# REGULATION OF OXIDATIVE PHOSPHORYLATION AS A POSSIBLE MEANS OF NORMALIZING BRAIN METABOLISM

V. I. Kresyun

UDC 616.45-001.1/.3:615.23

KEY WORDS: stress; psychotropic drugs; oxidative phosphorylation; brain.

Emotional-painful stress (EPS), like other systemic changes, causes a sharp disturbance of brain metabolism and, in particular, energy metabolism [4, 10]. In the stage of excess catabolism (or stage of exhaustion) during stress oxidative phosphorylation is inhibited and this leads to a disturbance of synthesis of high-energy compounds and to a decrease in their concentration in the brain [6, 7]. Psychotropic drugs are today the principal pharmacotherapeutic agents for regulating the course of stress reactions. Meanwhile there are other widely used drugs, in particular those of the benzodiazepine series, which themselves inhibit oxidative phosphorylation [5, 11, 12]. Accordingly, there is now an urgent need to find new therapeutic agents with a stress-protective action, through normalization of brain metabolism, and to study their pharmacology.

The object of this investigation was to study the effect of various metabolites created on the basis of nicotinic acid and GABA on oxidative phosphorylation processes in various brain structures of animals during chronic stress and its correction by drugs.

## EXPERIMENTAL METHOD

Experiments were carried out on 410 male albino Wistar rats weighing 200-220 g, kept on the standard animal house diet. After preliminary testing, highly emotional animals were chosen for the experiment [3]. Chronic EPS was produced in the form of an anxiety neurosis [13], in the writer's modification, consisting of prolonged (12 days, 2 h daily) exposure of the starved animals (deprived of food for 12 h before the experiment) to the stress-producing agent, and also arbitrary alternation of a conflict situation with immobilization and electrodermal stimulation. In this way reliable and stable changes in brain metabolism could be obtained, corresponding to the level of transition of the stage of stress from compensation to decompensation or excess catabolism, against the background of which the action of the drugs could be clearly distinguished. As protectors against stress, derivatives of natural metabolites (fenibut* and mebicar†) and also nicotinic acid derivatives created in the writer's laboratory (nicogamol and litonit), were used. For comparison the benzodiazepine derivative chlordiazepoxide and the propandiol derivative meprobamate were used. A course of intraperitoneal injections of the drugs in mean therapeutic doses, determined by anxiolytic tests (activity in a conflict situation, effect on external inhibition, nonreinforcement of actions, and the orienting reflex), was given. Physiological saline was given as the control. The intensity of tissue respiration and of oxidative phosphorylation was determined manometrically [15]. Inorganic phosphorus in the samples was determined by the method in [14]. The quantity of assimilated oxygen ($\Delta O$) and of bound inorganic phosphorus ($\Delta P$) was expressed in microatoms ($\mu at$) and calculated per 100 mg tissue for the incubation time. The ratio P/O, reflecting the degree of coupling of oxidation and phosphorylation, was calculated from the values of $\Delta O$ and $\Delta P$ obtained. The results were analyzed statistically by Minsk-22m computer.

*β-Phenyl-γ-aminobutyric acid.
†2,3,6,8-Tetramethyl-2,4,6,8-tetra-azobicyclo-(3,3,0)-octadione-3,7.

Department of Pharmacology, N. I. Pirogov Odessa Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 96, No. 12, pp. 37-40, December, 1983. Original article submitted June 28, 1983.

