The clinical pharmacology of biotechnology products
M M Reidenberg, editor

POTENTIAL USE OF PEPTIDE HORMONES IN SPORT

Jordi Segura, Rafael de la Torre and Roser Badia
Department of Pharmacology and Toxicology, Institut Municipal d'Investigació Mèdica, Passeig Marítim 25-29, 08003 Barcelona (Spain)

INTRODUCTION

The misuse of hormonal agents in sport dates back to the decade of 1960-70 where androgen-anabolic and corticoid steroids were known to be administered to athletes. These agents have continued to be used in sport although nowadays the percentage of steroids detection in sportsmen subjected to urine control is slightly declining. This is probably the result of educational and regulatory efforts but it could also be an indicator of changing trends in pharmacological abuse.

Advances in physiology and biochemistry have allowed to increase the knowledge on how the ultimate hormones are synthesized and regulated. The existence of hypophyseal and hypothalamic peptide factors is recognized together with the complicated mechanisms for the control of their blood and tissue concentrations. Human growth hormone is another hypophyseal peptide substance whose activity on the adult sportsmen has not been deeply substantiated but it is believed by some people to contribute to increase strength and endurance. The potential use of biologically active proteins and peptides has increased after the recent suggestion of sophistication in blood doping as is the misuse of erythropoietin in order to increase the number of red blood cells in athletes [1].

The availability of biological peptides has been relatively reduced until recently because the need for elaborated extraction procedures from natural sources or the complicated chemical synthetic procedures only available for small molecules. The introduction of DNA recombinant techniques is beginning to change the sources for obtaining such compounds. The misuse of such substances in healthy people will generate unknown problems to the delicate hormonal equilibrium of the human body. Taken all this into account the International Olympic Committee has included the peptidic hormones and analogs either from synthetic or recombinant origin among the list of forbidden substances in sport. This chapter presents a report about those hormones that may be potentially misused. Those that are of non-recombinant origin nowadays may become it in a few time.

ACTION ON GLUCOCORTICOID METABOLISM

Adrenocorticotropic hormone ACTH

Cortisol is a glucocorticoid synthesized in the adrenals which allows the body to face uncommon and stressful situations by affecting a number of reactions of intermediate metabolism. The

development of physical exercise is one of the stimulus that results in an increase of serum cortisol. Psychic pressure is known also as a factor resulting in increased cortisol levels. Recent research [2] has demonstrated that high level sport competitions makes the athlete to be confronted to this double mechanism in cortisol secretion. The results reported in Figure 1 demonstrated that only the competition, but not a normal training nor an exhaustion treadmill test generates the

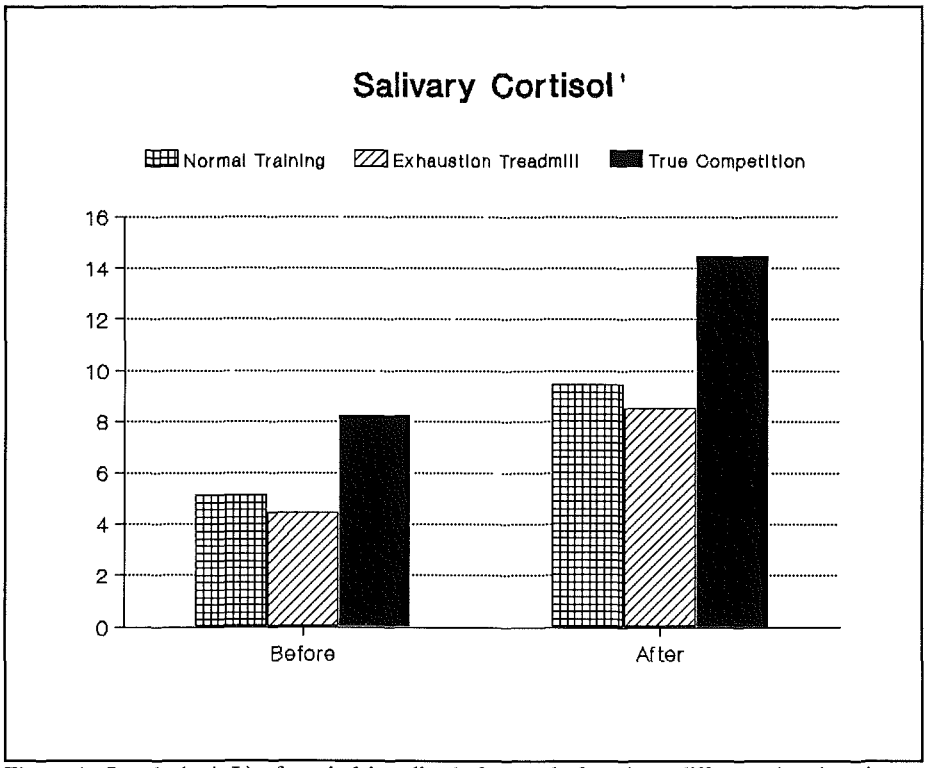

Figure 1.- Levels (ng/mL) of cortisol in saliva before and after three different situations in top level swimmers

high cortisol response. Thus the possibility exists of artificially increasing cortisol levels, specially in pre-competition situations, to artificially habituate the athlete to an actual competition setting.

