AMINO ACID COMPOSITION OF LYMPH AND BLOOD DURING A FEBRILE RESPONSE OF VARIED DURATION

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Experimental and clinical investigations have shown that a febrile response (FR) is accompanied by considerable changes in protein metabolism. However, these views are largely based on the study of the protein composition of the peripheral blood, and the role of the lymphatic system, an important transport factor of the intercellular connectivetissue spaces, in determination of the pattern of change and distribution of amino acids in the principal components of humoral transport, i.e., between the blood, tissue fluid, and lymph, remains largely unexplained.

With the widespread clinical use of preparations inducing FR, and also arising from views on the predominantly catabolic shifts in protein metabolism, it was decided to compare the amino acid composition of the lymph and blood at different times after FR of varied duration.

EXPERIMENTAL METHOD

Experiments were carried out on 80 chinchilla rabbits weighing from 2.2 to 3.4 kg. An FR was reproduced by intravenous injection of pyrogenal by the method described previously [4]. All the experimental animals were divided into four groups. Animals of group 1 received a single dose of pyrogenal, whereas the rabbits of groups 2, 3, and 4 received 3, 5, and 10 once-daily injections, respectively. Animals receiving injections of pyrogen-free physiological saline, made up in bidistilled water, served as the control.

Concentrations of free amino acids and total protein in lymph from the thoracic duct and in the venous blood were investigated after the following time intervals: in animals receiving 1 and 3 injections of pyrogenal, on the 4th, 6th, and 10 days; after 5 injections, on the 6th and 10th day; after 10 injections, on the 11th day of the experiment. The level of free amino acids in the lymph and blood serum was determined by liquid chromatography on the AAA 881 automatic amino-acid analyzer (Czechoslovakia).

EXPERIMENTAL RESULTS

The experiments showed (Table 1) that the blood amino acid levels in intact animals were significantly higher than in lymph. After only one injection of pyrogenal, concentrations of the amino acids valine, leucine, isoleucine, threonine, serine, tyrosine, and lysine in the lymph were increased at various times after the FR, whereas the proline level fell. Changes in concentrations of threonine, serine, tyrosine, and lysine under these circumstances were characteristic of the later stages of the investigation. The blood aminoacid pool was apparently more "stable," and changes in levels of threonine and glycine (an increase) and of arginine and leucine (a decrease) were not found until the late stages after ER.

Three injections of the lipopolysaccharide led to a marked increase in the total amino acid pool in the lymph (with the exception of phenylalanine, histidine, arginine, and cysteic acid). The most marked changes were found on the 4th and 6th days of the experiment. In the later stages only the levels of serine, valine, isoleucine, tyrosine, and lysine remained higher than initially. Changes in amino acid concentrations in the blood affected significantly fewer of the individual compounds than in the lymph, and in addition, they were

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TABLE 1. Concentrations of Total Protein (in g/liter) and Free Amino Acids (in μM) in Thoracic Duct Lymph and Blood Serum of Normal Rabbits and Rabbits Receiving Three Injections of Pyrogenal (M ± m)

Parameter	Intact animals	Day of experiment		
		4-h	6-h	10-h
Cysteic acid	18,240±1,485	19,065±0,742	23,027±1,815	23,109±2,228
	13,288±2,476	17,827±2,393	11,802±1,403	10,316±2,558
Aspartic acid	5,408±0,751	8,037±0,826*	9,089±0,751*	6,460±0,901
	20,507±2,403	20,658±1,352	22,536±2,028	25,766±1,502
Threonine	18,216±0,923	24,429±1,930***	23,925±1,343***	21,490±1,511
	58,512±6,548	61,450±8,562	61,030±6,212	59,939±4,197
Serine	15,699±0,570	18,744±0,761***	21,027±1,236****	24,643±1,522****
	52,711±3,520	55,185±3,235	43,577±3,901	36,488±4,471**
Proline	97,099±3,387	107,087±3,821*	107,521±4,169*	92,930±3,735
	158,329±7,469	182,647±8,077*	145,388±6,253	145,128±5,385
Glutamic acid	30,721±1,903	36,974±1,087*	37,110±0,339***	34,187±0,543
	43,974±4,485	56,548±4,349*	55,665±3,262*	32,556±4,146*
Glycine	26,375±1,065	34,367±1,864**	35,966±2,264***	29,572±1,198
	61,809±5,061	67,936±2,664	106,700±4,662****	104,036±9,857***
Alanine	28,507±1,346	40,179±2,244****	35,802±2,132***	27,721±1,795
	91,694±8,978	82,267±8,754	77,441±8,866*	64,983±10,886**
Valine	38,323±2,560	85,182±9,644****	86,633±8,962****	52,065±6,145*
	58,296±5,206	62,990±5,035	62,990±6,145	47,883±6,889
Methionine	6,165±0,603	15,547±1,474****	10,387±0,804***	7,639±0,603
	8,577±0,670	7,505±0,368*	6,567±0,603**	8,980±0,402
Isoleucine	6,479±0,762	16,237±2,820***	16,313±2,591****	13,721±1,753***
	26,680±4,573	33,613±2,896	24,089±3,659	31,559±3,811
Leucine	9,071±1,143	15,322±0,762****	16,313±1,372****	11,968±1,448
	19,286±1,524	21,854±2,439	24,394±2,515	24,241±1,143
Tyrosine	18,930±1,159	32,506±3,808****	32,064±3,311****	29,470±2,208****
	32,561±2,263	34,989±2,428	41,115±1,766***	43,764±2,208***
Phenylalanine	24,516±1,453	23,002±2,300	24,274±1,998	23,850±1,634
	40,012±2,543	48,971±2,542**	52,240±2,724***	31,961±2,421*
Lysine	23,257±1,573	20,658±1,231	48,566±4,241****	40,563±5,198**
	29,961±1,915	26,746±1,641	25,514±1,915	22,231±1,846*
Histidine	12,890±1,225	13,406±1,547	12,632±1,096	14,243±1,160
	21,462±2,771	29,389±2,449*	15,984±2,127	24,456±3,029
Arginine	42,994±1,837	45,807±1,492	41,215±2,181	40,411±1,779
	48,505±2,583	57,459±2,755*	55,967±3,329	45,864±2,583
Total protein	62,4±1,30	59,0±0,91*	56,3±1,39***	62,4±0,70
	68,0±1,30	67,8±2,24	61,4±0,54****	68,3±1,82

