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The anaerobic energy metabolism in the anterior byssus retractor muscle of *Mytilus edulis* during contraction and catch

Jochen Zange, Hans-Otto Pörtner, and Manfred K. Grieshaber

Institut für Zoologie, Lehrstuhl für Tierphysiologie, Heinrich-Heine-Universität, D-4000 Düsseldorf, Federal Republic of Germany

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Summary. The contribution of anaerobic metabolism in normoxic and hypoxic anterior byssus retractor muscles (ABRM) to ATP production during tonic and repeated phasic contractions under isotonic conditions was estimated by analysing changes in the levels of ATP, phospho-L-arginine, L-arginine, octopine, alanopine, strombine, L- and D-lactate, succinate, L-aspartate and L- and D-alanine.

1. During tonic contraction under normoxia, elicited by the application of acetylcholine and subsequent washout, muscles depleted the phospho-L-arginine store by 3 μmol·g wet wt^{-1}. The phosphagen was replenished during the maintenance of catch. Formation of anaerobic end products was not observed. The energy demand during repeated phasic contractions elicited in aerated media containing serotonin was not only indicated by a continuous breakdown of the phosphagen but also by decreasing ATP levels and the accumulation of octopine.

2. The energy demand during tonic contraction under hypoxic conditions ($P_{O_2} < 2$ Torr) was indicated by a breakdown of phosphagen as well as by ATP depletion. During catch, however, ATP was rapidly replenished whereas restoration of the phosphagen pool to control levels was delayed. Octopine and succinate were accumulated during the initial 30 min of catch and remained constant thereafter. The additional anaerobic ATP turnover (ATP_t) during the active phase of isotonic contraction was found to be 4.9 ± 1.4 μmol·g wet wt^{-1} accounting for the work done. An additional ATP turn-over during subsequent catch was not detectable.

3. Hypoxia ($P_{O_2} < 10$ Torr) did not change the contractility of ABRM during phasic contractions. Energy was provided by means of phosphagen and ATP depletion, by octopine and succinate formation and, in addition, some accumulation of alanopine.

Key words: Opine formation – Tonic contraction – Phasic contraction – Muscle performance – Energetic efficiency

Abbreviations: ABRM anterior byssus retractor muscle; *ATPt* additional anaerobic turn-over of ATP; *PLA* phospho-L-arginine

Introduction

The anterior byssus retractor muscles (ABRM) of *Mytilus edulis* L. contract in two different ways which are distinguished by the velocity of relaxation after cessation of contraction. The phasic contraction is characterized by rapid relaxation, whereas tonically contracted ABRM reach the catch state, where they relax very slowly (for review see Rüegg 1971). Catch is characterized by the maintenance of the inner tension, and by the ability of stretch resistance against external loads (viscosity). During catch inner tension can be abolished by longitudinal vibration of the ABRM, whereas stretch resistance is insensitive to mechanical disturbance of the muscle (Kobayashi et al. 1985). The neurotransmitter acetylcholine induces an active contraction of the ABRM, which is followed by catch when acetylcholine has been removed. Serotonin, dopamine and rectangular current pulses abolish catch and induce a rapid relaxation of the muscle (Winton 1937; Twarog 1954; Twarog and Cole 1972).

In their natural habitat common sea mussels, *Mytilus edulis,* are subjected to long periods of anaerobiosis during low tide. During these periods the animals must reduce their energy turn-over in order to avoid depletion of their energy stores (De Zwaan and Wijsman 1976). Catch muscles are known to perform active contraction more economically than other smooth muscles. In addition, the energy turn-over can very efficiently be reduced during catch (Rüegg 1971). Nevertheless, the question arises whether the maintenance of catch needs a slightly elevated energy supply compared to resting muscles in which catch is absent.

If maintenance of catch induced an extra ATP turn-over it would be considered an active process (e.g. by continuous crossbridge cycling), while an inactive process would be independent of any ATP-supply.

During catch, isometrically contracted ABRM or skinned fiber preparations of the ABRM fail to re-establish tension after their initial tension has been abolished by a quick release. Therefore, Jewell (1959) and Pfitzer and Rüegg (1982) concluded that catch is maintained by a passive mechanism. Nevertheless, the absence of a mechanical response after a quick release does not unequivocally prove, that the mechanism of catch is independent of extra ATP-consumption.

The energy demand of the ABRM during catch has long been a matter of great interest. Oxygen consumption and heat production have been analysed as parameters for energy consumption. Parnas (1910) measured the oxygen consumption of mussels in vivo and found only a negligible increase during shell closure against external loads. Abbott and Lowy (1955) postulated that the isolated ABRM of *Mytilus edulis* produces heat as long as muscle tension is maintained during an acetylcholine induced ‘tonus’. After treatment with serotonin heat production and tension drop. Unfortunately, Abbott and Lowy (1955) did not state whether acetylcholine had been removed after contraction to induce catch. Baguet and Gillis (1968) found enhanced oxygen consumption by isometrically contracted ABRM during ‘catch’ which was related to the maintained muscle tension. In these experiments, and possibly also in those described by Abbott and Lowy (1955), the ABRM was exposed to acetylcholine during the whole period of contraction. The question arises whether the muscles produced tension actively under these conditions due to the permanent presence of the contraction stimulus.

