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RECOMBINANT INTERFERON-ALPHA EFFECTS ON ELECTRICAL ACTIVITY AND METABOLISM OF THE MOUSE ISOLATED HEART

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Annotation. Were studied effects of recombinant interferon-α2b on the isolated mouse heart. Retrograde perfusion of the heart with Krebs-Henselite solution with dissolved interferon-α2b (with activity 2000 IU) caused decrease in the voltage of the R wave at the time of perfusion and ischemia (but during reperfusion there was an increase), an increase in the R-R' interval. At the same time, perfusion of the heart with solution of interferon-α2b was accompanied by a decrease in the volume rate of the coronary flow.

Passing of interferon-α2b through the heart led to a decrease in myocardial glucose consumption against the background of deposition of extracellular Ca2+ and an increase in the yield of the enzyme aspartate aminotransferase, a decrease in creatinine excretion, which indicates inhibition of creatinine phosphokinase activity. Due to the fact that the predominant amount of creatinine kinase is localized in mitochondrial membranes, the results obtained indicate the inhibitory effect of interferon on the activity of transport processes of macroergic compounds in the myocardium.

Key words: interferon-α2b, isolated heart, aspartate aminotransferase, creatinine.

Анотація. Проведено дослідження впливу рекомбінантного інтерферону-α2b на ізольоване серце миші. Ретроградна перфузія серця розчином Кребса-Хензеляйта із розчиненим інтерфероном-α2b у кількості 2000 МО спричиняла зниження вольтажу зубця R в момент проведення перфузії та ішемії (проте під час реперфузії спричиняє його підвищення), збільшення тривалості інтервалу R-R. Разом з тим, перфузія серця розчином інтерферону-α2b супроводжувалася зниженням об'ємної швидкості коронарного потоку. Пропускання інтерферону-α2b через серце спричиняє зниження споживання міокардом глюкози на тлі депонування позаклітинного Ca2+ та посилення виходу ферменту АсАт, зниження екскреції креатиніну, що свідчить про пригнічення активності креатинінфосфокінази. Оскільки переважна кількість креатинінкінази локалізована в мембранах мітохондрій, отримані результати свідчать про інгібуючий вплив інтерферону на активність процесів транспорту макроергічних сполук у міокарді.

Ключові слова: інтерферон-α2b, ізольоване серце, аспартатамінотрансфераза, креатинін.

Аннотация. Проведено исследование влияния рекомбинантного интерферона-α2b на изолированное сердце мыши. Ретроградная перфузия сердца раствором Кребса-Хензеляйта с растворенным интерфероном-α2b (2000 МЕ) вызывала снижение вольтажа зубца R в момент проведения перфузии и ишемии (но при реперфузии вызывала его повышение), увеличение продолжительности интервала R-R. Вместе с тем, перфузия сердца раствором интерферона-α2b сопровождалась снижением объемной скорости коронарного потока. Пропускание интерферона-α2b

через сердце приводит к снижению потребления миокардом глюкозы на фоне депонирования внеклеточного Са2+ и усиление выхода фермента АсАт, снижение экскреции креатинина, что свидетельствует об угнетении активности КФК. Поскольку подавляющее количество КФК локализовано в мембранах митохондрий, полученные результаты свидетельствуют об ингибирующем влиянии интерферона на активность процессов транспорта макроэргических соединений в миокарде.

Ключевые слова: интерферон-α2b, изолированное сердце, аспартатаминотрансфераза, креатинин.