Legend. Two rows of figures are given for each parameter. The first row corresponds to data obtained for lymph, the second to data for blood (here and in Table 2). The significance of differences is calculated compared with the control. *$p < 0.05$, **$p < 0.02$, ***$p < 0.01$, ****$p < 0.001$.

unstable and inconsistent in character depending on the time of investigation. For instance, in the early stages after FR (4th and 6th days of pyrogenal injections) an increase was observed in the concentrations of proline, phenylalanine, histidine, arginine, glycine, tyrosine, and glutamic acid. Elevation of the levels of these amino acids was accompanied by a fall in the blood levels of methionine and alanine in the early stages of the investigation, and of serine, phenylalanine, lysine, and glutamic acid in the later stages.

In the early stage of the investigation after 5 injections of pyrogenal (the 6th day of the experiment) qualitative changes in levels of individual amino acids in the thoracic duct lymph were mainly analogous to those described above after three injections of pyrogenal, i.e., their concentrations were increased (Table 2). However, the increases were quantitatively more marked, and in addition, levels of valine and alanine were considerably raised, and the histidine concentration was lowered. Meanwhile, in the late stages (10th day of the experiment) of FR, after 3 and 5 injections of pyrogenal, this "general pattern" of changes in the amino acid level in the lymph applied only to elevation of the lysine and tyrosine concentrations. At the same time, in the late stages after 5 injections of pyrogenal, the levels of threonine, glycine, methionine, alanine, and aspartic acid in the lymph remained high. In the blood serum, just as in the previous series of investigations, in the early stages after a five-day-long FR, elevation of the proline, tyrosine, phenylalanine, and arginine levels was accompanied by a fall in the alanine level. Qualitative differences in the content of the blood serum amino acid pool in this series of experiments were characterized also by elevation of the cysteic acid and lysine levels and a fall in the concentration of aspartic and glutamic acids. In the late stages of the investigation (10th day), not

TABLE 2. Concentration of Total Protein (in g/liter) and Free Amino Acids (in μM) in Thoracic Duct and Lymph and Blood Serum of Rabbits Receiving Five or Ten Injections of Pyrogenal (M ± m)

