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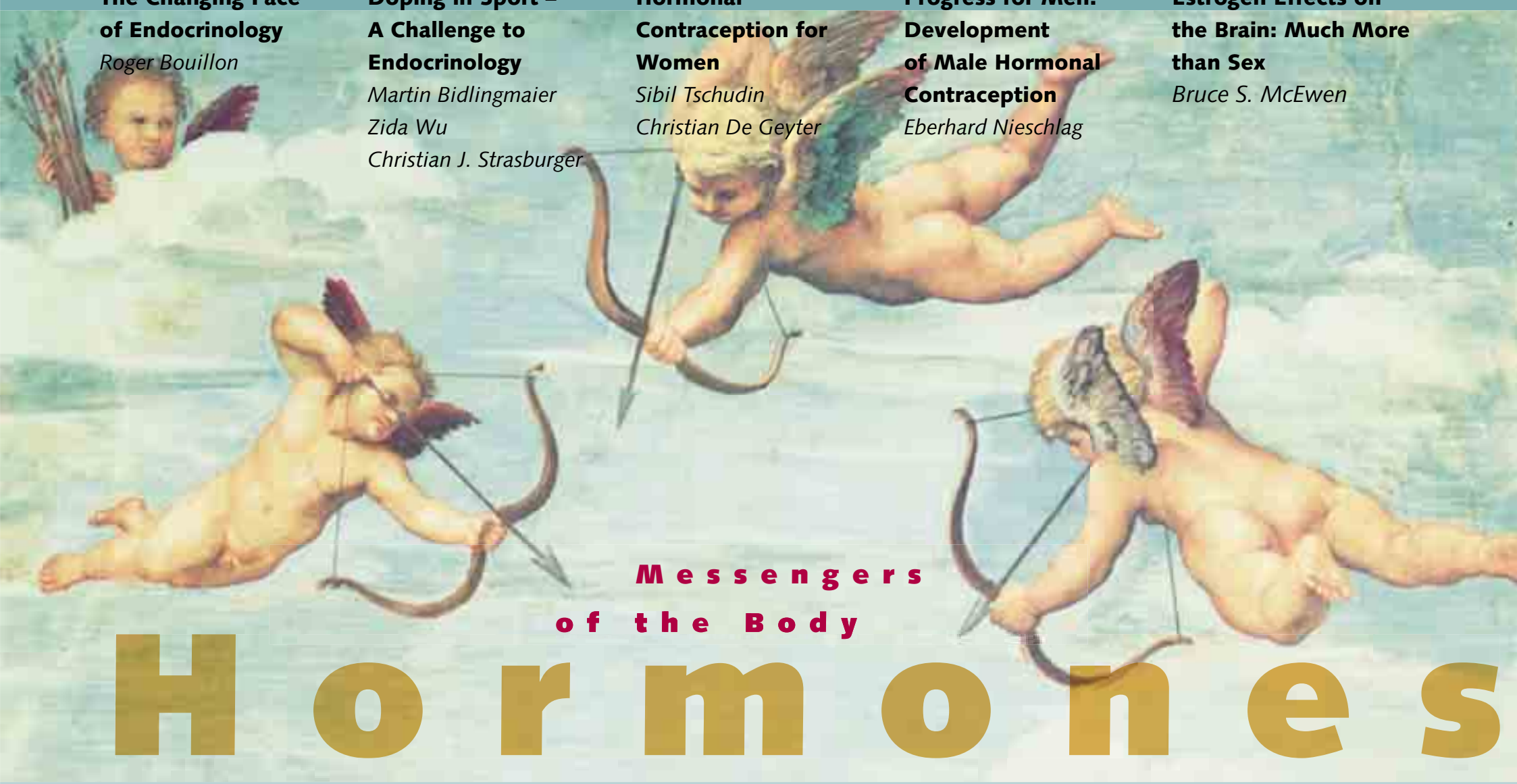

Messengers of the Body

Hormones

The Changing Face of Endocrinology

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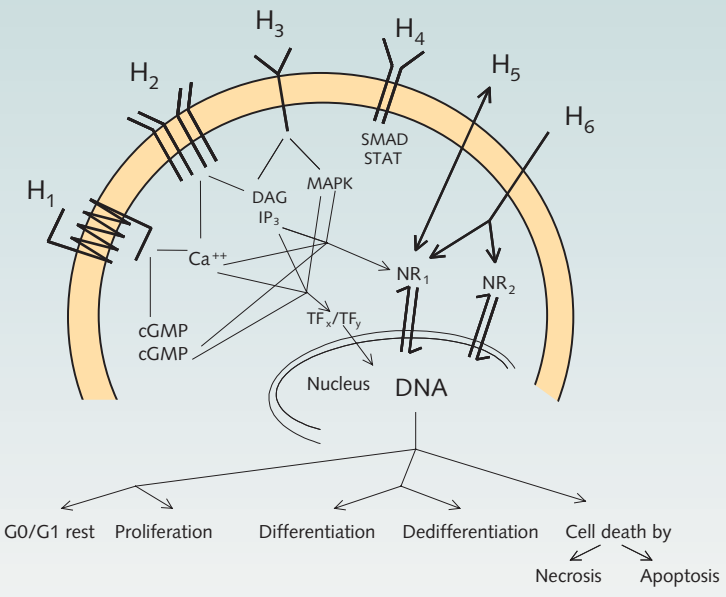

Fig. 1.
All cells are continuously exposed to a whole series of signals. These may come as hormones (H_n), produced in distant tissues or nearby cells (humoral signals such as cytokines and prostaglandins), may be synthesized internally (autocrine secretion), or arise from direct cell-cell contacts. All these signals are transduced into second signaling pathways of which cAMP, intracellular Ca^{2+}, and mitogen-activated protein kinases (MAPK) are the best known. The cell continuously integrates these extra → intracellular signals into vital decisions about its function. This can involve a simple variation in the intensity of functions of well-differentiated cells (e.g., more or less insulin production/secretion), or can result in dedifferentiation and resumption of cell cycling, or ultimately result in a suicidal apoptotic event.
NR = nuclear receptor
STAT = signal transduction and activation of transcription
DAG = diacylglycerol
IP_3 = inositol triphosphate
TF = transcription factor

"Hypotheses," Fuller Albright wrote, "are subject to changes without notice," an observation that is as applicable to the history of endocrinology as throughout the natural sciences. And, like other medical disciplines, endocrinology during the last century experienced the evolution of increasing specialization along with the generation of new preventive, diagnostic, and therapeutic possibilities that pose challenges to both our knowledge and clinical practice.

To think about what the future may bring, let me turn first to the past. Early concepts or paradigms in endocrinology, based on the facts available at the time, were logical in their simplicity (table 1). However, none of these concepts has withstood the test of time.

One gene does not simply code for one hormone. Different hormones may be generated from a 'mother gene' during processing at the mRNA or protein level, either by simple cleavage or by alternative splicing, producing a series of separate hormones (e.g., proopiomelanocortin giving rise to adrenocorticotropic hormone, α/β-melanocyte-stimulating hormone, lipocortin, and endorphins).

The idea that a single cell generates only one hormone has also been disproved: the β-cell, for example, produces not only insulin but also amylin and chromogranin, and many pituitary cells synthesize several hormones.

The notion that one hormone activates its dedicated receptor and then generates a coherent functional (in)activation of its target tissue has also had to be abandoned. One hormone can bind to several receptors [e.g., estrogen binding to estrogen receptor (ER)α, ERβ, and maybe even to a nongenomic receptor] and, conversely, many receptors behave promiscuously and bind several hormones. Moreover, many hormonal agonists are mimicked by natural hormone antagonists (e.g., agouti and agouti-related peptide compete with melanocortin for the melanocortin family receptors), while many ligands not only activate their natural receptor(s) but can also be lured by 'decoy receptors' (e.g., RANKL binding to the RANK receptor or osteoprotegerin).

Table 1.
'Expired' 20th-century paradigms in endocrinology

1 gene	→	1 hormone
1 cell	→	1 hormone
1 hormone	→	1 receptor
1 hormone	→	1 coherent function
Hormone concentration	=	hormonal response
1 hormone therapy	→	uniform predictable response in all patients

Indeed, most target cells express a wide variety of both nuclear and membrane receptors, all of which stimulate or modulate a variety of secondary signaling pathways. From this seemingly chaotic diversity of fluctuating information, the cell must choose between a set of programmes ranging from differentiation/dedifferentiation and proliferation to controlled cell death (fig. 1).

Table 2.
Origin of hormones

Classical endocrinology	Modern endocrinology		
Pituitary and hypothalamus	Liver and many other cells	→	insulin-like growth factor
Thyroid and C-cells	Gastrointestinal tract (GI)	→	numerous GI hormones and ghrelin
Parathyroid	Kidney	→	erythropoietin and 1,25(OH)$_2$D
Adrenal glands	Endothelium	→	endothelin, adrenomedullin
Ovary and testis	Skin/breast	→	PTHrP
Endocrine pancreas	Fat cell	→	leptin, resistin, adiponectin
	Brain	→	neuropeptides
	Bone	→	Phex → 'phosphatonin'

Table 3.
The past and future of endocrinology

	Thyroid hormone assays	Adrenal gland imaging	Diabetes therapy
1968	(1) protein-bound iodine/ butanol-extractable iodine	(1) X-ray tomography (2) pneumoretroperitoneum	(1) long-acting insulin with classic syringes (2) authority-based guidelines
2001	(1) free F_4/F_3 (2) third-generation thyroid-stimulating hormone assays	(1) dynamic computed tomography (2) nuclear magnetic resonance imaging (3) metaiodobenzylguanidine scan (4) positron emission tomography	(1) basal-bolus therapy (2) pump therapy (3) insulin analogues (4) evidence-based medicine (DCCT, UKPDS, ICU study)
2030–2040	(1) ? prediction/prevention of thyroid diseases (2) ? selective response of target tissues (heart, muscle, brain) (3) (selective) hormone agonist/ antagonist mimetics	(1) ? further functional imaging (2) ? steroid biosynthesis (3) cell-specific gene expression patterns (4) visualization of rate of cell division/death	(1) ? insulinomimetics (2) ? early detection of prediabetic state (3) ? β-cell engineering

DCCT = Diabetes Control and Complications Trial; UKPDS = United Kingdom Prospective Diabetes Study; ICU = Intensive Care Unit [4]

There are only about 50 nuclear receptors, but a large number (>1,000) of G-protein-coupled receptors, a small number of guanylyl-coupled receptors, and a large variety of phosphorylation-dependent receptors are encoded by the human genome and expressed on the cell surface or within the same cell. Moreover, a given signaling system may well employ more than one receptor, while one ligand-activated receptor may play roles in more than one signaling system (receptor cross-talk).

The concept that a hormone has a consistent and coherent physiological function also no longer holds true. Parathyroid-hormone-related protein (PTHrP), for example, probably arose from duplication of the gene for parathyroid hormone (PTH). Both these hormones bind to the same PTH receptor and regulate calcium homeostasis in a similar fashion. However, PTHrP has additional developmental functions such as regulating chondrocyte proliferation, and differentiation of the growth plate and cell-cell communication between epithelial and mesenchymal cells in hair and mammary gland development. PTHrP and PTH/PTHrP receptor knockout mice therefore have a completely different phenotype (with lethal growth, bone, and other organ abnormalities) when compared to PTH deficiency which produces only the expected hypocalcemic phenotype.

Finally, for a variety of still poorly characterized reasons, not all patients react in the same way to the same hormone, their responses depending on genetic heterogeneity and environmental differences.

On top of all this, the definition of what counts as a hormone has also become more complex, blurring the boundaries of both experimental and clinical endocrinology. The classical hormone concept (see box) has been extended to include humoral, paracrine, and autocrine factors, while the original fully endogenous hormone signal (e.g., insulin, growth hormone) has been extended to include nutrient-like signals (e.g., free fatty acids stimulating the peroxisome-proliferator-activated receptor), and even exogenous ligands such as light and pheromones can behave as hormonal signals.

So, it can be seen that although the original scope of classical endocrinology (founded by the description of thyroxin and epinephrin in 1895 and followed by secretin and gastrin in 1902) could be clearly defined by a restricted range of tissues of origin and cells/sites of action, we now know that nearly all cells can synthesize and produce hormonal signals, and, moreover, that several hormones originate from cells within organs with other functions (table 2). This 'new' endocrinology could never have been explored by ablation of the hormone-producing cells, and its contemporary form has only emerged with the introduction of modern techniques of gene manipulation employing, for example, knockouts and transgenes.

Definition of 'Hormone'

Classical definition

A chemical substance produced in a specialized gland, released into the blood stream, and transported to distant tissues to elicit a physiological response.

Modern definition

The classical definition of a hormone still holds true today, but modern endocrinology is much more complex because

1. most hormones are not produced in a specialized gland but either by specialized cells dispersed in other tissues (e.g., endocrine cells of the gut) or even by 'normal' cells (e.g., fat cells secreting leptin, adiponectin, and resistin).
2. other signals come from nearby cells or even from the responsive cell itself (paracrine and autocrine signals, respectively) or are of simple nutritional origin (e.g., calcium ions or cholesterol-derived bile acids).
3. many hormone receptors are present in cells not considered to be endocrine target cells.

The future of basic endocrinology

After a century of endocrinology research, are there still new hormones to be discovered? Analyzing the human genome using computer software techniques able to compare the sequence of DNA/RNA/amino acids, one should be able to detect new members of old hormone or hormone receptor families. Moreover, comparison of genomes from different species can reveal the previously unexpected existence of lower vertebrate or invertebrate hormone/hormone receptors in higher species (or vice versa) [1]. Using such techniques, there appear, at first sight, to be few unexpected hormonal signals, but this may be attributable to the limitations of such genomic screens. Many hormones arise from modifications of other structures (e.g., cholesterol, amino acids) which require multistep enzymatic reactions to generate the hormone, and therefore escape identification by genome analysis. Given that many human genes are still only identified as DNA sequences, a series of hormones and humoral factors is likely to be identified in the coming years by functional genomics, i.e., the systematic search to ascribe functions to such sequences.

Nevertheless, these 'in silico' techniques have already produced remarkable results. A beautiful example is the recent identification of a new member of the α subunit and of a new β subunit member of the pituitary glycoproteins. By homology comparisons of new genes identified in the Human Genome Project with known (pituitary) glycoprotein β subunits (thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, and chorionic gonadotropin), a new β subunit was found that combines with a new α subunit to create a new glycoprotein with potent thyroid-stimulating activity. This also implies the existence of a new G-protein-coupled receptor.

