CHANGES IN CYCLIC AMP CONTENT IN THE TISSUES OF RATS WITH INSULIN HYPOGLYCEMIA

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The role of cyclic nucleotides and mediators linking the cell with neurohormonal regulatory influences is exhibited particularly clearly in situations extremal for both cell and organism. Insulin hypoglycemia is a stress state activating the hypothalamus—pituitary system [6, 8]. Recently, despite the close attention which has been paid to the study of the effect of insulin *in vitro* on activity of the adenylate cyclase system, the effects of insulin *in vivo* have been judged with respect to blood cyclic AMP levels [5, 10]. However, although blood cyclic AMP levels reflect changes taking place in the pool of the principal nucleotide of the body, they do not allow the source of these fluctuations or the mechanisms lying at their basis to be identified. It seemed more productive to study the cyclic AMP content in the tissues at certain time intervals after administration of insulin.

The object of this investigation was to study the cyclic AMP concentration in the tissues and blood plasma during insulin hypoglycemia.

EXPERIMENTAL METHOD

Male rats weighing 180-220 g were used. Insulin was injected in a dose of 6 units/kg body weight, and the blood sugar of the animals was determined 15, 30, 60, and 120 min later by the orthotoluidine method, while the cyclic AMP concentration in the blood plasma and its content in weighed samples of tissues from the cerebral hemispheres, liver, adrenals, hypothalamus, pituitary, and epididymal fat were determined radioimmunologically by means of kits from the Radiochemical Centre, Amersham (England). Cyclic AMP was extracted from the tissues by the method of Steiner et al. [14].

EXPERIMENTAL RESULTS

Injection of insulin led to a significant decrease (by 33% compared with intact rats) in the blood sugar after 15 min, which reached a maximum (by 76.2%) after 30 min. The blood sugar returned almost to its level in intact animals by 120 min (Fig. 1).

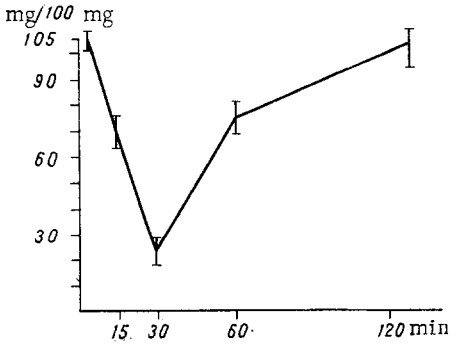

Fig. 1. Effect of insulin on blood sugar in rats.

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TABLE 1. Cyclic AMP Concentration (in ng/g) in Tissues of Rats with Insulin Hypoglycemia

Test tissue	Before injection of insulin (n = 10)	After injection of insulin			
		15 min (n=7)	30 min (n=7)	60 min (n=8)	120 min (n=7)
Blood (ng/ml)	14,0±2,0	10,0±1,7	9,0±1,5*	19,0±1,2*	19,4±2,1
Liver	588±3	422±44*	897±52*, *	547±34 †	665±35†
Adrenals	1877±102	2405±70*	1860±75†	2621±90*, †	2344±82*
Hypothalamus	1400±110	640±30*	1440±72†	794±56*, †	1262±91 †
Brain (cerebral hemisphere)	2090±114	858±25*	2851±130*, †	1050±52*, †	2805±170*, †
Pituitary	3990±190	2282±200*	3410±230 †	2712±140*, †	3337±270*, †
Epididymal fat	750±42	357±52*	874±56 †	682±72†	720±61 †

*P < 0.05 compared with control.

†P < 0.05 compared with previous time of investigation.

Investigation of the cyclic AMP concentration in the blood and tissues of rats with insulin hypoglycemia revealed phases in its changes in opposite directions at certain time intervals after administration of insulin (Table 1). For instance, after 15 min a significant decrease in the cyclic AMP concentration was found in all tissues except the blood in which the decrease was not significant, and in the adrenals in which the cyclic AMP concentration was increased. After 30 min, i.e., when the blood sugar reached its lowest level, the blood cyclic AMP concentration remained the same as after 15 min. The cyclic AMP concentration in the liver, adipose tissue, hypothalamus, pituitary, and brain was higher than the corresponding values obtained 15 min after injection of insulin. After 30 min of insulin hypoglycemia the cyclic AMP concentration in the above-mentioned tissues, after a significant decrease, was thus increased up to its level in intact animals, and in the case of brain tissue, its value was significantly higher (Table 1).

