

KONZENTRATION DER NIKOTINAMID-KOENZYME UND AKTIVITÄT EINIGER ENZYME DER ATMUNGSKETTE UNTER DEM EINFLUSS DER NITRITE
 N. Nestorov, N. Ivanov, J. Profirov
 Institut für Fleischwirtschaft - Sofia

Zusammenfassung

Es wurden Untersuchungen durchgeführt über die Gesamtzellenkonzentration der oxydierten Formen der Nikotinamid-Koenzyme (NAD^+ und NADP^+), den Sauerstoffverbrauch, die Aktivität der NADH-Zytochrom C Reduktase, der Sukzinat-Dehydrogenase und der Zytochromoxydase in von der Leber und von der femoralen Muskulatur isolierten Mitochondrien, sowie auch über den Prozentgehalt an Methemoglobin im Blut von Ratten, die während 30 Tage mit frischen, verschiedenen Natriumnitritmengen enthaltenden Brühwürsten verfüttert wurden.

Der Versuch wurde mit 3 Gruppen von je 10 weißen männlichen Ratten durchgeführt. Die erste Gruppe erhielt Würste, die kein NaNO_2 enthielten, die zweite Gruppe - Würste mit einem normalen NaNO_2 -Gehalt (8 mg %) und die dritte Gruppe - Würste mit einem 4-mal höheren NaNO_2 -Gehalt (32 mg %).

Die Verfütterung mit NaNO_2 enthaltenden Würsten verursachte eine Verminderung des Sauerstoffverbrauches des Leberhomogenates und eine Erhöhung der Aktivität der NADH-Zytochrom C-Reduktase und der Zytochrom-Reduktase in den Lebermitochondrien. Diese Ergebnisse sind nur bei der Gruppe, die mit Würsten mit einem 4-mal höheren NaNO_2 -Gehalt verfüttert wurde, mathematisch gesichert.

Die Aktivität der untersuchten Mitochondrienzymen ist ein Hinweis dafür, dass man den verringerten Sauerstoffverbrauch auf ein anderes angegriffene Glied der Atmungskette oder auf eine verringerte Zufuhr von zu oxydierenden Substraten zurückführen könnte.

Den erhöhten Gehalt an oxydierten Formen der Nikotinamid-Koenzyme kann man teilweise mit der NADH-Zytochrom C-Reduktase verbinden, sowie auch mit der Oxydation der reduzierten Koenzyme in einem System von Reaktionen, die zu einer Reduktion des NaNO_2 zu NO führen.

CONCENTRATION DES COENZYMES NICOTINAMIDIQUES ET ACTIVITE DE CERTAINS ENZYMES DE LA CHAINE RESPIRATOIRE SOUS L'INFLUENCE DES NITRITES

N. Nestorov, N. Ivanov, Y. Profirov
 Institut de recherches sur la viande - Sofia

Résumé

Des études ont été effectuées sur la concentration cellulaire globale des formes oxydées des coenzymes nicotinamidiques (NAD^+ et NADP^+), la consommation d'oxygène, l'activité de la NADH - cytochrome C - réductase, de la succinate - déhydrogénase, de la cytochrome - oxydase dans des mitochondres, isolées du foie et de la musculature fémorale, ainsi que le pourcentage de la méthémoglobine dans le sang de rats, nourris pendant 30 jours avec des saucissons cuits-fumés à une quantité différente du nitrite de sodium.

L'expérience a porté sur trois groupes de 10 rats blancs. Le premier groupe a reçu des saucissons ne contenant pas de NaNO_2 , le deuxième groupe - des saucissons contenant la quantité habituelle de NaNO_2 (8 mg %) et le troisième - des saucissons contenant une quantité de NaNO_2 quatre fois plus élevée (32 mg %).

L'alimentation à saucissons contenant du NaNO_2 provoque la diminution de la consommation d'oxygène de l'homogénat de foie et l'accroissement de l'activité de la NADH - cytochrome C - réductase et de la cytochromoreductase dans les mitochondres du foie. Ces résultats sont prouvés mathématiquement pour le groupe recevant des saucissons à une quantité de NaNO_2 quatre fois plus élevée.