Another problem linked to the interpretation of the available literature is that during periods of increased energy demand oxidative metabolism is frequently supported by anaerobic processes. It may thus be misleading to estimate the energy demand only from oxygen consumption measurements. Nauss and Davies (1966) described a breakdown of the phosphagen pool in ABRM under normoxia which was proportional to the work performed during contraction. The phosphagen pool was repleted during the first 5 min of subsequent catch. Devroede and Baguet (1982) focussed on octopine and alanine as glycolytic end products. Their results suggest that anaerobic glycolysis may not be involved during shortening and subsequent catch (tonic contraction).

The studies mentioned so far do not provide a complete picture of the energy metabolism and the anaerobic mechanisms during catch, since additional anaerobic end products have been discovered recently in *Mytilus edulis* (for review see De Zwaan and Dando 1984). Furthermore, some anaerobic processes may only start at the end of a contraction with the onset of recovery (Grieshaber 1978). In addition, the energy demand has to be determined not only during the active phase of contraction but also during the period of catch itself, taking all mechanisms of anaerobic energy production into account.

The present study was designed to investigate three areas of interest. First, the extent to which anaerobic metabolism is involved in ATP-supply during a single tonic contraction under normoxic conditions, distinguishing between the active phase of contraction and subsequent catch. Secondly, whether catch is maintained actively or passively. Thirdly, the anaerobic metabolism of normoxic and hypoxic ABRM during repeated phasic contractions.

Materials and methods

Animals. Mussels (*Mytilus edulis*) were obtained from the Biologische Anstalt Helgoland and were transported to the University of Düsseldorf within 12 h. The animals were kept in recirculated, filtered, artificial seawater (32 ‰) for 3–8 weeks at 15 ± 1 °C until used in the experiments.

Preparation and incubation. The mussel contains a pair of identically shaped anterior byssus retractor muscles. For each experiment both muscles were taken, one as a control and the other to be employed in the contraction experiment. The isolation of the ABRM followed the procedure of Hoyle and Lowy (1956). Intact muscles attached to a piece of shell and held by a silk thread tied to the byssal end of the muscles were used.

The muscles were mounted in incubation chambers filled with artificial seawater (32 ‰, Wiegand GmbH, Krefeld, FRG) which was equilibrated with air at 15 ± 1° C. In each experiment

both muscles were loaded with 0.1 N. Since freshly prepared ABRM were usually contracted, isolated muscles were subjected to electrical stimulation with rectangular pulses applied by platinum electrodes (2 Hz, 100 ms, 10 V; Winton 1937). Both muscles were then incubated for 4 h. For incubation under hypoxia ($P_{O_2}<2$ Torr) equilibration with 99.99% nitrogen started after 3.5 h. The oxygen tension was measured using a P_{O_2}-electrode (E 5046-0, Radiometer, Copenhagen) and a monitor (PHM 73, Radiometer, Copenhagen).

Recording of muscle movements. Muscle movements were followed by means of an isotonic transducer connected to a HF-modem and an amplifier (Hugo Sachs Elektronik, Freiburg, FRG) and were displayed on a chart recorder. The relaxation of control muscles during the initial phase of the incubation period was recorded using a simple lever arm, the position of which was marked on graph paper at regular time intervals.

Tonic contractions. Tonic contractions under isotonic conditions were elicited by the application of acetylcholine (0.5 m*M*; Nauss and Davies 1966). The ABRM contracted maximally within a minute. Two minutes after the addition of acetylcholine the incubation medium was replaced twice using thermostated (15±1 °C) seawater equilibrated with air or nitrogen. During this procedure the P_{O_2} rose to 20 Torr in the hypoxic medium but dropped rapidly to reach less than 2 Torr within 20 s. In two series of experiments normoxic and hypoxic ABRM were freeze-clamped (Wollenberger et al. 1960) after different periods of catch. In a first series sampling occured at 2, 7, and 30 min and in the second series at 1, 30 and 60 min after the beginning of contraction. Control muscles were sampled after incubation in normoxic or hypoxic sea water following the same time protocol. The animals used for the first series had been collected during winter and for the second series during summer.

Repeated phasic contractions. For the stimulation of isotonic, phasic contractions muscles were incubated in seawater containing 0.01 m*M* serotonin. Under these conditions electrical stimulation at the beginning of the preincubation period proved to be unnecessary. Contractions were again induced by the application of acetylcholine (0.5 m*M*) which was washed out two times, immediately at the peak of each contraction. Afterwards the muscles were allowed to relax for 2.5 min and were then stimulated again. Each ABRM was brought to contraction 9–10 times during a period of 31.1±1.0 min (normoxic conditions) or 30.9±1.0 min (hypoxic conditions), respectively, and was frozen either at the peak of the last contraction or after 10 min of relaxation. Since the hypoxic incubation medium had to be replaced several times the average oxygen tension was slightly higher than during the catch experiments ($P_{O_2}<10$ Torr). The incubation of control muscles followed the same time protocol in normoxic and hypoxic water.

Analysis of metabolites. The ABRM was ground under liquid nitrogen in a small mortar as described by Pette and Reichmann (1982). Concentrations of ATP, D- and L-lactate, L-aspartate, and D- and L-alanine were measured in neutralized perchloric acid extracts using standard procedures (Bergmeyer 1974). Octopine and lactate levels were determined in two steps using the same assay. First, nearly homogeneous octopine dehydrogenase was added followed by the addition of lactate dehydrogenase at the end of the octopine dehydrogenase reaction. Analysis of phospho-L-arginine and L-arginine was performed using the method of Grieshaber et al. (1978). Octopine dehydrogenase was prepared from the adductor muscle of *Pecten maximus* by the procedure of Gäde and Carlsson (1984). Succinate was measured according to Michal et al. (1976), strombine and alanopine according to Siegmund and Grieshaber (1983).

