Prolonged Corticotrophic Action of a Synthetic Substituted 1-18 ACTH

J. KEENAN, J. B. THOMPSON, M. A. CHAMBERLAIN, G. M. BESSER

British Medical Journal, 1971, 3, 742-743

Summary

The adrenocorticotrophic effects of a synthetic corticotrophin analogue, α^{1-18} ACTH with D-serine¹, lysine¹⁷, and lysine amide¹⁸ substitutions has been studied. It is effective after both intramuscular and subcutaneous administration and compared with tetracosactrin depot (Synacthen depot, Cortrosyn depot) it has a similarly prolonged time course of action—about 24 hours after 0.5 mg and 30 hours after 1 mg. Unlike tetracosactrin depot, however, it is well absorbed when given intranasally and does not produce painful reactions at the site of injection. Its prolonged time course of action does not depend on a formulation designed to delay its release from the injection site but most probably on a decreased rate of degradation in the circulation.

Introduction

Tetracosactrin (α^{1-24} ACTH,* Synacthen, Cortrosyn) is a synthetic polypeptide containing the first 24 amino-acids of ACTH in a sequence which is identical with that of all species so far fully investigated. It has potent corticotrophic properties but a length of action of only four to five hours when used in an unmodified form (Landon *et al.*, 1964). In order to produce a therapeutically useful preparation tetracosactrin has been adsorbed on to zinc phosphate to delay its absorption from the site of injection (tetracosactrin depot, Synacthen depot, Cortrosyn depot) and the adrenocorticotrophic actions of this compound last for 24 to 48 hours after an intramuscular injection of 1 mg (Besser *et al.*, 1967; Nelson *et al.*, 1968; Haupt *et al.*, 1969; Treadwell and Dennis, 1969). However, this preparation may produce painful local reactions if given subcutaneously or sometimes intramuscularly (Besser *et al.*, 1967; Nelson *et al.*, 1968; Treadwell and Dennis, 1969), and this seems to be due to the contained zinc complexes. Occasionally generalized allergic reactions after tetracosactrin depot occur though in general it can be used in patients who are hypersensitive to animal ACTH (El-Shaboury, 1968; Jensen and Sneddon, 1969).

An alternative corticotrophic polypeptide has been synthesized (Desaules *et al.*, 1969) which has a prolonged therapeutically useful time course of action without the addition of any agent to delay its absorption. The prolonged action results from modification of the ACTH peptide sequence and is presumably due to delayed degradation in the circulation. The polypeptide consists of the first (N-terminal) 18 amino-acids of natural ACTH with the following substitutions: D-

serine for L-serine in position 1 and lysine for the arginines in positions 17 and 18 with a C-terminal amide group (Fig. 1).

FIG. 1—Structure of tetracosactrin (α^{1-24} ACTH) compared with the substituted α^{1-18} ACTH. The amino-acid sequences between positions 1 and 15 are identical.

This peptide is referred to as α^{1-18} ACTH in this paper. We now report the potency and length of action of this compound after intramuscular, subcutaneous, intranasal, and sublingual administration to normal subjects.

Methods and Subjects

There were 12 normal subjects aged 20 to 40 years (2 were women). Intramuscular injections into the buttock of 0.5 and 1 mg and subcutaneous injections of 0.5 mg of α^{1-18} ACTH were compared in the same six subjects who had been given oral dexamethasone to suppress their endogenous ACTH secretion. The dexamethasone was given in a dose of 2 mg on retiring before and on waking on the day of the study, and then 1 mg each six hours until the blood sampling was complete. The three treatments were given according to a randomized Latin square design at intervals of at least seven days.

Intranasal administration was given to six other subjects who had not taken dexamethasone, a pressurized insufflator being used to deliver 0.9 mg of α^{1-18} ACTH as a powder in three doses.

Sublingual α^{1-18} ACTH (0.5 and 1 mg) was administered in gelatin lozenges to two subjects who had received oral dexamethasone as above.

The drug was given between 9 and 10 a.m. and blood was sampled before and at intervals for 30 hours afterwards. Plasma corticosteroids were measured by a fluorometric technique (Mattingly, 1962). The changes in corticosteroids from the starting control levels were calculated and the differences in the means at each time interval were compared with Student's *t* test (2-tailed). In addition, the maximum changes in plasma corticosteroids after each treatment, irrespective of the time interval at which they occurred, were calculated and the means compared.

Results

After intramuscular injection of 1 mg of α^{1-18} ACTH the mean plasma fluorogenic corticosteroid concentration rose by $45 \pm \text{S.E. } 4.7 \mu\text{g}/100 \text{ ml}$ to reach a peak at six hours and then

* The ACTH nomenclature of Li (1959) is used in this paper.

Middlesex Hospital, London W.1

J. KEENAN, M.B., M.R.C.PATH., Lecturer in Chemical Pathology, Court-aud Institute of Biochemistry

M. A. CHAMBERLAIN, M.B., M.R.C.P., Senior Registrar, Department of Rheumatology and Physical Medicine

Medical Professorial Unit, St. Bartholomew's Hospital, London EC1A 7BE

J. B. THOMPSON, M.B., M.R.C.P., Junior Registrar

G. M. BESSER, M.D., M.R.C.P., Senior Lecturer in Endocrinology

returned to the baseline in a little over 30 hours (Fig. 2). After 0.5 mg the mean corticosteroid level rose by 37 ± 2.7 $\mu\text{g}/100$ ml, reaching a peak at two hours, and remained raised for about 24 hours. After *subcutaneous* injections of 0.5 mg of α^{1-18} ACTH the mean plasma corticosteroids rose by 30 ± 1.9 $\mu\text{g}/100$ ml at two hours and returned to the baseline values between 16 and 20 hours. After *intranasal* administration (0.9 mg) the material was well absorbed and the mean plasma corticosteroids rose rapidly, reaching a peak at four hours of 37 ± 3.7 $\mu\text{g}/100$ ml above the mean control value and remained raised for about 24 hours. *Sublingual* administration of α^{1-18} ACTH did not result in any rise in plasma corticosteroids in either dose of 0.5 or 1 mg.

