PORCINE MALIGNANT HYPERThERMIA. V: FATAL HYPERThERMIA IN THE PIETRAIN PIG, ASSOCIATED WITH THE INFUSION OF \( \alpha \)-ADRENERGIC AGONISTS

G. M. HALL, J. N. LUCKE AND D. LISTER

SUMMARY

The effects of the administration of noradrenaline alone, and noradrenaline with either phentolamine or propranolol, on thermogenesis and substrate mobilization were investigated in six Pietrain (MH-susceptible) and six Large White (unsusceptible) pigs. The infusion of noradrenaline alone produced a significantly increased lipolytic response and a significantly decreased hyperglycaemic response in Pietrain pigs compared with the Large White breed. Although noradrenaline alone produced only a small increase in body temperature in both breeds, the administration of noradrenaline with propranolol in two Pietrain pigs was associated with the development of fatal hyperthermia. In a further experiment, phenylephrine or isoprenaline was infused into six Pietrain pigs. Three pigs, receiving phenylephrine, became hyperthermic and died, whereas isoprenaline had no effect on body temperature. The results demonstrate the importance of \( \alpha \)-adrenergic stimulation to heat production in MH-susceptible pigs.

Lister, Hall and Lucke (1974) reported that the plasma concentrations of catecholamines were increased greatly during porcine malignant hyperthermia (MH), and this observation was confirmed recently by Gronert and Theye (1976) and Van den Hende and others (1976). The importance of the catecholamine response was shown by the ability of an \( \alpha \)-adrenergic blockade to prevent suxamethonium-induced MH in the Pietrain pig (Lister, Hall and Lucke, 1976). The present investigation was undertaken to examine the heat production and substrate mobilization produced by the administration of noradrenaline alone, and noradrenaline with an \( \alpha \)- or \( \beta \)-adrenergic blocking drug. Noradrenaline was chosen as it was the predominant catecholamine during MH in the Pietrain pig (Lucke, Hall and Lister, 1976), and has mainly \( \alpha \)-adrenergic effects. Furthermore, Harrison and others (1969) and Hall, Trim and Woolf (1972) failed to demonstrate any increase in body temperature after an infusion of adrenaline in Landrace pigs, although the dose was not specified. Both Pietrain and Large White (unsusceptible) pigs were investigated, and two of the former breed, infused with noradrenaline and propranolol, became hyperthermic and died. Therefore, a further study was undertaken to examine the effects of the infusion of pure \( \alpha \)- and \( \beta \)-agonists, phenylephrine and isoprenaline respectively, on metabolic factors and thermogenesis in the Pietrain pig.

METHODS

Noradrenaline infusions

Six Pietrain and six Large White pigs with a mean body weight of 55 kg (SEM \( \pm \) 4 kg) were studied. General anaesthesia and the surgical preparation of the pig were undertaken as described by Lucke, Hall and Lister (1976). A 0.9% solution of sodium chloride was infused into a central vein at a rate of 30 ml h\(^{-1}\) using a calibrated pump (C. F. Palmer Ltd, London). Two venous samples were collected during a 40-min control period and then noradrenaline bitartrate (Sigma Chemical Co., Kingston upon Thames) was infused i.v. at a dose of 5 \( \mu \)g kg\(^{-1}\) min\(^{-1}\) for 50 min. The noradrenaline was dissolved in a solution of 0.9% sodium chloride in water immediately before use and contained 0.3% ascorbic acid to minimize oxidation of the amine. This dose of noradrenaline was chosen because it had been shown to produce maximal stimulation of lipolysis without irreversible cardiovascular damage in conscious pigs (J. Wood, personal communication). At the end of the infusion of noradrenaline, 0.9% sodium chloride solution was infused again for 1 h to allow the pigs to recover.

The measurements made throughout the noradrenaline and subsequent saline infusions are listed in table I and the methods used were those described by...
measurements during the infusions of noradrenaline, and noradrenaline plus adrenergic blocking drug

<table>
<thead>
<tr>
<th></th>
<th>Plasma glucose</th>
<th>Plasma free fatty acid (FFA)</th>
<th>Serum potassium (Pietrain pigs only)</th>
<th>Heart rate</th>
<th>Rectal temperature</th>
</tr>
</thead>
</table>

Lucke, Hall and Lister (1976). Plasma lactate concentrations in the first two pigs infused with noradrenaline increased by only a small extent to 2 mmol . litre⁻¹ so that further measurements were not performed in this group of animals. Plasma catecholamine concentrations were measured in six pigs at the end of the noradrenaline infusions.

