cumulating data on mediation of several neurotransmitter responses by cAMP [12, 14]. Two such neurotransmitters for *H. pomatia* are serotonin and dopamine [10], Further confirmation of this hypothesis is given by data on the ability of certain *H. pomatia* neurons to increase or reduce the amplitude of their responses to serotonin if it is applied repeatedly [6].

LITERATURE CITED

1. A. A. Aleksandrov, The Technique of Microelectrophoresis in Physiology [in Russian], Leningrad (1983).
2. N. I. Kononenko and A. D. Shcherbatko, in: Neurochemical Mechanisms of Regulation of Memory [in Russian], Pushchino (1984), p. 5.
3. E. A. Liberman, S. V. Minina, and N. E. Shklovskii-Kordi, Biofizika, 27, 542 (1982).
4. R. Pervis, Microelectrode Methods of Intracellular Recording and Iontophoresis [Russian translation], Moscow (1983).
5. D. A. Sakharov, The Genealogy of Neurons [in Russian], Moscow (1974).
6. E. I. Solntseva and L. V. Bezrukova, Dokl. Akad. Nauk SSSR, 256, 1499 (1981).
7. J. B. Aldenhoff, G. Hofmeier, H. D. Lux, et al., Brain Res., 276, 289 (1983).
8. P. Deterre, D. Paupardin-Tritsch, J. Bockaert, et al., Nature, 290, 783 (1981).
9. A. H. Drummond, J. A. Benson, and I. B. Levitan, Proc. Nat. Acad. Sci. USA, 77, 5013 (1980).
10. A. H. Drummond, Adv. Cycl. Nucl. Res., 15, 373 (1983).
11. R. Gilette and D. J. Green, Brain Res., 273, 384 (1983).
12. P. Greengard, Nature, 260, 101 (1976).
13. N. I. Kononenko, P. G. Kostyuk, and A. D. Shcherbatko (A. D. Scherbatko), Brain Res., 268, 321 (1983).
14. J. L. Nathanson, Physiol. Rev., 57, 157 (1977).

CAUSES OF DISTURBANCE OF FATTY ACID OXIDATION IN ISOLATED MITOCHONDRIA OF THE ISCHEMIC HEART

R. V. Balasevičius, A. I. Dagys, A. I. Toleikis, and A. K. Praškevičius

UDC 616.127-005.4-577.121.7

KEY WORDS: ischemia; mitochondria; fatty acid oxidation.

Activation of respiration of cardiac mitochondria (Mc) in state 3 with succinate by cytochrome c is considerably enhanced during ischemia [1]. Cytochrome activated oxidation of glutamate + malate is enhanced more than twofold during ischemia, but no activating effect of cytochrome c on fatty acid (FA) oxidation could be observed [7]. Activation of oxidation of pyruvate + malate (P + M) by cytochrome c was enhanced during ischemia by a greater degree than oxidation of 3-hydroxybutyrate [2]. These data show that cytochrome c did not activate respiration during ischemia, or activated it less, only in medium without malate or succinate. However, these dicarboxylates had no significant effect on FA oxidation under control conditions [6]. Inhibition of oxidation of palmitate was actually observed in another study [5] both in the control and during ischemia.

To examine this problem it was decided to study the effect of cytochrome c on FA oxidation under control and ischemic conditions in medium with and without malate. Depression of carnitine acyltransferase activity, which is observed even in the early stages of ischemia [8], may play an essential role in the disturbance of FA oxidation in ischemia. It has been suggested that injury to the outer membrane of Mc in ischemia can lower not only the cytochrome c content in Mc [1], but also external carnitine acyltransferase activity, and this fall must lead to a decrease in the ratio between velocities of oxidation of palmitoyl-CoA

Laboratory of Metabolism, Research Institute of Physiology and Pathology of the Cardiovascular System and Department of Biological and Organic Chemistry, Kaunas Medical Institute.

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 294-296, March, 1985. Original article submitted May 16, 1984.

0007-4888/85/9903-0282$09.50

TABLE 1. Effect of Ischemia for 30 min on Substrate Oxidation in Isolated Cardiac Mc (M ± m)

Experimental conditions	V_2	V_3	V_{2+C}	V_{3+C}	V_{3+C}/V_3
Control (PC)	31±1,5	218±9	37±1,4	275±13	1,25± 04
Ischemia (PC)	31±0,7	91±6*	38±1,3	127±16*	1,41±0,1
Control (H)	27±1	244±8	31±1	281±9	1,15±0,04
Ischemia (H)	28±1	102±4*	35±2	136±7*	1,36±0,1

Legend. Incubation medium without malate. ADP added after oxidation substrate (Fig. 1a). V_2 and V_3) Velocity of oxidation of substrates before and after addition of ADP respectively; V_{2+C} and V_{3+C}) the same in medium with cytochrome c. Number of experiments 5. *P < 0.001.