Interphenrons (IFN) are a family of cytokines with pleiotropic action, which includes inhibition of viral replication, cell proliferation. Interferon receptors are expressed on all cells in the body. These properties of interferons allow it to be used for infection, carcinogenesis. It is known that IFN acts on endothelium cells, causing an antiangiogenic effect [6; 9]. However, effects of the interferon on the heart muscle remain not fully disclosed. It is known that in individuals who have been using IFN for a long time, an increase in the voltage of the QRS complex is recorded [5]. There are reports of functional reactions of the heart during interferon therapy, in particular, the phenomena of arrhythmia, dilated cardiomyopathy, atrial extrasystole, symptoms of coronary heart disease, hyper- and hypotension are mentioned [8]. With the introduction of interferon-α to laboratory mice, ultrastructural changes of the cardiac capillaries are recorded: the thickness of endothelial cells increases with a corresponding decrease in their lumen. After 2-3 injections of high doses of interferon-α to rats, an increase in the time of ventricular repolarization and a decrease in the voltage of the R-wave on the ECG are observed. Some authors admit the ability of interferon molecules to activate heart β-adrenoreceptors, which most likely causes side effects from the cardiovascular system, which disappear after interferon therapy is stopped [7]. Thus, the direct effect of interferons on the heart remains undisclosed; the mechanisms of the formation of the aforementioned side effects of the cardiovascular system are not clear.

The aim of the work is to study the direct effect of recombinant interferon-α2b on an isolated mouse heart under ischemia-reperfusion.

Metrials and methods: The studies were conducted on the hearts of nonlinear laboratory mice (n = 20) 3-4 months old, weighing 20-25 g, in compliance with the standards of the European Convention for the Protection of Vertebrate Animals, which are used for experimental and other scientific purposes, the Council of Bioethics 1997 Convention The animals were fed on a standard diet.

Krebs-Henseleit solution (pH 7.3-7.4) with heparin after the cervical dislocation, the heart was isolated, which was placed in a cooled (+ 4° C). Through the cannula filed warm (+37^{0}C) Krebs-Henseleit solution under pressure102 ± 2 mm Hg (solution in mmol/l: NaCl - 118; KCl - 4.7; $MgSO_4$ - 1.2; KH_2PO_4 - 1.2; $CaCl_2$ - 2.5); glucose - 5.5; $NaHCO_3$ - 25). The solution was saturated with carbogen (95% O_2 and 5% CO_2).

It was in its natural state. The electrocardiogram registered by the Midas EK-1T. The amount of the flowing solution from the coronary vessels (ml/min). The resulting perfusate was used to determine the amount glucose, calcium, and aspartate aminotransferase (AsAt).

The first (control) group was a group of patients of the isolated heart (n = 10), through which the Krebs-Henseleite solution was passed. The second group consisted of hearts (n = 10), through which recombinant interferon α2b preparation (CHAP Biofarma, Ukraine) was dissolved.

Ischemia of an isolated heart in a thermostatically controlled container was passing for 20 minutes. The duration of the periods of perfusion and reperfusion was 20 minutes, respectively (Fig. 1).

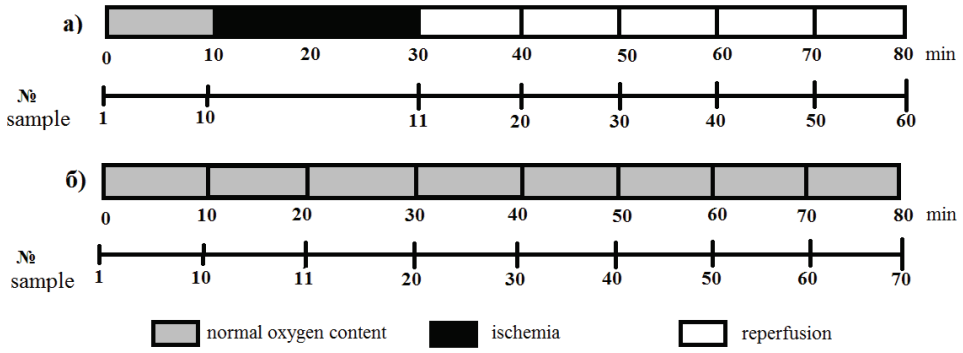

Fig. 1. Perfusion of isolated mouse heart

Note: a) perfusion with a stage of global ischemia-reperfusion; b) perfusion without a stage of global ischemia.

Statistical analysis of the results was performed using the program Statistica 6.0, the indicators were expressed as mean value and standard deviation. The significance of differences was determined using the Mann-Whitney test. Changes were considered significant when $p \leq 0.05$.

