Polymyxin B diminishes blood flow to brown adipose tissue and lactating mammary gland in the rat

Possible mechanism of its action to decrease the stimulation of lipogenesis on refeeding

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Polymyxin B, a cyclic decapeptide antibiotic, increased blood glucose and lactate, and inhibited the stimulation of lipogenesis in interscapular brown adipose tissue and lactating mammary gland of starved-refed virgin and lactating rats respectively. Lipogenesis was not inhibited in white adipose tissue or liver. The antibiotic increased the haematocrit. The relative blood flow to brown adipose tissue and lactating mammary gland was decreased by polymyxin B, and this was accompanied by a decrease in tissue ATP content. *In vitro* polymyxin B did not affect glucose utilization or conversion into lipid, nor the stimulation by insulin of these processes in brown-adipose-tissue slices. Treatment of rats *in vivo* with polymyxin B resulted in decreased utilization of glucose *in vitro* in brown-adipose-tissue slices. Similarly, acini from mammary glands of polymyxin B-treated lactating rats had decreased rates of conversion of [$1\text{-}^{14}\text{C}$]glucose to lipid. It is concluded that the effects of polymyxin B may be brought about by decreases in tissue blood flow. The possibility that these effects are secondary to inhibition of glucose utilization cannot be ruled out.

INTRODUCTION

Polymyxin B, a cyclic decapeptide, has been used since 1947 as a topical antibiotic. Recently, it has been shown to prevent insulin-induced hypoglycaemia in the mouse (Amir & Schechter, 1985; Grémeaux *et al.*, 1987) and the rat (Amir *et al.*, 1987). Studies *in vitro* indicate that polymyxin B inhibits insulin-stimulated glucose uptake into rat adipocytes, and 2-deoxyglucose uptake into rat (Amir *et al.*, 1987) and mouse (Grémeaux *et al.*, 1987) soleus muscle, whereas the insulin inhibition of isoprenaline-dependent lipolysis in adipose tissue is not affected by the antibiotic (Amir *et al.*, 1987). Similarly, polymyxin B has no effect on insulin binding to mouse soleus muscle (Grémeaux *et al.*, 1987) or on the activation *in vitro* of muscle glycogen synthase by the hormone (Grémeaux *et al.*, 1987). Colistin A, which differs from polymyxin B by one conservative substitution in the ring structure (D-phenylalanine replaced by D-leucine), does not show the effects of polymyxin B *in vivo* or *in vitro* (Amir *et al.*, 1987). It has therefore been concluded that polymyxin B is a highly specific inhibitor of insulin-stimulated glucose transport both *in vitro* and *in vivo* (Amir *et al.*, 1987; Grémeaux *et al.*, 1987). In addition, polymyxin B has been shown *in vitro* to be an inhibitor of protein kinase C (Wise *et al.*, 1982; Wrenn & Wooten, 1984), but this might be expected to potentiate the effects of insulin.

In the present work we have investigated the effects of polymyxin B and colistin administration on the stimulation of lipogenesis that occurs in brown adipose tissue (Sugden *et al.*, 1981) and lactating mammary gland (Munday & Williamson, 1981; Bussmann *et al.*, 1984; Mercer & Williamson, 1986) of the rat on refeeding after a period of starvation. It is likely that the increase of lipogenesis in both tissues is in part mediated by a

stimulation of glucose transport by insulin (Threadgold & Kuhn, 1984; Cooney *et al.*, 1985; Ferré *et al.*, 1986), and therefore we predicted that polymyxin B would inhibit the rate of lipogenesis. This work has been reported in a preliminary form (Tedstone *et al.*, 1989).

EXPERIMENTAL

Materials

Rats. Wistar rats were maintained at $22 \pm 2^\circ\text{C}$ in a room with a 12 h light/dark cycle (lights on at 07:00 h) and were allowed free access to stock diet and water. The diet (Special Diet Services, Witham, Essex, U.K.) consisted (by wt.) of 52% carbohydrate, 21% protein and 4% fat; the residue was non-digestible material. Lactating rats had 8–10 pups and were studied 10–12 days *post partum*. Virgin rats weighed 200 ± 5 g.