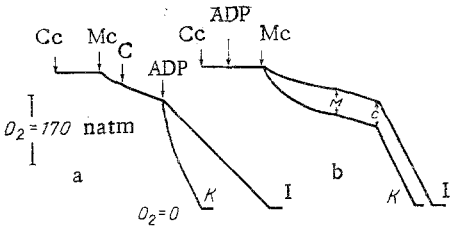

Fig. 1. Effect of ischemia on FA oxidation: a) measurement of oxidation without endogenous substrates (medium without malate); b) measurement of oxidation with exhaustion of endogenous substrates (medium with malate). K) Control, I) ischemia, C) fatty acid, Cc) cytochrome c, M) malate.

(P-CoA) and palmitoylcarnitine (PC). After ischemia for 30 min oxidation of PC, hexanoate (H), and P + M, and after 60 min of ischemia, oxidation of PC, P-CoA, and H, were investigated.

EXPERIMENTAL METHOD

Rabbits weighing 2-4 kg were used. Functions of Mc were investigated in the control and during total myocardial ischemia for up to 1 h. The heart was bathed in ice-cold 0.9% NaCl solution and divided into two parts. One part served as the control (Mc were isolated immediately after bathing), and the other part, bathed in warm (37°C) 0.9% NaCl solution, was subjected to autolysis in a glass chamber, saturated with vapor of 0.9% NaCl solution at 37°C [4]. The tissue, cut into pieces with scissors, was homogenized in a glass-Teflon homogenizer for 60 sec. Mc were isolated as described previously [1]. The velocity of oxidation of the substrates was measured polarographically at 37°C with an electrode of Clark type, in medium containing 150 mM KCl, 5 mM KH_2PO_4, and 10 mM Tris-HCl, pH 7.2. The oxidation substrates were: PC 20 μM, P-CoA 20 μM, H 300 μM, malate 100 μM, P + M 5 mM of each. Other reagents were cytochrome c from horse heart 30 μM, L-carnitine 1 mM, ADP 2 mM, carbonylcyanide-m-chlorophenylhydrazone 4 μM. The mitochondrial protein concentration for recording oxidation of P + M was 0.5 mg/ml, and otherwise 1 mg/ml. Protein was determined by the biuret method with extraction with diethyl ether [3]. The significance of changes was estimated by the method of paired comparisons.

EXPERIMENTAL RESULTS

The velocity of FA oxidation in state 3 was measured in two ways, differing in the order of addition of ADP and substrates (Fig. 1) and the presence of malate in the medium.

Measurement of FA oxidation without exhaustion of endogenous substrates showed that after 30 min of ischemia oxidation of PC and H was reduced equally, i.e., by 58% (Table 1; Fig. 1a). Cytochrome c activated respiration of control and ischemic Mc equally and caused hardly any change in the difference between control and experiment. When FA oxidation was

TABLE 2. Effect of Ischemia for 30 min on Substrate Oxidation in Isolated Cardiac Mc (M ± m)

Experimental conditions	V_3	V_{3+C}	V_{3+C}/V_3	V_U	V_{U+C}
Control (PC)	167±10	204±11	1,22±0,02		
Ischemia (PC)	114±11***	198±14	1,77±0,12*		
Control (H)	179±4	226±8	1,26±0,03		
Ischemia (H)	132±10*	221±12	1,68±0,06*		
Control (P + M)	438±17	560±18	1,28±0,02	402±17	560±20
Ischemia (P + M)	258±22***	490±24*	1,95±0,13**	249±25***	511±33

Legend. V_U, V_{U+C}) Velocity of uncoupled respiration in medium with and without cytochrome c respectively. Number of experiments: for PC 7, for H 5, for P + M 8, except V_U (6) and V_{U+C} (7). Velocities of respiration measured after exhaustion of endogenous substrates in medium with malate. *P < 0.01, **P < 0.002, ***P < 0.001. Remainder of legend as to Table 1.

TABLE 3. Effect of Ischemia for 60 min on FA Oxidation in State 3 in Isolated Cardiac Mc (M ± m)

Experimental conditions	P-CoA	PC	H
Control	200±16	236±20	219±22
Ischemia	168±20	193±21	189±23*

Legend. Incubation medium with cytochrome c, carnitine, and malate, pH 7.0. Number of experiments: for PC 9, for P-CoA 8, for H 6. *P < 0.05.

measured after exhaustion of endogenous substrates and with the addition of malate the velocity of oxidation of the same substrates was reduced much less in medium without cytochrome c, namely by about 30% (Table 2), whereas in medium with cytochrome c the values of activity were the same as in the control (Table 2; Fig. 1b). Under these conditions activation of PC and H by cytochrome c was significantly increased during ischemia by 33-45% (Table 2). During ischemia malate activated FA oxidation in medium with cytochrome c up to 60%, but in medium without it to only 30% (Tables 1 and 2). The results show that the decrease in activity of oxidation of PC and H after 30 min of ischemia was due mainly to a reduction in the concentration of intermediates of Krebs' cycle and cytochrome c in isolated Mc. A parallel study of oxidation of P + M (after exhaustion of endogenous substrates) in state 3 and in the uncoupled state, in medium without cytochrome c, showed that after 30 min of ischemia it was reduced by 41 and 38% respectively, i.e., by a very similar degree (Table 2). In medium with cytochrome c only respiration in state 3 was depressed by 12.5%. It will be clear that the velocity of respiration of Mc with P + M was more than twice that with PC and H. Consequently, activity of the Krebs cycle and electron transport in the respiratory chain, and also adenine nucleotide transport do not limit FA oxidation either in the control or during ischemia. However, a disturbance in the terminal part of the respiratory chain at the cytochrome c level plays an important role in the lowering of activity of PC and H oxidation during ischemia.