The results of the study and their discussion. It is known that interferon-α2b has the properties to influence the growth and differentiation of cells, causing changes in metabolic and synthetic processes, the accumulation and activation of mast cells in tissues [1, 3].

The registration of the biocurrents of the heart and the comparison of the average values of the strength of the R wave of the electrocardiogram showed that the transmission of the interferon solution determined a significant decrease in the voltage at the time of perfusion (0.48 mV) and ischemia (0.75 mV), but during reperfusion provokes its increase (0.6 mV) compared with the control (perfusion with Krebs-Henseleit solution) (Fig. 2).

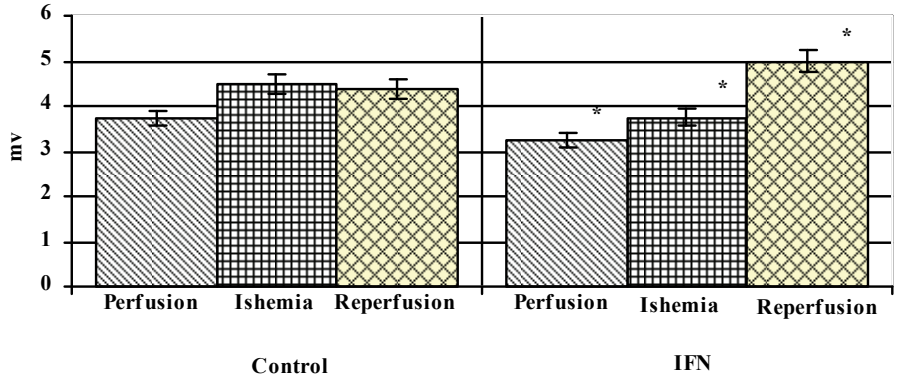

Fig. 2. Indicators of tooth strength R of electrocardiogram

Note: * - significant differences in comparison with the control ($p \leq 0.05$).

Comparison of the average duration of the R-R 'intervals in the control group showed no significant differences during the period of perfusion, ischemia and reperfusion (Fig. 3).

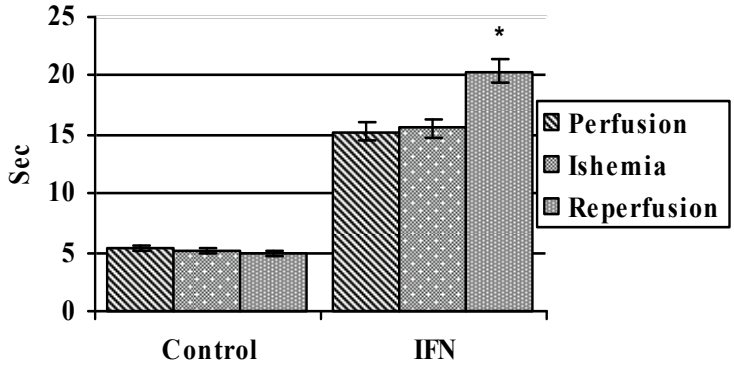

Fig. 3. Indicators of the duration of the intervals R-R '

Note: * - significant differences in comparison with the control ($p \leq 0.05$).

The indices obtained during the perfusion with the solution with interferon were significantly higher than the control group: the duration of the R-R 'interval increased by 15.3 s during the perfusion, 10.5 s during the ischemia, and 15.4 s during the reperfusion. Together with changes in the electrocardiogram, the corresponding changes in the coronary flow were recorded.

In the case of perfusion of an isolated heart with a Krebs-Henseleit solution with the addition of recombinant interferon-α2b, a significant ($p \leq 0.05$) decrease in the volumetric flow rate of the coronary flow is observed (Fig. 4).

Actually, before the onset of ischemia-reperfusion, the volumetric rate decreases by 58 ± 2.9% (1-10 min), After ischemia under reperfusion conditions — by 46 ± 2.3% (30-40 min), By 47 ± 2.4% (41 - 50 min.), 43 ± 2.2 (51-60 min. and 61 - 70 min.), And 41 ± 2% (71 - 80 min.). At the same time, in both cases, there is a compensatory increase in coronary flow immediately after ischemia, at the beginning of reperfusion (11 sample).