Changes in the contents of metabolites were calculated from the differences between the experimental muscle and the control muscle in each animal. The anaerobic ATP turnover (ATP_t) was calculated by summing up the changes of metabolite contents taking the ATP yields of the respective anaerobic pathways into account (Pörtner et al. 1984).

$$ATP_t = -\Delta ATP + (\Delta\text{L-Arg} + \Delta\text{octopine}) + 1.5\,(\Delta\text{octopine} + \Delta\text{alanopine} + \Delta\text{strombine}) + 2.5\,\Delta\text{succinate}$$

Metabolite levels and ATP turn-over are given as means ± standard deviations (SD). The significance of differences was evaluated using Student's *t*-test for paired samples. Data from different experimental series were compared using the *t*-test for unpaired samples. A value of $P<0.05$ was assumed to be indicative of statistically significant differences.

Table 1. Parameters of muscle performance during a single active contraction and subsequent catch in ABRM loaded with 0.1 N under normoxia (O_2) and hypoxia (N_2). An external work of 1 mJ was performed by a shortening of 1 cm

	Series 1		Series 2	
	O_2	N_2	O_2	N_2
n	15	14	15	14
Muscle length (cm)	2.1 ± 0.2	2.4 ± 0.2	2.6 ± 0.2	2.7 ± 0.2
Muscle shortening % muscle length	75 ± 7	77 ± 6	86 ± 6	88 ± 4
Work (mJ)	1.55 ± 0.26	1.82 ± 0.22	2.25 ± 0.23	2.35 ± 0.12
Wet weight (mg)	36 ± 7	37 ± 9	36 ± 8	38 ± 10
Work · g wet wt^{-1} (mJ · g^{-1})	43 ± 7	49 ± 10	63 ± 17	62 ± 25
(1) *t* (s)	61 ± 13	64 ± 18	45 ± 5	53 ± 9
n	10	9	9	9
(2) Rate of relaxation (mm · min^{-1})	0.07 ± 0.02	0.04 ± 0.02	0.08 ± 0.03	0.06 ± 0.03

(1), time for muscles to reach the maximum of the contraction
(2), constant rate of relaxation during catch, reached after an initial phase characterised by a decreasing relaxation velocity

Results

Performance during tonic contraction

Parameters of muscle performance during tonic contractions are listed in Table 1. Significant differences were observed between the two experimental series which were performed during different seasons of the year. In both cases, however, ABRM incubated under normoxia and hypoxia

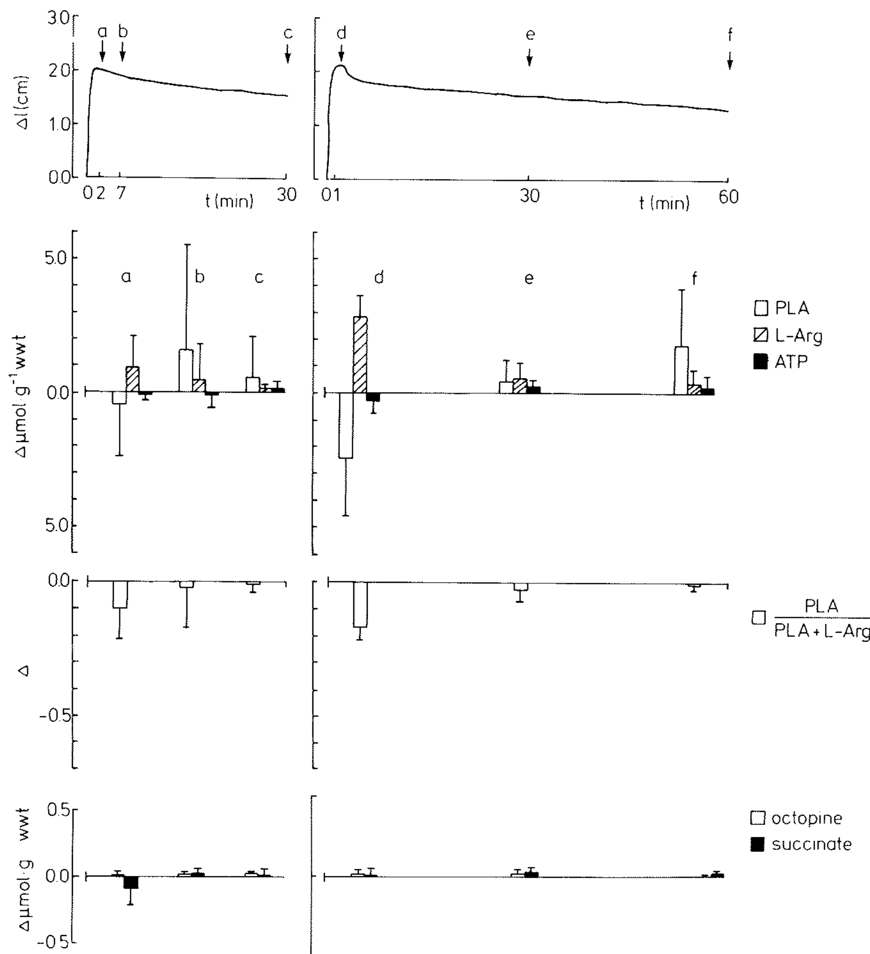

Fig. 1. Recordings of tonic contractions by ABRM under normoxia and changes in the contents of metabolites after different periods of contraction. Values are given as means of differences between contracted and paired control muscles ±SD. Positive values are indicative for an increase and negative values for a decrease in the levels of the metabolites (series 1: *a–c*, series 2: *d–f*)

performed the same amount of work during the actual phase of contraction.

