PLASMA STEROID RESPONSES TO CIRCADIAN-STAGE-SPECIFIED INJECTION OF DIFFERENT DOSES OF THE ACTH ANALOGUE ALSACTIDE (ACTH 1-17) IN HEALTHY ADULT HUMAN MALES

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There is now increasing evidence that glucocorticoid secretion and, in a broader sense, the activity of the so-called hypothalamo-pituitary-adrenal (HPA) axis is important for maintaining the appropriate circadian scale of numerous mammalian functions[2, 3, 5, 8, 13, 18, 19, 25]. Plasma cortisol levels have been suggested to represent an endogenous synchronizer in human subjects[1]. The heptadecapeptide ACTH-agonist (β-Ala[1],Lys[17]) ACTH 1-17-4-amino-N-butylamide (alsactide; Synchrodyn® 1-17) was demonstrated to have, both in animals and humans, higher potency and longer duration of action than other natural or synthetic ACTH preparations, without displaying any apparent immunogenicity[7, 11, 14, 29, 33]. In previous studies it was also shown that the molecule is very flexible in regulating the human adrenocortical response[4].

In this paper we present data obtained in healthy male volunteers who were injected serially with placebo and different intravenous (i.v.) or subcutaneous (s.c.) microdoses of alsactide at a circadian-stage-specified time, i.e. in the morning just before the endogenous cortisol would reach its maximum peak. This was made independently of data from the available literature on variations of the adrenocortical response to ACTH along the 24-h cycle[12, 16, 32]. Our aim was to provide data on the sensitivity threshold of different steroidogenetic patterns at this clinically most important time, which coincides with the beginning of the daily activity.

MATERIALS AND METHODS

Ten healthy medical students, aged from 21 to 24 years and homogeneous for height, weight and social habits, volunteered for the study. All subjects were informed of the aim and the sequence of experiments, which were per-

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formed at intervals of at least two weeks, and gave written consent. At the chosen days, subjects were assigned randomly to one of the following protocols: *placebo* i.v. or s.c.; *alsactide* 2 μg i.v. or s.c., 4 μg i.v. or s.c., 8 μg i.v. or s.c., 20 μg s.c. All subjects underwent these injections; six were also injected with alsactide 10 μg s.c. Placebo was isotonic saline, while the appropriate doses of the analogue were prepared immediately before injection from 10- or 100-μg dedicated ampoules. The injected volume amounted to 5 ml i.v. and 1 ml s.c. The study lasted from October 1984 to May 1985, and from October 1985 to February 1986.

The subjects were not hospitalized, but maintained roughly comparable habits throughout the study. They were diurnally active and nocturnally resting, i.e. asleep approximately from 23^{00} to 07^{00}. All tests were performed after an overnight fast by inserting an indwelling catheter into an antecubital vein at about 07^{00}; two blood samples were drawn at 15-min interval and served to assess the baseline hormone levels. Placebo or alsactide were injected at about 07^{15}, then blood samples were drawn at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min after the injection. Plasma was separated immediately and stored at −20 °C until assayed. Cortisol, progesterone, testosterone and aldosterone levels were measured using commercially available kits (Sorin Biomedica). For all variables, the intra-assay error measured as the coefficient of variation was always less than 10% between 20-80% displacement values. All samples from a single subject were run in a single assay for each hormone as well as in duplicate.

The basal value was defined as the mean of both values preceding the injection; the intensity of the steroid responses was evaluated as absolute and percentage increment above the basal level or as the integrated area below levels recorded until 360 min after the injection. The response area was calculated by subtracting the area corresponding to the basal level from the total area defined by the plotted curve, and was arbitrarily expressed in μg/dl/360 min (cortisol), ng/ml/360 min (testosterone) or pg/ml/360 min (progesterone and aldosterone). Statistical analysis was done using Student's *t*-test for paired data. All $p<0.05$ values were regarded as statistically significant.

RESULTS

No appreciable rise of plasma steroid levels was noted after placebo injection. As a consequence of their circadian stages, all the examined hormones were progressively lower in the peripheral plasma from 07^{00} onward. Also the s.c. injections of 2 and 4 μg alsactide resulted substantially ineffective in changing the hormonal pattern; when the individual curves were examined, the higher dose appeared to be at the sensitivity threshold of the adrenal glands in that changes of plasma cortisol and progesterone levels were apparent in 4 and 3 out of 10 subjects, respectively. As shown in figs 1-4, plasma steroid concentrations did appreciably change with respect to the basal value in the remaining experimental conditions. With regard to s.c. injections, the dose of 20 μg was associated with slightly lower testosterone levels during the 6h of observation, at variance with the comparable behavior observed after administration of placebo or other doses (fig. 4). In terms of maximum change above the basal level (Δmax), increasing doses by s.c. injection yielded a pro-

