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Comparative Investigation of Extracellular Space Volume and Na^+, K^+, Mg^{2+}, Ca^{2+} Content in Locust Locomotor Muscles

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ABSTRACT. Extracellular space volume (^{3}H-inulin was used) and Na^+, K^+, Mg^{2+}, Ca^{2+} content in locust thorax flight (dorsal longitudinalis 112, tergosternal 113, tergocoxal 119,120) and metathoracic leg (*flexor tibia, extensor tibia* and *retractor unguis*) muscles have been studied.

It is shown that the difference between water, Na^+, Mg^{2+} and Ca^{2+} content in locust flight and leg muscles was insignificant. As to the K^+ content, it varies in the range of 88.4 – 104.5 mM/kg (wet weight) in flight muscles and 115.5 – 129.1 mM/kg (wet weight) in leg muscles. In the mixed muscles (muscle 120, *flexor tibia, extensor tibia* and *retractor unguis*) K^+ content in them is high. The K^+ highest concentration 129.1 mM/kg (wet weight) has been shown in *extensor tibia*, the function of which is fast phasic contraction. Extracellular space volume is maximum in locust flight muscles and minimum in leg muscles, which must be connected with their functions. *© 2010 Bull. Georg. Natl. Acad. Sci.*

***Key words**: cations, flight muscles, leg muscles, locust.*

Introduction

The content of Na^+ and K^+ ions in the muscles of some species of insects has been investigated in a number of articles [1-3]. Interest in these ions was due to their participation in supporting of membrane potential and generation of potential actions [4,5] in muscle fibers. In the available literature we could not find data on the content of uni- and bivalent cations Mg^{2+} and Ca^{2+}, especially in the muscles with different functional specialization of the same species of insects. It must be noted that there are no data on the extracellular space volume of different muscles of insects either. The significance of this parameter is high, particularly in calculating the extracellular concentration of cations.

In previous studies the difference of locust flight muscles ultrastructure depending on their functional specialization has been shown [6-8]. In bifunctional muscles participating in the movement of locust wings and extremities myofibrils had longer sarcomeres and different ratio of thick and thin filaments, as compared with monofunctional muscles providing only wing movement of insects. It was interesting to ascertain if those functionally distinguished wing muscles differ from each other and other locomotor muscles in the maintaining of uni- and bivalent cations and water and in volumes of extracellular space.

The content of Na^+, K^+,Mg^{2+} and Ca^{2+} ions, water and extracellular space volume in bifunctional tergocoxal (119,120), monofunctional tergosternal 113 and dorsal longitudinalis 112 muscles of locust thorax have been investigated. The figural marking of muscles is given according to the anatomical nomenclature of Snodgrass [9]. Locust metathoracic leg muscles – flexor tibia, extensor tibia and retractor unguis have also been investigated. It must be mentioned that 112, 113, 119 muscles consist of structurally homogeneous phasic fibers and they do not differ in the activity of Ca^{2+} activated ATP-ase, succinate dehydrogenase and content of lipids [10]. The muscle 120, flexor tibia, extensor tibia and retractor unguis, besides the phasic fibers, maintains tonic and transit fibers differing in ultrastructure of contractile system, level of metabolic activity and content of myofibrillar proteins and intensity of DNA synthesis [11-16].

Materials and Methods

The experiments were conducted using the laboratory culture of locust (*Locusta migratoria migratorioides* R.F.). The insects were kept in the hothouse at 27- 28^{0}C. For standardization of experimental conditions special attention was paid to permanent presence of plentiful amount of green fodder (most of the experiments were carried out in the summer period).