The number of receptors by far exceeds the number of known hormones. Although there are an enormous number of G-protein-coupled receptors, only about 50 genes encoding nuclear receptors have been identified in the human genome. The large number of receptors with unknown ligands are called orphan receptors, but will, of course, lose their orphan status when their ligands are identified [2] or if they are found to act constitutively and lack a natural ligand. The large number of receptors in the human genome is a gift for the pharmaceutical and biotechnology companies because the search for ligands can begin using standard and new technologies such as combinatorial chemistry and reverse pharmacology. This phenomenon should not be underestimated, since about 52% of the targets of the current 500 major pharmaceutical agents are in fact modulating receptors [3].

The function of many familiar hormones has been extended by studies of natural mutations and receptor knockout and transgene experiments. For example, the vitamin D receptor has been found to play a role in hair follicle development, while estrogen and the estrogen receptors are essential for closure of the growth plate in males and females. So, we can expect to identify many more functions for 'old' hormones, and the limits of our knowledge are still on a distant horizon.

The future of clinical endocrinology

The rapid evolution of basic endocrinology has been reflected in the progress in clinical endocrinology, as I hope the following examples will show.

Whereas in the 1960s and 1970s, diagnosis of thyroid function was based on clinical examination and measurement of plasma-bound iodide (necessitating knowledge of a list of all possible iodide contaminants), thyroid diagnosis and imaging at the beginning of the 21st century look almost perfect (table 3). However, the next generation of endocrinologists may well be able to predict and possibly prevent far more thyroid diseases. Moreover, the wide discrepancy between free thyroid hormone concentrations and target tissue symptoms could be overcome by measuring hormone levels or activity in specific tissues.

Imaging of the endocrine gland is also likely to improve substantially (table 3), with the already impressive list of current techniques extended to more functional analyses visualizing hormone synthesis and release (fig. 2). Of great benefit would be to distinguish directly between iodide trapping, organification, and thyroglobulin conversion into thyroid hormones, or to be able to detect in vivo the rate of cell division in cold nodules or other endocrine tumors.

We are now beginning to look at tissue-specific gene expression patterns using powerful techniques such as microarrays and proteomics, and the identification of genes/proteins over- or underexpressed in diseased tissues will hopefully revolutionize our ability to diagnose accurately the benign or malignant nature of these cells.

Turning now to a specific pathology, the treatment of type 1 diabetes has evolved over the years from a therapy based on authority to one based more on evidence and a more physiological insulin replacement regime. However, the current therapy

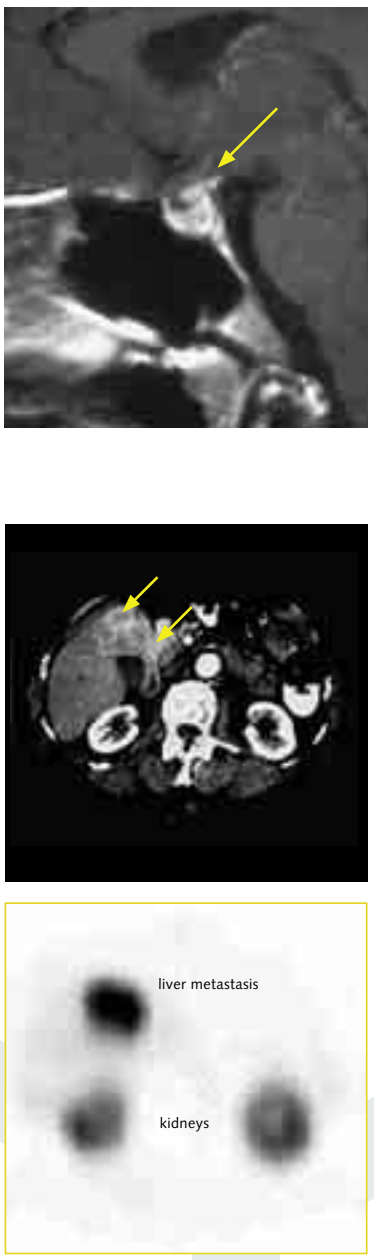

Fig. 2.
Endocrine imaging.
a. Infundibuloneurohypophysitis as revealed by MRI and subsequently confirmed by histology.
b. Insuloma with liver metastasis as revealed by radiolabeled octreotide scanning (below) compared with CT scan of the abdomen. Present-day imaging allows the detection of lesions based on their surface receptor expression, e.g, somatostatin receptors revealed in a tumor using the somatostatin analogue octreotide.

for diabetes is still far from ideal. But much research is underway (table 3), and we will hopefully resolve most of the problems of diabetic patients using insulin analogues or mimetics (acting at the postreceptor level), by early intervention in the disease process, or by replacement therapy of a natural or engineered β-cell.

So, several opportunities are opening out for endocrinologists of the future based on our ever-improving insight into the etiology and pathogenesis of known endocrine disorders and also an awareness of what new hormones/hormonal actions might achieve. This might also open up new fields, such as that of the endocrine abnormalities associated with aging, as I will discuss below.

A discipline in danger?

The endocrinologist and the field of clinical endocrinology face many challenges [5]. The pathogenesis of the diseases that endocrinologists manage is usually related to a tumor (of, e.g., the pituitary, parathyroid, or adrenal gland), or is due to autoimmune destruction and failure of the endocrine cell, or, occasionally, is a result of target resistance and particularly hormone resistance as in obesity and syndrome X, the metabolic syndrome of type 2 diabetes (table 4). We are only just beginning to understand the etiology and complete pathophysiology of these diseases. We have no real causal treatment, but tumor resection, endocrine therapy for endocrine tumors, and hormonal substitution in the cases of autoimmune destruction of a gland or in metabolic disorders are fairly practical and moderately efficient forms of intervention.

Within the next 20–30 years, the cell cycle defects leading to endocrine tumors are likely to be better defined and possibly amenable to correction. It may well be the oncologist, rather than the endocrinologist, who will be in the best position to diagnose and treat or prevent the cell cycle defects of these disorders. The same is true in the field of autoimmunity. If we were better able to understand the macrophage, Th1/Th2, and natural killer cell dysfunctions leading to autoimmune endocrine diseases, and were able to correct these defects or restore tolerance in these diseases, we would have totally new methods of treatment. Again, the clinical immunologist rather than the endocrinologist may well be better placed and more knowledgeable for this type of intervention.

Nevertheless, the endocrinologist is not out of a job yet, and I am fairly optimistic about the future. We will be able to make better diagnoses, use better imaging techniques to view endocrine cells, and will be better able to target hormone replacement therapy. I sincerely hope that some of today's major frustrations will be eased through the discovery of (1) a more physiological hormone replacement therapy for insulin or estrogen delivery, (2) a method to intervene and stop the autoimmune destruction of major endocrine cells, and, (3) causal and efficient treatments for hirsutism, obesity, and the metabolic syndrome X. Indeed, in treating such patients, I frequently have the feeling that we are not much better off than the psychiatrist in the 19th century treating insanity with physical constraint.

The endocrine patient

For the endocrine patient, the future looks bright. With a better understanding of basic endocrinology and an increasing number of medical specialists in the field, the endocrine patient can expect a healthier life. Not only patients with existing endocrine disease, but also those at risk for future disease will be better off if we are able to identify the risk in a timely manner and intervene early with primary or secondary prevention.

Although we have known for decades that genes and environment have a complex interaction in the development of disease, better understanding of this interaction may drastically improve our therapeutic attempts, allowing us to design drugs and drug regimens appropriately targeted for subgroups of patients (pharmacogenetics). Just one example: a high-fat (cafeteria) diet can induce obesity in rodents, yet this effect is markedly enhanced if such a diet is combined with specific gene mutations (e.g., knockout of all β-adrenergic receptors).

Along with our increasing competence, I can envision a new type of patient, one requesting 'comfort therapy,' asking, for example, for a particular height or body weight that s/he feels is deserved without attempting to achieve it by more 'natural' means. If appetite control by pharmaceutical intervention becomes possible – and this looks likely within the next 10–15 years – I predict a deluge of requests for this kind of therapy, as we are increasingly witnessing in other areas of endocrinology, e.g., in the endocrinology of reproduction and sexuality, and especially in those concerned with the effects of aging.

Geriatric endocrinology is something of an emerging phenomenon. One or two centuries ago, few people expected to live well beyond 50 years, let alone have a productive or healthy life after that age, and we now face a new series of health problems and questions that natural evolution never had to solve. Our endocrine system was not designed to let octagenarians live happily with appropriate hormone secretion and responses.

Hormone production and action are profoundly affected by aging – growth hormone, sex steroid hormone, and melatonin synthesis decrease, while that of others such as luteinizing hormone and follicle-stimulating hormone rise. It seems likely that we will in future be able to extend and improve the action profile of hormones, as we are currently witnessing with selective estrogen receptor modulators. For failing hormones we may be able to devise analogues or mimetics with a more selective mode of action than the natural hormone, for example, an androgen analogue which maintains a positive effect on bone, muscle, and libido, while having an antagonistic effect on prostate (cancer) cell growth and high-density lipoprotein concentration, or a selective vitamin D hormone analogue with favorable effects on intestinal calcium absorption and bone formation but devoid of osteoclastogenic effects. We are quite likely to witness novel hormone replacement therapies not only for failing ovarian function but for many other hormonal imbalances. Perhaps in Lucas Cranach's 1546 painting *The Fountain of Youth* (fig. 3), we can see a preview of a future where endocrinologists can rejuvenate the frail or ailing elderly with a combination of selective hormone analogues.

Thus the future for the endocrine patient in the wealthiest parts of the world looks 'bright', but s/he will have to work hard to pay for it. Meanwhile, we cannot turn a blind eye to discrepancies in the standards and possibilities of treatment throughout the world. Is it really acceptable, for example, that so many children and adults suffer from iodide, or vitamin A or D deficiency when simple and cheap interventions are possible?

Table 4.
Pathogenesis of classical endocrinologic diseases

Endocrine tissue	Tumor	Autoimmunity	Target resistance
Pituitary	++	+	rare
Thyroid	++	++	rare
Parathyroid	++	–	rare
Adrenal	+	+	rare
Endocrine pancreas	+	++	+++
Gonads	rare	–	rare

Fig. 3.
'The Fountain of Youth' by Lucas Cranach (1546, State Museums Berlin) illustrates mankind's ancient dream of overcoming the aging process and extending life. Could hormone replacement therapies for the elderly provide a modern 'elixir of life'? Whether such a scenario for clinical endocrinology is plausible or even desirable is a matter of considerable debate on both medical – there are no long-term data on the efficacy and safety of hormone replacement therapies – and ethical grounds, and is certainly a challenge for the future of endocrinology.

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About the author

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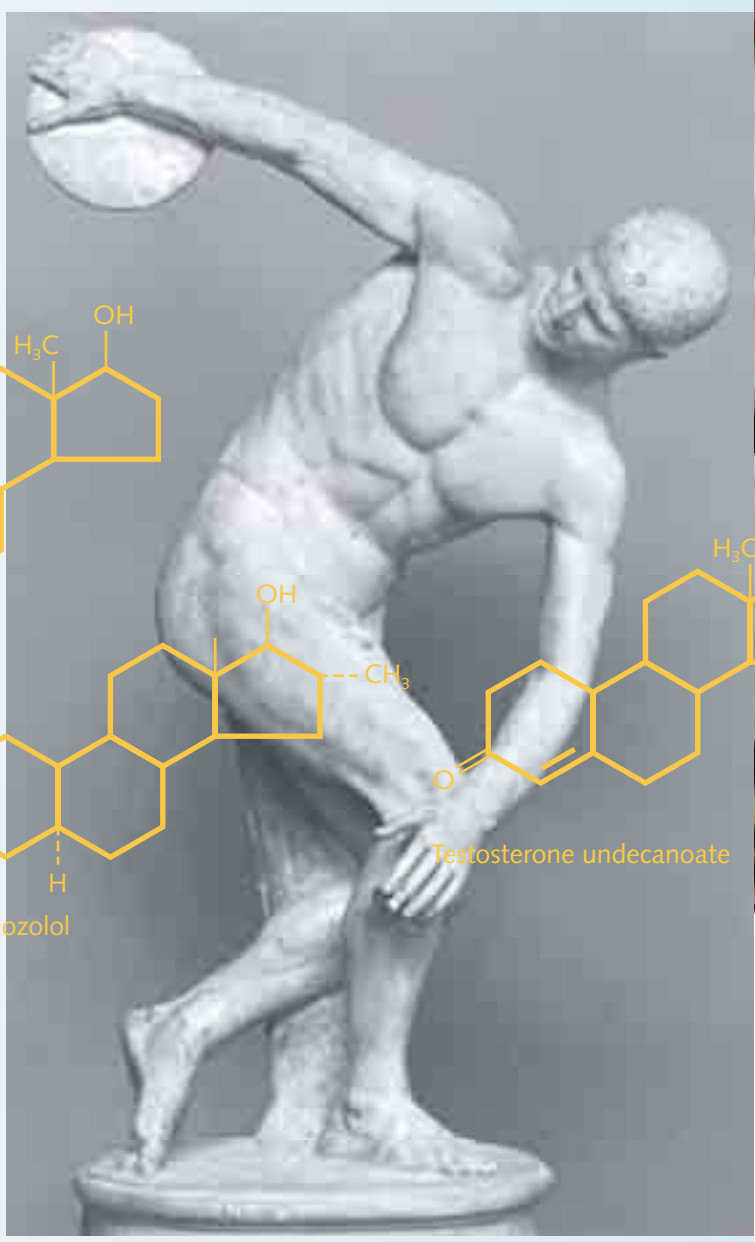

Doping in Sport – A Challenge to Endocrinology

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Although the use of performance-enhancing drugs in sport has gained much attention in recent years, the phenomenon is not new. Anecdotal reports from ancient Greece describe athletes consuming various substances and specific diets to improve their endurance and strength. Of course, their knowledge about physiology was poor, and nothing was known about hormones. The fact, however, that bulls' testes were among the athletes' preferred meals provides an amazing link to modern times, when the steroid hormone testosterone became one of the most popular doping substances.

