HORMONAL EFFECTS AND METABOLIC CLEARANCE RATE OF THE ENKEPHALIN ANALOGUE DAMME IN MAN

N. GILCHRIST, JENNIFER E. BOLTON, R.A. DONALD, J.H. LIVESSEY,
HELEN K. ROUD & M.G. NICHOLLS

Department of Endocrinology, The Princess Margaret Hospital, Christchurch, New Zealand

- 1 The enkephalin analogue DAMME (0.25 mg) and normal saline were administered by slow intravenous injection to six normal male subjects in a double-blind crossover study. Plasma DAMME concentrations were measured by a specific radioimmunoassay.
- 2 A significant rise in plasma growth hormone and prolactin and a significant fall in plasma corticotrophin and cortisol concentrations were observed. The fall in pancreatic polypeptide concentration just failed to reach significance.
- 3 No significant changes were observed in insulin, glucagon, glucose, adrenaline and noradrenaline.
- 4 Serum concentrations of DAMME were initially high (> 6.7 nmol/l) corresponding with the observed maximum plasma growth hormone and prolactin responses.
- 5 The serum DAMME concentration was readily detectable when plasma growth hormone and prolactin had returned to control levels, but corticotrophin and cortisol were still significantly depressed.

Keywords DAMME hormonal effects metabolic clearance

Introduction

The synthesis of an enkephalin analogue (FK 33-824, DAMME) with a prolonged action and resistance to hydrolysis has facilitated the study of the effect of enkephalins on hormone secretion from the pituitary gland (Rivier *et al.*, 1977), and pancreas (Reid & Yen, 1981; Stubbs *et al.*, 1978). The pharmacokinetics of this analogue have been reported in sheep (Bolton *et al.*, 1982) but we are not aware of any similar data in man.

In this study we report the effect of intravenously administered DAMME on the secretion of pituitary and pancreatic hormones and catecholamines, together with pharmacokinetic data in six healthy male volunteers.

Methods

A double-blind randomised crossover study of DAMME and normal saline was carried out in six healthy male volunteers (age range 26–48 years. After an overnight fast they were given either 0.25 mg DAMME or 0.9% saline intravenously over 2 min with an interval of at least 2 days between test injections. Cannulae were inserted into both arms while supine, and were kept open with heparinized saline. Blood samples (20 ml) for hormone assay were taken at

–30, –15, –5, 0, 5, 10, 20, 30, 60, 90, 120, 180, 210, 240 and 270 min and the test substance injected into the contra-lateral arm at time 0 min. DAMME was assayed in each of the post-zero samples (except 5 min) and also at 2, 4, 6, 8, 40, 50, 150 and 300 min. Urine samples were obtained before and at the end of the experiment.

Assays

Serum prolactin, growth hormone, insulin and glucagon were measured by methods described by Livesey & Donald (1982), Livesey *et al.* (1972), Albano & Ekins (1970) and Aguilar-Parada *et al.* (1969) respectively. Plasma pancreatic polypeptide was measured by radioimmunoassay (Bolton *et al.*, 1983). Corticotrophin (ACTH) was measured as described by Donald (1977), cortisol by the method of Murphy (1967), plasma adrenaline and noradrenaline by a radioenzymatic procedure (Peuler & Johnson, 1977), and glucose by Beckman glucose analyser 2.

Plasma concentrations of DAMME were measured by RIA as described by Bolton *et al.* (1982), although a different and more sensitive DAMME antiserum from that previously reported was used in the present study. The final dilution of this antiserum in the assay

incubate was 1:50,000. The minimum detectable concentration of a 0.1 ml samples of plasma was 25 pg ml⁻¹ (41 pmol l⁻¹) and the usable range of the standard curve extended up to 50 pg DAMME/tube, 50% displacement of radiolabelled DAMME being effected by 15 pg DAMME/tube. There was no cross reaction of antiserum with met- and leu- enkephalins, α -, β - and γ -endorphins, β - and γ -lipotrophins, ACTH and β -MSH for doses ranging from 50 to 10⁴ pg peptide/tube. The intra-assay coefficient of variation at a mean level of 101 pg DAMME ml⁻¹ was 6.3% ($n = 12$). The inter-assay coefficient of variation of samples of three separate pools of DAMME were 10.3, 15.1 and 7.5% for mean levels of 224, 86 and 49 pgml⁻¹ DAMME respectively.

