CHOLINERGIC MECHANISMS OF DEVELOPMENT OF HYDROLYTIC AND TRANSPORT FUNCTIONS OF THE SMALL INTESTINE OF INTACT AND STRESSED RATS DURING ONTOGENY

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Ontogenetic changes in functions of the digestive tract are associated not only with the local response of specialized digestive cells to changes in alimentary factors, but also with other levels of regulation, including the nervous and endocrine systems, and also the genetic apparatus of the cell [10-12]. Relations between substrate and nonsubstrate regulatory factors differ at different age periods [6, 9].

The aim of this investigation was to study the participation of cholinergic mechanisms at different levels in the development of hydrolytic and transport functions of the small intestine in early postnatal ontogeny in intact rats and rats exposed to a single period of heat stress.

EXPERIMENTAL METHOD

Four series of experiments were carried out on 756 laboratory albino rats of different ages. Animals of different sexes were chosen, weighing 150-180 g, to obtain progenies. The pregnant females were isolated in individual cages. Newborn rats were distributed at the rate of eight to one lactating female. The investigations were conducted at ages that are critical for growth and development of rats [1, 5]: 2nd-3rd day; 5th-6th day – the stage of active differentiation, when spontaneous phagocytosis of synaptic brain terminals takes place most rapidly; 13th-14th day – the period of opening of the eyes; 22nd-23rd day – the switch to definitive feeding; 30th day – emancipation from the mother, formation of skeletal muscle tone and mechanisms of temperature regulation; 60th day – the prepubertal period; 90th day – adult animals. For each experiment six rats from no fewer than three litters were used. In the experiments of series I the development of hydrolytic and transport functions in intact rats was studied. In series II the development of hydrolytic and transport functions was investigated after blockade of cholinergic structures at different levels by intraperitoneal injection of the following drugs twice, on the 2nd and 3rd, for the 5th and 6th, for the 13th and 14th, or the 22nd and 23rd days of life: the peripheral muscarinic cholinolytic atropine, the ganglion blocker benzohexonium, or the central muscarinic cholinolytic benactyzine, in doses equal to one-fifth of the dose of each of these drugs to produce a virtually complete cholinolytic effect on the corresponding cholinergic structures in adult rats. The acetylcholine receptor blocking dose of atropine for adult rats is 5 mg/kg, of benzohexonium 5 mg/kg, and of benactyzine 3 mg/kg body weight [2, 4]. Control animals received an intraperitoneal injection of the corresponding volume of distilled water. In the experiments of series III development of these same functions of the small intestine was studied after a single exposure of 2 h to heat stress (at a temperature of 40-41°C) on the 2nd, 6th, 14th, or 23rd day of life. Groups of young rats taken from their mother for 2 h and kept at a temperature close to that maintained in the nest by the lactating female under natural conditions (33-35°C), were used as the control.

In series IV, the single exposure to heat stress on the 2nd, 6th, 14th, or 23rd day of life followed a preventive injection of atropine, benzohexonium, or benactyzine

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TABLE 1. Effect of Cholinolytics, Injected Twice, on the 2nd and 3rd Day of Life, on Development of Transmembrane Transport of Glucose (in mmoles/liter) from Sucrose Solution in Albino Rat Duodenum (M ± m; n = 6)

Experimental conditions	Age, days					
	6	14	23	30	60	90
Control	0	0	0,45±0,06	3,59±0,22	4,47±0,11	4,52±0,24
Injection of atropine	0	0	0***	1,36±0,09***	2,51±0,16**	3,14±0,31*
Injection of benzohexonium	0	0	0,24±0,08*	0,63±0,10***	3,41±0,17*	4,46±0,32
Injection of benactyzine	0	0	0,36±0,08	1,79±0,20**	4,36±0,20	4,28±0,14

Legend. *p < 0.05, **p < 0.01, ***p < 0.001.

intraperitoneally, in doses of one-fifth of the adult dose, 30 min before hyperthermia. Relationships between contact hydrolysis and absorption, and also mechanisms of the initial stages of transport in four regions of the small intestine (duodenum, proximal, middle, and distal portions of the small intestine) were studied in vitro by a method using an accumulating preparation of the mucosa (APM) [7]. Accumulation of glucose (in mmoles/liter) in APM from 200 mg % oxygenated solutions of glucose, lactose, sucrose, and starch was estimated, in order to characterize the state of function of the transport mechanisms proper, and also the lactase-transport, saccharase-transport, and amylase-transport enzyme-carrier systems. The results were subjected to statistical analysis by Student's test.

