DO SUBUNITS OF LUTEINIZING HORMONE POSSESS ANY BIOLOGICAL ACTIVITY?

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Summary

Antisera raised against subunits of ovine luteinizing hormone (LH) were used to purify subunit preparations and then purified subunits were used to determine whether they possess any inherent biological activity. Reference preparations of ovine LH (NIH-LH-S18, 1 μg/ml), α-subunit of LH (21 μg/ml) or β-subunit of IH (23 μg/ml) were shown to stimulate cyclic AMP accumulation in immature rat ovaries in vitro. This action of the reference α-subunit preparation was completely abolished after absorption of any contamination of β-LH subunit as well as intact LH present in the preparation by treatment with an antiserum to the β-subunit of purified ovine LH (anti-β-LH). No change in its immunological properties were observed after the absorption. Similar results were obtained with the reference β-subunit preparation, which was not active in stimulation of cyclic AMP accumulation after its absorption with anti-αLH but still retained immunological properties. Thus none of the subunits of ovine LH has inherent biological activity. The activities of these subunits which were reported in the literature are probably due to the contamination of the subunit preparations by the intact hormone.

Introduction

Luteinizing hormone (LH), like other glycoprotein hormones of the pituitary gland, FSH and TSH, consists of two dissimilar subunits, α and β which are bound noncovalently[1]. The dissociation of LH and isolation of its subunits have been accomplished in bovine[2, 3], in ovine[4], in rat[5] and in human[6, 7]. The primary amino acid sequence of both subunits have been determined for ovine LH[8, 9]. It has been debated whether the biological activities of isolated LH subunits are intrinsic to the subunits or to contamination by the intact hormone. The wide range of biological potency (2–3%) for LH subunits have been reported[6, 10, 11, 12]. Nevertheless, the possibility that these biological activities of the subunits are due to contamination by the native hormone was not ruled out.

By the use of a specific antiserum to the β-subunit of LH (anti-β-LH) to absorb any traces of β-subunit or intact LH present in the α-subunit preparation, it is possible to determine whether the biological activities of this preparation are indeed intrinsic to the α-subunit. Adopting the same approach, a specific antiserum to the α-subunit (anti-αLH) can be used to probe the inherent biological properties of the β-subunit. The stimulation of cyclic AMP formation in rat ovaries was the parameter used to check biological activities of these subunits.

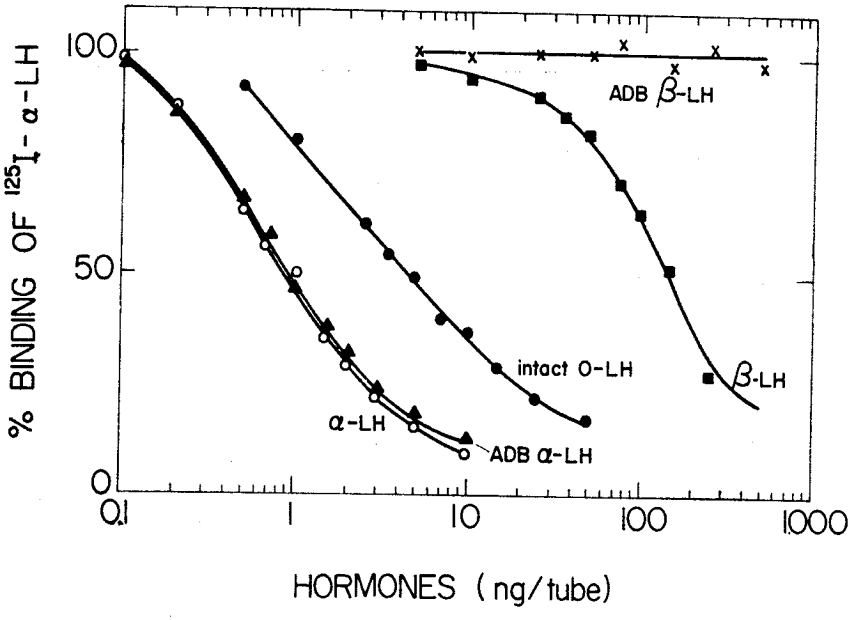

Fig. 1. Homologous α-LH radioimmunoassay system shows inhibition of binding of ^{125}I-α-LH to anti α-LH serum by intact LH and its subunits. ADB-α-LH (absorbed α-LH) and ADB-β-LH (absorbed β-LH) indicate incubation of α-LH or β-LH with anti-β-LH serum or with anti-α-LH serum, respectively at 37°C for 2 h, after which the anti rabbit gamma globulin serum was added. After precipitation, the supernatant fraction was used as absorbed subunits.