Immediately after acetylcholine was removed, the byssus retractor muscles started to relax. Between 2 and 13 min after the onset of contraction the relaxation velocity decreased until it reached a constant rate which characterized a linear phase of catch. The values of the relaxation velocity listed in Table 1 are only given for the latter period.

Tonic contractions under normoxia

At the maximum of their contraction normoxic muscles in series 2 had lifted a load of 0.1 N. The amount of shortening was 2.10 ± 0.23 cm ($n = 6$) corresponding to a work of 2.10 ± 0.23 mJ ($N \cdot mm^{-1}$) or 62 ± 20 $mJ \cdot g$ wet wt^{-1} (the average work performed by all muscles in series 2 was 63 ± 17 $mJ \cdot g$ wet wt^{-1}, $n = 15$, see Table 1). In these muscles a significant transphosphorylation of phosphagen occurred as indicated by a decrease of 2.34 ± 2.14 $\mu mol \cdot g$ wet wt^{-1} in phospho-L-arginine and an increase of 2.85 ± 0.78 $\mu mol \cdot g$ wet wt^{-1} in L-arginine contents, as compared to the corresponding non-contracting control muscles from the same animal. The ratio [PLA]/([PLA]+[L-Arg]) was 0.89 ± 0.03 in control muscles and had decreased by 0.17 ± 0.04 reaching 0.72 ± 0.03 in the experimental muscles.

About 1 min after the ABRM had reached its maximal contraction, i.e. after 2 min of incubation in water containing acetylcholine, differences in phosphagen concentrations between experimental and non-treated control muscles had become insignificant again, although the ratio of [PLA]/([PLA]+[L-Arg]) was still lowered by 0.10 ± 0.11 in experimental muscles ($n = 5$). After acetylcholine washout and 5 min of sustained catch phospho-L-arginine was completely restored. Glycolytic end products and succinate did not accumulate during tonic contraction under these conditions (see Fig. 1).

Table 2. The energy consumption of resting muscles during various periods of anaerobiosis is indicated by a breakdown of the phosphagen and an increase in octopine, alanopine and succinate levels ($\mu mol \cdot g$ wet wt^{-1})

	Series 1			Series 2		
t [min]	32	37	60	31	60	90
PLA	8.08 ±1.88	6.92 ±3.71	7.11 ±2.57	13.40 ±2.32	11.09 ±1.18	12.31 ±2.13
L-Arg	1.42 ±0.35	1.45 ±0.42	1.75 ±0.24	2.92 ±0.96	2.83 ±0.47	3.90 ±0.31
$\frac{[PLA]}{[PLA]+[L-Arg]}$	0.85 ±0.28	0.80 ±0.08	0.79 ±0.05	0.82 ±0.04	0.82 ±0.02	0.76 ±0.02
Octopine	0.09 ±0.06	0.18 ±0.09	0.24 ±0.12	0.16 ±0.04	0.23 ±0.07	0.23 ±0.07
Alanopine	0.02 ±0.03	0.02 ±0.01	0.04 ±0.02	0.01 ±0.01	0.04 ±0.01	0.07 ±0.04
Succinate	0.13 ±0.04	0.18 ±0.07	0.26 ±0.08	0.12 ±0.06	0.26 ±0.05	0.39 ±0.09

Tonic contractions under extreme hypoxia

Resting control muscles subjected to an oxygen tension of less than 2 Torr produced ATP by anaerobic processes resulting in octopine, alanopine and succinate formation, and showed a slow depletion of the phosphagen pool (see Table 2). Additional ATP was required to reach the maximum of an isotonic, tonic contraction as indicated by a decrease in phosphagen and ATP levels in experimental muscles exceeding the changes observed in control muscles. As shown by the experiments in series 2 the phospho-L-arginine content fell by 2.98±1.74 $\mu mol \cdot g$ wet wt^{-1} and ATP levels decreased by 1.02±0.82 $\mu mol \cdot g$ wet wt^{-1}, while L-arginine levels increased by 3.52±1.23 $\mu mol \cdot g$ wet wt^{-1} ($n=5$). The ratio of [PLA]/([PLA]+[L-Arg]) decreased by 0.21±0.03. The levels of glycolytic end products and of succinate did not rise significantly above the anaerobic resting levels. The additional ATP (ATP_t) required by isotonic contraction was calculated to be 4.9±1.4 $\mu mol \cdot g$ wet wt^{-1} equivalent to a mechanical work of 67.9±19.3 $mJ \cdot g$ wet wt^{-1} or 15.2±7.2 $mJ \cdot \mu mol^{-1}$ ATP. This corresponds to an average efficiency of 27% assuming that 56 kJ are equivalent to the splitting of 1 mol ATP (Sleep and Smith 1981).

During anaerobic catch ATP reached levels of anaerobic, quiescent control muscles within 5 min after acetylcholine washout. Resynthesis of the phosphagen occurred only very slowly. Resting levels were not restored within 58 min of catch. The small degree of phosphagen repletion was only indicated by a change in the ratio of [PLA]/([PLA]+[L-Arg]). After 58 min of catch it was still by 0.14±0.02 ($n=5$) lower than control values, but compared to the difference observed at the maximum of contraction the ratio was significantly reduced.

