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# PHOSPHOROUS COMPOUNDS STUDIED BY $^{31}P$ NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY IN THE TAENIA OF GUINEA-PIG CAECUM

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SUMMARY

1. In the isolated taenia (0·4–0·6 g) of guinea-pig caecum, the intracellular phosphorous compounds and pH were investigated using $^{31}P$ nuclear magnetic resonance (NMR) under various metabolic conditions.

2. The ratios of the intracellular concentration of phosphocreatine ([PCr]) and inorganic phosphate ([$P_i$]) to nucleotide triphosphate ([NTP]) were $1{\cdot}71 \pm 0{\cdot}14$ and $0{\cdot}58 \pm 0{\cdot}11$ ($n = 25$), respectively, in normal solution (32 °C). The intracellular pH estimated from the chemical shift of $P_i$ was $7{\cdot}05 \pm 0{\cdot}06$ ($n = 25$), agreeing well with those previously obtained.

3. In the absence of glucose, the [PCr] and [NTP] were decreased to almost a half after 150 min exposure to 40 mM-$K^+$ solution, while [$P_i$] was increased 3-fold. These changes were much faster than the rate of decline in tension. When glucose was readmitted, the contractile response to $K^+$ fully recovered in 50 min. However, this was accompanied with only a partial recovery of [PCr] and [$P_i$], but no recovery of [NTP]. The intracellular pH was lowered by about 0·2 of a unit, suggesting an increase in glycolysis.

4. In $Ca^{2+}$-free solution, respiratory inhibition with hypoxia or CN (1 mM) only decreased [PCr], leaving [NTP] nearly unchanged. On the other hand, respiratory inhibition in excess-$K^+$ solution containing $Ca^{2+}$ (2·4 mM) severely depleted PCr and decreased [NTP] to 40 %. Increasing glucose to 50 mM did not prevent these changes, although it increased tension development.

5. The simultaneous decrease of [NTP] and [PCr] during $K^+$ contracture suggests that the activity of creatine phosphokinase is low. The recovery from respiratory inhibition was much better for [PCr] than for [NTP]. Slow, but perfect, recovery of all NTP peaks was produced by adding 1 mM-adenosine to normal solution.

6. It was suggested that tension development is closely related to the turnover rate of ATP, and not to its concentration, and that deamination of adenosine is a limiting factor in the recovery of ATP after excessive consumption.

## INTRODUCTION

$^{31}P$ nuclear magnetic resonance (NMR) spectroscopy provides a unique non-destructive method for detecting intracellular phosphate compounds and has been successfully employed for metabolic studies in many tissues, including skeletal and cardiac muscles (cf. Gadian, 1983; Gupta, Gupta & Moore, 1984; Avison, Hetherington & Shulman, 1986). There are also several papers reporting NMR experiments on smooth muscles (the taenia of guinea-pig caecum: Vermuë & Nicolay, 1983; Vogel, Lilja & Hellstrand, 1983; rat uterus: Degani, Shaer, Victor & Kaye, 1984; Dawson & Wray, 1985; rabbit taenia, portal vein and urinary bladder: Hellstrand & Vogel, 1985; rabbit aorta: Carlier, Grandjean, Michel, Dorio & Rorive, 1985). These have confirmed the previous finding with conventional biochemical methods that the ATP and phosphocreatine content of smooth muscle is rather small compared with other muscles (Butler & Siegman, 1985). However, these NMR experiments were mainly carried out in smooth muscles relaxed in $Ca^{2+}$-free solution and at room temperature.

The advantage of NMR experiments is that metabolic changes can be continuously followed on the same preparation for a long time. Thus, the emphasis of the present experiments was on the comparison with previous metabolic studies on the taenia (Ashoori, Takai, Tokuno & Tomita, 1984; Ishida, Takagi & Urakawa, 1984; Takai, Tokuno & Tomita, 1985). In these studies, recovery of $K^+$ contracture with application of substrates was investigated following glycogen depletion caused by producing $K^+$ contracture in the absence of glucose, and, using a conventional biochemical analysis, some correlation was found between tension development and tissue content of ATP. To obtain more quantitative information, in the present experiments the changes in intracellular phosphate compounds were continuously monitored with NMR during and after the response to excess-$K^+$ medium under various conditions. Preliminary results have been communicated to the 30th Congress of the International Union of Physiological Sciences (Nakayama, Tomita, Seo & Watari, 1986).

## METHODS

Guinea-pigs of either sex were stunned and bled. The two entire strips of taenia of the caecum from three to four guinea-pigs were dissected. All preparations (0·4–0·6 g) were mounted isometrically in an NMR tube (10 mm in diameter) and superfused at a constant rate of 6 (for normal solution) or 12 ml/min (for 40 mM-$K^+$ solution), using a vinyl tube (5 m long, triple-walled) except for the last 1 m inside the magnet container. The temperature near the preparation was 32 °C. The inner tube was used for superfusing solution saturated with oxygen and pre-warmed to 34 °C, and the middle and outer tubes were for circulation of oxygen and warmed water (34 °C) respectively, to maintain the oxygen tension and temperature of the solution. The normal Krebs solution was of the following composition (mM): NaCl, 137·9; $KHCO_3$; $CaCl_2$, 2·4; $MgCl_2$, 1·2; glucose, 11·8; and HEPES (*N*-2-hydroxyethlypiperazine-*N'*-2-ethanesulphonic acid), 5; pH adjusted to 7·4 at 32 °C. Modification of solutions was made by isosmotic substitution of NaCl. For $Ca^{2+}$-free solution, $Ca^{2+}$ was simply omitted without a chelating agent. For hypoxic experiments, oxygen was replaced with nitrogen.

