

CYCLIC-AMP CONCENTRATION AND PSE PORK

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The mechanism which controls the abnormal rate of glycogenolysis associated with pale, soft, exudative (PSE) pig muscle is unknown. Our experiment was designed to test the significance of cyclic-AMP (c-AMP) concentrations in the formation of PSE muscles. Treatments included venous injection of saline (I), epinephrine (II), isobutyl methylxathine (IBMX) (III) or IBMX plus epinephrine (IV). At 3 min post-exsanguination, c-AMP concentrations in the longissimus muscles were 1437, 3118, 2404 and 6674 for I, II, III and IV, respectively; while pH of the muscles at 60 min were 6.25, 5.60, 5.80, and 5.62. All muscles in groups II, III and IV were PSE, according to reflectance measurements and visual evaluation. These results indicate that elevation of c-AMP, either by increased synthesis, as a result of elevation in epinephrine, or by decrease in degradation, which was produced artificially with IBMX in our experiment, will increase rate of glycogenolysis and thus produce PSE pork.

CONCENTRATION CYCLIQUE-AMP et PORC PSE

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Le mécanisme qui contrôle le taux anormal de glyco-génolyse qui produit chez le porc un muscle pâle, mou et exudatif est inconnu. Notre essai avait pour but de vérifier l'importance des concentrations de c-AMP (Cyclique-AMP) dans la formation de muscles PSE. Les traitements comportaient l'injection d'une solution saline (I), d'épinéphrine (II), d'isobutyl méthylxathine (IBMX) (III) ou d'IBMX et d'épinéphrine (IV). A 3 min de la post-exsanguination, des dosages de c-AMP dans les muscles longissimus se chiffraient à 1437, 3118, 2404 et 6674 respectivement pour les cas I, II, III et IV; tandis que le pH des muscles à 60 min était de 6,25, 5,60, 5,80 et 5,62. Tous les muscles des groupes II, III et IV étaient des PSE, selon les mesures de réflectance et l'évaluation visuelle. Ces conclusions indiquent que l'élévation de c-AMP, soit par synthèse accrue, à la suite de l'augmentation d'épinéphrine, soit par diminution de la dégradation, qui fut produite artificiellement au cours de l'expérience au moyen d'IBMX, augmente le taux de glyco-génolyse et produit ainsi de la viande de porc dite PSE (pale-soft-exudative).

KONZENTRATIONEN ZYKLISCHEN AMP UND BLASSES, WEICHES, EXUDATIVES (PSE) SCHWEINEFLEISCH

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Der die abnormale Zuckerspaltung im blassen, weichen, exudativen (PSE) SchweineMuskel kontrollierende Mechanismus ist nicht bekannt. Unser Experiment war dazu bestimmt, die Bedeutung der Konzentrationen von zyklischem AMP (c-AMP) bei der Bildung von PSE-Muskeln zu bestimmen. Die Verfahren umfassten intravenöse Einspritzungen von Salzlösung (I), Adrenalin (II), Isobutyl-Methylxanthin (IBMX) (III) oder IBMX mit Adrenalin (IV). Drei Minuten nach Ausblutung waren die c-AMP Konzentrationen im Longissimus für I, II, III, und IV resp. 1437, 3118, 2404 und 6674; die pH-Werte im Muskel nach 60 min waren dabei 6,25, 5,60, 5,60, 5,80 und 5,62. Sämtliche Muskeln der Gruppen II, III und IV waren nach Reflektierungsmessungen und visueller Auswertung blass, weich und exudativ (PSE). Die Ergebnisse weisen darauf hin, dass die Anhebung von c-AMP, entweder durch erhöhte Synthese mittels Adrenalin-erhöhung, oder durch verringerte Degradation (künstlich durch IBMX in unserem Experiment hervorgerufen), den Zuckerspaltungsprozess erhöht und somit PSE-Schweinefleisch erzeugt.

## КОНЦЕНТРАЦИИ ЦИКЛИЧЕСКОГО АДЕНОЗИН-МОНОФОСФАТА (АМФ) и БМЭ СВИНИНА

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Механизм, контролирующий ненормальную степень гидролиза гликогена связанного с бледным, мягким, эксудативным (БМЭ) свиным мускулом доселе неизвестен. В нашем эксперименте мы намеревались определить значение концентраций циклического АМФ (ц-АМФ) при формировании БМЭ мускулов. Применяемые методы включали внутривенное введение в вену солевого раствора (I), эpineфрина (II), изобутил-метилксатина (ИБМК) (III) или ИБМК с эpineфрином (IV). За 3 мин после обескровливания, значения концентраций ц-АМФ в musculus longissimus составляли 1437, 3118, 2404 и 6674 для I, II, III and IV групп соответственно; при этом значения pH после 60 мин составляли 6,25, 5,60, 5,80 и 5,62. Все мускулы в группах II, III, IV были БМЭ (согласно измерениям отражательной способности и визуальной оценке). Результаты показывают, что увеличение ц-АМФ либо путем усиленного синтеза (в результате увеличенного эpineфрина), либо посредством снижения деградации (вызванного искусственно в нашем эксперименте с помощью ИБМК), приводит к увеличению степени гидролиза гликогена и, тем самым, производит БМЭ свинину.

