METABOLITES OF CARBOHYDRATE METABOLISM AND ATP IN THE EYE TISSUE AFTER DISTURBANCE OF ITS INNERVATION

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The effect of injury to the trigeminal nerve and removal of the superior cervical sympathetic ganglion on the concentrations of glucose, lactate, pyruvate, and ATP in the tissues and fluids of the eye was investigated in rabbits. Deafferentation of the cornea was followed by a decrease in the concentrations of glucose, lactate and pyruvate and by a particularly sharp decrease in the ATP level. The glucose concentration also was significantly reduced in the aqueous and there was a tendency for the lactate concentration to rise. In the iris and lens the concentrations of these substances showed no significant change. Desympathization did not cause any marked changes in the concentrations of these substances but the glucose concentration in the cornea, on the other hand, was increased.

Investigation of the biochemistry of denervated tissues is important as a means of studying the nature of neurodystrophy [1, 2].

The eye is a convenient object in which to study the effect of the nervous system on the nutritional state of its tissues. After injury to the trigeminal nerve the changes which take place are most clearly manifested in the anterior segment of the eye, in the form of defects in the corneal epithelium, opacities, and edema of the cornea [3]. The normal course of metabolism is substantially disturbed, especially in the cornea: anaerobic glycolysis is slowed [4], the intensity of tissue respiration falls [5], the concentrations of RNA and DNA [6] and of mediators [9] decrease, the level of protein synthesis changes [7], the potassium concentration falls and the sodium concentration rises [8], and the thiamine content is reduced [10].

Considering the role of glucose in the nutrition of the avascular cornea and the role of carbohydrate metabolism, during which much energy is liberated, an investigation was carried out to study the changes in the concentrations of glucose, lactic acid (LA), pyruvic acid (PA), and ATP in the tissues and aqueous humor of the eye after injury to the trigeminal nerve and removal of the superior cervical sympathetic ganglion.

EXPERIMENTAL METHOD

Experiments were carried out on 49 chinchilla rabbits of which 17 formed the control group. The trigeminal nerve was injured in the middle cranial fossa by Zaiko's method [3]. Ten animals were studied 1 day after injury to the trigeminal nerve, when the initial degenerative changes appeared, 12 rabbits 6 days later when the trophic disturbances were fully developed, and 10 animals 1 day after desympathization.

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TABLE 1. Concentration of Products of Carbohydrate Metabolism and of ATP (in μmoles/g wet weight of tissue or /ml fluid) in the Eye of Control Rabbits, after Injury to the Trigeminal Nerve, and after Desympathization

Experimental conditions	Glucose				Lactate			
	cornea	aqueous	iris	lens	cornea	aqueous	iris	lens
Normal	3,30±0,12	5,65±0,62	4,05±0,37	0,55±0,01	8,67±0,48	6,83±0,57	6,95±0,41	9,70±0,22
Injury to trigeminal nerve (1 day)	2,44±0,35	4,74±0,48	3,63±0,50	0,53±0,02	7,86±0,73	6,36±0,54	4,08±0,58	8,26±0,43
P	<0,05	>0,2	>0,5	>0,5	>0,5	>0,5	<0,02	<0,05
Injury to trigeminal nerve (6 days)	1,89±0,40	3,51±0,44	3,65±0,37	0,52±0,05	5,43±0,77	7,91±0,94	4,45±0,33	8,92±0,69
P	<0,01	<0,02	>0,5	>0,5	<0,01	>0,05	<0,02	>0,5
Desympathization	4,16±0,24	4,98±0,26	3,79±0,43	0,44±0,05	7,81±0,56	4,93±0,21	6,65±0,68	9,80±0,41
P	<0,01	>0,5	>0,5	>0,5	>0,5	>0,05	>0,5	>0,5

Experimental conditions	Pyruvate				ATP		
	cornea	aqueous	iris	lens	cornea	iris	lens
Normal	0,35±0,04	0,34±0,07	0,35±0,05	0,25±0,05	0,59±0,05	0,48±0,03	0,88±0,07
Injury to trigeminal nerve (1 day)	0,30±0,07	0,25±0,06	0,27±0,05	0,22±0,02	0,26±0,06	0,38±0,05	0,83±0,09
P	>0,5	>0,2	>0,2	>0,5	<0,01	>0,1	>0,5
Injury to trigeminal nerve (6 days)	0,19±0,02	0,20±0,06	0,35±0,12	0,24±0,04	0,27±0,05	0,37±0,05	0,67±0,09
P	<0,01	>0,1	>0,5	>0,5	<0,01	>0,1	>0,2
Desympathization	0,27±0,02	0,29±0,08	0,23±0,02	0,21±0,02	0,50±0,04	0,32±0,02	0,87±0,04
P	>0,1	>0,5	<0,05	>0,5	>0,1	<0,01	>0,5