FIG. 2—Change in plasma fluorogenic corticosteroids after administration of α^{1-18} ACTH given intramuscularly, subcutaneously, or intranasally.

Comparison of Routes of Administration.—There was no significant difference between the mean maximum increments in corticosteroids achieved after 1 mg of α^{1-18} ACTH given intramuscularly and 0.9 mg given intranasally. However, the mean plasma corticosteroid increments were consistently lower after intranasal administration over the whole time course of action. The overall time courses of the compound administered both ways were the same, since the increment at 24 hours after the intramuscular dose was 10 ± 6.0 $\mu\text{g}/100$ ml and after the intranasal dose 6 ± 6.3 $\mu\text{g}/100$ ml and these were not significantly different. When the effects of 0.5 mg of α^{1-18} ACTH were compared the peak increments did not differ significantly whether the doses were given intramuscularly or subcutaneously (P just greater than 0.05). After the peak, however, the mean corticosteroid increments were lower after subcutaneous administration and the time course was shorter than after the intramuscular dose. The mean corticosteroid increments were virtually identical after 0.5 mg given intramuscularly and 0.9 mg given intranasally.

Side Effects.—Side effects were not encountered after administration of α^{1-18} ACTH when given intramuscularly or intranasally. When given subcutaneously two out of six subjects experienced a transient vascular flare at the site of the injection but there was no pain or induration.

Discussion

The corticotrophin analogue α^{1-18} ACTH with D-serine¹, lysine¹⁷, and lysine amide¹⁸ substitutions (Fig. 1) has been shown to be rapidly and effectively absorbed whether administered intramuscularly, subcutaneously, or intranasally but not from the buccal mucosa. There were no unpleasant side effects. It seems that the compound would be therapeutically valuable since it has a prolonged corticotrophic action—24 hours after 0.5 mg and 30 hours after 1 mg when given intramuscularly. These studies confirm the preliminary observations of Felber *et al.* (1969) and Walser *et al.* (1969). The prolonged effects are achieved by the modifications of the peptide sequence of natural ACTH designed to delay its degradation in the body, and hence a formulation to produce slow release from the site of injection is not required. A similarly prolonged time course is achieved when it is absorbed from the nasal mucosa.

The increments in plasma corticosteroids after intranasal administration are probably underestimated at four and eight hours after the dose, since the subjects in this group were not dexamethasone suppressed. The plasma corticosteroids normally decline during the second half of the day and would be expected to be 6 to 10 $\mu\text{g}/100$ ml lower than in the morning, but we have subtracted the control 9 a.m. pretreatment values from each subject's subsequent corticosteroid levels as control plasmas were not obtained later in the day.

The results contrast with those reported after tetracosactrin, for the length of action of this compound is only four to five hours after a 0.5-mg dose given intramuscularly (Landon *et al.*, 1964) or 1.5 mg intranasally (Keenan and Chamberlain, 1969), and to obtain a therapeutically useful duration of action this polypeptide was absorbed on to zinc phosphate to delay its absorption from the site of injection. This preparation of tetracosactrin cannot be given intranasally and produces painful reactions when injected subcutaneously or into muscles of small bulk (Besser *et al.*, 1967; Nelson *et al.*, 1968). The length of action of α^{1-18} ACTH is very similar to that of tetracosactrin depot despite the lack of a delayed-release formulation.

We conclude that α^{1-18} ACTH has advantages over tetracosactrin depot since it can be effectively administered subcutaneously and intranasally as well as intramuscularly without adverse local reactions, yet is equally potent and has a similar length of action.

We thank Dr. T. B. Binns, of Ciba Laboratories, for the supply of α^{1-18} ACTH, which is at present available only for research purposes.

Requests for reprints should be sent to Dr. G. M. Besser.

References

- Besser, G. M., Butler, P. W. P., and Plumpton, F. S. (1967). *British Medical Journal*, **4**, 391.
- Desaulles, P. A., Riniker, B., and Rittel, W. (1969). In *Protein and Polypeptide Hormones*, ed. M. Margoulies, p. 489. Amsterdam, Excerpta Medica Foundation.
- El-Shaboury, A. H. (1968). *British Medical Journal*, **3**, 653.
- Felber, J.-P., Aubert, M. L., and Deguillaume, R. (1969). *Experientia*, **25**, 1195.
- Haupt, E., Schemmel, K., and Leybold, K. (1969). *Pharmacologia Clinica*, **1**, 131.
- Jensen, N. E., and Sneddon, I. (1969). *British Medical Journal*, **2**, 383.
- Keenan, J., and Chamberlain, M. A. (1969). *British Medical Journal*, **4**, 407.
- Landon, J., James, V. H. T., Cryer, R. J., Wynn, V., and Frankland, A. W. (1964). *Journal of Clinical Endocrinology and Metabolism*, **24**, 1206.
- Li, C. H. (1959). *Science*, **129**, 969.
- Mattingly, D. (1962). *Journal of Clinical Pathology*, **15**, 374.
- Nelson, J. K., *et al.* (1968). *British Medical Journal*, **1**, 557.
- Treadwell, B. L. J., and Dennis, P. M. (1969). *British Medical Journal*, **4**, 720.
- Walser, A., Keller, R., and Herzog, H. (1969). *Schweizerische medizinische Wochenschrift*, **99**, 1726.