Appendix A to the Olympic Movement Anti-Doping Code lists banned substances and methods abused to enhance performance in sport (see table 1). To a large extent, this list comprises naturally occurring hormones and their metabolites or synthetic derivates. Apparently in use since the 1950s, anabolic steroids were added to the list of banned substances in 1975, after methods to detect these substances became available. However, a further 9 years passed until, at the 1984 Olympic Games, all samples were screened for anabolic steroids and the testosterone/epitestosterone ratio was introduced as an indicator of exogenous testosterone use. More recently, with the improved doping tests for steroids and the availability of recombinant peptide hormones, the abuse of peptide hormones has risen. Human growth hormone (hGH) (fig. 1), erythropoietin (EPO), and other peptide hormones were included on the IOC list in 1989. Because of the key role they play in a wide range of metabolic functions, hormones are highly attractive for athletes trying to improve their performance by cheating. Despite the lack of controlled and validated scientific studies on the performance-enhancing effects of hormones, physicians and endocrinologists have to accept that athletes are apparently convinced of their efficacy and frequently abuse them. Doping with hormones not only undermines fairness in sport, it also poses a serious threat to athletes' health.

Hormone abuse is not restricted to professional sports, nor – even more alarming – to adults. Because hormone abuse is illegal, establishing its prevalence is difficult. However, from anonymous surveys conducted in the 1990s, we know that between 2 and 7% of adolescents have used anabolic steroids at least once in their lives. In 1992, a survey revealed that about 5% of male American high-school students had used hGH as an anabolic agent at least once in their lives. Apparently, the frequency of hormone abusers is even higher in specific groups: in a survey among exercisers in commercial sports studios in northern Germany, 24% of males and 8% of females reported the use of prohibited drugs, mostly hormones. It is alarming to learn from this study that about 15% of the users of performance-enhancing substances had obtained a prescription from their physicians. Glamorous reports in the popular press about hormones as a 'fountain of youth' together with the trend toward an uncritical promotion of hormone-based 'anti-aging medicine' are expected to contribute to an increase in the prevalence of hormone abuse in the field of recreational sports.

Table 1.
IOC list of prohibited substances and methods

I. Prohibited classes of substances

a. Stimulants
b. Narcotics
c. Anabolic agents
   i. Anabolic steroids (testosterone and derivates)
   ii. Beta-2-agonists (e.g., clenbuterol)
d. Diuretics
e. Peptide hormones, mimetics and analogues (hGH, IGF-I, EPO, hCG, insulin)

II. Prohibited methods

a. Blood doping
b. Administering artificial oxygen carriers or plasma expanders
c. Pharmacological, chemical and physical manipulation

III. Classes of prohibited substances in certain circumstances

a. Alcohol
b. Cannabinoids
c. Local anesthetics
d. Glucocorticosteroids
e. Beta-blockers

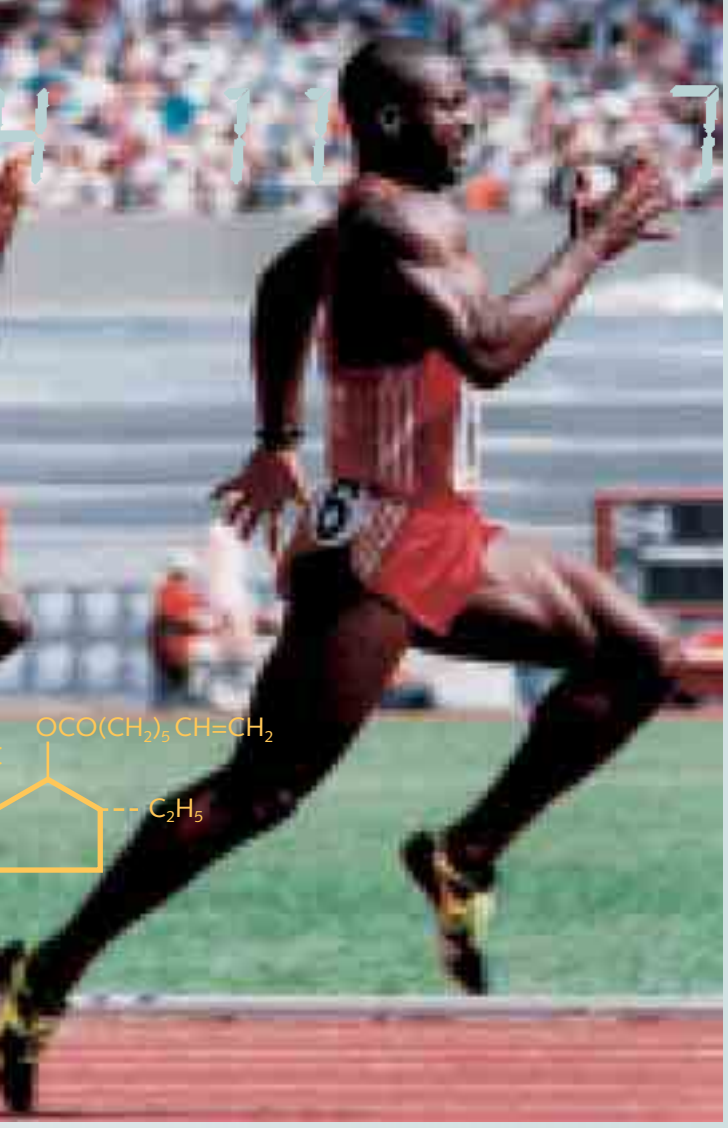
Anabolic steroids

From the annual statistics for drug testing by IOC-accredited laboratories, anabolic steroids seem to be the most widely used hormones in sport. Anabolics include testosterone and its synthetic derivates. Testosterone, nandrolone, stanozol, and metandienone are the most frequently used substances. However, in samples from athletes, doping test laboratories have also detected unusual derivates of testosterone which have never reached the official pharmaceutical market – there are apparently clandestine sources from which these steroids can be obtained. Charlie Francis, the former coach of Ben Johnson has written: "There are thousands of possible synthetic permutations of the testosterone molecule. The great majority of these steroids remain an unexplored frontier ... private laboratories stand ready to synthesize any number of these steroids – and keep the athletes ahead of the game." The detection of an increasing number of new anabolic steroids will remain a never-ending task for control institutions.

Fig. 1.
Human growth hormone, also known as somatotropin, is a peptide hormone of 191 amino acids arranged in a bundle of four α-helices. It is synthesized and secreted by cells of the anterior pituitary. [From S. Schwarz: Molecules of Life & Mutations, Karger, 2002.]

Testosterone possesses both anabolic and androgenic properties [1]. The synthetic derivates of testosterone are modifications of the hormone designed to enhance the anabolic action while reducing the androgenic effects. However, a derivate performing only the anabolic effects has not yet been synthesized – all anabolic steroids also possess some androgenic activity. Thus the term 'side effects' for the unwanted androgenic action is misleading – the effect on primary and secondary sexual characteristics is clearly one of the dominant effects of testosterone, and is performed by all known derivates. Testosterone acts on the development of external genitalia, induces sex-related peripubertal changes, and affects the distribution of hairs and muscles. Later in life, the inherent androgenic effects of anabolic steroids lead to virilization in females, and to acne and suppression of testicular function in males. Athletes try to benefit from the anabolic effects – an increase in protein synthesis, muscle growth, and erythropoiesis. Although large, validated scientific studies on the performance-enhancing effects of anabolic steroids are scarce, athletes do not doubt their efficacy. Furthermore, the doses used by cheating athletes are much higher than those used in the few published studies. For example, the daily dose of testosterone used by an athlete from the former German Democratic Republic was about 400 times (2,720 mg) the dose normally produced in healthy men (7 mg). For obvious ethical reasons, controlled studies in healthy subjects cannot test such high amounts of anabolic steroids.

The list of dangerous long-term effects caused by steroid hormone abuse is long: hypertension, changes in the lipid profile, atherosclerosis, and an increase in tendon damage are the most frequently observed. The main endocrine effect is the development of hypogonadotropic hypogonadism, characterized by low levels of gonadotrophins, suppression of testosterone production, and azoospermia in men. As prostate development and many prostate diseases are androgen dependent, the finding of prostate cancer after long-term abuse is not surprising. Chronic application of high doses of anabolic steroids in healthy young men has been shown to increase central prostate volume. In addition, through the physiological conversion of androgens to estradiol, gynecomastia (development of breasts) in males



is not uncommon. Originally, injectable forms of anabolic steroids were used. Today, anabolic steroids can be administered orally, offering several advantages to the cheating athlete, especially the shorter clearance time from the body. However, the orally active 17-alpha-methyl derivates of testosterone seem to be even more dangerous because they are first metabolized in the liver, increasing the risk of liver diseases such as hepatitis and hepatic carcinoma. Despite their awareness of most of these serious consequences of anabolic steroid abuse, athletes persist in taking these substances. Using performance-enhancing drugs to be competitive in sports seems to be accepted as an inevitable necessity, and the consequences, which might not appear for several years, are ignored.

Peptide hormones: hGH

Until the 1980s, when progress in biotechnology led to the production of large amounts of recombinant peptide hormones, their use as performance-enhancing drugs was very limited. For example, only cadaveric hGH extracted from pituitary glands had been available. These preparations were hard to obtain, expensive, and carried a high risk of disease transmission. However, as soon as recombinant hGH became available, it entered the doping scene, and in fact, the first publication proposing hGH as a powerful anabolic agent in adults was the Underground Steroid Handbook published by D. Duchaine in 1982, long before endocrinologists recognized its performance-enhancing power. At that time, physicians used hGH to treat children with growth defects, but not to treat adults. Knowledge about the anabolic effects of growth hormone in adults developed much faster in the body-building scene than among physicians and scientists. In the meantime we have learnt a lot about hGH effects on muscle and fat mass, tendons, the heart, and general well-being from controlled studies in hGH-deficient patients. Athletes seem to have anticipated these studies, and believe that hGH is a potent enhancer of training efficacy even in healthy subjects. Although this has not been verified by scientific studies, the effect, at least at high doses or in combination with other hormones, seems to be convincing. Otherwise, athletes would not take such an expensive drug. No test for hGH doping has yet been implemented, but there are many indirect hints pointing to widespread abuse. Several athletes have been arrested by customs officers when they tried to bring recombinant hGH to sports competitions. This happened for example at the 1998 Tour de France, when a huge number of ampoules containing recombinant hGH were detected in the possession of cyclists and accompanying physiotherapists. At the 1998 Swimming World Cup in Perth, Australia, a Chinese swimmer was caught smuggling recombinant hGH in her luggage. In Sydney in 2000, an athletics coach from Uzbekistan was fined for importing hGH, and a few months before the start of the Olympic Games, more than a thousand ampoules of an hGH preparation were stolen from a pharmacy in Australia.

Other peptide hormones

In addition to hGH, other peptide hormones like EPO, insulin, human chorionic gonadotrophin (hCG), and insulin-like growth-factor I (IGF-I) are known to be abused in sports today. Similar to the situation explained above for anabolic steroids, the rationale for their misuse is not always clear. Most of our knowledge on possible performance-enhancing effects is based on either an extrapolation from basic physiology or from findings in patients with an excess or a deficiency of these hormones. In addition, athletes may use more than one substance, increasing the likelihood of interactions and cumulative effects. Finally, very little is known about the doses and treatment regimes used. In the few cases where a so-called 'protocol' has been published, the doses were tremendously high: for hGH, up to 24 IU/day, way above the mean dose used in hypopituitary patients (1–2 IU/day).

EPO is used to enhance the number of red blood cells, which leads to an increase in aerobic strength, maximum oxygen uptake, and ventilatory threshold. Thus, the desired effect is comparable to that of high-altitude training, and the benefit might be most important in endurance athletes such as marathon runners or cyclists. Abuse of high doses of EPO may lead to hypertension, thrombosis, and pulmonary or cerebral embolism. Insulin might act as a performance-enhancing drug through the increase in glucose concentration in muscle cells, leading to greater muscle glycogen stores. In addition, there are reports on the use of short-acting insulin together with high-carbohydrate diets, which might lead to an increase in muscle bulk through inhibition of muscle protein breakdown. Little is known about the long-term consequences of insulin abuse, but in inexperienced hands, hypoglycemic events are very likely. By using hCG, athletes may hope to increase the body's own secretion of anabolic steroids, and most of the physiological effects and dangerous consequences are similar to those discussed above for anabolic steroids.

Developing tests for peptide hormone detection

While the detection methods for doping with steroid hormones have been standardized and well established in many doping control laboratories worldwide [2], methods to detect peptide hormone doping are poorly developed. The specific difficulties in developing tests for peptide hormone doping are as follows:

1 The amino acid sequence of recombinant peptide hormones like hGH or EPO is absolutely identical to the sequence of these hormones occurring naturally in the human body. Thus, for a long time, these recombinant hormones were believed to be undetectable after injection.

2 The physiologically circulating concentrations of hormones like hGH are highly variable within and between individuals, often exhibiting changes throughout the day or over a lifetime. Secretion of hGH is influenced by factors like nutrition, sleep, stress, or physical activity. It is therefore impossible to define a cut-off level above which the application of exogenous, recombinant hGH can be assumed.

3 Most of the accepted doping tests rely on urine samples (fig. 2). The concentrations of peptide hormones in urine are extremely low. For example, many efforts have been undertaken to apply urinary GH measurements for clinical use, but the majority of investigators have shown a poor discriminatory capacity of urinary GH measurements even under the conditions of controlled, clinical trials. Extrapolating these findings to possible doping control procedures makes it very likely that blood sampling must be introduced for reliable peptide hormone doping test methods.

Fig. 2.
A urine specimen is split into two samples, usually labeled 'A' and 'B', so that a confirmation test can be run if the initial test result shows the presence of a drug. (Photo by Daniel Käsermann.)

The recent development of methods to detect doping with recombinant EPO was a major step forward. Two main strategies have been pursued, and many sports organizations have approved these tests. The first, indirect, test involves measurement of many different EPO-dependent factors which change after recombinant EPO administration, e.g., hematocrit level, reticulocytes, serum transferrin receptors, and other markers of functional iron deficiency. A second, direct, test method utilizes the minimal differences that exist between the physicochemical properties of the hormone produced by the human body and the same hormone produced by recombinant technology. Recombinant EPO is produced by specifically engineered cells (Chinese hamster ovary and baby hamster kidney cells). The enzymatic equipment of these cells differs from that of human cells, leading to the lack of specific post-translational modifications in the recombinant proteins. In the case of EPO, the recombinant molecules do not have glycosylation sites typical for EPO produced in the human body. Using sophisticated methods, researchers were able to identify the presence of such differentially glycosylated EPO molecules in samples from cyclists known to have taken the banned drug [3]. The method has been established in some laboratories, and developed further. Thus, doping with EPO might be detectable in standard control procedures in the near future. However, cheating athletes seem to have already switched to other recombinant products to substitute EPO.