Synthesis of cortisol by the adrenal cortex is stimulated by ACTH, a polypeptide hormone secreted by cells of the anterior pituitary gland. Thus the exogenous administration of ACTH might be used to further stimulate cortisol secretion overriding feed-back inhibition or just generating an increase of cortisol over normal values. In either case it would be a non-physiological use of corticotropic hormone.

Human ACTH is composed of 30 aminoacids with a molecular weight of 4500. Actual sources of ACTH include extraction from animal pituitaries by chromatographic or electrophoretic techniques and the chemical synthesis of the entire human ACTH polypeptide. However, not all the aminoacid structure is needed for biological activity. The synthetic analog of the ACTH(1-24) is named Cosyntropin and is currently used by intramuscular or intravenous injection of about 0.25 mg for diagnostic purposes. ACTH(1-17) is also available.

Corticotropin-releasing hormone (CRH)

In addition to the direct negative feed-back mechanism, the secretion of ACTH is regulated by the CRH formed in the hypothalamus and released to the hypothalamic portal venous system, thus reaching the pituitary corticotropic cells. The structure of this factor (a 41 aminoacids polypeptide) was known in 1981. It is presently used for diagnostic purposes but it could be misused to artificially trigger the release of ACTH and consequently cortisol. It is obtained by isolation and purification from bovine hypothalami. Its synthesis by recombinant techniques might be developed in the near future.

ACTION ON ANDROGEN SECRETION

Gonadotropins

Luteinizing hormone (LH) and follicle stimulating hormone (FSH) are glycoproteins synthesized by the gonadotropes of the anterior pituitary gland. Human chorionic gonadotropin (HCG), also a glycoprotein, is synthesized by the placenta. Each glycoprotein is made of two dissimilar, glycosylated polypeptide chains, alpha and beta subunits, linked by hydrogen bonds. The alpha subunit is essentially common to LH, FSH, HCG and TSH (Thyroid-Stimulating hormone) and comprises 92 aminoacids . The beta subunit identifies the appropriate target tissue and confers their biological activity. The beta subunit has 121 aminoacids for LH,118 aminoacids for FSH and 145 aminoacids for HCG [3]. HCG beta-chain have a structural similarity with the beta-chain of LH; 97 aminoacids of HCG beta-chain are identical to those of beta-LH [4]. HCG has higher sugar content than LH, and has a longer half-life. The molecular weight of gonadotropins depends on carbohydrate composition and is about 30000 for LH, 36000 for FSH and 65000 for HCG.

HCG is not only produced by placenta. Trophoblastic tissues which can be found in hydatidiform mole, choriocarcinomas and germ cell tumors of the testes also produce HCG. It is also found in solid tumors of the ovaries, uterus, breast, lung, pancreas and stomach [5 6]. HCG can not be considered a foreign substance in the male, but the amounts found in normal men are minute. A recent study [7] concludes that HCG is produced in a pulsatile fashion, probably by the pituitary, in all normal adults. The amount found in serum samples from normal men averaged 8.9 pg/mL (range 3.0-160 pg/mL) (biologic potency 13.450 IU/mg).

Due to the similarity between HCG and LH beta chain, HCG injected in a male may bind to the LH receptors in the Leydig cell and stimulates the biosynthesis and excretion of testicular steroids, increasing testosterone and epitestosterone level. Brooks et al. have reported [8] that a single injection of testosterone heptanoate (Primoteston), 250 mg, produced a 3-5 fold increase in the plasma testosterone concentration but it declined fairly rapidly. Intramuscular injection of 5000 IU HCG, for four days, increases 2 to 3 fold testosterone level at the end of this period. With administration of HCG the maximum concentrations of testosterone in plasma were not as high as those obtained after Primoteston, although the rise was quite well sustained. This study also showed that HCG can be found in the urine at concentration above the cut-off limit for as long as the concentration of testosterone in the plasma is raised above the basal concentration.

Gonadotropin releasing hormone

(GnRH), also known as luteinizing hormone releasing hormone (LHRH), is a decapeptide that has a molecular weigh of 1182 and hat is synthesized by neurons in the brain. It is released from nerve terminals and is carried to the anterior pituitary by the hypophyseal portal vessels, where it stimulates the production and release of both LH and FSH. GnRH half-life is only of five minutes because is quickly metabolized by endopeptidases.

GnRH Analogs differ from the natural hormone in some peptide positions [9] [10]. They have more affinity for the hypophysis receptors and more stability in front of enzymatic degradation. Agonists analogs release more gonadotropins and in a more lasting time than natural hormone. Depending on the dose and way of administration of GnRH or its analogs, a stimulation or an inhibition in the pituitary is produced [11]. A pulsatile exogenous administration of GnRH increases gonadotropin production, while a continuous administration of GnRH inhibits gonadotropin release.

