LONG-LASTING CHANGES IN HORMONAL HOMEOSTASIS AFTER BRIEF INTERRUPTION OF THE CIRCULATION IN THE SMALL INTESTINE

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Total ischemia of the small intestine in rats of short duration (45-50 min) is followed by prolonged (up to 15 days or more) fluctuating changes in functional, biochemical, and morphological characteristics of the tissue (active transport, passive ionic permeability of the epithelium, activity of various oxidative and hydrolytic enzymes, the structure of the epithelial cover of the villi and crypts, mitotic index, mechanical properties and permeability of junctions between enterocytes, etc.) [2, 4]. Because of the fundamental importance of humoral influences for the activity of organs and tissues, it is clear that the study of the dynamics of the blood hormonal picture in animals after ischemia must shed light on the causes of these fluctuating processes in the mucous membrane, the mechanisms of which have not yet been explained.

In the investigation described below hormonal changes were studied in the blood of rats after ischemia of the small intestine.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 200-250 g. Total ischemia was produced by temporary ligation of the small intestine in the proximal portion and of the large intestine at the level of 2-3 cm beyond the iliocecal angle, followed by ligation of the cranial mesenteric arteries and, 2-3 min later, the veins. As a result the intestine was deprived of both inflow and outflow of blood for an assigned period (50 min), after which the ligatures were removed (first from the veins, then from the arteries and, finally, from the intestine itself). The abdominal wall was closed without drainage by a continuous suture.

The animals were divided into three groups: 1) control (intact rats), 2) rats undergoing only laparotomy for 50 min, and 3) rats in which total ischemia of the small intestine for 50 min was produced.

Blood samples were taken under ether anesthesia after decapitation from the animals of all groups simultaneously; after coagulation the serum was separated by centrifugation for 20 min at 2000 rpm.

To determine the serum levels of thyrotrophic hormone (TTH), tri-iodothyronine (T_3), thyroxine (T_4), insulin (IN), ACTH, and glucocorticoids (GC), radioimmunologic kits were used as follows: from Byk-Mallinckrodt (West Germany) for T_3, T_4, and TTH, from Corning (USA) for IN, from CEA-IRE-Sorin (France) for ACTH, and from the Radiochemical Centre, Amersham (England) for GC. Radioactivity was counted on a Gamma-Cord-II automatic gamma counter (from Ames, USA). The results were subjected to statistical analysis by means of the nonparametric Wilcoxon–Mann–Whitney criterion [1] and Student's t-test.

EXPERIMENTAL RESULTS

The results of measurement of the concentrations of the above-mentioned six hormones in the serum of animals of each group and of their statistical analysis are given in Table 1. The hormonal picture 2 h after ischemia and laparotomy was almost identical: a sharp increase in the GC concentration, a fall in the IN concentration, but no change in the ACTH, TTH, and T_4 levels compared with the control. However, there was

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TABLE 1. Serum Hormone Concentrations in Rats in the Postischemic Period

Time after operation	Conditions		ACTH, pg/ml	Cortisol, ng/ml	Insulin, micro-units/ml	TTH, micro-units/ml	Triiodo-thyronine ng/ml	Thyroxine, μg/100 ml
2 h	Ischemia		5300 (5)	377 (5)	13,2 (2)	1,37 (4)	0,66 (5)	1,66 (5)
	Laparotomy		7454 (5)	402 (5)	13,1 (2)	0,88 (5)	0,77 (5)	1,72 (5)
	Control		2548 (5)	89,4 (5)	20,5 (2)	1,63 (5)	0,86 (5)	1.13 (5)
	Ischemia/ control			3,86	0,65		0,77	
		P		<0,01	0,05		<0,05	
	Laparotomy/control			4,4	0,65			
		P		<0,01	0,05			
	Ischemia/laparotomy							
1 day	Ischemia		3653 (5)	148,2 (5)	14,7 (2)	1,94 (5)	0,47 (5)	0,48 (5)
	Laparotomy		1090 (5)	78,7 (5)	25,3 (2)	1,63 (5)	0,68 (5)	0,91 (5)
	Control		2304 (6)	78,4 (7)	24,3 (2)	1,84 (6)	1,04 (7)	2,57 (7)
	Ischemia/control				0,6		0,45	0,19
		P			0,05		<0,01	<0,01
	Laparotomy/control						0,66	0,35
		P					<0,01	<0,01
	Ischemia/laparotomy				0,6		0,7	
		P			0,05		<0,01	
4 days	Ischemia		182,5 (4)	61 (6)	24,7 (2)	1,53 (6)	0,69 (6)	0,82 (6)
	Laparotomy		2473 (6)	114 (7)	26,5 (2)	2,38 (7)	0,81 (7)	0,64 (7)
	Control		698 (6)	96 (7)	15,6 (2)	1,77 (7)	0,88 (6)	0,69 (7)
	Ischemia/ control			0,63	1,55			
		P		<0,01	<0,05			
	Laparotomy/control				1,77	1,35		
		P			<0,05	<0,05		
	Ischemia/laparotomy			0,53		0,64		
		P		<0,01		<0,01		
14 days	Ischemia		6097 (5)	167,8 (5)	12,8 (2)	1,71 (5)	0,75 (5)	3,12 (5)
	Laparotomy		3071 (7)	131,6 (7)	12,8 (2)	2,42 (7)	0,76 (7)	2,49 (7)
	Control		3666 (4)	166,2 (4)	11,4 (2)	1,74 (5)	0,76 (5)	3,37 (4)
	Ischemia/ control							
	Laparotomy/control							0,7
		P						<0,05
	Ischemia/laparotomy							

