PERFUSED ISOLATED MUSCLE TO STUDY ENVIRONMENTAL EFFECTS ON MUSCLE METABOLISM

T. Astruc, M. Foé-Essissima, X. Fernandez, A. Talmant, G. Monin
INRA, Station de Recherches sur la Viande, 63122 Saint Genès-Champanelle, France

Introduction

Slaughter of meat animals induces simultaneously muscle contractions and release of catecholamines. Both have been shown to strongly influence post mortem biochemical changes in muscle and subsequent meat quality (Bendall et Lawrie, 1962 ; Sair et al., 1970; Van der Wal, 1975). Accurate measurement of the effects of individual hormones is made difficult because hormonal systems interact. Moreover, experimentation requires deep anaesthesia of the animals, which is susceptible to strongly interfere with energetic metabolism or cell membrane function. Some authors overcame these difficulties by using perfused isolated muscle which allows to control both circulating medium composition and stimulation intensity (Miri et al., 1991 ; Monin et al., 1992). Death is then simulated by perfusion stop. These authors did not stimulate the muscle during perfusion, except a few twitches aimed to control the physiological state of the preparation. In fact, most animals have physical activity when waiting for slaughter and going to the slaughter post. In the present experiment, we intended to simulate this condition by inducing contraction twitches at a moderate rate (0.1 Hz) throughout the perfusion.

Material and Methods

Animals and muscle dissection

It was performed according to the technique described by Miri et al. (1991) slightly modified. Six New Zealand male rabbits weighing 2 to 3 kg were used. Rabbits were anesthetized using ketamine and sodium pentobarbital. The Biceps brachii muscle was isolated and perfused as described by Miri et al. (1991). However the isolated muscle was placed in a Krebs waterbath at 34°C instead of an oven as in Miri et al. (1991) (Fig. 1). The muscle was perfused for 10 min with Krebs added with 35g/l of albumin, 40 µg/l of gentamycine sulfate, 0.84 mM of papaverine chloride and 120 µIU/ml of insulin. Then it was perfused for 30 min with a suspension of washed bovine red cells (same medium added with 20 % red cells v/v)). The perfusion medium was oxygenated with 95 % O_2 – 5 % CO_2. In every experiment, one muscle (referred to as control) was stimulated (50 V, 0.1 Hz) in order to get 3 twitches at the beginning and again just before the end of the perfusion. The contralateral muscle (referred to as stimulated) was stimulated (50 V, 0.1 Hz) for 30 min.

Physical and chemical analyses

Muscle tension changes were monitored and contraction parameters were determined using a MacIntosh II equipped with a MacAdios card. Samples of 100-250 mg were taken off at different times (see Fig. 2), immediately frozen in liquid nitrogen, freeze-dried and trimmed of fat and blood under microscope before extraction with 0.6 M perchloric acid. Phosphocreatine (Pc), adenosine triphosphate (ATP) and inosine monophosphate (IMP) were determined from neutralized extracts by HPLC. Lactate was determined according to Bergmeyer (1974).

Results

The changes in contraction traits during red cells perfusion are shown in table 1. Regardless of stimulation pattern, the contraction time and the half relaxation time decreased. In control muscle, contraction strength decreased by 12 %. Continuous stimulation for 30 min generated a decrease of 33 % in the contraction force ($P < 0.05$). Changes in the concentrations of biochemical compounds during isolation and perfusion of muscle are shown on Fig. 2. During the isolation process small but significant decreases were observed in ATP ($P < 0.05$) and Pc ($P < 0.01$). During perfusion, lactate levels increased ($P < 0.05$ in both groups) but noticeably more in stimulated muscles ($P < 0.01$). Pc concentrations decreased ($P < 0.01$ in both groups), again more in stimulated muscles ($P < 0.05$). ATP level kept constant in control muscles and decreased ($P < 0.05$) in stimulated muscles.

Discussion

Miri et al. (1991) observed a decrease of 9 % in the contraction strength at the end of the perfusion when provoking 5 twitches at the beginning and end of perfusion. With a comparable stimulation pattern, we got a decrease of 12 %. This indicates that replacing the oven of Miri et al. by a Krebs bath maintained at 34°C did not affect noticeably the preparation.

The changes in energetic compounds and lactate during the muscle isolation process and perfusion without stimulation indicated a slight degradation of the muscle physiological state. It was probably not due to a change in the perfusion efficacy as perfusion pressure was maintained throughout the experiment (results not shown). Continuous electrical stimulation at 0.1 Hz induced some fatigue as indicated by the contraction force decrease as well as the two-fold lactate increase in stimulated muscle. The fatigue intensity could probably be modulated by varying the stimulation frequency.

Conclusion

The change from dry oven to waterbath for temperature control of the muscle preparation did not affect noticeably the quality of the preparation as described by Miri et al. (1991). Continuous electrical stimulation during perfusion led to a decrease in contraction force and Pc level with an increase in lactate level, which can be interpreted as muscular fatigue. We think that this preparation can be used as a model to study the effects of individualized environmental factors such as hormone levels, nervous stimulation, temperature, etc...on ante- and post mortem muscle metabolism, while simulating situations where animals have physical activity before slaughter.

