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GENERAL DISCUSSION

Stφa:

I want to make some brief remarks on 5α-pregnane-3,20-dione, or 5α-dihydroprogesterone, and its possible role not only as a progesterone metabolite, but also as a biologically active compound. The high affinity steroid-binding plasma protein of pregnant guinea pigs has recently been taken into use for quantitation of plasma progesterone. As shown in Fig. 1, the affinity of this protein for 5α-pregnanedione is equal to that for progesterone. On the other hand, the affinity for the 5β-epimer is much lower. On the basis of this rather specific protein binding, a method has been worked our for simultaneous determination of progesterone and 5α-pregnanedione in pregnancy plasma. Results of such assays in a group of normal pregnant women are shown in Fig. 2. The amount of 5α-pregnanedione averaged 34% of the progesterone concentration, and there was a positive correlation between the concentrations of the two steroids.

5α-pregnanedione has been suggested to play a role in the physiological action of progesterone. In this context, the similarity in the 5α-reduction of both progesterone and testosterone by their respective target tissues is interesting. In incubation experiments with human and guinea pig uterine tissue the capacity of these tissues converting progesterone to 5α-pregnanedione has been demonstrated. Furthermore, 5α-pregnanedione has been shown to be bound to the myometrial progesterone-binding cytosol receptor with high affinity. The relatively high amounts of preformed 5α-pregnanedione in circulating plasma may indicate a direct effect of this steroid on target organs, independent of 5α-reduction within the target cells.

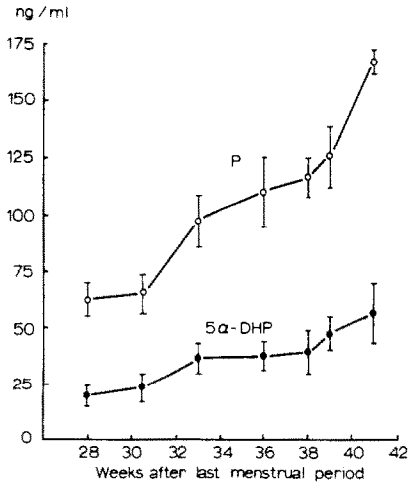

Fig. 1. The ability of the two pregnanediones to compete with progesterone for binding sites on the steroid-binding protein of plasma from pregnant guinea pigs. P: Progesterone. 5α-DHP: 5α-Pregnane-3,20-dione. 5β-DHP: 5β-Pregnane-3,20-dione.

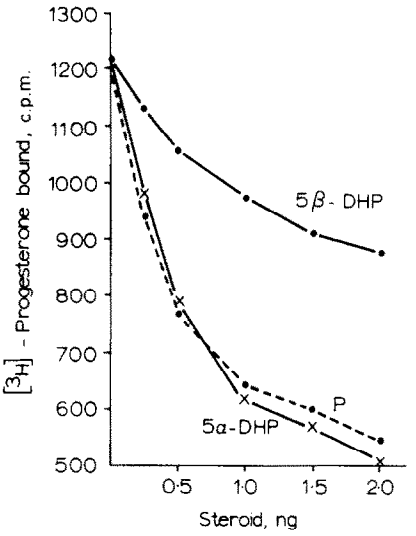

Fig. 2. Simultaneous determination of 5α-pregnanedione and progesterone in the plasma of normal pregnant women.

Lindner:

Dr. Støa, have you examined whether 5α-dihydroprogesterone has any biological activity in the guinea pig, because in the rat we find that this compound will not maintain pregnancy. Is this the case in the guinea pig?

Stφa:

No, we have not tried that. It has not yet been established if this steroid really has any biological activity in itself. It has not yet been demonstrated that it is concentrated in the nucleus. I think that the fact that it competes with the receptor proteins in the cytosol may show that it has some biological importance.

Sjövall:

When using the method I described this morning, we tried to look for 5α-dihydroprogesterone and so far have not found it. We have not looked too carefully, but with the concentrations mentioned here we ought to have found it. Also, we have failed to find it in bovine corpus luteum. (*Note added in proof.*) Further studies of plasma samples from pregnant women have revealed the presence of 5α-dihydroprogesterone although in lower concentrations and we can thus confirm Dr. Støa's finding regarding the identity of the compound.