Detection of doping with recombinant hGH

HGH has apparently gained much popularity among athletes, not least because there is still no official test for it. In contrast to EPO, hGH does not bear glycosylation sites, making the development of a direct test even more difficult. Accordingly, researchers from the consortium GH2000, an international collaborative research project, jointly funded by the EU and the IOC, investigated the suitability of a panel of hGH-dependent variables for the development of an indirect test method. Growth hormone exerts most of its effects through the generation of IGF-I. In the circulation, IGF-I is bound to specific binding proteins, namely IGF-binding protein 3 (IGFBP 3) and the acid-labile subunit (ALS). Like IGF-I, the concentration of these binding proteins is hGH dependent. In addition, several components involved in the regulation of collagen and bone turnover such as N-terminal peptide of type III procollagens (PIIIP), collagen I carboxy-terminal telopeptide (ICTP), osteocalcin, and collagen I carboxy-terminal propeptide (PICP) are known to be hGH sensitive. Increasing the hGH concentration by application of recombinant hGH induces changes in these factors clearly distinguishable from those induced by the increase in circulating hGH evoked by exercise. The advantage of such an indirect test method is that the half-life of the pharmacodynamic endpoints of hGH action is much longer than that of hGH itself. While hGH has a half-life of only 15 min, changes in the indirect parameters could be detected for up to 2 weeks after the last hGH administration. For PIIIP and osteocalcin, the elevation above pretreatment values persists for up to 84 days. These promising results have demonstrated the feasibility of a test method based on the measurement of pharmacodynamic endpoints of hGH action [4, 5]. Problems with this approach include a variety of possible confounding factors. For example, because injury is a frequent event in elite athletes, factors involved in bone and collagen turnover could be of limited utility. Furthermore, the influence of age, gender, and ethnic background on the hGH-dependent factors has to be investigated.

Independently, a second, more direct method was developed [6].

What Is Doping?

Doping is the use of a substance or method that is potentially harmful to the athlete's health and/or is capable of enhancing performance. It also refers to the presence in an athlete's body of a prohibited substance or evidence of the use of a prohibited training method.

Olympic Movement Anti-Doping Code

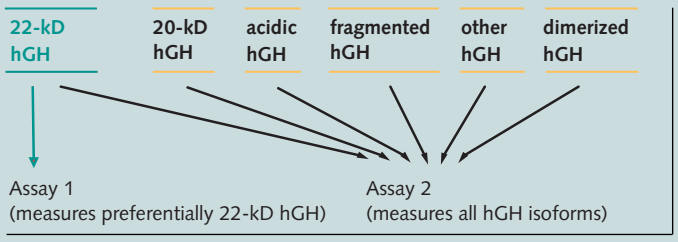


Fig. 3.
HGH isoforms: differential recognition by specific immunoassays.

Table 2.
Molecular isoforms of hGH in the circulation [modified from ref. 7]

22-kD monomer	48%
20-kD monomer	9%
Monomeric desamidated and acylated forms	5%
22-kD dimers	20%
20-kD dimers	7%
Dimeric desamidated and acylated forms	2%
Oligomers	13%
Fragments (17 kD, 12 kD, 5 kD, 30 kD)	variable

HGH produced by the body's pituitary gland is not a homogenous substance but consists of a variety of molecular isoforms (table 2). In contrast, recombinant hGH consists of 22-kD hGH only. Administration of recombinant hGH therefore raises the proportion of 22-kD hGH in the circulation. In addition, mediated through the negative feedback on pituitary gland hGH secretion, isoforms other than the 22-kD one are reduced in the circulation. These changes in the hGH isoform pattern after administration of recombinant hGH could be analyzed by the development of two specific immunoassays based on monoclonal antibodies. Assay 1 preferentially measures the 22-kD hGH isoform, whereas assay 2, a 'permissive' assay, measures all hGH isoforms (fig. 3). Analysis of a blood sample by both assays allows calculation of the relative abundance of the 22-kD hGH isoform in the sample. Studies confirmed that the relative abundance of 22-kD hGH is fairly constant in blood samples from healthy, untreated subjects, while administration of recombinant hGH leads to a massive increase in the abundance of the 22-kD isoform. In a series of more than 500 blinded samples (labeled by a number only), all subjects who had received hGH within 24 h prior to sample collection could be identified by this approach. This method of differential immunoassays proved to be highly reproducible. However, prior to introducing such a test into the official doping control procedures, large-scale studies have to be conducted to define a precise cut-off value above which the administration of recombinant hGH can be assumed with a high degree of probability. Furthermore, the time window of opportunity for detection has to be investigated. With the very short half-life of hGH in the human circulation, this window may be limited to 1–2 days. However, because doping with hGH requires daily injections, there should be no problem with a scenario of unannounced out-of-competition tests. Finally, the possible influence of age, gender, ethnic background, and different pathological conditions on the relative abundance of hGH isoforms remains to be thoroughly investigated, although these factors are not expected to influence this direct strategic approach.

Despite several problems, recombinant hGH doping can be detected by two independent methods. However, there is a long way to go from today's 'proof of principle' to a practicable and court-proof test. Further research, involving time-consuming and expensive large-scale studies, is needed to fulfill the strict legal requirements for acceptance of an official doping test. Keeping in mind that doping with hGH not only compromises fairness in sports but also represents a major risk factor for the athletes' health, funding such research should be a high priority for sports organizations.

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***Christian J. Strasburger** is lecturer and head of the Neuroendocrinology Research Group at the Medical University Hospital in Munich. Together with his coworkers **Martin Bidlingmaier** and **Zida Wu** he investigates both basic and clinical aspects of hGH, including GH deficiency in adults, GH receptor antagonists in the treatment of acromegaly, and neuroendocrine-immune system interaction. They were the first to develop a test to detect doping with hGH and are now working toward its implementation by the 2004 Olympics in Athens.*

Hormonal Contraception for Women

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The first oral contraceptive for clinical use was introduced in 1960 in the United States and 1961 in Europe. These historical events marked the beginning of a new era in the prevention of undesired pregnancy, and had a tremendous social impact, not least in contributing to the sexual revolution one decade later. Scientific milestones that had led to the development of hormonal contraception were the synthesis by Carl Djerassi of the first orally active progestogen, norethisterone, in 1951, and the detection of ovarian suppression by progesterone by Gregory Pincus in 1953.

Following the introduction of oral contraceptives, the number of induced abortions decreased steadily – in Switzerland between 1966 and 1998, for example, by 25%. However, improvements in sexual education and counseling may also have contributed to the lower rates of induced abortion in many countries. Today, approximately 90 million women worldwide use oral contraceptives [1].

In the earliest days of their use, the effectiveness and reliability of oral contraceptives were the issues most discussed by physicians and lay people. In the 1980s and '90s, the safety and potential health risks of oral contraceptives became the main focus of interest. Today, women can choose from a wide range of contraceptives, and for those who opt for hormonal contraception, a broad variety of preparations are available, varying in their mixture of estrogen and/or some specific progestogen, and their mode of application. In addition to oral contraceptives, hormones can be administered as depot preparations applied by injections, implants, vaginal rings, or intrauterine systems. While the injections on the market and the implants only contain progestogens, the other application forms as well as a solution for monthly subcutaneous injection contain both estrogen and a progestogen.

How hormonal contraceptives work

The first oral contraceptives contained mestranol, but today, ethinylestradiol (EE), a potent synthetic derivate of estradiol, has become the main estrogenic constituent of modern oral contraceptives. EE ensures adequate control over the menstrual cycle, maintaining stable and regular bleeding patterns in most users. In addition to the estrogen component of most hormonal contraceptives, a synthetic progestogen is added to suppress ovulation and prevent hyperplastic proliferation of the endometrium which can occur as a side effect. These progestogens are derivates of either progesterone, 19-nortestosterone, or, more recently, 17α-spirolactone. The gestagenic (progestational) activity of each compound is dose dependent and targeted to the endometrium, cervix, and ovary. Some progestogens may have additional effects, such as estrogenic, androgenic, antiandrogenic, or antimineralcorticoid activity. Some of these effects are clinically relevant in choosing the type of 'pill' most suitable for an individual woman.

Classification and types of hormonal contraceptives

The broad array of clinically available hormonal contraceptives can be divided into short-acting preparations (the conventional 'pill,' applied orally) and long-acting depot preparations, mostly applied by injection or implant, but also by vaginal ring and an intrauterine system. Oral contraceptives are divided into two groups. Those in the first group contain a progestogen only and are called 'POP's (progestogen-only pills or 'minipills'); those in the second group combine EE and some progestogen and are called 'COC's (combined oral contraceptive). COCs are the most commonly used form of hormonal contraceptive worldwide, mainly taken as a monophasic micropill. Micropills contain less than 50 μg (35–15 μg) of EE combined with one of the second- or third-generation progestogens which are derived from 19-nortestosterone. COCs are generally administered over a period of 21 days, followed by a pause of 1 week, when withdrawal bleeding should occur. In contrast to COCs, POPs must be administered continuously without a pause. POPs often trigger irregular bleeding patterns, which contribute to a high discontinuation rate. Because they do not normally inhibit ovulation but only change the mucus of the cervix and the endometrium and therefore act by inhibiting sperm ascension and the nidation of a fertilized ovum, POPs are less efficient in preventing an unwanted pregnancy. To guarantee effectivity, they ought to be taken at the same time every day, another factor that limits their use.

Hormonal contraceptives can also be given briefly, for example, after one single unprotected sexual intercourse or in cases when a barrier method fails. In such a situation, postcoital emergency contraception (more precisely, interception!) may be administered acutely following either the Yuzpe method (two doses of 100 μg EE and 300 μg Levonorgestrel) or using a progestogen-only preparation (two doses of 750 μg Levonorgestrel). Both methods must be applied within 72 h after unprotected intercourse.

Table 1.
Pearl index (= number of unwanted pregnancies per 100 years of use)

Method	Pearl index
Combined preparations	0.1–2.0
Minipill	0.4–4.3
Injectable progestogens	0.03–0.9
Implants	0–0.2
Levonorgestrel IUD	0.09–0.2

Effectiveness and reliability

The reliability of a contraceptive is generally measured and expressed by the Pearl index, which states the number of unwanted pregnancies per 100 years of use, or the number of pregnancies which may occur in 100 women who use the contraceptive method for 1 year. For hormonal contraceptives, the Pearl index ranges

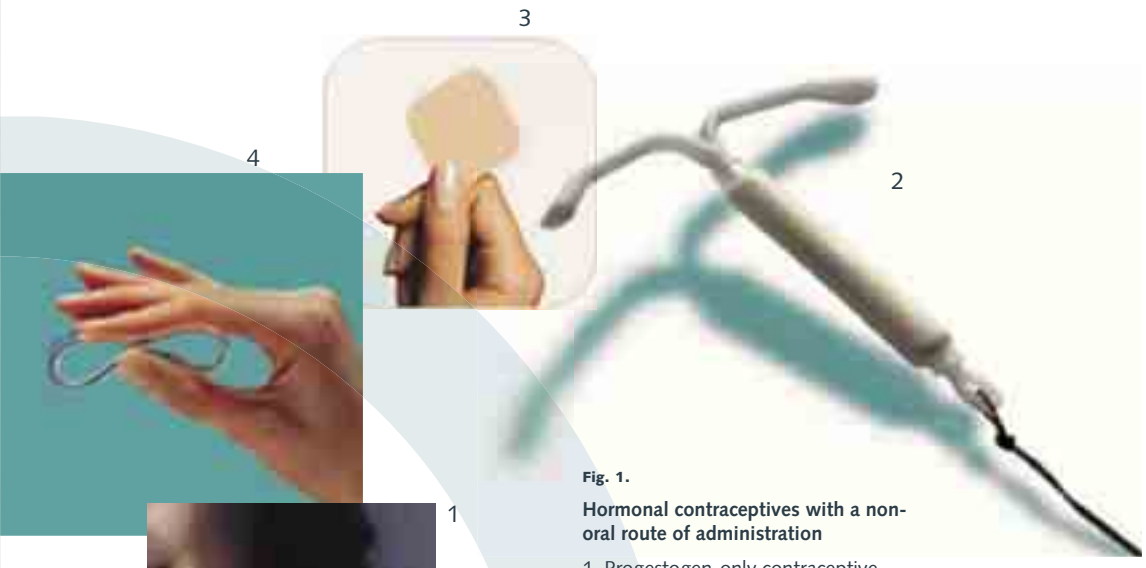

Fig. 1.

Hormonal contraceptives with a non-oral route of administration

1. Progestogen-only contraceptive implant which is positioned subdermally in the upper arm and is effective for 3 years.

2. Levonorgestrel-releasing intrauterine device which is inserted into the uterus by a gynecologist and is effective for up to 5 years. In contrast to conventional IUDs, it leads to a significant reduction in menstrual bleeding.

3. Skin patch delivering continuous levels of EE and progestogen through the skin and into the bloodstream. A patch is worn for 1 week and replaced on the same day of the week – for 3 consecutive weeks. The 4th week is patch free, and withdrawal bleeding occurs.

4. Vaginal ring containing a combination of EE and progestogen. The flexible, transparent ring measures 54 mm in diameter and 4 mm cross-sectionally. Inserted and removed by women themselves, it is placed in the vagina for 3 weeks and then removed for 7 days, during which withdrawal bleeding occurs.

from 0–0.2 for implants up to 0.4–4.3 for POPs (table 1).

Reliability depends on the modes of action and administration. The Pearl index is considerably better for preparations which inhibit ovulation (COCs and depot progestogens) and in those associated with little risk of administration failure (long-term contraceptives, such as depot injections, implants, and intrauterine systems).

Health risks

Hormonal contraceptives are taken by healthy women for prophylactic purposes. Their safety standard must therefore be higher than for drugs prescribed to cure disease. Potential risk factors are of major importance when assessing the quality of hormonal contraception. One of the major health risks due to COCs is an increased incidence of cardiovascular complications such as venous thromboembolism, myocardial infarction, and cerebrovascular insults due to increased blood clotting, mainly induced by their estrogen component. EE also has a negative impact on liver function. In addition to the role of EE, the effect of the gestagenic components of COCs on the pathogenesis of cardiovascular complications has been much discussed in the last few years. While this debate is still ongoing, other important health issues of long-term oral contraceptive use must be clarified, such as various types of cancer of the female genital tract and the breast, and the effect on bone metabolism.