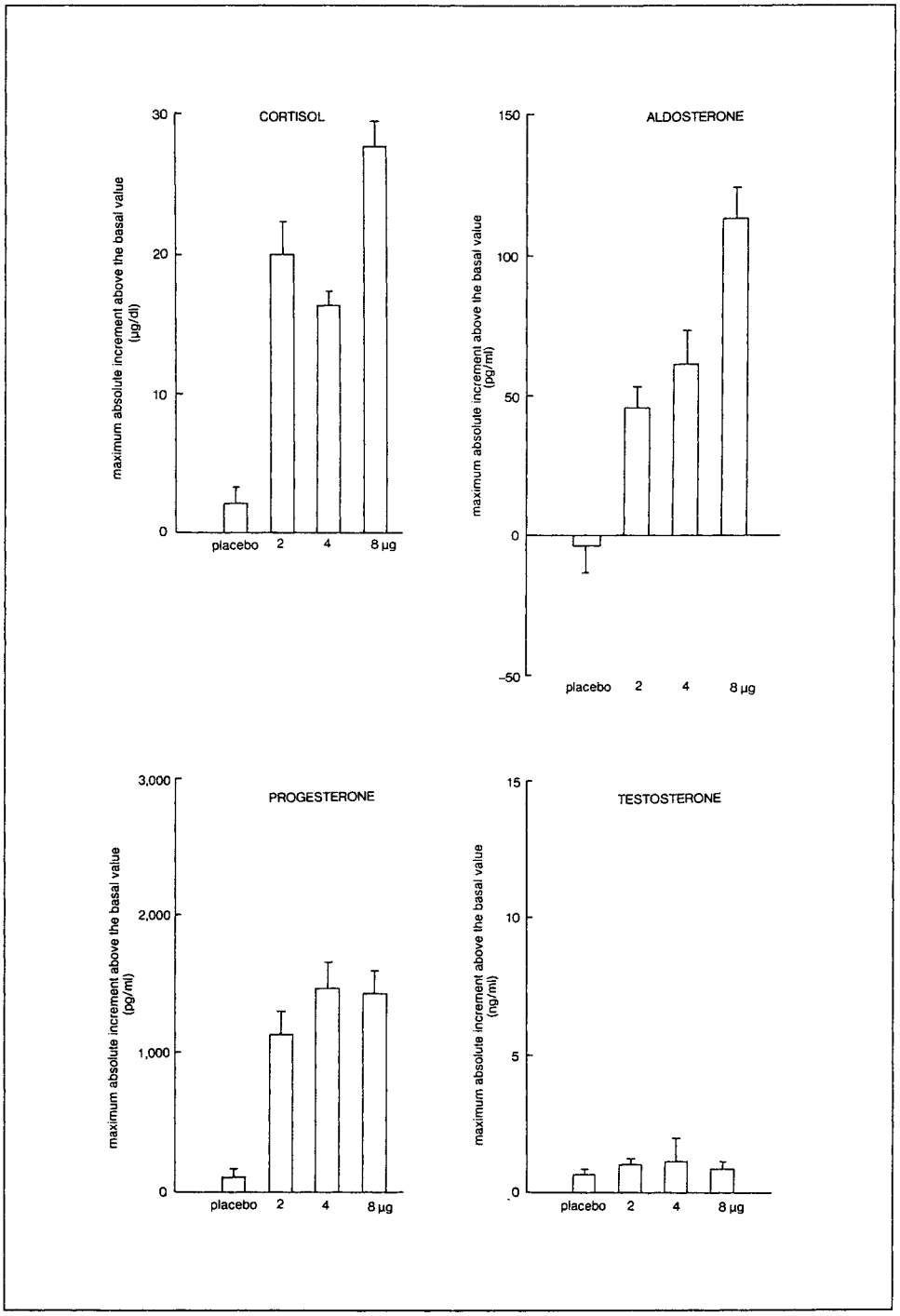


Fig. 1 - Effects of increasing doses of alsactide (ACTH 1-17) injected i.v. at about 07^{15} on plasma steroid concentrations in adult male volunteers. Ten subjects underwent injections of placebo (isotonic saline) or 2, 4 and 8 μg alsactide. Columns indicate the mean (± 1 SE) maximum absolute increment above the basal value.

