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EFFECT OF SINUSOIDAL MODULATED CURRENTS AND ACUTE
HYPOXIA ON CORTICOSTERONE CONTENT AND ACTIVITY OF
CERTAIN DEHYDROGENASES IN TISSUES OF DIFFERENT
RAT ORGANS DURING HYPOKINESIA

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By

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A lot of attention has been given to the problem of hypokinesia since re-
stricted motor activity, reduction in energy outlays are accompanied by disorders
in the physiological functions of the organism and the metabolic processes, which
leads to the development of different diseases (L. I. Kakurin, Z. I. Barbashova
et al., V.V. Parin, et al.).

/59**

In order to reduce the pathological disorders developing during lengthy
hypokinesia different physical factors can be used, including sinusoidal modulated
currents (SMC) that favorably affect the neuromuscular apparatus, circulation and
trophism of the tissues (V. G. Yasnogorodskiy, 1969, 1973). However, the mechanisms
for the effect of SMC during hypokinesia have almost not been studied, which limits
their purposeful use during lengthy hypodynamia. In particular, the effect of
SMC on the glucocorticoid function of the adrenal cortex and on the processes of
tissue respiration linked to hormonal regulation have not been studied; the
decrease in the intensity of tissue respiration processes plays an important role
in the pathogenesis of different disorders in hypokinesia (Ye. A. Kovalenko).

/60

We studied the corticosterone content and activity of a number of enzymes of
oxidizing metabolism--pyruvate-dehydrogenases and Krebs cycle dehydrogenase--in the
tissues of different organs during 30-day hypokinesia and the effect of SMC, as well as

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** Numbers in margin indicate pagination in original foreign text.

investigated the resistance of animals in the state of hypokinesia exposed to the effect of SMC to the extreme factor, acute hypoxia, with regard for the change in the corticosterone level and activity of the indicated enzymes.

The work was done on 115 male rats weighing 140-160 g, separated into 2 groups. The first group had 62 rats, 44 in the state of hypokinesia (25 of them were exposed to the effect of SMC) and 18 intact (control), the second--53 rats--36 in the state of hypokinesia (14 of them were exposed to the effect of SMC and 17 intact). All the animals of the second group at the end of the 30-day period of the experiment were exposed to the effect of acute hypoxia.

The state of hypokinesia in the rats was reproduced by keeping them for 30 days in special box cages that restricted the mobility of the animals in all directions. The effect of SMC began from the 15th day of hypokinesia with the help of the apparatus "Amplipul's-3" with carrier frequency of the current 5000 Hz, modulation frequency 30 Hz, depth 100%, current strength 2-3 mA (type of work-second). Electrodes 3 x 3 cm in size were placed on the region of both femurs with preliminarily cut fur. Procedures lasting 20 min. were carried out daily for 15 days, twice a day. The conditions of acute hypoxic (hyperbaric) hypoxia were reproduced in a vacuum chamber with the help of a Komovskiy pump during "elevation" to an "altitude" of 11,000 m (170 mm Hg) for 3-4 min. Here the reserve time (until the appearance of convulsions and agonized respiration) and the total duration of the life of the animals were recorded.

During the experiment the rats were weighed and their rectal temperature was measured with a TPEM-1 electric thermometer. In the adrenal glands, blood plasma, liver, heart and skeletal muscle the corticosterone content was determined by the fluorometric method (V. V. Davydov, Moor et al.) on a fluorometer of the firm "Hitachi" (Japan). In the liver and brain the activity of the pyruvate-dehydrogenase (PDG) and Krebs cycle enzymes: α -ketoglutarate-dehydrogenase (α -KDG), succinate-dehydrogenase (SDG) and malate-dehydrogenase (MDG) were determined by the method of Kun and Abood.

The study results demonstrated that under the influence of 30-day hypokinesia in rats the corticosterone content was significantly reduced in the blood plasma and tissues of all the studied organs (table 1), whereupon in the adrenal glands

the concentration of hormone was reduced by 32.6%, in the blood by 28.0%, in the liver, heart and skeletal muscle--by 39.5, 38.1 and 39.2% respectively. These hormonal shifts were combined with a reduction in the activity of PDG and SDG in the liver (respectively by 20.1 and 13.5%). In the brain the activity of α -KDG was reduced by 20.6% (table 2).

