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INSULIN CONTENT IN PANCREAS AND BLOOD PLASMA OF DECAPITATED ANE ENCEPHALECTOMIZED RAT FETUSES

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Previous observations on adult animals subjected to stimulation or destruction of certain hypothalamic zones suggested that this brain region is concerned with the regulation of pancreatic function [15]. These effects of the hypothalamus are considered to be mediated through the autonomic nervous system [7]. Meanwhile the insulin-stimulating and inhibitory activity of incubation media and homogenates of certain regions of the hypothalamus has been demonstrated and is evidence of the possible existence of a humoral pathway from hypothalamus to pancreas [5, 11].

Investigations into the connection between the pituitary and pancreatic function have shown that the plasma insulin level and glucose sensitivity of adult hypophysectomized animals are reduced and that proinsulin synthesis is inhibited [12]. However, there is as yet no clear understanding of the nature of the changes observed in the pancreas during disturbances in the hypothalamus–pituitary system.

The writer showed previously that decapitation and encephalectomy of fetuses in utero leads to loss of sensitivity of the pancreatic B cells to glucose. Subsequent replacement injection of hypothalamic homogenate, preincubation with the adenohypophysis, or injection of certain adenohypophyseal hormones (STH, ACTH) abolish the effects of the operations, and B-cell reactivity was restored after 30 min [10]. The writer postulated that this rapid recovery of reactivity may be due to the fact that removal of the hypothalamus and pituitary as a result of the operations inhibits development of structures of the B cell that are responsible for perception of glucose as an insulinotropic signal and does not affect fundamental processes of hormone biosynthesis and secretion.

The aim of this investigation was to study the role of the hypothalamus and pituitary in the development of insulin biosynthesis and its secretion into the blood stream in rat fetuses.

EXPERIMENTAL METHOD

Wistar albino rat fetuses were used. To remove the hypothalamus, the fetuses were encephalectomized in utero [1], and decapitation of the fetuses in utero was used as the experimental model of hypophysectomy [6]. Operations on the fetuses were performed after 17.5 days of pregnancy. After 21.5 days of pregnancy, the mother was anesthetized with pentobarbital and the fetuses removed; blood was taken quickly from the fetuses (by pipet from an incision in the heart) and the pancreas was removed. Blood from fetuses belonging to the same litter was pooled. The isolated glands from each fetus were weighed separately and placed in a plastic test tube with a drop of physiological saline on dry ice, where they were kept until the time of determination of immunoreactive insulin (IRI). The blood was collected in heparinized tubes and the plasma was separated by centrifugation and kept in plastic tubes in dry ice.

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TABLE 1. Content and Concentration of IRI in Rat Fetal Pancreas after Decapitation and Encephalectomy (M ± m)

Experimental conditions	Weight of gland, g	IRI in gland		IRI in plasma, μU/ml
		IU/gland	μU/mg	
Control	40,9±2,5 (14)	53,3±4,1	1,55±0,045 (14)	114,8±0,75 (44)
Decapitation	26±1,1 (10)**	25,9±2,5*	1,25±0,12 (10)	120,3±3,9 (12)
Encephalectomy	22,9±0,8 (11)**	33,2±1,95*	1,52±0,068 (11)	118,2±2,3 (15)

Legend. *P < 0.05 compared with control. Here and in Table 2, number of determinations shown in parentheses. [** — not identified in Russian original.]

In separate experiments the reaction of the pancreas of fetuses undergoing the above operation to glucose loading *in vivo* was determined in a separate experiment. For this purpose, after 21.5 days of development each fetus was given an intraperitoneal injection of 0.05 ml of 30% glucose solution. Blood was collected from the fetuses 30 min after the beginning of injection of the solutions, as described above.

To determine IRI in the pancreas of the rat fetuses, acid—ethanol extracts were prepared [2]. IRI was determined in tissue extracts and plasma of the fetuses by radioimmunoassay [4], using standard kits from CEA-IRE-Sorin (France) and the Isotopes Institute of the Hungarian Academy of Sciences.

Extracts for radioimmunoassay were used in dilutions of 1:50 and 1:100; the plasma was not diluted. To increase the accuracy of radioimmunoassay each value was obtained by measurement of three parallel samples. The results of the tests were assessed by Student's t test.

EXPERIMENTAL RESULTS

Investigation of the IRI concentration in the pancreas of 21.5-day fetuses revealed no difference between experimental and control fetuses (Table 1). This may indicate that insulin synthesis was not disturbed under these experimental conditions. Our results agree with those of a single study [3] in which no difference likewise was found in the IRI concentration in the pancreas of decapitated and control fetuses. The authors cited likewise found no difference in the percentage of islet tissue in the fetuses of these groups, whereas the percentage of exocrine tissue was significantly reduced in the decapitated fetuses. A decrease in the proportion of exocrine cells in the pancreas, grown in culture (i.e., in the absence of extrapancreatic regulating factors), also has been described in the literature [14].

No significant differences likewise were found in the plasma IRI levels of fetuses of the control and experimental groups, i.e., basal secretion was undisturbed. Weighing the pancreas showed (Table 1) that its weight was significantly lower in the decapitated fetuses than in the control. When the IRI content per weight of the whole gland was calculated, a significant decrease was found in the IRI content in both decapitated and encephalectomized fetuses. Meanwhile basal IRI secretion into the blood stream was undisturbed, and the IRI level in the plasma of the decapitated and encephalectomized fetuses was indistinguishable from the control values. This is evidently connected with inhibition of growth of the pancreas in fetuses undergoing these operations [14]. Since the principal parameters of the biosynthetic and secretory capacity of the pancreas, namely the hormone concentration (per milligram tissue and per milliliter plasma) were unchanged in the decapitated and encephalectomized fetuses, the results confirm the hypothesis that synthesis and basal secretion of insulin in the fetal pancreas takes place also in the absence of higher neuroendocrine centers.

The additional investigation showed that the increased insulin concentration in the plasma after injection of glucose into fetuses undergoing operation after 17.5 days and testing after 21.5 days was similar to that in intact fetuses (Table 2). Similar results on decapitated fetuses have also been obtained by other workers [9], who injected glucose into a pregnant female.

TABLE 2. Plasma IRI Level in Encephalectomized and Decapitated Rat Fetuses 30 min after Injection of Glucose (M ± m)

Experimental conditions	IRI in plasma, μU/ml		
	basal level	after glucose injection	after injection of physiological saline
Control	82,9±3,6 (39)	137,5±5,4 (15)*	91,8±3,2 (11)
Encephalectomy	87,0±7,8 (24)	135,4±6,8 (21)*	90,3±2,9 (14)
Decapitation	99,6±7,5 (22)	151,2±9,6 (21)*	—

Legend. Asterisk denotes significance of differences between basal IRI level and after glucose injection into fetuses ($P < 0.05$).

How can the opposite results obtained by the present writer previously *in vitro* [10] and in this investigation *in vivo* be explained? The results do not give an unequivocal answer to this question. However, on the basis of information in the literature on the potentiating effect of certain blood components it can be postulated that *in vivo* what was observed was not the pure effect of glucose on the fetal B cells, but an indirect effect mediated through mother and fetus [8, 13]. It can thus be tentatively suggested that the stimulating effect of glucose on IRI release from the pancreas of the encephalectomized and decapitated fetuses is connected with the potentiating action of one of these factors.

The results of the present investigation indicate that development of processes of biosynthesis, accumulation, and basal secretion of insulin in the prenatal period in rats are not controlled by the hypothalamus—pituitary system.

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