Aust. J. Biol. Sci., 1988, **41**, 463–74

Effects of Lys-β-urogastrone *in vivo*

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Abstract

Lys-β-urogastrone, an analogue of human β-urogastrone with an additional N-terminal lysine, was shown to have similar effects in mice and sheep to mouse epidermal growth factor (mEGF). Lys-β-urogastrone in doses of 0·18–3·24 μg g^{-1} body weight caused both precocious separation of eyelids and eruption of incisors in neonatal mice.

In 17 sheep, intravenous infusion of the urogastrone analogue over *c.* 24 h led, towards the end of infusion, to erythema of the muzzle, caused reductions in voluntary food intake (with doses ⩾50 μg kg^{-1}) and generally easier manual harvesting of the fleece (with infusions ⩾81 μg kg^{-1}), with spontaneous shedding of the fleece (*c.* 14 days after infusions of ⩾116 μg kg^{-1}). In five sheep infusions of 25, 38, 50, 83 and 118 μg kg^{-1} fleece-free body weight, plasma concentrations of lys-β-urogastrone were near maximal 20 h after the infusions started and were, respectively, 1·1, 1·7, 5·5, 18 and 79 μg l^{-1} plasma. Plasma concentrations of gastrin, somatostatin and pancreatic polypeptide were determined in these five sheep. Plasma gastrin rose sixfold by the end of infusions of 25 μg kg^{-1} of the urogastrone analogue, and tenfold with the higher doses of infusion.

Although plasma somatostatin concentrations were variable, a consistent trend was observed; lower levels were apparent during the lys-β-urogastrone infusions. There was no discernible trend in pancreatic polypeptide concentrations.

Introduction

Murine epidermal growth factor (mEGF) and human β-urogastrone have an homology of about 70% of amino acid sequences and a number of similar biological actions (Gregory 1975; Carpenter and Cohen 1979; Hollenberg and Gregory 1980; Smith *et al.* 1985). In the mouse, mEGF causes precocious eruption of the incisors and opening of the eyes. Effects of an analogue of β-urogastrone with an additional N-Terminal lysine, lys-β-urogastrone, have now been examined and are reported in this paper.

In sheep, mEGF may cause defleecing or facilitate plucking of wool; this has practical implications for use in alternative modes of wool harvesting. This defleecing activity has been shown in the foetus *in utero* (Thorburn *et al.* 1981) and in adult sheep (Moore and Panaretto 1981; Moore *et al.* 1982*a* and 1982*b*; Panaretto *et al.* 1982; McDonald *et al.* 1983). It appears to act *in vivo* by inhibiting DNA synthesis in the dermal region (Panaretto *et al.* 1984). This is associated with a decrease in cell division of the wool follicle bulb cells as measured by a decline in the mitotic indices of these cells (Moore *et al.* 1985), and consequent slowing of fibre production resulting in a fibre that is either weakened or shed completely depending on the dose of mEGF. The effects of lys-β-urogastrone on defleecing activity have also been investigated and are reported in this paper.

0004-9417/88/040463$03.00

Administration of mEGF to sheep also causes changes in voluntary food intake (Panaretto *et al.* 1982). This effect has also been examined in sheep given lys-β-urogastrone. In view of the actions of urogastrone in inhibiting gastric secretion of acid it was also of interest to examine the effects of lys-β-urogastrone on plasma concentrations of three of the peptides known to affect gastric acid secretion, since this phenomenon has been studied in sheep (see Titchen 1986; Titchen and Reid 1988).

Materials and Methods

Animals and Their Maintenance

The mice were derived from outbred white Swiss mice obtained from the Animal Resources Centre, Murdoch University, W.A. They were injected subcutaneously when 1 day old with standard volumes of 4 μl g^{-1} with or without added peptide in differing concentrations in distilled water.

Each litter of mice of mixed sex was reduced to nine and divided into three groups; one remained as a control group of mice each of which was injected with distilled water; mice in the other two groups were injected with lys-β-urogastrone. Control and treatment procedures were continued over 4 consecutive days. Inspections were made daily to assess separation of the eyelids, eruption of the incisors and effects of curvature of the hair and retardation of hair growth.

The sheep used were mature 40–50 kg Merino wethers kept in individual metabolism crates in a room maintained at 22 ± 2°C, 60 ± 10% humidity with 12 h light daily. They were given 1000 g 75 : 25% oaten : lucerne chaffs daily at 0830. Drinking water was freely available.

Materials

Lys-β-urogastrone (M.W. 6344) was kindly supplied by Dr H. Gregory (ICI-PLC, Pharmaceuticals Division). It was a side product resulting from the synthesis, cloning and expression of the gene for β-urogastrone (Smith *et al.* 1982; Franklin *et al.* 1986).

