Published in : APMIS. Supplementum (2001), vol. 109, suppl. 103, pp. ***174-7***
Status : Postprint (Author's version)

GENERAL DISCUSSION

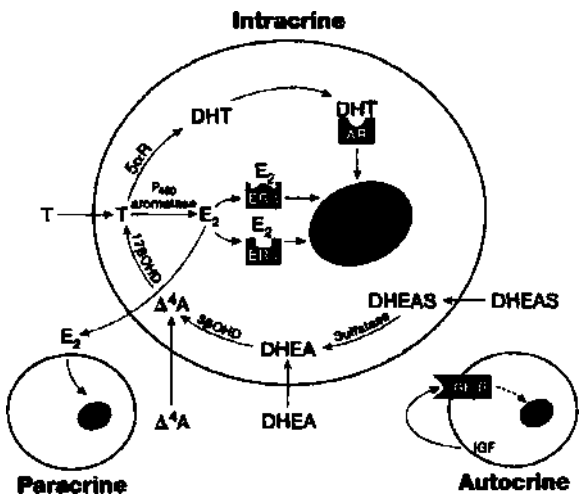
DISORDERS OF PUBERTY AND JUVENILE OBESITY

Moderator: Melvin Grumbach

Melvin Grumbach (San Francisco, USA) This figure (see below) shows the metabolism of hormones, in particular sex steroids in relation to their intracrine, paracrine and autocrine activities. Testosterone (T) enters the cell and can be converted to dihydrotestosterone (DHT), which interacts with the androgen receptor (AR), or it can undergo aromatisation and be converted to oestradiol (E2), which then can interact with the two known oestrogen receptors (ER). Dehydroepiandrosterone sulphate (DHEA-S) or dehydroepiandrosterone (DHEA) can also enter into this process after enzymic conversion to testosterone, and then to oestradiol. The E2 so formed can act within the cell or be secreted and act on a neighboring cell such as a mesenchymal cell, or an epithelial cell. There is a whole process of regulation of sex steroids within the cell.

Intracrine mechanisms refer, in this instance, to the synthesis in peripheral cells of oestradiol from testosterone and other 19-carbon precursors. T (testosterone) entering the cell from the circulation is converted to DHT (dihydrotestosterone) by 5aR (5a-reductase 1 or 2), which acts through binding to the AR (androgen receptor). T is converted to E_2 (17α-oestradiol) by CYP19, and the E_2 binds to either the Era or Er0 (oestrogen receptor α or β) forming homo- or heterodimers. Intracellular synthesized T, for example, can arise from Δ^4A (androstenedione) and DHEA or DHEAS (dehydroepiandrosterone or its sulphate conjugate).

Figure*. The complexity of extra-glandular synthesis of oestrogen hormones by the conversion of C19 androgens or androgen precursors to aromatic C18 oestrogens diagrammatically represented.*



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The desulphated DHEA is converted to Δ^4A by 3β- hydroxysteroid dehydrogenase and the Δ^4A is transformed into T by the 17β-hydroxysxteroid dehydrogenase (e.g., Type 3), which then can be converted to E_2, or androstenedione can be converted to oestrone. Some of the family of 170-hydroxysteroid dehydrogenase enzymes (e.g., Type II) can convert E_2 to oestrone, providing an additional mechanism of the regulation of oestrogen synthesis and metabolism.

The E_2 synthesized by intracrine mechanisms can be released to act on a neighbouring cell-a paracrine mechanism-through the neighbouring cell's oestrogen receptors; for example, oestradiol synthesized by a mesenchymal cell acting on a neighbouring epithelial cell. The E_2 also can enter the circulation, an endocrine role.

