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Adenosine Deaminase and Porcine Meat Quality

II. Effects of Adenosine Analogues on Plasma Free Fatty Acids, Glucose and Lactate in Pigs Representing High and Low Adenosine Deaminase Red Cell Activity

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Hyldgaard-Jensen, J.F.: Adenosine deaminase and porcine meat quality. II. Effects of adenosine analogues on plasma free fatty acids, glucose and lactate in pigs representing high and low adenosine deaminase red cell activity. Acta vet. scand. 1990, 31, 145-152. - The adenosine analogues 5'-(N-ethyl) carboxamidoadenosine (NECA) and N^6-(phenylisopropyl) adenosine (R-PIA) were shown to differ in their effect on the plasma level of free fatty acids (FFA), glucose and lactate in pigs representing low (Ada 0) and high (Ada A) red cell adenosine deaminase activity. At the same dosage range (0.001-0.005 mg/kg) R-PIA produced a much stronger suppression of the FFA level than NECA, indicating that A_1 adenosine receptors predominate in porcine adipose tissue. Pretreatment with 8-phenyltheophylline completely abolished the antilipolytic effect of both adenosine analogues. NECA in contrast to R-PIA elevated the blood glucose concentration, suggesting that A_2 adenosine receptors are involved in the stimulation of glycogenolysis. This effect of NECA was not altered by a β-adrenoceptor blockade providing evidence for a direct effect of adenosine on glycogenolysis. Whereas the changes in plasma FFA following NECA administration were of similar magnitude in Ada A and Ada 0 pigs, the changes in the blood glucose concentration were different in these two groups of pigs.

blood; metabolic regulation; receptors.

Introduction

Although the association in pigs between lack of red cell adenosine deaminase (Ada) and a type of inferior meat quality termed DFD has been known for some time, the underlying mechanism is still unknown. Biochemically the dominating feature of DFD muscles is a severe ante mortem glycogen depletion as a result of a stressful preslaughter treatment thus explaining the high ultimate pH observed post mortem (*Barton-Gade* 1979). As more recent observations point to a direct involvement of red cell Ada activity through its naturally occurring substrate - adenosine - studies of the possible regulatory role of adenosine on skeletal muscle energy metabolism have been initiated (*Hyldgaard-Jensen* 1990). Three major observations provide the basis for such studies, firstly a lowered ability to mobilize free fatty acids (FFA) as energy source has been linked to the occurrence of DFD meat in pigs (*Spencer et al.* 1983), secondly adenosine is proven to be a potent inhibitor of lipolysis (*Fredholm* 1981) and thirdly in pigs lacking red cell Ada activity (socalled Ada 0 type pigs) the catabolic rate of adenosine was found to be delayed (*Hyldgaard-Jensen* 1983).

That adenosine in vivo may interfere with rates of lipid and glucose mobilization gain support from an increasing number of in vitro observations demonstrating that adenosine apparently possesses both an antilipolytic and a glycogenolytic action (cf. *Ohisalo* 1987).

These metabolic actions of adenosine may also apply to porcine tissues as preliminary experiments, where dipyridamole - an adenosine uptake inhibitor - was given intravenously to conscious pigs, caused a marked suppression of the plasma FFA level and a distinct rise in both the glucose and lactate level (*Hyldgaard-Jensen* 1986, 1990). However, in these experiments it was unclear whether the observed changes in lipolytic and glycolytic intermediates could be ascribed entirely to a potentiated adenosine effect or there might be a direct effect of dipyridamole as well. Therefore new attempts, the results of which are shown in the present paper, were undertaken in order to show whether an infusion of adenosine analogues might result in similar changes in the blood and whether these changes differed according to type of analogue used and the red cell Ada activity of the pig.