The recovery of ATP and phosphagen levels during catch was accompanied by a transient synthesis of octopine and succinate. The amount of accumulated octopine differed between the two experimental series. In the first series the octopine level increased by 0.55±0.21 $\mu mol \cdot g$ wet wt^{-1} during the first 7 min of tonic contraction ($n=5$). Afterwards, the difference in octopine contents between control and experimental muscles remained constant. In the second series of experiments octopine increased only by 0.27±0.15 $\mu mol \cdot g$ wet wt^{-1} (30 min, $n=4$). During the first 28 min of catch under hypoxia, ABRM accumulated succinate (0.34±0.09 $\mu mol \cdot g$ wet wt^{-1} in series 1 ($n=4$) and 0.49±0.19 $\mu mol \cdot g$ wet wt^{-1} in series 2 ($n=4$)). Succinate accumulation stopped thereafter (see Fig. 2). The rate of alanopine accumulation was not increased above resting levels.

The additional ATP turn-over (ATP_t) calculated for anaerobic muscles frozen after different periods of catch (5–58 min) included the ATP_t during the active working phase of contraction plus the ATP_t during subsequent catch. The additional amount of ATP (ATP_t) consumed after different periods of catch was not significantly different from the quantity calculated for the maximum of contraction alone (see Fig. 2 and Table 3). Therefore, the additional ATP turn-over and all differences in metabolite contents between experimental and control muscles during the anaerobic, tonic contraction were caused by the actual working

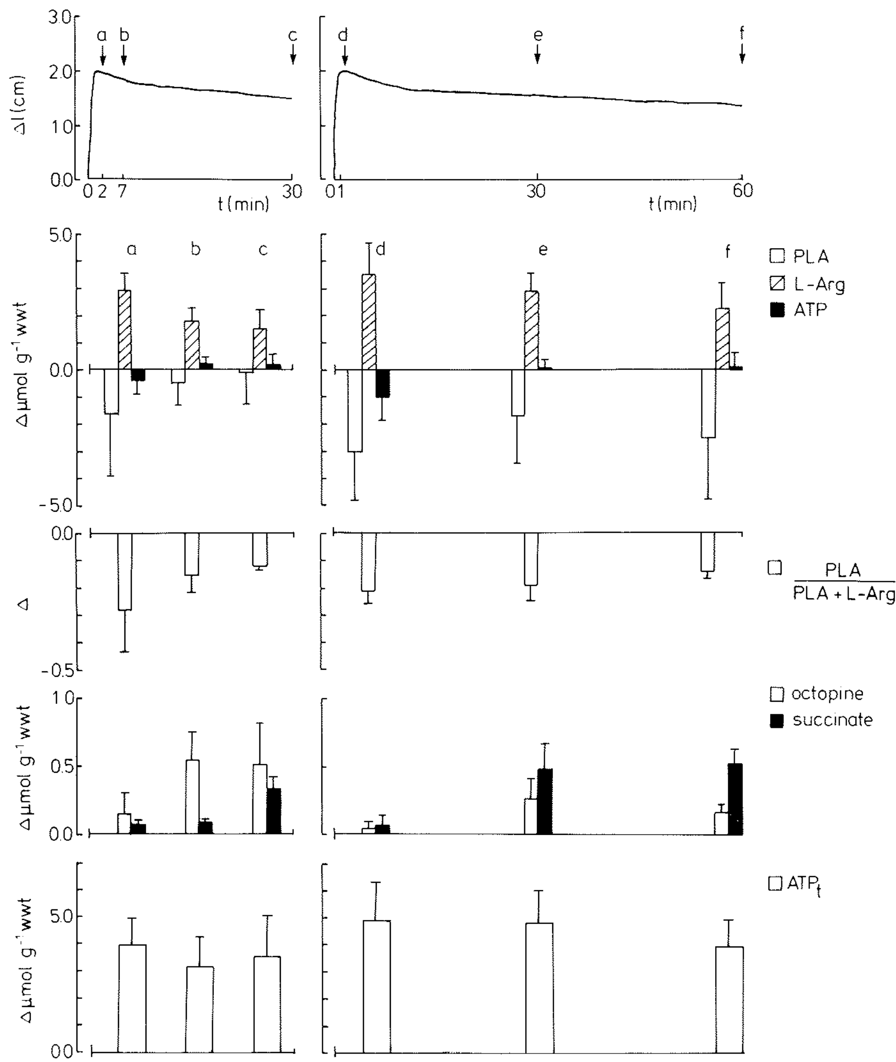

Fig. 2. Recordings of tonic contractions by ABRM under hypoxia (P_{O_2} < 2 Torr) compared to changes in the contents of metabolites and the additional consumption of ATP (ATP_t) after different periods of contraction (series 1: *a–c*, series 2: *d–f*)

Table 3. The relation between muscle performance and the additional ATP-turn-over (ATP_t) in hypoxic (P_{O_2} < 2 Torr) ABRM after different periods of an isotonic, tonic contraction. The results demonstrate that the ATP_t, which was estimated after different periods of catch, reflects only the ATP_t of the initial working phase, whereas a further ATP_t for the maintenance of catch was undetectable (At least a further ATP_t of 2–3 μmol · g wet wt^{-1} within 58 min of catch would have been significantly detectable). The efficiency by which mechanical work was performed was calculated assuming an energy release of 56 KJ by the splitting of 1 mol ATP (Sleep and Smith 1981)

Duration of tonic contraction [min]	Series 1			Series 2		
	2	7	30	1	30	60
n	5	5	4	5	4	4
Muscle shortening [cm]	1.65 ±0.19	1.85 ± 0.11	1.73 ± 0.12	2.26 ± 0.02	2.49 ± 0.17	2.32 ± 0.20
Work · g wet wt^{-1} [mJ · g^{-1}]	58.5 ±10.0	47.9 ±10.9	50.9 ± 2.1	67.9 ±19.2	64.3 ±20.3	58.4 ±11.3
ATPt [μmol · g wet wt^{-1}]	3.9 ± 1.1	3.2 ± 1.1	3.6 ± 1.5	4.9 ± 1.4	4.8 ± 1.2	3.9 ± 0.9
Work · ATPt^{-1} [mJ · μmol^{-1} ATP]	15.8 ± 7.2	17.3 ± 8.7	16.4 ± 7.1	15.1 ± 7.2	13.6 ± 3.4	15.5 ± 4.5
Efficiency (%)	28	31	29	27	24	28

phase. A further ATP consumption for the maintenance of catch could not be detected.