Legend. Mean values shown; level of significance and values for ratios given only when they differ significantly ($P \leq 0.05$) from 1; number of experiments shown in parentheses.

one difference: The T_3 concentration in the ischemized animals was lower than in the control rats, whereas in laparotomized animals statistically significant differences in the T_3 level compared with values obtained in intact animals were not found. Meanwhile, comparison of levels for animals of groups 2 and 3 showed no differences in the T_3 level, indicating the appearance of the typical stress reaction at this time after the operation.

After 1 day the IN, T_3, and T_4 concentrations in the ischemized rats were lower, whereas in the laparotomized animals only the T_3 and T_4 levels were lower; the change in the IN and T_3 concentrations, moreover, were greater in ischemia.

On the 4th day the blood levels of T_3, T_4, and TTH had returned to normal after ischemia, the IN level was high, but the GC concentration was low. At the same time after laparotomy, an increase was found also in the IN concentration but the GC level was unchanged and the TTH concentration, conversely, was increased. Concentrations of the other hormones were the same as in the control.

The results obtained 14 days after the operation indicate virtually complete recovery of hormonal homeostasis.

Analysis of the relations between changes in the properties of the mucous membrane of the small intestine on the one hand, and changes in hormonal homeostasis on the other hand, must evidently await fuller and more detailed information. However, the results of the present investigation are sufficient to indicate that this is a promising approach, and they reveal certain correlations. For instance, the hormonal changes are fluctuating in character and can be detected until 2 weeks after the operation, a fact which correlates with the duration of functional, biochemical, and morphological changes studied previously. This fact must be taken into account when the pathogenesis of various injuries to the gastrointestinal tract and their treatment are analyzed.

Correlation has also been found between changes in the mitotic index (MI) after ischemia [4] and the levels of thyroid and glucocorticoid hormones: A fall in MI after 4-24 h is accompanied by a fall in the T_3 and T_4 levels and a rise in the GC concentration, whereas an increase in MI on the 4th day is accompanied by a decrease in the GC level, in good agreement with data published previously on the effect of the hormone level on MI [5].

It has also been shown that ischemia of the small intestine is accompanied by specific hormonal changes in the blood, which differ from those observed after laparotomy. This fact may evidently reflect the much greater contribution of the intestinal hormonal (enterin) [3] system to the response of the tissue to injury, which interacts very closely through numerous feedback circuits with the general hormonal system.

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NEUROTROPHIC CONTROL OF FROG SKELETAL MUSCLE AFTER PARENTERAL INJECTION OF COLCHICINE

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Disturbance of axoplasmic transport (AT) of colchicine (COL) in motor nerve fibers of mammals and amphibians leads to the appearance of denervation-like changes in the skeletal muscle without any disturbance of neuromuscular transmission or exclusion of the muscle from motor activity [1, 4]. This suggests that substances (trophic factors), carried to the muscle by AT [6], participate in the mechanism of neurotrophic control of skeletal muscle fibers. Meanwhile investigations have shown that denervation-like changes also develop in mammalian muscles in direct contact with COL. In this case AT in motor nerve fibers is not appreciably affected [5, 7]. It can therefore be tentatively suggested that the development of the denervation-like syndrome in muscle fibers in experiments with COL is not necessarily the result of a disturbance of AT but may be the result of the direct action of the alkaloid on the muscle [5, 7]. However, the experiments described above did not solve the problem of how neuromuscular transmission is altered under these circumstances, and at the same time, we know that disturbance of neuromuscular transmission can lead to the appearance of changes of a denervation type in muscle [6].

Consequently, in experiments on frogs in which denervation changes in muscle fibers differ in certain respects from those in warm-blooded animals [8], it is interesting to study whether COL, through direct contact with the muscle, can cause the appearance of denervation-like changes in the muscle fibers and whether the character of myoneural transmission is altered under these circumstances.

The aim of the present investigation was to study the functional state of the pre- and postsynaptic membranes of the neuromuscular synapse after parenteral injection of COL into frogs.

EXPERIMENTAL METHOD

Experiments were carried out on a nerve–muscle preparation of the sciatic nerve and sartorius muscle of frogs (Rana ridibunda and Rana temporaria) in the winter period, using a standard microelectrode technique. Frogs of the experimental group received an injection of 0.1 ml of a 10 mM solution of COL (from Merck, West Germany), made up in Ringer's solution, into the dorsal lymph sac. The dose used is equivalent to the

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