INFLUENCE OF INJECTION OF α-BLOCKING AND β-BLOCKING AGENTS ON THE MUSCLE GLYCOGENOLYSIS DURING TRANSPORT STRESS IN LAMBS

G. MONIN and P. GIRE

Institut National de la Recherche Agronomique, Theix, 63110 BEAUMONT, France.

INTRODUCTION

pH is a very important characteristic of meat, since it influences many technological or organoleptical properties : waterbinding capacity, tenderness, colour, keeping ability. Muscle glycogen depletion due to preslaughter stress reduces post-mortem acidification of the muscle tissue during rigor mortis onset and leads to an abnormally high pH in meat (see LAWRIE, 1966). In cattle, in vivo glycogenolysis is believed to be produced essentially by adrenaline hypersecretion (HEDRICK et al., 1959 ; LAWRIE, 1966). However adrenaline injection and physical or psychic stress do not have identical effects on ultimate meat pH in the lambs : in fact FORREST et al. (1964) observed that adrenaline injection had a more pronounced effect on the Longissimus dorsi ultimate (48 hours post mortem) pH than on the Rectus abdominis ultimate pH, but the difference between the two muscles was much greater when the stress that the animals withstood was exhausting exercise or frightening by a dog (see table 1). We were also able to verify similar differences between effects of adrenaline and transport stress in some other sheep muscles (table 1). BENDALL and LAWRIE (1962) (cited by LAWRIE, 1966) found that noradrenaline, even in large doses, did not affect ultimate pH of the meat when injected before slaughter in the rabbit. However α effects of the catecholamines could induce modifications in the muscle glycogen metabolism, by modifying the blood flow in muscles and thus changing nutrient and oxygen supply to muscle tissue. LISTER et al. (1970) showed that α blocking agents modified glycolysis at slaughter and immediately post mortem in pigs. We designed experiments to study the effects of α or β adrenergic blocking agents on glycogen metabolism during preslaughter stress and subsequently on the pH of the meat, in lambs.

MATERIAL AND METHODS

1 - Animals

The animals, all male Limousin breed lambs, were bought from two farms two to four weeks before the experiments, and then fed with concentrate (cereal and pelleted hay - about 0.5 kg per animal per day) and hay (ad libitum). Each experiment involved animals from a single farm, and genetic origin (ram) was balanced as much as possible between experimental groups.

2 - Experimental design

Experiment 1 : fourteen 6 month old lambs were allotted to three groups. The day before the experiment, they were transported by truck from the farm to the meat laboratory (1 km from the farm). The animals were fitted with a polyethylene catheter (length : 60 mm ; diameter : 2 mm), in the jugular vein, to facilitate blood sampling and intravenous injections. The following morning, blood was taken from each animal by means of the catheter. Four animals were then slaughtered without other treatment. Five out of the 10 remaining lambs received a α blocker (Hygergine, Sandoz laboratories) injection (0.09 mg/kg liveweight). Hydergine , a mixture of three alcaloids extracted from rye ergot, blocks the action of catecholamines at the α adrenoreceptors and prevents noradrenaline release by sympathetic nerve endings (VON EULER and LISHAJKO, 1966). The 10 lambs were then transported by truck. After 2 hours of transport, a 30 minutes stop allowed blood sampling of each animal to take place, then the ride resumed. Transport plus stop lasted exactly four hours. Blood was again taken from each animal just after the end of the trip. The 10 animals were then slaughtered within 90 minutes after the end of transport, injected animals being alternated with non-injected ones. Control animals were killed in the morning, between 10 and 11 a.m. ; transported ones in the afternoon, between 2 and 3.30 p.m. Animals were referred to as follows : not transported as "control" ; transported without Hydergine injection as "transported control" ; transported with Hydergine injection as "transported treated".

Experiment 2 : fifteen 10 month old lambs were allotted to three treatment groups. Experimental design was exactly the same as for experiment 1, except that there were five "control" animals, and that the "transported treated" animals were injected with the β blocker propranolol (1 mg/kg liveweight) instead of Hydergine. The injection was not repeated as it was for Hydergine, after two hours of transport. Propranolol blocks the effect of adrenaline at the β adrenoreceptors.