Table 1.- Some Gonadotropin-related pharmaceutical Products

Chorionic Gonadotropin (from the urine of pregnant women)

Menotropin (LSH and FSH from urine of postmenopausal women)

Gonadorelin (synthetic Gonadotrophin releasing hormone)

Gonadotropin releasing hormone Analogs
- Buserelin Acetate
- Goserelin Acetate
- Leuprorelin Acetate
- Nafarelin Acetate
- Triptorelin

HCG components in urine

Several authors have identified different immunoreactive HCG components in urine [5]: Intact HCG, beta-subunit, alpha-subunit and fragments of the beta-subunit. There is a variability in sialylation and glycosylation such that the same polypeptide species may appear in several different molecular weight fractions.

Immunological methods of detection

Several immunological methods have been developed for the detection and quantitation of gonadotropins. Due to its structural similarity of alpha-subunit, exclusively antisera against the LH, FSH, HCG or TSH beta-subunit gives specificity to the method. The homology between LH and HCG beta-chain can result in cross-reactivity. In serum, proteins that bind to gonadotropins specific antibodies may cause aberrant test results [5].

The use of monoclonal antibodies has the advantage of very high specificity. However, they may miss some molecules with either slight modifications, denaturation, or steric hinderance at the specific binding site. The result is a diminution of sensitivity. Polyclonal antibodies react with more sites on the molecule, which should provide increased sensitivity and could result in detection of more types of fragments and metabolites. A disadvantage of the polyclonal systems may be a small increase in their ability to bind substances with structural homologies. So, test with polyclonal antibodies are less specific than test with monoclonal antibodies.

A great variety of immunoassays which used different labels such as radioisotopes, fluorescent molecules, enzymes or others are currently available. Also, there are immunoassays carried out in solution or in gel, with liquid phase or solid-phase reagents and so on [4]. A comparative study carried out by Brooks et al. [8] between various disposable commercial kits: Serono MAIA clone Immunoradiometric assay (IRMA), Serono Serozyme Enzymoimmunoassay (EIA) and Boehringer Enzyme linked immunoassay ELISA, showed a good performance of all of them. The specificity is ensured by three high affinity monoclonal antibodies to both the intact HCG molecule and to the beta subunit. The cut-off limit has been set far in excess of the level found in male urines from subjects who have not been given HCG.

Stenman et al. [12] had described a combination of chromatography with immunoassay method. It could be used in doping control [8]. The method uses gel chromatography on a TSK HW-50 (S) column and radioimmunoassay of the fractions using LH and HCG antisera.

GROWTH HORMONE AND ITS RELEASING FACTOR

Chemistry and Physiology

Growth Hormone (GH)

GH is an anterior pituitary hormone that has a molecular weight of 21000 and comprises 190

aminoacids in a simple chain with two intrachain disulphide bridges. GH acts throughout the body to stimulate the longitudinal growth of bones and to regulate glucose, amino acid, and fatty acid metabolism. Many of the actions of GH are mediated by other hormones released from the liver called somatomedines, or insulin-like growth factors [13].

Growth hormone release is controlled by two hypothalamic factors: the growth hormone releasing factor (GHRH), which stimulates GH secretion, and somatostatin, which inhibits GH secretion. The GH-RH molecule consist of 44 aminoacids. Other isolated shorter fragments 1-37 and 1-40 are also biologically active. A number of metabolic factors influence the secretion of GH. Increased plasma levels of substances such as glucose, non-sterified fatty acids and ketone bodies inhibit GH release, whereas hypoglycemia stimulates GH secretion. Consistent with these observations is the elevation of GH secretion during strenuous exercise.

Growth hormone in use in therapeutics must be from human origin because other animal sources are biologically inactive. The source of GH used in clinical situations in the past has been cadaver pituitaries. Severe controls are needed to reduce bacterial and viral contamination of preparations of GH from human pituitaries. So, in some countries GH preparations from recombinant DNA techniques are preferred. Growth hormone preparations from recombinant DNA methodologies are produced after the insertion of the GH messenger RNA gene in a plasmid and its expression either in procaryotes (E. coli, Somatrem) or mammalian cell lines [14].

Therapeutic use of GH-RH is mainly restricted to dwarfism of hypothalamic origin. Little is known about side effects in normal adults and mimetically we associate those related with clinical situations where there is an hypersecretion of GH. Side effects of hypersecretion of GH in adults are those associated with acromegaly. The most prominent are myopathy, peripheral neuropathy, coronary artery disease and cardiomyophaty [15].

Table 2.- Growth Hormone/Growth Hormone-Releasing Hormone Preparations

1.-Growth Hormone Preparations [16 17]

Somatropin Refers to preparations of GH with the same sequence of the natural human GH either pituitary-derived or synthesized by means of DNA recombinant techniques.

Somatrem Refers to a synthetic methionyl analogue of GH.

2.-Growth Hormone-Releasing Hormone Preparations [16]

Somatorelin Refers to preparations of GH-RH with the same sequence of the natural human GH-RH either hypothalamic-derived or synthesized by means of DNA recombinant techniques. Usually the DNA recombinant preparation consist on the 1-40 aminoacid sequence of the naturally occurring GH-RH.

Sermorelin Refers to a synthetic version of GH-RH with the first 29 aminoacids of its natural sequence.

