Effects of Glucose and Glutamine on the Intensity of NAD Precursor Utilization in Rats with Transplanted Tumors

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The rate of nicotinamide utilization for the biosynthesis of NAD both in the liver and kidneys and in tumor tissue of rats with Walker's carcinosarcoma and Pliss' lymphosarcoma is shown to be limited by glucose. Glucose and glutamine increase the utilization of nicotinic acid in the liver and kidneys of rats with Walker's carcinosarcoma, in the kidneys of rats with Pliss' lymphosarcoma, and, to a lesser extent, in Pliss' lymphosarcoma tumor tissue, but do not affect the utilization of nicotinic acid in Walker's carcinosarcoma. It is concluded that certain stages of the NAD biosynthesis pathway are impaired.

Key Words: *NAD; nicotinamide; nicotinic acid; glucose; glutamine; rat tumors*

The resistance of cells to injurious agents depends on the synchronous increase in the activities of closed-loop enzymes, where NAD is utilized for the biosynthesis of poly(ADP-ribose), and the nicotinamide released is again involved in the biosynthesis of NAD. Nicotinamide may be used for NAD biosynthesis directly (emergency pathway) or after preliminary deamidization with the formation of nicotinic acid (NA), which, turning into a mononucleotide, is utilized after the main pathway of NAD biosynthesis by forming deamido-NAD. Previously we reported that experimental tumors differ in the rate of NA and nicotinamide utilization, and the intensity of the major pathway of NAD biosynthesis in some organs of animals with tumors is reduced [2,3]. This may be due to a deficiency of ATP and glutamine. Resynthesis of ATP in tumors is mainly accomplished by glucose oxidation. For this reason, we investigated the effects of glucose on the rate of NA and nicotinamide utilization and the effect of glutamine on NA utilization in rats with Walker's carcinosarcoma and Pliss' lymphosarcoma.

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MATERIALS AND METHODS

Experiments were carried out with 336 white outbred rats weighing 100 to 140 g with subcutaneously transplanted Walker's carcinosarcoma and Pliss' lymphosarcoma. Each of the tumors was transplanted to 168 animals, which were then divided into 7 groups, with 24 animals in each. On day 6-7 after transplantation the animals were intraperitoneally injected glucose at 300 mg/kg in group 1, nicotinamide at 100 mg/kg in group 2, glucose+nicotinamide in the same doses in group 3, NA at 100 mg/kg (the solution was neutralized before injection) in group 4, glucose+NA in the same doses in group 5, glutamine at 100 mg/kg in group 6, and NA+ +glutamine in the same doses in group 7. The animals were decapitated 0, 1, 2, 3, 4, 5, 6, and 7 h after injection. NAD concentrations were measured in the organs and tumor tissue removed, as described previously [3]. All the measurements were carried out in two parallel samplings of 3 animals per period. The results were statistically processed after Student [1]. Only reliable ($p<0.05$) differences in the content of NAD are presented; in case of borderline states ($p=0.05$) only a tendency is spoken of.

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TABLE 1. Effects of Glucose and Glutamine on the Rate of NAD Precursor Utilization in the Liver, Kidneys, and Tumor Tissue of Rats with Walker's Carcinosarcoma

Time postinjection, h	Glucose	Nicotinamide	Glucose+ nicotinamide	NA	Glucose +NA	Glutamine	Glutamine +NA
	μg NAD/g tissue						
Liver							
0	259±45	322±45	255±47	266±46	221±45	221±47	255±69
1	132±46	587±13	316±34	310±46	222±22	266±23	377±23
2	132±46	577±13	489±12	476±55	344±33	233±23	567±34
3	99±22	599±46	622±23	476±52	414±10	209±12	522±46
4	121±11	510±58	622±23	454±88	666±66	211±69	577±23
5	165±33	688±69	944±80	510±60	844±44	267±34	467±138
6	254±22	510±69	1133±46	433±103	844±44	281±12	756±92
7	188±58	410±58	995±46	366±34	666±66	255±34	622±23
Kidneys							
0	190±69	288±12	211±58	200±68	244±11	188±12	222±23
1	221±11	368±12	300±69	154±34	533±33	199±23	277±45
2	266±12	367±80	355±38	132±0	466±31	266±23	347±46
3	332±11	344±23	533±38	208±45	400±44	421±23	589±57
4	77±12	275±34	477±92	142±10	400±44	433±34	844±45
5	77±23	385±34	544±39	268±63	400±44	389±81	777±23
6	99±22	332±22	311±69	257±45	466±66	399±34	676±39
7	99±22	344±34	400±63	210±58	500±60	267±34	611±11
Tumor							
0	221±69	262±46	200±10	210±56	221±66	155±23	199±46
1	142±11	277±92	194±12	110±23	200±0	132±22	221±47
2	121±11	355±46	287±46	99±23	165±23	233±23	211±58
3	89±12	310±46	177±23	121±53	165±23	187±46	199±46
4	89±12	277±12	288±138	99±58	199±46	143±11	188±34
5	99±23	294±12	521±11	131±21	233±33	121±11	199±34
6	110±20	288±46	555±12	131±21	277±41	121±22	210±35
7	142±11	222±23	520±30	121±46	210±11	132±22	177±23