3 - Slaughter, muscle sampling and analytical techniques

Animals were killed by severing all major neck veins and arteries. Between 15 and 20 minutes after slaughter, samples were taken from Rectus abdominis (RA), Pectoralis profundus (PP), Semitendinosus (ST) and Supraspinatus (SS) muscles and from the liver (in three standardized locations) and were put into liquid nitrogen and kept frozen (- 20° C) for subsequent glycogen and lactic acid determinations. 60 minutes after slaughter, carcasses were put into a + 10° C room. 24 to 26 hours later, pH was measured using a Radiometer 29 pH meter and inserting the probe electrode into the intact muscle tissue on the Longissimus dorsi (LD), Adductor (A), Semimembranosus (SM), Biceps femoris (BF), Triceps brachii (TB), Psoas major (PM), Infraspinatus (IS), SS, PP, ST and RA muscles. The pH value obtained was referred to as ultimate pH. Frozen samples were ground in a Waring blendor (for liver, the three samples were mixed). The freezing was maintained during grinding with liquid nitrogen. 2 g of muscle powder were homogeneized in 18 ml 0.005 M sodium iodoacetate and pH was measured in this homogenate using a Radiometer 29 pH meter. 3 g of muscle powder were homogeneized in 25 ml 0.6 M cold perchloric acid. Glycogen was determined in ali-

quots of this homogenate according to the technique of DALRYMPLE and HAMM (1973) with minor modification. Homogenate was neutralized by 3 M K_2CO_3 filtered, and lactic acid was determined in the filtrate (HOHORST, 1963). To correct glycogen values for the loss due to post mortem glycogenolysis between death and sampling time, lactic acid level was added to the glycogen level as measured at sampling, and the sum referred to as "muscle glycogen" (GIRE and MONIN, 1979). Liver glycogen was determined in a homogenate of 5 g of liver powder in 25 ml 0.6 M perchloric acid, using the technique of DALRYMPLE and HAMM (1973) with minor modification.

Blood was deproteinized immediately after sampling by addition of two volumes of 0.6 M perchloric acid and subsequent centrifugation. Lactic acid and glucose were determined in the supernatant (respectively HOHORST (1963) and TRINDER (1969) methods).

RESULTS AND DISCUSSION

1 - Effect of the α blocking agent

Transport stress alone increased blood glucose ($P < 0.01$ at 2 hours) and lactic acid ($P < 0.05$ at 2 and 4 hours), as shown in fig. 1. Glycogen level was decreased by transport stress alone in PP, ST and SS muscles ($P < 0.05$ in the three muscles). Ultimate pH increased significantly in SS ($P < 0.01$), IS ($P < 0.01$) and A ($P < 0.05$) muscles. Hepatic glycogen was not affected by transport treatment alone. Hydergine injection prevented a rise in blood lactic acid during transport stress ($P < 0.05$ at 2 and 4 hours) but not hyperglycemia (fig. 1). Hydergine had no effect on the muscle glycogen mobilization nor on ultimate pH of any muscle (Fig. 2). Liver glycogen level was lower in "transported treated" animals than in "control" animals ($P < 0.05$) (Fig. 2).

The decrease in lactic acid blood level due to Hydergine injection could be explained by reduced muscle glycogenolysis, since glycogen levels were similar in "transported treated" and in "transported control" animals. Instead, the drop might be due to an increased lactic acid uptake in liver as a result of the α blocking agent's vasodilatory effect (inhibition of hepatic, splanchnic and peripheric vasoconstriction allowing a better blood flow). Surprisingly, liver glycogen was lowered in "transported treated" animals, since glycogenolysis in liver is controlled by α effects (ADNIT, 1969). We were indeed able to verify that Hydergine prevents liver glycogenolysis produced by adrenaline perfusion in lambs very efficiently (unpublished results). The inefficiency of α blocking agent to inhibit in any extent muscle glycogen mobilization during transport stress indicates that α effects of catecholamines do not play a major role in DFD meat occurence in lambs. This agrees with the report of BENDALL and LAWRIE (1962) that noradrenaline is not able to produce DFD meat.