Pharmacokinetics

After the administration of recombinant GH by the intravenous route the mean half-life is 9 minutes [18] although some reports presents longer half-lives estimations (around 25 minutes). Maximal effects are reached between 3 and 5 hours depending on the administration route (3 hours IM, 4-5 hours S.C.) [18 19]. When interpreting these pharmacokinetic data, we must take into account that some effects of GH are mediated by hepatic somatomedines (half life between 3 and 4 hours) and that the effects last for longer periods of time. Reports show that recombinant GH is safe and well tolerated by the intravenous, intramuscular and subcutaneous routes in short term periods [20]. The preparations somatropin and somatrem are bioequivalent [21].

Most of the pharmacokinetic data of GH-RH preparations have been evaluated on the basis of they maximal effects on GH secretion. Different administration routes have been assayed including the intranasal, intravenous and intramuscular. Maximal effects appear between 15 and 40 minutes after the administration irrespectively of using sermorelin or somatorelin [22 23 24]. Both preparations are bioequivalent [24].

Assessment of GH and GH-RH in body fluids

1.-Assay

Radioimmunoassay (RIA) and other non-isotopic immunological techniques have replaced bioassays and are routinely used in the assessment of GH concentrations in patients sera and for physiological studies on GH secretion [25 26 27 28 29 30].

Important differences have been found between techniques using monoclonal and polyclonal antibodies. Most of these techniques are standardized with GH from pituitary origin but interestingly there was more than a fivefold variation in cross reactivities against dimeric GH from DNA recombinant techniques.

If natural sequences of GH and GH-RH are administered no assay will distinguish between endogenous and exogenous materials because antigenically they are indistinguishable. Short after the administration of these substances levels of GH and GH-RH may be unnaturally high in plasma. At present is not known what kind of changes we can expect in urine. If analogs of GH or GH-RH are administered, there is a chance of developing immunoassays to detect them. Other possibilities are the detection, with very sensitive immunoassays, of antibodies against these substances.

Alternatively it has been proposed to use assays of somatomedine C and the insulin growth

factor 1 as a measure of the degree of exposure to biologically active GH in humans [15,31].

Some techniques using mass spectrometry coupled to high performance liquid chromatography have been used successfully for the characterization of natural and biosynthetic GH, and are promising for a future application in biological fluids [32].

2.-Urinary excretion of GH

Growth hormone and the growth hormone binding protein (GH-BP) can be detected in urine using current immunological analytical techniques [33 34]. The detection of GH-RH in urine has not been reported yet.

Some aspects are still pending before starting urinary controls of GH. In one hand normal values for normal people -but most specifically in highly trained/physically stressed athletes- must be validated. In the other hand we must establish which compounds GH like are being detected in urine. Detection of GH in urine is based on cross reactivities with different antibodies raised against plasma products. Some preliminary results show that compounds detected in urine are not the parent compounds GH and GH-BP but these compounds metabolized to a certain extent [35].

ERYTHROPOIETIN

One way of affecting the blood components is the use of hematopoietic growth factors. Many of them are now available in large quantities through recombinant DNA technology [36,37]. Erythropoietin was the first factor studied and is the only clinically available at present. It acts on the mature red cell precursors to induce their proliferation and differentiation. The concurrent use of erythropoietin with other growth factors such as Interleukin-3 (IL-3) or the Growth factor for granulocytes / macrophages (GM-CSF) might be used in the future to stimulate even those other early developing cell precursors.

The Product

Erythropoietin is a glycoprotein of about 30400 amu whose aminoacid chain has 165 residues (molecular weight 18224 amu). The protein contains 2 disulphide bonds (positions 7-161 and 29-33), 3 asparagine N-glycosylations (positions 24,38 and 83) and 1 serine O-glycosylation (position 126). Glycosylation is not, however, necessary for biological activity but protects the protein molecule from a rapid degradation. Characterization of EPO structure followed the observation of its presence in urine [38] and its subsequents isolation [39].

Present availability of rHuEPO is consequence of cloning and expressing the responsible gen (7q 11-22). Initial synthesis using E.coli cells have been substituted by mammal cells (Chinese Hamster Ovary) in order to afford complete glycosylation. Only a few pharmaceutical companies have

developed the recombinant EPO product. Under license it is being approved in a number of countries.

Physiology

Erythropoietin is produced mainly in the kidney (about 90%). The production of erythropoietin is regulated by the concentration of oxygen in the blood. EPO stimulates the final differentiation of committed erythroid progenitors, increases hemoglobin synthesis and promotes the release of marrow reticulocytes into the circulation.

Main clinical indication of rHuEPO is for those anaemias due to low EPO availability such is the chronic renal failure [40]. Experiences in other kind of anaemias of inflammatory or neoplastic origin is being accumulated. It has also been suggested to use EPO for increasing the number of erythrocytes for those patients to be bled and to be further autotransfused. A similar misuse in sport would generate a double incorrect process (EPO+blood doping).

Hypertension is the major problem in EPO treated patients. In fact, increase in blood pressure has also been observed in healthy people treated with EPO [41]. Some other side effects described in treated patients are seizures and increments in serum potassium and urea and in the thrombocyte count (clotting may be at increased risk). An unexplained influenza-like syndrome related to EPO administration has rarely appeared.