The rabbits were killed by air embolism and the eyes were quickly removed and immersed in liquid nitrogen for determination of the metabolites. The cornea, iris, lens, and aqueous were separated from the frozen eye. The tissues were weighed, ground into a powder, and the protein was precipitated with perchloric acid in the proportion of 1 : 9. The concentrations of metabolites and ATP were determined by specific methods using coenzymes and crystalline enzymes by Warburg's UV-test [11] on the Spectromom-201 spectrophotometer at 340 nm and 25°C. The concentrations of glucose [12] and ATP [13] were determined with NADP (Reanal), hexokinase (Ferak), and glucose-6-phosphate dehydrogenase (Fluka), and LA [14] and PA [15] were determined with NAD ($NAD \cdot H_2$, respectively) and lactate dehydrogenase (Reanal). Three metabolites and ATP were determined simultaneously in one weighed sample. The content of water in the cornea was determined gravimetrically after drying at 105°C.

EXPERIMENTAL RESULTS AND DISCUSSION

The glucose concentration in the cornea was significantly reduced ($P < 0.05$ in the early period after injury to the trigeminal nerve (Table 1) and was only just over half the normal level after 6 days ($P < 0.01$). The concentration of glucose in the aqueous, the principal way by which substances enter the cornea, showed a similar change: 24 h after deafferentation it showed a tendency to decrease, while in the late period the glucose concentration was further and significantly reduced ($P < 0.02$).

The LA concentration in the cornea began to fall in the early stage. After 6 days the decrease was more marked and was significant ($P < 0.01$). The LA level in the aqueous in the early period after injury was almost unchanged, but in the late period, by contrast, its concentration showed a tendency to rise.

The PA concentration in the cornea also fell, especially 6 days after injury, when it was reduced by almost half the normal value ($P < 0.01$). A tendency for the PA concentration in the aqueous to fall appeared, but the decrease was not statistically significant. The ATP concentration in the cornea in the early period after deafferentation was reduced by more than half the normal level ($P < 0.01$), and later it still remained low ($P < 0.01$).

The glucose and PA concentrations in the iris were slightly reduced in the early and late stages after injury to the trigeminal nerve but the decrease was not statistically significant; the LA concentration, however, was significantly reduced ($P < 0.02$) and the ATP concentration showed a tendency to diminish.

No significant abnormalities were found in the concentrations of these substances in the lens after deafferentation, evidently because the lens has neither nerves nor blood vessels.

The results show a severe disturbance of carbohydrate and energy metabolism in the denervated cornea. A decrease was observed in the concentrations of glucose, the initial substrate of glycolysis, and of LA and PA, the end products of glycolysis, matching the reduced respiration [5] and anaerobic glycolysis [4] of the cornea. A reflection of these disturbances was the very sharp decrease in the accumulation of energy in the high-energy bonds of ATP. This evidently must account to some extent for the onset of opacity and edema of the cornea after trigeminal nerve injury, for the transparency of the cornea depends on the normal processes of active transport and the connected processes of hydration and dehydration of the cornea, which cannot take place properly without maintenance of the necessary level of ATP. Whereas the water content in the cornea of the control rabbits was 73.2%, 1 day after deafferentation it was 79.5% and 6 days after it was 78.4%.

The intracranial method of injuring the trigeminal nerve used in these experiments also caused injury to sympathetic fibers running to the eye from the superior sympathetic ganglion in a common trunk with the trigeminal nerve [16]. It has been suggested that the neurodystrophy of the eye arising under these circumstances is the result of injury to the sympathetic fibers only [17] or to the fibers of the trigeminal nerve only [3].

In the present experiment desympathization did not cause such severe changes in the concentration of metabolites in the cornea and aqueous as injury to the trigeminal nerve. Indeed, the glucose concentration in the cornea was higher than normal, the LA and PA concentrations in the aqueous and cornea were reduced only a little, and the ATP level in the cornea showed a tendency to decrease. After desympathization neither edema (water content 72.2%) nor opacity of the cornea developed, possibly as a result of the slight disturbances of metabolism and maintenance of the ATP concentration at a high level.

The chief cause of the neurotrophic disturbances and of the corresponding metabolic changes in the eye was thus injury to the trigeminal nerve itself, although injury to the sympathetic fibers may also play an admittedly much less important role.

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