UDC 612.3+591.1

Liver electrical activity upon epinephrine action

P.K. Tsapenko[1], T.P. Liashchenko[2], S.P. Vesel'sky[3]

[1]Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv;
[2]Educational and Scientific Center "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv;
[3]Chemical faculty of Taras Shevchenko National University of Kyiv; e-mail: p.tsapenko@biph.kiev.ua

The electrical potential of the liver is an integrative indicator the activity of the cells in this organ. Here we tried to elucidate a link between liver functional state and its electrical activity. For that, electrical potential of the liver, the content of bile acid and blood glucose concentration were measured in control conditions and after intravenous epinephrine administration. Epinephrine at dose 0.2 μg/kg body weight caused short-term increase in blood glucose concentration and a decrease in hydroxylation ratio of bile acids. The administration also resulted in an increase in power spectral density (PSD) of liver electric potential in a frequency range of 1.6-2.5 Hz. In addition, a trend to increasing in PSD at 0.6-1.5 and 2.6-10 Hz ranges and decreasing in conjugation ratio of bile acids were shown. Correlation between liver electrical activity and blood glucose concentration observed in control conditions vanished upon epinephrine administration. In contrast, epinephrine administration potentiated correlation between liver electrical activity and content of bile acids. Thus, we conclude that the liver electrical activity could reflect its secretory processes.
Key words: liver; electrical activity; power spectral density; bile acids; epinephrine; glucose.

INTRODUCTION

One of the promising methods of researching the liver functional state is the measurement of the total electrical potential. On the one hand, the electrical potential of an organ is a reflection of the functional state of its cells, and on the other hand, it can be measured non-invasively (or almost non-invasively) [1]. Liver electrical activity was investigated earlier and normal electrohepatogram was characterized in a canine model [2, 3]. In these studies interlobular electrical waves were analyzed, which reflect irradiation of liver electrical activity. But source of electrical waves and its link with functional state of organ could be determined with monopolar measurements. And it is why our aim was to analyze a total liver electrical potential recorded using monopolar measurement. A complication of the analysis of the liver total electrical potential is the need to determine which part of the organ contributes to the electrical activity: parenchymal cells, smooth muscle cells, or nerves? And what liver function would be indicated by electrical potential changes? So, the main task of this work was to establish the relationship between the liver electrical activity and its main functions - bile and glucose secretion.

We were also interested in how a change in the functional activity of the liver will affect its electrical potential. For this, it is advisable to use a compound that affects different subpopulations of liver cells, while involving different intracellular processes. One of these compounds is epinephrine, which under physiological conditions can act on liver cells as a neurotransmitter and a hormone of the medulla of the adrenal glands [4]. Epinephrine can act through activating adrenergic and dopaminergic receptors both. In normal liver there are expressed DRD1 to DRD5 and ADRA1A, ADRA2A, and ADRB2 receptors [5]. Activation of adrenergic receptors effects on intracellular calcium concentration and, in accordance, calcium-dependent processes: bile secretion, carbohydrates metabolism, detoxi-

fication function of the liver etc. Epinephrine influence on liver functions could be both by direct action on hepatocytes and indirectly (through changes in liver blood flow). The latter may be caused by local effect of epinephrine on liver blood vessels' smooth muscle cells and its systemic effect on cardiac output [6, 7]. Also epinephrine can affect mitochondria of normal liver hepatocytes [8].

Thus, epinephrine has a significant effect on the functional state of the liver, which allows us to use it to deepen the understanding of the nature of the electrical potential of the gland.

METHODS

Research was conducted in compliance with the provisions of the Council of Europe Convention on Bioethics (1997), the Helsinki Declaration of the World Medical Association (1996), the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, 1986), general ethical principles of scientific studies approved by the First National Congress of Ukraine on Bioethics (September 2001), Law of Ukraine No. 3447-IV "On the Protection of Animals from Cruelty" (2006), other international agreements and national legislation in this field.

Experiment was carried out on 12 laboratory white male rats weighting 200-250 g under acute experimental conditions. The animals were divided into 2 groups of 6 animals each. The first group was the control group; the second group was the animals injected with epinephrine.

