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MARIANNA SIKORSKA, WŁODZIMIERZ BICZ, MIECZYSŁAW ŚMIAŁEK
MIROSŁAW J. MOSSAKOWSKI

ATP, ADP, AMP CONCENTRATIONS IN RAT BRAIN FOLLOWING CARBON MONOXIDE INTOXICATION AND IN EXPERIMENTAL ISCHEMIA

Department of Neuropathology of the Experimental and Clinical Medical Research Centre of the Polish Academy of Sciences
Head: Prof. M. J. Mossakowski, M. D.

The phenomenon of transient glycogen accumulation and the increase of UDP glucose:glycogen glucosyltransferase (E.C. 2.4.1.11) activity in the brain was observed to accompany experimental perinatal asphyxia in Macacca mulata rhesus, experimental brain ischemia in rats (Mossakowski et al., 1968; Pronaszko-Kurczyńska et al., 1972, Śmiałek et al., 1971) as well as carbon monoxide intoxication (Śmiałek et al., 1973).

In view of the well known facts pointing to a close relationship between the adenine nucleotides system and glycogen metabolism, it seemed reasonable to devote attention to the correlation between the level of these nucleotides and the phenomenon of glycogen accumulation in the central nervous system after hypoxia.

The fluctuations in ATP, ADP and AMP levels in the condition of brain hypoxia and ischemia may be one of the factors responsible for the changes in the glycogen metabolism (Broniszewska-Ardelt, Jongkind, 1971; Kirsch, Leitner, 1967; Lowry et al., 1964). Gatfield et al. (1966) demonstrated a decrease of the ATP level in some of the mouse brain structures under the influence of anesthetic drugs and ischemia. Brosnan et al. (1970) observed a decrease of the ATP level, with a concomitant rise of ADP and AMP concentrations in rat liver during ischemia. The effects of a number of anesthetics, hyperthermia and ischemia on the adenine nucleotides level in mouse brain were observed and discussed by Goldberg et al. (1966).

The aim of the present work was to compare the possible changes in ATP, ADP and AMP levels caused by carbon monoxide intoxication and in an experimental ischemic model.

MATERIAL AND METHODS

In the experiments concerning carbon monoxide intoxication, 60 Wistar rats of both sexes aged 6 weeks were used. The animals were placed in a 60 — 1 chamber for 90 min. Air (1 l/min) with an admixture of CO was passed through the chamber continously. During the first 30 min the air contained 1% CO.

At that time the blood carboxyhemoglobin level, as determined by the method of Whithead and Worthington (1961) reached the value of 76.6 ± 0.9%. The blood HbCO level was maintained by CO inflow regulation up to the 60th min. Then it was interrupted and the rats were kept in the chamber with a continuous flow of air reducing CO concentration to the 90th min so that the blood HbCO level decreased to about 50% at the end of the experiment.

The animals were examined after 20, 30, 60 and 90 minutes stay in the chamber and after 1, 2, 4, 6 and 24 hrs they were transferred to normal conditions. The control group consisted of rats maintained in a normal atmosphere.

In experimental ischemia, 120 Wistar rats of both sexes, aged 4 weeks were used. The animals of the experimental groups were subjected to bilateral ligation of the carotid arteries under ether anesthesia. Besides, three control groups were used: a) animals not subjected to any treatment, b) animals under the same ether anesthesia as the operated rats and c) animals anesthetized and then subjected to operation, consisting in isolation of the common carotid arteries without ligation. The animals were examined directly after the experimental or control treatments and 1, 6, 24, 48 and 72 hrs later.

In both experimental models, the rats were sacrificed by decapitation and the heads were immediately frozen in liquid nitrogen.