EXPERIMENTAL RESULTS

In intact rats in the suckling period the mechanisms of intestinal assimilation of carbohydrates with different degrees of polymerization are unequally developed: the small intestine possesses definite transport activity for "free" glucose; of the disaccharidase transport assemblies the lactase-transport system is functioning, but its activity falls sharply toward the time of transition to definitive feeding; transport of glucose included in the structure of sucrose and starch occurs but at a very low level. The formation of mechanisms of glucose transport from sucrose and starch solutions takes place toward the time of switching of the animals to definitive feeding, and they attain their final development at the time of emancipation from the mother, i.e., by the age of 30 days.

During postnatal development considerable changes take place in the character of the spatial distribution of the hydrolytic and transport functions among the small intestine. The maximum of transport of glucose in the composition of starch, sucrose, and lactose, and also of free glucose in the suckling period is located in the middle and distal portions of the gut, but after transition to definitive feeding it shifts in the proximal direction. Blockade of peripheral muscarinic acetylcholine receptors by atropine at different periods of early postnatal ontogeny delays any further formation of purely transport, and also of hydrolytic-transport mechanisms in the small intestine. Blockade of the ganglia or central muscarinic cholinergic structures leads to selective defects in the hydrolytic-transport pools of the intestine, mainly not affecting the transport mechanism proper (Table 1). After blockade of cholinergic structures at different levels changes are observed in the formation of the topography of carbohydrate accumulation along the intestine. More disturbances of formation of hydrolytic-transport functions of the small intestine are observed after administration of cholinolytics to rats aged 2 and 6 days than in the older animals. Higher resistance of the lactase transport mechanism to blockade of cholinergic structures at different levels is observed compared with the transport mechanism proper, and also with saccharase- and amylase-transport mechanisms.

Heat stress in the early stages after birth (2nd, 6th, and 14th days of life) has virtually no effect on development of the mechanisms of transport of "free" glucose, it induces earlier transport of glucose than normally from solutions of sucrose and starch, and represses active transport of glucose from lactose solution. Hyperthermia on the 23rd day of life, while not affecting further development of mechanisms of transport of "free" glucose, and also of the lactase transport mechanism, inhibits the development of mechanisms responsible for transmembrane transport of glucose from sucrose and starch.

The writer's previous investigations on adult rats [3] showed that cholinolytics, especially centrally acting (benactyzine) largely correct disturbances of digestion due to stress. As regards growing animals, in relation to active carbohydrate transport, the consequences of hyperthermia against the background of cholinometics were manifested very variably depending on age. Thus neither atropine nor benzohexonium prevented disturbances of carbohydrate accumulation due to stress on the 2nd or 6th days of life.

Against the background of these preparations, both the intensity of assimilation of free glucose and of glucose from solutions of sucrose and starch in different parts of the intestine, and also the distribution of these processes along the intestine as a whole, remained altered. The protective effect was manifested by these preparations only with respect to the lactase transport mechanism.

The central cholinolytic benactyzine completely corrected disturbances of working of the digestive-transport conveyor after heat stress only at these times, however. If administered before stress on the 14th day of life, none of the cholinolytics chosen could abolish completely the negative effect of stress on carbohydrate accumulation. In hyperthermia on the 23rd day of life atropine exhibited a correcting action, whereas benzohexonium and benactyzine had no such effect.

However, individual rats [3] exhibited certain differences: on the 14th and 23rd days of life benzohexonium and benactyzine completely abolished the negative action of stress on carbohydrate accumulation in these animals. The negative effect of blockade of cholinergic structures at different levels in the early periods of postnatal ontogeny on subsequent development of hydrolytic-transport functions of the small intestine was determined by the important role which these mechanisms played in regulating the functioning of biological membranes, the cyclic nucleotide system, enzyme protein synthesis, and the genetic apparatus of the cell [8]. Data obtained on age differences in responses of the digestive organs and mechanisms of their regulation in stress situations help to some degree to reveal the causes and to organize the prevention and treatment of certain diseases of the gastrointestinal tract in growing organisms.

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