 0007-4888/83/9612-1692$07.50 

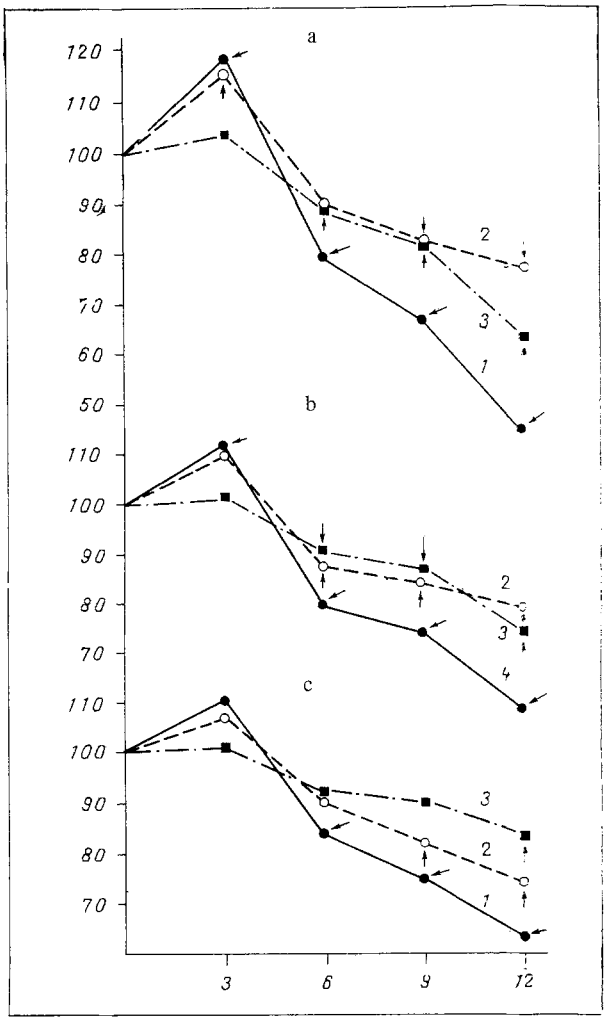


Fig. 1. Dynamics of changes in tissue respiration, oxidative phosphorylation, and their coupling in the brain at different times of development of chronic EPS. Abscissa, duration of stress (in days); ordinate, changes (in %). 1) Phosphorylation; 2) respiration; 3) P/O. a) Cortex, b) limbic region, c) medulla. Arrows indicate statistically significant differences at $P < 0.05$ level.

## EXPERIMENTAL RESULTS

Chronic EPS induced by this method causes marked changes in oxidative phosphorylation of the brain (Fig. 1). Whereas in the early stages (up to 3 days) activation of oxidative phosphorylation was observed, later it was inhibited, and inhibition was most marked by the 12th day of development of stress (more than doubled). Depression of respiration and phosphorylation, and also their uncoupling, affected the cortex first, then the structure of the limbic system (in these experiments, the hippocampus, hypothalamus, amygdala, and septal region, and to a lesser degree, the medulla). Similar changes were obtained previously for concentrations of high-energy compounds (ATP, ADP, AMP). Phosphorylation was inhibited most of all by stress. For example, by the 12th day in the cortex $\Delta P$ was $9.24 \pm 0.32$ μat/100 mg tissue compared with $20.37 \pm 0.54$ μat in the control ($P < 0.001$; the corresponding values of $\Delta O$ were $8.41 \pm 0.24$ μat compared with $10.57 \pm 0.27$ μat in the control ($P < 0.01$). The ratio P/O also fell from $1.91 \pm 0.03$ to $1.20 \pm 0.2$ ($P < 0.001$). Although oxidative phosphorylation was inhibited primarily in the cortex and limbic region, these changes in the medulla, while less marked, were nevertheless significant and stable. This is evidence that stress universally depresses tissue respiration and phosphorylation processes in the brain and uncouples them. Inhibition of the key reactions of energy production for the brain,

TABLE 1. Changes in Oxidative Phosphorylation (in μat/100 mg tissue/h) of Brain Structures of Intact and Stressed Animals during a Course of Tranquilizers (M ± m)