Pharmacokinetics

A single dose application to patients by intravenous route resulted in a serum half-life of 5.4 hours (dose 50 UI/Kg) or 7.6 hours (dose 150 UI/Kg) [42]. Other reports about serum EPO half-life in patients range from 4 to 11 hours [40] [43]. The concentration of serum EPO after subcutaneous administration is many times lower (5-10%) with a concentration peak obtainable between 12 and 24 hours [40] [44].

In spite of the pharmacokinetics described, a dose of 50 IU/Kg 3 times weekly is usually used and further titration for each patient is done according to the respective response. Higher doses may reach around 450 IU/Kg weekly. Once a target hemoglobin level has been reached reductions in dose frequency to twice or even once weekly are possible [40].

Reports from EPO administration to healthy people as it would be the situation in athletes are scarce in the literature. A recent report [45] evaluates the pharmacokinetics after both IV or SC administration in six healthy people. Volume of distribution and half life after iv route were 76 ml/kg and 4.5 hours respectively. Clearance was 64 ml/kg which is slightly higher than in patients, probably because a more efficient urinary excretion in healthy people. However, recent data on renal excretion of rHuEPO in healthy subjects [43] indicate that total urinary excretion represented less than 5% of the dose at all dose levels. Bioavailability by subcutaneous administration was 36% over a period of 72 hours and half-life was estimated to be around 46 hours. Subcutaneous

administration originates smaller fluctuations and a sustained increase over baseline levels.

When there is no EPO administration, basal levels of EPO in serum in healthy people are about 10-50 mU/ml. Under treatment with rHuEPO there appear to be a proportional relationship between serum concentrations and single dose administrations. Levels in the range of 20 mU/ml to 30 U/ml have been reported [43] after intravenous dosages (10-1000 IU/Kg). Levels substantially higher than normal (up to 5-30 U/ml) are found also in acute and aplastic leukemia and in myelodisplastic disorders [46].

Effects on exercise related variables

a) Patients

Since initial trials with hemodialysis patients [47] it was clear that in addition to correct anaemia, exercise capacity and perhaps cardiac function could be improved by EPO treatment. A multicenter,placebo controlled, clinical trial with hemodialysis patients where functional capacity was measured on a treadmill [48] lead to the conclusion that EPO causes a marked statistically and clinically significant improvement towards fatigue and physical symptoms. EPO appeared to cause an improvement in exercise capacity, but the magnitude of the change was not as great.

b) Athletes and healthy people

With regard to EPO in healthy adults there is little clinical information available. Main data come from a study performed at the Karolinska Institute [41] (to be published) where young gymnastics were treated with either rHuEPO, autologous blood or placebo [49]. In regards to the reponse elicited by EPO [41], an increment of oxygen capacity and endurance to treadmill running was observed. Hemoglobin content and the hematocrit was also increased but not the total blood volume. Maximum aerobic power returned to normal after 2 weeks of cessation of EPO treatment.

Detection of rHuEPO

Theoretically there appear to be different ways for trying to identify the misuse of rHuEPO in sport although none of them has been successfully applied to date. Some of them are listed below:

a) Speculations about slight differences between the recombinant product and the natural hormone are the base for the suggestion of finding specific antibodies to the recombinant product as an index of previous use. So far no antibodies to the artificial product have been found in patients receiving the product [50].

b) Detection of specific modifications of the red cell biochemistry and morphology that may follow EPO treatment [51].

c) To request regulatory bodies to ask companies involved in producing rHuEPO to add an

internal marker compound to the marketed products [52].

d) Detection of urinary excretion of rHuEPO. Obviously some critical quantitative cut-off point after population studies would be needed to distinguish, if possible, excretion of exogenous rHuEPO from normal endogenous EPO excretion because no biochemical distinction is expected.

e) Determination of the blood EPO concentrations. Higher than normal values may be found in rHuEPO users, but only for a few hours after intravenous, and for a few days after subcutaneous administration [51]. Unambiguous cut-off distinction would be needed as stated above. Traditional research methods to detect EPO in biological samples are based in bioassays. The in vivo bioassays [53] are the most reliable assays for biologic EPO activity and they should be used as reference to compare immunoreactivity and bio-activity of any other methodology [54]. In vitro bioassays [53] appear to have too many pitfalls (i.e. false positive reactions) that render them inappropriate for their application [54].

The developments of immunological methods will allow routine determination of blood EPO in the near future. The limit of detection for some RIA assays [45 46 55 56] range from 2-10 mU/ml with coefficients of variation around 6-7%. The alternative use of non isotope labeling is used in recently developed ELISA assays [57 50] with similar technical characteristics.