RESULTS

Table 1 shows that glucose injection to rats with Walker's carcinosarcoma caused a gradual reduction of the NAD concentration in the liver, which reached the minimal level 3-4 h postinjection and then showed a trend toward a recovery of the initial level. Injection of nicotinamide alone or NA alone brought about an increase of the NAD concentration. When glucose was injected with nicotinamide alone or with NA, the concentrations of NAD were higher than after injection of nicotinamide or NA alone. These results indicate that the biosynthesis of NAD in the liver of rats with Walker's carcinosarcoma is limited by the presence of the pyridine nucleotide precursor, because only after its administration did glucose cause an increase of NAD biosynthesis. The absence of changes in the NAD concentration in the liver of rats after glutamine injection indicates that deamido-NAD did not accumulate in the liver. Injection of glutamine with NA led to an increase of the NAD concentration.

The results suggest that both the main and the emergency pathway of NAD biosynthesis are preserved in the liver of rats with Walker's carcinosarcoma, as is the regulation of the rate of NAD biosynthesis.

Similarly as in the liver, glucose caused a decrease of the NAD concentration in the kidneys of rats with Walker's carcinosarcoma. After combined injection of glucose with NA or nicotinamide, the concentration of NAD was higher than after injection of NA or nicotinamide alone. In contrast to its effect in the liver, in the kidneys glutamine caused an increase of the NAD concentration. The rise of the NAD concentration in the kidneys after injection of glutamine with NA was higher than after injection of glucose with nicotinamide.

Hence, the main pathway of NAD biosynthesis is used more intensively than the emergency pathway in the kidneys of animals with tumors.

As in the liver and kidneys, in the tumor tissue injection of glucose led to a decrease of the NAD concentration followed by a tendency toward its recovery. In contrast to the case with the liver

TABLE 2. Effects of Glucose and Glutamine on the Rate of NAD Precursor Utilization in the Liver, Kidneys, and Tumor Tissue of Rats with Pliss' Lymphosarcoma

Time postinjection, h	Glucose	Nicotinamide	Glucose+ nicotinamide	NA	Glucose +NA	Glutamine	Glutamine +NA
	μg NAD/g tissue						
Liver							
0	244±23	300±69	355±103	210±58	210±35	243±69	221±47
1	220±35	322±34	500±0	333±34	222±23	266±69	277±46
2	249±58	533±200	622±23	355±46	399±46	288±69	321±46
3	232±58	284±56	644±23	288±12	369±23	377±23	255±34
4	222±23	287±34	788±34	277±45	333±68	277±44	232±35
5	255±34	422±37	844±45	300±69	310±68	300±66	244±32
6	299±23	622±125	866±45	266±34	310±68	300±66	199±46
7	265±34	366±138	955±46	266±34	380±68	300±66	244±42
Kidneys							
0	210±35	266±34	300±69	177±28	176±23	177±23	154±23
1	222±23	666±69	633±103	187±13	199±46	266±34	221±49
2	311±11	579±47	533±34	288±12	432±35	288±12	722±126
3	333±34	712±69	600±142	588±12	433±34	288±23	866±45
4	344±69	600±0	633±103	588±12	354±68	444±45	844±45
5	333±69	355±92	567±34	533±69	299±12	466±34	555±46
6	333±34	233±34	433±34	400±10	261±62	410±126	442±95
7	267±34	210±58	433±34	434±34	277±62	366±34	422±115
Tumor							
0	188±12	173±23	165±23	177±23	154±23	188±16	177±23
1	177±23	174±13	199±93	200±1	255±34	188±16	344±34
2	267±34	220±47	500±19	177±23	255±34	165±23	254±47
3	300±10	254±58	350±34	210±47	177±23	300±69	121±12
4	244±45	255±53	350±34	266±34	199±46	244±39	220±20
5	190±10	366±34	444±34	233±34	199±46	354±34	210±35
6	233±23	522±126	477±45	187±13	222±23	288±12	210±35
7	244±34	366±100	400±0	154±34	222±23	235±35	220±21