After the 1 h of recovery, each pig was infused with either 2, 10 or 20 μg . kg⁻¹ . min⁻¹ of phentolamine mesylate or 2, 10 or 20 μg . kg⁻¹ . min⁻¹ of propranolol hydrochloride for 20 min. Then noradrenaline bitartrate 5 μg . kg⁻¹ . min⁻¹ was given into a different central vein and both compounds were administered concomitantly for 50 min. At the end of the combined infusion, 0.9% sodium chloride solution was infused for a final 60-min period. The same metabolic and physiological measurements were made as for the infusion of noradrenaline alone (table I).

Noradrenaline alone was given to four Pietrain and four Large White pigs and, for these animals, the results were expressed as mean values (+ SEM). The significance of the difference between the two means was assessed by the Student’s t test for unpaired data. For the combined infusions, results were obtained from one pig of either breed at each dose of adrenergic blocking drug.

**Results**

**Infusions of noradrenaline**

The mean concentration of glucose was significantly greater (P<0.01) in the Large White pigs than in the Pietrain breed after 25, 35 and 50 min of the infusion of noradrenaline. At the end of the infusion the plasma glucose concentration was 22.9 mmol . litre⁻¹ in the Large White animals, but only 16.5 mmol . litre⁻¹ in the Pietrain pigs (fig. 1). The lipolytic response, on the other hand, was significantly greater (P<0.01) at the 25-, 35- and 50-min samples in the Pietrain breed. The maximum plasma FFA concentration was 2.29 mmol . litre⁻¹ in the Pietrain pigs (fig. 1). The lipolytic response, on the other hand, was significantly greater (P<0.01) at the 25-, 35- and 50-min samples in the Pietrain breed. The maximum plasma FFA concentration was 2.29 mmol . litre⁻¹ in the Pietrain pigs (fig. 1).

There was a slow increase in the serum potassium concentration of the Pietrain pigs, throughout the infusion of noradrenaline, to 7.1 mmol . litre⁻¹ at the end of the infusion (fig. 2). The increase in rectal temperature was 0.7 °C in both groups during the infusion, and a further increase in the subsequent recovery period produced changes from 38.5 °C to 39.6 °C in Pietrain pigs and from 38.4 °C to 39.3 °C in phenylephrine hydrochloride 25 μg . kg⁻¹ . min⁻¹ (Boots Co. Ltd, Nottingham) or isoprenaline sulphate 0.5 μg . kg⁻¹ . min⁻¹ (Macarthys Ltd, Eire) were infused for 50 min. A solution of 0.9% sodium chloride was given during a recovery period of 1 h, and the phenylephrine or isoprenaline solution was repeated for another 50 min. A second recovery period of 40 min, during which a 0.9% sodium chloride solution was infused, completed the experiment. The metabolic and physiological factors measured during the experiment are listed in table II. The methods used were described previously (Lucke, Hall and Lister, 1976).

In this experiment it was considered essential to record changes in muscle temperature and plasma concentrations of lactate, because, in the previous experiment with noradrenaline and propranolol, there was no direct evidence that the hyperthermia was secondary to the stimulation of muscle metabolism.

**Results**

**Infusions of phenylephrine and isoprenaline**

A fatal hyperthermic response was found in the two Pietrain pigs in which noradrenaline was infused with propranolol 10 and 20 μg . kg⁻¹ . min⁻¹. Therefore, in this experiment, the effects of an infusion of the α-agonist, phenylephrine, were compared with the administration of the β-agonist, isoprenaline, in the Pietrain pig. Because the two pigs had received a noradrenaline infusion earlier in the experiment, the same protocol of two infusions separated by a saline recovery period was used in this study also.

Six Pietrain pigs with a mean body weight of 60 kg (SEM ± 5 kg) were studied. Three were given phenylephrine, and the remainder received isoprenaline. Control measurements were made during the infusion of 0.9% sodium chloride solution, and either
in Large White pigs (fig. 2). Tachycardia was produced by the infusion of catecholamine in both breeds of pig showing that the high dose of noradrenaline stimulated myocardial \(\beta\)-receptors in addition to an \(\alpha\)-adrenergic effect (fig. 2). The mean plasma noradrenaline value at the end of the infusion was 13.3 \(\mu\)g.litre\(^{-1}\) (SEM \(\pm\) 3.6 \(\mu\)g.litre\(^{-1}\)) and virtually no adrenaline was detectable.