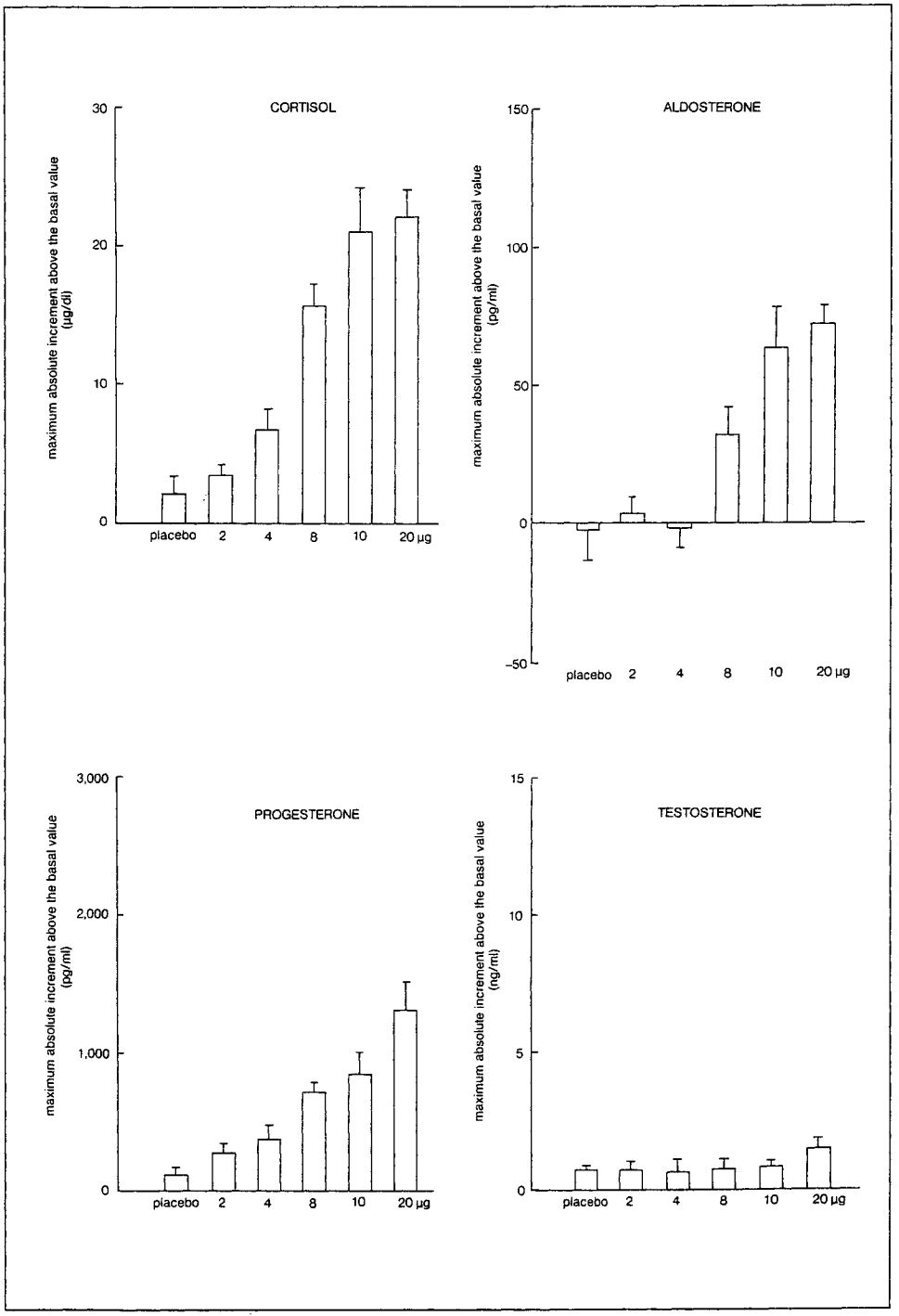
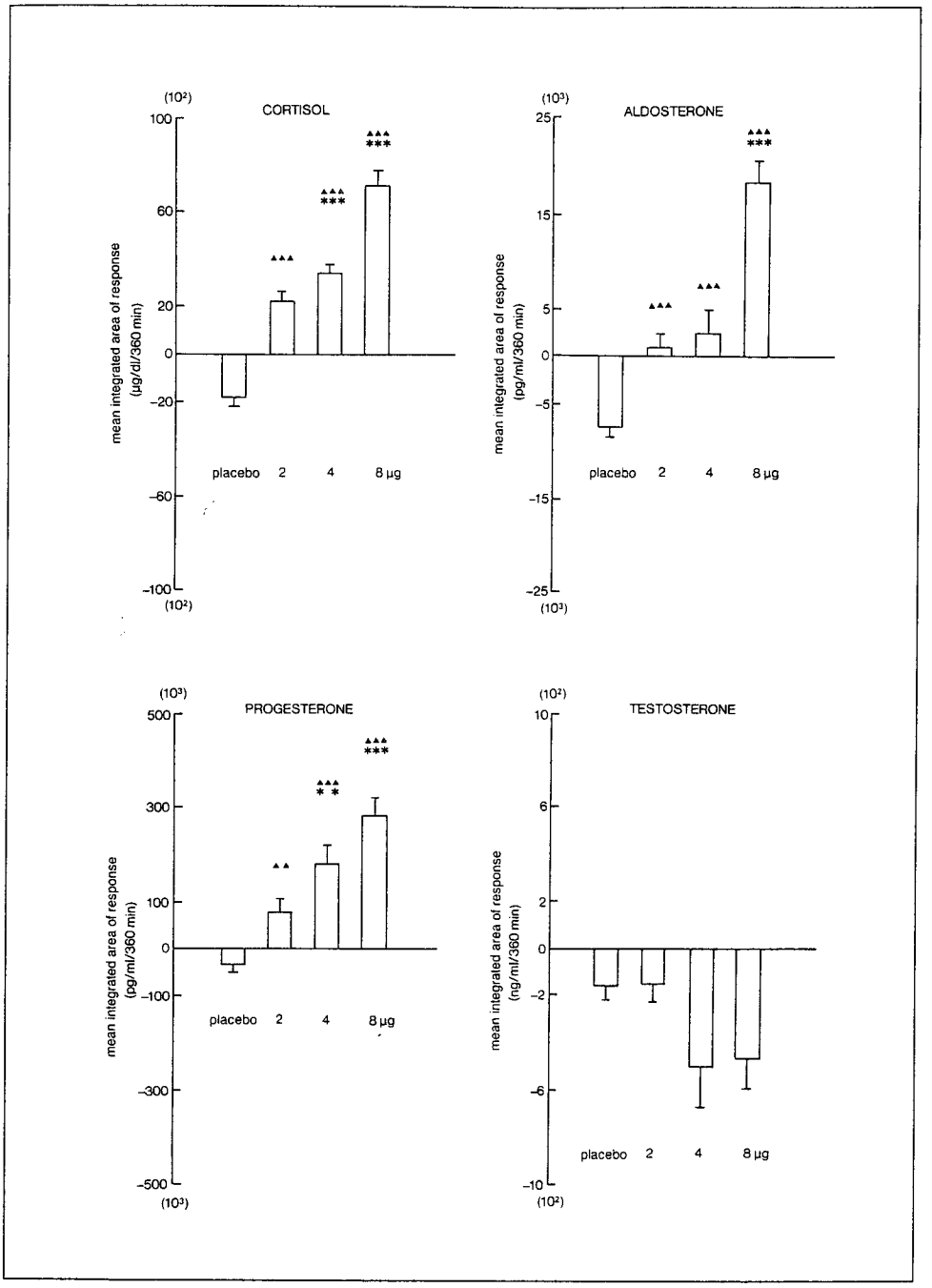