Surgical procedure. Before the start of the experiment, the rats were starved for 12 h with free access to water. Laparotomy was performed on anesthetized rats (sodium thiopental at a dose of 5 mg/100 g of body weight intraperitoneal), and a thin cannula connected to a micropipette was inserted into the common bile duct, and a graphite electrode was placed on the surface of the left liver lobe. Graphite was chosen because this material does not interact with biological tissues, does not exhibit toxic properties, but at the same time is one of the best conductors; its characteristics are close to those of gold [7]. The indifferent electrode was connected to the animal's tail using a salt bridge. The salt bridge ensured constant contact with the skin. Putting an indifferent electrode on the tail made it possible to maximally reduce the mutual influence of the electrodes and minimize the polarization between them [1]. After applying the electrodes, the abdominal wound was closed.

Drugs administration. After surgery rats were administered different drugs depending on the group. After a thirty-minute period, 0.1 ml/100 g of body weight of an isotonic solution of sodium chloride (8.9 g/l) was administered intraperitoneal to control group. After the first thirty minutes, experimental group rats were injected with epinephrine ("Sigma", USA) in vena porta hepatica at a dose of 0.2 μg/kg body weight through thin intravenous cannula. At the end of each animal recording concentrated KCl solution (2.5 M, 0.1 ml/100 g of body weight) was applied on liver surface by syringe. To prevent contact of KCl with other organs liver lobe was covered by polyethylene film. After stabilization of electrical potential (through less than 30 sec) data were recorded.

The liver electrical activity measurements. The electrical potential of the liver was measured during 2 min through every 10 min using the amplifier with R-C filters, which allows recording the active component of the electrical potential [10]. The recording was performed with a time constant τ of 2.2 sec. The signal from the amplifier was fed through the analog-to-digital converter ACC (NPO "RKS", Ukraine) to the computer; the recording took place at a sampling frequency of 32 Hz. Fourier analysis was performed and oscillation with frequencies above 32 Hz were filtered out. Power spectral density (PSD) was calculated and then root mean square (RMS) of PSD for chosen frequency range was analyzed by statistical methods.

Bile acids content and glucose concentration analysis. In each 10 min sample the volume of bile was measured. In each half-hour bile

sample, the qualitative and quantitative analysis was performed. Qualitative analysis of bile acids in each 30-minute bile sample was determined chromatographically according to Veselskyi et al. as previously described [8]. Bile acids were distributed as follows: taurocholic acid, a mixture of taurochenodeoxycholic and taurodeoxycholic acids, glycocholic acid, a mixture of glycochenodeoxycholic and glycodeoxycholic acids, cholic acid, a mixture of chenodeoxycholic and deoxycholic acids. To quantify the content of bile acids, the chromatograms were pre-sprayed with dyes: 15 ml of glacial acetic acid, 1 g of phosphoromolybdic acid, 1 ml of sulfuric acid and 5 ml of a 50% solution of trichloroacetic acid. Chromatograms were developed at a temperature of 60-70°C for 5 min and the content of bile acids was determined on a DO-1m densitometer at a wavelength of 620 nm. Conjugation quotient (the ratio of the conjugated cholates sum to the amount of free bile acids) and hydroxylation quotient (ratio of the trioxycholates sum to the sum of deoxycholates) were calculated [11]. We also determined the glucose concentration in tail blood samples every ten minutes by the Optium Xceed glucometer.

Statistical analysis. Statistical analysis was performed using "GraphPad Prism" 8.0. Data were checked for normality of distribution using the Shapiro-Wilk test. Data were compares using two-way ANOVA-repeated measure test with post-hoc analysis by Sidak. For correlation analysis correlation matrices were created: first, root mean square of power spectral density for each record at each frequency range was added to analyze; then each sample of glucose concentration/total concentration of conjugated bile acids/total concentration of deoxycholic bile acids/total concentration of trioxycholic bile acids/each meaning of conjugation ratio/each meaning of hydroxylation ratio were added too. Correlation analysis between RMS of PSD at each frequency range and above mentioned indicators was performed using the Spearman method separately for control and experimental groups. Differences at $P < 0.05$ were considered significant.