Sample of the frozen brain hemispheres were then homogenized with 3 ml of cold 1.5 M PCA. The homogenate was centrifuged for 30 min at 15 000 rpm at 2°C. The supernatant was neutralized with 2 M potassium carbonate solution containing 0.5 M triethanolamine hydrochloride and thereafter supplemented with 0.1 M triethanolamine buffer (pH 7.5) to a final volume of 6 ml. The mixture was kept in ice for 10 min and centrifuged again for 30 min at 15 000 rpm at +2°C. ATP, ADP and AMP were assayed in the supernatant by the method of Adams (1963). Extinction at 340 nm was monitored on a Spectromom 202 spectrophotometer.

RESULTS

In the brains of 6-week rats, which served as the control group for the CO intoxication model, the respective mean concentrations of adenine

nucleotides were found to be as follows: ATP — 6.91 ± 0.16, ADP — 1.96 ± 0.07 and AMP — 0.32 ± 0.04 μmoles/g fresh tissue.

In the group of animals subjected to the action of CO for 20 minutes, the mean ATP concentration in the brain was 5.58 ± 0.50 μmoles/g fresh tissue. As compared with the control group, a statistically significant decrease of ATP concentration was noted ($p \leqslant 0.05$), which parallelled the statistically insignificant rise in the ADP (2.06 ± 0.19 μmoles/g fresh tissue) and AMP (0.41 ± 0.03 μmoles/g fresh tissue).

After 30 minutes' exposure to CO, the brain concentrations of all the nucleotides approached the control values and were found to be: ATP — 6.68 ± 0.47, ADP — 1.90 ± 0.06 and AMP — 0.36 ± 0.05 μmoles/g fresh tissue.

From the 60th minute on, a statistically significant increase of ATP concentration could be observed (7.99 ± 0.28 μmoles/g fresh tissue, $p \leqslant 0.01$).). At that time, the concentrations of ADP and AMP were insignificantly lower than in the control brains and amounted to 1.75 ± 0.04 and 0.30 ± 0.03 μmoles/g fresh tissue respectively.

In the two following experimental groups — kept in the chamber for 90 minutes and those which after 90 minutes exposure were left for 1 hr in a normal atmosphere, the ATP concentration remained at the level determined after 60 min exposure to CO. The concentration of ADP did not differ from normal, whereas the AMP level increased slightly but statistically insignificantly.

In the group of rats examined 2 hrs after removal from the chamber the mean ATP concentration in the brain was the highest as compared with that in all other groups amounting to 8.87 ± 0.37; the increase was statistically significant in relation to the control ($p \leqslant 0.001$). The ADP concentration in this group was 1.98 ± 0.08 and that of AMP 0.38 ± 0.04 μmoles/g fresh tissue.

The highest AMP level (0.49 ± 0.05 μmoles/g fresh tissue) was found in the group which after exposure to CO remained for 4 hrs outside the chamber. This increase was statistically significant in relation to the control ($p \leqslant 0.05$). The mean ATP concentration in this group 7.81 ± 0.10 μmoles/g fresh tissue was lower than in the group of rats examined 2 hrs after exposure to CO, but still higher than in the control group ($p \leqslant 0.01$). The ADP level in this group did not show a statistically significant deviation as compared with the control.

In the rats examined 6 hrs after they were taken out of the chamber the ATP level was slightly, but statistically insignificantly higher than in the control animals (7.23 ± 0.16). At that time mean concentrations of ADP and AMP also approximated the control values and were respectively: 1.81 ± 0.04 and 0.34 ± 0.03 μmoles/g fresh tissue.

Table 1. ATP, ADP, AMP concentrations in rat brain following carbon monoxide intoxication /(μmoles/g fresh tissue)

Tabela 1. Stężenie ATP, ADP, AMP w mózgu szczura po zatruciu tlenkiem węgla (μmole/g świeżej tkanki)

