THE EFFECTS OF ACTH AND OF CORTISOL ON SERUM CERULOPLASMIN IN RABBITS

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Received 24 August 1971

1. Introduction

Ceruloplasmin, a copper containing blue colored protein (molecular weight 160,000), has moderate amounts of oxidase activity towards a variety of polyamines and polyphenols [1]. It has been known for quite some time that estrogen in the form of ethinyl estradiol [2] or stilbestrol [3] causes a marked increase in serum copper levels (which reflects serum ceruloplasmin levels). More recently some reports have appeared showing increased serum ceruloplasmin [4] and copper [5] levels in women taking oral contraceptives. The authors attributed this effect to the estrogen component present. However, in our laboratory altogether different results were obtained in rabbits; while progesterone had profound influence in increasing serum ceruloplasmin activity, similar to stilbestrol, estradiol, after causing a slight increase in the first week, caused a small but steady decrease [6]. There is also evidence that cortisol [7] causes a decrease in serum copper levels in man. Administration of cortisol, it is established, suppresses the secretion of endogenous ACTH thereby adrenal steroidogenesis is also suppressed. This can lead to a lowering in the serum of some of the steroids at the beginning steps of adrenal steroidogenesis, like progesterone or 17-hydroxypregnenolone [8] and, presumably, the latter could be the reason for the lowering of serum copper. It could then be hypothesized that administration of ACTH could result in elevation of serum ceruloplasmin, and that ACTH and cortisol could have opposing effects in this respect. To test this hypothesis the following experiment was done.

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2. Methods

Six healthy male and 2 healthy non-pregnant female rabbits weighing about 1–1.5 kg were used. In 3 males and in the 2 females 7 I.U. of ACTH (Acton) per rabbit were administered subcutaneously. Blood samples were drawn before, and one week after, administration of the hormone. In the other 3 males 25 mg of hydrocortisone acetate (Wycort) per rabbit were given subcutaneously in four injections (1st, 3rd, 6th and 10th day), and blood samples were also drawn before, 1 week, and 2 weeks after the first injection. Serum ceruloplasmin activity was estimated on the following day always simultaneously with two serum samples of control rabbits using *p*-phenylenediamine (PPD) oxidation, adopting the method of Ravin [9. 10].

Acetate buffer, 8 ml (pH 5.5), 0.1 ml serum and 1.0 ml 0.5% substrate (PPD) were incubated at 37° for 1 hr. The oxidation reaction was arrested by 1 ml 0.5% sodium azide, and after cooling to 10–15° for 30 min, the colour intensity read in a photoelectric colorimeter; an experimental control (other than the serum samples of two control rabbits) for each serum sample was also set up simultaneously by adding sodium azide before adding PPD. The values are expressed as the optical density, without converting to mg ceruloplasmin per 100 ml by using a conversion

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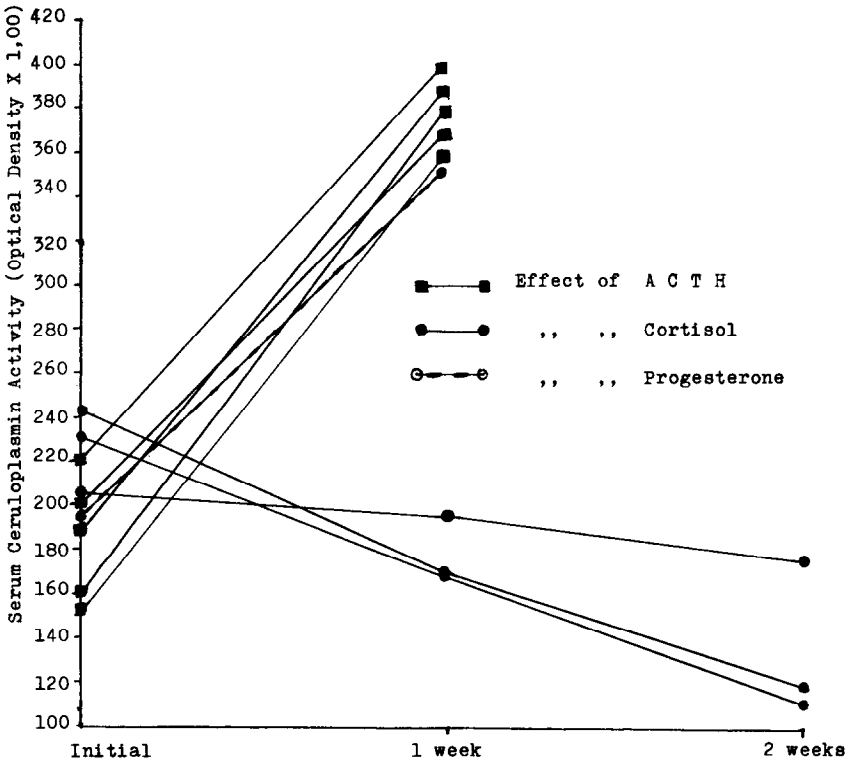