MISUSE IN SPORT

Gonadotropins

The abuse of testosterone may be detected by measuring testosterone to epitestosterone ratio (T/ET) with the established GC/MS techniques [58]. But this ratio remains normal after HCG abuse. In 1986, 42 samples were screened using T/LH ratio [8]. Two of these samples, that come from male, were found to have low T/LH ratio suggesting that HCG might have been administered. Assayed by the MAIA clone method for HCG, these two samples were found to have values of 242 and 154 IU/L, while the normal range for this method is 1.2-3.9 IU/L.
In 1987, 740 male sport urines were tested by this method and 21 positive urines having HCG concentrations in the range 30-119 IU/L were found [8]. In 1988 one HCG positive urine had been reported and 3 cases have been reported in 1989.
A recent study [59] suggests that HCG could be used as a masking agent after testosterone abuse, if only urinary T/ET ratio is used as a doping criterion. This ratio remains normal in individuals injected with testosterone coupled with HCG. T/LH ratio appears effective as an additional marker for the detection of testosterone coupled with HCG doping.
Since now no case of GnRH or its analogs has been reported.

Growth hormone

The ergogenic properties of growth hormone have been the basis of its use in sports. Even if there are some reports that show an increase in muscular development after GH administration,

this muscular hypertrophy is not accompanied by an increase in strength. Despite the fact that any scientific report has been able to demonstrate an increase in athletic performance its consumption is becoming widespread within athletes that believe that with the current doping control procedures it cannot be detected.There are anecdotal reports of misuse of GH in sports. Some cases of athletes with early signs of acromegaly have been detected [15 60].

There are some reports that suggest that GH is used in very short periods of time at the end of a cycle of treatment with anabolic steroids. A synergistic effect between anabolic steroids and GH and/or GHRH has been proposed.

A new possible form of "GH misuse" is to increase indirectly GH secretion by the ingestion of some drugs and aminoacids [60].

Erythropoietin

The misuse of erythropoietin has not been substantially reported although speculations are abundant among the non technical press [61]. Rumors have been mainly focused on distance runners and cyclists. In fact it appears to have been considered, inter alia, as a potential explanation for some fatalities studied by the Royal Dutch Cycling Federation. Because the ergogenic potential of its misuse, similar to blood doping, some sport federations (i.e. cross country skying) are considering its control by blood analysis.

Final remarks on detection

The control of non-physiological use of peptide hormones in sport is additionally complicated by different factors:

a) They have a very short plasma half-life and therefore they are excreted, if any, in very small quantities in urine (the normal fluids for antidoping analysis). Even the exact chemical structures of the products excreted in urine, coming from metabolism in the body, are not usually known.

b) The majority of analytical techniques are based on radioimmunoassay which means a relative non-specificity of the antibodies used and the possibility of cross-reactivity with endogenous compounds. Different specificity of antibodies or mixtures of them used by different techniques is additionally complicated by their monoclonal or polyclonal origin. Availability of Internationally acceptable Reference Materials in order to standardize results is absent for some of the hormones. Concentrations reported by using a given methodology may not usually be compared to other assays for the same hormone.

c) Even when specifically and sensitively detected it is not easy to establish criteria to distinguish an exogenous administration from a normal endogenous concentration.

d) Finally, the total confirmation of the structure of the peptide or protein detected should ideally be done by mass spectrometry. Only recently new techniques (i.e.electrospray, fast atom bombardment) are becoming potentially available to be used as confirmation assays.

Acknowledgements

The authors especially appreciate the suggestions and informations received from Prof. A.H.Beckett (London). Additional informations received from Prof. F.Conconi (Ferrara), Prof. C.Bauer (Zurich) and Dr. Wieczorek (Mannheim) are also deeply acknowledged.

REFERENCES

1. Wadler G.I.,Hainline B. (1989) in Drugs and the Athlete, F.A.Davis Co., Philadelphia 172-177

2. Aubets J., Torres M., de la Torre R., Camí J., Segura J. (1989), VIIth European Congress in Sports Medicine, Barcelona

3. Stockell A. (1989) Multiple Forms of Pituitary and Placental Gonadotropins. Oxf Rev Reprod Biol 11:147-177.

4. Kiburis J. (1989) IInd I.A.F.World Symposium in Sport. Monte Carlo.

5. Rundell C.A. (1986) Journal of Clinical Immunoassay 9(2):89-95.

6. Hussa R.O. (1981) Ligand Rev 3:6-44

7. Odell W.D., Griffin J. (1987) The New England Journal of Medicine 317(27):1688-1691

8. Brooks R.V., Collyer S.P., Kicman A.T., Southan G.J., Wheeler M.A. (1989) IInd I.A.F. World Symposium on Doping in Sport. Monte Carlo.

9. Schally A.U., Arimura A. Coy D.H. (1981) Vitam Horm 38:257

10. Delfosse M. (1989) J Pharm Belg 44(2):137-142

11. Calaf J., Guinot M., Domingo N., (1988) JANO 34(815):1689-1693

12. Stenman H., Alfthan H., Ranta T., Vartiainen E., Jalkanen J., Seppala M. (1987) J Clin Endocrin Metabol 64:730-736

13. Brown B.L., Forsling M.L., Gould R.P. (1982) In: O'Riordan J.L.H., Malan P.G., Gould R.P. eds. Essentials of Endocrinology. London: Blackwell Sci. Publ.,25-57.