Material and methods

Experiments were carried out on 8 catheterized (jugular vein) Landrace pigs representing high (Ada A) and low (Ada 0) red cell Ada activity. The pigs originating from 2 litters were fed twice daily using a 16% protein pig ration. At 50-60 kg body weight pigs were fitted with a venous catheter connected by polyvinyl chloride tubing to syringes so that infusions and blood collection could be done without disturbing the pig. The effects of adenosine analogues on blood FFA, glucose and lactate were determined using a cumulative type injection procedure. Two incremental doses of adenosine analogues were injected at 30 min intervals starting with the low dose succeeded by a higher dose 30 min later. All experiments were carried out on 22 h fasted pigs at a time where pigs have been found to show a steady increase and decline in rates of lipolysis and glycogenolysis, respectively (*Hyldgaard-Jensen* 1990).

The treatments were as follows:

1. NECA (5'-N-ethylcarboxamidoadenosine) to fasted pigs: following a fasting period of 22 h pigs received a low (0.001 mg/kg) dose succeeded by a higher (0.005 mg/kg) dose 30 min later. The experiment was repeated in which pigs before the analogue infusion received a bolus injection of 8-phynyltheophylline (1 mg/kg) or propranolol HCl (10 μg/kg). The drugs were given 10 min before the first injection of NECA (low dose).
2. R-PIA (N^6-R-phenylisopropyladenosine) to fasted pigs: experimental conditions the same as described for NECA except that drug combinations were not used in this case.

Drugs

The drugs used were: NECA (Boehringer), R-PIA (Boehringer), 8-phenyltheophylline (Sigma) and propranolol HCl (Sigma). Adenosine was purchased from Boehringer. Analogues of adenosine were dissolved in 0.2 N HCl solution and then diluted to the desired concentrations with sterile 0.9% saline. The other drugs were dissolved in sterile 0.9% saline.

Blood samples

Blood samples were taken prior to treatment and at short intervals up to 70 min after the start of the infusion.

Analytical methods

FFA determination was done on EDTA

plasma using the Wako-NEFA C test kit (Wako Chemicals GmbH). Glucose and lactate were determined enzymatically using the Sigma kits No. 16-UV and No. 826-UV, respectively.

Results

Control values (i.e. values before analogue administration) of blood FFA, glucose and lactate in the non-pretreated, the 8-phenyltheophylline pretreated and the propranolol pretreated animals are given in Table 1. No significant differences in control values were observed between the non-pretreated and 8-phenyltheophylline pretreated groups. Although not significant, the FFA level in the latter group was higher than in the non-pretreated group. In the propranolol-pretreated group, control values of FFA and lactate were significantly lower than in the non-pretreated group.

Effects of adenosine analogues without pretreatment

The low dose of NECA (0.001 mg/kg) produced a short-lasting, moderate suppression of the plasma FFA content (Fig. 1). At the end of the 30 min period just before the second, higher dose was injected, the FFA level had reached the preinfusion level. The higher dose of NECA (0.005 mg/kg) suppressed the FFA level more markedly and for a longer time. Still at the end of the experimental period the FFA level was clearly increasing towards the preinfusion value. Using the same dosage of R-PIA a much more marked suppression of the FFA, level was observed (Fig. 1). NECA infusion resulted in a distinct rise in the glucose level, whereas R-PIA had no effect. The rise in the plasma glucose level was produced especially by the low NECA dose, as no further increase was seen following the infusion of the high dose. NECA administration also created a rise in the lactate level whereas this was unaffected by R-PIA.

8-phenyltheophylline-pretreatment

The suppression of plasma FFA caused by NECA was completely abolished following 8-phenyltheophylline pretreatment (Fig. 1). Similarly, the elevation in the blood glucose and lactate levels was significantly diminis-

Table 1. Control values of plasma FFA, glucose and lactate in the non-pretreated, the 8-phenyltheophylline-pretreated and the propranolol-pretreated pigs.