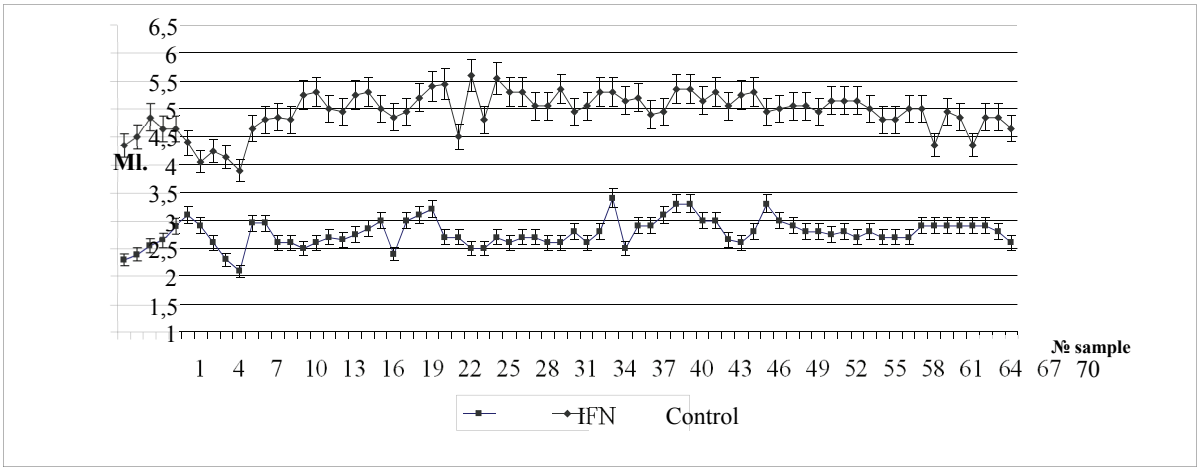

Fig. 4. Volumetric rate of the coronary flow of an isolated mouse heart under ischemia-reperfusion.

Interest caused by indicators of glucose in the perfusion solution, which flowed from the heart. In comparison with the control, during perfusion of the heart with a Krebs-Henznleit solution with dissolved recombinant IFN-α2b, there is a decrease in glucose uptake by the myocardium. Before the onset of ischemia, this indicator decreased by 3.6 ± 0.2 μmol/l, after ischemia — by 3.7 ± 0.2 μmol/l, and at the end of reperfusion it reached 4.0 ± 0.2 μmol/l (Fig. 5).

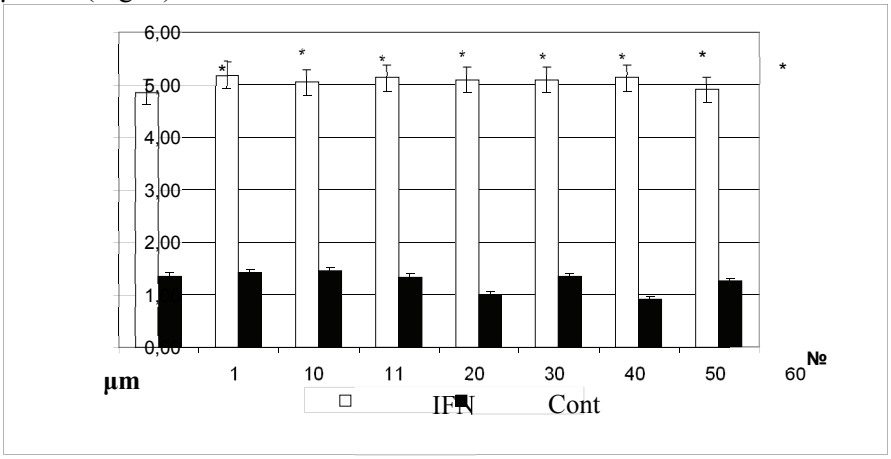

Fig. 5. Glucose content in the samples of the perfusion solution, which flowed from the heart
Note: * - significant differences in comparison with the control ($p \leq 0.05$).