For comparison, an autocrine mechanism is illustrated. IGF-I generated in and released by a peripheral cell can act on the same cell through the cell's surface IGF-I receptors. Recent studies have emphasized the importance of the autocrine/paracrine role of IGF-I in body growth in contrast to the endocrine role of IGF- I synthesized and released into the circulation by the liver, the major contributor to plasma IGF-I. Mice with a selectively and totally deleted hepatic IGF-I gene have greatly reduced circulating IGF-I but normal postnatal bone and body growth; these observations challenge the widely held somatomedin hypothesis. Similarly, even though oestrogens produced by extraglandular synthesis are a major source of circulating oestrogen in the male and the post-menopausal woman, especially, the intracrine and paracrine role of oestrogen in its diverse and specialized functions in specific tissues needs to be considered. Endocrine, paracrine, and intracrine oestrogen can also act rapidly on cell surface receptors, a non-genomic action. (Grumbach et al., J Clin Endocrinol Metab, 1999; 84:4677-94).

In a recent issue of 'Endocrinology' there is an important paper on oestrogen sulfotransferase whereby a sulfonate radical is transferred to the 3-OH group of oestrogens conjugated with an oestrogen such as E2 to yield oestradiol sulphate (E2S): there are endocrine disrupters which interfere with the conversion of E2 to E2S and thereby could increase oestradiol action by inhibiting one of the cytosolic pathways of metabolic inactivation (i.e., conjugation) (Kester et al., Endocrinol 2000;141:1897-1900, Song & Melner, Endocrinol 2000;141:1587-89 [editorial]). The same applies to agents compromising glucuronidation and a variety of other metabolic pathways, which inactivate potent steroid hormones. We therefore not only have a system of conversion of sex steroid precursors to more active substances, but also within the same cell mechanisms, which lead to their inactivation.

A further point that I would like to make concerns oestrogens in humans during childhood. The prepubertal males have serum oestradiol concentration of 0.08 pg/ml whereas the mean value in prepubertal girls is 0.6 pg/ml (Klein et al., J Clin Invest 1994;94:2475-80). The 11-year-old girl has a bone age equivalent to that of a 13 year old boy. This difference in bone maturation is quite likely related to the higher prepubertal concentration of oestradiol in girls. We know that oestrogen in the human, in contrast to testosterone, has a critical role in the pubertal growth spurt and skeletal maturation in both boys and girls (Grumbach & Auchus, J Clin Endocrinol Metab 1999;84: 4677-94). Oestrogens have a very limited effect on growth in Turner's syndrome: it produces a small blip in

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growth, which is not maintained. I want to emphasize that less oestrogen synthesis is required to increase the rate of linear growth and skeletal maturation than required to develop female secondary sex characteristics.

The measurement of oestrogens is fraught with difficulties. Many years ago we tried to measure serum oestradiol in prepubertal girls but the method was not sufficiently sensitive. We used solvent extraction and thin layer chromatography before radioimmunoassay (RIA) which gave reproducible and specific values in girls beginning with Tanner Stage 2 breast development (Jenner et al., J Clin Endocrinol Metab 1972;34:521-30). Serum oestradiol values must therefore be interpreted with caution if measured on neat serum by RIA without fractionation. This also applies to oestrogen conjugates.

Peter Part (European Commission) I have a question on early menarche in girls. We know that in many wild animals, which live in social groups, the oestrus in females is under strict social control. If we look at how girls have been growing up in Europe and USA over the last 100 years, in my home country of Sweden in the 1960s, girls and boys were separated into different classes at school. The girls had very little contact with the opposite sex. At the end of the 1960s there was more mixing of the sexes at school. Do girl groups suppress menarche, while mixed groups stimulate menarche in girls? Is there pheromonal activity in humans affecting the age of menarche?

Melvin Grumbach You are referring to social chemistry! There is evidence that "pheromones" may play a role in coordinating cyclical menstruation in young woman roommates, but I am not aware of data associating "pheromonal activity" with the age of menarche.

Annie Sasco (Lyon, France) I am attracted to your hypothesis that contact with the opposite sex may influence the time of onset of puberty, but I have no supportive data. In my presentation, I pointed out the difference between girls in rural and urban areas (A. Sasco, p. S80), and we must consider that rural girls are living closer to nature, and have a more natural contact with sexuality and giving birth. This may be having an influence. A more concerning factor is the possible influence of sexual abuse on earlier maturation.