L'activité des enzymes mitochondriques étudiés montre que la consommation réduite d'oxygène peut être due à l'atteinte d'un autre maillon de la chaîne respiratoire ou à l'apport diminué des substrats à oxyder. La teneur élevée des formes oxydées des coenzymes nicotinamidiques peut être liée partiellement avec la NADH cytochrome C - réductase et l'oxydation des coenzymes diminuées dans un système de réactions qui mènent à la réduction de NaNO_2 au NO.

CONCENTRATION OF NICOTINEAMIDE COENZYME AND ACTIVITY OF SOME ENZYMES FROM THE RESPIRATORY CHAIN UNDER THE ACTION OF NITRITES

N. Nestorov, N. Ivanov, I. Profirov
 Meat Industry Institute - Sofia

Summary

Studied was the allcellular concentration of oxidized forms of nicotineamide coenzymes (NAD^+ and NADP^+), oxygen demand, activity of NADH-cytochrome C-reductase, succinatdehydrogenase and cytochromoxydase in mitochondria isolated from liver and thigh muscles and percentages of metchaemoglobin in the blood of rats fed for 30 days with fresh coocked sausages, containing different quantities of sodium nitrite.

The test was made with three groups, each of 10 white male rats. The first group received sausages without NaNO_2 , the second with normal quantity of NaNO_2 (8 mg %) and the third with four times higher content of NaNO_2 (32 mg %).

Feeding with sausages containing NaNO_2 evoked a lowering of oxygen demand of the liver homogenate and an increase of NADH-cytochrome C-reductase activity as well as the cytochromereductase in the liver mitochondria which was mathematically proved only with the group receiving four times higher content of NaNO_2 .

The activity of the investigated mitochondrial enzymes shows that the diminished oxygen need could be the result of on impairment of another link in the respiratory chain or a lower inflow of substrates for oxydation. The increased content of the oxidized forms of the nicotineamide coenzymes we could partly link with the NADH cytochrome C-reductase and on oxydation of the reduced coenzymes in a system of reactions, leading to a reduction of NaNO_2 to NO.

КОНЦЕНТРАЦИЯ НИКОТИНАМИДНЫХ КОЭНЗИМОВ И АКТИВНОСТЬ НЕКОТОРЫХ ЭНЗИМОВ ДЫХАТЕЛЬНОЙ ЦЕПИ ПРИ ДЕЙСТВИИ НИТРИТОВ

Н. Несторов, Н. Иванов, Я. Профиров
 Институт мясной промышленности - София

Аннотация

Исследована общеклеточная концентрация окисленных форм никотинамидных коэнзимов (NAD^+ и NADP^+), потребление кислорода, активность цитохром C-редуктазы, сукцинатдегидрогеназы и цитохромоксидазы в митохондриях, изолированных из печени и бедренной мускулатуры, и процент метгемоглобина в крови крыс, откормленных в течение 30 дней вареными колбасами с различным содержанием нитрита натрия.

Опыт проведен на трех группах самцов-крыс по 10 животных в каждой. I группа получала колбасы без NaNO_2 , II группа - нормальное количество NaNO_2 (8 мг %) и III - в 4 раза большее содержание (32 мг %) NaNO_2 .

Питание крыс колбасами, содержащими NaNO_2 , вызвало уменьшение потребления кислорода гомогенатом печени и увеличение активности цитохром C-редуктазы и цитохромредуктазы в митохондриях печени, что доказано математически только у крыс, получающих колбасы с большим содержанием нитрита (III группа).

Активность исследованных митохондриальных энзимов показывает, что уменьшение потребления кислорода происходит, вероятно, в результате повреждения некоторого другого звена дыхательной цепи или обрывается уменьшением поступления субстрата для окисления. Увеличенное содержание окисленных форм никотинамидных коэнзимов, в частности, мы можем связать с NADH-цитохром C-редуктазой и с окислительно-восстановительными коэнзимами в системе реакций, восстанавливающими NaNO_2 в NO.

