6 mM, 7.7 mM and 2.8 mM, respectively from the NMR spectrum shown in fig.2A.

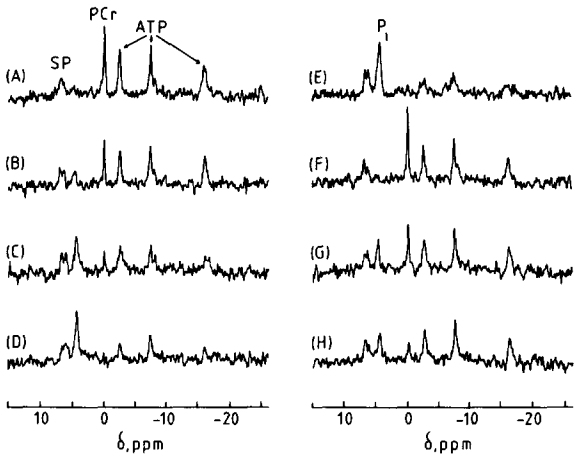

Fig.2. ^{31}P-NMR spectra of 8 taenia caeci at 23°C. The peaks of sugar phosphates (SP), orthophosphates (P_i), phosphocreatine (PCr) and ATP can be seen. Each spectrum represents the time-average of 2000 scans (33 min) accumulated in the following media: (A) Ca^{2+}-free solution; (B–E) 30 mM K^+-Krebs solution; (F) Ca^{2+}-free solution; (G–H) Krebs solution.

Upon stimulation to maximal contraction both the [PCr] and [ATP] decreased (fig2B–E and fig.3). After 1.5 h of stimulation PCr could no longer be detected in the spectra. Initially the ATP level decreased only slowly, but this decrease accelerated approaching PCr exhaustion. ATP was, however, not completely depleted, but stabilized at about 1.5 mM. Due to the breakdown of PCr and ATP the [P_i] increased to 7.4 mM final conc. At the same time a measurable free ADP concentration of about 0.7 mM could be detected. The level of SP did not change during stimulation.

Although the energy content of the muscles had been severely exhausted (fig.2E), this situation was quickly reversed on relaxation (fig.2F). From spectra obtained with 500 scans, it could be determined

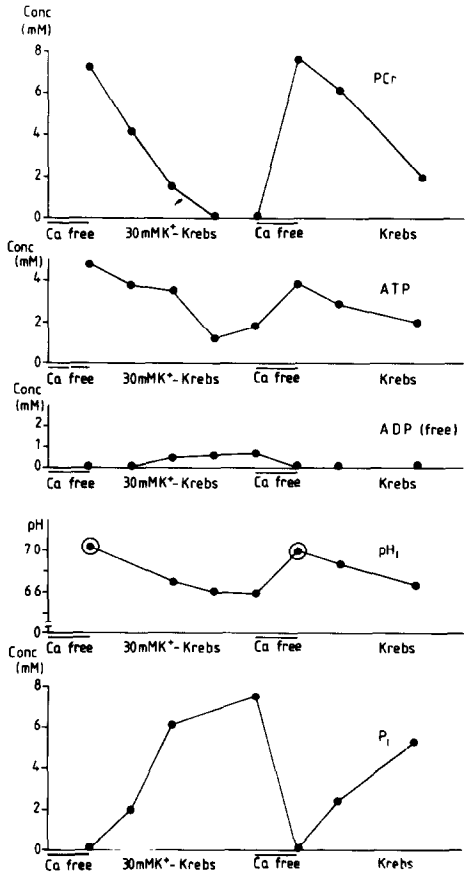

Fig.3. The concentrations of the phosphorylated metabolites PCr, ATP, free ADP, P_i and pH_i determined from spectra as shown in fig.2. The encircled values in the pH_i curve were determined by interpolation as described in the text.

from the level of PCr, that equilibrium had been reached within 9 min. The ATP concentration did not completely recover to the initial level. This is assumed to be due to some loss of P_i during the long-lasting contraction period, under the influence of a large P_i gradient across the muscle membranes.

As has been demonstrated in fig.1 taenia caeci become spontaneously active in Krebs solution after about 30 min following a Ca-free period. As a consequence of this spontaneous activity the levels of PCr and ATP decreased (fig.2,3). The breakdown of energy-rich phosphates did not occur as quickly as during maximal contraction. However, the energetic consequences of the two types of muscular activity were very similar. It is remarkable that during spontaneous activity the PCr strongly decreases. Again, the SP level did not change, nor was any significant amount of free ADP formed.

When the experiments were repeated at 37°C, no essential differences were revealed by ^{31}P-NMR.

3.3. *Internal pH*

During maximal stimulation the internal pH (pH_i) could be measured from the chemical shift of the evoked P_i and was found to stabilize at 6.5. Since no P_i was present in relaxed muscles, pH_i in this condition could not be directly determined. However, an estimation could be obtained by interpolation of the pH_i-values obtained from the first 4 spectra of 500 scans accumulated during stimulation directly following a Ca-free period. Thus, it was found that in relaxed muscle the pH_i is 7.05.

The significant acidification observed during maximal contraction was quickly reversed in Ca-free solution (fig.3). It is interesting to observe that during both maximal contraction and spontaneous activity a substantial acidification occurs in the smooth muscle taenia caecum.

4. DISCUSSION

When the concentrations of phosphorylated metabolites in relaxed taenia caeci are compared with striated muscle [12], it is found that the concentrations of SP and ATP are essentially the same. By contrast, the PCr level of 7.7 mM in taenia caeci is 3–4-times lower. As a consequence of the relatively low PCr concentration, the ATP buffering capacity of the PCr/Cr system is relatively low in the smooth muscle taenia caecum. Also it seems likely that the prominent role of PCr for intracellular energy transport in striated muscle, that has been proposed in [13], will be of less importance in the smooth muscle taenia caecum.

Upon contraction, both the PCr and ATP levels decrease. This suggests a lower creatine kinase activity in taenia caecum than that in striated frog muscle [14], in which ATP decreased only at very low PCr levels. The PCr and ATP content can be severely exhausted without apparently damaging the preparation, because complete recovery was observed during relaxation.

Changes in the level of SP during contraction and relaxation, which have been described for heart muscle [15], were not found. This implies that in taenia caeci the uptake of extracellular glucose and its conversion to SP in no way limit the energy supply, even though no insulin was present in the Krebs solutions.

pH_i in smooth muscle has been studied with pH selective micro-electrodes in vas deferens [16] and in trachea [17] and has been found to be 7.08 and 7.03, respectively. Our value of pH_i 7.05 in relaxed taenia caecum shows excellent agreement with the results of the micro-electrode experiments. The patterns of spontaneous activity at 37°C in vitro and in vivo are considered to be similar [18]. Consequently, the acidification, which was found in spontaneous active muscles, can also be expected in vivo. Hence, strict pH homeostasis appears to be of minor importance for taenia caecum.

Under the condition that the muscles are severely exhausted, ATP and PCr are strongly decreased and 0.7 mM free ADP can be measured. Hence, the free energy change at the hydrolysis of ATP is diminished, which is promoted by the low pH_i. However, both the low pH_i and the formed ADP inhibit the myosin light chain kinase [19]. Consequently, it cannot be concluded from this study whether the contraction is limited by the energy supply or by the influence on the kinase. Further, the results show that there is only a small difference in energetics of spontaneous active and maximally contracted muscles, but that under both conditions the energetics have substantially changed as compared with the completely relaxed condition of the taenia caeci.

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