Experimental conditions Warunki doświadczenia			ATP		ADP		AMP		Number of animals Liczba zwierząt	$\frac{[ATP]\,[AMP]}{[ADP]^2\,[K]}$
A	B	C	$\bar{x}\pm m$*)	p**)	$\bar{x}\pm m$	p	$\bar{x}\pm m$	p		
Control min	Kontrola min	hrs godz.	6.91±0.16		1.96±0.07		0.32±0.04		8	0.55
20			5.58±0.50	≤0.05	2.06±0.19	≥0.05	0.41±0.03	≥0.05	8	0.54
30			6.68±0.47	≥0.05	1.90±0.06	≥0.05	0.36±0.05	≥0.05	6	0.66
60			7.99±0.28	≤0.01	1.75±0.04	≥0.05	0.30±0.03	≥0.05	6	0.79
60	30		7.94±0.56	≥0.05	1.86±0.09	≥0.05	0.34±0.03	≥0.05	6	0.78
60	30	1	7.55±0.37	≥0.05	2.05±0.16	≥0.05	0.41±0.05	≥0.05	6	0.74
60	30	2	8.87±0.37	≤0.001	1.98±0.08	≥0.05	0.38±0.04	≥0.05	8	0.86
60	30	4	7.81±0.10	≤0.01	1.73±0.06	≥0.05	0.49±0.05	≤0.05	5	1.27
60	30	6	7.23±0.16	≥0.05	1.81±0.04	≥0.05	0.34±0.03	≥0.05	6	0.75
60	30	24	6.95±0.12	≥0.05	1.94±0.03	≥0.05	0.36±0.02	≥0.05	5	0.66

A — time of exposure to CO
czas ekspozycji CO

B — time after cessation of CO
czas po wyłączeniu CO

C — time of survival after exposure to CO
czas przeżycia zwierząt po ekspozycji CO

*) $\bar{x}\pm m$ arithmetic mean ± mean error of the mean
średnia artmetyczna ± średni błąd średniej

**) p probability
prawdopodobieństwo

In the group of animals examined 24 hrs after cessation of exposure to CO, the mean concentrations of all the adenine nucleotides did not differ from the values obtained for the control group and were: ATP — 6.95 ± 0.12, ADP — 1.94 ± 0.03 and AMP — 0.36 ± 0.02 μmoles/g fresh tissue.

On the basis of the obtained values an equilibrium constant was calculated expressed by the following formula:

$$K = \frac{(ATP)\ (AMP)}{(ADP)^2}$$

The K value for the control group was 0.55. After a 20 min exposure to CO the K value remained practically unchanged (0.54). From the 60th minute on, up to 4 hrs after cessation of exposure to CO, the K value increased up to 1.27. In the group examined 6 hrs after the animals were taken out of the chamber, K was already much lower and amounted to 0.75 and after 24 hrs it reached the value of 0.66, this approximating the values obtained for the control group.

In the group of rats aged 4-weeks, which were not subjected to any operation and formed the control group in the model of experimental ischemia, the mean concentrations of ATP, ADP and AMP in the brain were: 6.34 ± 0.39, 1.88 ± 0.06 and 0.36 ± 0.04 μmoles/g fresh tissue respectively. Anesthesia itself was found to increase the ATP level in the brain. This was most pronounced after 1 hr (8.80 ± 0.41 μmoles/g fresh tissue). The effect of anesthesia on the ATP brain level disappeared within 24 hrs. The control operation did not produce significant changes in the adenine nucleotides levels as compared with normal values. In the time intervals examined the brain ATP level in rats of this group remained within the range of 6.32 ± 0.30 — 7.35 ± 0.48 μmoles/g fresh tissue, thus none of the values showed a statistically significant difference as compared with normal and with the group subjected to anesthesia.

As to the experimental groups which comprised the animals examined 1, 6, 12, 24, 48 and 72 hrs from the moment of ligation, a marked tendency of ATP and ADP to decrease and of AMP to increase could be observed only after 6 hrs. These changes were very pronounced in 50% of cases. The mean brain ATP concentration in this group was 4.70 ± 0.74, that of ADP 1.60 ± 0.1 and of AMP 0.53 ± 0.07 μmoles/g fresh tissue. In the remaining animals, the nucleotides concentration was found to approximate the values in the control group. The significant dispersions between the results for individual animals in all these groups do not allow a statistical analysis of these observations.