CONCENTRATION OF NICOTINEAMIDE COENZYMES AND ACTIVITY OF SOME ENZYMES FROM THE RESPIRATORY CHAIN UNDER THE ACTION OF NITRITES

N.Nestorov, N.Ivanov, I.Porfirov

Meat Technology Research Institute - Sofia

The widespread use of nitrites in the food industry, impedes deeper investigations of their action on the different biochemical processes in the organism.

Better information for the technological use of nitrates and nitrites, and the metabolic action of nitrosamines, could be seen in the review of Wolf and Wasserman, 1972.

In the present paper, we tried to investigate the influence of the increased quantities of nitrites and coenzymes having connection with the biological oxydation.

Material and Methods

The test was made with three groups of young male white rats equal in age, and having a liveweight of 150 g each. The first group received a determined quantity of fresh cooked and smoked sausages without nitrite, the second group - the same sausage containing 8 mg% NaNO₂, the third - sausage containing fourfold quantity nitrite - 32 mg%. Feeding of the rats was made only with the said fresh sausages and continued for 30 - 35 days. During this period the animals did not receive any other food. On the 30 - 35 day the rats were killed, and the oxygen need of liver and muscle tissue was established, the allcellular concentration of oxydised forms of the nicotineamide coenzymes and the activity of cytochromoxydase succinadehydrogenase, and NADH-cytochrom-C-reductase in the mitochondria of liver and thigh muscles. Also, the percentage of methaemoglobin was established.

The oxygen consumption of liver and thigh muscles was established manometrically with the Warburg apparatus by incubation of homogenate for 30 min at 37°C in media containing 120 mM of NaCl, 20 mM of tris-Cl with pH = 7.4, 3 mM of K₂HPO₄, 1 mM of MgCl₂ and of CaCl₂. The vessels contained 2.8 ml buffer and 0.2 ml 10% KOH in the side branch. The concentration of NAD⁺ and NADH⁺ was established spectrophotometrically after the method of Ciotti and Kaplan, 1956. Mitochondria were isolated after the method of Schneider

and Hageboom (1950) in 0.25 M saccharose and centrifugation at 8,500 x g. The activity of cytochrome oxidase, succinatehydrogenase and NADH-cytochrom-C reduction was determined spectrophotometrically on the bases of changes in extinction (E), measured at 550 NM following the oxydation, respectively the reduction of cytochrom C (Cooperstein and Lazarov, 1951; Goulidova and Srokina, 1967; Mahler, 1955). The reduction of the cytochrom C was made with sodium hydrosulfite. The methaemoglobin in the blood was determined after the method of Evelyn and Malloy (1938).

Results and Diskussion

After evaluation of the quantity of sausage used for feeding the experimental animals, it was established, that the rats of the second group had consumed about 10 mg nitrite to a kilogram live-weight, and those of the third group - about 40 mg. While feeding with sausages after the accepted pattern, the weight gains of the rats from the different groups was almost one and the same, with a certain tendency of an increase for the second group. For 30 days the rats from group one gained 66 g, from group two - 72 g and from group three - 65 g.

Pathologic-anatomical changes in the liver and stomach were not observed.

From table 1 is seen, that feeding with sausages, containing nitrite leads to a decrease of oxygen consumption of the liver tissue almost with 16%, which is mathematically true only for the third group, receiving an increased amount of nitrite. The oxygen consumption of muscle tissue from the animals receiving nitrite, showed a slight tendency towards decrease. The higher level of nitrite increases the concentration of nicotineamid coenzymes oxyforms (table 2) while normal quantities do not.

The activity of cytochromoxydase (table 3) in the mitochondria of the liver is increased under the influence of feeding sausages with about 30%, but mathematically this is true only for the difference between the control group and the group receiving higher nitrite quantities. In the thigh muscles, the activity of the enzyme does not change significantly.

The activity of succinate dehydrogenase (table 4) in the liver and thigh muscle mitochondria does not follow any significant change in any of the groups.