Experiments were carried out with a Bruker WM-360wb NMR spectrometer, operating at 145·8 MHz with a field strength of 8·45 T. Radiofrequency pulses of 10 $\mu$s (30 deg pulses) were repeated at 0·6 s intervals, except when obtaining saturation factors. Spectra were usually obtained by accumulating 1500 or 2500 free induction decays. Line broadening of 15 Hz was used to reduce

noise in the spectra. Spectral peak positions were measured in p.p.m. taking phosphocreatine (PCr) as 0 p.p.m. The peaks were assigned on the basis of their resonance position. The intracellular concentration of phosphorous compounds was obtained by integrating the spectral peak area and correcting with the saturation factors experimentally obtained using pulse repetition of 6 s intervals. The pulse interval of 6 s was considered to be sufficient, since no change in spectrum was observed when the interval was further increased to 12 s. The saturation factor was 1·15 for the $\beta$-peak of nucleotide triphosphate (NTP), 1·56 for PCr, 1·77 for inorganic phosphate ($P_i$).

Intracellular pH was estimated from the chemical shift of the $P_i$ resonance, as applied to skeletal muscle (Dawson, Gadian & Wilkie, 1977; Seo, Murakami, Watari, Imai, Yoshizaki, Nishikawa & Morimoto, 1983). The $pK_a$ value used for this estimation was 6·70 as obtained from a pH titration of $P_i$ at 32 °C in a model solution (mM): KCl, 160; $MgCl_2$, 2·0; $P_i$, 10; PCr, 10. The chemical shifts of $H_2PO_4^-$ and $HPO_4^{2-}$ were found to be 3·15 and 5·72.

Isometric tension was separately measured in an organ bath (1 ml), using a strain gauge and a potentiometric pen recorder. A small muscle strip (about 7 mm) of taenia was set up in the bath, which was superfused at a constant rate of 3 ml/min.

The numerical data were expressed as means ± standard error of means. Differences were evaluated by paired or unpaired $t$ tests, and a probability of less than 0·05 was taken as a statistically significant difference.

## RESULTS

### *Characteristics of the NMR spectrum in normal solution*

The preparations were equilibrated in normal solution for about 30 min before starting the accumulation of signals. Under this condition, the preparations usually generated spontaneous mechanical activity in separate organ bath experiments. Figure 1 shows a typical example of an NMR spectrum obtained in normal solution by accumulating 5000 scans for 50 min. As previously shown, the spectrum consisted of five major peaks (Vogel *et al.* 1983; Vermuë & Nicolay, 1983). Their chemical shifts indicate that they arise from phosphomonoester (PME, 6–7 p.p.m.), phosphocreatine (PCr, 0 p.p.m.) and $\gamma$- (−2·4 p.p.m.), $\alpha$- (−7·5 p.p.m.) and $\beta$-phosphorous atoms (−16·2 p.p.m.) of nucleotide trisphosphate (NTP). The relative concentration of adenosine compounds in NTP is not known in this tissue, but according to the chemical analysis on the urinary bladder and uterus (Kushmerick, Dillon, Meyer, Brown, Krisanda & Sweeny, 1986), 73–78% of NTP was ATP. The concentration ratio of PCr and NTP ([PCr]/[NTP]), estimated from the spectral area corrected for the saturation factor, was 1·71 ± 0·14 (mean ± S.D., $n = 12$). The peak for inorganic phosphate ($P_i$) was usually clearly noticeable, as shown in this Figure. The concentration ratio of $P_i$ and NTP ([$P_i$]/[NTP]) was 0·58 ± 0·11 ($n = 25$). The chemical shift of the $P_i$ peak was 4·93 ± 0·06 p.p.m. at 32 °C, and from this value the intracellular pH ($pH_i$) was calculated to be 7·05 ± 0·06.

Two separate peaks were distinguishable in the PME region. The peak 1 at 6·75 p.p.m. is probably due to phosphoethanolamine, as identified in the rabbit urinary bladder and uterus (Kushmerick *et al.* 1986), and the peak 2 at 6·3 p.p.m. partly to adenosine monophosphate (AMP) and inosine monophosphate (IMP), as shown for the skeletal muscle (Meyer, Brown & Kushmerick, 1985).

The spectrum pattern remained practically the same in normal solution for about 10 h provided that the flow rate of perfusate was kept constant at 6 ml/min or more. The variation of the spectral area of PCr and NTP measured at 25 min intervals was within 4% during prolonged measurement under this condition.

*Effects of substrate removal and readmission*

Simple removal of glucose from the normal solution had a minor effect on the NMR spectrum. The NTP peak and $pH_i$ remained the same, but the [PCr] was slowly reduced to about 80% after 150 min and conversely the [$P_i$] nearly doubled. These changes recovered completely on glucose (11·8 mM) readmission. Similar recovery was observed with 11·8 mM-$\beta$-hydroxybutyrate ($\beta$-HB), which is a substrate metabolized directly through oxidative phosphorylation and has been shown to be capable of substituting for glucose in this tissue (Bueding, Bülbring, Gercken, Hawkins & Kuriyama, 1967; Ashoori *et al.* 1984; Takai *et al.* 1985).