Stφa:

We feel quite confident that we have measured the right compound. It has also been identified by mass spectrometry.

Saez:

I have a comment and a question concerning the paper by Dr. Ungar. We have also found a cholesterol carrier protein in adrenals and in testis. The sedimentation coefficient of this protein is about 2.7–3. This protein also binds squalene and pregnenolone but not DHA. At what temperature do you run your Sephadex column? Perhaps there is dissociation of the cholesterol-protein complex in the Sephadex columns and all the cholesterol dissociates. The dissociation constant is about 10^{-5} M. Maybe this is the reason.

Ungar:

We have run our Sephadex column both at room temperature and at 4°C and we found no difference. The cholesterol binds to the protein and most of it comes off at the major peak, but there is a small but significant amount that comes off at the peak that has the stimulatory activity. I might say that cholesterol binding to certain lipo-proteins in blood is also known and some of these proteins have higher affinity. I don't think we can say very much about the specific binding of cholesterol to protein at this time.

Cooke:

I would like to ask Dr. Ungar three questions. First of all, I noticed that you isolated the protein factor from the adrenal mitochondria, whereas from the corpus luteum it was the cell sap. Does this mean, in fact, that there is a different subcellular distribution of the protein? Secondly do you know anything about the kinetics of the formation of the protein factor? Thirdly is it stimulated by ACTH in the adrenal and LH in the corpus luteum?

Ungar:

We used hypophysectomized animals and compared the adrenal factor in the untreated animals with that of ACTH-treated animals. By the time you go through the process of isolating mitochondria, getting an acetone powder and an extract of this, the chances of finding a quantitative difference would not be good. We could not find any difference between the hypox and the ACTH-treated animal as far as increasing the amount of the protein. The level of activity in the hypox animal is lower, of course, but we could not demonstrate any relationship between ACTH stimulation and this factor. As to the first question on the subcellular distribution, other people have found that the acetone powder preparation of mitochondria of corpus luteum is almost inactive. We did find a small amount of activity there. It's quite possible in the preparation of corpus luteum that mitochondrial material might have been solubilized into the cytosol fraction. It is also quite possible that the material in the corpus luteum is analogous to the liver SCP which is a soluble material. There might be, I think, a number of these lipo-proteins that have the ability to bind cholesterol and they might have an effect on the cholesterol cleavage activity in various tissues.

Schrader:

I'd like to make a comment with reference to Dr. Støa's comments on 5α-pregnane 3,20-dione activity. Work by Dr. Glasser at Vanderbilt University has also demonstrated the fact that apparently 5α-dihydroprogesterone is inactive in the rat in maintaining pregnancy. In the chick, however, dihydroprogesterone will induce avidin biosynthesis. We've been studying the progesterone receptor in the chick and dihydroprogesterone does bind to that molecule. However, I think that we have to make a distinction here between obligatory processes *in vivo* and those processes which can go on *in vitro*. If receptor complexes containing progesterone only are incubated with nuclei for up to one hour *in vitro* the receptors which are retained by the nuclei still have only progesterone bound and not any metabolites. In the case of androgens, for example, you would expect to find the dihydro derivative. But in the chick, the receptors still retain the unmetabolized progesterone.

Munck:

I would like to comment on what Dr. Schrader and Dr. Støa said. It seems that if dihydroprogesterone does bind and is inactive, it should be an anti-progestational compound. Has anyone tested it for that kind of activity?

Lindner:

It is not anti-progestational in the rat. I think you may have misunderstood Dr. Schrader here. He said it did have biological activity, but that it was not an obligatory intermediate of progesterone. It could substitute for it pharmacologically, if you like, but progesterone doesn't have to undergo this transformation in order to exert its effect at the nuclear level.

Vihko:

This 5α-pregnane-3,20-dione is bound by certain mammalian receptors, too, those which are induced with estradiol in non-pregnant human, rabbit, sheep and guinea pig myometrium. It is bound with a relative affinity of 25–55% that of progesterone. In addition, it is bound to progesterone-binding protein which appears in guinea pig plasma and guinea pig myometrium during pregnancy with a relative affinity about the same as that of progesterone.