Radiochemicals and biochemicals. $^3\text{H}_2\text{O}$ was obtained from Amersham International, and [^{14}C]DDT [1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane] from Sigma Chemical Co. All biochemicals were purchased from Boehringer Corp. (London). Colistin and polymyxin B were purchased from Sigma.

Methods

Treatment of rats. All experiments were commenced at 09:00 h. Starved rats had their food withdrawn for 18 h before the experiments. Virgin rats were refed by gastric intubation of glucose (4 mmol; 2 ml) without anaesthesia, and lactating rats were given 5 g of normal diet (Mercer & Williamson, 1986). These refeeding protocols were chosen because virgin rats will not readily eat 5 g of diet within 30 min. Colistin or polymyxin B (1 mg/kg body wt. in 0.9% NaCl) were injected subcutaneously

10 min before the glucose administration in virgin rats or 10 min after the start of refeeding of the lactating rats. $^3\text{H}_2\text{O}$ (0.3 ml; 10 mCi/ml) was administered intraperitoneally 30 min after the start of refeeding, and rats were killed 60 min later. The procedure for fed virgin or lactating rats was the same, except that no glucose was administered. For measurement of metabolites, tissues were rapidly excised from anaesthetized (pentobarbital; 60 mg/kg body wt.) rats and freeze-clamped (Wollenberger *et al.*, 1960). The interscapular brown adipose tissue was carefully freed *in situ* of visible white adipose tissue. The gastrointestinal tract (tissue plus contents) was treated as previously described (Mercer & Williamson, 1987).

Measurement of lipogenesis and relative blood flow. Lipogenesis (with $^3\text{H}_2\text{O}$) and relative blood flow (with [^{14}C]DDT) were measured in conscious rats by a combined procedure (Mercer & Williamson, 1986) based on individual methods previously described [Robinson *et al.* (1978) and Rofe & Williamson (1983) respectively]. Rats had polyethylene cannulae (internal diameter 0.63 mm and external diameter 0.96 mm) inserted into the left jugular vein under anaesthesia (Jones *et al.*, 1984). Three days later, when food intake and/or litter-weight gain had returned to normal, the rats were used for the experiments. Simultaneously, $^3\text{H}_2\text{O}$ (0.3 ml; 10 mCi/ml) was injected intraperitoneally, and [^{14}C]DDT [10 $\mu\text{Ci}/\text{ml}$ per kg body wt. in 0.9% NaCl containing 2.5% (v/v) Tween 20] was infused (1 min) via the jugular-vein catheter. Arterial blood samples were taken 60 min later (pentobarbital anaesthesia 5 min earlier). Tissues were quickly removed, weighed and saponified, and the lipid was extracted with light petroleum (Stansbie *et al.*, 1976) to determine the incorporation of ^3H *in vivo* into lipid and the accumulation of [^{14}C]DDT. A dual-label program was used with a LKB scintillation counter.

Brown-adipose-tissue slices. Rats were killed by cervical dislocation, and the interscapular brown adipose tissue was removed, freed of adhering white adipose tissue and connective tissue and placed in Krebs–Henseleit (1932) saline. The tissue was then sliced free-hand with a razor blade. The slices were weighed (about 50 mg; four slices), incubated in 2 ml of Krebs–Henseleit saline containing 4% (w/v) essentially fatty acid-free albumin, [$1\text{-}^{14}\text{C}$]glucose (10 mM), and with or without insulin (50 munits/ml; Actrapid, Novo, Copenhagen) or other additions as indicated. The incubations were carried out in 50 ml conical flasks fitted with a centre well. The flasks were gassed with O_2/CO_2 (19:1), sealed with Subaseals and shaken at 37 °C for 60 min. Metabolism was stopped by injection of HClO_4 (0.2 ml; 20%, v/v) into the incubation medium. NaOH (0.3 ml; 1 M) was added to the centre well to collect $^{14}\text{CO}_2$ and the flasks were shaken for 2 h at 20 °C. The contents of the centre well were transferred into a scintillation vial, and absorbed $^{14}\text{CO}_2$ was measured (Williamson *et al.*, 1975). The acidified medium was then centrifuged at 3000 rev./min for 10 min. The supernatant was decanted and treated as described by Robinson & Williamson (1977). Any solid material remaining in the flask was removed with two washes each of 2 ml of ethanol (50%, v/v) and added to the pellet, which was then treated as described by Robinson & Williamson (1977) to measure incorporation of ^{14}C into saponified lipid.