During the effect on the hypokinetic rats of SMC in them as compared to the control group there was a considerable increase in the corticosterone concentration in the adrenal glands (by 124.4%) and almost 2-fold increase in the hormone content in the blood, liver, heart and skeletal muscle (see table 1). In addition to this the activity of the studied dehydrogenases in the liver and brain was increased to normal (or even above normal) (see table 2).

TABLE 1. CONTENT OF CORTICOSTERONE (in $\mu\text{g}\%$) IN TISSUES OF RATS OF DIFFERENT GROUPS (M+m)

Group of rats	Adrenals	Blood plasma	Liver	Heart	Skeletal muscles
Intact	2712,6 \pm 106,1	17,5 \pm 0,44	51,7 \pm 1,54	44,3 \pm 1,50	35,9 \pm 1,81
Hypokinetic	1829,3 \pm 132,2	12,6 \pm 1,20	31,3 \pm 1,86	27,4 \pm 0,87	21,8 \pm 0,84
	<0,001	<0,01	<0,001	<0,001	<0,001
Hypokinetic + SMC	4050,1 \pm 145,3	24,4 \pm 1,46	65,3 \pm 2,24	65,0 \pm 1,51	40,1 \pm 1,49
	<0,001	<0,001	<0,001	<0,001	<0,001

The findings indicate that on the 30th day of hypokinesia a decrease occurs in the biosynthesis of corticosterone in the adrenal glands, level of its secretion in the blood and entrance into the tissue of different organs. This probably is due to the reduction in the need of the tissues for glucocorticoids that can be linked to the change in the processes of oxidizing metabolism which is indicated by the decrease in the activity of a number of oxidizing enzymes. /61

The decrease in PDG activity in the liver reflects the reduction in intensity of pyruvate oxidation in the acetyl coenzyme A, by means of which the carbohydrates are included into the Krebs cycle.. There are data according to which during lengthy hypokinesia anaerobic (glycolytic) path of carbohydrate oxidation is activated (V. V. Portugalov and O. G. Gazenko; Konishi) which can be maintained by a reduction in the glucocorticoid activity in the organism, since the glucocorticoids inhibit the processes of glycolysis (E. F. Derkachev et al.).

The change in the processes of tissue respiration during hypokinesia is indicated also by the reduction in the activity of α -KDG and SDG--enzymes that

catalyze individual reactions in the Krebs cycle (oxidation of α -ketoglutarate and succinate). Here the role of the reduced level of glucocorticoids can be determined by the diminished entrance into the cycle of oxidation substrates. Therefore evidently glucocorticoid insufficiency promotes a decrease in the intensity of oxidative phosphorylation (K. S. Klimenko), formation of energy-rich phosphorous compounds, the demand for which is significantly reduced during lengthy hypokinesia (Ye. A. Kovalenko).

The effect of SMC during hypokinesia is manifest by stimulation of the glucocorticoid function of the adrenal cortex, increase in the level of corticosterone in different tissues, which affects the activation of the processes of tissue respiration. This is indicated by the correlation between the increase in concentration of the hormone and the activity of dehydrogenase, in particular, in the liver that plays an important role in the organism, especially during hypokinesia (M. R. Mogendovich).

The increase in PDG activity indicates the intensification of the processes of oxidation of carbohydrates in the Krebs cycle, while the activity of α -KDG, SDG and MDG--the stimulation of the rate of the cycle reaction, which induces acceleration in the transfer of electrons over the respiratory chain, and consequently, the increase in intensity of oxidative phosphorylation--ATP resynthesis (A. Lenindzher).