Isolated muscles were weighed on teflon mats by means of micro analytic scales SARTORIUS-4431(FRG) with precision ±1mg. After weighing samples were dried at 105^{0}C up to permanent weight. Dried muscles were mineralized in quartz test-tubes by distilled concentrated nitric acid in air bath at 100^{o}C. After the ashing samples were dried and the residuum was dissolved in 0.05 M HCl (ch.p.). Concentrations of Na^+ and K^+ ions in obtained solutions were determined by means of flame photometer FLAPHO-4, and Mg^{2+} and Ca^{2+} - using the atomic absorptive spectrophotometer AAS-1 (Karl Zeiss, Jenna, GDR). Concentrations of Na^+, K^+, Mg^{2+} and Ca^{2+} ions in hemolymph were determined using the same methods after the dilution of hemolymph picked into the calibrated glass capillary by distilled water 1:100. The hemolymph was picked into the capillary from the cut in the head capsule of an insect. The volume of extracellular space of investigated insects was determined by using ^{3}H-inulin, which was injected into the belly under the cuticle (1 mCi). After exposure for 2 hours, when balanced distribution of ^{3}H-inulin was attained in the locust organism (Fig.) the samples of hemolymph and investigated muscles were picked for analysis. Radioactivity of preparations was measured by means of automatic liquid scintillation counter SL-4000 (Intertechnics, France) or RACKBETA -1209 (LKB, Sweden).

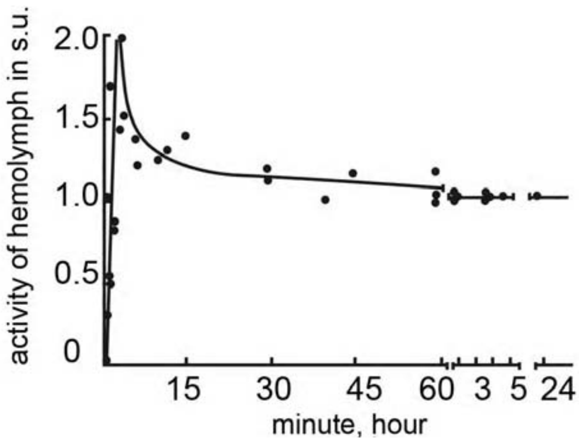

Fig. Kinetics of ^{3}H-inulin distribution after the single injection into the belly cavity of an insect (represents 3 experiments)

Results and Discussion

The investigation of cation content in hemolymph (Table 1) showed that the locust belongs to the group of insects with high content of Na-ions and low content of K-ions in hemolymph. These results are in good accordance with literature data of a number of authors [1, 17]. All the investigated locust wing and leg muscles differ slightly one from another in the content of water, Na^+, Mg^{2+} and Ca^{2+}. In different series of tests essential variation in Ca^{2+}-concentration was observed, affecting the dispersion of experimental data (Table 1). The reasons causing such fluctuation must be studied. The K^+ distribution among hemolymph and different muscles of locust undoubtedly is subject to the general rules for the whole organism – the tissue concentration of this cation correlates with the functional peculiarities of muscles. The highest K^+ concentration was detected in leg muscles, especially in exstensor tibia – the muscle intended for realization of fast phasic contractions. A similar relation between the parameters of K^+ and Na^+ distribution and contractile properties of muscles was shown for vertebrates [18,19] and in a number of invertebrates [20]. Our investigation of extracellular space volume, carried out by using ^{3}H-inulin as a marker, allowed to show that inulin space in locust flight muscles is distinctly greater than in leg muscles. The extracellular space volume is minimal in exstensor tibia, which probably must have functional significance. Using the data on extracellular space dimensions, we calculated intracellular K^+ concentrations (Table 2). The difference in extracellular concentrations of that cation is more significant in comparison with tissue concentrations.

It is interesting that all compound muscles – muscle 120, flexor tibia, extensor tibia and retractor

Table 1

The volume of extracellular space and content of water, Na^+, K^+, Mg^{2+} and Ca^{2+} in locust locomotor muscles