In series 2 of the experiments changes in D- and L-alanine as well as L-aspartate were monitored during tonic contractions under hypoxia. L-Aspartate was degraded during the same time period when succinate accumulated. The amino acid was metabolized by 0.75 ± 0.45 μmol·g wet wt^{-1} during 28 min of catch. An accumulation of L-alanine was indicated by a difference of 2.32 ± 1.37 μmol·g wet wt^{-1} between control and experimental muscle being significant after 28 min of catch. Otherwise, standard deviations of L-alanine differences were too large to allow quantitative conclusions. D-Alanine was not detected at all (<0.03 μmol·g wet wt^{-1}).

Phasic contractions

Muscular performance during isotonic contractions elicited in media containing serotonin was not affected by the concentration of oxygen in incubation media. During the ninth contraction normoxic ABRM reached only $37 \pm 18\%$ and hypoxic muscles $36 \pm 19\%$ of the initial amount of shortening, indicating a similar degree of fatigue.

At the maximum of the last contraction phosphagen and ATP were found to be depleted and octopine accumulated in normoxic ABRM as compared to resting normoxic control muscles (PLA: -2.31 ± 1.52 μmol·g wet wt^{-1}; L-Arg: $+2.03 \pm 0.89$ μmol·g wet wt^{-1}; [PLA]/([PLA]+[L-Arg]): -0.21 ± 0.08; ATP: -0.11 ± 0.09 μmol·g wet wt^{-1}; octopine: $+0.24 \pm 0.14$ μmol·g wet wt^{-1}; $n=6$).

Another group of muscles was allowed to relax for 10 min after the last contraction. During this period ATP reached control values. Phospho-L-arginine was partly resynthesized. The amount of accumulated octopine remained constant. Additionally, succinate was slightly increased by 0.09 ± 0.06 μmol·g wet wt^{-1}.

Hypoxic exposure accelerated the depletion of phospho-L-arginine and of ATP and the accumulation of octopine (PLA: -5.73 ± 2.36 μmol·g wet wt^{-1}; L-Arg: $+2.19 \pm 1.25$ μmol·g wet wt^{-1}; [PLA]/([PLA]+[L-Arg]): -0.37 ± 0.09; ATP: -0.60 ± 0.22 μmol·g wet wt^{-1}; octopine: $+2.72 \pm 0.91$ μmol·g wet wt^{-1}). In addition, 0.66 ± 0.46 μmol·g wet wt^{-1} succinate and 0.13 ± 0.06 μmol·g wet wt^{-1} alanopine were formed. During 10 min of relaxation under hypoxia phospho-L-arginine and ATP contents were incompletely restored, while the levels of succinate and alanopine doubled. Octopine levels remained unchanged (see Fig. 3). Generally, L-lactate and strombine were found in concentrations below 0.10 μmol·g wet wt^{-1} and did not change significantly during contraction. D-Lactate was not detectable (<0.03 μmol·g wet wt^{-1}).

Discussion

The complete analysis of anaerobic energy metabolism in ABRM during tonic contraction under normoxia (Fig. 1) confirmed the observations of Nauss and Davies (1966) and of Devroede and Baguet (1982). A single active contraction leading to catch (tonic contraction) was accompanied by a transient utilization of the phosphagen pool. Neither an additional accumulation of glycolytic end products or succinate nor a decrease in ATP contents would be observed. Obviously, anaerobic ATP production from glycogenolysis or aspartate depletion are not required for the build-up of tension and catch under normoxia. The transient transphosphorylation from phosphagen only contributed to ATP production when the muscles were actually shortening. These changes were reversed during the subsequent period of catch. The transient buffering of ATP levels by phosphagen and the capacity of aerobic pathways is sufficient to allow the muscles to shorten and remain in catch.

During incubation under nitrogen ($P_{O_2} <$ 2 Torr) resting muscles produced ATP from the phosphagen pool as well as by anaerobic glycolysis, as judged from the significant accumulation of octopine, alanopine and succinate during short periods of hypoxia (maximum 90 min). Additional ATP turn-over caused by muscle action is, therefore, only supported by anaerobic processes under these conditions. Consequently, the comparison of relaxed anaerobic control muscles with anaerobic muscles remaining in catch should reveal whether catch causes the ATP requirements to be elevated above the metabolic needs in resting muscle.