2 - Effect of the β blocking agent

In experiment 2, transport stress alone increased blood glucose ($P < 0.01$ at 2 and 4 hours) and blood lactic acid ($P < 0.01$ at 4 hours), as shown in fig. 3. Liver glycogen was similar in "control" and "transported control" animals, but muscle glycogen decreased in PP ($P < 0.05$) and SS ($P < 0.01$) muscles of the latter (Fig. 4). Ultimate pH was increased in SS ($P < 0.01$), IS ($P < 0.05$), TB ($P < 0.01$), PP ($P < 0.05$), ST ($P < 0.05$) and BF ($P < 0.05$) muscles of the "transported control", compared to "control" animals (Fig. 4).

Propranolol injection had no significant effect on blood glucose level, but blood lactic acid level tended to lessen ($0.10 < P < 0.05$) in propranolol-treated animals (fig. 3) compared to "transported control" ones. Glycogen level decrease due to transport stress was prevented by β blocker injection in SS muscle ($P < 0.05$) and to some extent in PP muscle ($0.10 < P < 0.05$) (fig. 4). Ultimate pH was lower in SS, IS and TB muscles ($P < 0.05$ in the three muscles) of "transported treated" animals, compared to "transported control" animals. Il was similar in "transported treated" and in "control" animals for all the muscles under investigation (fig. 4). Liver glycogen level was lower ($P < 0.05$) in "transported treated" animals than in "control" ones.

Although it reduced glycogenolysis markedly in muscles, propranolol affected blood lactic acid increase due to stress only to a small extent. According to LABORIT (1972) this could be explained by a decrease in tissue oxygen consumption, due to inhibition of adrenaline's vasodilatory effect (β effect) : this would lead to a vasoconstriction and subsequent hypoxia, increasing anaerobic glycolysis and lactic acid production . Depletion of liver glycogen in propranolol-injected animals could be due not only to a reduction in blood flow in the liver, but also to a potentialization by the β blocker of the α effects of catecholamines in the liver (BOISSIER and GIUDICELLI, 1968), increasing liver glycogenolysis. This agrees with the trend to higher blood glucose level in propranolol-treated animals than in "transported control animals (although the difference is not significant).

PEARSON et al. (1973) reported an increase in ultimate pH in meat from ewes that had been injected with propranolol (1 mg/kg liveweight, as in the present experiment) 60 minutes before slaughter. However, they let their animals rest between injection and slaughter, and this difference in preslaughter treatments may be responsible for the discrepancy between their results and our own results. ASHMORE and coworkers found that propranolol was very efficient in preventing meat pH increase due to adrenaline injection in sheep (ASHMORE et al., 1973 b) and in cattle (ASHMORE et al., 1973 a).

Our results indicate that β effects of catecholamines play a predominant role in muscle glycogen mobilization observed during transport stress in lambs. However catecholamines probably act synergistically with other unknown factors, since adrenaline and transport stress effects are not identical in individual muscles, as noted in the introduction. Muscular exercise probably plays no role in our experimental conditions, since, as we could observe , lambs were generally very quiet during all the transport treatment, and they had only short walks (a few meters). Muscle metabolic type could have a great influence on muscle reaction to transport stress. For example, lamb LD and A muscles which are sensitive to adrenaline and rather insensitive to transport stress (in our conditions - see table 1) could be classified as predominantly composed of "fast-twitch-red" fibers (or αR fibers according to ASHMORE and DOERR, 1971) according to histochemical (LACOURT et ARNAL, 1974) and biochemical (TALMANT, 1979) studies. On the other hand, lamb TB and IS muscles, which may be considered to be predominantly "slow-twitch-red" (TALMANT, 1979), are, likewise, sensitive to stress or adrenaline (table 1). The differential response of individual muscles to stress or adrenaline could thus be related to differences in enzymatic equipment. Moreover, it may be due to different changes in blood flow during stress, since REIS and WOOTEN (1970) showed that blood flow rises selectively in white muscles during excitement in cats.