References

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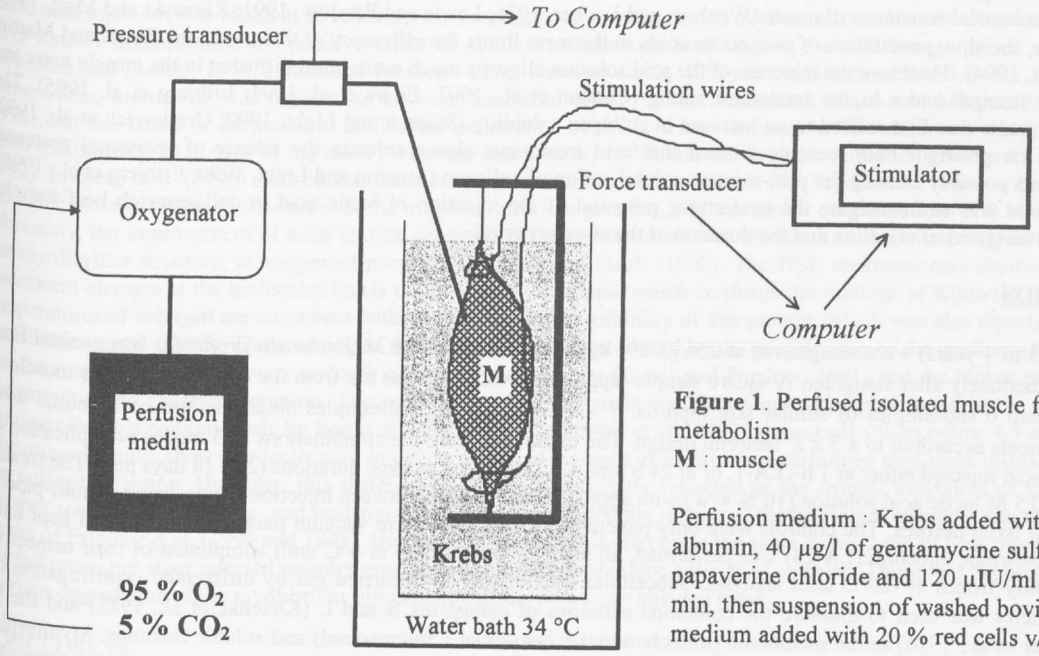

Figure 1. Perfused isolated muscle for studying muscle metabolism
M : muscle

Perfusion medium : Krebs added with 35g/l of albumin, 40 µg/l of gentamycine sulfate, 0.84 mM of papaverine chloride and 120 µIU/ml of insulin, for 10 min, then suspension of washed bovine red cells (same medium added with 20 % red cells v/v) for 30 min.

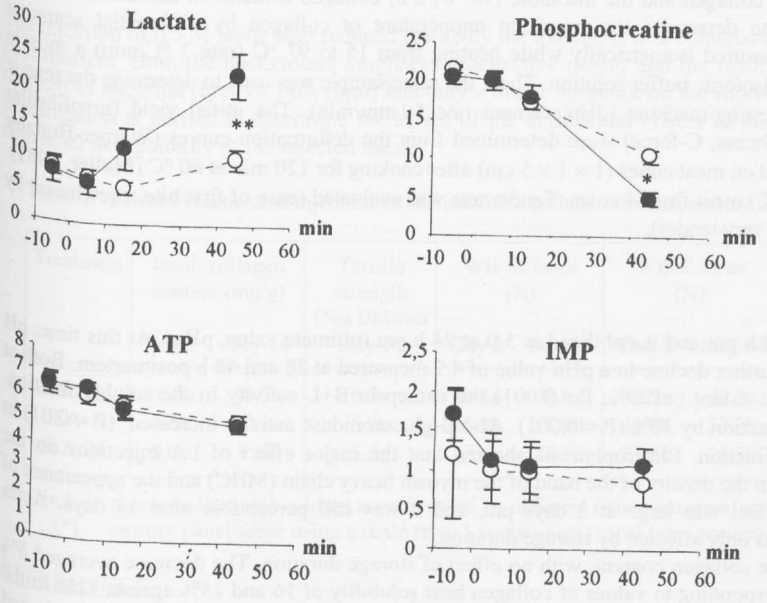

Fig 2. Changes in concentration of metabolic compounds (concentrations expressed as µmol/g of fresh muscle)

Significant difference, P<0.05 *, and P<0.01 **
Muscle was put in the bath at time = 15 min.
Stimulation and perfusion were stopped at time = 45 min

	Stimulation pattern	Start of perfusion	End of perfusion	Significance
Contraction time (ms)	6 twitches	40.3 ± 2.3	35.7 ± 2.9	0.06
	180 twitches	37.5 ± 1.0	32.3 ± 1.7	0.02
Half relaxation time (ms)	6 twitches	62.3 ± 2.6	58.5 ± 3.1	0.01
	180 twitches	57.0 ± 1.4	52.7 ± 2.5	0.03
Contraction strength (ms)	6 twitches	100	88 ± 7.4	0.16
	180 twitches	100	67 ± 9.5	0.02

Table 1. Contraction parameters during perfusion