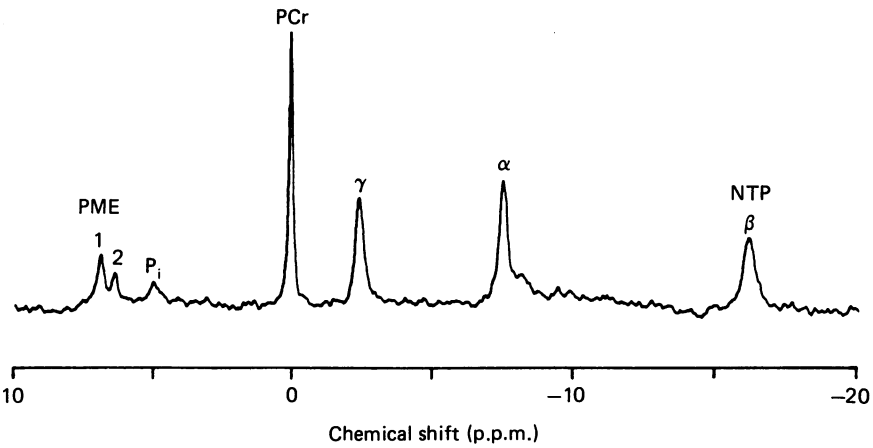


Fig. 1. $^{31}P$ NMR spectrum of the guinea-pig taenia (0·43 g) in normal solution (32 °C) obtained by accumulating 5000 signals at 0·6 s intervals in a 10 mm tube at a superfusion rate of 6 ml/min. Six main peaks are detectable: PME, phosphomonoesters; $P_i$, inorganic phosphate; PCr, phosphocreatine; $\alpha$-, $\beta$-, and $\gamma$-NTP, $\alpha$-, $\beta$-, and $\gamma$-phosphorus of nucleotide triphosphate. See text for further explanation.

The tension development caused by excess $K^+$ is known to be reduced by glucose removal, accompanied by depletion of the tissue glycogen (Axelsson, Högberg & Timms, 1965; Pfaffman, Urakawa & Holland, 1965; Ashoori *et al.* 1984). An example of the effects of glucose removal and reapplication on $K^+$ contracture produced by 40 mM-$K^+$ is shown in Fig. 2*A*. In the absence of glucose the tension slowly decreased to $17 \pm 6$% of the maximum of tonic contraction ($n = 8$) in 150 min. It recovered nearly completely within 25–50 min following glucose (11·8 mM) readmission. Under the same condition, the NMR spectra were measured, as shown in Fig. 2*B*. When 40 mM-$K^+$ solution was superfused, the rate of flow was always kept at 12 ml/min which was found to supply enough oxygen in separate experiments. The [PCr] and [NTP] were decreased to $72 \pm 16$ and $54 \pm 4$% of the control [NTP], respectively, and [$P_i$] increased to $213 \pm 38$% ($n = 5$) after 150 min exposure to 40 mM-$K^+$ solution containing no glucose. These changes were significantly greater than those observed after simple removal of glucose for the same period, probably resulting from stronger depletion of glycogen store, as observed previously (Ashoori *et al.* 1984).

On glucose readmission after 150 min exposure to glucose-free 40 mM-$K^+$ solution,