REFERENCES

1/ ADNIT P.J., 1969 - Bioch. Pharmac., 18, 2599 -- 2/ ASHMORE C.R., CARROL F., DOERR L., 1973 a - J. Anim. Sci., 37, 435 -- 3/ ASHMORE C.R., CARROL R., DOERR L., TOMPKINS G., STOKES M., DARLER W., 1973 b - J. Anim. Sci., 36, 33 -- 4/ ASHMORE C.R., DOERR L., 1971 - Exptl. Neurol., 31, 408 -- 5/ BENDALL J.R., LAWRIE R.A., 1962 - J. Comp. Path., 72, 118 -- 6/ BOISSIER J.R., GIUDICELLI J.F., 1968 - Prod. Prob. Pharm., 23, 585 -- 7/ DALRYMPLE R.H., HAMM R., 1973 - J. Food Technol., 8, 439 -- 8/ FORREST J.C., MERKEL R.A., MACKINTOSH D.L., 1964 - J. Anim. Sci., 23, 551 -- 9/ GIRE P., MONIN G., 1979 - Ann. Technol. Agric., 28 (in press) -- 10/ HEDRICK H.B., BOILLOT J.B., BRADY D.E., NAUMANN M.D., 1959 - Univ. Missouri Coll. Agr. Res. Bull. n° 717 -- 11/ HOHORST H.J., 1963 in Methods in enzymatic analysis (ed. H.U. BERGMEYER) Academic Press, New-York -- 12/ LABORIT H., 1972 - Agressologie, 13, 1 -- 13/ LACOURT A., ARNAL M., 1974 - 20th European meeting of Meat Research Workers, N 3, Dublin, 15-20 September -- 14/ LAWRIE R.A., 1966 in Physiology and biochemistry of muscle as a Food, p 137 (eds. E.J. BRISKEY, R.G. CASSENS, J.C. TRAUTMAN) Univ. Wisconsin Press, Madison -- 15/ LISTER D., SAIR R.A., WILL J.A., SCHMIDT G.R., CASSENS R.G., HOEKSTRA W.G., BRISKEY E.J., 1970 - Am. J. Physiol., 218, 102 -- 16/ PEARSON A.M., CARSE A.W., VENHAM L.M., FAIRSBAIRN S.J., LOCKER R.H., JURY K.E., 1973 - J. Anim. Sci., 36, 500 -- 17/ REIS D.J., WOOTEN G.F., 1970 - J. Physiol., London, 210, 121 -- 18/ TALMANT A., 1979 - Thèse d'Université, Université de Clermont 2, n° 56 -- 19/ TRINDER P., 1969 - J. Clin. Path., 22, 158 -- 20/ VON EULER U.S., LISHAJKO F., 1966 - Acta Physiol. Scandin., 68, 257.

Workers	Treatments	Number of animals	MUSCLES Longissimus dorsi	Rectus abdominis	Adductor	Triceps brachii	Infraspinatus
FORREST et al. 1964	Control	4	5.54	6.06			
	Exercise (treadmill)	4	5.53	6.90			
	Exercise (dog)	4	5.83	6.87			
	Adrenaline (intramuscular)	4	6.12	6.98			
	For experimental details, see FORREST et al., 1964)						
MONIN and GIRE	Control	5	5.60	6.0	5.60	5.70	5.70
	Transport stress (a)	5	5.70	6.20	5.80	6.40	6.40
	Adrenaline (b)	5	6.30	6.90	6.20	6.30	6.20

Table 1 : COMPARISON OF EFFECTS OF STRESS OR ADRENALINE ADMINISTRATION ON MUSCLE ULTIMATE pH IN VARIOUS LAMB MUSCLES
a = four hour transport by truck before slaughter b = intravenous infusion for 4 hours before slaughter

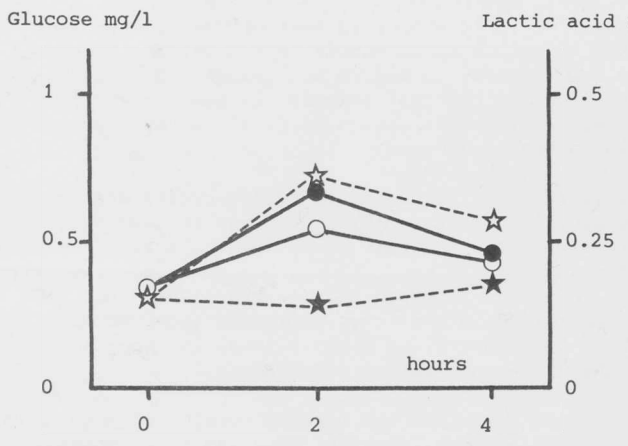

Figure 1 : INFLUENCE OF HYDERGINE ADMINISTRATION ON EVOLUTION OF BLOOD GLUCOSE AND BLOOD LACTIC ACID DURING TRANSPORT STRESS IN LAMBS