Parameter	Five injections		Ten injections
	sixth day	tenth day	11th day
Cysteic acid	19,148±1,568	21,624±1,815	26,411±2,393**
	29,712±4,539*	14,196±1,073	19,808±2,641***
Aspartic acid	10,141±1,277****	10,742±1,201****	16,676±2,028*
	10,441±2,178****	9,915±2,178****	11,268±2,479****
Threonine	24,177±1,595***	21,239±1,259****	25,100±1,343*
	66,991±3,274	67,830±3,106	92,092±8,730***
Serine	17,982±0,761****	17,316±0,856	21,122±0,856*
	42,816±4,757	39,581±2,854****	56,517±3,615
Proline	115,946±3,474*	99,097±4,516	109,519±5,645****
	179,260±7,469****	164,148±6,601	200,452±7,469*
Glutamic acid	36,838±0,747***	34,595±0,611	35,207±0,543****
	34,799±3,806****	43,634±2,039	37,313±5,097
Glycine	36,499±1,465***	35,433±1,998**	32,503±1,465**
	71,266±3,330	123,884±7,060*	66,737±3,263
Alanine	40,853±1,234*	32,884±1,571****	47,699±3,254*
	76,431±8,754****	67,115±6,397***	111,896±9,090
Valine	89,621±5,974*	47,456±5,206	81,341±1,195*
	45,578±5,718	107,203±11,010*	113,605±10,157*
Methionine	13,068±1,742***	10,990±0,603	22,450±2,479*
	10,387±0,938	7,639±1,005	5,428±0,536*
Isoleucine	11,633±1,219*	8,080±0,609	12,730±2,210***
	27,671±2,591	59,079±5,564*	30,568±3,582
Leucine	13,492±1,219***	8,385±0,914	25,232±2,820*
	17,380±1,677	27,671±3,277**	16,619±1,448
Tyrosine	35,265±1,821*	23,400±1,656****	30,077±3,035***
	42,384±2,208***	29,470±1,490	28,698±2,428
Phenylalanine	23,184±2,300	24,274±1,755	28,632±1,816
	51,513±2,724***	38,317±2,663	41,041±2,845
Lysine	21,136±1,162	54,381±3,557*	56,501±4,172*
	43,299±6,293****	62,247±6,293*	63,000±7,729*
Histidine	8,056±0,644***	14,823±1,289	20,882±1,805*
	26,682±2,127	21,011±1,869	24,813±2,256
Arginine	47,414±2,353****	39,550±1,894	53,900±1,148*
	62,166±3,157**	53,613±3,616	58,209±3,846***
Total protein	66,0±0,89**	64,6±1,03	66,7±0,80***

Legend. *p < 0.001, **p < 0.02, ***p < 0.01, ****p < 0.05.

only did the lysine level remain high, but concentrations of glycine, valine, isoleucine, and leucine were considerably increased, and there was a further decrease in the concentrations of alanine, serine, and aspartic acid.

FR lasting 10 days was accompanied by an increase in the concentration of all the free amino acids studied in the lymph except phenylalanine. The level of some of them (methionine, aspartic acid, valine, isoleucine, leucine, lysine), moreover, was raised more than two-threefold. During FR lasting 10 days, and also in the immediate periods after 5 daily injections of pyrogenal, elevation of the lysine, proline, arginine, and cysteic acid levels took place in the blood serum, whereas the aspartic acid concentration fell. In addition, there was a marked increase in the valine and threonine concentrations and a decrease in the methionine concentration.

The total protein level in the lymph and blood showed changes only after repeated injections of pyrogenal: during FR lasting three days it fell, but after a longer period of injections of pyrogenal it rose in the lymph and fell in the blood.

These investigations of the amino acid spectrum in the lymph and blood after FR showed no correlation between changes in their level and structure: concentrations of amino acids belonging to different classes showed similar changes, whereas levels of amino acids belonging to the same class showed opposite changes. The manifestations of the "individualism" of changes in each amino acid in the body fluids require further study, both qualitative and quantitative, with particular reference to the specific features of their metabolism, their presence in larger quantities in particular tissues, their involvement in FR, and so on.

In the course of transmembrane transport of amino acids and protein biosynthesis, complex (competitive and synergistic) relations are established between them. A deficiency of some amino acids and a relative excess of others in the amino acid pool are reflected in the

quality of the synthesized proteins. It has been suggested that an excess of a particular amino acid may inhibit incorporation of some amino acids and, at the same time, it may stimulate the incorporation of others. All this may lead to a serious change in the primary structure of proteins and to the appearance of, not only biologically active, but also of biologically inactive proteins, with subsequent loss of physiologically essential functions by subcellular structures and disturbance of the function and structure in tissues and organs [1, 7, 8].

It can also be postulated that the biological significance of these changes we found in amino acid levels in different components of humoral transport (especially during a long-term FR) reflect a process of redistribution of amino acids in the body in favor of organs and systems that are in a state of "metabolic stress."

The FR is accompanied by activation of the glucocorticoid function of the adrenal cortex [3, 9], which mobilizes amino acids from the tissue, especially lymphoid tissue, and stimulates their utilization in the liver for gluconeogenesis and protein biosynthesis in the hepatocytes [2, 5, 6, 8]. An early and substantial increase in the amino acid concentration in the lymph compared with that in the blood in the present experiment may probably be evidence of their lymphoid origin, and also of a disturbance of their turnover and inadequate utilization in the liver. These views are in agreement with the notion that the biochemical composition of the thoracic duct lymph is mainly determined by the functional activity of the liver.

The results of this investigation are evidence that changes in protein metabolism during FR are manifested primarily in the amino acid composition of the lymph. Irrespective of the duration of FR, their concentrations in the lymph are predominantly in one direction, namely an increase, confirming the view that catabolic processes predominate in protein metabolism during FR. The absence of conditions for their "cumulation" in the blood, dilution of the amino acid in a large circulating blood volume, and also enhancement of vascular permeability (including a selected change of permeability relative to different amino acids at the level of the microcirculatory bed of different organs and tissues) are causes of the less marked manifestations of the "catabolic reserves" of the body during FR in the blood than in the lymph.

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