Mathematical treatment of data:

The exponential function $y = a_1 e^{-k_1 t} + a_2 e^{-k_2 t}$, where y is the concentration of DAMME, t is the time after injection and a_1 , a_2 , k_1 and k_2 are unknown parameters, was fitted by weighted non-linear least squares (Snedecor & Cochran, 1967) to the 18 measured plasma concentrations of DAMME following the bolus. The variance of each concentration was assumed proportional to the square of its magnitude. From the exponential approximation to the bolus data for each subject, the metabolic clearance rate (MCR), mean residence time (t) and apparent volume of distribution (V) were calculated by the non-compartmental method of Oppenheimer *et al.* (1975). An approximate half-life based on the value of k_2 (denoted by $t_{1/2}(k_2)$) was taken to be $0.693/k_2$.

Results are expressed as mean values $\pm$ s.e. mean of absolute values and comparisons were made using Student's t -test for paired samples.

Results

Hormone concentrations

An increase in hormone concentration was arbitrarily defined as a two-fold rise or greater above baseline values whereas a 50% fall compared with baseline was interpreted as a decrease. For glucose, changes of 1 mmol/l were considered to be significant.

Prolactin An immediate increase in plasma prolactin concentration was observed in all subjects following i.v. injection of DAMME with a peak observed at 45 min, being highly significant ($P < 0.001$) compared with controls and still significantly different ($P < 0.05$) from the controls at 120 min (see Figure 1).

Growth hormone Plasma growth hormone also increased immediately in all subjects following i.v. injection of DAMME with a peak at 45 min being

Figure 1 Mean ($\pm$ s.e. mean) changes in plasma concentrations of GH and prolactin in response to 0.25 mg of DAMME (○) or 0.9% saline (●). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

significantly ($P < 0.05$) different from control subjects but not significant ($P < 0.1$) at the 60 min interval (see Figure 1).

Cortisol Plasma cortisol gradually fell in all subjects after i.v. injection of DAMME, the mean cortisol concentrations at 180 min being highly significantly ($P < 0.001$) less than that of the control subjects and still being significantly ($P < 0.001$) lower than the control group at 240 min (see Figure 2).

ACTH The mean plasma ACTH concentration also fell gradually in all subjects and was significantly lower than that of the control group at 60 min ($P < 0.001$) and 120 min ($P < 0.01$) (Figure 2).

Other hormones Of the other hormones measured there was no statistically significant difference at the $P < 0.05$ level between the test and control groups for insulin, glucagon, pancreatic polypeptide, adrenaline

Figure 2 Mean ($\pm$ s.e. mean) changes in plasma concentrations of cortisol and ACTH in response to 0.25 mg of DAMME (o) or 0.9% saline (●). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

and noradrenaline, at any time between -30 and 270 min although pancreatic polypeptide levels tended to be lower after administration of DAMME ($P = 0.07$, 0.08 and 0.10 at 10, 120 and 180 min respectively). There was no significant change in plasma glucose.

Kinetics of DAMME in the circulation

Figure 3 shows mean plasma concentrations of DAMME following injection and the fitted exponential line. The means of the values of MCR, $\bar{t}$, V and $t_{1/2}$ (k_2), calculated individually for the six subjects are given in Table 1. In the four subjects in whom it was measured, $11 \pm 3\%$ of the administered DAMME was excreted in the urine by 5 h following injection. The mean concentration of DAMME, calculated from the fitted equation, was found to have fallen to 50% of its initial value by 19 min, to 28% at 45 min, 17% at 90 min and to 8% at 180 min. These times correspond to the maximum observed hormone changes.

Figure 3 Plasma concentrations from 2 to 300 min following intravenous injection of 0.25 mg of DAMME. Mean $\pm$ s.e. mean of logarithmic values for six subjects. Points at 4 and 8 min omitted for clarity. The line is that fitted by weighted non-linear least-squares and is given by $y = 15.62e^{-0.06228t} + 8.16e^{-0.00783t}$

Table 1 Kinetic parameters (mean $\pm$ s.e. mean) calculated from DAMME plasma concentrations in six subjects.