Preparation and incubation of mammary-gland acini. The preparation, incubation and further treatment of acini were essentially as described by Robinson & Williamson (1977).

Measurement of metabolites. The following metabolites were determined in deproteinized and neutralized tissue or blood samples by standard enzymic techniques: glucose (Slein, 1963), lactate (Hohorst, 1963), glycogen (Keppler & Decker, 1974), ATP (Lamprecht & Trautschold, 1963), ADP and AMP (Adam, 1963).

Plasma insulin. Plasma insulin was measured by radioimmunoassay with a rat insulin standard (Albano *et al.*, 1972).

RESULTS AND DISCUSSION

Changes in blood glucose and lactate, and haematocrit

Polymyxin B caused a significant increase in whole-blood glucose in fed and refed virgin and lactating rats (Table 1). This hyperglycaemia can be interpreted as indicating inhibition of glucose utilization, and supports the hypothesis that polymyxin B inhibits insulin-stimulated glucose transport (Amir *et al.*, 1987; Grémeaux *et al.*, 1987). In contrast with the present finding, no increase in glycaemia was seen when polymyxin B was administered to fed mice (Amir & Schecter, 1985). Alternatively, the increase in blood glucose might be due to a decrease in plasma insulin, because polymyxin inhibits secretion of the hormone by isolated rat islets (Stutchfield *et al.*, 1986). Measurements of plasma insulin in virgin refed rats showed that polymyxin B-treated rats had higher values (polymyxin B, 13.5 ± 3.9 $\mu\text{units}/\text{ml}$; colistin, 6.5 ± 0.7 $\mu\text{units}/\text{ml}$; $n = 5$), possibly in response to the hyperglycaemia. Surprisingly, the hyperglycaemia was accompanied by an increase in blood lactate, except in the fed virgin rats (Table 1). In the absence of anoxia and/or ischaemia, inhibition of glucose utilization might be expected to decrease blood lactate, whereas decreased oxygenation (see below) may lead to a variable relationship between blood glucose and lactate, depending on the relative changes in tissue glucose removal and lactate accumulation. In addition, there was a decrease in plasma volume indicated by an increase in haematocrit, which may have contributed to the increases in blood glucose and lactate. This possibility is supported by a significant increase in plasma glucose/whole-blood glucose ratio in polymyxin B-treated rats (results not shown). As far as we are aware, other workers have not reported this change in haematocrit, and it is notable that Amir *et al.* (1987) measured the glucose in plasma in their experiments, which would have tended to accentuate the effects of polymyxin on glycaemia. In the refed virgin rats the increase in blood glucose might be explained by an increased rate of intestinal absorption. However, measurements of residual glucose in the gastrointestinal tract 90 min after oral glucose administration to starved virgin rats indicated inhibition of glucose removal by polymyxin B (colistin-treated, 1070 ± 110 μmol of glucose; polymyxin B-treated, 2115 ± 175 ; both $n = 9$; $P < 0.01$).