/62

One of the indices for the activation of the energy metabolism in hypokinetic rats under the influence of SMC was also the increase (normalization) of the reduced body temperature. The rectal temperature in 30-day hypokinesia was $35.9 \pm 0.4^\circ\text{C}$, in the hypokinetic rats exposed to the effect of SMC-- $36.8 \pm 0.3^\circ\text{C}$ ($P < 0.1$), in the intact animals $36.9 \pm 0.3^\circ\text{C}$. Evidently, stimulation by SMC of the energy metabolism led also to intensification of the anabolic (synthetic) processes reduced during hypokinesia. This is indicated by the lower degree of body weight loss during hypokinesia with the use of SMC as compared to the "pure" hypokinesia (respectively 8.4% -- $P > 0.1$ and 17.4% -- $P < 0.001$).

Further studies demonstrated that the course of effects of SMC as if normalize the resistance of the hypokinetic rats to acute hypoxia. Under conditions of acute hypoxia the duration of life of the hypokinetic rats was almost two times greater

TABLE 2. ACTIVITY OF A NUMBER OF DEHYDROGENASES (IN UNIT. OPTIC DENSITY OF FORMAZAN PER 1 g OF TISSUE IN 1 h AT 37°C) IN TISSUES OF RATS OF DIFFERENT GROUPS (M±m)

Group of rats	Liver			Brain				
	PDG	c-KDG	SDG	MDG	PDG	α-KDG	SDG	MDG
Intact	0,441±0,02	0,335±0,02	0,401±0,02	0,253±0,02	0,151±0,02	0,146±0,01	0,156±0,02	0,116±0,01
Hypokinetic	0,352±0,01	0,309±0,01	0,347±0,004	0,276±0,02	0,136±0,02	0,116±0,01	0,137±0,02	0,108±0,01
Hypokinetic + SMC	<0,002	>0,1	<0,025	>0,1	>0,1	=0,05	>0,1	>0,1
D	0,420±0,01	0,354±0,02	0,415±0,02	0,326±0,02	0,197±0,01	0,183±0,02	0,199±0,02	0,156±0,01
	<0,001	=0,05	<0,025	<0,1	<0,01	<0,01	<0,05	<0,01

TABLE 3. CHANGE IN CORTICOSTERONE CONTENT (in %) IN TISSUES AND ACTIVITY OF ENZYMES (IN UNIT. OF OPTIC DENSITY OF FORMAZAN PER 1 g OF TISSUE IN 1 h AT 37°C) IN LIVER OF RATS OF DIFFERENT GROUPS UNDER THE INFLUENCE OF ACUTE HYPOXIA

Group of rats	Corticosterone content			Activity of enzymes			
	adrenals	heart	liver	PDG	α-KDG	SDG	MDG
Intact	2712,0±106,1	44,3±1,50	51,7±1,54	0,441±0,02	0,335±0,02	0,401±0,02	0,253±0,02
Intact + hypoxia	2909,8±294,9	51,3±2,0	57,8±2,20	0,210±0,03	0,177±0,02	0,312±0,02	0,175±0,02
Hypokinetic	>0,1	<0,025	<0,05	<0,001	<0,001	<0,01	<0,025
Hypokinetic + hypoxia	1829,3±132,2	27,4±0,87	31,3±1,86	0,352±0,01	0,309±0,01	0,347±0,04	0,276±0,02
D	3313,9±232,5	55,2±1,60	62,0±1,60	0,242±0,03	0,199±0,03	0,454±0,02	0,239±0,03
Hypokinetic + SMC	<0,001	<0,001	<0,001	<0,01	<0,01	<0,001	>0,1
D	4050,0±145,3	65,0±1,50	65,3±2,24	0,420±0,01	0,354±0,02	0,415±0,02	0,326±0,02
Hypokinetic + SMC + hypoxia	2587,6±233,8	60,2±2,30	60,2±2,30	0,234±0,04	0,198±0,04	0,313±0,03	0,215±0,03
D	<0,001	>0,1	<0,1	<0,001	<0,001	<0,01	<0,002

than the control animals (respectively 18.7 ± 4.9 and 8.5 ± 1.7 min; $P=0.05$). At the same time this index in the hypokinetic rats exposed to the effect of SMC does not differ from the control (9.0 ± 2.7 min; $P>0.1$). The resistance of the animals in the indicated groups to the acute hypoxia depended on the peculiarities of the change in glucocorticoid function of the adrenal cortex and the activity of the studied enzymes. These peculiarities were manifest also in the relationship of the noted normalizing effect of SMC.