14. Haynes S.P. (1987) In: Belloti P., Benzi G., Ljungqvist A. eds. Official Proceedings. Ist I.A.F. World Symposium on Doping in Sport. Florence,175-92.

15. Wadler G.I.,Hainline B. (1989) in Drugs and the Athlete, F.A.Davis Co., Philadelphia 70-74

16. Martindale, The Extra Pharmacopeia (1989) 29th Edition. The Pharmaceutical Press,London 1148-51

17. Catalogo de Especialidades Farmaceuticas (1990) Consejo General de Colegios de Farmaceuticos eds. Madrid

18. Hindmarsh P.C., Matthews D.R., Brain C.E., Pringle P.J., di Silvio L., Kurtz A.B., Brook C.G. (1989) 30:443-50

19. Ho K.Y., Weissberger A.J., Stuart M.C., Day R.O., Lazarus L. Clin Endocrinol 1989;30:335-45.

20. Sietnieks A., Wilton P. III World Conference on Clinical Pharmacology & Therapeutics. Stockholm,1986:333.

21. Wilton P., Widlund L., Guilbaud O. (1987) Acta Paediatr Scand Suppl 337:118-21

22. Albin H., Auziere J., Bedjaoui A., Angibeau M., Morre M. (1986) III World Conference on Clinical Pharmacology and Thearpeutics. Stockholm, 334.

23. Colle M., Frangin G., Jacob C., Angibeau R., Coude F.X. (1986) III World Conference on Clinical Pharmacology and Toxicology. Stockholm, 314.

24. Vance M.L., Evans W.S., Kaiser D.L., Burke R.L., Rivier J., Vale W., Thorner M.O. 1986 Clin Pharmacol Ther 40:627-33.

25. Evans A.J., Wood P.J. (1989) Ann Clin Biochem 26:353-7

26. Hashida S., Ishikawa E., Mohri Z., Nakanishi T., Noguchi H., Murakami Y. (1988) Endocrinol Jpn 35:171-80

27. Hourd P., Edwards R. (1989) J Endocrinol 121:167-75

28. Brostedt P., Luthman M., Wide L., Werner S., Roos P. (1990) Acta Endocrinol 122:241-8

29. Tomita H., Ogawa M., Kamijo T., Mori O., Ishikawa E., Mohri Z., Murakami Y. (1989) Acta Endocrinol 121:513-9

30. Bowsher R.R., Apathy J.M., Ferguson A.L., Riggin R.M., Henry D.P. (1990) Clin Chem 136:362-6

31. Hizuka N., Takano K., Asakawa K., Sukegawa., Horikawa R., Yoshizawa Y., Saito S., Shizume K. (1988) Acta Paediatr Scand Suppl 347:127-33

32. Nakazawa H. (1988) Chem Pharm Bull 36:988-93

33. Hattori N., Shimatsu A., Kato Y., Imura H. (1990) Kidney Int. 37:951-954

34. Hattori N., Shimatsu A., Kato Y., Koshiyama H., Ishikawa Y., Tanoh T., Assadian H., Imura H. (1989) Acta Endocrinol 121:533-7

35. Hashida S., Ishikawa E., Mohri Z., Nakanishi T., Noguchi H., Murakami Y. (1988) Endocrinol Jpn 35:171-80

36. Cheson B.D. (1990) Drug News Perspect. 3:154-160

37. Whetton A.D. (1990) TIPS 11:285-289

38. Finne P.H. (1965) Br Med J 697-699

39. Miyake T., Kung C., Goldwasser E. (1977) J Biol Chem 252:5558-64

40. Winearls C.G. (1989) Drugs 38:342-345

41. B.Ekblom (1990) IOC Medical Commission Meeting, Laussanne 7-8 April

42. Nielsen O.J. (1990) Pharmacol Toxicol 66:83-86

43. Flaharty K.K.,Caro J.,Ersley A.,Whalen J.J.,Morris E.M.,Bjornsson T.D.,Vlasses P.H. (1990) Clin Pharmacol Ther 47:557-563