and kidneys, injection of nicotinamide did not bring about a rise of the NAD level, and injection of NA led to a reduction of the NAD concentration in the tumor. After injection of glucose together with nicotinamide the concentration of NAD increased, whereas after injection of glucose together with NA it remained unchanged. Glutamine did not intensify the utilization of NA in the tumor tissue.

Hence, glucose and glutamine did not increase the rate of NA utilization in tumor tissue. Therefore, unlike in the liver and kidneys, the main pathway of NAD biosynthesis is disrupted in tumor tissue of rats with Walker's carcinosarcoma.

Table 2 shows that glucose did not cause a reduction of the NAD concentration in the liver of rats with Pliss' lymphosarcoma, whereas glutamine tended to raise the NAD level. The latter fact indicates the presence of deamido-NAD in the liver of rats, which forms NAD after glutamine injection. Moreover, the increase of the NAD concentration after combined injection of glucose and nicotinamide is more pronounced than after injection of nicotinamide alone. Combined injection of glucose and NA caused a moderate increase of the NAD level, whereas glutamine combined with NA did not lead to its increase.

The emergency pathway of NAD biosynthesis is more intensively utilized in the liver of rats with Pliss' lymphosarcoma. The low intensity of the main pathway is not due to glucose or glutamine deficiency.

The NAD concentration rises in the kidneys of rats with Pliss' lymphosarcoma after glucose administration, as well as after glutamine administration. Hence, there is a precursor of pyridine nucleotide in the kidneys of rats with this tumor, and NAD biosynthesis is realized after the main pathway, but limited at the stage of deamido-NAD formation because of glutamine deficit. Further evidence of this is the increased concentration of NAD following injection of glutamine together with NA in comparison with that after injection of NA alone.

Injection of glucose or glutamine to rats with Pliss' lymphosarcoma led to an increase of the NAD level in the tumor tissue. This indicates the presence of pyridine nucleotide precursor and

deamido-NAD. Injection of nicotinamide alone and of glucose with nicotinamide led to an increase of the NAD concentration. On the other hand, NA did not cause an increase in the NAD concentration in the tumor tissue. Injection of glucose with NA slightly raised the NAD level only during the first few hours postinjection.

The NAD concentration rose one hour after injection of glutamine with NA, after which the NAD level returned to the initial value.

Hence, the emergency pathway of NAD biosynthesis is utilized mainly in Pliss' lymphosarcoma tissue. Similarly as in the liver, the low intensity of the main pathway of NAD biosynthesis is not caused by a deficit of glucose or glutamine.

The data demonstrate that the use of the emergency pathway of NAD biosynthesis is intrinsic tor tumor tissue of Walker's carcinosarcoma and Pliss' lymphosarcoma, the intensity of nicotinamide utilization being limited by glucose. The fact that glucose and glutamine did not intensify NA utilization in Walker's carcinosarcoma tissue and only negligibly increased NAD biosynthesis in Pliss' lymphosarcoma is evidence of the impairment of certain stages in the main pathway of NAD biosynthesis with partial or complete disruption of NA utilization. This is proven by the failure of the NAD concentrations in these tumors to rise following injection of quinolinic acid, a precursor of the main pathway of NAD biosynthesis [2].

The presence of tumors does not influence the utilization of both pathways of NAD biosynthesis in the kidneys; the same is true for the liver of animals with Walker's carcinosarcoma. In Pliss' lymphosarcoma the intensity of the main pathway is decreased in the liver. Both pathways of NAD biosynthesis are utilized in the liver and kidneys of intact animals [2].

The results lead us to assume that blocking of the emergency pathway of NAD biosynthesis in tumors may lead to their selective damage.

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