The results of the administration of noradrenaline with different doses of either phentolamine or propranolol are shown in table III. The most important finding was that two Pietrain pigs became hyperthermic and died when given noradrenaline plus propranolol 10 and 20 \(\mu\)g.kg\(^{-1}\).min\(^{-1}\). Phentolamine had little effect on the lipolytic response, but reduced the hyperglycaemia, particularly in the Large White pigs. Propranolol progressively inhibited both the tachycardia and lipolysis, but had no effect on the increase in plasma glucose concentration in those pigs which did not die from hyperthermia. The changes in rectal temperature during the combined infusions were similar to the increase of 0.7 °C observed for noradrenaline alone (fig. 2), but the administration of noradrenaline and propranolol was particularly effective in increasing the temperature of the Pietrain pigs.

**Phenylephrine and isoprenaline infusions**

The changes in muscle and rectal temperatures, and heart rate, in the pigs treated with phenylephrine and isoprenaline are shown in figure 3. The three which were given phenylephrine became hyperthermic and died, either during or shortly after the second infusion of catecholamine, whereas isoprenaline had no effect on body temperature. The increase in muscle temperature of the pigs infused with phenylephrine preceded the increase in rectal temperature by as much as 2 °C, which indicated that increased muscle metabolism was an important source of heat production in these animals. A heart rate greater than 200
TABLE III. Maximum values of plasma FFA and glucose concentrations, heart rate and increase in rectal temperature of each pig during the infusion of noradrenaline (NA) plus adrenergic blocking drug in Large White and Pietrain pigs

<table>
<thead>
<tr>
<th>Infusion</th>
<th>Plasma FFA (mmol. litre$^{-1}$)</th>
<th>Plasma glucose (mmol. litre$^{-1}$)</th>
<th>Heart rate (beat/min)</th>
<th>Increase in rectal temperature ($^\circ$C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large White pigs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA + phentolamine</td>
<td>2 μg.kg$^{-1}$.min$^{-1}$</td>
<td>1.61</td>
<td>10.4</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>10 μg.kg$^{-1}$.min$^{-1}$</td>
<td>1.88</td>
<td>5.7</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>20 μg.kg$^{-1}$.min$^{-1}$</td>
<td>1.76</td>
<td>5.6</td>
<td>220</td>
</tr>
<tr>
<td>NA + propranolol</td>
<td>2 μg.kg$^{-1}$.min$^{-1}$</td>
<td>0.80</td>
<td>23.0</td>
<td>152</td>
</tr>
<tr>
<td></td>
<td>10 μg.kg$^{-1}$.min$^{-1}$</td>
<td>0.47</td>
<td>15.4</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>20 μg.kg$^{-1}$.min$^{-1}$</td>
<td>0.36</td>
<td>17.0</td>
<td>128</td>
</tr>
<tr>
<td><strong>Pietrain pigs</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NA + phentolamine</td>
<td>2 μg.kg$^{-1}$.min$^{-1}$</td>
<td>3.01</td>
<td>13.3</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>10 μg.kg$^{-1}$.min$^{-1}$</td>
<td>3.10</td>
<td>8.4</td>
<td>250</td>
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<tr>
<td></td>
<td>20 μg.kg$^{-1}$.min$^{-1}$</td>
<td>2.33</td>
<td>10.7</td>
<td>240</td>
</tr>
<tr>
<td>NA + propranolol</td>
<td>2 μg.kg$^{-1}$.min$^{-1}$</td>
<td>0.70</td>
<td>14.0</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>10 μg.kg$^{-1}$.min$^{-1}$*</td>
<td>0.63</td>
<td>7.9</td>
<td>132</td>
</tr>
<tr>
<td></td>
<td>20 μg.kg$^{-1}$.min$^{-1}$*</td>
<td>0.22</td>
<td>6.8</td>
<td>120</td>
</tr>
</tbody>
</table>

* Pig died.

FIG. 3. Mean (± SEM) heart rate and mean muscle and rectal temperature ($^\circ$C) during infusions of isoprenaline and phenylephrine in Pietrain pigs.

beat/min was observed with both infusions of isoprenaline, but phenylephrine produced a decrease in heart rate before severe hyperthermia occurred. Bradycardia was probably a reflex response to the systemic hypertension caused by the infusion of phenylephrine. Systolic arterial pressures greater than 200 mm Hg were recorded when the $\alpha$-agonist was infused, but isoprenaline caused a decrease in systolic pressure to 75–80 mm Hg (results not shown).