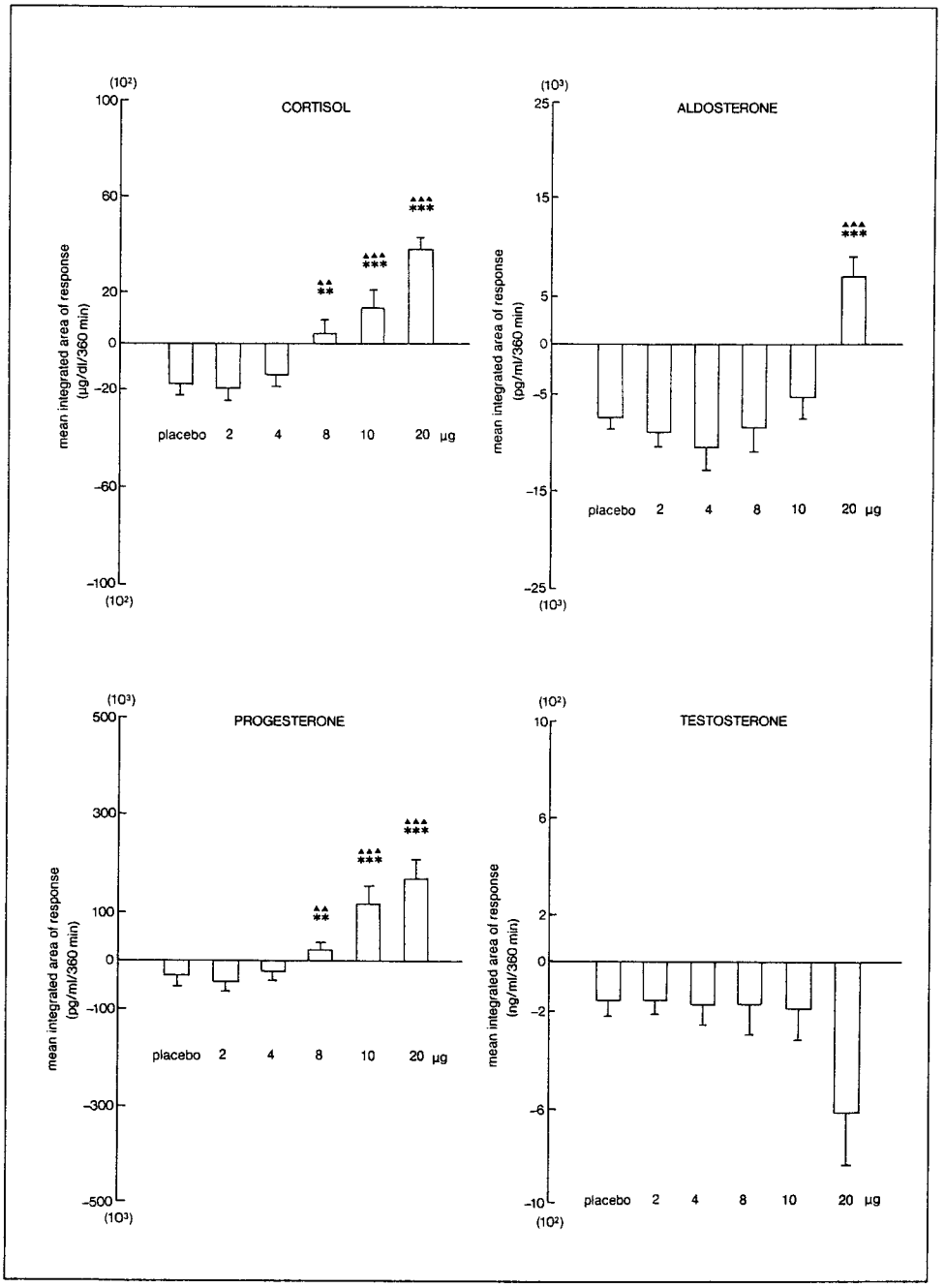