	Control values		
	Untreated pigs	8-phenyltheophylline pretreated pigs	Propranolol pretreated pigs
FFA (mmol/l)	1.084±0.098	1.342±0.107	0.406±0.062***
Glucose (mmol/l)	3.07±0.31	3.18±0.28	3.26±0.32
Lactate (mmol/l)	1.70±0.34	1.31±0.26	0.94±0.24*

Significance symbols: * $0.05 > p > 0.01$; ** $0.01 > p > 0.001$; *** $p < 0.001$

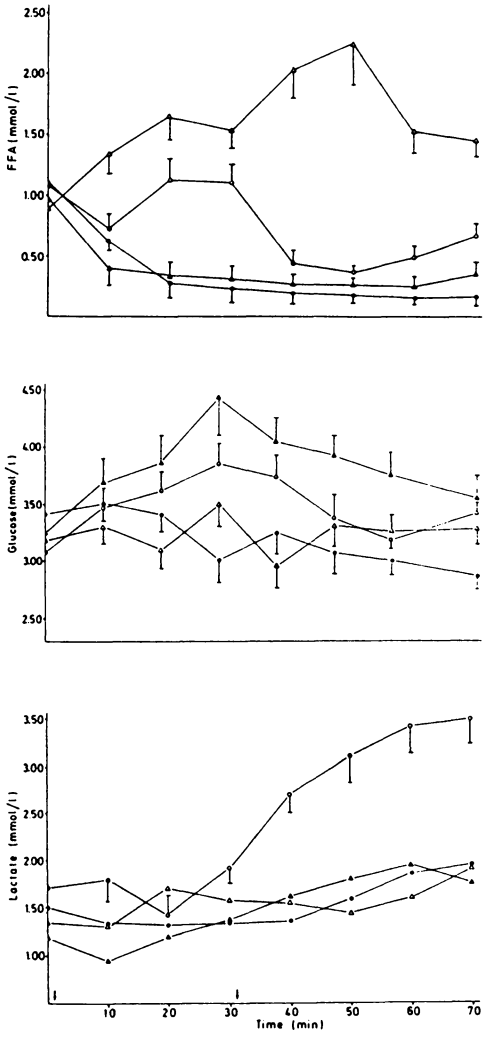

Figure 1. Effect of (•-•) R-PIA and (○-○) NECA on plasma FFA, glucose and lactate. The influence of pretreatment with 8-phenyltheophylline (△-△) and propranolol (▲-▲) following NECA administration is also shown. Overnight fasted pigs were infused a low (0.001 mg/kg) dose of R-PIA and NECA succeeded by a higher (0.005 mg/kg) dose 30 min. later (see arrows). Ten min. before NECA administration pigs were given a bolus injection of 8-phenyltheophylline (1 μg/kg) or propranolol HCl (10 μg/kg). The results are the mean±S.E.M. of 8 pigs.

hed when animals were pretreated with 8-phenyltheophylline.

Propranolol-pretreatment

Propranolol pretreatment caused a more pronounced fall in the FFA level than when NECA was given without pretreatment (Fig. 1). The rise in plasma glucose concentration produced by NECA in the propranolol-pretreated animals was of similar magnitude or even more pronounced than in the non-pretreated animals. The increase in plasma lactate level caused by NECA was practically abolished by the propranolol pretreatment.

Effect of red cell Ada activity

The effect of adenosine analogues on the plasma FFA and lactate level did not seem to depend on the pig's red cell Ada activity (data not shown). As shown in Fig. 2, however, the NECA effect on blood glucose was found to be related to the pig's Ada type. Thus in Ada A pigs the maximal increase in blood glucose was observed already 10-20 min following infusion of the low NECA dose, whereas this increase in Ada 0 pigs appeared distinctly later about 10 min after the second, higher dose was given.

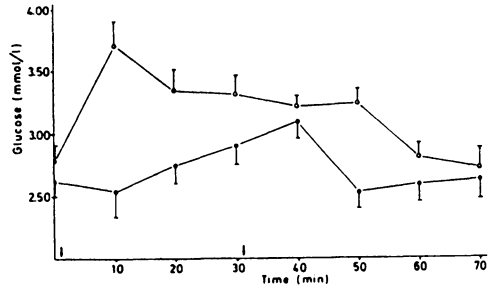

Figure 2. Effect of NECA on plasma glucose in Ada A (○-○) and Ada 0 (•-•) pigs. NECA administration as described in text to Fig. 1.