Rates of lipogenesis in tissues

The rates of lipogenesis *in vivo* in white adipose tissue

Table 1. Effects of polymyxin B on blood glucose and lactate, and on haematocrit in virgin and lactating rats

For full details see the Experimental section. All rats were injected subcutaneously with either colistin or polymyxin B (1 mg/kg body wt.). The fasted virgin rats were given an intragastric load of glucose (2 ml; 2 M), and the fasted lactating rats were allowed to eat 5 g of chow diet. The results are mean values $\pm$ S.E.M., with the numbers of rats in parentheses. Values for polymyxin B-treated rats that are significantly different from those for colistin-treated rats by Student's *t* test are indicated by: **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

Experimental group	Blood glucose (μ mol/ml)		Blood lactate (μ mol/l)		Haematocrit	
	Colistin	Polymyxin B	Colistin	Polymyxin B	Colistin	Polymyxin B
Virgin rats						
Fed	6.64 $\pm$ 0.04 (5)	8.64 $\pm$ 0.53** (5)	1.54 $\pm$ 0.16 (5)	1.85 $\pm$ 0.35 (5)	0.48 $\pm$ 0.01 (5)	0.65 $\pm$ 0.02* (5)
Fasted (24 h) and refed (90 min)	6.84 $\pm$ 0.17 (9)	15.41 $\pm$ 2.04* (9)	2.55 $\pm$ 0.30 (9)	4.00 $\pm$ 0.21* (9)	0.49 $\pm$ 0.01 (9)	0.63 $\pm$ 0.02*** (9)
Lactating rats						
Fed	4.00 $\pm$ 0.22 (5)	6.22 $\pm$ 0.50** (7)	1.76 $\pm$ 0.11 (5)	3.91 $\pm$ 0.60** (7)	0.48 $\pm$ 0.01 (3)	0.62 $\pm$ 0.03** (5)
Fasted (24 h) and refed (90 min)	5.07 $\pm$ 0.27 (9)	6.78 $\pm$ 0.50* (12)	2.52 $\pm$ 0.21 (9)	6.37 $\pm$ 0.50*** (10)	0.50 $\pm$ 0.02 (8)	0.67 $\pm$ 0.03* (10)

Table 2. Effects of polymyxin B on lipogenesis in tissues of virgin and lactating rats

For full details see the Experimental section. All rats were injected subcutaneously with either colistin or polymyxin B (1 mg/kg body wt.). The fasted virgin rats were given an intragastric load of glucose (2 ml; 2 M) and the fasted lactating rats were allowed to eat 5 g of chow diet. The results are mean values $\pm$ S.E.M., with the numbers of rats in parentheses, and are expressed as μ mol of $^3\text{H}_2\text{O}$ incorporated into saponified lipid/h per g wet wt. Values for polymyxin B-treated rats that are significantly different from colistin-treated rats by Student's *t* test are indicated by: **P* < 0.01, ****P* < 0.001.

Experimental group...	Fed virgin rats (5)		Refed virgin rats (15)		Refed lactating rats (15)	
	Colistin	Polymyxin B	Colistin	Polymyxin B	Colistin	Polymyxin B
Liver	—	—	6.1 $\pm$ 0.3	5.7 $\pm$ 0.3	7.5 $\pm$ 1.0	7.4 $\pm$ 0.8
White adipose tissue	21.7 $\pm$ 6.0	27.9 $\pm$ 5.8	6.3 $\pm$ 2.2	4.8 $\pm$ 1.4	—	—
Brown adipose tissue	140 $\pm$ 2.2	31.5 $\pm$ 12.3***	138 $\pm$ 17	18.8 $\pm$ 3.4***	28.2 $\pm$ 8.7	12.0 $\pm$ 3.2
Mammary gland	—	—	—	—	74 $\pm$ 10	17.3 $\pm$ 4.0***

and liver of fed or fasted-refed virgin or lactating rats were not altered by polymyxin B treatment (Table 2). In contrast, the high rates of lipogenesis in brown adipose tissue of fed and refed virgin rats were greatly decreased (about 80%) by polymyxin B, as was the increased rate of lipogenesis in lactating mammary gland on refeeding (Table 2). As expected, the rate of lipogenesis in interscapular brown adipose tissue of the control lactating rats was low (Agius & Williamson, 1980), and polymyxin B did not significantly inhibit the rate (Table 2). With the notable exception of the results on white adipose tissue, these findings are in accord with the view that polymyxin B inhibits insulin-stimulated glucose transport and consequently inhibits the rate of lipogenesis. Insulin stimulation of glucose transport has been demonstrated in brown adipose tissue (Ferré *et al.*, 1986; Greco-Perotto *et al.*, 1987), and refeeding after starvation increases glucose transport in lactating mammary gland of the mouse (Prosser, 1988) and rat (Threadgold & Kuhn, 1984). It is, however, surprising that the rate of lipogenesis in white adipose tissue, where glucose transport is stimulated by insulin (see Simpson & Cushman, 1986), was unaffected by polymyxin B (Table 2), particularly as this process, measured as deoxyglucose

