EFFECT OF SODIUM AMYTAL ON THE ACTIVITY OF MUSCLE TISSUE PHOSPHORYLASE DURING LOCAL ISCHEMIA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 9, pp. 85-89, September, 1960
Original article submitted January 22, 1960

Previous research has shown that the phosphorolysis of glycogen in the limb muscles of rabbits is severely retarded after the application and removal of a hemostatic tourniquet [1]. The principal pathogenetic factors causing metabolic changes in the tissues isolated by the tourniquet are hemostasis and disturbance of the nervous control of the tissues of that part of the limb below the site of constriction. It has been shown that the application of a tourniquet to a limb is accompanied by severe functional disturbances of the nerves of the limb compressed by the tourniquet [2, 6, and others]. D. M. Gzgzyan [3, 4, 5], Biró, Büki, and Kovách [8], and other authors have established that marked changes take place in animals as a result of the application and removal of tourniquets, including effects on the functional state of the central nervous system.

Since the intensity of tissue metabolism depends on the regulating influence of the central nervous system, we considered it profitable to investigate the pathogenesis of the disturbances of the process of phosphorolysis in the muscles after the application and removal of a tourniquet, and to study the activity of the muscle phosphorylase in these conditions in relation to the initial functional state of the central nervous system. The effect of application and removal of a tourniquet on the activity of the muscle phosphorylase in rabbits was studied in two series of experiments in different functional states of the central nervous system: when awake, and during drug-induced sleep following administration of sodium-amytal.

METHOD

Experiments were carried out on 42 rabbits weighing 2-3 kg. The phosphorylase activity was determined by estimating the amount of inorganic phosphorus utilized in the formation of hexosephosphate esters during the phosphorolysis of glycogen added to a muscle extract. The incubation mixture consisted of 1 ml of muscle extract, 2 ml of a M/15 solution of phosphate buffer (pH 7.5), 0.2 ml of a 0.5 M solution of fluoride, and 0.5 ml of glycogen solution (40 mg). To control flasks an equal volume (3.7 ml) of 10% trichloroacetic acid solution was at once added. The precipitated proteins were separated by filtration, and the inorganic phosphorus content of the filtrate was determined by the method of Lowry and Lopez. The experimental flasks were placed in a water bath at 38° for 30 minutes, and after incubation were treated in the same way as the controls. The phosphorylase activity was judged by the amount of inorganic phosphorus used up from the reagent mixture, expressed in mg. The reliability of the mean values obtained in all the series of experiments was determined by means of the method of variational statistics [7].

RESULTS

In the first series of experiments we investigated the phosphorylase activity in an extract prepared from the symmetrical muscles of the right and left femoral regions of normal rabbits. These investigations showed that the magnitude of the decrease in inorganic phosphorus in the sample under the influence of muscle extracts of symmetrical limbs of normal rabbits was insignificant. The decrease in inorganic phosphorus during incubation for 30 minutes of an extract obtained from the muscles of the right limb, for instance, was 8.13 mg, and that during incubation of an extract from the left limb, 8 mg. The individual variations in the phosphorylase activity in the various animals were fairly considerable (the decrease in the inorganic phsophorus in the incubation medium varied between 6.48 and 10.16 mg.

Having established the mean value of the activity of the muscle tissue phosphorylase in normal rabbits, in the next series of experiments we determined the changes in these values in an extract of the limb muscles of rab-

Effect of Application and Removal of a Tourniquet on the Phosphorylase Activity of an Extract of the Limb Muscles of Rabbits

No. of experiments	Application of tourniquet to limb (in hr)	Recovery period (in hr)	Limb to which tourniquet was applied				Intact limb			Diff. bet. phosphorylase activity of muscle extracts from tourniqueted limb and intact limb
			Inorganic P in sample (in mg)		decrease of P (mg/ml of extract)	change (as % of normal)	Inorganic P in sample (in mg)		decrease of P (mg/ml of extract)	change (as % of normal)	
			before incubation	after incubation			before incubation	after incubation			
Sodium amytal not administered											
12	0	0	12,20	4,07	8,13±0,35	±0	12,20	4,20	8,00	±0	+ 2
5	4	0	9,63	5,57	4,11±0,19	—49	7,97	2,65	5,32	—33	—23
5	4	3	6,74	4,15	2,59±0,23	—68	7,67	3.19	4,48	—44	—42
5	4	24	13,93	7,29	6,64±0,19	—18	13,88	6,61	7,27	—9	—8
Sodium amytal administered											
5	4	0	11,89	7,30	4,59±0,31	—43	11,42	3,38	8,04	+0,5	—43
5	4	3	12,10	7,42	4,68±0,27	—41	10,85	3,20	7,65	—4	—39
5	4	24	12,28	6,69	5,59±0,10	—31	11,59	4,65	6,94	—13	—19

bits subjected to application of the tourniquet. As a tourniquet, we used an elastic rubber tube with a diameter of about 0.5 cm. In all the experiments the tourniquet was applied to the middle third of the right thigh for 4 hours, some animals being in a waking state and others in a state of sleep induced by the subcutaneous injection of sodium amytal (in a dose of 100 mg/kg body weight). The phosphorylase activity was determined both during compression of the limb with the tourniquet and after removal of the tourniquet. During the period of restoration of the phosphorolytic activity of the muscle extracts, this was investigated 3 and 24 hours after removal of the tourniquet.

In all the experiments the phosphorylase activity was determined in the extract of the muscles of the right thigh, on which the tourniquet was applied, and also of the left, intact thigh, acting as a control for each animal. The results obtained (mean values) are shown in the form of a comprehensive table. The mean values were deduced from 5-7 individual experiments, and their reliability was determined by treating the experimental results by the method of variational statistics [7].