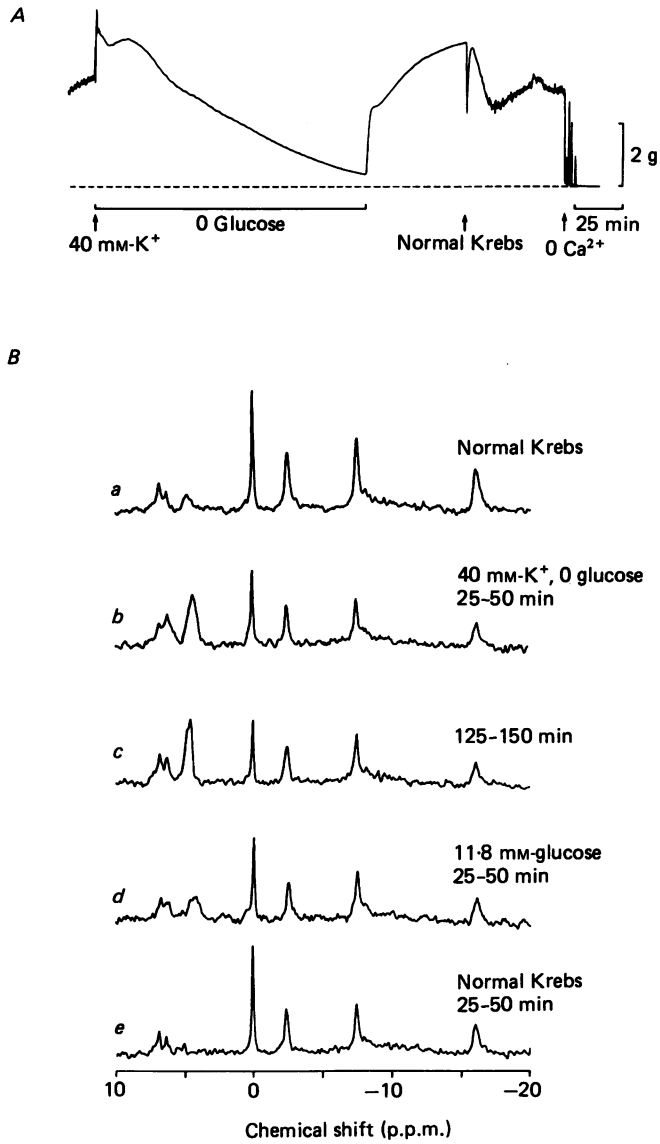


Fig. 2. *A*, tension response to 40 mM-$K^+$ solution containing no glucose and readmission of glucose (11·8 mM). After equilibrating the preparation in normal solution for 1 h, 40 mM-$K^+$ was applied for 200 min, but glucose was omitted for the first 150 min. At the end, $Ca^{2+}$ was removed from normal solution to produce complete relaxation. *B*, NMR spectra obtained separately, but during a similar experiment to that shown in *A*. Spectrum *a* was obtained in normal solution 0–25 min before superfusing with glucose-free 40 mM-$K^+$ solution. Each spectrum was obtained by accumulation of 2500 signals (0·53 g tissue). The dashed line in this and the following Figures indicates the initial position of the $P_i$ peak.

the [PCr] and [$P_i$] partially recovered to 94 ± 24 and 165 ± 34 % ($n = 5$) of the control [NTP], respectively. These changes were statistically significant ($P < 0.05$). However, the recovery of [NTP] was negligible, although [PCr]/[NTP] recovered to 1·68. The decrease in [$P_i$] was accompanied by a decrease in pH to 6·56 ± 0·09, suggesting an increase in glucose utilization. When normal solution was introduced, the [PCr] recovered to 156 % (89 % of the control [PCr]), but all NTP peaks were still less than 60 % of the control.

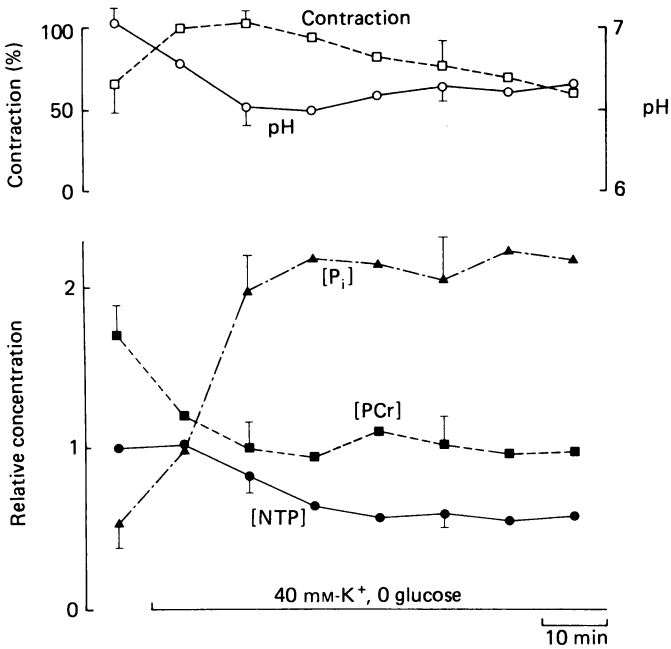


Fig. 3. Time course of changes in tension, intracellular pH, [$P_i$], [PCr] and [NTP] during the first 70 min exposure to glucose-free 40 mM-$K^+$ solution (average of five experiments). Maximum contraction was taken as 100 % and concentrations of phosphorous compounds (corrected by saturation factors) were expressed relative to [NTP] in normal solution. Vertical bars show S.E. of means.

Figure 3 shows the time course of changes in tension, [PCr], [NTP], [$P_i$] and $pH_i$, measured every 10 min, during the first 70 min exposure to glucose-free 40 mM-$K^+$ solution ($n = 5$). The decrease in tension was much slower than the changes in other parameters. After 30 min when the changes in phosphorous compounds had already reached maximum, the tension started to decline.

The $P_i$ peak moved to the right in the early phase of the contracture (25–50 min), but then partially recovered (125–150 min). These changes correspond to pH of 6·63 and 6·75, respectively. The recovery of acidification may be due to the gradual depletion of glycogen and a concomitant decrease in lactate production. There was also a transient rise in the PME peak 2 at 25–50 min, suggesting the break-down of ATP to AMP.

*Effects of respiratory inhibition*

In order to study effects of more severe energy depletion, oxidative phosphorylation was inhibited. Under this condition, ATP would be supplied mainly through the glycolytic process. Effects of hypoxia were first observed in a relaxed