Infusions and Sampling

Catheters for intravenous infusions were placed under sterile conditions in a jugular vein 3–4 days before infusions were made. The infusions of lys-β-urogastrone were made intravenously at 2·1 ml h^{-1} with Braun, Perfusor Secura pumps, with the dose of peptide dissolved in 50 m 0·9% (w/v) saline and sterilized by passage through a 0·2 μm filter. Doses given on a $\mu g\ kg^{-1}$ basis were estimated from fleece-free body weights and converted to actual fleece-free body weights after the fleece was removed and weighed at the end of the experiment. Doses were also expressed on metabolic body weight ($kg^{0·75}$) as described by Kleiber (1965). For each animal infused with the peptide another was infused simultaneously using the same pump and with the same volume of saline. Samples of blood (10 ml) were taken from the jugular vein not used for infusion and added to chilled tubes containing 5000 k.i.u. aprotinin (Trasylol^{R}, Bayer Australia Ltd) and 12 mg EDTA. The samples were centrifuged at 1000 *g* for 15 min at 4°C. Plasma was separated into aliquots, snap frozen and stored at −20°C until transported (on dry ice) to the University of Sydney for assay of hormones and lys-β-urogastrone. Infusions were started 2·5 h after fresh food was provided for the day. This precluded observations on food intake being made on the day of infusion. Some sheep had eaten all or most of their food by the time the infusions were started.

Measurements

Wool growth

Wool growth was determined in sheep by sampling at approximately fortnightly intervals from a pre-marked site (100 cm^2) on the skin of the left mid-side region. The weight of clean dry wool was determined for each sample as described by Reis (1967). Three or four periods of growth were measured before and after each infusion.

Plucking force

The force (in N $ktex^{-1}$) to pluck wool staples was determined from mid-dorsal sites of sheep using the method of Gordon and Pallister (1980). Plucking force measurements (five replications at each time) were made four times during the 10-day period before infusion and four times from day 9 to day 25 after infusion unless the fleece was cast.

Gastrin

Gastrin was assayed by radioimmunoassay as described by Hansky *et al.* (1971) using antisera kindly provided by J. Hansky (Prince Henry's Hospital, Melbourne, Australia) at a 1 : 50 000 final dilution. Under these conditions the interassay variation was 11·8% at 20 pmol l^{-1}, 6·3% at 50 pmol l^{-1} and 6·4% at 100 pmol l^{-1}. Intra-assay variation was 4·3% at 20 pmol l^{-1}, 7·8% at 50 pmol l^{-1} and 2·4% at 100 pmol l^{-1}. Sensitivity of the assay was 5 pmol l^{-1}.

Pancreatic polypeptide

Pancreatic polypeptide was assayed by radioimmunoassay using the method described by Chance *et al.* (1979) and standard and antiserum kindly provided by Dr R. E. Chace, Eli Lily, Indianapolis, Ill., U.S.A. The antiserum was used at 1 : 10^6 dilution. In our hands, the inter-assay variation was 12·1% at 20 pmol l^{-1}, 7·6% at 45 pmol l^{-1} and 8·1% at 90 pmol l^{-1}. Intra-assay variation was 9·8% at 20 pmol l^{-1}, 2·1% at 45 pmol l^{-1} and 2·8% at 90 pmol l^{-1}. Sensitivity of the assay was 8 pmol l^{-1}.

Somatostatin

Somatostatin was assayed according to the procedure described by Darvodelsky *et al.* (1988). It involved extraction of plasma with acetone and ether prior to radioimmunoassay. Under these conditions, recovery of somatostatin from plasma was 60–65%. Interassay variation, including extraction, was 7·7% at 20 pmol l^{-1}, 5% at 50 pmol l^{-1} and 8·6% at 100 pmol l^{-1}. Intra-assay variation was 7·4% at 20 pmol l^{-1}, 5·7% at 50 pmol l^{-1} and 2·6% at 100 pmol l^{-1}. Sensitivity of the assay was 3 pmol l^{-1}.

Lys-β-urogastrone

The radioimmunoassay for lys-β-urogastrone was carried out essentially as described for β-urogastrone by Gregory *et al.* (1977) using standard and antisera kindly provided by Gregory (ICI-PLC Pharmaceuticals Division). However the lys-β-urogastrone was iodinated using chloramine T (Hunter and Greenwood 1962). Antiserum was used at 1 : 10^4 dilution. Separation of antibody-bound and free hormone was by addition of Sca-Cel (donkey anti-rabbit antibody coated cellulose, Wellcome Reagent Ltd, Beckenham, England, BR3 3BS). The interassay variation was 3·4% at 2·5 μg l^{-1} and the intra-assay variation was 7%. the sensitivity of the assay was 0·3 μg l^{-1}.

Units

In order to facilitate cross referencing to the relevant reports in the literature, concentrations of lys-β-urogastrone have been presented relative to mass (c.f. mEGF, Panaretto *et al.* 1982). Results of hormone assays are presented as molar concentrations.

Results

Effects in Suckling Mice

The comparative potency of mEGF and lys-β-urogastrone was assessed concomitantly in a limited bio-assay in mice. Both molecules produced similar effects with similar doses. The most sensitive parameters were precocious separation of the eyelids and eruption of incisors (Table 1); the responses were dose dependent. At higher levels effects were discernible on hair structure or growth; thus at 1·62 μg g^{-1} clear effects on hair curvature were evident. In experiments at even higher doses (10 μg g^{-1}) there was retardation in hair growth of treated mice relative to their litter-mate controls; such an effect for density of hair cover was scored as 2 on a scale ranging from 0 (no effect) to 4 (nude).