The investigated subjects	Inulin space, %wet weight	H_2O Kg/kg.dr.w	mM/kg. wet weight			
			Na^+	K^+	Mg^{2+}	Ca^{2+}
Flight muscles						
Muscle 112	11.5 ± 1.9 (10)	2.47 ± 0.13 (5)	15.6 ± 3.5 (5)	90.1 ± 3.9 (5)	15.6 ± 0.9 (5)	2.8 ± 1.6 (5)
Muscle 113	19.3 ± 3.3 (14)	2.43 ± 0.20 (21)	13.9 ± 5.4 (25)	88.4 ±10.4 (25)	15.2 ± 3.5 (25)	2.2 ± 1.8 (25)
Muscle 119	9.8 ± 1.8 (10)	2.34 ± 0.11 (17)	13.2 ± 4.0 (21)	99.8 ± 8.4 (21)	16.5 ± 1.9 (16)	3.0 ± 1.2 (16)
Muscle 120	14.0 ± 4.3 (17)	2.49 ± 0.37 (24)	17.0 ± 9.5 (18)	104.5 ± 8.5 (18)	15.3 ± 2.2 (18)	2.7 ± 1/8 (18)
Leg muscles						
Flexor tibia	9.8 ± 3.4 (12)	2.43 ± 0.13 (14)	14.7 ± 3.8 (14)	115.5±10.7 (14)	16.7 ± 1.5 (14)	3.4 ± 0.9 (14)
Extensor tibia	8.9 ± 3.5 (12)	2.42 ± 0.14 (14)	21.3 ± 8.6 (14)	129,1 ±9.8 (14)	20.4 ± 1.6 (14)	2.5 ± 0.9 (14)
Retractor unguis	10.6 ± 3.3 (6)	1.70 ± 0,40 (7)	9.4 ± 2.9 (7)	125.5±13.1 (7)	19.2 ± 3.8 (7)	2.3± 4.2 (7)
			mM/L			
Hemolymph	23.4±4.5 (17)	-	118.4±19.1 (17)	6.8±2.5 (21)	11.9±2.1 (16)	5.6±1.8 (16)

Note: The Table represents X ± σ (in round brackets – number of definitions)

Table 2

Intracellular concentration of K^+ in locust locomotor muscles

The investigated subjects	K^+ concentration mM/kg wet .weight
Flight muscles	
Muscle 112	100.9 ± 4.4 (5)
Muscle 113	107.9 ± 12.8 (25)
Muscle 119	109.9 ± 9.3 (21)
Muscle 120	120.4 ± 9.8 (18)
Leg muscles	
Flexor tibia	127.3 ± 11.8 (14)
Extensor tibia	141.1 ± 10.7 (14)
Retractor unguis	139.6 ± 14.6 (7)

Note: The Table represents arithmetic mean ±average square deviation (in round brackets – number of definitions)

unguis, besides phasic fibers, maintain tonic and transit fibers, the K^+ content was the highest. Earlier Huddart showed [21] that a significant part of K^+ -ions in locust muscles was bound to mitochondrium and miofibrillar fractions. Probably, in compound muscles consisting of phasic, tonic and transit fibers and characterized by different morphology; there are definite differences in K^+-distribution among definite cell structures. Nevertheless, any other explanation should not be excluded. For definitive elucidation of the reason of high K^+ content in locust compound locomotor muscles special investigations to study the distribution of this cation in definite cell structures must be carried out. The membrane potentials of isolated fibers, measured *in vitro,* should also be compared with calculated ones.

ბიოფიზიკა

კალიის ლოკომოტორულ კუნთებში უჯრედშორისი სივრცის მოცულობისა და Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} შემცველობის შედარებითი შესწავლა

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(წარმოდგენილია აკადემიკოს მ. ზაალიშვილის მიერ)

შესწავლილია უჯრედშორისი სივრცის მოცულობა (გამოყენებული იყო ^{3}H-ინულინი) და Na^{+}, K^{+}, Mg^{2+}, Ca^{2+} კათიონების შემცველობა კალიის თორაქსის საფრენ (ზურგის სიგრძივი 112, ტერგოსტერნული 113 და ტერგოკოქსული 119, 120) და მეტათორაკალურ ფეხის კუნთებში (წვივის მომხრელი, წვივის გამშლელი, ბრჭყალის შემწევი).