uptake, appears to be inhibited in rat adipocytes *in vitro* by the antibiotic (Amir *et al.*, 1987). Glucose transport into liver is not affected by insulin, and therefore the absence of a polymyxin B effect is not unexpected.

The decrease in lipogenesis in brown adipose tissue of fed virgin rats treated with polymyxin B was 108 μ mol of $^3\text{H}_2\text{O}$ incorporated into lipid/h per g of tissue (Table 2). Assuming that the only lipogenic substrate for this tissue is glucose, this means that the decrease in glucose utilization was at least 54 μ mol of glucose/h per g or 81 μ mol per total mass (1.5 g/200 g rat) of brown adipose tissue. The increment in glycaemia in this group of rats was 2.0 μ mol of glucose/ml of whole blood over 60 min (Table 1) or 40 μ mol per rat (assuming a blood volume of 10% of body wt.). Thus inhibition of glucose utilization in brown adipose tissue by polymyxin B can explain the increase in glycaemia. In the untreated virgin rat, the utilization of glucose for lipogenesis in brown adipose tissue is 1.3 μ mol/min or 9% of the glucose turnover in non-anaesthetized rats (19.5 μ mol/200 g rat; Issad *et al.*, 1987). Similar calculations indicate that inhibition of lipogenesis in lactating mammary gland can account for the increase in glycaemia after polymyxin B treatment in lactating rats. In starved-refed virgin rats

Table 3. Effects of polymyxin B on relative blood flow to tissues of virgin and lactating rats

For full details see the Experimental section. Where stated rats were injected subcutaneously with either colistin or polymyxin B (1 mg/kg body wt.). The fasted virgin rats were given an intragastric load of glucose (2 ml; 2 M) and the fasted lactating rats were allowed to eat 5 g of chow diet. The results are mean values $\pm$ S.E.M. for five rats, expressed as % of administered dose of [14 C]DDT per g wet wt. Values for polymyxin B-treated rats that are significantly different from colistin-treated rats by Student's *t* test are indicated by: ****P* < 0.001.

Tissue	Experimental group . . . Fasted virgin rats —	Refed virgin rats		Refed lactating rats	
		Colistin	Polymyxin B	Colistin	Polymyxin B
Liver	1.57 $\pm$ 0.28	1.16 $\pm$ 0.30	1.03 $\pm$ 0.22	0.94 $\pm$ 0.14	0.74 $\pm$ 0.07
Heart	0.39 $\pm$ 0.21	0.30 $\pm$ 0.08	0.38 $\pm$ 0.08	0.36 $\pm$ 0.06	0.38 $\pm$ 0.06
Muscle	—	0.12 $\pm$ 0.02	0.31 $\pm$ 0.18	—	—
White adipose tissue	0.21 $\pm$ 0.02	0.13 $\pm$ 0.02	0.16 $\pm$ 0.08	0.23 $\pm$ 0.04	0.19 $\pm$ 0.06
Brown adipose tissue	3.04 $\pm$ 0.45	2.13 $\pm$ 0.41	0.48 $\pm$ 0.08***	1.08 $\pm$ 0.17	0.71 $\pm$ 0.13
Mammary gland	—	—	—	0.61 $\pm$ 0.07	0.28 $\pm$ 0.04***

Table 4. Effects of polymyxin B on tissue metabolites in liver, mammary gland and brown adipose tissue on refeeding