## CYCLIC-AMP CONCENTRATION AND PALE, SOFT AND EXUDATIVE PORK

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## INTRODUCTION

The mechanism that controls the abnormal rate of glycogenolysis associated with pale, soft, exudative (PSE) pork is unknown. Cyclic-AMP controls the rate of glycogenolysis by serving as a mediator for the stress hormone, epinephrine (Sutherland *et al.*, 1965; 1966). Results from our laboratory (Ono, *et al.*, 1976), indicated that cyclic-AMP is higher in muscles from stress-susceptible (SS) than in muscles from normal pigs. Since postmortem the muscles from SS pigs tend to become PSE, the effect of artificially elevating c-AMP levels might provide insight into the mechanism that is responsible for PSE muscle. We now describe the effects of elevating c-AMP levels either by increasing the rate of synthesis through epinephrine stimulation or by decreasing the rate of degradation by use of isobutyl methylxanthine (IBMX) which is a powerful inhibitor for phosphodiesterase (Beavo, *et al.*, 1971).

## MATERIALS AND METHODS

Sixteen cross-bred pigs, each weighing about 90 kilograms, were screened for stress-susceptibility by determining their serum creatine phosphokinase (CPK) activities (Allen and Patterson, 1971). The CPK values ranged from 7 to 69 Sigma units which is within the normal range. All pigs were placed on a high carbohydrate diet (25% dextrose), *ad libitum*, for 4 to 5 days prior to slaughter. The pigs were randomly assigned to four groups, and treated, before slaughter, as follows: Group I received saline injections at 15 and 5 min pre-exsanguination; Group II received saline at 15 and epinephrine (5.5 mg/kg) at 5 min; Group III received IBMX (10 mg/kg) at 15 and saline at 5 min; Group IV received IBMX and epinephrine at 15 and 5 min, respectively.

Longissimus muscles were excised at 3, 10, 30, and 60 min post-exsanguination and frozen immediately in liquid nitrogen. The entire frozen samples were pulverized with a Sorvall Omnimixer homogenizer and kept at  $-45^{\circ}\text{C}$  until analysis.

Cyclic-AMP was determined by the competitive protein-binding technique (Gilman, 1970). Protein kinase and protein kinase inhibitor were obtained from Sigma Chemical Company, St. Louis, Missouri, U.S.A. Glycogen was determined by the method of Somogyi (1934). One g of the powdered frozen muscle was blended for 10 sec in 10 ml of .05M iodoacetate and the resulting pH was determined with a combination electrode. Reflectance of the longissimus muscle excised at 24 hours post-exsanguination was measured in the blue spectral region by use of a TriColorPhoto instrument.

## RESULTS AND DISCUSSION

Pre-exsanguination administration of epinephrine, IBMX, or a combination of both, significantly elevated c-AMP levels at 3 min post-exsanguination (Table 1).

Table 1. Effects of epinephrine and isobutyl methylxanthine (IBMX) on cyclic-AMP levels in pig longissimus muscle at 3 min post-exsanguination

Treatment	Picomoles c-AMP/g muscle
Saline	1437 $\pm$ 115 <sup>a</sup>
Epinephrine	3118 $\pm$ 304 <sup>c</sup>
IBMX	2404 $\pm$ 318 <sup>b</sup>
IBMX plus Epinephrine	6674 $\pm$ 207 <sup>d</sup>

a,b,c,d Means  $\pm$  SEM having different superscripts are significantly different from each other ( $P=.05$ ).

The increases in c-AMP concentrations were accompanied by abnormally rapid rates of glycogenolysis as indicated by the glycogen values that were lower at 3 and 60 min for the epinephrine and IBMX-treated than for saline-treated pigs (Table 2). Also 60 min pH values were significantly lower for the epinephrine and IBMX-treated pigs than for saline-treated pigs.



Table 2. Glycogen and pH values in longissimus muscles

Treatment	Glycogen ( $\mu$ moles glucosyl units/g muscle)		pH 60 min
	3 min	60 min	
Saline	60.4 $\pm$ 5.5 <sup>b</sup>	29.8 $\pm$ 6.2 <sup>c</sup>	6.25 $\pm$ .21 <sup>c</sup>
Epinephrine	37.5 $\pm$ 4.3 <sup>a</sup>	9.0 $\pm$ 1.3 <sup>a</sup>	5.60 $\pm$ .06 <sup>a</sup>
IBMX	40.0 $\pm$ 4.8 <sup>a</sup>	15.7 $\pm$ 4.0 <sup>b</sup>	5.80 $\pm$ .12 <sup>b</sup>
IBMX plus Epinephrine	40.6 $\pm$ 3.5 <sup>a</sup>	11.4 $\pm$ 2.3 <sup>b</sup>	5.62 $\pm$ .05 <sup>a</sup>

There were 4 pigs per treatment.

<sup>a,b,c</sup> Means  $\pm$  SEM having different superscripts, in the same column, are significantly different from each other (P=.05).

Rapid decrease in muscle pH value is a symptom of PSE pork. Reflectance measurement and visual evaluation indicated that all muscles treated with epinephrine or IBMX were PSE (Table 3). The one saline-treated pigs which turned PSE had c-AMP values of 1600 picomoles/g muscle, which was the highest in this group.

Table 3. Reflectance and sensory evaluation of longissimus muscle

Treatment	Reflectance	Sensory
Saline	20.2 (3) <sup>1</sup>	Normal (3)
	30.0 (1)	PSE (1)
Epinephrine	27.8 (4)	PSE (4)
IBMX	27.4 (4)	PSE (4)
IBMX plus epinephrine	29.0 (4)	PSE (4)

<sup>1</sup> Numbers in parenthesis denote number of pigs.

Our experiment shows that PSE pork can be produced consistently either by increasing rate of synthesis or by decreasing rate of degradation of c-AMP. The results imply that one mechanism in the formation of PSE muscle involves the factor that controls cyclic AMP levels.

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