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EFFECTS OF ANTIBODIES TO ESTRADIOL-17β AND TO PROGESTERONE ON THE PLACENTAL WEIGHT AND PREGNANCY IN RATS—A QUANTITATIVE STUDY

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SUMMARY

Antibodies to estradiol or progesterone were injected to pregnant rats. The observed biological changes were correlated to the concentration of estradiol or of progesterone which remains unbound to the injected antibody.

Effects of antibodies to estradiol (E_2) and to progesterone (P) in pregnant rats have been studied previously by others [1,2] and by ourselves [3,4]. To interpret the results we carried out a quantitative study: we measured the concentrations of the antibodies in the immunsera used (anti-E_2 (A–E_2) and anti-P (A–P)), and after the injection of antibodies to pregnant rats, the concentration of the circulating A–E_2 and A–P, total E_2 and P (E_{2t}, P_t), E_2 and P bound to A–E_2 or A–P (E_{2b}, P_b), and E_2 and P unbound to A–E_2 or A–P (E_{2u}, P_u).

Highly specific antibodies were raised to E_2 and to P by immunizing rabbits with E_2-6-carboxymethyloxime-BSA [5] or P-11α-hemisuccinate-BSA, resp. The antibodies were partially purified and concentrated by ammonium sulfate precipitation to obtain the stock solutions used throughout this study. The "concentration" of the antibodies was defined by measuring the total concentration of hormone-binding sites, i.e. the "Binding Capacity" (BC) of the antibody, using tritiated hormones and the dextrane-coated charcoal technique. Scatchard plots showed that the BC at saturation of A–E_2 was 12 μg/ml and that of A–P 3 μg/ml in the stock solutions. 1 ml A–E_2 or 2 ml A–P was injected i.p. to pregnant rats and blood was sequentially collected to measure the concentrations of circulating A–E_2, E_{2t}, E_{2b}, E_{2u} and A–P, P_t, P_b, P_u.

A–E_2 and A–P were measured by determining their BC at saturation with the charcoal technique. E_{2t} and P_t were determined by radioimmunoassay, E_{2b} and E_{2u} or P_b and P_u were either estimated directly or calculated. Estimation: aliquots of plasma-samples were equilibrated at 37°C with tracer quantities of the corresponding tritiated hormone, then the antibodies were adsorbed on a solid immunoadsorbent and radioactivity bound and unbound to the antibodies was measured. Calculation: as total concentration of the circulating antibody and hormone have been measured, the concentration of hormone bound and unbound to antibodies can be calculated if the *in vivo* binding-characteristics of the antibody are known as well. Therefore we measured the binding of tritiated E_2 and P to A–E_2 and P respectively with the double antibody technique in presence of undiluted rat plasma, i.e. under conditions very close to those present *in vivo*.

Previous experiments [3] have shown already that if A–E_2 was injected to pregnant rats, placental weights increased, in comparison with control animals, an observation similar to that obtained with ovariectomised rats [6]. The present experiments furnished the quantitative background to this observation: 6 hours after the injection of 1 ml A–E_2 to rats on the 6th or 10th day of pregnancy, the BC of circulating A–E_2 was 650 ng/ml, E_{2u} decreased from 40 pg/ml before the injection to 2 pg/ml and was still less than 15 pg/ml at the end of the 7–10 day observation period.

In a subsequent experiment we injected 2 ml A–P to rats either at day 10 of gestation, (these animals aborted in about 48 h), or at day 6 (these animals had normal pregnancy) 6 h after the injection of 2 ml A–P, A–P_i the BC of the circulating A–P was identical in both groups: 330 ng/ml, but while in the first group (abortion) P_u decreased from 45 ng/ml before the injection of A–P to 15 ng/ml (and continued to decrease during the 4 days observation period) in the second group (normal pregnancy) P_u decreased from 65 ng/ml before the injection to only 45 ng/ml (and increased to 60 ng/ml during the 10 days observation period). The simplest explanation of this finding would be that more P is synthesized at day 6 than at day 10 therefore the relatively low BC (6 μgP) of the injected A–P was insufficient to reduce the concentration of the biologically active P (P_u) to a critical level. This suggestion is substantiated by the finding that A–P injection to unilaterally ovariectomized rats

provokes abortion even at day 6 of pregnancy [4], and we hope to be able to furnish final proof: provoking abortion in the intact rat at the 6th day of pregnancy by injecting purified A–P of higher BC than that used in the present studies. The concepts and techniques developed during the present studies might have a general use for the quantitative examination of the various components of the regulatory mechanism of pregnancy.

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