General Effects in Sheep

Erythema of the skin, bare of wool, around the muzzle was clearly seen 20–22 h after starting the infusions especially in sheep dosed with 50 μg kg^{-1} or greater and it was more severe in animals that received larger doses. Erythema was transient and had disappeared completely by 48 h from the start of infusion. Voluntary food intake was reduced on the day following the infusion by as much as 86% in 14 of 17 sheep in which it was studied

Table 1. Effects of subcutaneous injections of lys-β-urogastrone and mEGF in suckling mice (n = 3 for each treatment)

Dose (μg g^{-1} body wt)	Day of eruption of both incisors		Day of opening of both eyelids	
	Lys-β-urogastrone	mEGF	Lys-β-urogastrone	mEGF
0	11	10	14	13
0·02	10	10	14	13
0·06	10	10	14	13
0	11	11	15	14
0·18	10	10	14	13
0·54	10	10	13	12
0	11	11	14	14
1·62	9	8	12	11
3·24	8	8	9	10

(Table 2). In one of the sheep that was given 134 μg kg^{-1}, food intake remained depressed for 4 days. Six of the 14 had returned to normal food intake on the second day after the infusion; all but one of the rest showed full recovery on day 3.

Biological Defleecing Activity and Depilation Forces

Lys-β-urogastrone has clear defleecing activity (Table 3). In animals given 107 μg kg^{-1} or more, only one post-treatment plucking-force measurement was taken on day 9 or 10 as the fleece was cast thereafter. This defleecing activity was manifested as a decrease in the post-treatment plucking-force measurements relative to those pre-treatment in the same animal. The extent of this decrease in plucking force was greater with greater doses.

In the treated animals, three classes of responses were identified. The strongest effects were characterized by a complete break in the wool fibre and casting of the fleece. This occurred at doses of 116–154 μg kg^{-1} and facilitated manual removal of the fleece. In these responses the plucking force after treatment was less than 1 N ktex^{-1}.

With doses of 50–110 μg kg^{-1} partial weakness developed in the fibres, spontaneous casting of the fleece did not occur and greater difficulty was experienced in plucking (plucking force $\geqslant$0·7 N ktex^{-1}) and longer time (8–60 min) was needed for one person to remove the fleece manually. Mechanical shearing was needed in some sheep dosed with 50–110 μg lys-β-urogastrone kg^{-1}, and was necessary in all given lesser amounts.

Wool Growth

In three of the four sheep in which it was examined lys-β-urogastrone reduced wool growth. This is shown in Fig. 1 where the reduction in wool growth measured at 14 days was 92%, 44% and 32% in animals given 118, 83 and 50 μg kg^{-1} respectively. A dose of 38 μg kg^{-1} was without effect.

Plasma Concentrations of Lys-β-urogastrone

In the five sheep in which plasma was analysed for lys-β-urogastrone it could be detected in the plasma within 1–2 h from the start of the infusion, depending on the dose given. The highest concentrations were measured towards the end of the infusion (Fig. 2); they were 79, 18, 5·5, 1·7 and 1·1 μg l^{-1} at 22·3, 19·3, 19·6, 22·8, and 24 h from the start of infusion in sheep given 118, 83, 50, 38 and 25 μg lys-β-urogastrone kg^{-1} respectively.

The plasma concentration rapidly fell when infusions ended. In sheep infused with 118, 83, 50, 38 and 25 μg lys-β-urogastrone kg^{-1}, plasma levels at 2·4, 2·5, 3·7, 2, and 2 h post-infusion were 4·6%, 3%, 9·6%, 14%, and 34% of the highest concentrations measured during infusion. In the same animals by 5, 4·4, 8·7, 4, and 4 h post-infusion the levels

Table 2. Food consumption in merino wethers infused intravenously for 24 h with lys-β-urogastrone

Sheep No.	Lys-β-urogastrone ($\mu g\ kg^{-1}$)[A]	Pre-dose food intake (g)[B]	Food consumed (% pre-dose intake) +1[C]	+2[C]	+3[C]	+4[C]	+5[C]
104	154	1000	52	90	102	100	100
111	134	740	14	27	54	81	122
960	118	1000	19	95	100	100	100
107	116	1000	25	40	100	100	100
115	110	1000	85	90	100	100	100
126	107	1000	73	100	100	100	100
110	103	854	56	117	117	117	117
068	98	1000	80	100	100	100	100
108	95	1000	60	100	100	100	100
969	83	1000	49	84	94	100	100
116	81	933	46	75	107	107	107
113	73	1000	85	100	100	100	100
117	65	1000	100	100	100	100	100
106	56	970	80	103	103	93	103
961	50	1000	88	88	100	100	100
970	38	1000	100	100	100	100	100
974	25	980	102	82	102	102	102

[A] Dose is expressed per kg fleece-free body weight.
[B] Pre-dose food intake is the average daily consumption for 5 consecutive days before dosing.
[C] Days from start of infusion.

had further declined and were 1·2%, 1·5%, undetectable, 5%, and undetectable of the highest concentrations respectively.

