Letters to the Editor should be typed double-spaced (including references) with conventional margins. The overall length is limited to five manuscript pages, including not more than one figure or one table.

"Hook-Effect" in a Patient with a Gonadotropin-Secreting Tumor

To the Editor:

Two-site or "sandwich" immunoradiometric assays (IRMA) offer theoretical advantages over competitive immunoassay systems for sensitivity, precision, and rapid incubation. However, practical realization of these advantages has been limited by the phenomenon of the "high-dose hook effect," in which high concentrations of an analyte give similar responses to those of much-lower concentrations. Here we report a patient with excessive secretion of lutropin (LH) and follitropin (FSH), which was masked by spuriously low assay values for the gonadotropins.

In June 1988, a 25-year-old woman was referred to the Department of Neurosurgery for bitemporal visual field impairments due to a large intracranial suprasellar tumor. Her previous medical history included ovarectomies in 1982 and 1983 due to increasing cystic tumors in both ovaries. Since then, she had been on constant estrogen replacement therapy (1.25 mg of conjugated estrogens per day). She was otherwise asymptomatic, and the suprasellar tumor was removed by cranial surgery.

The pre-operative assessment of the endocrine pituitary function revealed increased basal values for LH and FSH combined with a paradoxical decrease in LH concentration after stimulation with gonadotropin (GnRH, 0.1 mg, intravenously). Concentrations of other pituitary hormones were normal in the basal and stimulated states. The estradiol concentration was 283 ng/L. We determined LH and FSH with "MAIA-clone" kits supplied by Serono, Freiburg, F.R.G., according to the manufacturer's instructions. The kits are IRMAs with two monoclonal antibodies that recognize the respective epitopes of both hormones. Standard curves are linear up to 200 and 150 inte.units/L, respectively. Values exceeding this range are identified and samples must be diluted and the assay repeated. In the kit instructions the manufacturer indicates the possibility of a high-dose hook effect for LH and FSH above a concentration range of 400 and 1000 inte.units/L, respectively.

Figure 1 illustrates LH and FSH concentrations in the patient's serum, diluted and undiluted. Although the FSH values of the undiluted specimens are registered as being outside the standard curve, the measured LH values are decreasing. Only the diluted specimens show clearly that the true concentrations are 15 to 20 times higher than values for the undiluted ones. After GnRH administration these values increase considerably.

The initially determined results for basal and stimulated LH and FSH concerned us, because they revealed a quite unusual situation. The increased basal values could be explained by the functional menopause after bilateral ovarectomy and estrogen replacement treatment. However, the paradoxical decrease in LH hinted at a method-dependent hook effect. When we repeated the determination with 20-fold-diluted samples, the results for the basal and stimulated LH and FSH values revealed excessive gonadotropin secretion. These laboratory findings, together with the clinical symptoms, confirmed the diagnosis of a gonadotropin-producing tumor of the pituitary gland. Retrospectively, we can speculate that the patient's bilateral cystic ovarian tumors were already a pathological consequence of the endogenous gonadotropin excess induced by the pituitary adenoma; however, hormone data from the time of ovarectomy are not available.

The hook effect is gaining importance as more and more monoclonal antibodies are being used in modern test systems designed as "sandwich" assays. Various theories as to the causes of the hook effect and various methods to avoid it have been developed (surveyed in 1). In general, this artifact can be prevented by decreasing the sample volume or by modifying the one-step assays to a two-step procedure (2).

The possibility of a method-dependent hook effect must be considered carefully, because this methodological problem might contribute to a delayed diagnosis, with hazardous clinical consequences.

References


Nicolaus Dahlmann
Karl A. Bresing
Dietrich Klingmüller
Frank Bidlingmaier

Instit. für Klinische Biochemie
Universität Bonn
Siegfried-Freud-Straße 25
5300 Bonn 1, F.R.G.