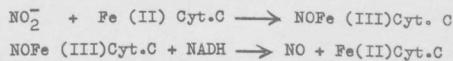
Feeding sausages containing a higher quantity of nitrite has evoked a verywell expressed increase in the activity of NADH-cytochrom-C-reductase in liver mitochondria, amounting to 32% (table 5). The activity is increased also in the second group - with 19%, but the difference is not mathematically true. In the thigh muscles the activity of this enzyme does not change.

Under the influence of the nitrite contained in the sausages fed to the animals (table 6) there is a slight tendency for increase the percentage of methaemoglobin in the blood compared to total haemoglobin.

From the obtained results, it is clearly seen, that the sodium nitrit has evoked biochemical changes only in the liver, which are mathematically true in the group experimental animals receiving a higher dose of NaNO₂. In the muscles, nitrite has not established a clear effect. This is easily explained having in mind, that after resorption these substances in the liver are detoxicated. That is why the nitrites would influence predominantly in the liver. From our results is seen, that the decreased oxygen consumption of the liver is not a result of the decreased activity of the investigated enzymes of the respiratory chain as the activity of the succinadehydrogenase is unchanged, but to the other two - cytochromoxydase and NADH-cytochrom-C-reductase, which are even increased. Even the oxygen supply of liver is not affected, as the percentage of methaemoglobin remains unchanged. We believe, that the supply of hydrogen to the respiratory chain is limited, as a great part of it shall be used for reduction of formed, under the influence of the sodium nitrite, methaemoglobin and metmyoglobin as well as for the reduction of nitrite to nitrogen oxyde. The reduction of the methaemoglobins by the different reductors in this number the nicotineamid coenzymes,

flavine coenzymes, ascorbic acid and others, which can be an enzymatic or nonenzymatic process is a well known fact (Fox, 1966; Brown and Snyder, 1969), but for the liver the expenditure of hydrogen following this pattern, has no big significance. We believe, that in it, there is a deviation of H from the respiratory chain, mainly following the second pattern - reduction of nitrite to nitrogen oxyde.

The formation of NO from nitrite with the participation of NADH is shown by Evans and McAuliffe (1956), and Fox and Ackerman (1968), while Koizumi and Brown (1971) prove that this process could be effected also with the participation of NADFH. The reduction could be enzymatic and nonenzymatic. Walter et al. (1968) propose the following scheme for reduction of nitrite by the intermediary of reduced cytochrom C and reduced NADH.



It is established also, that nitrite and NO interact with SH groups (Mirna et al., 1969, 1974) which could also reflect on the biological oxydation.

The increased concentration of oxydised forms of nicotineamid coenzymes, which we establish in feeding to rats, we explain namely with oxydation of their reduced forms by the said patterns and as a result of the higher activity of NADH-cytochrom-C-reductase.

Conclusion

Feeding rats for one month with fresh cooked and smoked sausages containing 8 and 32 % of sodium nitrite, evokes a decrease of oxygen consumption of liver and an increase of the activity of cytochromoxydase and NADH-cytochrom-C-reductase in liver mitochondria, which is mathematically true only for the groups receiving sausages with a fourfold contents of nitrite in comparison with the control. In the group is increased the content

of NAD⁺ and NADF. The activity of succinate dehydrogenase does not change under the influence of nitrite.

The thigh muscles do not exhibit any changes for the different groups of rats. The percentage of methaemoglobin in the blood does not exhibit any changes also.

The activity of the investigated mitochondrial enzymes show, that the decreased oxygen consumption of the liver is a result of an effect from some other link in the respiratory chain or there is a limit to H₂ abundance.

We believe, that a part of the hydrogen is deviated, and instead of being used in the respiratory chain it is used for the reduction of nitrate to NO. The increased content of oxydised forms of nicotinamide coenzymes we assign namely to this and the higher activity of NADH-cytochrome C-reductase.