The first infusion of phenylephrine caused a large increase in plasma glucose to 17.1 mmol . litre$^{-1}$, but this change was not repeated during the second
infusion (fig. 4). The diminished response to the second infusion was associated with the development of hyperthermia, and probably represented a reduction in the glycogen content of the liver, with an increase in the peripheral utilization of glucose. Isoprenaline, however, produced a mild hyperglycaemia during both infusions of 9.7 mmol. litre\(^{-1}\) and 9.2 mmol. litre\(^{-1}\) respectively. Plasma FFA results were available only for the pigs infused with isoprenaline in which maximum values of 1.02 mmol. litre\(^{-1}\) and 0.94 mmol. litre\(^{-1}\) were recorded (fig. 5). The first infusion of isoprenaline produced a larger increase in plasma lactate concentration than the infusion of phenylephrine—4.6 mmol. litre\(^{-1}\) compared with 3.0 mmol. litre\(^{-1}\) (fig. 6). The second infusion of isoprenaline increased the lactate concentration further to 6.5 mmol. litre\(^{-1}\), but this was less than the 12.5 mmol. litre\(^{-1}\) of the hyperthermic pigs infused with phenylephrine. The large standard errors of the mean lactate concentrations of the phenylephrine-treated pigs (fig. 6) reflected the different rates of increased muscle metabolism in the three animals.

![Fig. 4. Mean (± SEM) plasma glucose concentrations during infusions of phenylephrine and isoprenaline in Pietrain pigs.](attachment:image4)

![Fig. 5. Mean (± SEM) plasma FFA concentrations during infusions of isoprenaline in Pietrain pigs.](attachment:image5)
DISCUSSION

The occurrence of fatal hyperthermia in the Pietrain pig, in response to an infusion of either phenylephrine or noradrenaline with large doses of propranolol, confirmed the importance of the \( \alpha \)-adrenergic stimulus to heat production in this breed (Lister, Hall and Lucke, 1976). The results of infusions of noradrenaline plus propranolol suggested that the sensitivity to \( \alpha \)-adrenergic stimulation was not found in Large White pigs (table III). This was confirmed in a separate experiment in which an increase in rectal temperature of only 1 °C was found in two Large White pigs infused with phenylephrine according to the protocol described in the present experiment (personal observations).

The dose of noradrenaline given to the Pietrain and Large White pigs was determined by the body weight of each animal. However, at a given body weight the Pietrain pig is more muscular than is the Large White breed. If the amount of noradrenaline infused was based on the lean body mass, the Pietrain pig would have required 8% more noradrenaline (estimated from Meat Research Institute carcass data). Thus, the use of body weight rather than lean body mass for calculating the dose of noradrenaline underestimated slightly the effects of this compound on muscle metabolism in the Pietrain pig.

The metabolic data recorded during the noradrenaline plus propranolol treatment failed to indicate the site of the increased heat production. However, an increase in muscle tone was a prominent feature of the hyperthermic episode and it seems likely that the genesis was derived principally from skeletal muscle. Muscle temperatures and plasma lactate concentrations were measured when the pigs were infused with phenylephrine in an attempt to demonstrate the presumed increase in muscle metabolism. Although the increase in muscle temperature always occurred considerably in advance of the rectal temperature (fig. 3), a large increase in plasma lactate concentration only occurred when the hyperthermia was well established (fig. 6). Furthermore, the mean plasma lactate concentration of the pigs infused with isoprenaline was greater than that of the \( \alpha \)-stimulated animals until the muscle temperature of the latter group had reached 41.7 °C. This indicated that the increase in muscle temperature preceded the increase in plasma lactate, in contrast to the situation found in suxamethonium-induced MH (Lucke, Hall and Lister, 1976).
Thus, at first sight there appeared to be a fundamental difference between suxamethonium-induced MH and catecholine-induced hyperthermia. However, it is important to note that in MH the initial suxamethonium stimulation caused a large increase in plasma lactate to more than 10 mmol litre⁻¹ (Lucke, Hall and Lister, 1976), and there was then little further change until the terminal stages of the response. Furthermore, a direct comparison between the metabolic effects of an infused catecholine and endogenously released catecholamines on muscle metabolism may not be valid as important differences between the two methods of stimulation have been found in adipose tissue, which is under adrenergic control also (Hjemdahl and Fredholm, 1974). A possible explanation for the apparently anomalous effects of isoprenaline and phenylephrine on muscle temperature and plasma lactate concentration is that the two catecholamines stimulate mainly anaerobic and aerobic metabolism respectively. Some support for this hypothesis was provided by the oxygen consumption rate in one pig given phenylephrine which showed a two- to three-fold increase above control values (unpublished results).