In a comparison of the dynamics of the studied indices in rats of different groups exposed to the effect of acute hypoxia it was established that in the hypokinetic rats as compared to the control the activity of SDG and MDG in the liver was increased (respectively by 45.5% -- $P<0.001$ and by 36.6% -- $P<0.1$), as well as SDG in the brain (by 36.5% ; $P<0.1$). Here in the hypokinetic rats preliminarily exposed to the effect of SMC the activity of the dehydrogenases was not significantly altered, and the content of corticosterone in the adrenals was the lowest, 2587.6 ± 233.8 $\mu\text{g}\%$ (in the hypokinetic without the effect of SMC-- 3313.9 ± 232.5 $\mu\text{g}\%$, $P=0.05$).

At the same time the peculiarities of the change in the level of corticosterone and the activity of enzymes to a greater measure were revealed in the comparison of the hormonal and enzymatic shifts in the groups of rats that died under conditions of acute hypoxia, and in the analogous groups of rats not exposed to the effect of this extreme factor.

As is apparent from table 3, in the intact rats after acute hypoxia the content of corticosterone in the adrenals was practically not altered, although the level of the hormone in other organs was somewhat increased--in the heart and liver only by 15.8 and 11.8% respectively. In the liver there was a significant reduction in the activity of all the studied enzymes. In contrast to this, in the hypokinetic rats under conditions of acute hypoxia there was an almost 2-fold rise in the initially reduced concentration of corticosterone both in the adrenal glands, and in the heart and liver in which an increase in the activity of SDG was observed on the background of a decrease in the activity of PDG, and α -KDG. In the hypokinetic rats exposed to the effect of SMC the subsequent effect of acute hypoxia led to a significant reduction in the increased content of corticosterone in the adrenal glands and to a lower degree--in the liver and heart. In the liver, as in the

intact rats, an inhibition was noted of the activity of all the studied dehydrogenases (see table 3), which occurred also in the brain ($P < 0.1$ and < 0.01).

On the whole the findings indicate that the resistance of the rats of different groups to acute hypoxia depends on the directivity of the change in the processes of tissue respiration. The resistance of the intact (control) and hypokinetic rats preliminarily exposed to the effect of SMC that is less pronounced and similar in duration of survival is linked to the reduction in activity of the dehydrogenases in the Krebs cycle, i.e., with inhibition of the aerobic oxidation occurring under conditions of oxygen insufficiency. Activation of the anaerobic path of oxidation, which is indirectly indicated by the reduction in PDG activity, cannot increase the resistance of the organism to acute hypoxia, since glycolysis, even with an oxygen deficit is not the main source of the energy formation (K. P. Ivanov). /63

The more pronounced resistance to acute hypoxia of the hypokinetic rats is evidently linked to the realization of the defense metabolic mechanism that was not manifest in the rats of other groups. This concerns the increase in SDG activity indicating the stimulation of the succinate-oxidase branch of the respiratory chain. Here, probably, a "turn" of the Krebs cycle occurs (decrease in the activity of α -KDG and increase in the activity of MDG) directed towards intensification of succinate synthesis. "Switching" of the metabolism to primary oxidation of succinate has great importance in the compensatory adaptation of the oxidizing metabolism to the acute oxygen deficiency, since succinate as compared to NAD-dependent substrates possesses a greater rate of oxidation and guarantees to a greater degree the supply of energy-rich compounds to the respiratory chain (M. N. Kondrashova). /64

The value of activation of oxidation of succinate in increasing the resistance of rats to acute hypoxia can be illustrated by the correlations between the periods of survival under conditions of an oxygen deficit and SDG activity. In the hypokinetic rats the degree of connection between these indices was the most significant ($r=0.64 \pm 0.18$), in the intact rats--weakly pronounced ($r=0.36 \pm 0.015$), and in the hypokinetic, exposed to the effect of SMC, the indicated bond practically was missing ($r=0.09 \pm 0.033$).