Table 3. Effects of intravenous infusion of lys-β-urogastrone on the plucking force needed to remove wool samples from merino wethers

Sheep No.	Dose ($\mu g\ kg^{-1}$)	Dose ($\mu g\ kg^{-0.75}$)	Plucking force N $ktex^{-1}$) Pre-dose	Post-dose	Time for manual defleecing[A] (min)
104	154	348	9·3±1·6	0·6±0·2	<5
111	134	292	8·0±1·2	0·5±0·1	<5
960	118	283	9·7±1·7	0·5±0·2	<5
107	116	283	10·8±2·8	0·5±0·1	<5
115	110	248	8·2±1·0	0·9±0·1	15
126	107	233	7·6±1·0	0·9±0·2	8
110	103	223	9·1±1·4	2·3±0·5	20
068	98	240	9·3±0·9	0·7±0·1	14
108	95	206	9·1±1·0	4·2±0·4	S
969	83	201	9·6±1·2	1·4±0·4	50–60
116	81	186	8·7±1·2	2·8±0·3	15
113	73	164	7·5±1·4	2·9±0·4	S
117	65	161	10·0±1·2	4·2±0·4	S
106	56	122	7·6±0·9	6·8±0·7	S
961	50	144	20·6±3·4	6·8±0·8	S
970	38	92	9·7±1·5	8·9±1·7	S
974	25	61	10·4±1·4	10·9±1·5	S

[A] S indicates mechanical shearing.

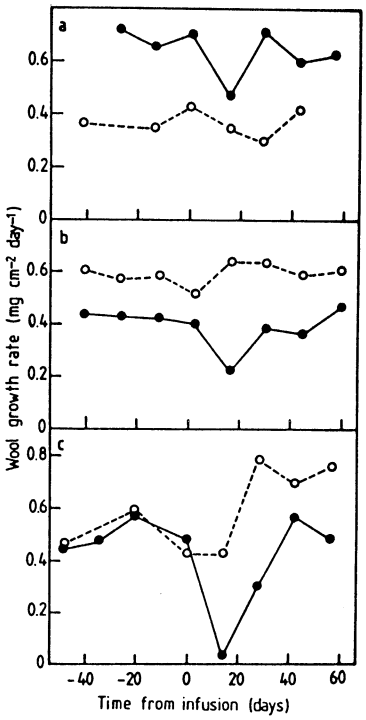

Fig. 1. Wool growth rate in sheep infused with saline (○-------○) or lys-β-urogastrone (●——●) at (*a*) 50 μg kg^{-1}, (*b*) 83 μg kg^{-1} and (*c*) 118 μg kg^{-1}.

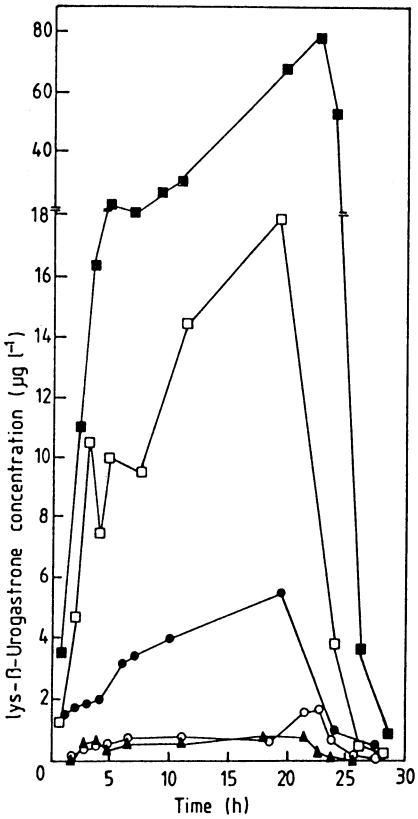

Fig. 2. Lys-β-urogastrone concentration in the plasma of sheep infused with lys-β-urogastrone at 25 μg kg^{-1} (▲), 38 μg kg^{-1} (○), 50 μg kg^{-1} (●), 83 μg kg^{-1} (□) and 118 μg kg^{-1} (■).

Plasma Concentrations of Gastrin, Somatostatin and Pancreatic Polypeptide

In the five sheep infused with lys-β-urogastrone in which plasma levels of the urogastrone analogue were measured, plasma gastrin, somatostatin and pancreatic polypeptide concentrations were determined. With infusions of lys-β-urogastrone of 38 μg kg^{-1} or above, the plasma gastrin concentrations had risen at least threefold over base levels within 3 h of the start of infusion and remained relatively constant at about fivefold higher than base levels until late in infusion (19–24 h) when plasma gastrin increased to approximately 10 times the base level. After the infusions ceased, plasma gastrin fell and in all instances had returned to normal within 24 h of the last administration of lys-β-urogastrone. Fig. 3 shows the plasma gastrin levels with infusion of lys-β-urogastrone.

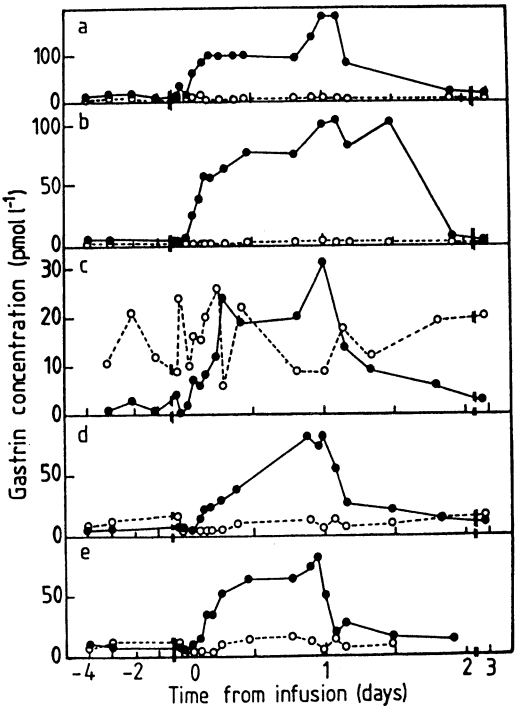

Fig. 3. Gastrin concentration in the plasma of sheep infused with lys-β-urogastrone (●——●) or saline (○-------○) at (*a*) 118 μg kg^{-1}, (*b*) 83 μg kg^{-1}, (*c*) 50 μg kg^{-1}, (*d*) 38 μg kg^{-1} and (*e*) 25 μg kg^{-1}. Note the different scales used.