DISCUSSION

The model of carbon monoxide intoxication applied in these studies did not differ from that used in the previous work (Śmiałek et al., 1973). The degree of intoxication of the organism was controlled by the HbCO concentration in blood and the cytochrome oxidase activity of the crude brain mitochondrial fraction. Maximum inhibition (23%) of the enzyme activity was noted at the moment of transfer of the animals from the chamber to normal air.

The action of CO on the rats during the first 20 min resulted in a decrease of ATP concentration in the brain. A similar decrease of the level of high-energy compounds and among others ATP, both in brain and in other tissues, was observed to occur in the conditions of hypoxia (Gatfield et al., 1966; Brosnan et al., 1970). Comparison of the $K = \frac{(ATP)\ (AMP)}{(ADP)^2}$ coefficient for the control group and that of animals examined after 20-min exposure to CO indicates that the observed decreases of ATP concentration and the shifts in the concentrations of AMP and ADP proceeded within the system controlled by the adenyl kinase (Brosnan (et. al., 1970). In the subsequent time intervals, that is from the 60th minute of CO action up to 2 hrs removal of the rats from the chamber, the ATP concentration increased, whereas those of ADP and AMP remained within the limits of the control group. The increased K value found at that time suggests, that in this case the changes in ATP concentration may have resulted not only from shifts within the system controlled by adenyl kinase, but also from disturbances of equilibrium within other metabolic pathways in the brain which involve ATP. This increase of ATP level may be caused among others factors by a reduced utilization of this nucleotide in protein and nucleic acid synthesis (Albrecht, 1972, 1973) or in the synthesis of fatty acids (Strosznajder et al., 1972; Łazarewicz et al., 1972).

In our further studies on the carbon monoxide intoxication model we noticed that the highest ATP level which became apparent 2 hrs after the rats had been taken out of the chamber, coincides in time which the maximum increase of UDP-glucose: glycogen glucosyltransferase activity, but preceedes the highest increment of the glycogen level taking place two hours later. These facts suggest, that the changes in the glycogen level in rat brains following hypoxia may be causally related to the changes in ATP concentration. At present it is difficult to judge which enzymatic step of glycogen biosynthesis is stimulated by higher ATP concentration. It is probable that the reaction of the G-6-P-independent I form of glycogen synthetase in involved in this case. On the other hand, the possibility of phosphofructokinase inhibition which might cause

a shift of the concentration of the glycogen pathway metabolites in the direction facilitating the glycogen biosynthesis has to be taken into account. Another possibility is the activation of the phosphorylase involved in glycogen metabolism by increased AMP concentration, which could readily be observed in the 4th hr after the animals were removed from the chamber. The results obtained by Breckenridge et al. (1965) support this inference. When comparing the results in the two applied different models of central nervous system hypoxia, it is to be noted, that the dynamics of the transient glycogen accumulation, increase of the UDPglucose:glycogen glucosyltransferase activity, as well as of changes in ATP level are different in these two models.

Carbon monoxide causes a reduction of oxygen transport into brain of rats which is due to the block of hemoglobin. Damage to the respiratory chain through inhibition of the cytochrome oxidase activity strongly related to the O_2/CO molecular concentration ratio (Chance et al., 1970), cannot be excluded. Insofar, there are not enough data available to distinguish the molecular mechanism in both models.