MCR ($\text{ml min}^{-1} \text{kg}^{-1}$)	$\bar{t}$ (min)	V (ml/kg)	$t_{1/2}$ (k_2) (min)
4.41 ± 0.48	108 ± 5	481 ± 70	92 ± 4

Discussion

We have confirmed that DAMME causes a rise in plasma growth hormone and prolactin (Stubbs *et al.*, 1978; del Pozo *et al.*, 1980) and a gradual fall of plasma ACTH (del Pozo *et al.*, 1980) which was maximal at 60 min and sustained to 120 min. The fall in plasma cortisol was most marked at 180 min and still sustained at 240 min. We observed no clear change in any of the other hormones measure, although the pancreatic polypeptide concentration tended to fall. Although the fall was of uncertain statistical significance ($0.05 < P < 0.1$) this result contrasts with that observed in sheep (Bolton *et al.*, 1983) in which pan-

creatic polypeptide was observed to rise after intravenous administration of DAMME.

The fall of plasma ACTH may indicate a direct effect of DAMME at the hypothalamic level (Watson *et al.*, 1978) causing an inhibition of corticotrophin releasing factor (CRF) although a direct inhibitory action on the pituitary cannot be ruled out (del Pozo *et al.*, 1980). The clinical use of DAMME (FK33-824) has been studied (Allolio *et al.*, 1982; Gaillard *et al.*, 1981) in situations of chronic ACTH hypersecretion either due to ACTH dependant Cushing's syndrome or Addison's disease. Although the results have been conflicting, there is some indication that ACTH secretion can be inhibited by opiates.

The development of a radioimmunoassay for DAMME (Bolton *et al.*, 1982) has allowed us to study the inter-relationship between the plasma concentrations of DAMME and anterior pituitary hormone secretion. The kinetic data shows that 50% of the DAMME has disappeared by 19 min, 72% by 45 min and almost all (92%) by 180 min. These times correspond to maximal differences for the hormonal changes. Comparing the pharmacokinetics of DAMME in humans with those we have found previously in sheep (Bolton *et al.*, 1982) humans have a somewhat higher metabolic clearance rate and mean residence time and significantly greater volume of distribution. The $t_{1/2}$ (k_2) is also significantly ($P <$

0.02) longer in humans (92 ± 4 min) than sheep (38 ± 11 min) and this may explain the apparent earlier rise of growth hormone and prolactin observed in sheep.

The delayed suppression of ACTH secretion relative to the growth hormone and prolactin responses is unlikely to be due to the different clearance rates of the three hormones as the plasma disappearance half time of ACTH is relatively rapid (Donald, 1977). It seems likely that the suppression of ACTH secretion involves a different mechanism than stimulation of growth hormone and prolactin release. It may be relevant that the suppression of ACTH secretion by dexamethasone involves protein synthesis (Fleischer & Battarbee, 1967), and hence a time delay might be expected if DAMME modified the inhibitory effect of glucocorticoids. It is possible that DAMME could also affect ACTH release by inhibiting the secretion of corticotrophin releasing factor (CRF), and that a noradrenergic mechanism may be involved (Grossman & Besser, 1982). However, the regulation of CRF secretion is not well understood (Yasuda *et al.*, 1982), and any action of DAMME on CRF secretion is speculative at present.

We wish to thank H. Duff, J. Brett, J. Braithwaite, J. Smith, N. Marsh, K. Miles, H. Legge, A. Toth and J. Lawson for technical assistance, Sandoz Pharmaceuticals for the gift of DAMME, the New Zealand Medical Research Council for funding this study and P. Gilson for typing the manuscript.