Fig. 2 - Effects of increasing doses of alsactide (ACTH 1-17) injected s.c. at about 07^{15} on plasma steroid concentrations in adult male volunteers. Ten subjects underwent injections of placebo (isotonic saline) or 2, 4, 8 and 20 µg alsactide; 6 subjects received also a 10-µg alsactide injection. Columns indicate the mean (± 1 SE) maximum absolute increment above the basal value.



▲▲ and ▲▲▲ = $p<0.01$ and $p<0.001$, respectively, *vs.* placebo; ** and *** = $p<0.01$ and $p<0.001$, respectively, *vs.* the immediately lower dose.

Fig. 3 - Effects of increasing doses of alsactide (ACTH 1-17) injected i.v. at about 07^{15} on plasma steroid concentrations in adult male volunteers. Ten subjects underwent injections of placebo (isotonic saline) or 2, 4 and 8 µg alsactide. Columns indicate the mean (± 1 SE) integrated area of response, calculated from concentrations measured for 360 min after injection.



▲▲ and ▲▲▲ = $p<0.01$ and $p<0.001$, respectively, *vs.* placebo; ** and *** = $p<0.01$ and $p<0.001$, respectively, *vs.* the immediately lower dose.

Fig. 4 - Effects of increasing doses of alsactide (ACTH 1-17) injected s.c. at about 07^{15} on plasma steroid concentrations in adult male volunteers. Ten subjects underwent injections of placebo (isotonic saline) or 2, 4, 8 and 20 µg alsactide; 6 subjects received also a 10-µg alsactide injection. Columns indicate the mean (± 1 SE) integrated area of response, calculated from concentrations measured for 360 min after injection.

gressive rise of the values, but the differential sensitivity of the mineralocorticoid line and the linearity of the dose-response ratio for cortisol and progesterone were more apparent when data were expressed as integrated area of response than Δmax or Δ%max (maximum percentage change) (figs 2 and 4).

The i.v. injections of 2, 4 and 8 μg alsactide were all effective; the highest dose did cause a sustained rise of plasma cortisol, progesterone and aldosterone, but also the other doses were able to stimulate an appreciable mineralocorticoid secretion (figs 1 and 3). Notwithstanding the considerable interindividual variability, mean testosterone levels after 4 and 8 μg i.v. injections were lower than after placebo injection, without progression towards a more pronounced effect by doubling the dose of the analogue. Plasma testosterone behaved roughly in the same way after 20 μg s.c. as well as after 4 and 8 μg i.v. alsactide injections (figs 3 and 4).