44. Lui S.F., Chung W.W., Leung C.B., Chan K., Lai K.N. (1990) Clin Nephrol 33:47-51

45. Salmonson T.,Danielson B.G.,Wikstrom B. (1990) Br J Clin Pharmacol 29:709-713

46. Hamazaki H. et al. (1990) Kinki University School of Medicine 23:975-985

47. Esbach J., Egrie J., Downing M., Browne J., Adamson J. (1987) New Engl J Med 316:73-78

48. Mandin H. (1989) IInd I.A.F. World Symposium on Doping in Sport, Monte Carlo

49. Wieczoreck L. (1990) personal communication

50. Kientsch-Engel R.,Hallermayer K., Dessauer A. (1989) Contrib. Nephrol. 76:100-105

51. Conconi F. (1990) IOC Medical Commission Meeting, Laussanne 7-8 April

52. Subcommission Doping and Biochemistry of Sport of IOC Medical Commission (1989) Position paper on Erythropoietin

53. Kurtz A., Eckardt K-U. (1989) Nephron 51(suppl 1):11-14

54. Bauer C. (1990) personal communication

55. Eckardt K.-U., Kurtz A., Hirtz P., Scigalla P., Wieczorek L., Bauer C. (1988) Klinische Wochenschrift 66:241-245

56. Matsubara K.,Yoshimura T., Kamachi S., Fukushima M., Hino M.,Morli H. (1989) Clin Chim Acta 185:177-184

57. EPO-ELISA Test (Medac Diagnostika)

58. Massé R., Ayotte C., Dugal R. (1989) J Chromatogr 489:23-50

59. Ueki M. (1990) personal communication

60. Macyntire J.G. Growth Hormone and Athletes. Sports Med 1987;4:129-42.

61. Walker R., Brown A. (1988) Calgary Herald, Feb 17
Jereski L. (1989) Business Week, Dec 11
Temple C. (1990) The Sunday Times, Jan 7
Unknown (1990) El País, Jan 17
Specter M. (1990) Herald Tribune, Apr 3
Highfield R. (1990) Daily Telegraph, Apr 12

Discussion - POTENTIAL USE OF PEPTIDE HORMONES IN SPORT

M.M. Reidenberg

A couple of questions. First, is there any evidence that gonadotropin used actually improves performance. From what I have read the modest doses of androgens make little difference. The people who bulk up take enormous doses of androgen and that is most unlikely to be able to come endogenously no matter how much gonadotropin you give the testis. Secondly, I can see that cortisol given in an acute situation could theoretically help, but I think there is no data to suggest it. If one gave ACTH chronically during training its antianabolic effect ought to be detrimental to performance rather than beneficial. And so I am puzzled why these pituitary hormones would be used at all for athletes; they ought to be detrimental if anything.

J. Segura

The appearance of some compounds as banned substances is not always substantiated by scientific studies. Some of the substances are there because of logical reasons, but not just because they have been proved to be useful to athletes. Releasing factors are included probably to complete the list of hormones and main factors affecting them. I agree that ACTH administration without a very special knowledge of its effects could even be detrimental to the athlete.

M.M. Reidenberg

But then if there is reason to suspect it will be detrimental why does the Olympic Committee add to the burden of the analytical chemist by banning it and forcing you to develop methods to measure compounds that don't matter whether the athlete takes or not, so far as the potential performance is concerned.

L. Gauci

Most of the drugs that you are talking about have to be given by injection. How many injection needle marks should we accept from a normal healthy athlete?

A.J.H. Gearing

Can you also indicate what the major source of these drugs is? Are they normal pharmaceuticals that have been purloined from pharmacies or are they research grade materials? And where is the normal source?

J. Segura

This is something that really puzzles. The majority of these sophisticated drugs are released in a very controlled environment. In many countries there are even regulatory bodies that control the distribution of the drug on a patient's name basis. So, it seems difficult that these drugs could be diverted into the black market, but obviously this is the case for some of them.

W. Aulitzky

The use of androgens has two purposes. First of all, the increase of muscle weight which is important during the training period. Second, and this is a much more important reason, it causes aggressive behaviour. We know that intensive training results in a decrease of pituitary hormone release and to cope with that people are using LHRH or ACTH to overcome those periods of fatigue and bad performance. As a matter of fact we are in the stage where we may loose sports as one of the major educational tools because some sports are totally dominated by pharmacology, biochemistry or medicine.

D.C. Brater

Concerning the use of internal markers, I would think that a relatively safe way to incorporate internal markers would be to put into some of the synthetic steps some stable isotopes, which could be readily detected by mass spectrometry. I don't know what the regulatory implications of that would be, but I personally wouldn't be bothered by taking something that had a stable isotope in it. In fact some of your detection methods might be made very easily; if it was a stable carbon you could even do breath analysis, and you wouldn't have to wait in the locker room until they could generate a urine sample.

J. Segura

The incorporation of stable isotopes is one of the markers that have been suggested. But it is really difficult to state if the small percentage of the drug that will be misused justifies that inclusion into the product. There are probably alternative ways to cope with that problem of hormone misuse in sport other than the incorporation of these expensive and not totally physiological markers to a molecule released for a wider clinical use.

J. Mous

Talking about growth hormone, if I remember correctly, many of the effects of

growth hormone are mediated through the induction of insulin-like growth factors. Should these not be taken up into the list of forbidden products?

J. Segura

Really I don't know. It would depend on the availability of the products. Do they exist as a product that can be administered? In fact, it has been suggested that we should monitor insulin-like growth factors as indicators of human growth hormone abuse.

J. Bigorra

I don't know whether it makes sense but I wonder whether some very simple biological parameters such as hematocrit or reticulocyte count could provide a clue for the pre-screening of EPO. I mean not as detection method or as a confirmatory measure, but only to decide whether further testing is indicated or not.

J. Segura

Well, for instance, to detect blood doping there is already a strategy that, I would say, is quite similar to what you suggest. We check the blood levels of EPO, the haemoglobin level and the content of iron. And these three parameters together can indicate probably with 60% exactitude that some autologous or heterologous blood doping has been done. But the problem is that in doping control, because there is some punishment later on, we can not cope with a probability. We have to resort to very specific methodology that can allow us to claim with more or less 100% security that this or that has been consumed or not.