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М. Сикорска, М. Смялек, В. Бич, М. Я. Моссаковски

ИЗМЕНЕНИЯ КОНЦЕНТРАЦИЙ АДЕНИНОВЫХ НУКЛЕОТИДОВ АТФ, АДФ И АМФ В МОЗГЕ КРЫС ПОСЛЕ ОТРАВЛЕНИЯ СО И ПРИ ЭКСПЕРИМЕНТАЛЬНОЙ ИШЕМИИ

Резюме

Целью работы было исследование уровня адениновых нуклеотидов АТФ, АДФ и АМФ в мозге крыс при отравлении СО и при эксперимнетальной ишемии, вызванной двусторонней перевязкой общих сонных артерий. В модели с окисью углерода было использовано 60 крыс линии Вистар обоих полов в возрасте 6 недель. Животные исследовались через 20, 30, 60 и 90 мин. экспозиции в СО, и далее через 1, 2, 4, 6 и 24 часа после выхода животных из экспериментальной камеры.

В модели экспериментальной ишемии исследования проводились на 120 крысах линии Вистор, обоих полов в возрасте 4 недель. Уровень нуклеотидов исследовался через 1, 6, 24, 48 и 72 часа с момента перевязки артерий. Уровень нуклеотидов определялся по методу Адама (1963).

В модели с окисью углерода было обнаружено через 20 мин после воздействия СО статистически значимое падение уровня АТФ и далее постепенный его рост, наиболее выраженный через 2 часа. По истечении 24 часов уровень АТФ не отличался от нормы. Уровень АДФ не изменялся ни в одном из временных интервалов, — а в случае АМФ через 4 часа было обнаружено статистически значимое увеличение уровня этого нуклеитида.

Изменения уровня адениновых нуклеотидов не контролировались системой аденилат-киназы, поскольку величина К изменялась в реакции, контролируемой этим ферментом.

В модели экспериментальной ишемии отмечалось в 50% исследуемых случаев и только в группе животных, исследуемых через 6 часов с момента перевязки артерии, падение уровня АТФ и АДФ с одновременным ростом уровня АМФ.

M. Sikorska, W. Bicz, M Śmiałek, M. J. Mossakowski

STĘŻENIA ATP, ADP I AMP W MÓZGACH SZCZURÓW PO ZATRUCIU TLENKIEM WĘGLA I W DOŚWIADCZALNEJ ISCHEMII

Streszczenie

Celem pracy było przebadanie poziomu ATP, ADP i AMP w mózgach szczurów po zatruciu CO i w doświadczalnej ischemii wywołanej obustronnym podwiązaniem tętnic szyjnych wspólnych.

W modelu tlenkowęglowym szczury rasy Wistar badano po 20, 30, 60 i 90 min. ekspozycji CO, a następnie po 1, 2, 4, 6 i 24 godz. od momentu wyjęcia zwierząt z komory doświadczalnej.

W modelu doświadczalnej ischemii poziom nukleotydów w mózgach szczurów rasy Wistar badano po upływie 1, 6, 24, 48 i 72 godz. od momentu podwiązania tętnic. Ilościowe oznaczanie stężeń nukleotydów wykonywano metodami enzymatycznymi wg Adama (1963).

W modelu tlenkowęglowym stwierdzono statystycznie znamienny spadek poziomu ATP po 20 min działania CO, a następnie stopniowy jego wzrost, najwyraźniej zaznaczony po 2 godz. Po upływie 24 godz. poziom ATP nie różnił się od normy. Poziom ADP nie ulegał zmianie we wszystkich badanych czasach, natomiast w przypadku AMP stwierdzono statystycznie znamienny przyrost poziomu nukleotydu tylko po 4 godz. Wartość stałej równowagi (K) dla reakcji kontrolowanej przez układ kinazy adenylowej ulegała zmianie, co może wskazywać, że zmiany w poziomie nukleotydów adeninowych były kontrolowane nie tylko przez system tego układu enzymatycznego.

W modelu doświadczalnej ischemii jedynie w grupie zwierząt badanych po 6 godz. od momentu podwiązania tętnic w 50% badanych przypadków stwierdzono spadek poziomu ATP i ADP z równoczesnym wzrostem poziomu AMP.

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Authors adress: Department of Neuropathology, Experimental and Clinical Medical Research Centre, 00-784 Warszawa, 3 Dworkowa str.