The phenomenon of a decrease in glucose metabolism in the heart under influence of interferon is consistent with indicators of a decrease in volumetric rate of coronary flow.

It is known that work of contractile apparatus of the muscle fiber is brought to an active state due to Ca^{2+} ions, while these ions cause incoming current during the generation of the action potential [2]. Since part of the Ca^{2+} ions, which initiates the contraction of myofibrils, enters the cells from the intercellular fluid via "slow" Na^{+}-Ca^{2+} membrane channels, the activity of the heart to absorb Ca^{2+} ions from the perfusion solution under influence of recombinant IFN-α2b during ischemia-reperfusion was investigated.

During perfusion of the heart with a Krebs-Henseleite solution with addition of recombinant IFN-α2b, an increase in myocardial deposition of extracellular calcium observed. In fig. 6 depicts a comparison of calcium in samples of perfusion solution.

Thus, at the beginning of the perfusion of an isolated heart with a Krebs-Henzeleite solution with addition of recombinant IFN-α2b, the content of Ca^{2+} in the first sample was reduced 2.4 times in comparison with the control.

Before the onset of ischemia, in the 10th minute of perfusion, this indicator was also reduced 2.3 times. At the beginning of reperfusion, the indicator of the amount of Ca^{2+} absorbed was 1.5 times higher, at the end - 2.4 times. So, it is undoubted that the perfusion of an isolated heart with a solution that contained IFN-α2b causes an increase in the absorption of Ca^{2+} ions by the myocardium both during ischemia and during reperfusion.

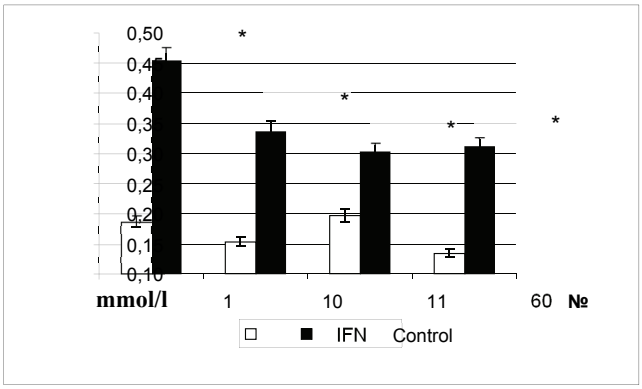

Fig. 6. Content of Ca2 + in samples of perfusion solution, which flowed from the heart
Note: * - significant differences in comparison with the control (p≤0.05).

It is known that 3 mechanisms of energy supply are distinguished in muscle bioenergy: aerobic, glycolytic and creatine phosphokinase (alactic). In our case, when studying the content of creatinine, interferon caused a decrease in its excretion into the perfusion solution. At the beginning of the perfusion, the level of creatinine was reduced 1.3 times (compared to the control), in the 10th test - 1.7 times. During the reperfusion period, the fixed index was reduced by 1.6 times (11 samples), at the tenth minute of reperfusion, this indicator was reduced by 1.2 times, at the twentieth - by 1.7 times, the thirtieth - by 1.6 times, the fortieth - 1.2 times, at the end of reperfusion, 1.7 times (compared to control) (Fig. 7).

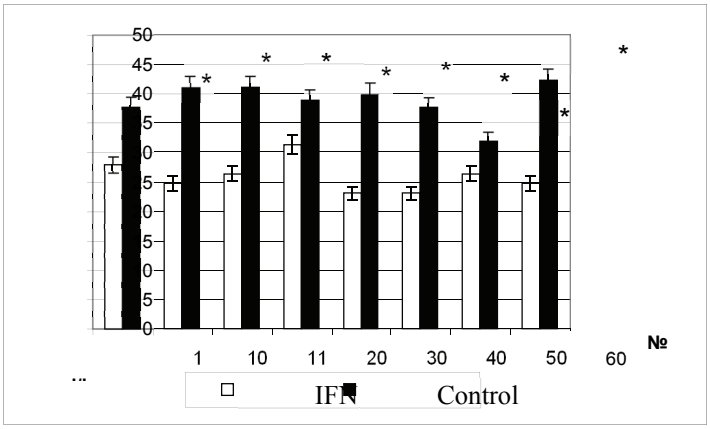

Fig. 7. Content of creatinine in the samples of perfusion solution, which flowed from the heart
Note: * - significant differences in comparison with the control (p≤0.05).