RESULTS

Our research showed that the liver electrical potential measured from organ' surface oscillates at different frequencies (Fig. 1). Power spectral density (PSD) was calculated to determine what frequencies are presented at these oscillations.

Analysis of PSD suggests there are several peaks at some frequencies (Fig. 2A), so we divide frequency's range into smaller ranges with a step of 1 Hz (except of smallest range): 0.04-0.08 Hz, 0.6-1.5 Hz, 1.6-2.5 Hz, 2.6-3.5 Hz, 3.6-4.5 Hz, 4.6-5.5 Hz, 5.6-6.5 Hz, 6.6-7.5 Hz, 7.6-8.5 Hz, 8.6-9.5 Hz, 9.6-10.5 Hz, 10.6-11.5 Hz, 11.6-12.5 Hz, 12.6-13.5 Hz, 13.6-14.5 Hz and 14.6-15.5 Hz. For these ranges RMS was calculated to provide statistical analysis.

To exclude influence on liver electrical potential of other organs (heart, skeletal muscles etc.) concentrated solution of KCl was applied on liver surface at the end of each recording (Fig. 1). Concentrated KCl solution depolarizes cell's membranes and prevents liver cells electrical activity but not conduction of electrical currents from other organs. Absence of electrical potential oscillation of liver after KCl application was proved by PSD calculation (Fig. 2D). It means the source of recorded electrical activity could be the liver only. So, our results suggest that recorded electrical activity was generated by cells of very gland. But what cell type generate which oscillation? It's still unclear.

To establish relationship between liver electrical potential oscillation and bile acids concentration correlational analysis by Spearman was provided. In control group correlation between free bile acids concentration and RMS of PSD at 0.04-0.08 Hz range was shown ($r = 0.7619$, $P = 0.0368$). There was no correlation between other bile acids concentration (conjugated, deoxycholates, trioxycholates) and any range of electrical potential oscillation. Also there were no correlation between RMS of PSD and conjugation and hydroxylation ratios. These data suggest that there is a link between free bile acid secretion (and possibly primal bile acid