During anaerobiosis ABRM contracted as well as, or even better than, under normoxic conditions. At the maximum of tonic contraction under hypoxic conditions, phosphagen and ATP levels were depleted to a larger extent than under normoxia. During the active phase of contraction 15.8 ± 7.2 mJ of mechanical work was performed by the splitting of 1 μmol of ATP. Assuming an energy-release of 56 kJ by the splitting of 1 mol ATP (Sleep and Smith 1981), mechanical work was performed with an efficiency of about 28%. Nauss and Davies (1966) estimated a mechanical work of 23 mJ per μmol ATP corresponding to a distinctly higher efficiency of 43%. Nauss and Davies

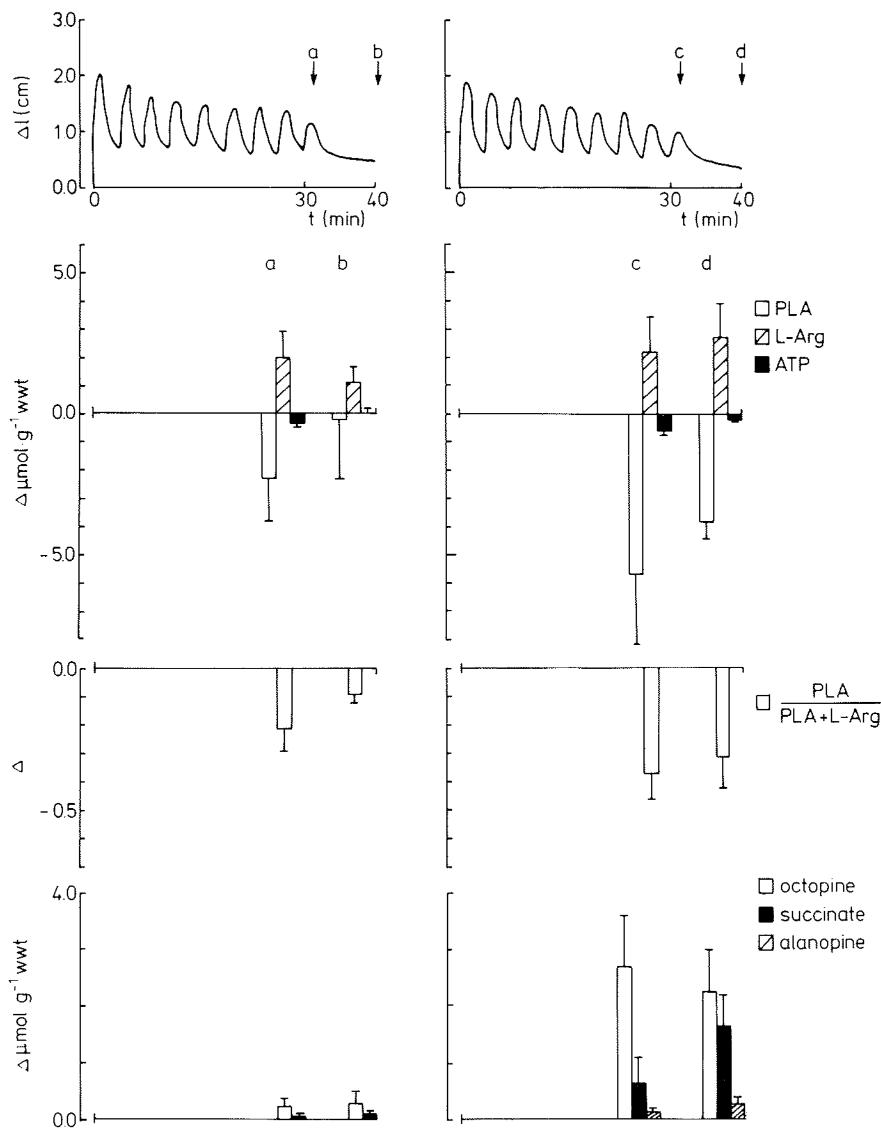

Fig. 3. Recordings of phasic contractions by ABRM under normoxia (*a* and *b*) and hypoxia (P_{O_2}<10 Torr; *c* and *d*) and changes in the contents of metabolites at the different times of contraction

(1966) only measured the phosphagen breakdown of the ABRM, which obviously reflects only part of the ATP-demand during an active contraction.

During subsequent catch a transient, additional accumulation of octopine and succinate occurred in hypoxic ABRM, while ATP levels were restored to anaerobic resting levels. The difference in phosphagen contents decreased slightly. Succinate was most probably formed from aspartate and not from glycogen, as indicated by the concomitant depletion of L-aspartate and the accumulation of L-alanine. It is remarkable that octopine and succinate fermentation returned to the anaerobic resting rate before the repletion of phospho-L-arginine had been accomplished. Both the recovery of phosphagen and the reduction in anaerobic metabolic rate indicate a drop of energy requirements during catch.

The calculated value of ATP_t comprises the additional ATP turn-over during the whole period of tonic contraction. Consequently, in all groups of muscles an additional ATP turnover was found during the period of shortening, i.e. when the ABRM performed mechanical work. There were no significant differences between the values of ATP_t calculated for muscles which had been sampled at the maximum of contraction or at different times after the induction of catch. As a corollary no further ATP turn-over for the maintenance of catch could be detected. Consequently, the excess ATP turn-over during catch is less than 2–3 µmol · g wet wt^{-1} within 58 min, which would have been significantly detectable.

Baguet and Gillis (1968) found that the excess respiration of a muscle during catch is directly proportional to the sustained isometric muscle tension

(P [$Kg \cdot cm^{-2}$]). The additional oxygen consumption was calculated by Baguet and Gillis (1968) using the following equation:

$\dot{M}_{O_2}$ [$nmol \cdot min^{-1} \cdot g$ wet wt^{-1}] $= 16.9(\pm 0.5)$ [$nmol \cdot min^{-1} \cdot g$ wet wt^{-1}] $+ P \cdot 6.8$ (± 0.5) [$nmol \cdot cm^2 \cdot Kg^{-1} \cdot min^{-1} \cdot g$ wet wt^{-1}].

