

CONCAVALIN A INHIBITS THE BASAL AND STIMULATED HORMONE RELEASE FROM PITUITARY CELLS IN MONOLAYER CULTURES

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1. Introduction

Many of the properties of the plasma membrane glycoproteins have been investigated by using several lectins, including concanavalin A (con A). Although the precise mechanism of lectin action on the cell surface is not known, interaction of con A with plasma membrane involves the recognition of sugar residues [1]. Moreover, the binding of con A to cell surface macromolecules can be specifically reversed by the addition of α -methyl-D-mannoside or α -methyl-D-glucoside [2]. The interactions of con A with plasma membranes have been shown to cause a number of biological effects including cell agglutination [3], transformation of lymphocytes [4] and mitotic induction [5] and to affect membrane enzyme activities such as 5'-nucleotidase [6], ATPase [7] and sialyltransferase [8]. Con A has also been found to modify the binding [9–11] or the activity [12–14] of certain hormones, and to mimic several hormonal effects [15,16].

Here we demonstrate that con A is able to inhibit the basal and stimulated hormone release by rat pituitary cells in culture.

2. Materials and methods

Culture medium and fetal calf serum were obtained from GibcoBiocult (Paisley). [³H]Leucine, [³H]-glucosamine and [³H]thymidine were from CEA (Saclay). Plastic Petri dishes were purchased from

Falcon Plastics Co. (Oxnard, CA). Thyrotropin releasing hormone (TRH) was obtained from UCB (Brussels) and GnRH was a generous gift of Dr R. Studer from Hoffman La Roche (Basel). Con A was from Pharmacia (Uppsala) and α -methyl-D-mannoside from Sigma (St Louis, MO). All other chemicals were of reagent grade.

2.1. Cell culture and incubations

Dispersed rat anterior pituitary cells in monolayer cultures were prepared according to [17]. After dispersion, the cells were suspended in medium F-10 with the addition of fetal calf serum (2.5%), rat serum (15%), *N*,2-hydroxyethylpiperazine-*N*,2-ethane sulfonic acid (Hepes, 25 mM) and a mixture of antibiotics (50 IU penicillin/ml, 50 μ g streptomycin/ml and 2.5 μ g fungizone/ml). Each Petri dish was seeded with $4-8 \times 10^5$ cells in 1 ml/culture medium. Usually 5 dishes/group were used. The cells were used on day 5 of the culture when adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), prolactin (PRL) and growth hormone (GH) release were studied. For follicle stimulating hormone (FSH) and luteinizing hormone (LH) studies, the cultures were used on day 7 with a change of medium on day 4. After washing the cells, the incubations were carried out in the synthetic medium F-10 for 4 h. For the studies with high K⁺, the cells were incubated in Krebs-Ringer bicarbonate buffer containing 59 mM K⁺ (instead of Na⁺), 16 mM glucose and 15 mM Hepes.

2.2. Radioimmunoassays

Radioimmunoassays for rat TSH, PRL and GH were performed using kits provided by the NIAMDD rat pituitary hormone distribution program. ACTH

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was radioimmunoassayed according to [18], using antiserum kindly provided by Dr Vaudry. LH and FSH were estimated by radioimmunoassay as in [19], using anti-ovine LH β and anti-ovine FSH obtained from Dr Kerdelhué (this laboratory). Our own preparations of rat LH ($1.26 \times \text{NIH-LH-S}_1$) and rat FSH ($37.60 \times \text{NIH-FSH-S}_1$) were used as standard preparations.

2.3. Incorporation of labelled precursors

After incubation in the presence of labelled precursors ($2.5 \mu\text{Ci}/\text{dish}$), the cells were washed and 0.5 ml water were added. The dishes were frozen and thawed. The broken cells were detached with a rubber policeman, transferred into test tubes and centrifuged at $2000 \times g$ for 20 min. The clear supernatants were used for measuring the incorporation of [^3H]leucine and [^3H]glucosamine into trichloroacetic acid-precipitable proteins and for measuring the incorporation of [^3H]thymidine into deoxyribonucleic acid (DNA) [20].

2.4. Hypothalamic extracts

Lyophilised hypothalamic extracts (HE) used for ACTH studies [21] were dissolved in phosphate-buffered saline so that the equivalent of 0.2 hypothalamus/dish was added.

3. Results

As shown in fig.1, both basal and GnRH-stimulated FSH release were inhibited by con A in a dose-related manner. At $100 \mu\text{g}/\text{ml}$, con A was able to decrease the basal FSH release by $\sim 90\%$ and to completely inhibit the releasing action of GnRH. The inhibition patterns of the basal and stimulated release of LH, TSH, ACTH, GH and PRL, observed in the presence of con A, were dose-related in a similar way (not shown).

Whatever the secretagogue used, physiological (TRH, GnRH, HE) or pharmacological (8 Br-cAMP, high K^+), con A was able to decrease the release of each pituitary hormone tested (fig.2). The effect of con A was partially reversed by α -methyl-D-mannoside (1 mg/ml). In contrast, con A was unable to modify the incorporation of [^3H]leucine and [^3H]glucosamine into trichloroacetic acid-precipitable proteins and the incorporation of [^3H]thymidine into DNA (table 1).