○—● glucose ☆○ "transported control"

☆---★ lactic acid ★● "transported treated"

n = 5 lambs in each group

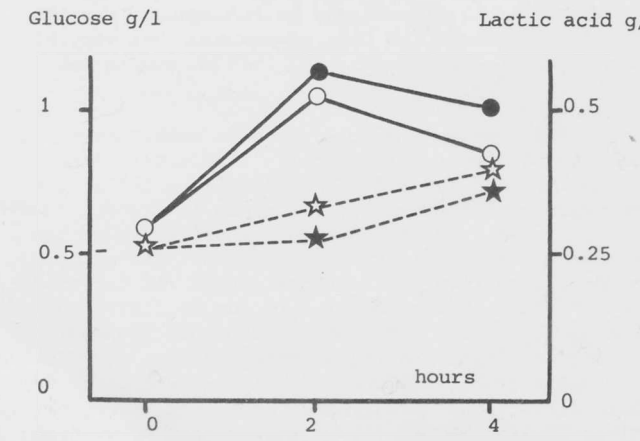

Figure 3 : INFLUENCE OF PROPRANOLOL INJECTION ON EVOLUTION OF BLOOD GLUCOSE AND BLOOD LACTIC ACID DURING TRANSPORT STRESS IN LAMBS

○—● glucose ☆○ "transported control"

☆---★ lactic acid ★● "transported treated"