Finally, it is to be said that none of the subjects of our series experienced any unpleasant side effect after administration of the analogue at the tested doses. Heart rate and blood pressure were also unaffected.

DISCUSSION

The comparison of responses obtained using two different routes of administration together with previously published data[4, 11] allows to say that acute injections of 8-10 μg s.c. or 1-2 μg i.v. alsactide in the morning are equally effective in enhancing transiently the circulating cortisol pool without significant changes, at least in normal adult men, of the aldosterone levels. Higher doses seem to loose the advantage of stimulating selectively the glucocorticoid line.

Our data confirm that human adrenocortical cells are exquisitely sensitive to the N-terminal sequence of ACTH. The analogue used consists of the first (N-terminal) 17 amino acids of the natural ACTH in which serine in position 1 is replaced by β-alanine and arginine in position 17 is replaced by lysine followed by the terminal butylamide group. It is assumed that the prolonged action depends on the delayed degradation[17], but a peculiar behavior at the target cell level cannot be excluded. In any case, the overall time courses of the steroid responses, the differences between the two routes of administration and the linearity of the dose-response ratio for cortisol in the range close to the adrenocortical threshold sensitivity were in agreement with previous data on other synthetic ACTH preparations[10, 15, 21, 22, 33]. More specifically, we have confirmed in humans the observations of SANDOW et al.[29], who found that i.v. injection of alsactide in rats was 8-10 times more effective than the s.c. injection. In humans too it appears that the effectiveness of s.c. administration of the analogue is roughly comparable with that of the intramuscular (i.m.) administration (CAVAGNINI, personal communication).

Relevant additional considerations regard the observed effects on plasma progesterone and testosterone. Plasma progesterone could be considered a very sensitive marker of adrenal steroidogenesis in the adult male; changes of the hormone after dexamethasone inhibition and gonadotropin stimulation need reevaluation in order to better assess the testicular contribution to the circulating pool. As regards testosterone, an appropriate timing of administration of the analogue was not associated with deleterious effects on the Ley-

dig's cell function. Only a slight accentuation of the downward diurnal slope of plasma testosterone was observed in our subjects. Also REINBERG et al.[27] did not find an inhibitory effect upon testosterone secretion after i.m. injection of 100 μg alsactide. On the contrary, injection at 07^{00} was followed by a transient rise of the androgen levels; this issue is however controversial[28, 31]. The results of recent observations are compatible with the view that the HPA axis has a dual effect on plasma testosterone, i.e. physiological cortisol concentrations could act as a synchronizer for the circadian rhythm of testosterone and favour a higher release from the Leydig's cells in the morning hours, while supraphysiological concentrations could inhibit the testicular function[3, 23]. If this is true, then the use of the analogue alsactide could be beneficial in cases with impaired testicular function as a result of dose and timing of administration, as suggested by an encouraging preliminary trial[24].

Manipulation of the endogenous rhythmicities with the aim to gain tolerance of an exogenous substance (e.g., an antimitotic drug) is an important area of the chronobiological research. Not surprisingly, some of the most valuable results have been obtained in mice with the use of alsactide[9, 19, 30]. Thus, it was logical to think that an adequate function of the HPA axis along the circadian cycle could be important in the tolerance of oncostatic chemotherapy[20]. Some attempts have been made in this perspective by using ampoules containing 100 μg of the i.m. injected analogue; since a few years, this dosage form is commercially available (Synchrodyn® 1-17) as a diagnostic tool to test the adrenocortical function in patients with potential adrenocortical insufficiency or as a drug for corticotrophic therapy. Beneficial effects of the morning injection of alsactide were noted with regard to the side effects associated with the administration of cytotoxic drugs in studies performed in patients with advanced cancer[6, 26].

The consistently lower doses that were demonstrated to enhance selectively the morning plasma cortisol (e.g., the s.c. injection of 10 μg alsactide) will allow the routine use of repeated daily administrations in a large number of individuals. We suggest application in elderly subjects and patients undergoing oncostatic chemotherapy, who conceivably are more prone to rhythm desynchronization as a consequence of disease and/or treatment.