The cardiovascular risk of COCs. During their reproductive life, the risk for cardiovascular incidents in women is low, but it increases with age. Venous thromboembolism occurs in 32–59 out of 1,000,000 premenopausal women every year. COC use increases the risk of venous thromboembolism in all age groups.

Smoking also contributes to the cardiovascular risk of COCs (see table 2), and thus COCs are definitely contraindicated in smoking women older than 35 years. Other risk factors for cardiovascular disease in COC users include hereditary thrombophilia (a tendency to excessive blood clotting as may occur in individuals with a genetically altered blood clotting system), high blood pressure, migraine, and chronic diseases such as diabetes.

Third-generation progestogens are associated with a slightly elevated risk of venous thromboembolism compared to the first- and second-generation progestogens. However, the absolute risk remains very low and may be counterbalanced by a somewhat lower prevalence of myocardial infarction [2].

The risk of cancer in COC users. Long-term use of COCs is associated with a slight increase in the relative risk (RR) of breast cancer (RR 1.07) [3], whereas the relative risks of endometrial and ovarian cancer are considerably lower (0.5 and 0.4, respectively) in COC users. However, cervical cancer is significantly more common in COC users (RR 1.5). Apart from the particular effect of estrogens in the induction of cervical cancer, various epidemiological aspects must also be considered. For example, in women carrying the human papilloma virus, the risk of invasive cervical cancer is higher in COC users than in non-COC users [4]. The excess incidence of invasive cervical cancer is outweighed by the lower prevalence of other uterine and ovarian cancers.

COCs and bone metabolism. Bone metabolism is positively influenced by circulating estrogen levels. Ovarian suppression by COCs or depot progestogens may reduce circulating estrogen levels, thereby down-regulating bone metabolism. This has been demonstrated in women of all ages taking COCs containing 20–30 μg EE. However, there is at present no indication for an adverse effect due to long-term use of COCs in women over 30 years, and even a benefit in perimenopausal women. In adolescents, intake of COCs containing as little as 20 μg and less may be associated with an impairment of peak bone mass [5]. We therefore recommend that COCs containing 15 μg of EE should not be prescribed to women of this age group.

Therapeutic use of oral contraceptives

COCs exert desirable effects on the uterine bleeding pattern, body weight, mood, skin disorders, and bone metabolism. Bleeding disturbances such as hypermenorrhea can be influenced positively. Even a temporarily limited amenorrhea can be induced if necessary and desired, for example, in patients with extreme dysmenorrhea (painful menstruation) who do not respond to other treatment options.

Use of COCs in the menstrual cycle before the actual treatment with assisted reproduction is associated with a better response to ovarian hyperstimulation and a lower incidence of ovarian cysts [6]. Although often prescribed for this indication, COCs do not exert a positive effect on endometriosis. The antimineralcorticoid property of the new progestogen drospirenone has a positive impact on the fluid retention experienced premenstrually by many women. Not only somatic symptoms, but, to a lesser extent, premenstrual mood changes can be alleviated by COCs. The antiandrogenic potency of the progesterone-derived progestogens offers an option for the treatment of acne. Even in women suffering from polycystic ovarian disease and hyperinsulinism, oral contraceptives containing the antiandrogenic progestogen cyproteronacetate have proved to be superior to insulin sensitizers in counteracting hyperandrogenism. Perimenopausal women, in particular, might benefit from COCs due to the positive effect on bone density and the normalizing effect on the menstrual cycle. If there are no specific risk factors and after exclusion of high blood pressure and an irregular lipid profile, liver function, and glucose metabolism, low-dose COCs can be prescribed up to the age of 50.

New trends in hormonal contraception

The appropriate choice of a hormonal contraceptive is based on its efficacy, safety, and also on its practicability in daily use. Beside the well-established pills, implants, and progestogen-containing intrauterine systems, new methods of application have recently become available including a vaginal ring, which can be worn for a period of 3 weeks, and a skin patch for weekly use (fig. 1). Both of these applications contain a combination of EE and a progestogen and act as ovulation inhibitors.

Women preferring parenterally applied contraceptives may now self-administer once monthly a subcutaneously injectable contraceptive which replaces the often painful intramuscular injections. In contrast to the latter, this contraceptive contains a combination of EE and a progestogen and acts in the same way as a COC, i.e., it normally causes a regular cyclic bleeding pattern. While the vaginal ring and the skin patch seem to be as reliable as COCs, studies concerning the reliability of the monthly subcutanous injections are not yet conclusive.

The effectiveness of a contraceptive will always be offset by possible side effects, and a failure in user compliance. However, a broad array of various types of hormonal contraceptives offers a woman the opportunity to make a contraceptive choice best suited for her individual lifestyle and health needs. It is still too early to tell whether the new application types (patch, vaginal ring, subcutaneous injection) will find general acceptance, and the well-established conventional pill might remain the most used hormonal contraceptive for the time being.

However, research into new types of contraception, both hormonal and nonhormonal, i.e., mechanically and chemically acting contraceptives as well as male-initiated methods, remains important.

The recent increase in distinctive contraceptive methods can also put more strain on the decision process of the women and couples involved. High-quality counseling therefore becomes increasingly important, taking into account the individual's preferences and needs and providing honest information about the range of contraceptive options.

Table 2.

Cardiovascular risk with and without COCs in relation to age and smoking
[from ref. 1]

	Events per one million women years					
	without COCs		with COCs		with COCs + smoking	
Age (years)	20–24	40–44	20–24	40–44	20–24	40–44
Myocardial infarction	0.1	21	0.2	32	1.7	255
Ischemic insult	6	16	9	24	18	48
Hemorrhagic insult	13	46	13	93	38	232
Venous thromboembolism	32	59	97	178	97	178

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Progress for Men: Development of Male Hormonal Contraception

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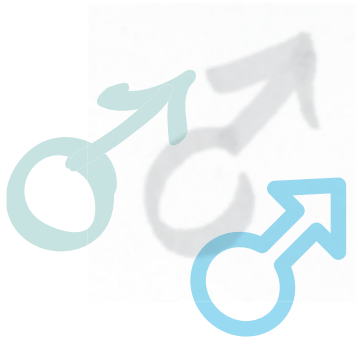


The invention of the 'pill' for women was arguably one of the most significant medical and cultural events of the 20th century. Although nature has sweetened procreation with the pleasures of sex to guarantee human reproduction, the pill was the culmination of a millennial-long development of methods to disentangle procreation from sex, and has had a substantial impact on society – e.g., on family planning, sexual mores, and demography.

An equivalent pharmacological male method is not yet available. This article will discuss why, and the prospects for its introduction sometime during the 21st century.

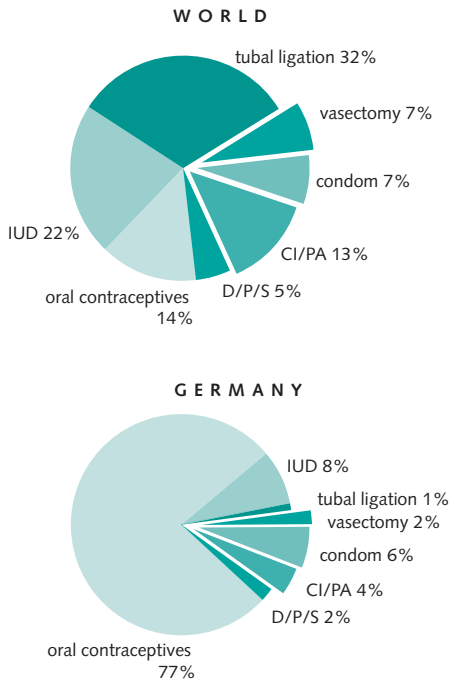


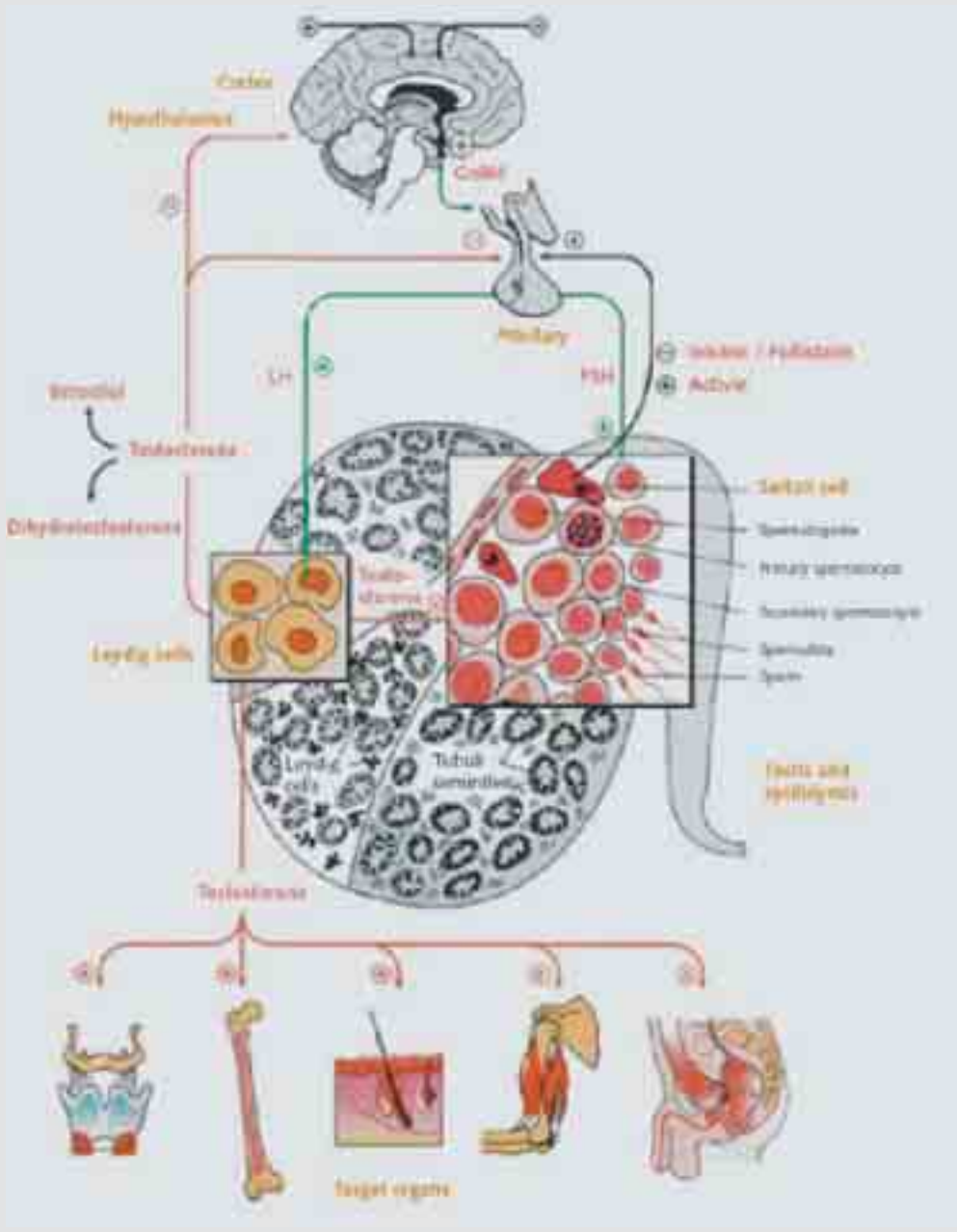
Fig. 1.
Use of contraceptive methods worldwide and in Germany. D/P/S diaphragms, cervical cap, spermicides etc; CI/PA coitus interruptus, periodic abstinence; IUD intrauterine device (UN Population Division, New York, June 1998.)

Why male contraception at all?

Female contraception is very effective. Nevertheless, 50% of the 1,000,000 conceptions occurring every day worldwide remain unplanned, of which 150,000 are terminated by abortion, an intervention that will end fatally for 500 of these women. Although improved distribution and utilization of female contraceptive methods might ameliorate this situation, the contribution of a male contraceptive is well worth considering (fig. 1). Men enjoy the pleasures of sex, but can do little to contribute to the tasks of family planning – a pharmacological male contraceptive is perhaps long overdue. In addition, the risks of contraception would also be more fairly shared between women and men. Representative surveys have shown that a pharmacological male contraceptive would be acceptable to large segments of the population in industrial nations, and would thus contribute to further stabilization of population dynamics. It might also help developing countries whose exponential population growth endangers economic, social, and medical progress. Last but not least, male contraception can be considered an outstanding issue in the political field of gender equality.

Castration and abstinence

For the male, there are ways to eliminate both procreation and sex at the same time. Such methods have been used in the past and are still being practiced on a limited scale. Castration has been employed since ancient times to destroy enemies by abolishing their ability to reproduce and transmit their genes. Until the end of the imperial period in China (1912), men were willing to sacrifice their testicles (and often with them their lives) in return for high-ranking positions and political influence at the emperor's court. Meanwhile, in the West, up until almost the same time, some promising boys were forced to give up their manhood for the sake of preserving their pre-pubertal voice and achieving fame as singers, often without success. Abstinence is a less bloody means of eliminating procreation, but few men are willing to give up both sex and procreation for extended periods of time, let alone their entire lives (fig. 2).



Existing methods

Traditional male methods of contraception such as periodic abstinence or coitus interruptus are associated with a relatively high rate of unwanted pregnancy and also cause a disturbance in sexual activity. Condoms are the oldest barrier method available. However, conception rates when using condoms are relatively high, with 12 out of 100 couples conceiving during the first year of use (Pearl index = 12). Condom use has increased since the beginning of the AIDS epidemic, but more for protection from HIV infection and other sexually transmitted diseases than for contraceptive purposes.

Vasectomy is a safe and surgically relatively simple method for male contraception. The rate of unwanted pregnancies after vasectomy is less than 1%. The drawback to vasectomy is that it is not easily reversible. Achieving fatherhood after vasectomy requires either surgical reversion or sperm extraction from a testicular biopsy and intracytoplasmic sperm injection into the ovum. Only about 50% of these men will become fathers in the end.

