Free and conjugated C_{19}-steroids in plasma protein fractions

Steroid fraction	Cpm in protein fraction				
	Albumin	α_1-globulin	α_2-globulin	β-globulin	γ-globulin
Free	2,144	3,556	2,201	1,336	2,861
Sulphoconjugates	36,520	20,350	4,810	214	82
Dehydroepiandrosterone	26,190	14,650	1,040	–	–
Androsterone	4,850	3,230	410	–	–
Etiocholanolone	2,090	1,230	221	–	–
Androstenedione	870	312	108	–	–
Androstandione / Etiocholandione	631	358	76	–	–
Androstenediol	1,078	834	146	–	–
Androstenetriol	311	148	18	–	–
Glucuronosides	839	2,692	172	29	36
Dehydroepiandrosterone	69	241	–	–	–
Androsterone	352	1,110	–	–	–
Etiocholanolone	109	817	–	–	–

Androstenedione = 4-androstene-3,17-dione. Androstandione = 5α-androstan-3,17-dione. Etiocholandione = 5β-androstan-3,17-dione.
Androstenediol = 5-androstene-3β,17β-diol. Androstenetriol = 5-androstene-3β,16α,17β-triol.

hydrazones[4] or acetates and rechromatographed in suitable solvent systems. ^{3}H-activity of individual compounds was determined in a Packard Tricarb Spectrometer Mod. 3310. Final identification of compounds was achieved by purification to constant specific activity, eventually after reverse isotope dilution with standard material.

As indicated by the Table, free C_{19}-steroids were randomly distributed over the various protein fractions, whereas the sulphoconjugates prevailed in the albumin and α_1-globulin fractions as observed in earlier experiments[1]. On the other hand, almost 70% of ^{3}H-labelled C_{19}-steroid glucuronosides could be isolated from the fraction of α_1-globulins. On the basis of these findings and previous data it is suggested that endogenous lipophile C_{19}-steroid sulphoconjugates are transported preferably by a post-albumin. C_{19}-steroid glucuronosides, however, which in this experiment were formed through metabolism of free dehydroepiandrosterone, appear to be associated rather with an α_1-globulin. The composition of the different conjugate fractions is quite comparable to that detected in other investigations[5]. In contrast to the fraction of sulphoconjugates, where dehydroepiandrosterone represented the predominant C_{19}-steroid, the fraction of glucuronosides contained primarily 3α-hydroxy-5α-androstan-17-one (androsterone) and 3α-hydroxy-5β-androstan-17-one (etiocholanolone) as the major metabolites of the aforementioned substrate.

Zusammenfassung. Nach i.v. Gabe von 7α-^{3}H-Dehydroepiandrosteron wurde menschliches Plasma einer präparativen Zonenelektrophorese unterworfen und in Albumine, α_1-, α_2-, β- und γ-Globuline zerlegt. Aus den einzelnen Proteinfraktionen trennte man freie Steroide, Steroid-Sulfokonjugate und -Glukuronoside ab und untersuchte letztere Fraktionen auf ihren Gehalt an markierten C_{19}-Steroiden. Es zeigte sich, dass die Steroid-Sulfokonjugate vornehmlich in der Proteinfraktion der Albumine, die Steroid-Glukuronoside dagegen bevorzugt in der Proteinfraktion der α_1-Globuline auftraten.

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Interactions Between α-MSH and Sex Steroids on the Preputial Glands of Female Rats

The preputial glands, which can be regarded as giant sebaceous glands, are structures of epidermic origin whose precise biological significance has still not been clarified. Various studies have been published showing that their development and their secretory function are dependent upon certain sex steroids[1,2] and polypeptides such as STH, LTH, and ACTH[3,4]. Since melanocyte-stimulating hormone (MSH) exerts effects which are essentially of an epidermotropic character, it was felt that it would be interesting to determine to what extent and under what conditions this hormone might also be capable of stimulating growth of the preputial glands.

Method. Sprague-Dawley rats of female sex, weighing 180 g, were given treatment with synthetic α-MSH and sex steroids by the s.c. route for 2 weeks; the treatment

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was commenced 2 weeks after the animals had been castrated and immediately after they had undergone adrenalectomy or hypophysectomy. The α-MSH was injected twice daily in aqueous solution, and the sex steroids once a day in oily solution. At autopsy, which took place the day after the last injection, the preputial glands were removed and their fresh weight determined.

Results. When given in daily doses of between 0.01 and 1.0 mg/kg, α-MSH produces changes in the weight of the preputial glands, these changes varying depending upon the endocrine conditions prevailing in the animal (Figure 1). In the intact female rat, a steady and progressive increase in the weight of these glands is observed when the doses are raised; this increase, which is statistically significant, amounts to +45 mg ($p < 0.05$) in response to 0.03 mg/kg daily and rises to +78 mg ($p < 0.01$) in response to a dose of 1.0 mg/kg daily. Following castration, which produces only a slight decrease in the weight of the preputial glands in control animals, α-MSH exerts little effect, the weight of the glands increasing only very slightly as the dose is raised; even daily doses of α-MSH as high as 1 mg/kg elicit an increase in weight (+34 mg) which is only just significant.