Table 1

Oxygen consumption of liver and thigh muscles homogenates (ml/30 mg) fresh tissue					
Group	fed with sausages	no NaNO ₂	8 mg/ % NaNO ₂	32 mg/ % NaNO ₂	n
Tissue					
Liver		355 100 %	297 83,7 %	296* 83,4 %	8
Thigh muscles		81 100 %	74 91,4 %	73 90,1 %	9

* $p < 0.05$

All cellular concentration of NAD⁺ and NADP⁺ /mg/g fresh tissue

Group fed with sausages		no NaNO ₂	8 mg/ % NaNO ₂	32 mg/ % NaNO ₂	n
Tissue					
L i v e r		735 100 %	731 99,5 %	812* 111 %	8

Thigh muscles	49 100 %	45 91,8 %	45 91,8 %	9
---------------	-------------	--------------	--------------	---

* $p < 0,05$

Table 6

Group fed with sausages		no NaNO ₂	8 mg % NaNO ₂	32 mg % NaNO ₂	n
Tissue					
L i v e r		2,10 100 %	2,40 114,3 %	2,29 109,1 %	9

Percentage of methaemoglobin to total haemoglobin in the blood

Thigh muscles	363 100 %	384 106,1 %	364 100,1 %	9
---------------	--------------	----------------	----------------	---

* $p < 0,05$

Table 3

Activity of cytochromoxydase /n moles/mg mitochondrial protein					
Tissue	Group fed with sausages	no NaNO ₂	8 mg % NaNO ₂	32 mg % NaNO ₂	n
L i v e r		81 100 %	105 130 %	104* 128 %	8
Thigh muscles		93 100 %	96 103,2 %	84 90,3 %	9

* $p < 0,05$

Table 4

Activity of succinate dehydrogenase /n moles/mg mitochondrial protein				
Group fed with sausages	no NaNO ₂	8 mg % NaNO ₂	32 mg % NaNO ₂	n
Tissue				
L i v e r	29 100 %	26 89,6 %	30 103,5 %	8
Thigh muscles	19 100 %	16 84,2 %	17 89,5 %	9

Table 5

Activity of NADH-cytochrome-C-reductase /n moles/mg mitochondrial protein				
Group fed with sausages	no NaNO ₂	8 mg/ % NaNO ₂	32 mg/ % NaNO ₂	n
Tissue				
L i v e r	110 100%	131 119 %	145° 132 %	8

Literature

1. Goulidova, G., I. Sorokina, Buletin experimentalnoi biologii i medizini, 1967, 32, 41
 2. Brown, W.D., H.E.Snyder, J. Biol. Chem., 1969, 244, 6702
 3. Ciotti, M.M., N.O.Kaplan, Methods in Enzymology, 1957, 3, 891
 4. Cooperstein, S., A.Lazarov, J.Biol.Chem., 1951, 189, 665
 5. Evans, H.J., C.McAuliffe in "A Symposium on Inorganic Nitrogen Metabolism", p.1 89 ed. Mc Elroy W.D. and Glass E., The Johns Hopkins Press, Baltimore, Md. 1956
 6. Evelyn, K.H., H.J.Malloy, J.Biol. Chem., 1938, 126, 655.
 7. Fox, J.B., J. Agric. Food Chemm., 1966, 14, 207
 8. Fox, J.B., S.A. Ackerman, J. Food. Sci., 1 968, 33, 364
 9. Koizumi, O., W.D.Brown, J. Food.Sci., 1971 , 36, 1105
 10. Mahler, H.R., Methods in Enzymology, 1955, 2, 688
 11. Mirna, A., K.Hofmann, Kulmbach, Die Fleischwirtschaft, 1963, 10, 1361 and 1366
 12. Mirna, A., K. Coretti, Die Fleischwirtschaft, 1974, 3, 507
 13. Schneider, W., H. Hogeboom, J.Biol.Chem., 1950, 183, 123
 14. Walter, C.L., A.Mc Taylor, R.J.Casselden, N.Ray, British Food Manufacturing Industries Research Association, Research Report No 139, 1968
 15. Wolff, I.A., A.E.Wassermann, Science, 1972, 177, 9.