The activity of the enzyme aspartate aminotransferase (AsAT) was also compared. It is known that AsAt plays an important role in the synchronization of energy and nitrogen metabolism, which is carried out at level of mitochondria [4]. Functioning of the enzyme is associated with mechanisms of exchange of nitrogenous and nitrogen-free compounds between the mitochondrial matrix and the cytoplasm [10].

In general, level activity of AsAT in perfusion solution, which flows from the heart during perfusion with Krebs solution with dissolved interferon preparation, was significantly ($p \leq 0.05$) higher for control. Interestingly, during reperfusion period, there is a decrease in activity of AsAT in control after 10 minutes after ischemia, and in conditions of cardiac reperfusion with a solution with IFN, a decrease in tindices was observed after 20 minutes. Only at the end of reperfusion with a Krebs solution with IFN did a decrease in activity of AsAT occur, whereas this was not observed in the control group (Fig. 8).

Analysis of the results showed that addition of recombinant interferon-α2b to perfusion solution causes metabolic changes in the myocardium. Given that interferon is a proinflammatory cytokine, biological activity of which is realized by antiviral and antitumor activity, its effect on the myocardium causes metabolic changes. The latter are manifested in a decrease in glucose uptake, an increase in Ca^{2+} absorption, a decrease in creatinine excretion, and an increase in the yield of AsAt by cardiomyocytes against the background of a reduced volumetric rate.

In our case, the determination of the activity of the alactate pathway is an important indicator of the activity of creatinine phosphakinase and the state of the myocardium. After perfusion of an isolated heart, it

was found that the transmission of the interferon solution was the cause of the decrease in creatinine excretion from the myocardium, which in turn indicates inhibition of activity of creatine phosphokinase. It is known that an increase in activity of this enzyme is a marker of damage to cardiomyocytes. The concentration of creatine phosphate in the muscles is 3-4 times higher in comparison with content of ATP. A decrease in creatine excretion also indicates a decrease in creatinine phosphate reserves. By indicators of creatinine release can be judged on the content of creatinine phosphate in the muscles. It is known that the synthesis of creatine phosphate in myocytes occurs during the rest period by interaction of creatine with an excess of ATP. Due to the fact that overwhelming amount of CPK is localized in mitochondrial membranes, the results indicate an inhibitory effect of interferon on activity of transport of high-energy compounds in the myocardium.

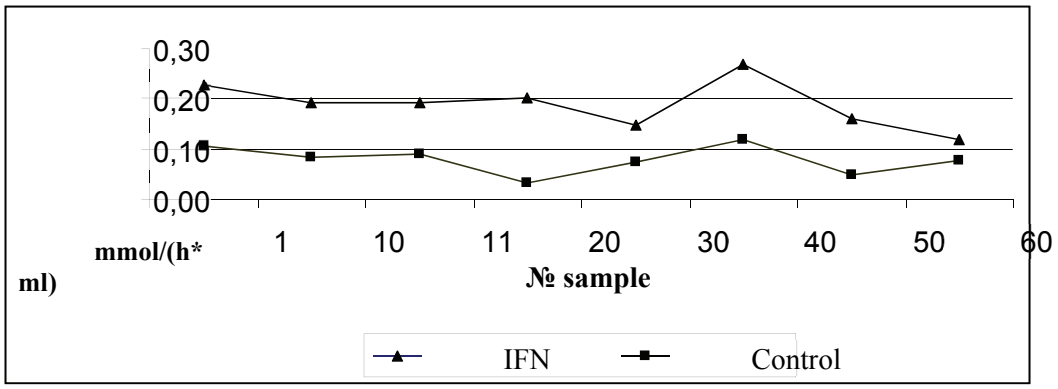

Fig. 8. Activity of AsAt in samples of perfusion solution, which flowed from the heart