The figures given in the table show that the phosphorylase activity in the extracts of the limb muscles of the rabbits, to which a tourniquet was applied (for 4 hours) in a state of sleep, was reduced. The decrease in inorganic phosphorus in the sample, after incubation of glycogen, with extract of the muscles below the site of application of the tourniquet, was, on the average, 4.59 mg, and with extract of intact muscles – 8.04 mg. The phosphorolytic activity of the muscle extracts of the experimental limb was decreased by 43% by comparison with those of the intact and normal limbs. If the results of the experiments with and without sodium amytal are compared, it will be seen that the difference in the phosphorolytic activity of the extracts of muscles below the site of application of the tourniquet between the two groups of rabbits was insignificant (the decrease in inorganic phosphorus in the first case was, on the average, 4.59 mg, and in the second case, 4.11 mg). Consequently, the inhibition of the central nervous system caused by administration of the hypnotic was not essentially reflected in changes in the phosphorylase activity of the muscle extract obtained after compression for 4 hours. Comparison of the phosphorylase activity of the muscle extracts of the intact limbs, on the other hand, shows that this value differed considerably in the sleeping and waking animals. For instance, whereas the phosphorolytic activity of the muscle extract of the intact limb in the experiments in which the animals did not receive sodium amytal was appreciably smaller (by 33%) than in normal rabbits, in the experiments in which the hypnotic was given, this decrease was not observed. In the recovery period in the first few hours after removal of the tourniquet (after 3 hours), the phosphorylase activity of the muscle extract from the experimental limb remained at roughly the same level as

at the end of the fourth hour of hemostasis (the amount of inorganic phosphorus used up from the sample was, on the average, 4.68 mg). In these conditions, as in the period of compression, the phosphorolytic activity of the muscle extracts of the intact limb remained undisturbed (the average decrease of inorganic phosphorus in the sample was 7.65 mg).

It is clear from the figures given that the phosphorolytic activity of the muscle extract of the experimental limb was lower than that of the intact limb by 39%, and than that of the normal limb, by 41%. In those animals on which the tourniquet was applied in the normal manner (without the hypnotic), the fall in the phosphorylase activity of the extract of the muscles below the site of application of the tourniquet was more marked -(68% less, compared with the normal value). It must be pointed out that, in the rabbits not receiving the hypnotic, the phosphorylase activity in the muscle extract from the intact limb was also greatly reduced by comparison with that in normal rabbits (on the average, by 44%). This demonstrates that, at the beginning of the recovery period, the phosphorylase activity, in the muscle extract from the limb in which the central nervous influences were cut off during the time of compression by the tourniquet, was disturbed to a far less extent than that in the extract of muscles compressed without administration of sodium amytal.

We also obtained similar results from determination of the phosphorolytic activity of muscle extracts from the intact limb in experiments with and without the use of sodium amytal. Prolongation of the recovery period to 24 hours, after compression for 4 hours, in experiments in which the hypnotic was given caused an increase in the phosphorylase activity in the muscle extract of the experimental limb and a decrease in the muscle extract of the intact limb. The decrease in inorganic phosphorus in the incubation mixture under the influence of extract of the injured muscles was, on the average, 5.59 mg, and in the symmetrical muscles , 6.94 mg (the difference between the phosphorolytic activity of the muscle extracts was, on the average, 19%). By comparison with the results obtained in the experiments on normal rabbits, the phosphorolytic activity at this stage of the recovery period was reduced, not only in the extract of the traumatized muscles, but also in the symmetrical muscles.

It may be seen that, by comparison with the normal value, the phosphorylase activity of the muscle extract from the experimental limb was decreased by 31%, and that of the extract from the intact muscles, by 13%. In the experiments carried out on the waking animals, 24 hours after removal of the tourniquet from the limb, the decrease in the inorganic phosphorus in the incubation mixture containing the muscle extract from the experimental, and also the intact limb, was slightly less pronounced than in the experiments on the sleeping animals. In our experimental conditions, the difference which was determined in the phosphorylase activity of the muscle extracts from the limbs of rabbits compressed by the tourniquet, with and without administration of sodium amytal, was thus also observed 24 hours after removal of the tourniquet.

In the analysis of the results showing the effect of drug-induced sleep on the phosphorylase activity of muscle tissue after application and removal of the tourniquet, it must be pointed out that the changes in the phosphorylase activity in the sleeping and waking animals bore the same character. The phosphorolytic activity of the extracts from muscles below the site of application of the tourniquet was reduced in all animals; the decrease was more marked in the period of compression and at the beginning of the recovery period in the animals in which the tourniquet was applied without the use of a hypnotic. The phosphorylase activity of the extract of intact muscles, which was lowered after application of the tourniquet and 3 hours after its removal in experiments on waking animals, was undisturbed in the experiments in which the hypnotic was used. The application of a hemostatic tourniquet to rabbits during inhibition of the central nervous system was thus accompanied by less marked changes in the phosphorylase activity of the extract obtained from the muscles of both limbs, both during hemostasis and in the first few hours after its removal. With a lengthening of the recovery period to 24 hours, the decrease in the phosphorylase activity of the muscle extract from the limb freed from the tourniquet, on the other hand, was less marked in the experiments in which sodium amytal was not used.

SUMMARY

The results clearly show that the phosphorylase activity of a muscle extract of the limbs in rabbits undergoes changes after application of a tourniquet in the waking and sleeping states, and that the changes, although in the same direction, are different in degree. This difference in the changes of enzyme activity in the experiments with and without the use of sodium amytal is proof that the functional state of the central nervous system plays an important part in the metabolic process taking place in the tissues of the part of the limb demarcated by the tourniquet.

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