n = 5 lambs in each group

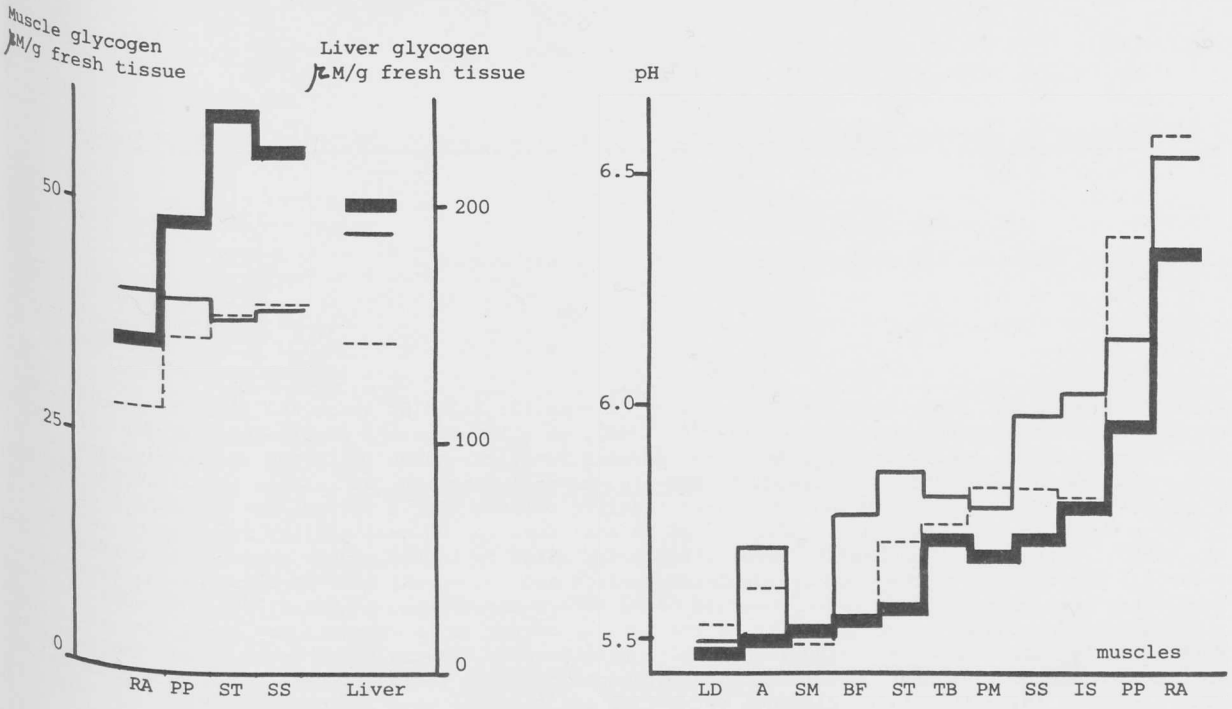

Figure 2 : INFLUENCE OF HYDERGINE ADMINISTRATION BEFORE AND DURING TRANSPORTATION ON GLYCOGEN MOBILIZATION AND MEAT pH CHANGES DUE TO TRANSPORT STRESS IN LAMBS

"control" (n = 4) "transported control" (n = 5) ----- "transported treated (n = 5)

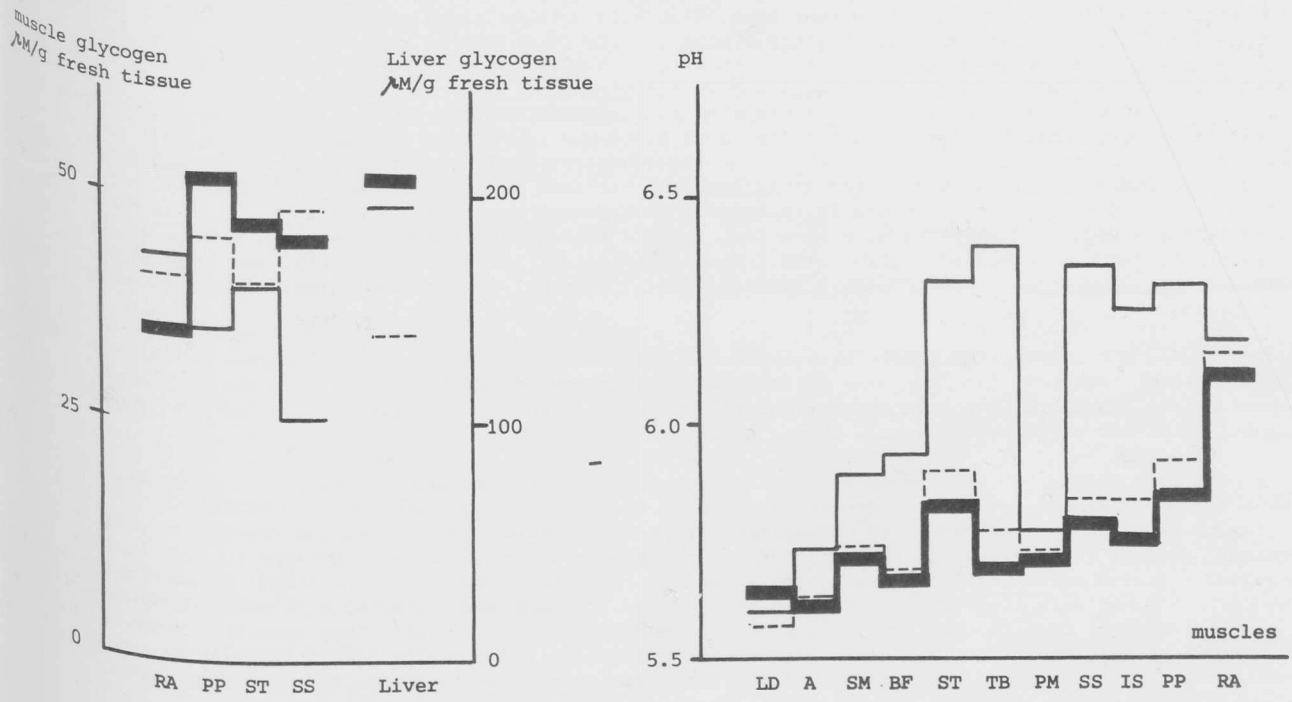

Figure 4 : INFLUENCE OF PROPRANOLOL INJECTION BEFORE TRANSPORTATION ON GLYCOGEN MOBILIZATION AND MEAT pH CHANGES DUE TO TRANSPORT STRESS IN LAMBS (n = 5 lambs in each group)

control "transported control" ----- "transported treated"