Cord Serum Thyrotropin and Birth Weight in a Normal Japanese Population

To the Editor:

Orinda et al. (1) described an inverse relationship between thyrotropin (TSH) concentration in cord blood and birth weight of normal newborn infants in Kenya. They suggested that the high TSH in babies with low birth weight...
may reflect the stress to the fetal hypothalamic–pituitary–thyroid system, and that thyrotropin–releasing factor, TRF during labor may be responsible for increased TSH in babies. More recently, Delange et al. (2) suggested a different possibility: moderate iodine deficiency during the perinatal period in Kenya.

In Japan there is no area of iodine deficiency, and Japanese people generally ingest iodine-rich foods frequently. Thus we examined the relation between TSH concentrations in cord blood and birth weight in Osaka, Japan. We measured TSH, free thyroxin (FT₄), and free triiodothyronine (FT₃) concentrations in cord blood from a normal Japanese population. Values for deviation of birth weight from the mean more accurately reflect neonatal development than does net birth weight. Therefore, we not only examined net weight (in grams) but also values for the deviation from the mean. We found no significant relation between birth weight and TSH (Figure 1). FT₄ or FT₃.

We think that the result for the Kenyan population may be related to iodine deficiency and cannot be generalized as a physiological phenomenon.

References

Nobuaki Mitsuda
Haruo Tamaki
Nobuyuki Amino

1-1-50 Fukushima, Fukushima-ku
Osaka 553, Japan

Shifting the "Hook Effect" In One-Step Immunometric Assays

To the Editor:

One of the major limitations of the one-step "sandwich-type" assay has been the high-dose "hook effect," a decrease in the response of the assay to increasing concentrations of the analyte. Consequently, falsely low values may be observed when samples with high concentrations are tested. To avoid this problem, most workers have performed two-site immunometric assays in a two-step format in which the test sample first reacts with the solid-phase (capture) antibody and then, after an incubation and washing step, with the labeled (detection) antibody. Although under optimized conditions a two-step modification is free from the hook effect, the assay design has the disadvantages of an additional step and a longer incubation. Furthermore, the two-step format does not always eliminate the high-dose hook effect.

In one-step immunometric assays involving monoclonal antibodies, the hook effect occurs because the concentration of the detection antibody is limited. In samples with increasing analyte concentrations, the excess antigen progressively saturates both the solid-phase and the detection antibodies, thus preventing them from forming the "sandwich." Thus, we initially recommended that sandwich-type assays for analytes such as chorionic gonadotropin (hCG), which could be present in very high concentrations, be performed in a two-step assay format. The effect of excess antigen can also be reversed by increasing the concentration of the detection antibody to be sufficient to saturate both the excess antigen and those captured by the solid-phase antibody. Under such conditions, the assay response would be expected to plateau at analyte concentrations that otherwise would result in the hook effect.

I have examined this possibility by testing the response of the "FitAgent" (CyberFluor Inc., Toronto, Canada) time-resolved immunofluorometric assays (1) to increasing concentrations of hCG in the presence of various amounts of the detection (biotinylated) antibody. Similar to our previous findings (1), samples with hCG concentrations up to 500 kilo-int. units/L (preparation 75/537) did not exhibit the hook effect in the two-step assay (data not shown). In contrast, the hook effect was significant at hCG concentrations >10 kilo-int. units/L when the samples were tested according to the one-step procedure. However, the critical concentration at which the hook effect occurred was directly related to the amount of labeled antibody used (Figure 1). Increasing the antibody concentration proportionately increased the assay's tolerance to the hook effect. In the presence of 2.0 μg of the detection antibody per well, the hook effect for concentrations up to 500 kilo-int. units of hCG per liter was completely eliminated (Figure 1, curve D).

These results complement previous findings (1, 2) in which a two-step modification of the immunometric assays eliminated the high-dose hook effect. However, as in the two-step assays (4), inadequate concentrations of the labeled antibody appear to be the major cause of the hook effect in one-step assays. By optimizing the concentration of the labeled antibody, one can take advantage of the simplicity of operation and the speed of the one-step assay, while avoiding the high-dose hook effect. The feasibility of this approach should be evaluated in other immunometric assays.

References

CLINICAL CHEMISTRY, Vol. 36, No. 1, 1990 189