If the stimulation of aerobic and anaerobic muscle metabolism can be ascribed to two different adrenergic receptors, then either there is some method of separating the two types of metabolism within each cell or, more likely, there is a preponderance of one receptor on a particular muscle fibre-type. In the latter instance, the obvious division is for the β-adrenergic receptors subserving anaerobic metabolism to be located on the white, fast-fibres, and the α-receptors stimulating aerobic metabolism to be present on the red, slow-fibres. It is of interest that Bowman and Nott (1969) observed that β-agonists generally shortened the duration of the active state of slow-contracting muscle fibres, but no results were presented for the action of pure α-agonists on such fibres.

An increase in body temperature accompanied by an increased oxygen consumption in response to the administration of a catecholamine is described commonly as the “calorigenic effect” of these compounds (Griffith, 1951; Ellis, 1956). Himms-Hagen (1967, 1976) reviewed the nature of the calorigenic action of the catecholamines and concluded that the underlying mechanism was not known. She considered that it was possible for the amines to activate muscle contraction to an extent that did not produce any visible change. This idea is particularly attractive when related to the hypothesis of the mode of action of α-adrenergic agonists described above.

Intense peripheral vasoconstriction and pilo-erection were seen in all the pigs infused with phenylephrine, and this was occasionally associated with peripheral cyanosis. Therefore, loss of heat from the skin surface was impaired, and this was undoubtedly an additional factor contributing to the hyperthermia. The mobilization of substrates such as glucose, FFA and lactate did not make an important contribution to the thermogenesis found in the pigs infused with noradrenaline plus propranolol and phenylephrine. Table III and figures 3, 4, 5 and 6 show that there was little glycolysis or lipolysis during the hyperthermia, and that although isoprenaline increased the plasma glucose, FFA and lactate concentrations, it had no effect on body temperature.

The infusions of noradrenaline demonstrated a significantly increased lipolytic and decreased hyperglycaemic response in the Pietrain pigs compared with the Large White breed (fig. 1). Wood and colleagues (1976) have discussed the mechanism of this differential response in detail, and they favoured an increased sensitivity of the lipolytic response to a given dose of catecholamine in the Pietrain breed. The enhanced lipolytic response may be responsible for the lack of subcutaneous adipose tissue in this breed, and hence the mesomorphic body conformation.

Measurement of the serum potassium ion concentrations during the infusions of noradrenaline was undertaken in an attempt to estimate the contribution of the increased muscle metabolism and other metabolic processes to the hyperkalaemia found in porcine MH. The concentration of 7.1 mmol litre⁻¹ present at the end of the infusion (fig. 2) resulted mainly from two factors. Hepatic glycogenolysis caused a shift of potassium ions from the liver to the extracellular fluid, and α-receptor stimulation, per se, produced an increase in efflux of potassium from the cells of many other tissues (Daniel et al., 1970). These results indicated that a 40% increase in the serum potassium concentration was produced by noradrenaline in the presence of only a small increase in muscle temperature.

In conclusion, the present study shows that Pietrain pigs are extremely sensitive to α-adrenergic stimulation and develop a fatal hyperthermia. The cause of this sensitivity is as yet unknown, but has been demonstrated also in other tissues under adrenergic control in this breed (Gregory, Wood and Lister, 1977). The mechanism by which striated muscle increases its heat production in response to α-agonists cannot be elucidated from this experiment. However, the results presented indicate that the large increase in
circulating catecholamines observed in porcine MH is capable of an important contribution to the thermogenesis found in this syndrome.

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REFERENCES


HIPERTERMIA MALIGNA DE PORCINOS. V: HIPERTERMIA FATAL EN EL CERDO PIETRAIN ASOCIADA CON LA INFUSION DE AGONISTAS ADRENERGICOS-α

SUMARIO
Los efectos de la administración de noradrenalina sola y noradrenalina ya sea con fentolamina o propranolol, sobre la termogénesis y movilización del substrato fueron investigados en seis cerdos Pietrain (susceptibles a HM) y seis Large White (no susceptibles). La infusión de noradrenalina sola produjo una respuesta lipolítica aumentada en forma significativa y una respuesta hiperglicémica disminuida en forma significativa en los cerdos Pietrain en comparación con los de la raza Large White. Aunque la noradrenalina sola produjo solamente un pequeño aumento en la temperatura del cuerpo en ambas razas, la administración de noradrenalina con propranolol en dos cerdos Pietrain se asoció con el fomento de hipertermia fatal. En otro experimento, se infundió fenilefrina o isoprenalina a seis cerdos Pietrain. Tres cerdos, al recibir fenilefrina, sufrieron hipertermia y murieron, mientras que la isoprenalina no ejerció efecto alguno sobre la temperatura del cuerpo. Los resultados demuestran la importancia del estímulo adrenérgico-α a la producción de calor en cerdos susceptibles a HM.