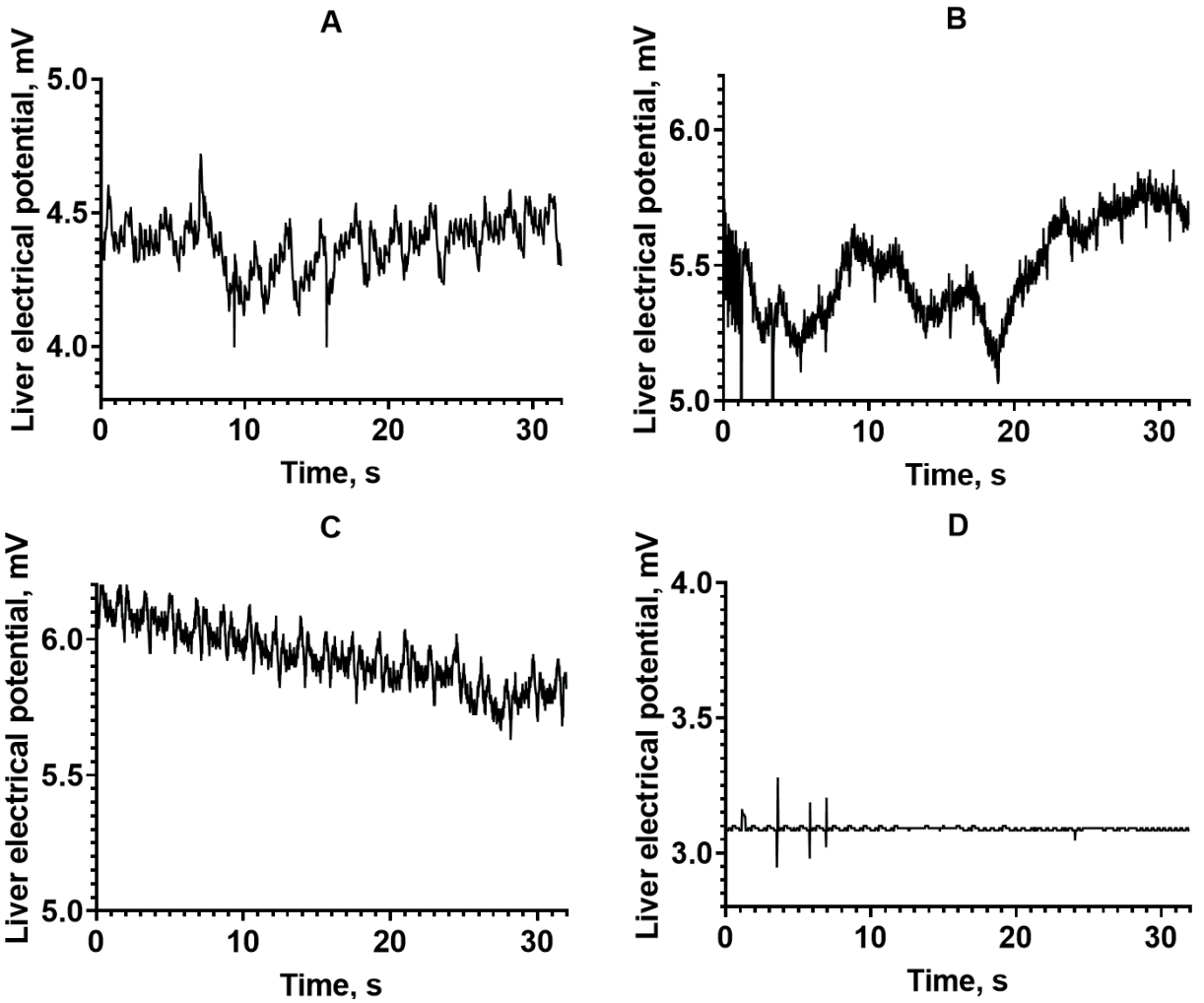

Fig. 1. Electrical potential of the liver, fragments of original records after drugs administration. A - liver electrical activity recorded 10 min after 0.89% NaCl intravenous administration, control group. B - liver electrical activity recorded 10 min after epinephrine intravenous administration. C - liver electrical activity recorded 20 min after epinephrine intravenous administration. D - liver electrical activity recorded 30 sec after 2.5 M KCl application on liver surface

synthesis) and liver electrical potential oscillation at lowest frequency range.

Glucose concentration in control group was ~4.8 mM without drastic oscillations through experiment time. There was negative correlation between blood glucose level and RMS of PSD at 0.04-0.08 Hz range ($r = -0.5421$, $P = 0.0135$); and positive correlation at ranges 1 Hz and higher but not at ranges 1.6-2.5, 5.6-7.5, 8.6-9.5 and 11.6-16 Hz (Table 1). So, electrical activity of the liver correlates with glucose level more than with bile acids concentration.

Epinephrine administration did not cause changes in RMS of PSD at ranges 0.04-0.08 and 0.6-1.5 Hz (Fig. 3). At range 1.6-2.5 Hz RMS of PSD significantly increased through 10 min and then returned to control values. ANOVA test results show influence of epinephrine ($F = 13.10$, $P = 0.0111$). It indicates fast epinephrine effect and could be related with electrical activity of liver sympathetic nerves either smooth muscles of blood vessels. Hypothetically electrical oscillation at this range could be provided by hepatocytes membrane potential changes. At