When castration is followed by adrenalectomy, the weight of the preputial glands in the controls decreases a little, whereas after castration and hypophysectomy it shows a marked decrease; in both instances, however, α-MSH – in the doses employed – no longer exerts any perceptible influence on growth of the preputial glands.

It would therefore appear that, to produce a stimulant action on this receptor, α-MSH requires an endocrine balance different from that in which STH and ACTH are able to exert their effects, since STH proves active in castrated and hypophysectomized male rats[5] and ACTH is active in castrated and adrenalectomized female rats[4].

The difference observed in this initial experiment between the results obtained with α-MSH in intact as compared with castrated animals prompted us to examine the effect of this peptide when administered in combination with sex steroids. It was known that certain of the sex steroids are in themselves capable of stimulating growth of the preputial glands: testosterone, for example, has a very marked effect in this respect, and progesterone a weaker one, whereas oestradiol displays no such activity[2,5].

In the castrated animal, a dose of progesterone (10 mg/kg daily) which alone would exert only a subliminal action, produces considerable growth of the preputial glands when combined with varying doses of α-MSH (Figure 2). The resultant increase in the weight of the glands is statistically significant, amounting to +60 mg when α-MSH is given in a daily dose of 0.01 mg/kg and rising to +186 mg when the dose of α-MSH is raised to 0.3 mg/kg daily; the dose-effect curve thus becomes much steeper, i.e. it rises far more sharply than the dose-effect curve for α-MSH alone, both in the castrated and in the intact animal. A comparable response can be obtained by administering testosterone (1 mg/kg daily) together with α-MSH, although here the degree of potentiation is not quite so great as with progesterone, the increase in weight at the 0.3 mg/kg level being +100 mg. When on the other hand, α-MSH is given in combination with oestradiol (0.1 mg/kg daily), the increase in the weight of the glands is barely perceptible and is not even statistically significant when the dose of α-MSH is raised to the 0.3 mg/kg level.

Progesterone, given in the same daily dose of 10 mg/kg, is also capable of enhancing the response to α-MSH in castrated animals following adrenalectomy and hypo-

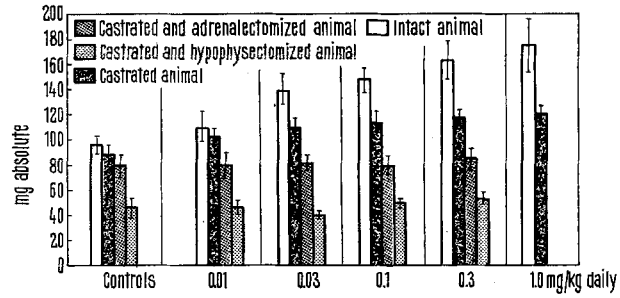

Fig. 1. Action of α-MSH alone on the weight of the preputial glands in various endocrine conditions.

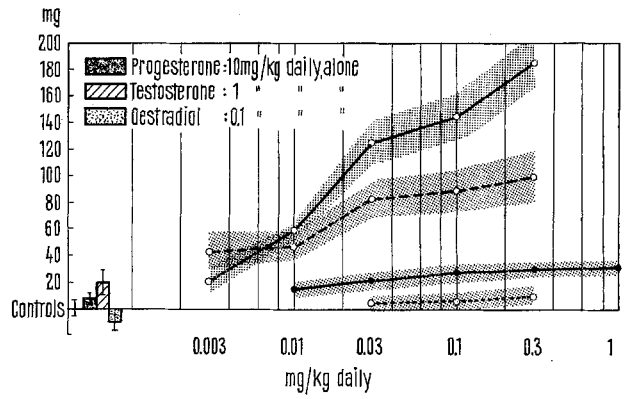

Fig. 2. Action of α-MSH and sex steroids, administered alone and in combination, on the weight of the preputial glands in the castrated animal. Difference as compared with controls. ●—— α-MSH alone; ○—— α-MSH + Progesterone (10 mg/kg daily); ○– – – α-MSH + Testosterone (1 mg/kg daily); ○ ..... α-MSH + Oestradiol (0.1 mg/kg daily).

Increase in weight of the preputial glands produced by addition of progesterone (10 mg/kg daily) to α-MSH in various endocrine conditions

Doses of α-MSH mg/kg/daily	Animals castrated		Animals castrated and adrenalectomized		Animals castrated and hypophysectomized	
	mg[a]	%[b]	mg[a]	%[b]	mg[a]	%[b]
Controls	+ 7	+ 8	+ 7	+ 9	0	0
0.01	+ 44[c]	+ 42	+17	+21	+ 7	+ 14
0.03	+104[d]	+ 94	+31	+38	+22	+ 55
0.1	+117[d]	+100	+48[c]	+61	+44[c]	+ 90
0.3	+156[d]	+135	+84[d]	+99	+84[d]	+150

[a] Difference of the weight obtained with each dose of α-MSH combined with progesterone and the weight obtained with the same dose of α-MSH alone. [b] Difference expressed in relation to the weight recorded at the corresponding dosage level of α-MSH. Statistically significant increase. [c] $p < 0.05$. [d] $p < 0.01$.