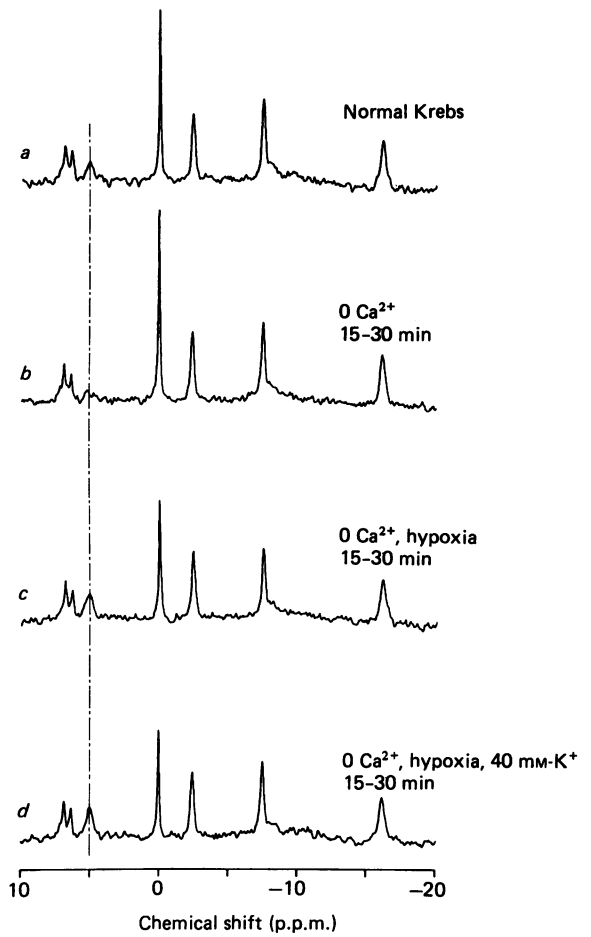


Fig. 4. Effects of hypoxia in $Ca^{2+}$-free solution on NMR spectra (accumulation of 1500 signals, 0·58 g). *a*, control spectrum in normal solution; *b*, between 15 and 30 min after exposing to $Ca^{2+}$-free solution; *c*, 15–30 min in hypoxic $Ca^{2+}$-free solution; *d*, 15–30 min after $K^+$ concentration was increased to 40 mM in hypoxic $Ca^{2+}$-free solution.

state in $Ca^{2+}$-free solution. When spontaneous mechanical activity was stopped by $Ca^{2+}$-removal, [PCr] was increased by $9 \pm 4\%$ ($n = 3$) and [$P_i$] decreased, but [NTP] was not significantly affected, as shown in Fig. 4*b*. Hypoxia caused by replacing $O_2$ with $N_2$ markedly decreased [PCr] with an increase in [$P_i$], but without a significant effect on [NTP] (Fig. 4*c*), as previously reported by Vogel *et al.* (1983). On average, the [PCr] decreased to $37 \pm 9\%$ of the control obtained in $Ca^{2+}$-free solution saturated with $O_2$. In the absence of $Ca^{2+}$, the effects of increasing the $K^+$

concentration to 40 mM were weak (Fig. 4*d*). Application of 1 mM-cyanide (CN) produced similar results to those of hypoxia.

Under normoxic condition, the spectrum was kept constant during a 60 min exposure to $Ca^{2+}$-free solution, when studied in a separate experiment. Simple removal of $Ca^{2+}$ from normal solution containing 1·2 mM-$Mg^{2+}$ was shown to have little effect on intracellular ionic concentration for at least 60 min (Casteels, 1970).

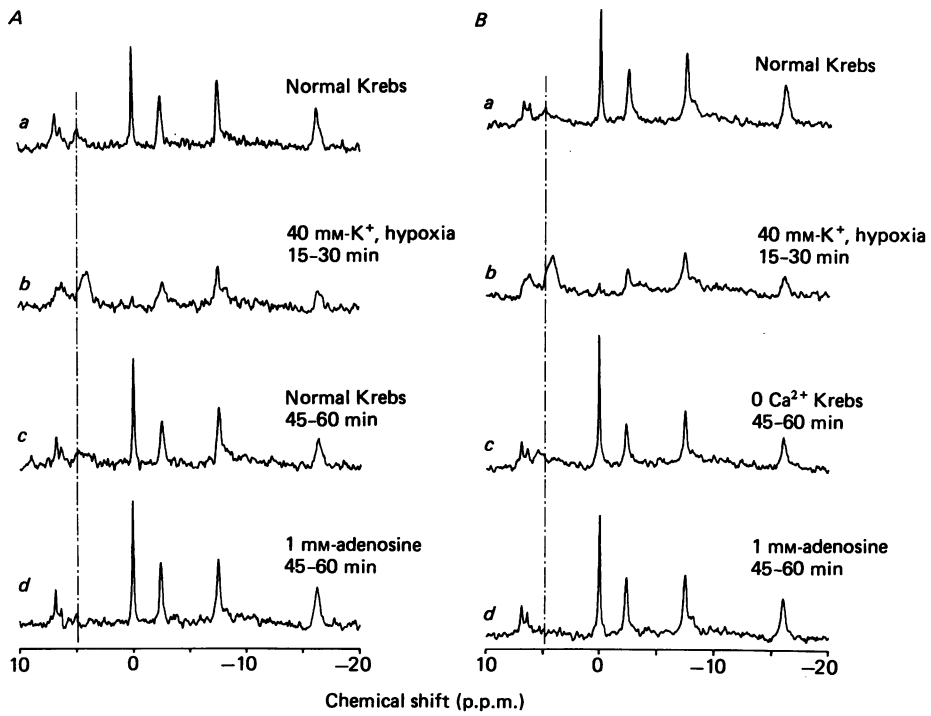


Fig. 5. *A*, effects of hypoxia in 40 mM-$K^+$ solution containing normal $Ca^{2+}$ (2·4 mM). *a*, control spectrum (accumulation of 1500 signals) in normal solution; *b*, between 15 and 30 min after superfusing hypoxic 40 mM-$K^+$ solution; *c*, 45–60 min after returning to oxygenated normal solution, *d*, 45–60 min after addition of adenosine (1 mM). *B*, similar to *A*, but recovery was observed in the absence of $Ca^{2+}$. Total tissue weight was 0·47 g in *A* and 0·59 g in *B*.