With infusion at 25 μg kg^{-1}, a similar profile was obtained. However, increases of approximately threefold were not recorded until 3·7 h and, after a plateau at about four times base level, the plasma gastrin concentration increased to sixfold at 22–24 h, and had returned to normal within 24 h.

In control sheep, infused with saline, fluctuations in plasma gastrin were small and generally showed decreases in the 1–2 h immediately after feeding. In no sheep were levels increased above 25% of the base level (Fig. 3).

Somatostatin concentrations in plasma were variable in both the test animals and the controls but were always lower relative to base levels in animals infused with lys-β-urogastrone than in animals infused with saline. Decreases in somatostatin levels were seen from 4·8 h after the start of infusion of lys-β-urogastrone, when levels decreased to about 40% of base levels. At higher doses (118 μg kg^{-1}) levels of somatostatin decreased to 23% of the base levels at 22–26 h, when gastrin levels were highest. Fig. 4 shows somatostatin levels in the sheep infused with lys-β-urogastrone at 118 μg kg^{-1} (Fig. 4*a*) and 25 μg kg^{-1} (Fig. 4*b*).

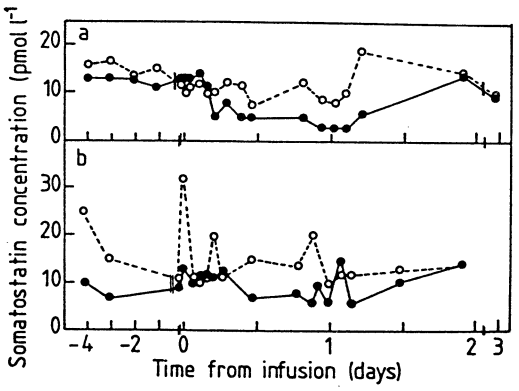

Fig. 4. Somatostatin concentration in the plasma of sheep infused with lys-β-urogastrone (●——●) or saline (○-------○) at (*a*) 118 μg kg^{-1} and (*b*) 25 μg kg^{-1}.

Pancreatic polypeptide concentrations show large diurnal variation, falling as much as tenfold in the early morning and rising slowly during the day. Fig. 5 shows pancreatic polypeptide levels in sheep infused at the lowest dose of lys-β-urogastrone (25 μg kg^{-1}) and the highest dose (118 μg kg^{-1}). Although Fig. 5 shows differences between the animals infused with lys-β-urogastrone and saline, there were no overall consistent changes.

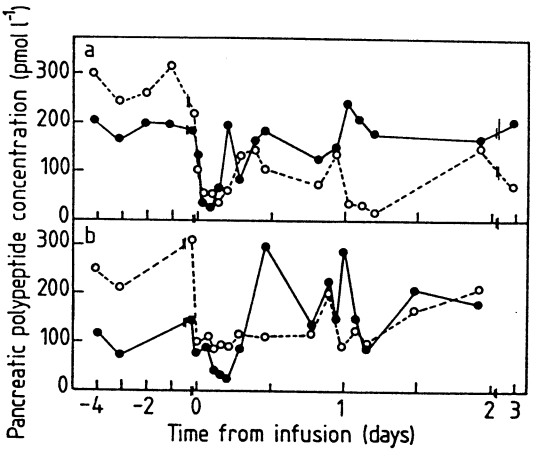

Fig. 5. Pancreatic polypeptide concentration in the plasma of sheep infused with lys-β-urogastrone (●——●) or saline (○-------○) at (*a*) 118 μg kg^{-1} and (*b*) 25 μg kg^{-1}.

Discussion

Of the parameters investigated, it appears lys-β-urogastrone has similar actions and potency to those of the closely related molecule mEGF. In suckling mice, both promote early eyelid separation and incisor eruption (Table 1). In sheep, not only do they cause facial erythema and transient inappetance (Table 2), but also temporary inhibition of wool growth (Fig. 1) and a dose-related development of a partial or complete weakness in the fleece (Table 3).