When the effect of ischemia for 60 min was investigated, oxidation of PC, P-CoA, and H was measured only after exhaustion of endogenous substrates in medium containing cytochrome c, carnitine, and malate (Table 3). An equal decrease in oxidation (state 3) of all the above-mentioned substrates was observed during ischemia (by 16-18%, a significant decrease was observed only for H). This can be explained by increased injury to the inner membrane of Mc after ischemia and by an increase in its permeability for low-molecular-weight compounds [1], including for NADH [2]. The ratios between velocities of oxidation of P-CoA and PC were unchanged during ischemia. Hence it follows that no significant change in activity of external carnitine-palmitoyltransferase for P-CoA oxidation took place during 60 min of ischemia.

The main causes of the decrease in velocity of FA oxidation in the early stages of myocardial ischemia are therefore reductions in the concentrations of intermediates of the Krebs' cycle and of cytochrome c in isolated Mc.

The results in Fig. 1a show that when respiration of Mc with PC and H was recorded without exhaustion of endogenous substrates there was a gradual fall in the velocity of respiration, probably due to oxidation and, consequently, to a decrease in the concentration of endogenous substrates. When respiration was recorded after preliminary oxidation of endogenous substrates, the measured velocity was constant. For that reason, the initial velocity of FA oxidation measured in the presence of endogenous substrates in Mc in the control was 25-30% greater than after their preliminary oxidation. During ischemia the quantity of oxygen consumed to oxidize endogenous substrates and, consequently, their concentration in Mc, fell (Fig. 1b): by 31% after 30 min and by 52% after 60 min ($P < 0.001$). That is why the opposite picture is observed in ischemia: The velocity of respiration was lower in experiments without oxidation of endogenous substrates than in experiments with their preliminary oxidation. Measurement of the true velocities of FA oxidation is possible only after complete oxidation of endogenous substrates. This must be taken into account not only in ischemia, but also when comparing results of investigations carried out on different animals, whose Mc differ considerably in their concentration of endogenous substrates.

LITERATURE CITED

1. R. V. Balasevičius, A. I. Toleikis, and A. K. Praškevičius, Byull. Éksp. Biol. Med., No. 1, 40 (1984).
2. R. V. Balasevičius, A. I. Toleikis, and A. K. Praškevičius, Vopr. Med. Khimii, No. 2, 130 (1984).
3. P. P. Dzeja, A. I. Toleikis, and A. K. Praškevičius, Vopr. Med. Khimii, No. 6, 731 (1980).
4. L. C. Armiger, R. N. Seele, V. M. Carnell, et al., Lab. Invest., 34, 357 (1976).
5. J. Bremer, J. Biol. Chem., 237, 362B (1962).
6. J. B. Fritz and K. T. K. Yue, J. Lipid Res., 4, 479 (1963).
7. A. Schwartz, J. M. Wood, J. C. Allen, et al., Amer. J. Cardiol., 32, 46 (1973).
8. J. M. Wood, L. A. Sordahl, R. M. Lewis, et al., Circulat. Res., 32, 340 (1973).

CELLULAR AND REGIONAL LOCALIZATION OF NEUROSPECIFIC ANTIGEN 10-40-4 IN THE HUMAN BRAIN

T. P. Klyushnik, S. G. Kushner, M. B. Aksenova, and G. Sh. Burbaeva

UDC 612.124.017.1-08

KEY WORDS: immunoenzyme assay; immunofluorescence assay; nonspecific antigen.

Neurospecific protein (NSP) 10-40-4, isolated and characterized in the writers' laboratory, has a molecular weight of 76 ± 2 kilodaltons and an isoelectric point of 4.7. The antigen contains no carbohydrate or lipid components and is precipitated by ammonium sulfate at 40-60% saturation [1]. The specificity of protein 10-40-4 for nerve tissue has been demonstrated by immunodiffusion and immunoenzyme analysis. Specimens of NSP 10-40-4 from different species of mammals are not immunologically identical [2].

The aim of this investigation was to study the cellular and regional localization of NSP 10-40-4 by the indirect immunofluorescence method of Coons and also to determine it quantitatively in extracts of various structures of the human brain by immunoenzyme assay (ELISA).

Laboratory of Neurochemistry and Chemistry of Physiologically Active Substances, All-Union Mental Health Research Center, Academy of Medical Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Snezhnevskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 99, No. 3, pp. 296-298, March, 1985. Original article submitted April 6, 1984.