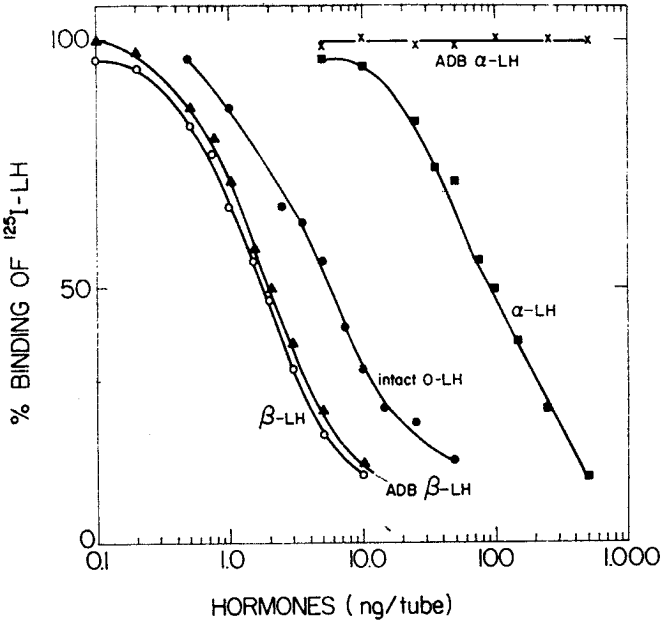

Fig. 2. Inhibition of binding of ^{125}I-LH to anti β-LH by intact hormone and its subunits. Absorbed-α or β-LH was obtained as indicated in Fig. 1

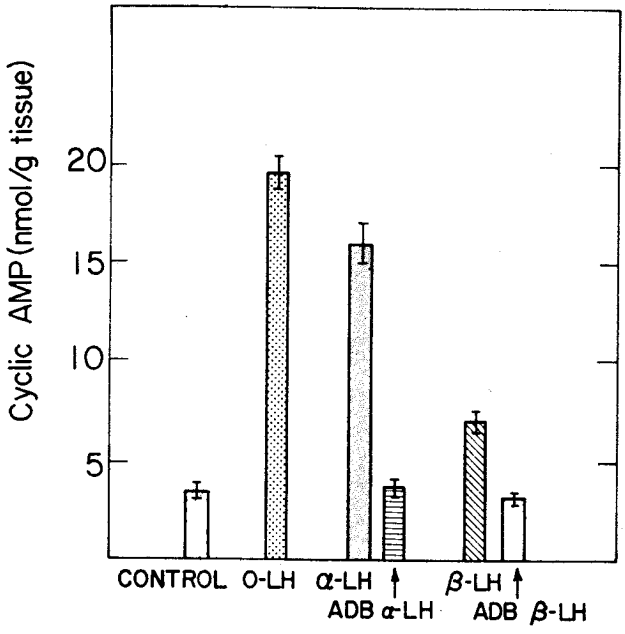

Fig. 3. Effect of LH and its subunits on cyclic AMP accumulation in immature rat ovaries during 40 min incubation with 10^{-2}M theophylline at 37 °C. Control, no hormone added; LH (1 μg/ml medium); α-LH (21 μg/ml medium); β-LH (23 μg/ml medium); ADB α-LH (absorbed α-LH: α-LH 21 μg preincubated with anti-β-LH) and ADB β-LH (absorbed β-LH: β-LH 23 μg preincubated with anti-α-LH). Vertical brackets indicate ± SEM (N = 12).