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physectomy (Table). But the differences in the absolute weights recorded at each dosage level of α-MSH with and without progesterone appear to be a little less marked than in the case of the animals which had undergone castration only; the relative differences, however, i.e. the differences expressed in percentages of the weight of the gland recorded with α-MSH alone, are much the same following castration as following castration and hypophysectomy.

Discussion. The results outlined above deserve comparison with those published by LORINCZ and LANCASTER[6], who demonstrated that a synergistic effect on the preputial glands was exerted by progesterone and a pituitary extract from which the STH, ACTH, and TSH fractions had been eliminated, but whose hormonal composition had not been defined. As regards its influence on the preputial glands, it could seem that STH, while not displaying any synergistic effect with progesterone[6], does exert a synergistic action with testosterone[5]. This type of action, moreover, is not confined to the preputial glands, and a recent study has shown that the ventral prostate can also be stimulated by administering progesterone in combination with LTH[7].

The data obtained in the present series of experiments, coupled with the findings quoted above, serve to highlight the role which peptide hormones may play in modulating the response of certain peripheral receptors to the steroids.

Résumé. L'α-MSH stimule la croissance des glandes préputiales de la rate intacte, cet effet est moins marqué après l'ablation des ovaires et s'atténue encore après castration et surrénalectomie ou castration et hypophysectomie. Chez l'animal castré, en doses liminaires, la progestérone accroît considérablement la stimulation produite par l'α-MSH tandis que la testostérone exerce un effet comparable mais moins accentué. Le synergisme de la progestérone et de l'α-MSH est encore observé, mais légèrement réduit, chez l'animal castré et surrénalectomisé ou castré et hypophysectomisé.

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Administration by Nasal Spray of an 18 Amino Acid Synthetic Polypeptide with Corticotropic Action

The clinical use of ACTH has hitherto been limited by the fact that it could only be given by intramuscular or intravenous injection. Attempts to administer the hormone orally have proved unsuccessful, since, like other proteins, it is denatured by the proteolytic enzymes of the intestinal tract.

For a long number of years, the antidiuretic hormone of the posterior pituitary has been administered by the nasal route. In 1952, SMITH et al.[1] described observations on the effectiveness of nasal insufflation of extractive ACTH. In these investigations, the corticotropic action of ACTH given in powder form was measured indirectly on the basis of the eosinopenic response of the blood.

In the study reported here, a new synthetic polypeptide derived from the ACTH molecule, CIBA 41,795-Ba, was given by nasal spray to normal volunteers, and its corticotropic action was measured directly by reference to the rise in plasma coriticosteroids.

Material and methods. The synthetic polypeptide CIBA 41,795-Ba [D-Ser[1], Lys[17,18]]-β-corticotropin-(1-18)-octadecapeptide amide, was synthesized by RINIKER and RITTEL[2]. It contains the amino acid sequence of the 18 N-terminal amino-acids of ACTH, with the following changes: the first amino acid, serine, is in the D-form instead of the L-form, arginine in positions 17 and 18 is replaced by lysine, and an amide group is present at the carboxyl end of arginine in position 18.

The pharmacology of this polypeptide has been studied by DESAULLES et al.[3,4]. Its action when given by injection to human subjects has been described by WALSER[5,6]; i.m. administration of 1 mg leads to an increase in plasma corticosteroids lasting 24 h.

The subjects taking part in these experiments were normal volunteers, aged between 20–30 years. They had rested for 1 h before the beginning of the tests. In all cases the dose was given at 08.00 h.

The polypeptide administered as a liquid suspension by means of a nebulizing flask which automatically controlled the dose. 6 subjects received a single dose of 0.1 mg. The effect on plasma corticosteroids was followed over a period of 8 h. 5 subjects received 0.3 mg and plasma corticosteroids were subsequently followed over a 6-h period. 12 subjects were treated with a dose of 1 mg. In this group plasma corticosteroids were followed for 4 h in 6 cases and for 12 h in the other 6.

Blood was removed under resting conditions, before and at various intervals after the administration of the product. Plasma corticosteroids were measured according to the method described by PETERSON, KARRER and GUERRA[7].

Results. The means of the plasma corticosteroid levels determined in these subjects after the administration of the various doses are shown in the Table. In the Figure these values are compared with the results obtained in subjects in the same age group receiving no treatment.

As can be seen from these curves, the nasal administration of the 1-18 polypeptide was followed by rise in

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