During the contracture produced by 40 mM-$K^+$ in the presence of 2·4 mM-$Ca^{2+}$, hypoxia decreased both [PCr] and [NTP] and reduced tension to about 10% of the maximum. In the experiments shown in Fig. 5*A*, the preparations were superfused for 30 min with hypoxic 40 mM-$K^+$ solution. The [PCr] and [NTP] were reduced to $11 \pm 6$ and $42 \pm 7$%, respectively, and [$P_i$] was increased to $280 \pm 42$% of the control [NTP] ($n = 3$). The PME peak 2 was usually increased slightly. This increase was less than that observed in the experiments shown in Fig. 2*B*, probably due to severe acidosis in hypoxic conditions in the presence of glucose. When normal Krebs solution with $O_2$ was applied (Fig. 5*A*), [PCr] recovered completely within 45–60 min, but [NTP] recovered only partially from 42 to $74 \pm 11$% of the control ($n = 3$), even after 120 min, as already shown in Fig. 2*B*.

*Effects of adenosine on the recovery*

The poor recovery of [NTP] could be the result of a decrease in adenosine due to deamination. Therefore, adenosine (1 mM) was applied during the recovery from hypoxia. When adenosine (1 mM) was added to the superfusing solution the [NTP] was restored nearly completely, as shown in Fig. 5*Ad* and *Bd* (95%, $n = 2$).

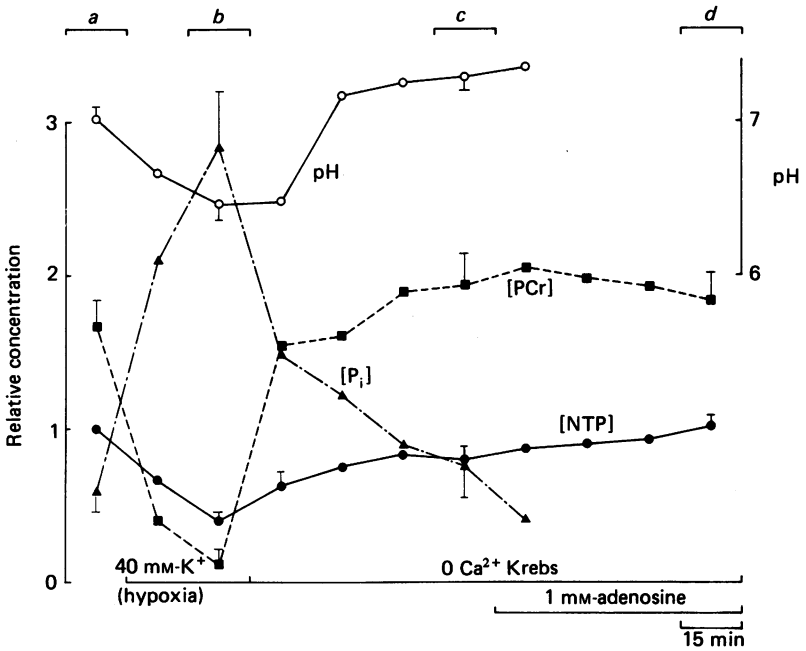


Fig. 6. Time course of changes in pH (top) and phosphorous compounds during experiments similar to that shown in Fig. 5*B*. [PCr] and [$P_i$] were expressed relative to control [NTP]. Vertical bars show S.E. of means ($n = 5$).

Since adenosine is known to relax the taenia caeci by stimulating the purinergic receptor (Burnstock, 1981), the experiment was repeated with adenosine applied with the tissue already relaxed in $Ca^{2+}$-free solution, to avoid the secondary effect of purinergic relaxation, as shown in Fig. 5*B*. The results of five experiments are plotted in Fig. 6. In $Ca^{2+}$-free Krebs solution saturated with $O_2$, [PCr] recovered quickly, and then gradually increased to $203 \pm 21$% of the control [NTP] in 60 min. On the other hand, [NTP] slowly reached $80 \pm 9$% of the control, and showed no further recovery after 60 min. Full recovery ($102 \pm 9$%) of [NTP] was observed with adenosine in 60 min. The effect of adenosine was statistically significant ($P < 0{\cdot}05$). On application of adenosine there was a transient further increase in [PCr], but then it slowly returned to $184 \pm 18$% of the control [NTP] in 60 min.