For full details see the Experimental section. All rats were injected subcutaneously with either colistin or polymyxin B (1 mg/kg body wt.). The fasted virgin rats were given an intragastric load of glucose (2 ml; 2 M) and the fasted lactating rats were allowed to eat 5 g of chow diet. The results are mean values $\pm$ S.E.M., with the numbers of rats in parentheses, and are expressed as μ mol of metabolite/g of tissue. The values for brown adipose tissue refer to virgin rats, and those for liver and mammary gland are for lactating rats. Values for polymyxin B-treated rats that are significantly different from colistin-treated rats by Student's *t* test are indicated by: **P* < 0.05, ***P* < 0.01, ****P* < 0.005.

Metabolite	Brown adipose tissue		Liver		Mammary gland	
	Colistin (4)	Polymyxin B (4)	Colistin (8)	Polymyxin B (10)	Colistin (8)	Polymyxin B (10)
Glucose	2.39 $\pm$ 0.21	5.57 $\pm$ 0.53**	6.22 $\pm$ 0.32	13.44 $\pm$ 1.60***	0.79 $\pm$ 0.19	1.41 $\pm$ 0.42
Lactate	1.88 $\pm$ 0.46	2.53 $\pm$ 0.70	1.41 $\pm$ 0.06	4.46 $\pm$ 0.80**	1.46 $\pm$ 0.12	7.14 $\pm$ 0.70***
Glycogen	—	—	79.3 $\pm$ 18.4	41.9 $\pm$ 9.9	—	—
ATP	2.19 $\pm$ 0.31	1.12 $\pm$ 0.17*	1.82 $\pm$ 0.07	1.19 $\pm$ 1.14**	0.97 $\pm$ 0.19	0.30 $\pm$ 0.07**
ADP	0.79 $\pm$ 0.10	0.76 $\pm$ 0.09	1.37 $\pm$ 0.21	1.51 $\pm$ 0.13	0.50 $\pm$ 0.08	0.46 $\pm$ 0.06
AMP	0.42 $\pm$ 0.17	0.48 $\pm$ 0.15	0.36 $\pm$ 0.07	0.63 $\pm$ 0.15	0.16 $\pm$ 0.02	0.29 $\pm$ 0.05
Total adenine nucleotides	3.50 $\pm$ 0.36	2.36 $\pm$ 0.22	3.55 $\pm$ 0.30	3.33 $\pm$ 0.20	1.62 $\pm$ 0.29	1.08 $\pm$ 0.09

the calculations indicate that 50% of the increment in the glucose pool on polymyxin B treatment can be explained by decreased lipogenesis in brown adipose tissue. This suggests that in this situation glucose removal is also inhibited in another tissue, possibly muscle.

Relative tissue blood flow

It was observed on dissection of interscapular brown adipose tissue from polymyxin B-treated virgin rats that there were signs of ischaemia and that the weight of tissue was significantly decreased (polymyxin B-treated, 125 $\pm$ 10 mg; colistin-treated, 197 $\pm$ 10 mg; *n* = 4; *P* < 0.01). The relative blood flow of liver, heart, muscle or white adipose tissue measured with [14 C]DDT (see Rofe & Williamson, 1983) was not affected by polymyxin B treatment in either virgin or lactating rats that had been fasted and refed with glucose or chow respectively (Table 3). However, that of mammary gland of lactating rats and interscapular brown adipose tissue of virgin rats was considerably decreased by polymyxin B treatment (Table 3). Insulin administration to diabetic rats increases relative blood flow to interscapular brown adipose tissue by 100% and decreases it (58%) to white

adipose tissue (Jamal & Saggerson, 1988). Madsen & Malchow-Moller (1983) observed decreases in blood flow to white adipose tissue on infusion of glucose to normal rats, and suggested this was secondary to an increase in plasma insulin. Whether these and the present changes in blood flow are secondary to alterations in tissue metabolism is not known.