Jean-Pierre Bourguignon (Liège, Belgium) A recent report in Nature (Stern & McClintock, Nature 1998;392:177-9) indicated that the length of the follicular phase could be modulated differentially in women by placing Scotch tape under their noses impregnated with extracts from apocrine secretions collected at different times during the menstrual cycle. The apocrine secretion was collected either during follicular phase or luteal phase and this indicated that a pheromone effect was acting in humans. There is, however, no evidence as yet that age of menarche may be influenced by such olfactory signalling from female or male associates.

Heinrich Meyer (Freising- Weihenstephan, Germany) We have information from farm animals including chickens in the 1950s and 1960s, that oestrogens and androgens have similar effects on animals and humans. Also, in the 1980s similar effects were seen as a result of illicit use of steroids. I received the best explanation from a calf producer concerning the different effects of androgens and

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oestrogens, and he knew exactly when to use nortestosterone and when to use oestradiol.

The misuse of anabolic agents in Europe was rife, but has diminished since the 1980s when their control became more effective. However, is it possible that the accidental ingestion of injection sites for growth promoters in animals has had an effect on the timing of the onset of puberty in humans? Is it sufficient for a single intake of exogenous oestrogen to accelerate the onset of puberty, or should exposure last for several days or weeks, and what concentrations are required to affect puberty or growth regulation?

Jean-Pierre Bourguignon It is difficult to answer this question because in the human, there is a physiological range of 4-5 years between early and late maturers. We cannot predict whether an individual girl will start puberty earlier or later. Data from epidemics of early puberty suggest that we might anticipate that the larche can occur within a relatively short period of time after exposure. However, we are usually unable to identify the responsible agent or agents, and therefore it is not possible to measure the concentration or the potency of the substances involved. These epidemics relate to accidental exposures and therefore the conditions are non-experimental and non-standardised. Hopefully such accidents can be eliminated so that we shall never be able to ascertain the doses and frequencies required to affect the onset of puberty.

Rainer Stephany (Bilthoven, The Netherlands) In response to Heinrich Meyer's question about the probability of an effect on growth by a single oral hormonal episode caused by hormones in meat. In The Netherlands in the mid 1980s we had many court cases about hormones in meat such as the illegal use of liquid hormone preparations in veal calves. The Dutch authorities wanted to shift the accusation from an economic offence to the issue of endangering life or public health, because this would then be a criminal offence and they could send the offenders to jail. The defence documented many young children who had consumed their mother's oral contraceptive pills, or their father's potency pills, and there was never any long lasting effect demonstrated. In these cases in The Netherlands, there was never any conviction. There should be many such cases worldwide, and it should be possible to follow up the children involved.

Melvin Grumbach Sometimes there is an unfortunate exposure of a child *to* either an androgen or an oestrogen; for example, accidentally to an oestrogen containing ointment, or given therapeutically for growth promotion in the bygone days when so-called "pituitary extracts" contained methyltestosterone. Recently, instances have been described in which young boys and girls were exposed to a testosterone gel skin preparation used by their father, which induced virilization. When the agent is stopped, the boy or the girl may go into spontaneous puberty, but this depends on the bone age. If the bone age is advanced to 10 years, the girl will enter spontaneous puberty after cessation of the agent. If the girl is 4 years when exposure is stopped and the bone age is advanced to 8 years or less, the child will eventually enter early puberty after a few years delay. Therefore, the sex steroids do have an effect but whether or not these stimulate spontaneous puberty depends on the degree of general maturation as qualitatively reflected in the bone age.

Fred vom Saal (Columbia, USA) An interesting feature of the presentations at this Workshop is that

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we see dramatic changes in body growth in males and we should therefore expect to see a large effect on puberty. There is abundant ecological literature indicating that sexual maturation and reproductive performance in general in male mammals is resource-independent (independent of nutritional status) whereas ecological data suggest that females are exquisitely resource-dependent in terms of reproductive parameters such as sexual maturation, or turning on and off LH, and ovulation. These suggest an environmental effect acting more on females. It must be recognised that the physiology of the male and the female are dramatically different with respect to nutrition and reproduction, and therefore the relationship between body weight and sexual maturation in males and females may be very different.