The $pH_i$ fell to 6·47 during the hypoxic contracture in the presence of $Ca^{2+}$. When exposed to $Ca^{2+}$-free Krebs solution, the $pH_i$ slowly increased to 7·27. However, the peak of the $P_i$ spectrum accumulated for the first 15 min in $Ca^{2+}$-free solution did not show clear recovery. This is due to the fact that the position of the largest peak

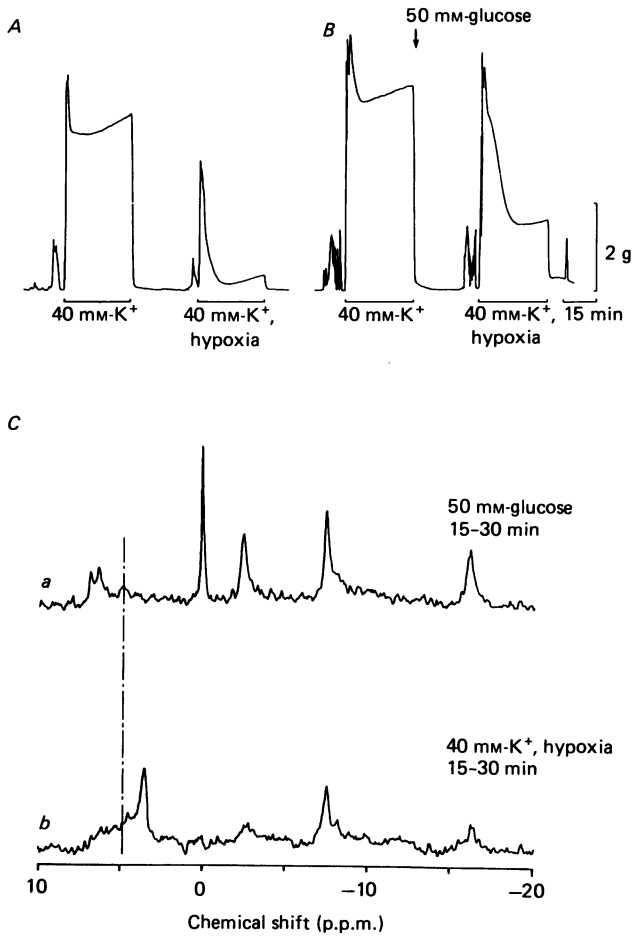


Fig. 7. *A*, effects of hypoxia on tension development caused by 40 mM-$K^+$ in the presence of 11·8 mM-glucose. Hypoxic 40 mM-$K^+$ solution was applied 30 min after observing the control response. *B*, similar experiment in a different preparation, but with the glucose concentration increased to 50 mM immediately after the control response, as indicated. *C*, NMR spectra obtained during an experimental procedure similar to that shown in *B*. *a*, 15–30 min in a solution in which glucose was increased to 50 mM by isosmotic replacement of NaCl; *b*, 15–30 min in hypoxic 40 mM-$K^+$ solution containing 50 mM-glucose (tissue weight, 0·51 g).

obtained immediately after exposure to $Ca^{2+}$-free solution dominated the $P_i$ position. In the presence of adenosine, [$P_i$] decreased to a very low level and the estimation of the pH became difficult.

*Effects of increasing glucose concentration*

It was shown in the guinea-pig taenia caeci that the $K^+$ contracture depressed by hypoxia was partially antagonized by raising the glucose concentration to 50 mM (Ishida *et al.* 1984). This was confirmed in the experiments shown in Fig. 7*A* and *B*. $K^+$ contractures were produced by applying 40 mM-$K^+$ for 30 min at 30 min

intervals. After observing reproducible responses, hypoxic 40 mM-$K^+$ solution was applied. This produced a very small tonic contracture in the presence of 11·8 mM-glucose. The average tension at the end of the contracture was 17·7 ± 7% of the control ($n = 6$). The experiments were repeated, but glucose was increased to 50 mM 30 min before applying hypoxic 40 mM-$K^+$ solution (Fig. 7*B*). In the presence of 50 mM-glucose, the $K^+$ contracture during hypoxia was significantly larger (33 ± 10%, $P < 0·05$, unpaired $t$ test).

The NMR spectra shown in Fig. 5*Ab* and *Bb* were taken during the $K^+$ contracture under hypoxic conditions in the presence of 11·8 mM-glucose. The same experiment performed in the presence of 50 mM-glucose is shown in Fig. 7*C*. The changes in spectral pattern were not essentially affected by increasing glucose concentration. In the presence of 50 mM-glucose, [NTP] was decreased to 36% and [$P_i$] was increased to 350% of the initial [NTP] ($n = 4$) during the hypoxic contracture. The PCr peak disappeared. The $pH_i$ was more acidic (5·9) with 50 mM-glucose, probably due to increased production of lactate (Ishida *et al.* 1984). Full recovery of the phosphorous peaks was obtained when the tissues were treated with $Ca^{2+}$-free solution containing 1 mM-adenosine.

The lack of the effect of increasing glucose from 11·8 to 50 mM on the concentration of high-energy phosphates was confirmed in another experiment in which glucose was increased during continuous perfusion with hypoxic 40 mM-$K^+$ solution. Nearly identical results were also obtained when oxidative phosphorylation was suppressed by 1 mM-CN, i.e. the contents of PCr and NTP were not increased by increasing the glucose concentration in the presence of cyanide.

## DISCUSSION

In the present experiments, using pieces of guinea-pig taenia weighing 0·4–0·6 g, a stable NMR spectrum could be consistently obtained with a reasonable time resolution (15–25 min) in normal medium at 32 °C. The spectrum is essentially the same as that previously reported for the same tissue by Vogel *et al.* (1983) and Vermuë & Nicolay (1983). In these studies, however, the control spectrum was obtained with the tissue in a relaxed state in $Ca^{2+}$-free medium at 23 °C, and almost no $P_i$ peak was detected. On the other hand, in the present experiments, a small but clear $P_i$ peak is usually observed. This difference may be due to spontaneous activity in the present experiments, since this occurs in the presence of $Ca^{2+}$ and a higher temperature (32 °C). When $Ca^{2+}$ is removed, [PCr] is only slightly increased (9%), accompanied by a decrease in [$P_i$], but no change in the $\beta$-ATP peak is observed. Therefore, under our experimental conditions, spontaneous mechanical activity does not seem to affect significantly the content of high-energy phosphate compounds, and the amount of tissue detected with the NMR coils is practically unaltered by the mechanical activity.