Tissue metabolites

The decreases in blood flow to brown adipose tissue and lactating mammary gland of polymyxin B-treated rats may result in relative hypoxia in these tissues. The concentrations of glucose and lactate in liver, mammary gland and brown adipose tissue in polymyxin B-treated rats paralleled the changes found in whole blood (Table 4). However, the ratio of mammary-gland [glucose] to plasma [glucose] did not alter in the polymyxin B-treated rats (results not shown), whereas it would be expected to decrease if glucose transport had been inhibited. The high concentration of lactate in the mammary gland of the polymyxin B-treated rats (Table 4) suggests decreased efflux of lactate or increased glycolysis and/or decreased oxidation of pyruvate. In both mammary gland and

Table 5. Effects of polymyxin B and insulin on the metabolism of [1-¹⁴C]glucose in brown adipose tissue slices

For full details see the Experimental section. One group of fed virgin rats was injected subcutaneously with polymyxin B (1 mg/kg body wt.) and the remainder were injected with the same volume of 0.9% NaCl. The concentration of colistin or polymyxin in the incubation medium was 200 µg/ml, and that of insulin was 50 munits/ml. The results are mean values ± S.E.M. for at least six observations and are expressed as µmol of glucose metabolized/h per g of brown adipose tissue. Values for insulin-containing incubations that are significantly different by Student's *t* test from the control incubations are indicated by: **P* < 0.05, ***P* < 0.01, ****P* < 0.001. Values for rats treated with polymyxin *in vivo* that are significantly different from untreated rats are indicated by: †*P* < 0.05.

Measurement	Pretreatment of rat... Incubation medium...	None Colistin	None Polymyxin B	Polymyxin B Colistin
[1- ¹⁴ C]glucose → ¹⁴ CO ₂				
Control		2.28 ± 0.27	2.83 ± 0.60	1.50 ± 0.19†
+ Insulin		6.38 ± 0.72**	5.60 ± 0.65**	5.21 ± 0.90***
[1- ¹⁴ C]glucose → ¹⁴ C-labelled lipid (saponified)				
Control		0.56 ± 0.11	0.62 ± 0.13	0.32 ± 0.11
+ Insulin		1.66 ± 0.29**	1.33 ± 0.32*	1.10 ± 0.30*

Table 6. Effects of polymyxin B treatment *in vivo* on subsequent metabolism of [1-¹⁴C]glucose *in vitro* in acini from refed lactating rats

For full details see the Experimental section. Groups of starved (24 h) lactating rats were injected subcutaneously with either colistin or polymyxin B (1 mg/kg body wt.) and then refed with 5 g of chow. Mammary gland was removed 90 min later and acini were prepared. The results are mean values ± S.E.M. for three experiments. Values that are significantly different from the colistin group are indicated by: **P* < 0.05.

Pretreatment of rat <i>in vivo</i>	Metabolite changes (µmol/min per 100 mg defatted dry wt.)			
	Glucose removed	Lactate produced	[1- ¹⁴ C]Glucose → ¹⁴ CO ₂	[1- ¹⁴ C]Glucose → ¹⁴ C- labelled lipid
Colistin	0.84 ± 0.18	0.78 ± 0.22	0.16 ± 0.04	0.17 ± 0.04
Polymyxin B	0.51 ± 0.18	0.29 ± 0.07	0.028 ± 0.005*	0.054 ± 0.001*

interscapular brown adipose tissue there was an appreciable decrease in ATP content, but the decrease in the total adenine nucleotide content was only significant in brown adipose tissue (Table 4). This decrease in tissue ATP content might be due to the relative ischaemia and lack of oxygen. However, there was also a decrease in ATP content of the liver, which suggests that polymyxin B might also have effects on ATP synthesis, because there was no evidence for decreased hepatic blood flow (Table 3).