In our isotonic experiments the ABRM remained shortened against an external load of 0.1 N ($\approx$10 g). As the diameter of the muscles during catch was found to be within a range of 2–3 mm, the ABRM maintained a constant, average tension of about $2\ N \cdot cm^{-2}$ ($\approx 0.2\ Kg \cdot cm^{-2}$). Assuming that 6 moles of ATP are produced per mole of oxygen (O_2) consumed the additional ATP turnover of a muscle holding $0.2\ g \cdot cm^{-2}$ for 58 min would be about $6.4\ \mu mol \cdot g$ wet wt^{-1} according to the results of Baguet and Gillis (1968). Even with regard to the different experimental conditions, such an increase would have been detectable in the present study. No such increase in mean ATP turn-over could be substantiated in our experiments. The equation put forward by Baguet and Gillis (1968) included a significant term independent from the maintained tension ($16.9\ nmol \cdot min^{-1} \cdot g$ wet wt^{-1}). In our opinion, Baguet and Gillis (1968) found this tension-independent elevation in oxygen consumption of ABRM during catch because they incubated the muscles continuously in a solution containing acetylcholine, possibly leading to a stimulation of metabolism during the whole period of maintained tonic contraction. If the tension-independent term of the equation of Baguet and Gillis (1968) was ignored, then the tension-dependent term ($P \cdot 6.8\ nmol \cdot cm^2 \cdot g^{-1} \cdot g$ wet wt^{-1}) would be equivalent to an ATP turnover of $0.5\ \mu mol \cdot g$ wet wt^{-1} in ABRM holding a tension of $0.2\ g \cdot cm^{-2}$ over a period of 58 min. This very small ATP turnover for the maintenance of tension during catch would not have been detectable in the present study.

Güth et al. (1984) induced contractions of skinned fibers of the ABRM by increasing the concentration of Ca^{2+} in the medium. When they induced a catch-like state by removal of Ca^{2+}, ATP-hydrolysis dropped rapidly to the resting level while tension was held for some minutes. The latter observation supports the conclusion of the present study that maintenance of catch does not involve an increase in the need for energy. The underlying mechanism must be dependent upon this precondition. Fixation of actin-attached crossbridges (Lowy et al. 1964; Takahashi et al. 1988) or cross-linking of non-contractile filaments, for instance the thick, paramyosin containing filaments (Rüegg 1971; Hauck and Achazi 1987), would meet this requirement.

An elevation of energy expenditure during active contraction could be substantiated during repeated phasic stimulations. A total of 9–10 phasic contractions elicited signs of fatigue in ABRM under normoxia, as indicated by the decrease in the amount of work done. These changes were not only accompanied by decreased phosphagen contents but also by a depletion of the ATP pool and an accumulation of octopine, indicating that some energy is supplied by anaerobic glycolysis. During recovery from phasic contractions the glycolytic pathway is not important for the repletion of the phospho-L-arginine. Only a small increase in succinate levels is observed, possibly linked to an increase of the aerobic metabolic rate.

The performance of hypoxic ($P_{O_2} < 10$ Torr) ABRM during phasic contractions at a frequency of about $0.3\ min^{-1}$ was not reduced compared with normoxic muscles, although the phosphagen was almost depleted after the first contraction and repletion of the phosphagen during relaxation occurred more slowly than in aerobic muscles. The energy demand for the subsequent contractions was supported by anaerobic glycolysis, as shown by the accumulation of octopine and a small increase in alanopine levels. The predominance of octopine as the major end product of anaerobic glycolysis is in accordance with the mechanism of opine formation as outlined by Kreutzer et al. (in press). Moreover, succinate reached higher levels than in anaerobic controls. To provoke a sequence of phasic contractions the medium had to be changed repeatedly. Since an increase in P_{O_2} could not be avoided, it cannot be assumed that changes in ATP turn-over during these experiments were solely reflected by the accumulation of anaerobic end products. The larger changes in metabolite levels are even more indicative of the increased energy demand during phasic as compared to tonic contractions.

The anterior and posterior byssus retractor muscles regulate the tension of the byssal threads by which the mussel is fixed to the substrate. When byssus retractor muscles are relaxed, enlarged interstices between the animals living in colonies facilitate the filtration of seawater. When the animals are at risk of being dislodged from their substrate by wave action during low tide, or by enemy attack, the contraction of the byssus retractor muscles brings the mussels closer to the surface of the substrate. In addition, low tide and enemy attack, i.e. by a starfish, induce long periods of shell closure which provoke hypoxia (De Zwaan and Wijsman 1976). During hypoxia a reduction in energy

turn-over is an important feature of survival (Shick et al. 1983). Accordingly, maintenance of catch must be seen as an adaptation which allows the byssus retractor muscles to hold an animal close to the substrate and the adductor muscles to keep the shell closed without additional energy cost for a long period. As muscles are passively elongated during catch by external forces such as the weight of the animal or the tension exerted by the ligament, active contractions at regular long intervals are needed. Therefore, muscles must be able to increase ATP turn-over to some extent regardless of the amount of available oxygen. Our study shows that normal function of the ABRM does not depend on the amount of oxygen dissolved in the hemolymph. During normoxia, the energy demand of the working ABRM is supported by oxidative metabolism and transient transphosphorylation of phospho-L-arginine. Only when ATP turn-over is increased above a certain threshold does octopine fermentation occur. During anaerobiosis the reduction in oxidative ATP production is compensated for by octopine, alanopine and succinate formation.

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