| Experimental conditions | | Intact animals receiving 12-day course of tranquilizers | | | Chronic stress in animals receiving 12-day course of tranquilizers | | |
|---|---|---|---|---|---|---|---|
| | | ΔP | ΔO | P/O | ΔP | ΔO | P/O |
| **Cortex** | | | | | | | |
| Control | | 20,37±0,54 | 10,57±0,27 | 1,91±0,03 | 20,37±0,54 | 10,57±0,27 | 1,91±0,3 |
| Stress | | — | — | — | 9,24±0,65* | 8,41±0,41* | 1,20±0,3* |
| Nicogamol | (10) | 29,04±0,90* | 14,87±0,38* | 1,95±0,2 | 20,11±0,39† | 11,56±0,15† | 1,74±0,02† |
| Litonit | (10) | 29,95±0,75* | 15,15±0,33* | 1,98±0,01 | 21,81±1,34† | 12,02±0,53† | 1,80±0,3† |
| Fenibut | (10) | 25,82±0,73* | 13,88±0,33* | 1,86±0,01 | 18,16±1,08† | 11,01±0,54† | 1,65±0,03† |
| Mebicar | (30) | 13,50±0,46* | 7,99±0,29* | 1,69±0,1* | 14,03±1,17† | 11,23±0,70† | 1,25±0,03 |
| Meprobamate | (20) | 11,30±0,52* | 7,50±0,42* | 1,51±0,02* | 11,68±0,63† | 10,12±0,47† | 1,15±0,3 |
| Chlordiazepoxide | (2) | 8,39±0,30* | 6,52±0,26* | 1,29±0,02* | 9,90±0,45 | 9,75±0,34† | 1,01±0,2† |
| **Limbic region** | | | | | | | |
| Control | | 19,92±1,15 | 10,79±0,73 | 1,85±0,026 | 19,92±1,15 | 10,79±0,73 | 1,85±0,03 |
| Stress | | — | — | — | 11,29±0,38* | 8,13±0,32* | 1,39±0,2* |
| Nicogamol | (10) | 26,02±0,66* | 13,70±0,36* | 1,90±0,02 | 20,68±0,81† | 11,17±0,42† | 1,85±0,01† |
| Litonit | (10) | 26,77±0,72* | 14,30±0,32* | 1,87±0,01 | 23,65±1,14† | 12,45±0,52† | 1,90±0,03† |
| Fenibut | (10) | 24,85±0,71* | 13,15±0,29* | 1,89±0,02 | 19,90±1,22† | 11,41±0,48† | 1,74±0,02† |
| Mebicar | (30) | 13,93±0,44* | 8,21±0,29* | 1,70±0,01* | 15,17±0,90† | 10,87±0,57† | 1,39±0,02 |
| Meprobamate | (20) | 11,94±0,69* | 7,81±0,48* | 1,53±0,01* | 13,24±0,85 | 10,49±0,61† | 1,26±0,04 |
| Chlordiazepoxide | (2) | 8,36±0,35* | 6,40±0,34* | 1,31±0,02* | 11,20±0,23 | 9,50±0,16† | 1,18±0,03† |
| **Medulla** | | | | | | | |
| Control | | 20,96±1,33 | 11,84±0,84 | 1,78±0,04 | 20,96±1,33 | 11,84±0,84 | 1,78±0,04 |
| Stress | | — | — | — | 12,41±1,06* | 8,32±0,55* | 1,48±0,33* |
| Nicogamol | (10) | 23,00±0,65 | 12,30±0,40 | 1,87±0,01 | 21,72±1,57† | 12,49±1,05† | 1,74±0,02† |
| Litonit | (10) | 25,22±0,70* | 12,28±0,42 | 1,90±0,01* | 22,68±1,39† | 12,73±0,60† | 1,78±0,03† |
| Fenibut | (10) | 23,82±0,48 | 12,97±0,37 | 1,84±0,02 | 22,45±1,59† | 13,20±0,92† | 1,70±0,01† |
| Mebicar | (30) | 14,02±0,43* | 8,11±0,26* | 1,73±0,02 | 16,38±0,23† | 11,40±0,55† | 1,44±0,02 |
| Meprobamate | (20) | 11,52±0,59* | 7,45±0,46* | 1,55±0,02* | 15,75±0,49† | 11,25±0,52† | 1,40±0,03 |
| Chlordiazepoxide | (2) | 8,78±0,31* | 6,37±0,25* | 1,38±0,01* | 12,23—0,30 | 9,14—0,26 | 1,34—0,04† |

Legend. In all experiments number of observations 9-10, in control 15-20; doses (in mg/kg) given in parentheses. *$P < 0.05$ compared with control, †$P < 0.05$ compared with stress.

namely oxidative phosphorylation, seriously upsets coordination of energy metabolism and leads to metabolic disadaptation. The results are in agreement with data in the literature [1, 2] on the profound pathological changes in tissues in a state of transition from stress to decompensation or exhaustion. Under these conditions the only correct course is not by aggravating metabolic disturbances by administration of foreign therapeutic substances, which themselves inhibit oxidative phosphorylation, but to optimize them. Recently investigations indicating the beneficial effect of sodium hydroxybutyrate in stress have been published [8, 9]. Yet sodium hydroxybutyrate, despite its marked protective action in stress, has a powerful depressant effect accompanied by muscle relaxation, disturbance of movement coordination, and so on, which limits its application, especially under outpatient conditions.