ნაჩვენებია, რომ კალიის საფრენ და ფეხის კუნთებში წყლის, Na^{+}, Mg^{2+} და Ca^{2+}-ის შემცველობაში განსხვავება უმნიშვნელოა. რაც შეეხება კალიუმს, მისი შემცველობა საფრენ კუნთებში მერყეობს 88,4-104,5 mM/კგ (ნედლი წონა), ფეხის კუნთებში კი 115,5-129,1 mM/კგ (ნედლი წონა) ფარგლებში. შერეულ კუნთებში (კუნთი 120, წვივის მომხრელი, წვივის გამშლელი, ბრჭყალის შემწევი) კალიუმის შემცველობა მაღალია. კალიუმის იონების ყველაზე მაღალი კონცენტრაცია 129,1 mM/კგ (ნედლი წონა) აღმოჩნდა წვივის გამშლელ კუნთში, რომლის ფუნქციაა სწრაფი, ფაზური, შეკუმშვა. უჯრედშორისი სივრცის მოცულობა მაქსიმალურია საფრენ კუნთებში და მინიმალურია ფეხის კუნთებში, რაც, ალბათ, მათ ფუნქციასთან არის დაკავშირებული.

REFERENCES

1. *P.N.R. Usherwood* (1969), Adv.Ins.Physiol., 6: 205-278.
2. *T. Piek, K.D. Njio* (1979), Adv. Ins. Physiol.,14: 185-250.
3. *D.W. Wood* (1965), Comp.Biochem.Physiol., 15: 303-312.
4. *H. Huddart* (1966), Arch.Inter.Physiol. Biochem., 74: 592-602.
5. *I. Zakhar, D. Zakharova, M. Gencheket et al.* (1978), Zh. Evol. Biokhimii i Fiziol., 14: 467-474 (in Russian).
6. *G.P. Papidze, Yu.E. Mandelshtam* (1987), Soobshcheniya AN GSSR., **126**: 169-172 (in Russian).
7. *G. Papidze* (1988), In: Abstracts of IV International Congress of Cell Biology. Montreal, Canada, 120.
8. *G.P. Papidze* (2003), Izvestiya AN Gruzii, Ser. Biol., ser. A, 29: 705-711 (in Russian).
9. *R.F. Snodgrass* (1935), Principles of insect morphology. N.Y., 667.
10. *I.A. Shumova* (1976), Zh. Evol. Biochimii i Fiziol., 12: 475-476 (in Russian).
11. *G. Hoyle* (1978), J. Exp. Biol., 73: 205-233.
12. *Yu.E. Mandelshtam* (1983), Neiron i myshtsa nasekomogo. Leningrad, 168s. (in Russian).
13. *Yu.E. Mandelshtam, G.P. Papidze* (1987), Zh. Evol. Biochimii i Fiziol., 23: 780-785 (in Russian)
14. *M. Novotova, Yu. Mandelshtam, E. Lipskaya et al.* (1989), Zh. Evol. Biochimii i Fiziol., 25: 360-366 (in Russian).

15. *G. Papidze, N. Gachechiladze, T. Toriashvili, A. Chubinidze* (2006), Proc. Georg. Acad. Sci. Biol. Ser. B, 4: 19-23.
16. *G. Papidze, Ts. Sumbadze, N. Gachechiladze* (2009), Proc. Georg. Acad. Sci. Biol. Ser. B, 7: 72-75.
17. *Yu.V. Natochin, R.G. Parnova* (1987), Comp. Biokhem. Phys., 88A: 563-570.
18. *Yu. A. Sculsci* (1969), Zh. Evol. Biokhimii i Fiziol., 6: 249-254 (in Russian).
19. *V.P. Nesterov, A.V. Lapin* (1970), Zh. Evol. Biokhimii i Fiziol., 6: 580-583 (in Russian).
20. *V.P. Nesterov, Yu.A. Sculsci* (1965), Zh. Evol. Biokhimii i Fiziol., l: 151-157 (in Russian).
21. *H. Huddart* (1971), Comp. Biochem. Physiol., 38: 715-721.

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