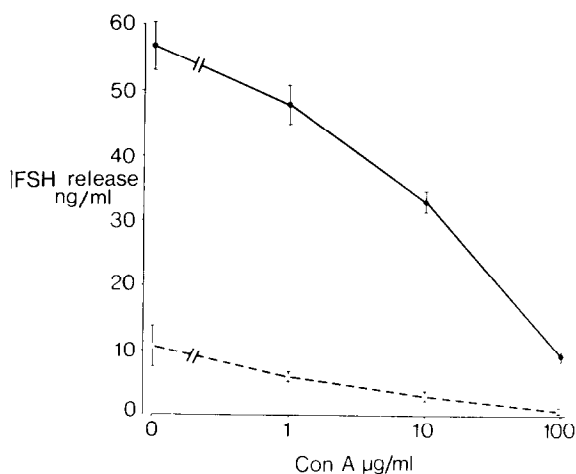


Fig.1. Effect of increasing concentrations of con A on the basal (○—○) and the GnRH (9 ng/ml) (●—●) induced release of FSH by pituitary cells in culture, during a 4 h incubation.

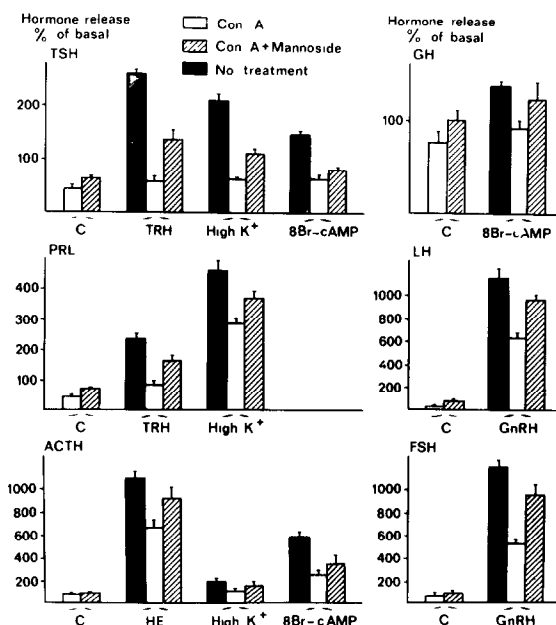


Fig.2. Effect of con A ($50 \mu\text{g}/\text{ml}$) on the basal and stimulated hormone release by pituitary cells in culture during 4 h incubations. LH and FSH release was stimulated by GnRH (9 ng/ml), that of TSH by TRH (10 ng/ml), 59 mM K^+ and 8 Br-cAMP (1 mM), that of ACTH by HE (0.2 hypothalamus/dish), 59 mM K^+ and 8 Br-cAMP (3 mM), that of PRL by TRH (10 ng/ml) and 59 mM K^+ and that of GH by 8 Br-cAMP (1 mM). The concentration of α -methyl-D-mannoside was 1 mg/ml.

Table 1
Lack of effect of con A (100 µg/ml) on the incorporation of [³H]leucine and [³H]glucosamine into trichloroacetic acid-precipitable proteins and on the incorporation of [³H]thymidine into DNA

Group	Incorporation of		
	[³ H]Leucine into proteins (cpm)	[³ H]Glucosamine into proteins (cpm)	[³ H]Thymidine into DNA (cpm)
Control	135 502 ± 9363	13 855 ± 1182	88 186 ± 3749
Con A (100 µg/ml)	148 832 ± 13 157	17 351 ± 372	86 895 ± 6815

The incubations were carried out for 4 h in the presence of the labelled precursors (2.5 µCi/ml)

4. Discussion

This paper shows that con A is able to decrease the hormone release from rat pituitary cell cultures. This was true for every hormone tested. The effect of con A was dose-related and partially reversed by α -methyl-D-mannoside.

Con A has been demonstrated to inhibit the binding of prolactin to mammary tumors [9] and to particulate fractions of rat liver [10]. The binding of insulin to both intact cells and solubilized receptors was also inhibited by con A [11]. In contrast, con A had a protective effect on the stimulation of the adenylate cyclase activity of adrenocortical plasma membranes, by ACTH [12], whereas it facilitated [13,14] or decreased [14] the in vitro thyroid activation by TSH, depending on lectin concentration. Taking these results into account, one could postulate that the inhibition of the pituitary hormone release observed here was due to an alteration by con A of the binding of the hypothalamic releasing hormones tested (TRH, GnRH and CRF from HE) to their specific receptors. However this explanation cannot account for the inhibition of the hormone release stimulated by either 59 mM K⁺ or 8 Br-cAMP. It could be thought that the permeability of the pituitary cells to both K⁺ and 8 Br-cAMP was decreased because of a major alteration of the plasma membrane transport processes by con A. This is unlikely since our results showed that the incorporation of [³H]leucine and [³H]glucosamine into trichloroacetic acid-precipitable proteins, and the incorporation of [³H]-thymidine into DNA were not altered by con A,

indicating that the membrane transport of amino acids, carbohydrates and nucleotides were not modified. Since the release of each pituitary hormone tested was decreased by con A, whatever the secretagogues used, an alternative explanation for our results would be that con A acted at the hormone release level. This would be in good agreement with the fact that the basal hormone release was also inhibited by the lectin. Therefore, our results suggest that in addition to its effect on the interactions of certain hormones with their receptors [9–14], con A may alter the function of other carbohydrate containing membrane components involved in the transport of hormones across the plasma membrane, during their release.

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