

OVERLAP OF CONCAVALIN-A AND INSULIN IMPRINTING IN RAT THYMOCYTES

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Received July 8, 1988

Accepted February 7, 1989

The thymocytic insulin binding in rats treated with the hormone neonatally on a single occasion increased considerably compared to the untreated control by 3 months of age. Treatment with Concanavalin-A also accounted for an increase in adult insulin binding, whereas neonatal treatment with insulin did not alter the binding relations of Concanavalin-A in adulthood.

Keywords: imprinting, insulin, Concanavalin-A, thymocytes, cytofluorimetry

The primary interaction of the hormone with its target cell gives rise to hormonal imprinting, which completes the maturation of the genetically encoded, preformed receptor structures [2]. With imprinting the binding capacity of the receptor, i.e. the responsiveness of the cell increases, whereas without it the cellular response to a certain hormone will remain incomplete. The immature(plastic) receptors can be influenced not only by the adequate hormone, but also by foreign molecules similar to it (related hormones, hormone analogues), which then imprint the target cell either normally or abnormally provoking a relatively stronger or weaker response [4].

Evidence has been accumulating that lectins binding to special sugar groups may also overlap on certain membrane-associated hormone receptors. Costlow and Gallagher [1] demonstrated inhibition of prolactin binding, whereas Sandra et al [8] described reversion of insulin effect by Con-A-treatment. An insulin-like action of Con-A was also reported by Cuatrecasas and Tell [6], and Smith and Liu [10] observed activation of tyrosine aminotransferase (TAT) by Con-A. The latter finding was later substantiated by Sorimachi and Yasumura [11]. Suzuki et al. [12] reported activation of insulin-sensitive phosphodiesterase by Con-A. We [5, 7] demonstrated insulin and Con-A overlap on the insulin receptor in earlier studies on *Tetrahymena*. These experimental observations prompted us to study the impact of a single neonatal Con-A treatment on insulin binding in adulthood and vice versa.

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Materials and methods

Thirty male Wistar CFY rats were used. One part of the rats was treated with 0.2 I. U. insulin (Semilente MC, Novo, Copenhagen) when newborn, in a single occasion, the other part remained untreated serving as control. No lethal effect following insulin treatment was observed. The rats of another group received 100 μ g Concanavalin-A (Con-A, Serva, Heidelberg), and the Con-A control received saline. At 3 months of age the thymic glands of the rats were removed under ether anaesthesia, washed in saline, and the thymocytes were recovered by shaking in saline. Samples of the thymocyte suspension were spread on slides and fixed in ethanol for 3 min.

The hormone or lectin binding of the membrane was estimated from the binding relations of fluorescein-isothiocyanate-(FITC, BDH, England)-labeled insulin and Con-A. (The FITC: protein ratio was 0.14 and 1.33, the protein content 0.2 and 1.0 mg/ml, respectively, in the FITC-insulin and Con-A-insulin conjugates.) The fixed cells were incubated for 1 h in presence of the conjugates, in a moist chamber. The binding of label was assessed by cytofluorimetry in a Zeiss Fluoval cytofluorimeter, which was connected with a HP41CX calculator for statistical evaluation, including determination of the mean values, standard deviation and significance of inter-group differences (by analysis of variance and Student's t-test). Twenty cells were assayed for each animal.

Results and discussion

In every case, treatment with insulin or Con-A was performed on a single occasion, within 24 h after birth. A single exposure in the critical period of receptor development was sufficient to alter the binding capacity of the cells in adulthood considerably, as assessed at 3 months of age. The FITC-insulin binding of the thymocytes in the neonatally insulin-treated rats increased significantly compared to the control. This accorded well with the

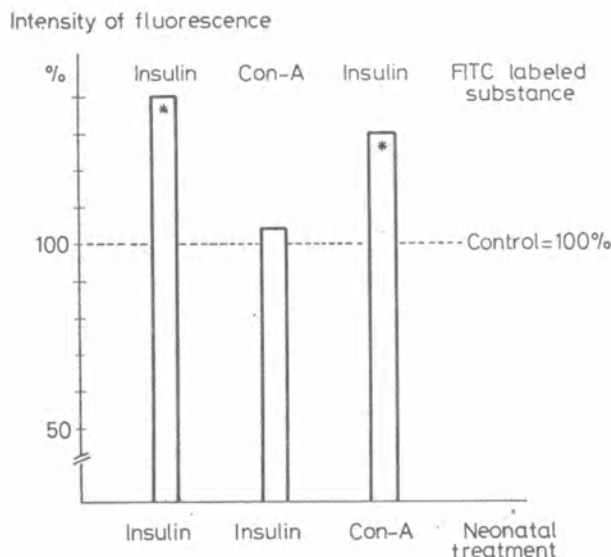


Fig. 1 Labeled insulin and Con-A binding to neonatally treated animal's thymocytes related to the untreated control as 100%. * = $p < 0.01$ related to the control

earlier observation [3, 4] that the hepatocellular receptors, too, were markedly influenced by neonatal insulin exposure. However, while the hepatocytes of the males always showed a decrease, the thymocytes showed a marked increase in the insulin binding capacity. This difference can be explained by the disparate behaviour of insulin receptors on the different target cells [13]. The thymocytic Con-A binding in the same animals was practically not altered by neonatal insulin treatment. Neonatal Con-A treatment, on the other hand, accounted for a significant increase of insulin binding in adulthood.

It follows that Con-A imprinted the cells exactly as insulin for insulin, whereas insulin imprinted them exclusively for itself, but not for Con-A (Fig. 1). The Con-A and the insulin molecules differ considerably in size and structure; their only common feature seems to be the substructures responsible for interaction with the sugar component of the receptor, as indicated also by the circumstance that the binding of insulin to the glycoprotein-like insulin receptor can only be partly inhibited with Con-A. At the same time, dissolved insulin receptors can be adsorbed into a Con-A-agarose column, and can be eluted from it with a simple sugar (mannose) [9]. Thus insulin binds to the complete receptor, whereas Con-A binds only to a part — the sugar component — of the insulin receptor. It appears that the interaction of Con-A with the insulin receptor of the neonate has the same effect as genuine insulin imprinting. The fact that insulin, on the other hand, cannot effectively imprint for Con-A is not surprising, because receptors for Con-A are abundantly present in the cell membrane, and occur not only as parts of insulin receptors. Exactly therefore, the binding of Con-A is a more general phenomenon, in which the binding increase induced by insulin imprinting forms only a practically immeasurable part, especially in the case of lymphoid cells, whose ready Con-A binding is generally known.

The present experimental results support the conclusion that the receptor-level overlap of insulin and Con-A, which has been substantiated in many systems [1, 5, 6, 7, 9, 10, 11, 12], also applies to imprinting in mammalian cells. In addition they call the attention to the high sensitivity of hormone receptors at neonatal age, as a single treatment caused the above mentioned effect measured in adult animals.

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