Fig. 1. Effects of ACTH and of cortisol on serum ceruloplasmin in rabbits. 3 male and 2 female rabbits were each given a single subcutaneous injection of 7 I.U. of ACTH. Serum ceruloplasmin activity was estimated before (initial) and one week after the injection. 3 male rabbits were each given subcutaneously 25 mg hydrocortisone acetate on 1st, 3rd, 6th and 10th day of the experiment. Ceruloplasmin was estimated before, 1 week, and 2 weeks after the first injection. All individual values are shown. The effect of progesterone (o - - o) is the average response of 8 rabbits on giving 20 mg of progesterone in divided doses over 1 week. The latter is quoted from Indian J. Exptl. Biol. [6], by the kind permission of the editor.

factor [9]; this is equally dependable [9, 10] and moreover was felt safer since no useful standard was available. The substrate solution was always prepared fresh to get the optical density reading of the control between 0.050 and 0.090.

3. Results and discussion

The ceruloplasmin values of each rabbit in response to ACTH and to cortisol are shown in fig. 1; for comparison the corresponding mean response of 8 rabbits to progesterone also is shown. It appears convincing that ACTH has profound influence in increasing serum ceruloplasmin, since in all 5 rabbits a marked increase was noted in 1 week in response to a single injection of 7 I.U. of ACTH. The decrease in ceruloplasmin levels in response to hydrocortisone is probably due to suppression of ACTH production. The long term influence of ACTH, however, was not tested. There is evidence [11] that in leukemic patients treatment with ACTH for 1–1½ months causes a decrease in serum copper levels. The sustained elevation of cortisol and of other terminal steroids in the adrenal steroidogenesis as a result of the continual stimulation by ACTH might have caused this. It could also be possible that the ratio between steroids in the beginning and final steps of steroidogenesis influences the ceruloplasmin levels. However, the long term response to ACTH need not be the same in normal humans or rabbits as in leukemic patients.

The results of this and of the previous communication [6] suggest that there could be steroids other than progesterone that have this influence, and that some of them may be much more potent than progesterone, since ACTH is apparantly more potent than the former (see fig. 1). It appears, therefore, worthwhile to screen all steroids to find out the relative potency of each with regard to this property and to delineate the minimal structural requirement for the same. For instance, a side chain at C-17 may be essential since ethinyl estradiol [2], having the ethinyl side chain at C-17, does cause an increase while estradiol having no side chain at C-17 does not have this influence [6]. However, cortisol having a side chain at C-17 has no positive influence; the other aspects of its structure, like the configuration at C-11, may be responsible for this. It would also be worthwhile to correlate this property of the steroids with their biological functions. A further step could be that some of the most potent steroids in this respect could be tried in the treatment of Wilson's disease; they may be able to mobilize the tissue copper for the synthesis of serum ceruloplasmin. Although at this stage it appears far-fetched, a speculation may be made that Wilson's disease might be the result of a genetic defect in the synthesis of a special steroid, powerful like aldosterone for the synthesis of serum ceruloplasmin; a hypothetical equilibrium between serum copper bound to ceruloplasmin and tissue copper is shifted to the right by this.

Acknowledgement

I am grateful to Mr. A.K. Ulahannan, Dr. K. Balasaraswathy and Professor R. Raghunandana Rao for their help and co-operation in this work.

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