Melvin Grumbach An important aspect that we have not yet considered is the effects on the male. Endocrine disrupters with oestrogenic activity would be expected to produce breast development or advanced bone age in boys because they too are very sensitive to oestrogen. If this occurs, it must be rare as we have not seen an epidemic of gynaecomastia or advanced skeletal maturation in boys. We have heard of the effects of prenatal anti-androgens, but I am also concerned with postnatal exposure. Adopted girls in Belgium from less developed countries have advanced puberty, but why do adopted boys not have the same advanced puberty?

Jean-Pierre Bourguignon There is a general tendency for girls to start puberty earlier than boys. The mechanism for precocious puberty in adopted children follows the same pathway as physiological puberty. I would expect to see the same sex difference in earlier puberty in the adopted children as we see in physiological puberty, and also in other causes of precocious puberty.

Melvin Grumbach The age of onset of puberty in boys and girls is similar, only differing by about half a year. The first sign in the boy is testicular enlargement due to an FSH effect, and this occurs before Leydig cell stimulation, so the secondary sex characteristics are only seen at a later stage, but the age of onset of hypothalamic LHRH reactivation is not very different than in girls. I am surprised, therefore, that Dr Bourguignon does not see earlier puberty in his study of adopted boys in Belgium from less well developed countries.

Niels Skakkebcek (Copenhagen, Denmark) Dr Grumbach has a great deal of experience with precocious puberty and should be able to explain to Dr Bourguignon why girls have a higher incidence of precocious puberty than boys.

Melvin Grumbach We do not really understand this sex difference, but it depends on prenatal influences, and especially the balance between LH and FSH. The effect of FSH is to drive the granulosa cell (although LH receptors are required) and FSH causes an increase in oestradiol from an immature human ovary. In the male, FSH causes a subtle testicular enlargement, but no increase in the sex steroids for secondary sexual manifestations; for the latter LH is required to stimulate the Leydig cells. We do not understand why idiopathic true precocious puberty occurs 8 times more commonly in girls than in boys. It appears to be related to as yet poorly understood and characterized fundamental sex differences in the hypothalamic-pituitary-gonadal apparatus.

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Pete Myers (Charlottesville, USA) There is a dramatic difference in the rates of precocious puberty in boys and girls in the immigrant population to Belgium. Are there cultural differences between the reporting of male and female precocious puberty, with perhaps fathers being less likely to consult about early development of their sons?

Melvin Grumbach We have encountered fathers proud of their son's advanced physical maturation due to the classic form of virilizing congenital adrenal hyperplasia. As a consequence, the diagnosis is delayed until the discrepancy between chronological age and maturation is very apparent.

Jean-Pierre Bourguignon There is no evidence that cultural background may explain the striking gender difference in consultation for precocious puberty (PP). The few affected boys who are seen for PP are very disturbed psychologically, and clearly they are worried about their condition. Moreover, delayed puberty is conversely much more frequent in boys than in girls, and this adds further support to the concept of sexual dimorphism. The sexual dimorphism in PP in adopted or foreign children follows the same pattern as in other aetiological groups of central PP, whether organic or idiopathic. This suggests that these different forms follow the same pathway of hypothalamo-pituitary activation.

Howard Kulin (Hershy, USA) We are struggling to identify in young populations the end organs which are most sensitive to oestrogen from whatever source. We have relevant data on bone and breast, but we struggle with the brain to ascertain any changes. Perhaps, we are looking at the wrong end of the age spectrum for CNS effects: in fact the ageing brain may be a better target for identifying oestrogen induced changes.

Jean-Pierre Bourguignon Ultimately we need to know more about the genetic control of the onset of puberty. The oncogene encoding for the promoters of TGF-a may give an insight into the control of the brain and how it could be modulated differently in males and females.