SUMMARY

Plasma cortisol, progesterone, testosterone and aldosterone levels were measured on serial blood samples drawn in 10 healthy adult human males up to 6h after single administration at about 07^{15} of increasing amounts of the short-chain analogue ACTH-agonist alsactide (Synchrodyn® 1-17). The following doses were employed: 2, 4, 8, 10 and 20 μg subcutaneously (s.c.), as well as 2, 4 and 8 μg intravenously (i.v.). Data were compared with those obtained by placebo (isotonic saline) injections. The s.c. injections of 2 and 4 μg resulted to be ineffective in changing the hormonal pattern. A significant rise of cortisol and progesterone, but not of aldosterone and testosterone, followed the s.c. injections of 8 and 10 μg. The differential pattern of the glucocorticoid *vs.* the mineralocorticoid response was also apparent after the s.c. injection of 20 μg alsactide; when compared with placebo, this dose was able to elicit a significant increase of all examined hormones except testosterone. All i.v. injections of 2, 4 and 8 μg alsactide were effective; the highest dose did cause a sustained rise of plasma cortisol, progesterone and aldosterone, but also the other doses were able to change significantly the mineralocorticoid levels. These results provide evidence that circadian-stage-specified s.c. or i.v. administration of the analogue can be employed in the clinical practice for enhancing selectively and transiently the morning glucocorticoid secretion.

REFERENCES

1. ANGELI A.: Glucocorticoid secretion: a circadian synchronizer of the human temporal structure - J. Steroid Biochem. *19*, 545, 1983.
2. ANGELI A., CARANDENTE F., DAMMACCO F., MARTINI L.: Progress in chronophysiology of peptide hormones with emphasis on clinical application of analogues - Chronobiologia *12*, 293, 1985.
3. ANGELI A., GATTI G., CAVALLO R., CARIGNOLA R.: Chrononeuroendocrinology of the hypothalamo-pituitary-adrenal system and testicular function. In: PANCHERI P., ZICHELLA A. (Eds): Biorhythms and stress in the pathophysiology of reproduction. Hemisphere Publ. Co., New York, 1988. (In press).
4. ANGELI A., PACCOTTI P., ORLANDI F., GAIDANO G., CERESA F.: Differential patterns of plasma cortisol and aldosterone following stimulation with increasing doses of the synthetic analogue (β-Ala^1,Lys^{17}) ACTH 1-17-4-amino-N-butylamide - Hormone metabol. Res. *13*, 24, 1981.
5. BARTTER F. C., DELEA C. S., HALBERG F.: A map of blood and urinary changes related to circadian variations in adrenal corticoid function in normal subjects - Ann. N.Y. Acad. Sci. *98*, 969, 1962.
6. BATTELLI T., MATTIOLI R., MANOCCHI P., GIUSTINI L., GINNETTI A., BURATTINI L.: ACTH 1-17 effects in oncology - La Ricerca Clin. Lab. *14*, 205, 1984.
7. BROWN H., HALBERG F., HAUS E., LAKATUA D., BERG H., SACKETT L., MELBY J., WILSON T.: Circadian-stage-specified effects of a synthetic short chain ACTH-1-17 (HOE 433) on blood leukocytes and corticosterone secretion in mice - Chronobiologia *7*, 21, 1980.
8. BUREAU J. P., GARRELLY L., COUPÉ M., LABRECQUE G.: Circadian rhythm studies on BCG-induced migration of PMN in normal and adrenalectomized mice - Ann. Rev. Chronopharmacol. *1*, 333, 1984.
9. CARANDENTE F.: Heptadecapeptide ACTH 1-17: the first analogue in chronobiology - La Ricerca Clin. Lab. *14*, 111, 1984.
10. CERESA F., ANGELI A., BOCCUZZI G., BISBOCCI D., FRAIRIA R.: Caratteristiche della risposta cortisolemica iniziale dopo stimolazione endovenosa impulsiva con corticotropina sintetica β 1-24 - Boll. Soc. ital. Biol. sper. *47*, 122, 1971.
11. CERESA F., PACCOTTI P., ORLANDI F., TABASSO R., ANGELI A.: Evaluation d'une nouvelle corticotrophine de synthèse: (β-Ala^1,Lys^{17}) ACTH 1-17-4-amino-N-butylamide - Ann. Endocrinol. (Paris) *41*, 54c, 1980.
12. DALLMAN M. F., ENGELAND W. C., ROSE J. C., WILKINSON C. W., SHINSAKO J., SIEDENBURG F.: Nycthemeral rhythm in adrenal responsiveness to ACTH - Amer. J. Physiol. *235*, R210, 1978.
13. DALY J. R., EVANS J. I.: Daily rhythms of steroid and associated pituitary hormones in man and their relationship to sleep - Advanc. Steroid Biochem. Pharmacol. *4*, 61, 1974.
14. DAMMACCO F., CAMPOBASSO N., ALTOMARE E., IODICE G.: Analogues in immunology - La Ricerca Clin. Lab. *14*, 137, 1984.
15. DURAN F., TORRES J., CHARRO A., ORIOL BOSCH A.: The plasmatic 11-hydroxycorticosteroid response in the human to a synthetic octadecapeptide corticotropin (C-41795 Ba) - Hormone metabol. Res. *7*, 499, 1975.
16. FERRARI E., BOSSOLO P. A., CARNEVALE SCHIANCA G. P., SOLERTE S. B., FIORAVANTI M., NASCIMBENE M.: Adrenocortical responsiveness to the synthetic ACTH 1-17 analogue given at different circadian stages - Chronobiologia *9*, 133, 1982.
17. GEIGER R., SCHRÖDER H. G.: New short-chain corticotrophin analogues with high corticotrophic activity. In: LANDE S. (Ed.): Progress in peptide research. Gordon and Breach, New York-London-Paris, 1972; vol. 2, p. 273.
18. HALBERG F.: Some physiological and clinical aspects of 24-hour periodicity - J. Lancet (USA) *73*, 20, 1953.
19. HALBERG F., HALBERG E., HEROLD M., VECSEI P., GÜNTHER R., REINBERG A.: Toward a clinospectrometry of conventional and novel effects of ACTH 1-17 Synchrodyn® in rodents and human beings. In: TAKAHASHI R., HALBERG F., WALKER C. (Eds): Toward chronopharmacology. Proc. of the 8th IUPHAR Congress and Satellite Symposia, Nagasaki, July 27-28, 1981. Pergamon Press, Oxford and New York, 1982; p. 119.