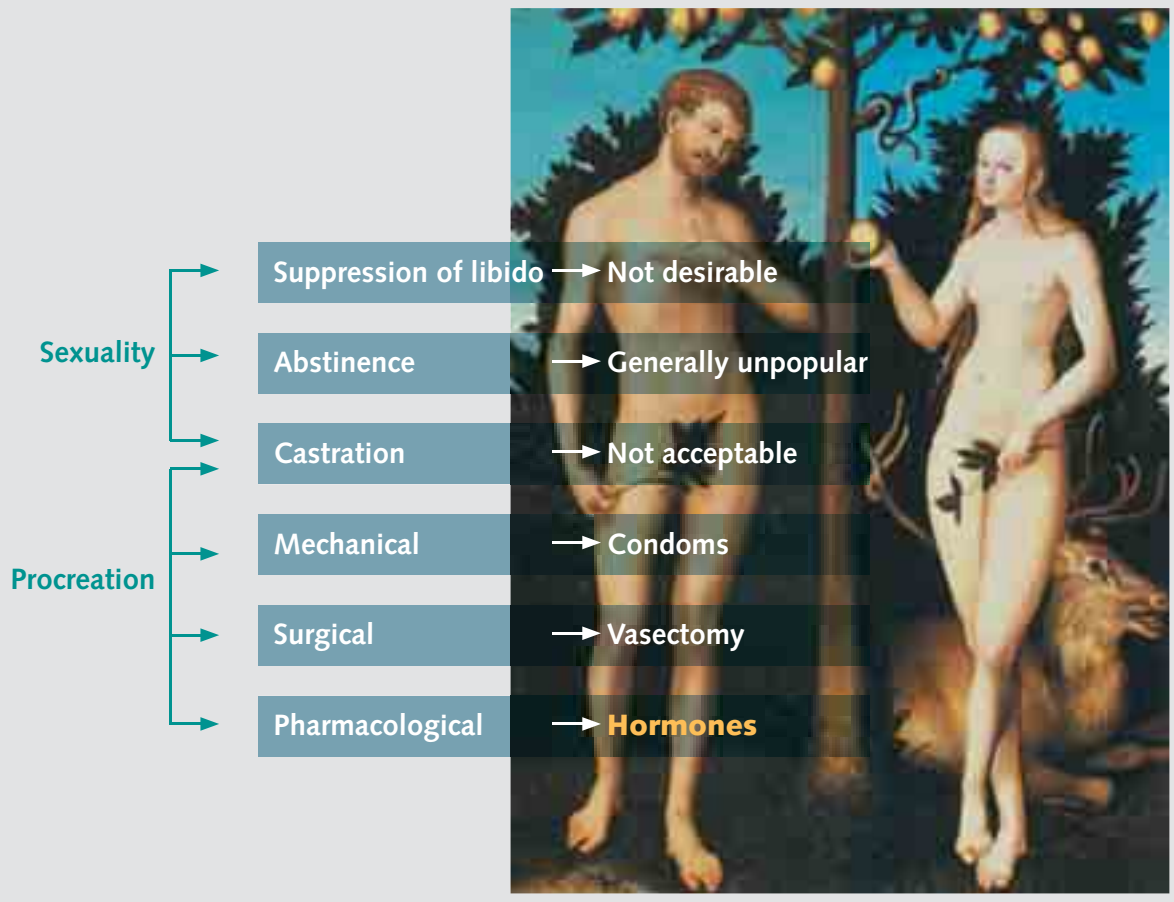


Fig. 2.
Approaches to male contraception. Eliminating procreation together with sexuality or sexuality alone is considered unacceptable. Surgical vasectomy has to be considered irreversible for all practical purposes. Of the pharmacological possibilities, only hormones have reached clinical trials. [Background painting: 'Adam and Eve' by Lucas Cranach (1533), Museum of Fine Arts, Leipzig].

Fig. 3.
Hormonal regulation of testicular function and effects of androgens. Key hormones are luteinizing hormone (LH) and follicle-stimulating hormone (FSH), synthesized and secreted under hypothalamic control of gonadotropin-releasing hormone (GnRH). Leydig cells are located between the seminiferous tubules and synthesize and secrete testosterone under the control of LH. Testosterone stimulates the maturation of germ cells in seminiferous tubules. FSH acts directly on the seminiferous tubules. In the germinal epithelium, only Sertoli cells possess receptors for testosterone and FSH. The trophic effects of testosterone/FSH on gametogenesis are therefore believed to be mediated via somatic Sertoli cells. The testis and the hypothalamo-pituitary system communicate through steroids and protein hormones. Testosterone inhibits the secretion of GnRH and gonadotropins. Inhibin B and follistatin selectively suppress the release of FSH from the pituitary gland, while activin stimulates this process. Beside the effects on gametogenesis, testosterone plays an important role in hair growth, bone metabolism, muscle mass and distribution, secondary sexual characteristics and function of the male reproductive organs. (Adapted from Nieschlag E et al. in: Deetjen & Speckmann (ed): Physiologie. München, Urban & Fischer, 1999.)

Given the disadvantages of these mechanical male methods, what then are the prerequisites for an ideal male contraceptive? It should

- be applied independently of the sexual act
- be acceptable for both partners
- not interfere with libido, potency, or sexual activity
- have neither short- nor long-term toxic side effects
- have no impact on eventual offspring
- be rapidly effective and fully reversible
- be as effective as comparable female methods

New approaches

Despite attempts to improve existing methods, e.g., vas occlusion instead of surgical dissection, or the introduction of new materials (e.g., polyurethane) for condoms, the inherent disadvantages of these mechanical methods preventing sperm transport into the female tract persist, and must be replaced and/or supplemented by pharmacological methods. Posttesticular approaches to male contraception are still in the preclinical phase. By investigating the molecular physiology of sperm maturation and epididymal function, the aim is to identify processes that might be blocked by specific pharmacological agents with a rapid onset of action. However, all substances investigated so far have shown toxic side effects when interfering effectively with sperm function. At the moment then, only hormonal methods fulfill most of the requirements for a male contraceptive and are currently under clinical development.

Principle of hormonal male contraception

The general goal of hormonal male contraception is to suppress sperm production in the testes without impairing testicular function. As in female oral contraception, the principle of hormonal suppression of spermatogenesis is based on influencing the endocrine feedback mechanism between hypothalamus, pituitary, and testes (fig. 3). Women have physiologically infertile periods and contraceptive methods are designed to eliminate cyclically occurring fertility peaks. Men, however, produce sperm and are fertile continuously. A safe, reversible, and acceptable contraceptive method interrupting this state must achieve either azoospermia or severe oligozoospermia with dysfunction of any remaining sperm.

To reach this goal, the pituitary gonadotropins luteinizing hormone and follicle-stimulating hormone (FSH) must be completely suppressed, because residual FSH and intratesticular testosterone activity would suffice to maintain spermatogenesis. However, testosterone must be available in sufficient amounts outside the testes to maintain the many other functions dependent on that hormone, e.g., erythropoiesis, protein, mineral and bone metabolism, as well as libido and potency, cognitive functions, and male personality (see box).

Modalities of hormonal male contraception

All these effects can be achieved with testosterone itself. The effectiveness of exogenous testosterone for contraception was first shown in clinical multicenter studies performed under the aegis of WHO using injections of testosterone enanthate. However, reactions to testosterone vary among ethnic groups: whereas almost 100% of East Asian men respond to testosterone with azoospermia, only 60% of Caucasian men reach this goal. The causes of this variable response to hormonal suppression of spermatogenesis have not been fully clarified, but there is evidence that genetic polymorphism of the hormone receptors, especially the androgen receptor, influences individual responses to the contraceptive steroid dose.

So, the first hormonal male contraceptive may be marketed in China, following the recent resolution of another problem associated with testosterone enanthate. Testosterone enanthate requires weekly injections, rendering it impractical for general use. A search for longer-acting testosterone preparations identified several modalities of which testosterone undecanoate, testosterone pellets, and subdermal silastic implants with 7α-methyl-19-nortestosterone appear to be the most promising. Nevertheless, these testosterone preparations used as single entities may only be effective in East Asian men; Caucasians will require an additional substance to achieve contraceptive protection.

General principle of male hormonal contraception

1. **Suppression of FSH and LH**
   (Depletion of intratesticular testosterone)
2. **Replacement of peripheral testosterone**

Among the promising additives to testosterone are gonadotropin-releasing hormone (GnRH) antagonists. They block pituitary GnRH receptors so that the pituitary can no longer produce gonadotropins under the influence of hypothalamic GnRH. GnRH antagonists in combination with testosterone lead to rapid and complete suppression of sperm, but the preparations currently available require daily or weekly injections and are expensive. However, new depot preparations are being developed for the treatment of prostate carcinoma, and the antagonists may only be required during an induction phase. Studies in monkeys and humans suggest that testosterone alone may then maintain sperm suppression. The GnRH antagonist/testosterone combination could well become a feasible approach to male contraception.

Another option to enhance the effectiveness of testosterone is the addition of progestins. Progestins, derivatives of natural progesterone, have been mainly developed for female contraception and have been tested only unsystematically as possible male contraceptives. Depot medroxyprogesterone acetate has the longest history in male contraceptive trials and has recently been shown to be highly effective in combination with subdermal testosterone pellets. Levonorgestrel and etonorgestrel (both used in implants for female contraception) are further options and appear to be most effective when used as silastic implants. In research at my institute, a combination of testosterone undecanoate with noresthisterone enanthate was most effective and both substances can be combined in one injection given at 8-week intervals. An alternative to injections would be long-acting implants, effective for one year or longer. The combination of an implant with an injection might be less desirable, since the use of two modalities would be impractical.

Attempts to create a self-administered contraceptive such as an oral or transdermal steroid have not yet been successful. The steroids show fluctuating pharmacokinetics when administered orally or transdermally, while constant serum levels seem to be an absolute prerequisite for effectiveness. It therefore looks as if 'the male pill' will be an injection or an implant, at least for the time being.

Side effects such as moderate weight gain, mild gynecomastia or acne were mainly observed during the early phases in the development of hormonal male contraception. In newer studies steroid levels in the circulation have been lower, their medium-range kinetics have been more constant, and side effects are less pronounced. Effects on libido, potency and psychological variables are very rare. In studies with progestins, increased sweating was encountered as an undesirable side effect, probably caused by the thermogenetic effect of progestins, which also occurs in women. In all volunteers participating in clinical trials for hormonal male contraception to date, suppression of spermatogenesis was fully reversible.

Fig. 4.
Location of centers worldwide active in research on hormonal male contraception.

Outlook

Although the scientific basis of hormonal male contraception had been established and practical modalities developed, drug companies were hesitant to engage in clinical development. Leading scientists in the field of male contraception therefore met in 1997 and issued the 'Weimar Manifesto on Male Contraception,' challenging the drug industry to become more actively involved in this field (fig. 4). The pharmaceutical companies did respond, initiated programs for male contraception, and a healthy competition between firms has finally emerged. In addition, the Population Council (New York), WHO's Department of Reproductive Health and Research and a few philanthropic organizations are also devoting part of their activities to male contraception. The pharmaceutical companies have finally discovered male contraception as a potentially profitable area, while governmental and philanthropic organizers believe that male contraception will help contribute to a reduction in world population growth.

The field has now progressed to the point that strategies for the approval of male hormonal contraceptives are being considered. At the Sixth Summit Meeting on Hormonal Male Contraception in 2002, guidelines were drafted for consideration and discussion with the regulatory authorities, preparing the path toward registration of the first hormonal male contraceptive. Although it has taken 30 years to get this far and there is still no male contraceptive on the market, one should remember that hormonal female contraceptives also required many years to develop, and regulatory agencies are more stringent today than 40 years ago when the first hormonal female contraceptives were registered for general use. Nevertheless, the first hormonal male contraceptive may be available for general use in 2006, in China, and a few years later in the West.

Suggested further reading

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About the author

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Estrogen Effects on the Brain: Much More than Sex

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Estradiol is a versatile hormone that does much more than regulate reproductive function in female animals. First recognized as products of the ovaries, we now know that estrogens are also produced from androgens by the brain and by body fat deposits, and that they are synthesized in both males and females, along with the estrogen receptors (ERs) that mediate their effects. Moreover, although originally recognized to regulate reproductive processes during adult life, estrogens also regulate sexual differentiation of the brain, and have protective effects on bone, the cardiovascular system, and the brain. In the nervous system, estrogens affect brain functions ranging from fine motor control to pain sensitivity, memory, and protection from Alzheimer's disease and stroke damage. This article presents a brief update on this emerging story.

Where in the brain do estrogens act and what do they do?

Estrogens are generated from cholesterol via testosterone in the ovaries, but also by the brain and by body fat deposits in both males and females [1]. Gonadal testosterone as well as adrenal androgens are the sources of estrogen formation in fat tissue and brain (fig. 1). Both males and females express intracellular ERs throughout the body and in many parts of the brain (see below).

We have known for more than 40 years that estrogens target the brain of experimental animals. The first animal studies focused on estrogen actions on the hypothalamus affecting ovulation and reproductive behavior. Only recently has it become apparent that estrogens exert many actions on brain areas that are important for learning, memory, emotions, and affective state as well as motor coordination and pain sensitivity [2]. Table 1 summarizes some of these estrogen effects.

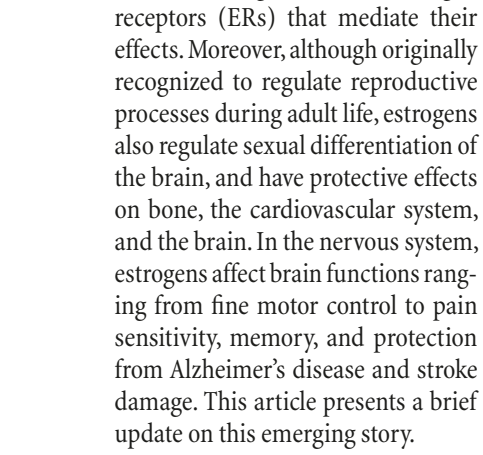

Fig. 1.
Testosterone is converted to estradiol via aromatizing enzymes and to 5-alpha dihydrotestosterone via 5-alpha reductase.

Where in the brain do estrogens exert these actions? As summarized in table 2, many widely projecting neural systems such as the basal forebrain cholinergic system, the midbrain serotonin and dopamine systems, and the brainstem cholinergic and noradrenergic systems are targets of estrogen action (fig. 2). In addition, the hippocampus, a structure important for declarative, episodic, and spatial learning and memory, is also responsive to estrogens (fig. 3), as are cerebral blood vessels and glial cells.