The study of oxidative phosphorylation in intact animals during a course (2 weeks) of the drug chosen for study revealed distinct differences in their action. Nicotinic acid and GABA derivatives, nicogamol, litonit, and fenibut, stimulated tissue respiration and phosphorylation, whereas mebicar, meprobamate, and chlordiazepoxide inhibited them and, at the same time, uncoupled them (Table 1). In the strength of their stimulating action the drugs were arranged in the following order: nicotinic acid derivatives nicogamol and litonit, then the GABA derivative fenibut. Whereas their stimulating effect in the cortex and limbic region was significant, in the medulla there was merely a tendency toward stimulation of oxidative phosphorylation. The inhibitory action of chlordiazepoxide and, to a lesser degree, of meprobamate, and less still, of mebicar was exhibited significantly in all structures. Chronic stress, superposed on a course of injections of these psychotropic drugs, caused less marked changes in oxidative phosphorylation and its uncoupling (Table 1). Under these conditions the action of all the drugs tested except chlordiazepoxide was in the same direction. Chlordiazepoxide potentiated the effect of stress on oxidative phosphorylation. As regards the strength of their protective action against stress, these drugs differed. Whereas nicogamol, litonit, and fenibut restored tissue respiration and oxidative phosphorylation virtually to their initial values, and sometimes even higher, and normalized the P/O ratio, mebicar and

meprobamate, while stimulating oxidative phosphorylation, by no means returned it to its initial level and, most important of all, did not couple these processes (the P/O ratio remained low and virtually indistinguishable from that during stress). Injection of chlordiazepoxide into animals under stress caused even stronger inhibition of phosphorylation and, in particular, of respiration compared with stress; the value of P/O was reduced. These changes were more marked in the cortex and limbic region. Even though in the medulla a tendency was observed for oxidative phosphorylation to be inhibited under the influence of chlordiazepoxide, the P/O ratio fell significantly to 1.34 ± 0.04 compared with 1.48 ± 0.03 during stress ($P < 0.01$) and 1.78 ± 0.4 in the control.

The results show that nicotinic acid and GABA derivatives restore brain energy metabolism to normal by stimulating oxidative phosphorylation when inhibited by chronic stress. The action of mebicar and meprobamate, while in the same direction, is much weaker. Chlordiazepoxide, on the other hand, potentiates the effect of stress on tissue respiration and oxidative phosphorylation. Its protective action against stress is evidently realized at the level of synaptic transmission, and not at the level of normalization of oxidative phosphorylation of brain neurons.

## LITERATURE CITED


1. A. A. Ananenko, V. I. Gereshkovich, A. M. Timofeeva, et al., Vopr. Med. Khim., No. 4, 361 (1966).
2. R. N. Akhmerov and I. R. Saakyan, in: Biophysics of the Living Cell [in Russian], Pushchino (1973), No. 4, p. 51.
3. N. A. Bondarenko, A. V. Val'dman, and V. A. Kamysheva, Byull. Éksp. Biol. Med., No. 7, 35 (1981).
4. A. V. Val'dman, M. M. Kozlovskaya, and O. S. Medvedev, Pharmacologic Regulation of Emotional Stress [in Russian], Moscow (1979).
5. A. M. Zubovskaya and R. U. Ostrovskaya, Byull. Éksp. Biol. Med., No. 6, 41 (1972).
6. V. I. Kresyun, in: Pharmacology of γ-Aminobutyric Acid Derivatives [in Russian], Tartu (1983), p. 74.
7. V. I. Kresyun, in: Biochemistry and Medicine [in Russian], Odessa (1981), p. 102.
8. F. Z. Meerson, R. I. Lifshits, V. I. Pavlova, et al., Dokl. Akad. Nauk SSSR, 239, 743 (1978).
9. F. Z. Meerson, L. M. Giber, G. I. Markovskaya, et al., Dokl. Akad. Nauk SSSR, 237, 1230 (1977).
10. K. I. Pogodaev, in: Current Problems in Stress [in Russian], Kishinev (1976), p. 211.
11. Z. I. Savchenko, in: Problems in Psychoneurology [in Russian], Moscow (1966), p. 311.
12. S. Cotev and M. N. Shalit, Anesthesiology, 43, 117 (1975).
13. O. Desiderato, J. R. MacKinen, and H. Hisson, J. Comp. Physiol. Psychol., 87, 208 (1974).
14. O. H. Lowry and J. A. Lopez, J. Biol. Chem., 162, 421 (1964).
15. W. W. Umbreit, R. H. Buris and J. F. Stauffer, Manometric Techniques in Biochemistry, Minneapolis (1972).