In our experiments, direct comparison was made between lys-β-urogastrone and mEGF in suckling mice. This was not possible in sheep and we compare our results with the effects of mEGF in sheep infused subcutaneously for 26–28 h with mEGF (Moore *et al.* 1982*b*), the dose in these experiments being calculated on metabolic body weight (Kleiber 1965). For this reason our data are presented in metabolic body weight (Table 3). Moore *et al.* (1982*b*) referred to a comparison between one observation on plucking force before administration and one 9 days after mEGF was infused. It must be noted, however, that the two measures are not the same. Before treatment, the depilation force is measured,

whereas after treatment the measure may be of staple strength. For comparison, our data are similarly presented. Moore *et al.* (1982*b*) found that after subcutaneous infusion (26–28 h) of mEGF at doses >250 μg $kg^{-0.75}$ there was a decline in plucking force of greater than 90% and complete shedding of the fleece occurred; at lower doses, from 100–230 μg $kg^{-0.75}$, a partial weakness or break developed in the fleece and this was correlated with dose-dependent decreases in plucking force in the range 40–90%; no effect was seen after infusion of 60 μg $kg^{-0.75}$. Administration of lys-β-urogastrone at >283 μg $kg^{-0.75}$ resulted in a decline in plucking force of more than 90% and complete defleecing, whereas infusion of lower doses, 122–248 μg $kg^{-0.75}$, resulted in the production of weakened wool (Table 3); the extent of the weakness depended on the dose and this was reflected by the decline in plucking-force measurements. At doses <92 μg $kg^{-0.75}$ no effect was detectable (Table 3). As it appears that the biological defleecing response of sheep infused with lys-β-urogastrone is similar to that of mEGF there is no compelling reason to suspect that there would be any great difference in the morphological changes or sequence of changes between those that occur after treatment with mEGF (Hollis *et al.* 1983; McDonald *et al.* 1983). Moreover, it is likely that lys-β-urogastrone exerts its effect at the cellular level in the same manner as mEGF by inhibition of DNA synthesis (Panaretto *et al.* 1984) and a reduction in mitotic indices of the bulb cells of the wool follicles (Moore *et al.* 1985).

The profile of lys-β-urogastrone plasma concentrations after its infusion (Fig. 2) was similar to that for mEGF given intravenously (Moore *et al.* 1984; Panaretto *et al.* 1982, 1984). During infusion the plasma levels of both molecules rose steadily to reach the highest detectable concentrations towards the end of infusion and thereafter clearance from the circulation occurred so that, within 4–5 h, post-infusion levels were often considerably lower than 10% of the highest concentration measured during infusion.

Comparisons can also be made between the biological defleecing response and persistence of either mEGF or lys-β-urogastrone at different circulating concentrations. Panaretto *et al.* (1982) concluded that when the plasma mEGF concentration in adult Merino wethers was sustained at >20 μg l^{-1} for 15–24 h, complete shedding of the fleece resulted, whereas concentrations of 10 μg l^{-1} for 15 h resulted only in a break (Moore *et al.* 1982*a*). The average concentration of lys-β-urogastrone calculated over the last 20 h of infusion was 39 μg l^{-1} in a sheep given 118 μg kg^{-1} in which the fleece was subsequently cast; over this period the concentration did not fall below 10 μg l^{-1}. Lower average plasma concentrations (3 and 10·5 μg l^{-1}) for the same period resulted in the development of weakened wool in sheep receiving 50 and 83 μg kg^{-1} respectively. At average concentrations of <1 μg l^{-1} for this period, in a sheep given lys-β-urogastrone at 38 μg kg^{-1} no biological defleecing response was seen.

The transient erythema and inappetance in sheep infused with lys-β-urogastrone appear to be similar to those in sheep infused with mEGF. In sheep given mEGF, erythema was observed around the muzzle, lips and eyes but no report was made of any dose dependency (Panaretto *et al.* 1982); in sheep treated with lys-β-urogastrone the muzzle was the site most clearly affected and the response was dose dependent. For both molecules the effects subsided with the cessation of infusion. The erythema may reflect the transient dermal haemorrhage caused by dilation of capillaries as reported in histological studies of the wool-bearing regions of sheep treated with mEGF; these effects were seen within the first 3 h from the start of infusion and had disappeared by 24 h (Hollis *et al.* 1983). Feed refusals in sheep dosed with mEGF were of varying magnitude and duration and not necessarily dose related (Panaretto *et al.* 1982, 1984); like those elicited by lys-β-urogastrone they were most pronounced in the first 3 days from the start of infusion and had returned to pre-treatment levels by the fifth day (Table 2).

A dose-dependent temporary inhibition of wool growth was found in sheep infused with either peptide. Despite differences in the route of administration, the magnitude of the response was comparable over a similar dose range but the timing of the response was generally delayed in sheep given mEGF subcutaneously compared with those receiving

lys-β-urogastrone intravenously. For example, Moore *et al.* (1982*b*) found that subcutaneous infusion of mEGF for 28 h at 310, 170 and 60 μg $kg^{-0.75}$ resulted in the development of maximal inhibition at 4 weeks post-infusion of about 80%, 55% and −5% of pre-treatment wool growth rates; full recovery to pre-treatment levels, or greater, was not evident until 6 weeks post-treatment. By comparison, intravenous infusion of lys-β-urogastrone at 284, 201 and 120 μg $kg^{-0.75}$ resulted in the development of maximal inhibition (of 92%, 44% and 32% respectively) at 2 weeks post-treatment and either substantial or full recovery to pre-treatment levels 2 weeks later; no effect was evident at 61 μg $kg^{-0.75}$. The earlier response of wool growth to lys-β-urogastrone can be ascribed to the intravenous route of administration, for Panaretto *et al.* (1982, 1984) found that intravenous infusion of defleecing doses of mEGF (120–140 μg kg^{-1}) caused maximal inhibition of wool growth of 75–95% earlier (at 16–23 days) than that which developed after subcutaneous administration; subsequent recovery to levels >70% pre-treatment values was correspondingly earlier (4 weeks). It is noteworthy that the magnitude and timing of response of wool growth to the highest dose of lys-β-urogastrone (118 μg kg^{-1}) was very similar to that for the defleecing doses of mEGF given intravenously for 24–26 h (Panaretto *et al.* 1982, 1984).