ERs and molecular mechanisms of estrogen action in the brain

Intracellular ERs were first identified in the 1960s by binding of tritiated estradiol: they are proteins that bind to DNA in the cell nucleus when estrogens are bound to them (fig. 4). Found initially in the reproductive tract, putative ERs were subsequently identified in the pituitary gland and hypothalamus. These were studied for many years because they were the most obvious and also the most obviously related to estrogen actions on reproduction. More recently, estrogen-sensitive brain regions have been identified, not so much because of the localization of cell nuclear ERs but because of the effects that estrogens produce. This, in turn, has led to the recognition that estrogens produce their effects via a variety of intracellular mechanisms and sites of action.

With the development of antibodies to ERs and ER cloning, intracellular ERs themselves or their mRNAs could be measured by immunocytochemistry and in situ hybridization histochemistry. The classical intracellular ER is ERα, while a new form, ERβ, has recently been identified and cloned: it is found in some tissues and brain regions not previously known as estrogen targets, while also existing with ERα in other tissues and brain regions.

Alongside these discoveries with intracellular ERs, studies of estrogen effects on nerve cell activity and neuroprotection have uncovered rapid actions of these hormones that, either because they are so fast or from what we know of their structure-activity profile in relation to the specificity of known intracellular ERs, cannot involve activation or repression of gene expression (fig. 4). These 'nongenomic' actions of estrogens operate in many cases at or near the cell surface and affect the excitability of nerve and smooth muscle cells and the movement of the sodium, potassium, and calcium ions that create a nerve impulse. However, we know very little about the molecular characteristics and the mechanism of action of these 'nongenomic' receptors in cell membranes, though several recent studies have shown that intracellular ERs of the nuclear type may be expressed in small numbers at or near the cell surface where they could regulate second-messenger systems [3].

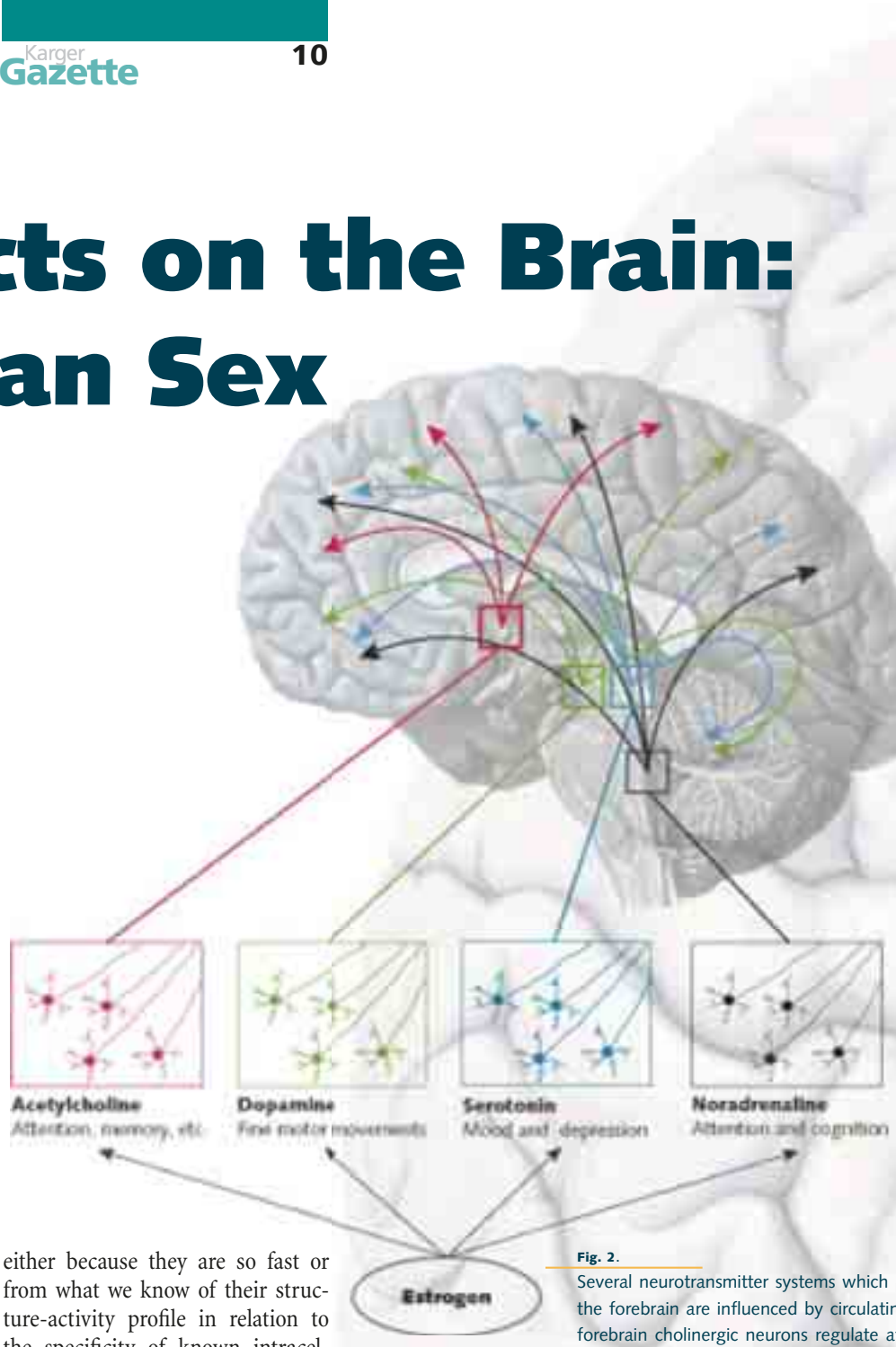

Fig. 2.
Several neurotransmitter systems which project widely into the forebrain are influenced by circulating estrogens. Basal forebrain cholinergic neurons regulate attention and other functions. Midbrain and brainstem monoamine-producing cells (serotonin, dopamine, and noradrenaline) are involved in attention, mood, memory, motor activity, and fine motor skills.

Estrogen effects in the hippocampus: genomic and nongenomic effects

Estrogen treatment increases dendritic spine density on CA1 pyramidal neurons in the female rat hippocampus, whereas progesterone treatment acutely enhances spine formation, but causes the down-regulation of estradiol-induced synapses over the next 24 h (fig. 3) [4]. The mechanism of estrogen action involves both genomic and nongenomic effects. Moreover, estrogens do not act alone, and ongoing excitatory neurotransmission involving NMDA receptors is required for synapse induction: estradiol treatment increases NMDA receptor density in the CA1 region of the hippocampus by a mechanism that may not involve transcriptional regulation by estradiol.

Where are the ERs that mediate these effects? Adult CA1 pyramidal cells of the dorsal hippocampus do not express detectable cell nuclear ER. Instead, immunocytochemistry for ERα showed cell nuclear ERs in sparsely distributed interneurons in the CA1 region and other regions of Ammon's horn. Beside cell nuclear ERs, evidence is increasing for nonnuclear ERs that interact with second-messenger pathways. Using electron microscopic immunocytochemistry, ERα-immunoreactivity (IR) was detected in dendritic spines of principal cells, where it was often associated with spine apparati and/or postsynaptic densities, suggesting that estradiol might act locally to regulate calcium availability, phosphorylation, or protein synthesis. Other ERα-IR was found

Table 1.

Gonadal hormone effects on clinically relevant nonreproductive functions

Affective state and mood. Estrogens affect the serotonergic, noradrenergic, dopaminergic, and cholinergic systems, all of which play a role in affective state and mood. Two disorders are particularly noteworthy, premenstrual syndrome (PMS) and depressive illness. For PMS, suppression of ovarian cyclicity reduces mood swings, although specific hormonal mechanisms are not known. High doses of estrogens have antidepressant effects in human subjects, and estrogen treatment influences the response to antidepressant drugs in animal models.

Cognitive function. Estrogens influence short-term verbal memory as well as performance on tests of fine motor skills and spatial ability. Sex differences exist in humans and in animals for strategies used in solving spatial navigation problems.

Dementia. Estrogen therapy in open trials has been reported to prospectively benefit cognitive function in nondemented women. There is a reportedly lower prevalance of Alzheimer's disease as a cause of death in elderly women who receive estrogen replacement therapy postmenopausally.

Motor coordination and movement disorders. Estrogens modulate activity of the cerebellum and the nigrostriatal and mesolimbic dopaminergic systems and have effects on normal and abnormal locomotor activity. High levels of estrogens antagonize the dopamine system and are recognized to exacerbate symptoms of Parkinson's disease, whereas low estrogen levels facilitate dopaminergic function.

Excitability and epilepsy. Catamenial epilepsy varies according to the menstrual cycle, with a peak frequency corresponding to the lowest ratio of progesterone to estradiol during the cycle. Three potential mechanisms are recognized: (1) estrogen induction of excitatory synapses in hippocampus, leading to decreased seizure thresholds; (2) progesterone actions via the steroid metabolites which act via the GABAa receptor to decrease excitability; (3) hormone actions on the liver to increase clearance rates of antiseizure medication.

Pain. Recent studies in mice indicate that males and females use functionally distinct pain pathways, and that gonadal steroids, particularly estrogens, play a major role in regulating these pathways.

Stroke. Estrogens protect against damage produced by ischemia in experimental models of stroke.

For review, see ref. 2.

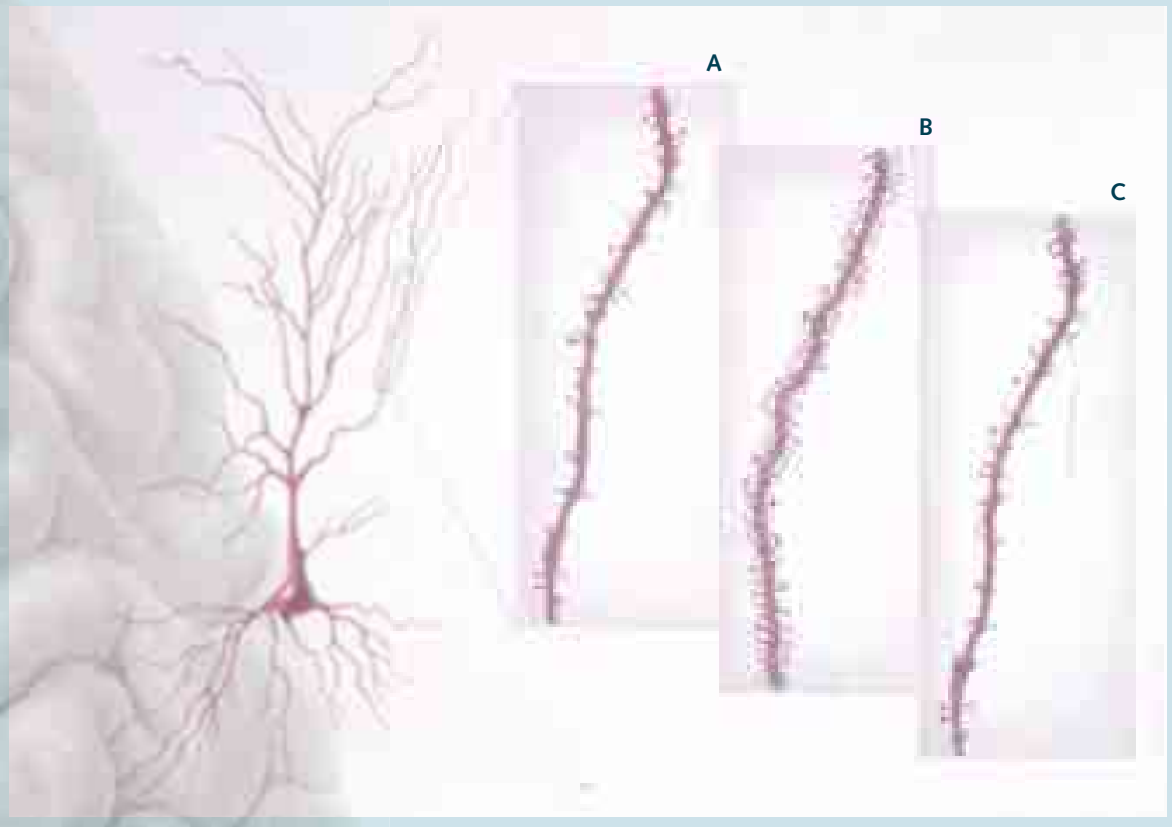

Fig. 3.
Spine synapses on hippocampal pyramidal neurons, which are sites of excitatory neurotransmission important for learning and memory, are replaced during the 4- to 5-day estrous cycle of a female rat under the influence of estradiol and progesterone. Hippocampal-dependent memory processes are affected in parallel with the increases and decreases in synapse density. **A**. Diestrus – the beginning of the cycle when estradiol levels are low. **B**. Proestrus – the day when ovulation occurs and sexual receptivity is shown. **C**. Estrus – the day after proestrus, when the system is beginning to reset itself for the next cycle.

in unmyelinated axons and axon terminals containing small synaptic vesicles, where it may be involved in regulating neurotransmitter release. The close association between ERα-IR and dendritic spines supports a possible local, nongenomic role for this ER in regulating dendritic spine density via second-messenger systems (see fig. 4, 5).

How can nuclear and nonnuclear actions of estradiol work together in the hippocampus (fig. 5)? Acting on the ERα-containing inhibitory interneurons, estradiol decreases GABA inhibition of excitatory neurons where the new spine synapses are generated. Concurrently, ERs in dendritic spines may be associated with the activation of mRNA translation from polyribosomes or endomembrane structures found in spines. In addition, other second-messenger signaling effects might include the phosphorylation of neurotransmitter receptors or ion channels. ERs in certain presynaptic terminals might modulate neurotransmitter release or reuptake. Moreover, ER-mediated activation of second-messenger systems in dendritic spines and presynaptic endings might lead to retrograde signal transduction back to the cell nucleus, providing another pathway through which estradiol could regulate gene expression.

Aging female brain

With an increasing life expectancy, women may live a substantial part of their lives with greatly reduced estrogen levels after the menopause. For many women, hot flushes are the most dramatic and noticeable consequence of ovarian hormone loss. But there are other less obvious and more gradual changes, such as loss of bone calcium and osteoporosis, that have led many women to take hormone replacement therapy (HRT) at the menopause. Furthermore, the loss of protection of coronary arteries by estrogens, increasing the risk for cardiovascular disease, has also reinforced the value of HRT. The brain also suffers from the loss of this circulating hormone. There are two aspects to this loss, namely, reversible changes in cognitive function and an irreversible decline associated with the onset of dementia.