In the manifestation of varying resistance to acute hypoxia linked to the noted changes in the activity of respiratory enzymes a certain role was played

by the dynamics of the level of corticosterone. As noted above, during 30-day hypokinesia the glucocorticoid activity of the adrenal cortex is reduced. This is accompanied by the preservation and even increase in the "reserves" of glucocorticoids (S. K. Kalandarov et al., 1973) that can be mobilized in extreme conditions--in acute hypoxia. Therefore, on the background of an intensification in biosynthesis of corticosterone the discharge and entrance of the hormone are significantly intensified, in particular, into the liver where the SDG activity is significantly increased. The correlation between the increase in the level of corticosterone and the activity of the enzyme indicates the definite "contribution" of the hormone to stimulation of succinate oxidation, which is governed by substrate activation of SDG due to the intensification of succinate entrance into the mitochondria due to the increase membrane permeability (P. V. Sergeev et al., G. G. Vol'skiy and L. M. Osadchaya).

The reaction of the adrenal cortex to acute hypoxia in the intact rats was essentially missing, and the relatively small increase in the level of corticosterone in the liver, evidently was insufficient to activate the succinate-oxidase branch of the respiratory chain. In the hypokinetic rats the preliminary effect of SMC already lead to a significant increase in the glucocorticoid activity in the organism, and evidently, therefore in the subsequent effect of acute hypoxia a reduction was observed in steroid-genesis, absence of the increase in the content of corticosterone in the liver, and on this background the inhibition in it of the processes of tissue respiration. Consequently, the normalizing effect of SMC in relation to the resistance of the hypokinetic rats to acute hypoxia to a certain measure is linked to the pronounced stimulating effect of currents on the glucocorticoid function of the adrenal cortex.

Thus, the results of our studies showed that the effects of SMC during lengthy (30-day) hypokinesia in rats increase the reduced glucocorticoid activity in the organism and in addition intensify the processes of oxidizing metabolism in the liver and brain. Here the resistance of the hypokinetic rats to acute hypoxia is normalized, which is governed by certain peculiarities in the changes of the glucocorticoid function of the adrenal cortex and the activity of a number of dehydrogenases in the Krebs cycle.

The findings, revealing certain previously unstudied aspects of the mechanism for SMC action can promote the development of indications for the

broader use of this factor, not only during hypodynamia, but also in different types of functional hypocorticoidism, and reduction in the intensity of the oxidation-reduction processes. An outlook is also created for the use of the extreme effects (acute hypoxia and others) in an extreme evaluation of the effect of different physical factors employed for a treatment or preventive purpose.

Conclusions

/65

1. During 30-day hypokinesia in rats the content of corticosterone is reduced in different tissues, as well as the activity of pyruvate- and succinate dehydrogenase (PDG and SDG) in the liver and α -ketoglutarate-dehydrogenase (α -KDG) in the brain.

2. The effect of sinusoidal modulated currents (SMC) during 30-day hypokinesia is manifest in an increase in glucocorticoid activity in the organism and parallel activation of PDG and enzymes of the Krebs cycle-- α -KDG, SDG and malate-dehydrogenase (MDG) in the liver and brain, which indicates the intensification in the oxidative (energy) metabolism.

3. In rats who spent 30 days in a state of hypokinesia the resistance to acute hypoxic hypoxia is increased. This is linked to the considerable rise in the reduced level of corticosterone in different organs and the SDG activity in the liver and brain. The latter indicates the primary oxidation of succinate, which has great importance in the adaptation of the oxidative metabolism to acute oxygen insufficiency.

4. The use of SMC in the period of hypokinesia promotes normalization of the indices for resistance of the rats to acute hypoxia, under whose conditions the glucocorticoid function of the adrenal cortex and the activity of PDG, α -KDG, SDG and MDG is reduced in the liver and brain.

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