α-LH preparation with anti-β-LH (ADB α-LH) or absorption of reference β-LH preparation with anti-α-LH (ADB β-LH), resulted in abolition of activities of these subunits (Fig. 3).

Discussion

Numerous reports claimed different biological potencies for subunits of LH. Using the OAAD assay, it was found by Reichert *et al.*[2] that the α and β subunits of LH possessed 30% and 4% of the activity of the intact hormone, while Pierce *et al.*[10] showed that each subunit possessed about 6% of the original activity. Using other parameters (e.g. ovulation, lipolysis and radioligand), activities ranging between 2-25% of the intact hormone were reported for LH subunits[6, 11, 12]. The question whether the reported biological activity of LH subunits is intrinsic to the subunit, or due to contamination of the subunit preparation with the intact hormone, remained to be clarified.

In a homologous α-subunit radioimmunoassay system, intact LH and β-subunit showed 17% and 0.5% cross reaction respectively (Fig. 1). Absorbed α-LH lost its biological activity, as determined by cyclic AMP accumulation in immature rat ovaries (Fig. 3). while its immunological activity was retained in a homologous α-subunit radioimmunoassay system (Fig. 1). In contrast, absorbed β-LH, failed to inhibit the binding in the same system (Fig. 1), showing that this cross reaction is likely due to the presence of α-subunit in the β-subunit preparation. Similarly, absorbed β-LH retained its immunological properties (Fig. 2). while the biological activity was abolished (Fig. 3).

These observations suggest that both the α-and the β-subunits were slightly contaminated and thus the chance that the antisera produced may react with these contaminants has to be kept in mind. However, it is also possible that such a minute amount of contamination does not provoke any antibody production. The anti-β-LH preparation used in this experiment had been previously characterized to be specific for the β-subunit of LH[17] and had been used to neutralize biological actions of LH e.g. induced maturation of follicle-enclosed oocytes, steroid release and cyclic AMP accumulation by cultured Graafian follicles and ovulation[18]. The iodinated LH has been used as an iodinated β-LH (Fig. 2), since β-LH possesses an immunological properties of intact LH[16, 19].

From the above results, it is concluded that the subunits of ovine LH have no detectable biological activity. The reported biological activities of the subunits of LH[6, 10, 12] are probably due to the contamination of the subunit preparations with the corresponding subunit or with the intact hormone. Similar conclusions were also offered for the β-subunit of human chorionic gonadotrophin (hCG), using antisera specefic for the β-subunit of hCG[20] and for hCG and LH subunits by using testosterone production by the rat testes homogenate as the assay system for hCG and LH activities[21].

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บทคัดย่อ

ฮอร์โมนที่จำเป็นในการตกไข่ หรือ luteinizing hormone (LH) จำนวน 1 ไมโครกรัม/มิลลิลิตร และ subunits ของมัน ทั้ง α-LH (21 ไมโครกรัม/มิลลิลิตร) หรือ β-LH (23 ไมโครกรัม/มิลลิลิตร) ต่างก็สามารถกระตุ้นให้เกิดการสะสมของ cyclic AMP ในรังไข่หนูเมื่อทดลองในระบบ in vitro แต่เมื่อ α-LH ถูกทำให้บริสุทธิ์โดยใส่ anti β-LH เข้าไปเพื่อทำลาย β-LH ที่หลงเหลืออยู่ใน α-LH แล้วจะไม่สามารถให้ผลดังกล่าว ในทำนองเดียวกันก็พบว่า β-LH เมื่อทำให้บริสุทธิ์โดยใส่ anti α-LH เข้าไป ก็จะไม่ทำให้จำนวน cyclic AMP ในรังไข่หนูเพิ่มขึ้น จึงสรุปได้ว่า subunits ทั้งสองของ LH ไม่มีความสามารถทางชีววิทยาโดยตัวของมันเอง รายงานอื่น ๆ ที่ว่า subunits ทั้งสองมีความสามารถทางชีววิทยานั้น คงเนื่องมาจากความไม่บริสุทธิ์ของ subunits ที่ใช้ในการทดลอง คือมี LH ปนอยู่นั่นเอง