From the chemical shift of the $P_i$ peak, the $pH_i$ is estimated to be about 7·0 in normal solution, and this agrees well with previous values obtained from various types of smooth muscle with NMR (Vermuë & Nicolay, 1983; Vogel *et al.* 1983; Hellstrand & Vogel, 1985; Dawson & Wray, 1985) and also with intracellular pH-sensitive electrodes (Aickin, 1984).

When the muscle is relaxed in $Ca^{2+}$-free solution, inhibition of oxidative phosphorylation with hypoxia or CN reduces [PCr] with little decrease in [NTP]. Prolonged exposure to glucose-free solution containing the normal concentration of $Ca^{2+}$ has a similar effect. Thus, when energy expenditure is limited, or metabolic inhibition is very mild, the ATP concentration can be maintained at the expense of PCr by the Lohman reaction. However, both [PCr] and [NTP] are clearly decreased during contracture caused by excess $K^+$, particularly in the absence of substrate or under metabolic inhibition, as previously observed with NMR in guinea-pig taenia (Vermuë & Nicolay, 1983) and bull-frog stomach (Yoshizaki, Radda, Inubushi & Chance, 1987). Therefore, it seems that when energy consumption is increased, enough ATP cannot be supplied by PCr, probably due to an insufficient activity of creatine phosphokinase during $K^+$ contracture.

The decrease in [ATP] during the contracture, even in the presence of a sufficient amount of PCr, may also be explained by the compartmentalization of ATP, as assumed for the bull-frog stomach (Yoshizaki *et al.* 1987), or by limited supply of ADP to the creatine kinase reaction, probably resulting from deamination of adenosine. In the rat skeletal (fast-twitch white) muscle, it has been shown that deamination is increased during sustained contraction, and that reamination occurs primarily during recovery at rest (Meyer & Terjung, 1980). In the taenia caeci, the recovery of [ATP] also tends to be better when the preparations are relaxed in $Ca^{2+}$-free solution before returning to the normal solution. When complete recovery of [ATP] is not observed after treatment with hypoxic 40 mM-$K^+$ solution, addition of adenosine leads to full recovery. This suggests that excessive deamination during high energy expenditure is responsible for the poor recovery of [ATP]. A transient rise in the PME peak 2 observed at 25–50 min during $K^+$ contracture also supports the idea of degradation of AMP to hypoxanthine due to deamination.

Another characteristic feature of the NMR spectrum during $K^+$ contracture is a marked increase in $[P_i]$. It has been reported that in smooth muscles an increase in $[P_i]$ inhibits contraction by interfering with the contractile protein (Itoh, Kanmura & Kuriyama, 1986; Gagelmann & Güth, 1987). When the tension development during $K^+$ contracture has been reduced in the absence of glucose, it recovers after glucose readmission (Ashoori *et al.* 1984). The NMR spectrum, obtained in the present experiment, shows a marked decrease in $[P_i]$, with a marginal increase in [NTP], on readmission of glucose to 40 mM-$K^+$ solution. This might suggest that the increase in $[P_i]$ is responsible for the inhibition of tension development. However, during the early phase of $K^+$ contracture in the absence of glucose, $[P_i]$ increases much earlier than the fall of tension. Furthermore, under hypoxic condition or in the presence of CN, the restoration of tension caused by increasing the glucose concentration to 50 mM occurs without much change in $[P_i]$. These results suggest that intracellular $P_i$ does not inhibit contraction significantly, at least under these experimental conditions.

No clear correlation between tension development and [NTP] measured with the NMR is observed in the present experiments. In the absence of glucose, the $K^+$ contracture can be maintained even when [NTP] declines by nearly a half. Glucose readmission, after a prolonged exposure to glucose-free 40 mM-$K^+$ solution, causes recovery of tension which is not accompanied by an increase in [NTP]. On the other

hand, it has been shown that, when the tension recovers on glucose readmission, there is a concomitant increase in oxygen consumption (Ashoori *et al.* 1984), strongly suggesting an increase in ATP production.

The effects of 40 mM-$K^+$ in hypoxia or in the presence of 1 mM-CN are very similar in the presence of 11·8 and 50 mM-glucose, i.e. a large reduction in high-energy phosphates and an increase in $[P_i]$ are not prevented by increasing glucose to 50 mM. The lack of effects of excess glucose on the spectrum is unlikely to be due to tissue damage, because good recovery is observed, particularly in $Ca^{2+}$-free solution containing adenosine. Since intracellular acidification, estimated from the chemical shift of the $P_i$ peak is much stronger with 50 mM-glucose than with 11·8 mM-glucose, it is very likely that glycolysis is facilitated by increasing the glucose concentration. This agrees with the increased production of lactate (Ishida *et al.* 1984). Therefore, the partial recovery of contraction is likely to be caused by an increase in ATP turnover rate, but this is not reflected in the ATP concentration.

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