Glucose metabolism in brown adipose tissue *in vitro*

To test whether the effects of polymyxin B *in vivo* could be demonstrated in the absence of a blood supply, its action on [1-¹⁴C]glucose metabolism in brown adipose tissue slices was examined (Table 5). The concentration of polymyxin B per ml of medium was approximately equal to the amount injected per rat, to allow for possible concentration of the antibiotic in the tissue *in vivo*. The basal rate of [1-¹⁴C]glucose conversion into ¹⁴CO₂ or ¹⁴C-labelled saponified lipid by brown-adipose-tissue slices from fed virgin rats was not altered by inclusion of polymyxin B in the medium when the tissue was taken from untreated rats (Table 5). Similarly, the insulin-stimulated increase in [1-¹⁴C]glucose oxidation and conversion into ¹⁴C-labelled saponified lipid was not significantly decreased by polymyxin B addition, although there was a tendency to lower values (Table 5). Pretreatment of the rats with polymyxin B decreased the

basal rates, but did not alter the stimulation of glucose metabolism by insulin (Table 5). Use of such preparations of brown adipose tissue *in vitro* has been criticized, in part because of loss of ATP and possible poor oxygenation (McCormack, 1982). Therefore the present results must be viewed with some caution; however, the slices demonstrated a definite insulin-stimulation of glucose utilization similar to that observed in brown adipocytes (Saggerson *et al.*, 1988).

Glucose metabolism in lactating-mammary-gland acini *in vitro*

Polymyxin B (200 µg/ml) did not inhibit [1-¹⁴C]glucose utilization or its conversion into ¹⁴CO₂ or ¹⁴C-labelled lipid when incubated with acini from fed lactating rats (results not shown). Treatment of starved-refed lactating rats *in vivo* with polymyxin B and subsequent isolation of mammary-gland acini resulted in a tendency for decreased [1-¹⁴C]glucose utilization and lactate formation, and a significant decrease in ¹⁴CO₂ production and ¹⁴C-labelled lipid synthesis, compared with acini from colistin-treated rats (Table 6). The finding that lactate accumulation as a percentage of glucose utilization (expressed as C₃ units) decreased from 46% to 28% in acini from polymyxin B-treated rats suggests that pyruvate dehydrogenase was not inhibited further by the antibiotic (see Munday & Williamson, 1981). Acetyl-CoA carboxylase, another key enzyme in lipogenesis, is inactivated by complete ligation of

mammary-gland blood vessels for 12 min (Clegg & Calvert, 1988). The decreased blood flow to the mammary gland of polymyxin B-treated rats may result in a similar inactivation and, together with the decrease in glucose utilization, may explain the inhibition of lipogenesis *in vitro* (Table 6).

Concluding remarks

The absence of any effect of polymyxin B on glucose metabolism or insulin action *in vitro* in brown adipose tissue suggests that either polymyxin B is not itself the active substance *in vivo* or its effects *in vivo* are unlikely to be solely via inhibition of insulin-stimulated glucose utilization in these tissues. Although the mechanism is unclear, the marked depression of blood flow in the brown adipose tissue and mammary gland, together with the decrease in ATP content, may be sufficient to explain the inhibition of lipogenesis by the antibiotic. The absence of effects *in vitro* would support this view. However, the relationship between blood flow and provision of glucose for tissue metabolism is complex, and a primary effect of the antibiotic on glucose transport cannot be ruled out. It is of interest that, in the present experiments, the effects of polymyxin B on lipogenesis appear to be restricted to brown adipose tissue and lactating mammary gland, which are highly responsive to insulin in virgin (McCormack & Denton, 1977; Agius & Williamson, 1980; Sugden *et al.*, 1982; Burnol *et al.*, 1983; Ferré *et al.*, 1986) and lactating rats (Robinson *et al.*, 1978; Burnol *et al.*, 1983; Mercer & Williamson, 1986) respectively. The high blood flow to these two tissues may result in accumulation of polymyxin B and thus explain its marked effects. The fact that basal rates of glucose metabolism are decreased *in vitro* in preparations of brown adipose tissue and mammary gland after treatment of the rats *in vivo* with the antibiotic suggests that irreversible damage occurs. The multiple effects of polymyxin B administration *in vivo*, particularly the alterations in haematocrit and blood flow, would seem to raise sufficient uncertainties to preclude its use as a specific tool for studying insulin-stimulated glucose transport *in vivo*.

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