References

- ALBANO, J. & EKINS, R.P. (1970). The attainment of high sensitivity and precision in radioimmunoassay techniques as exemplified in a simple assay of serum insulin. In *In vitro procedures with radioisotopes in medicine*. I.A.E.A., Vienna 491.
- ALLOLIO, B., WINKELMAN, W., HIPPE, F.X., KAULEN, D. & MIES, R. (1982). Effects of a met-enkephalin analog on adrenocorticotrophin (ACTH), growth hormone and prolactin in patients with ACTH hypersecretion. *J. clin. Endocrinol. Metab.*, **55**, 1-7.
- AQUILAR-PARADA, E., EISENTRAUT, A.M. & UNGER, R.G. (1969). Pancreatic glucagon secretion in normal and diabetic subjects. *Am. J. med. Sci.*, **257**, 415-419.
- BOLTON, J.E., LIVESEY, J.H. & DONALD, R.A. (1982). Metabolic clearance rate in sheep of a met-enkephalin analogue estimated by radioimmunoassay. *J. Endocrinol.*, **93**, 427-433.
- BOLTON, J.E., ROUD, H.K., REDEKOPP, C., LIVESEY, J.H. & NICHOLLS, M.G. (1983). Effects of an enkephalin analogue (DAMME) on pituitary and pancreatic function in sheep. *Hormone Metab. Res.* (in press).
- DEL POZO, E., VAN GRAFFENRIED, B., BRONWELL, J., DERRER, F. & MARBACK, P. (1980). Endocrine effect of a methionine-enkephalin derivative (FK 33-824) in man. *Hormone Res.*, **13**, 90-97.
- DONALD, R.A. (1977). Radioimmunoassay of corticotrophin (ACTH). In *Handbook of radioimmunoassay*, ed. Abrahams, G., p. 319. Marcel Dekker: New York and Basel.
- FLEISCHER, N. & BATTARBEE, H. (1967). Inhibition of dexamethasone suppression of ACTH secretion *in vivo* by actinomycin D. *Proc. Soc. exp. Biol. Med.*, **126**, 922-925.
- GAILLARD, R.C., GROSSMAN, A., SMITH, R., REES, L.H. & BESSER, G.M. (1981). The effects of a met-enkephalin analogue on ACTH, β -LPH, β -endorphin and met-enkephalin in patients with adrenocortical disease. *Clin. Endocrinol.*, **14**, 471-478.
- GROSSMAN, A. & BESSER, G.M. (1982). Opiates control ACTH through a noradrenergic mechanism. *Clin. Endocrinol.*, **17**, 287-290.
- LIVESEY, J.H. & DONALD, R.A. (1982). Prevention of adsorption losses during radioimmunoassay of polypeptide hormones: effectiveness of albumins, gelatin, caseins, Tween 20 and plasma. *Clin. Chim. Acta*, **123**, 193-198.
- LIVESEY, J.H., DONALD, R.A., ESPINER, E.A. & SALAMONSEN, L.A. (1972). Measurement of pituitary hormones: Clinical applications 3: Growth Hormone. *N.Z. med. J.*, **76**, 261-265.
- MURPHY, B.E.P. (1967). Some studies of the protein binding of steroids and their routine micro and ultra micro measurements of various steroids in body fluids by competitive protein-binding radioassay. *J. clin. Endocrinol. Metab.*, **27**, 973-990.
- OPPENHEIMER, J.H., SCHWARTZ, H.L. & SURKS, M.I. (1975). Determination of common parameters of iodothyroxine metabolism and distribution in man by non-

- compartmental analysis. *J. clin. Endocrinol. Metab.*, **41**, 319–324.
- PEULER, J.D. & JOHNSON, G.A. (1977). Simultaneous single isotope radio-enzymatic assay of plasma norepinephrine, epinephrine and dopamine. *Life Sci.*, **21**, 625–633.
- REID, R.L. & YEN, S.S.C. (1981). β -endorphin stimulates the secretion of insulin and glucagon in humans. *J. clin. Endocrinol. Metab.*, **52**, 592–594.
- RIVIER, G., VALE, W., LING, N., BROWN, M. & GUILLEMIN, R. (1977). Stimulation *in vivo* of the secretion of prolactin and growth hormone by β -endorphin. *Endocrinology*, **100**, 238–241.
- SKOWSKY, W.R. & FISHER, D.A. (1972). The use of thyroglobulin to induce antigenicity to small molecules. *J. lab. clin. Med.*, **80**, 134–144.
- SNEDECOR, G.W. & COCHRAN, W.G. (1967). *Statistical methods*, p. 465. 6th Ed. Ames, U.S.A.: Iowa State University Press.
- STUBBS, W.A., DELITALIA, G., JONES, A., JEFFCOATE, W.J., EDWARDS, C.R.W., RATTER, S.J., BESSER, G.M., BLOOM, S.R. & ALBERTI, K.G.M.M. (1978). Hormonal and metabolic responses to an enkephalin analogue in normal man. *Lancet*, **ii**, 1225–1227.
- WATSON, S.J., AKIL, H., RICHARD, C.W. & BARCHES, J.D. (1978). Evidence for two separate opiate peptide neuronal systems. *Nature*, **275**, 226.
- YASUDA, N., GREER, M.A. & AIZAWA, T. (1982). Corticotropin releasing factor. *Endocrine Reviews*, **3**, 123–140.

(Received March 4, 1983,
accepted June 27, 1983)