Discussion

Previously it was found that dipyridamole – an adenosine uptake inhibitor – markedly suppressed the plasma FFA level and increased the blood lactate level when given into the circulation of conscious pigs (*Hyldgaard-Jensen* 1986, 1990). Although this action appeared to be mediated by a potentiating effect of endogenously adenosine, a direct effect of dipyridamole could not be excluded. The present study which show that adenosine analogues produce similar effects on the plasma FFA, glucose and lactate levels following intravenous administration extend these findings and support the notion that adenosine per se is the principal modulator of rates of lipolysis and glycogenolysis in pigs. Both NECA and R-PIA produced dose related effects, however, the magnitude and duration of these effects varied considerably, depending on the analogue examined. Furthermore as shown in the case of NECA, pretreatment of animals with an adenosine antagonist – 8-phenyltheophylline – distinctly altered the effect of this analogue. The changes in plasma FFA and glucose levels were quantitatively similar following NECA administration irrespective of propranolol pretreatment. In contrast the rise in plasma lactate was abolished by propranolol pretreatment.

Both R-PIA and NECA were shown to possess an antilipolytic effect in pigs, however, at the same doses R-PIA produced a much stronger and prolonged suppression of the plasma FFA level than NECA. These results thus support the predominant role af A_1 receptors in porcine adipose tissue, as it is known that R-PIA has a higher affinity for A_1 than for A_2 whereas NECA is relatively specific for A_2 receptors (*Daly* 1985). Following 8-phenyltheophylline pretreatment the antilipolytic effect was abolished a finding in agreement with in vitro observations that theophylline and other methylxanthines reverse the inhibitory action of adenosine on lipolysis in isolated fat cells (*Fredholm* 1981). 8-phenyltheophylline was chosen as this compound possesses more adenosine receptor antagonist activity and much less phosphodiesterase inhibiting activity than theophylline (*Scotini et al.* 1983).

Catecholamines are well known to produce increased levels of plasma FFA, glucose and lactate levels via adrenoceptors. As shown in the present study β-adrenoceptor blockade following propranolol pretreatment did not significantly influence the effects of NECA on plasma FFA and glucose levels. The slightly more suppressed FFA levels following NECA in the propranolol pretreated animals than in the non-pretreated animals agree well with the fact that β-adrenoceptor blockade per se produces a decrease in plasma FFA levels (*Schütz et al.* 1978). In the case of plasma glucose the rise produced by NECA was of similar magnitude whether propranolol pretreatment was used or not. Therefore these results indicate a direct inhibitory and stimulatory effect of NECA hence of adenosine on rates of lipolysis and glycogenolysis, respectively. Furthermore the possibility that the changes observed were due to increased sympathicotonus caused by a possible decrease in blood pressure could be excluded. Adenosine analogues are known as highly vasoactive agents (*Fredholm & Sollevi* 1986, *Böhm* 1987), however, as shown previously at low doses as used in the present study NECA does not lower the arterial blood pressure significantly (*Coffin & Spealman* 1987, *Barraco et al.* 1987).

R-PIA apparently did not elevate the blood glucose level as shown for NECA. This finding suggests the existence of an A_2 adenosine receptor in the mediation of glycogenolysis in pigs. An increasing number of in vitro observations using isolated hepatocytes or

perfused livers support such notion as adenosine through receptors of the A_2 subtype was found markedly to stimulate glycogenolysis (*Hoffer & Lowenstein* 1986, *Stanley et al.* 1987, *Buxton et al.* 1987). In the present in vivo experiment the source of the increased blood glucose level in the NECA treated pigs is unknown.

From above mentioned in vitro observations, however, it appears likely that the extra glucose was released from the liver due to an increase in c-AMP and activation of glycogen phosphorylase effected by administration of NECA. Another possible explanation could be that adenosine indirectly via an increased secretion of glucagon (*Loubatieres-Mariani & Chapal* 1988) caused the observed hyperglycemic state. The glucagon-releasing activity of adenosine analogues appears to occur through adenosine receptors of the A_2 subtype and over a particular low-dose range of adenosine (*Bacher et al.* 1982). Irrespective of mechanisms involved, the present finding that NECA at low doses increases the blood glucose level is noteworthy and underlines the need for further studies of the effect of adenosine on glucose homeostasis and its physiological and pathophysiological significance. Such studies should not least include the skeletal muscles, as it recently was found that adenosine may be an important modulator of skeletal muscle sensitivity of the rate of glucose utilization to insulin (*Challis et al.* 1984, *Leighton et al.* 1988). Dipyridamole was earlier shown to increase the blood lactate level in pigs (*Hyldgaard-Jensen* 1990).