20. HRUSHESKY W. W. M.: The clinical application of chronobiology to oncology - Amer. J. Anat. *168*, 519, 1983.

21. KEENAN J., THOMPSON J. B., CHAMBERLAIN M. A., BESSER G. M.: Prolonged corticotrophic action of a synthetic substituted 1-18 ACTH - Brit. med. J. *ii*, 742, 1971.

22. LANDON J., JAMES V. H. T., WHARTON M. J., FRIEDMAN M.: Threshold adrenocortical sensitivity in man and its possible application to corticotrophin bioassay - Lancet *ii*, 697, 1967.

23. LEJEUNE-LENAIN C., VAN CAUTER E., DESIR D., BEYLOOS M., FRANCKSON J. R. M.: Control of circadian and episodic variations of adrenal androgen secretion in man - J. endocrinol. Invest. *10*, 267, 1987.

24. MARRAMA P., CARANI C., CELANI M. F., BARAGHINI G. F., CAVANI D., MONTANINI V.: ACTH 1-17 effects in psychogenic impotence - La Ricerca Clin. Lab. *14*, 233, 1984.

25. MINORS D. S., WATERHOUSE J. M.: The kidney and hormones affecting it. In: Circadian rhythms and the human. John Wright and Sons, Bristol-London-Boston, 1981; p. 68.

26. PIAZZA E., CATTANEO M. T., COLOMBO F., LIBRETTI A.: ACTH analogue application to oncostatic chemotherapy. A double blind approach - La Ricerca Clin. Lab. *14*, 257, 1984.

27. REINBERG A., DUPONT W., TOUITOU Y., LAGOGUEY M., BOURGEOIS P., TOUITOU C., MURIAUX G., PRZYROWSKY D., GUILLEMANT S., GUILLEMANT J., BRIÈRE L., ZEAU B.: Clinical chronopharmacology of ACTH 1-17 (Synchrodyn® 1-17). II. Effects on plasma testosterone, plasma aldosterone, plasma and urinary electrolytes (K, Na, Ca and Mg) - Chronobiologia *8*, 11, 1981.

28. RIVAROLA M. A., SAEZ J. M., MEYER H. E., MIGEON C.: Metabolic clearance rate and blood production rate of testosterone and androst-4-ene-3,17-dione under basal conditions, ACTH and HCG stimulation - J. clin. Endocrinol. *26*, 1208, 1966.

29. SANDOW J., GEIGER R., VOGEL H. G.: Pharmacological effects of a short chain ACTH analogue - Naunyn-Schmiedeberg's Arch. exp. Pathol. Pharmakol. *297* (Suppl. 2), R41, 1977.

30. SCHEVING L. E., TSAI T. H., PAULY J. E.: Chronotoxicology and chronopharmacology with emphasis on carcinostatic agents - Ann. Rev. Chronopharmacol. *2*, 177, 1986.

31. SORCINI G., SCIARRA F., CONCOLINO G., RASCIO L.: Effect of ACTH on plasma testosterone in normal human subjects - Folia endocrinol. (Roma) *16*, 449, 1983.

32. UNGAR F., HALBERG F.: Circadian rhythm of the *in vitro* response of mouse adrenal to adrenocorticotropic hormone - Science *137*, 1058, 1962.

33. VIERHAPPER H., WALDHÄUS L. W.: Comparison of the corticotropic action of two synthetic, substituted analogues of ACTH: ACTH 1-17 and ACTH 1-18 - J. clin. Endocrinol. *47*, 208, 1978.

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