As there is no substantial difference in potency of the molecules with respect to wool growth it is likely that the values of 2–5% estimated for the clean wool production lost due to mEGF treatment (Moore *et al.* 1982*a*) would apply to losses due to the use of lys-β-urogastrone for biological defleecing.

A physiological effect of urogastrone is reduced secretion of acid (Gregory 1955). In humans, urogastrone infusion at 0·25 μg kg^{-1} h^{-1} inhibited within 15–30 min both the volume and acid concentration of gastric secretion previously induced by pentagastrin infusion. However, there was no increase in plasma gastrin concentration after infusion for 1 h (Elder *et al.* 1975). In sheep, such a reduction in gastric secretion would give rise to an increase in abomasal pH and indirectly stimulate gastrin secretion. This may explain the increase in plasma gastrin after 2–3 h of infusion of lys-β-urogastrone at 1–5 μg kg^{-1} h^{-1}. However, the possibility has been raised that urogastrone interferes with the normal binding of gastrin to its receptors (H. Gregory, personal communication).

This investigation supports previous observations that despite differences of about 30% in the primary sequence of urogastrone (and thus lys-β-urogastrone) and mEGF, this does not apparently affect the tertiary structure sufficiently to cause substantial differences in biological activity both *in vitro* and *in vivo* (Gregory 1975; Hollenberg and Gregory 1980; Smith *et al.* 1985). Even with differences in homology of 56% and 67%, which have been noted for human and murine EGF (Marquardt *et al.* 1984) and rat TGF-α (transforming growth factor), similar patterns of activity have been observed *in vitro* (Massague 1983) and *in vivo* (Smith *et al.* 1985; Tam 1985). However, it is of interest to note that under our experimental conditions recombinant TGF-α was without defleecing activity when infused intravenously for 24 h at 123 and 396 μg kg^{-1} fleece-free body weight. At the higher dose a transient decrease in plucking force was observed at day 7 after the start of infusion but no weakness developed in the wool. The same preparation had similar potency to that of mEGF when tested in the suckling mouse bioassay for precocious eyelid separation and tooth eruption (S. S. Adams and A. J. Campbell, unpublished) and was found to be appropriately mitogenic in a fibroblast assay (H. Gregory, personal communication). Thus, despite conservation of the disulfide bonds in TGF-α, it appears there are specific areas in β-lys-urogastrone and mEGF that are sufficiently different in TGF-α not to elicit a defleecing response.

Acknowledgments

The work was supported by a grant from the Wool Research Development Council on the recommendation of the Australian Wool Corporation. We are grateful to Dr H. Gregory, Pharmaceuticals Division, ICI-PLC for the supply of lys-β-urogastrone.

We thank M. Christie, A. Darvodelsky, T. Ferraro and D. Wallace for valuable technical assistance and V. and W. Davin for their reliable care of the animals.

References

Carpenter, G., and Cohen, S. (1979). Epidermal growth factor. *Annu. Rev. Biochem.* **48**, 193–216.

Chance, R. E., Moon, N. E., and Johnson, M. G. (1979). Human pancreatic polypeptide (HPP) and bovine pancreatic polypeptide (BPP). In 'Methods of Hormone Radioimmunoassay'. (Eds B. M. Jaffe and H. R. Behrman.) pp. 657–72. (Academic Press: New York.)

Darvodelsky, A. M., Davey, M. W., Reid, A. M., Titchen, D. A., and Wang, X. (1988). Immunochemical characterisation of somatostatin in ruminants. *Regul. Pep.* **20**, 161–70.

Elder, J. B., Ganguli, P. C., Gillespie, I. E., Gerrying, E. L., and Gregory, H. (1975). Effect of urogastrone on gastric secretion and plasma gastrin levels in normal subjects. *Gut* **16**, 887–93.

Franklin, T. J., Greogry, H., and Morris, W. P. (1986). Acceleration of wound healing by recombinant human urogastrone (epidermal growth factor). *J. Lab. Clin. Med.* **108**, 103–8.

Gordon, A. J., and Pallister, E. (1980). A hand-held device for the rapid measurement of wool-staple thickness. *J. Text. Inst.* **72**, 145–6.

Gregory, H. (1975). Isolation and structure of urogastrone and its relationship to epidermal growth factor. *Nature (Lond.)* **275**, 325–7.

Gregory, H., Holmes, J. E., and Willshire, I. R. (1977). Urogastrone levels in the urine of normal adult humans. *J. Clin. Endocrinol. & Metab.* **45**, 668–72.

Gregory, R. A. (1955). A new method for the preparation of urogastrone. *J. Physiol.* **129**, 528–46.

Hansky, J., Soveny, C., and Korman, M. G. (1971). Effect of secretion on serum gastrin as measured by radioimmunoassay. *Gastroenterology* **61**, 62–8.