Loss of estrogens following suppression of ovarian function with gonadotropin-releasing hormone agonists, or the loss of ovarian hormones as a result of surgical and natural menopause, leads to generally reversible decreases in declarative memory and motor coordination that respond to estrogen replacement therapy. Estrogen actions in the hippocampus are suspected to underlie the declarative-memory deficits. A long-term consequence of estrogen loss at menopause is an increased risk for Alzheimer's disease that can be reduced by estrogen replacement therapy, although treatment with estrogens once the disease is clearly established apparently has no beneficial effect [5]. Estrogens could protect the brain from neurodegeneration in at least two ways. As discussed above, estrogens maintain the function of key neural structures such as the hippocampus and basal forebrain and the widely projecting dopaminergic, serotonergic, and noradrenergic systems (tables 1, 2). As estrogen levels decline over the menopause, these systems and the cognitive and other behavioral processes that depend upon them also decline, at least functionally, but appear to respond to estrogen replacement. Estrogens not only maintain function but may also confer resilience against neural damage maintaining synaptic connections and promoting the activity of these important neural systems.

Estrogens may also directly block the actions of neurotoxic agents or inhibit their generation. The A ring of the estrogen molecule appears to have special properties with respect to the formation of free radicals, and special protective effects on cells in culture deprived of serum or exposed to free radical generators. In addition, estrogen treatment of cultured nerves decreases formation of the toxic form of the beta-amyloid protein. Estrogen treatment also interferes with the toxic effects of the beta-amyloid protein and the HIV coat protein, gp120, both of which act via free radical generation.

Hormone replacement therapy

The recent termination of the arm of the HRT trial of the Women's Health Initiative (WHI) that involved administration of the estrogen-progestin combination known as PremPro (Premarin and Provera) brings the issue of HRT into sharp focus [6]. The purported benefits of HRT for the brain include improved cognitive function, motor coordination, and reaction times, protection from brain damage due to stroke, and reduced risk of Alzheimer's disease.

It is important to note that the estrogen-only arm of the WHI trial was not terminated, thus focusing attention not on the efficacy of estrogen replacement per se, but rather on the failure of PremPro to have beneficial effects for cardiovascular disease. Indeed, the progestin component of the HRT, in this case medroxyprogesterone acetate (Provera), and the way it was administered appear to have been the major culprits. Because Provera is a synthetic progestin, it interacts with glucocorticoid and androgen receptors as well as progesterone receptors. Moreover, in the most common form of HRT, the combination of Premarin and Provera were given concurrently, contrary to the natural secretion pattern of estradiol and progesterone which are sequential. Thus the failure of the PremPro form of HRT is an indictment of Premarin and Provera and their concurrent administration, rather than a failure of HRT in general, a distinction that, unfortunately, has not emerged in much of the press coverage of the termination of the WHI trial.

The distinction between the actions of Provera and progesterone are quite apparent in studies on nerve cells and brain tissue. In nerve cells in culture, estradiol, together with progesterone and 19-norprogesterone, exerts protective effects against toxicity by glutamate; in contrast, Provera did not protect against glutamate neurotoxicity and attenuated the neuroprotective effects of estradiol. For the serotonin system, estrogen administration increases synthesis of the neurotransmitter serotonin by inducing the rate-limiting enzyme, tryptophan hydroxylase, in midbrain raphe nucleus neurons; progesterone administration after estrogen priming does not alter this induction, but Provera treatment reverses the estrogen effect [7].

An alternative to replacement with estradiol and progestins is the use of so-called selective estrogen response modulators (SERMs), which have the advantage of antagonizing proliferative effects of estrogen on estrogen-dependent cancer and are, at the same time, partial agonists for some estradiol effects. For example, the SERM raloxifene mimics estradiol in inducing tryptophan hydroxylase in the midbrain raphe, and another SERM, CI-628, mimics estradiol effects to induce a rate-limiting enzyme for acetylcholine formation, choline acetyltransferase, in the basal forebrain. At the same time, SERMs antagonize some effects of estradiol: e.g., CI-628 blocks estradiol induction of new spine synapses in the hippocampus of the female rat. Therefore, SERMs may enhance some, but not all, of the beneficial actions of estradiol on the brain and other systems.

Comparison with the male brain

Males show a lesser decline of gonadal function with increasing age and do not suffer from a substantial loss of androgens, and so we know much less about the effects of androgen or estrogen replacement therapy on cognitive function or protection from Alzheimer's disease in men. In one study, androgen reduced beta-amyloid formation. However, since in animal models there are developmentally programmed sex differences in many of the neural

Table 2.

Brain regions affected by estrogens

Basal forebrain cholinergic system. Estradiol treatment up-regulates cholinergic markers and nerve growth factor receptors, promoting neuronal survival; there are sex differences programmed during early development.

Midbrain serotonergic system. Estrogen treatment regulates tryptophan hydroxylase, serotonin transporters, and certain serotonin receptor subtypes; there are sex differences in progestin receptor expression and in serotonin turnover.

Midbrain and hypothalamic dopamine system and projections. Incertohypothalamic dopamine neurons show developmentally programmed sex differences in neuron number and function and respond to prolactin and estrogen treatment. In contrast, nigrostriatal and mesolimbic dopamine neurons fail to express detectable intracellular ER; however, estrogen facilitates amphetamine- or apomorphine-stimulated dopamine release and locomotor activity in rats.

Brainstem catecholaminergic systems. Estradiol regulates tyrosine hydroxylase gene and immediate early gene expression, and does so apparently via intracellular ERs.

Hippocampus. Estrogen treatment induces de novo synapse formation on pyramidal neurons, involving the participation of NMDA receptors.

Spinal cord. There are sex differences and estrogen modulation of nociception in humans and animals.

Glial cells. Estradiol regulates specific genes such as glial fibrillary acidic protein and apolipoprotein E within astrocytes and microglia via intracellular ERs, and these changes may reflect a role of glial cells in normal synaptic plasticity as well as lesion-induced plasticity.

Cerebral vasculature. Some intracellular ERs are expressed in central nervous system endothelia, and estrogen treatment regulates glucose utilization, possibly by inducing glucose transporter 1 in endothelial cells of the blood-brain barrier.

For review, see ref. 2.

systems that respond to estrogens (tables 1, 2) and androgens, estrogen and androgen actions in men and women are unlikely to be equivalent. Although the normal physiological effects of estrogens on the widespread estrogen-responsive systems of the brain (tables 1, 2) may show sex differences, the antioxidant and related neuroprotective effects of estrogens summarized above will probably not differ between men and women. Considering the fact that androgens may themselves have beneficial effects on the brain, and that testosterone is converted not only to 5 alpha-reduced androgens but also to estrogens in the brain and fat tissue, testosterone replacement in males may bring benefits, even if the estrogens produced by aromatization may not do exactly the same things in males as estrogens in females.

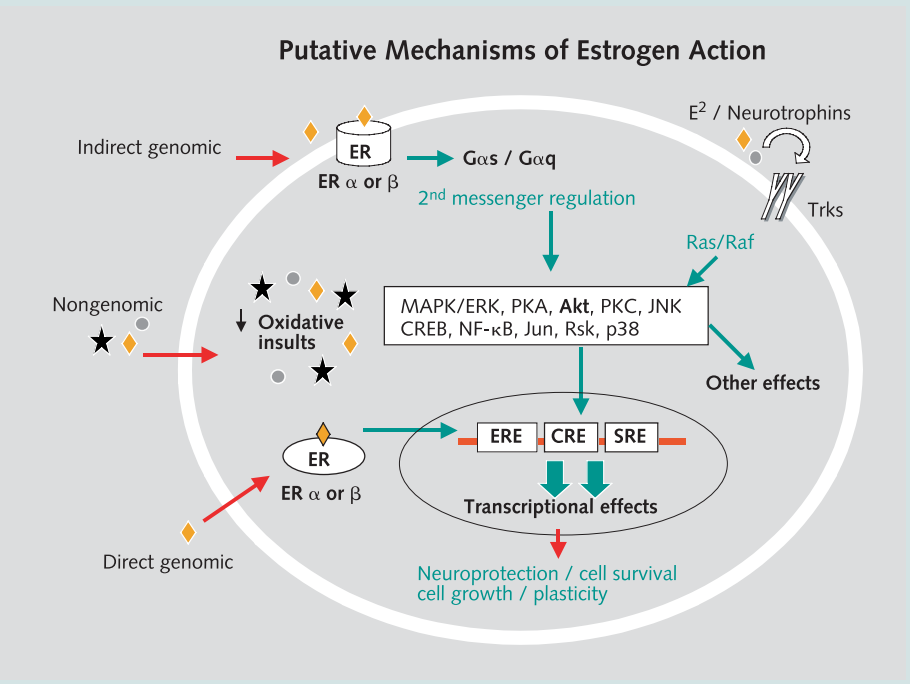

Fig. 4.
Putative mechanisms of estrogen action affecting cell growth, neuroprotection, and cell survival, and structural plasticity. In the **direct genomic mechanism**, the nuclear form of ERα or ERβ associates with either the estrogen response element (ERE) on DNA or fos/jun heterodimers that bind, in turn, to AP-1 sites on DNA. **Indirect genomic mechanisms** include the activation of an estrogen receptor linked to second-messenger systems, such as protein kinase C (PKC), cyclic AMP and protein kinase A (cAMP/PKA) and mitogen-activated protein kinase (MAPK/ERK), converging with the genomic pathway. In one of these pathways, Ras activates Raf, which leads to sequential phosphorylation and activation of MAPK/ERK. Activated ERK then translocates into the nucleus to interact directly with nuclear transcription factors (e.g., CREB, cfos/cjun), and indirectly through the activation of intermediary signaling proteins (e.g., Rsk, p38, JNK) to bind to the DNA regulatory regions, cAMP response element (CRE), and serum response element (SRE). Neurotrophins and estrogens may influence each other's actions by regulating receptors and/or ligand availability through reciprocal regulation at the genomic level. **Nongenomic estrogen effects** at high concentrations involve antioxidant effects not mediated by known intracellular estrogen receptors. **Terminology**: ERE, AP-1, SRE, and CRE are regulatory regions in DNA sequences that are recognized by specific gene regulatory proteins. ERE is recognized by estrogen-ER complexes; AP-1 is recognized by fos/jun heterodimers; CRE is recognized by phospho-CREB (phosphorylated by PKA in response to a rise in cAMP levels); SRE is recognized by the SRF-Elk-1 complex phosphorylated by MAPK/ERK. MAPK/ERK migrates from the cytoplasm to the nucleus and phosphorylates Elk-1, thereby activating it to turn on transcription of the fos gene. MAPK/ERK and PKC can phosphorylate jun protein, which combines with the newly formed fos to form heterodimers that ultimately bind to AP-1.
★, estriol; ♦, 17α-estradiol; ●, 17β-estradiol [reprinted from ref. 8 with permission].

Future challenges

Estrogen effects on the brain must now be regarded in a broad arena that encompasses many aspects of brain function, including mood, motor control, pain, higher cognitive processes, and the new domain of neuroprotection. Recognition of multiple ER types and intracellular mechanisms of action has opened the door to the study of estrogen effects in brain regions not previously recognized to be estrogen sensitive. The multiple mechanisms of estrogen action present new opportunities and challenges for pharmaceutical strategies directed toward developing agents targeted to specific processes such as reduction of free-radical-related damage in the brain while minimizing the risks associated with estrogen replacement therapy. At the same time, study of androgen actions and the potentially beneficial effects of androgens may help to counter the loss of cognitive function and risk for dementia in aging males. Study of gender differences in the actions of gonadal hormones has now moved beyond strictly reproductive functions into their roles in major functions of the nervous system in health and disease.

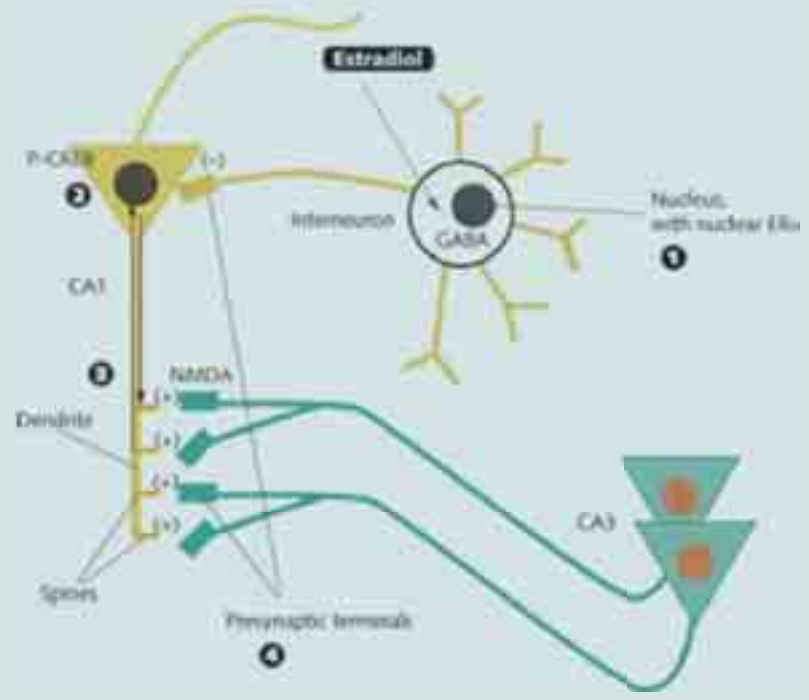

Fig. 5.
In the rodent hippocampus, estrogens operate via both genomic and nongenomic ERs to regulate the formation of excitatory spine synapses. (1) Estradiol works via genomic ERs in inhibitory interneurons to depress GABA activity. (2) Estradiol regulates the phosphorylation of CREB via a second-messenger pathway in CA1 pyramidal neurons where spine formation occurs, and pCREB is involved in regulating expression of genes that remain to be determined. (3) Estradiol regulates local protein synthesis in dendrites from mRNA that has been transported into the dendrites; the Akt signaling pathway is involved. (4) Estradiol may also regulate the release of neurotransmitters such as acetylcholine from presynaptic terminals via ERs located in the terminals.

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