A similar rise was produced by NECA but not by R-PIA. This indicates that A_2 receptors were involved in this stimulation of glycolysis. Since the rise in lactate caused by NECA was considerably diminished in propranolol-pretreated pigs, it appears that NECA only partly was responsible for the observed elevation in the blood lactate level.

Whereas the changes in plasma FFA and lactate following NECA administration were of similar magnitude in Ada A and Ada 0 pigs, the trend of changes in plasma glucose differed distinctly between these two groups of pigs. Thus in Ada 0 pigs the peak level appeared later than in the Ada A and were elicited by the high dose of NECA. The reason why Ada 0 pigs showed a delayed response in the plasma glucose level is not clear, however, it cannot be excluded that this might have been caused by a higher tolerance towards adenosine in these pigs. The possibility thus exist that Ada 0 pigs known to possess a delayed catabolism of adenosine have developed a certain tolerance to adenosine. Such phenomenon is generally known to occur by a prolonged exposure to a hormone, neurotransmitter or drug and could also be valid for adenosine (*Ohisalo* 1987).

The results of the present work suggest that adenosine in vivo may act as a modulator of both the free fatty acid - and glucose metabolism in pigs. This action seems to occur through specific adenosine receptors so that a receptor of the A_1 subtype mainly controls rates of lipolysis and a receptor of the A_2 subtype rates of glycogenolysis. The fact that the NECA induced rise in the blood glucose level varied according to the dose given and the red cell Ada activity makes it attractive to speculate that a difference in sensitivity to adenosine could be involved i the way the glucose metabolism is regulated in Ada 0 and Ada A pigs.

Acknowledgements

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Sammenfatning

Adenosin deaminase og kødkvalitet hos svin: Effekt af adenosinanaloger på plasma frie fede syrer (FFA), glukose og laktat hos grise repræsenterende høj og lav erytrocyt adenosin deaminase aktivitet.

Som led i at forklare den fundne sammenhæng mel-

lem det polymorfe enzymsystem adenosin deaminase (Ada) og svinets kødkvalitet er en mulig betydning af adenosin for fedt- og glukosemobiliseringen søgt underbygget. Landracegrise repræsenterende høj (Ada A) og lav (Ada 0) erytrocyt Ada aktivitet tilførtes via kateter indlagt i V. jugularis to forskellige adenosin analoger (5'-N-ethylcarboxamidoadenosin, NECA og N^6-phenylisopropyl-adenosin, R-PIA) for at belyse effekten af adenosin på blodets indhold af FFA, glukose og laktat. Med 30 min interval infunderedes to doser (0,001 og 0,005 mg/kg) af NECA og R-PIA på et tidspunkt, hvor de fastede grise viste en stigende og faldende hastighed af henholdsvis lipolysen og glykogenolysen. Forsøgene blev gentaget hvor der forud for NECA infusionen tilførtes dels en adenosin antagonist (8-phenyltheophyllin) og dels en β-adrenoceptor blocker (propranolol). Resultaterne viste at R-PIA ved samme dosering fremkalder et betydeligt kraftigere og længerevarende fald i plasma FFA end NECA. Det omvendte forhold sås for plasma glukose, hvor NECA fremkaldte en distinkt stigning, medens R-PIA var uden effekt. Tilførsel af adenosin receptor antagonisten 8-phenyltheophyllin ophævede fuldstændigt effekten af NECA på plasma glukoseindholdet. Tilførsel af β-blockeren propranolol forud for NECA tilførslen ændrede ikke den NECA fremkaldte stigning af plasma glukoseindholdet. Resultaterne tyder på, at adenosin in vivo kan virke som en regulator af såvel FFA- som glukoseomsætningen hos svin. Virkningen synes for lipolysens vedkommende at ske via A_1 typen af adenosinreceptorer og for glykogenolysens vedkommende via A_2 typen.

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