Hollenberg, M. D., and Gregory, H. (1980). Epidermal growth factor—urogastrone: biological activity and receptor binding of derivatives. *Mol. Pharmacol.* **17**, 314–20.

Hollis, D. E., Chapman, B. A., Panaretto, B. A., and Moore, G. P. M. (1983). Morphological changes in the skin and wool fibres of merino sheep infused with mouse epidermal growth factor. *Aust. J. Biol. Sci.* **36**, 419–34.

Hunter, W. M., and Greenwood, F. C. (1962). Preparation of iodine-131 labelled human growth hormone of high specific activity. *Nature (Lond.)* **194**, 495–6.

Kleiber, M. (1965). Metabolic body size. In 'Energy Metabolism'. (Ed. K. L. Blaxter.) European Assoc. Anim. Prod. Publ. No. 11 pp. 427–35. (Academic Press: London.)

Marquardt, H., Hunkapiller, M. W., Hood, L. E., and Todaro, G. J. (1984). Rat transforming growth factor type 1: structure and relation to epidermal growth factor. *Science* **223**, 1079–82.

Massague, J. (1983). Epidermal growth factor-like transforming growth factor. *J. Biol. Chem.* **258**, 13614–20.

McDonald, B. J., Waters, M. J., Richards, M. D., Thorburn, G. D., and Hopkins, P. S. (1983). Effect of epidermal growth factor on wool fibre morphology and skin histology. *Res. Vet. Sci.* **35**, 91–9.

Moore, G. P. M., and Panaretto, B. A. (1981). Epidermal growth factor causes shedding of the fleece of merino sheep. *Search* **12**, 128–9.

Moore, G. P. M., Panaretto, B. A., and Carter, N. B. (1985). Epidermal hyperplasia and wool follicle regression in sheep infused with epidermal growth factor. *J. Invest. Dermatol.* **84**, 172–5.

Moore, G. P. M., Panaretto, B. A., and Robertson, D. (1982*a*). Inhibition of wool growth in merino sheep by epidermal growth factor; production of breaks in the fleece. In Proc. 2nd Nat. Conf. on Wool Harvesting Research and Development, Sydney, 1981. (Ed. P. R. W. Hudson.) pp. 57–65. (Australian Wool Council.)

Moore, G. P. M., Panaretto, B. A., and Robertson, D. (1982*b*). Inhibition of wool growth in merino sheep following administration of mouse epidermal growth factor and a derivative. *Aust. J. Biol. Sci.* **35**, 163–72.

Moore, G. P. M., Panaretto, B. A., and Wallace, A. L. C. (1984). Treatment of ewes at different stages of pregnancy with epidermal growth factor; effects on wool growth and plasma concentrations of growth hormone, prolactin, placental lactogen and thyroxine and on foetal development. *Acta Endocrinol.* **105**, 558–66.

Panaretto, B. A., Leish, Z., Moore, G. P. M., and Robertson, D. M. (1984). Inhibition of DNA synthesis in dermal tissue of merino sheep treated with depilatory doses of mouse epidermal growth factor. *J. Endocrinol.* **100**, 25–31.

Panaretto, B. A., Moore, G. P. M., and Robertson, D. M. (1982). Plasma concentrations and urinary excretion of mouse epidermal growth factor associated with the inhibition of food consumption and of wool growth in Merino wethers. *J. Endocrinol.* **94**, 191–202.

Reis, P. J. (1967). The growth and composition of wool. IV. The differential response of growth and of sulphur content of wool to the level of sulphur-containing amino acids given per abomasum. *Aust. J. Biol. Sci.* **20**, 809–25.

Smith, J., Cook, E., Fotheringham, I., Pheby, S., Derbyshire, R., Eaton, M. A. W., Doel, M., Lilley, D. M. J., Pardon, J. F., Patel, T., Lewis, H., and Bell, L. D. (1982). Chemical synthesis and cloning of a gene for human β-urogastrone. *Nucl. Acids Res.* **10**, 4467–82.

Smith, J. M., Sporn, M. B., Roberts, A. B., Derynck, R., Winkler, M. E., and Gregory, H. (1985). Human transforming growth factor-α causes precocious eyelid opening in newborn mice. *Nature (Lond.)* **315**, 515–16.

Tam, J. P. (1985). Physiological effects of transforming growth factor in the newborn mouse. *Science* **229**, 673–5.

Thorburn, G. D., Waters, M. J., Young, I. R., Dolling, M., Buntine, D., and Hopkins, P. G. (1981). Epidermal growth factor: a critical factor in fetal maturation? *Ciba Found. Sym.* **86**, 172–98.

Titchen, D. A. (1986). Gastro-intestinal peptide hormone distribution, release and action in ruminants. In 'Control of Digestion and Metabolism in Ruminants'. (Eds L. P. Milligan, W. L. Grovum and A. Dobson.) pp. 227–48. (Prentice-Hall: New Jersey.)

Titchen, D. A., and Reid, A. R. (1988). Putative roles of peptides in the genesis and control of parasitic diseases. In 'Aspects of Digestive Physiology in Ruminants'. (Eds A. and M. J. Dobson.) pp. 217–37. (Cornell University Press: Ithaca, New York.)

Manuscript received 24 November 1987, revised 6 April 1988, accepted 2 June 1988