After 60 min a significant decrease was observed in the cyclic AMP concentration in the brain (cerebral hemispheres), hypothalamus, and pituitary compared with the corresponding values both in intact rats and 30 min after injection of insulin. In the liver and epididymal fat after 60 min of insulin hypoglycemia the cyclic AMP concentration was lower than 30 min after injection of insulin, but practically indistinguishable from its level in the intact rats. In the adrenals and blood the cyclic AMP concentration 60 min after injection of insulin was significantly higher than 30 min after its injection and in intact rats.

By the 120th minute after injection of insulin, when the blood sugar had returned almost to its initial level, the cyclic AMP concentration in all tissues studied was significantly higher than 60 min after injection, except in the adrenals, where it remained within the same limits. An increase in the cyclic AMP concentration in some tissues 120 min after injection of insulin was significant, but in the liver, fat, and blood it was not significant, compared with its level in intact animals (Table 1).

Analysis of the results thus shows that administration of insulin in doses leading to the development of marked hypoglycemia is accompanied by significant changes in the cyclic AMP concentration in the tissues and blood. Changes in the cyclic AMP concentration in the tissues of rats with insulin hypoglycemia were due both to the direct action of insulin and to disturbances in the regulation of systemic biochemical and physiological processes and the products thus formed. Similar changes are observed in all stress situations to which the organism responds by activation of the hypothalamo-hypophyseoadrenal system.

Evidence in support of this view is given by the fact that 15 min after injection of insulin distinct and significant changes in the cyclic AMP level were found in the liver, fat, brain, hypothalamus, and pituitary (a decrease), on the one hand, and in the adrenal tissue (an increase), on the other hand. At subsequent times after injection of insulin and in the course of formation of the stress reaction, changes in the cyclic AMP concentrations in these tissues were of the opposite kind and their relative values returned to those found at the 15th minute. The decrease in the cyclic AMP concentration in the test tissues at the 15th minute is linked with the direct inhibitory action of insulin on adenylate cyclase and activation of the tissue phosphodiesterase [12, 13].

The increase in the cyclic AMP concentration in the adrenals 15 min after injection of insulin was evidently due to the action of ACTH and specific stimulation of cyclic AMP [9].

This suggestion is supported by the increase in the blood ACTH level at the 15th minute of the stress reaction [2, 7, 11] and the decrease in the cyclic AMP concentration in the cerebral cortex, hypothalamus, and pituitary compared with its level in intact animals (Table 1).

The explanation of these facts must take into account that neurohormonal regulation in cells, including cells of the central and peripheral nervous system, is effected through both cyclic AMP and cyclic GMP, whose levels do not change simultaneously, but which are in a state of dynamic and functional equilibrium. Consequently it may be that at the 15th minute after injection of insulin its action in the brain structures studied is mediated through cyclic GMP, whereas in the adenylate cyclase–cyclic AMP system the response is extinguished. Similar results have been obtained by the use of several stressor agents including acute hypoxia and swimming in cold water [5, 10].

In the next time interval (30 min after injection of insulin), when the blood sugar level was down to its minimum, the development of a typical stress reaction was observed in the animal. Liberation of adrenalin into the blood stream stimulates the tissue adenylate cyclase and leads to an increase in the cyclic AMP level in the tissue cells. The only exception is adrenal tissue, in which the cyclic AMP concentration falls compared with that after 15 min, and remains at the characteristic level for intact rats, evidently on account of fluctuations in the ACTH concentration [1]. This is in agreement with data showing an increase in the cyclic AMP concentration in different parts of the brain, evidence of a phase of excitation and formation of specific secretions.

Changes in the catecholamine concentrations in the body during insulin hypoglycemia, reaching its lowest level after 30 min, bring about a fresh liberation of endogenous insulin, although much less than the quantity of exogenous insulin injected [3, 4]. This, in turn, leads to the development of certain physiological processes that are accompanied by changes in the cyclic AMP concentration in the tissues.

The cyclic character of reactions to changes in the cyclic AMP concentration in the tissues and blood is repeated relatively clearly at subsequent times after injection of insulin (60 and 120 min). However, the intensity of the processes gradually declines, as is shown by smaller fluctuations in the tissue cyclic AMP concentrations.

The results thus indicate that under extremal conditions (insulin hypoglycemia) cyclic nucleotides react to the stimulus rapidly, but in different directions. This reaction must be regarded as mobilization of the internal resources in order to ensure adaptation of the animal to the pathological agent.

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