Confirmation of foregoing is established reduction in glucose consumption of the heart muscle under influence of IFN-α2b. It is well known that calcium is necessary for activation of mitochondrial enzymes and stimulation of energy production. In our study, deposition of Ca^{2+} by cardiomyocytes was recorded, which was most pronounced after ischemia - at beginning of reperfusion. At the same time, we believe that increased release of AsAt is also associated with accumulation of Ca^{2+} in cardiomyocytes. Consequently, these indicators are interrelated, since it is known that an overload of mitochondria with calcium leads to changes in the level of mitochondrial AsAt (with participation of Ca^{2+}-sensitive proteases) and launch of mitoptosis.

The above phenomena were accompanied by changes in the electrical activity of the heart, causing a significant decrease in the voltage at the time of perfusion with interferon, but during reperfusion its increase was observed. Duration of R-R' intervals with interferon perfusion was significantly higher than control, manifested by a corresponding decrease in coronary flow.

Findings

1. Solution of interferon-α2b causes changes in the electrogram of an isolated mouse heart - a decrease in voltage of the R wave at the time of perfusion and ischemia (but during reperfusion causes its increase), an increase in duration of the R-R' interval in all cases. Changes in the indices of the electrogram of an isolated heart under the influence of interferon-α2b are consistent with a decrease in volumetric flow rates of the coronary flow, especially during reperfusion period.

2. Interferon α2b causes metabolic changes in an isolated heart, which is manifested by a decrease in glucose consumption against background of deposition of extracellular Ca^{2+} by myocardium and an increase in yield of the enzyme AsAt in a perfusion solution. At the same time, transmission of interferon solution through the heart showed a decrease in excretion of creatinine from the myocardium, which indicates inhibition of CPK activity. Due to the fact that overwhelming amount of CPK is localized in mitochondrial membranes, obtained results indicate inhibitory effect of recombinant interferon-α2b on activity of transport of high-energy compounds in the myocardium.

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ОСОБЛИВОСТІ СТАНУ МОЗКОВОГО КРОВООБІГУ У МОЛОДШИХ ШКОЛЯРІВ ІЗ ДИТЯЧІМ ЦЕРЕБРАЛЬНИМ ПАРАЛІЧЕМ

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Анотація, Використовуючи метод реоенцефалографії досліджували особливості церебрального кровотоку у дітей з дитячим церебральним паралічем. Встановлено, що мозкова гемодинаміка дітей з ДЦП характеризується суттєвим зниженням артеріального кровонаповнення та об'ємної швидкості кровотоку, утрудненим венозним відтоком у каротидній та вертабро-базилярный системах. Виявлені статеві особливості церебральної гемодинаміки дітей з ДЦП: у каротидній системі дівчат у порівнянні з хлопцями підвищення периферичного опору, тонусу судин артеріального типу дрібного калібру та об'ємної швидкості кровотоку правої гемісфери; зниження тонусу судин венозного типу дрібного калібру та підвищення артеріального кровонаповнення та об'ємної швидкості кровотоку лівої гемісфери. З'ясовано, що у вертебро-базилярній системі дівчат нижчі показники периферичного опору та об'ємної швидкості кровотоку в обох гемісферах, а тонусу судин артеріального типу дрібного калібру та артеріального кровонаповнення – у правій гемісфері. Отримані результати свідчать про певні ускладнення кровопостачання веретебро-базилярної системи дітей з ДЦП.

Ключові слова: реоенцефалограма, мозковий кровообіг, дитячий церебральний параліч.

Аннотация. Используя метод реоэнцефалографии, исследовали особенности церебрального кровотока у детей с детским церебральным параличом. Установлено, что мозговая гемодинамика детей с ДЦП характеризуется существенным снижением артериального кровенаполнения и