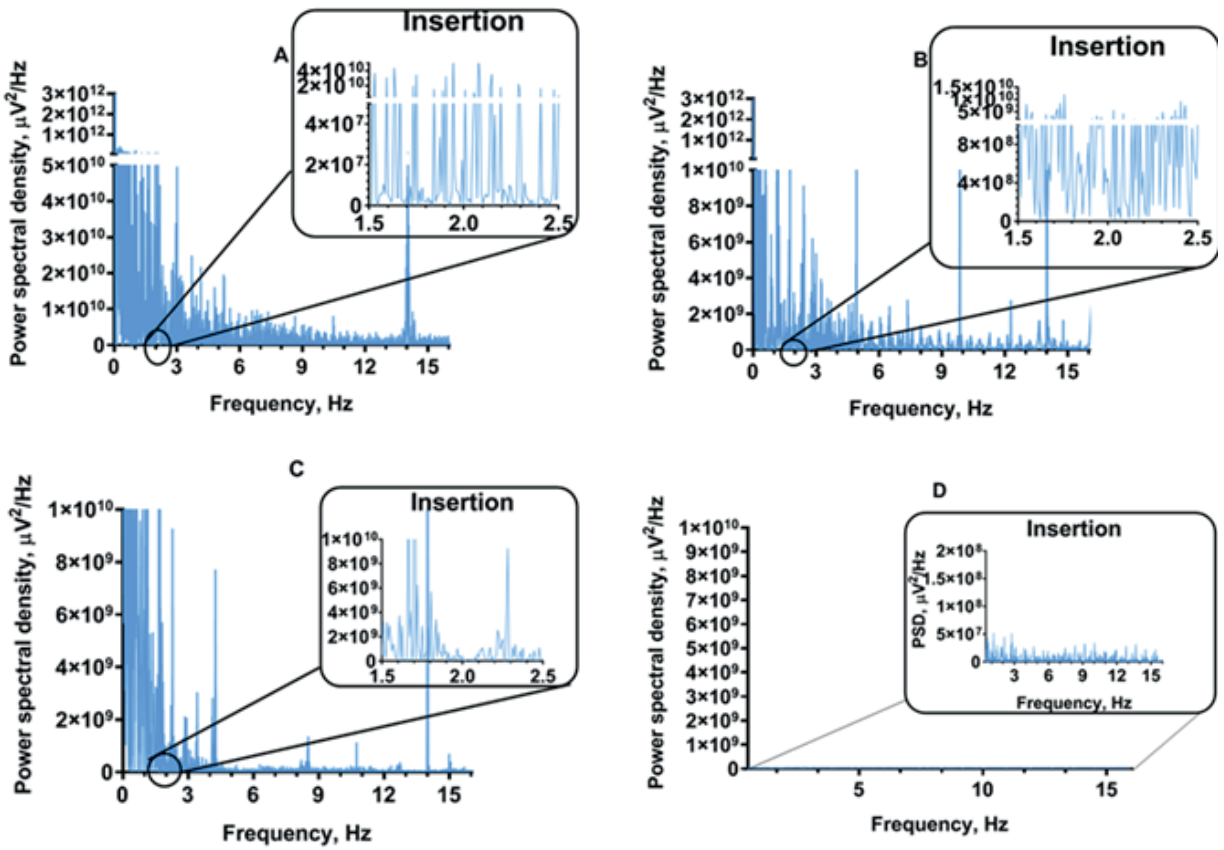

Fig. 2. Power spectral density in control animal after NaCl administration in control animal (A), 10 min after epinephrine administration (B), 20 min after epinephrine administration (C), and after KCl application (D). A range of 1.6-2.5 Hz was highlighted since it was most substantially changed due to epinephrine administration

Table 1. Correlational matrix between glucose concentration and PSD of liver electrical activity

Index	Frequency range	Spearman (r)	P
glucose concentration	0.04-0.08 Hz	-0.5421	0.0135
	0.6-1.5 Hz	0.6023	0.005
	1.6-2.5 Hz	0.3304	0.1547
	2.6-3.5 Hz	0.5063	0.0227
	3.6-4.5 Hz	0.5421	0.0135
	4.6-5.5 Hz	0.6982	0.0006
	5.6-6.5 Hz	0.2315	0.3262
	6.6-7.5 Hz	0.2558	0.2763
	7.6-8.5 Hz	0.5155	0.02
	8.6-9.5 Hz	0.2939	0.2085
	9.6-10.5 Hz	0.6632	0.0014
	10.6-11.5 Hz	0.5157	0.02
	11.6-12.5 Hz	0.1858	0.4329
	12.6-13.5 Hz	0.067	0.779
	13.6-14.5 Hz	-0.410	0.0722
	14.6-16 Hz	0.2269	0.3361

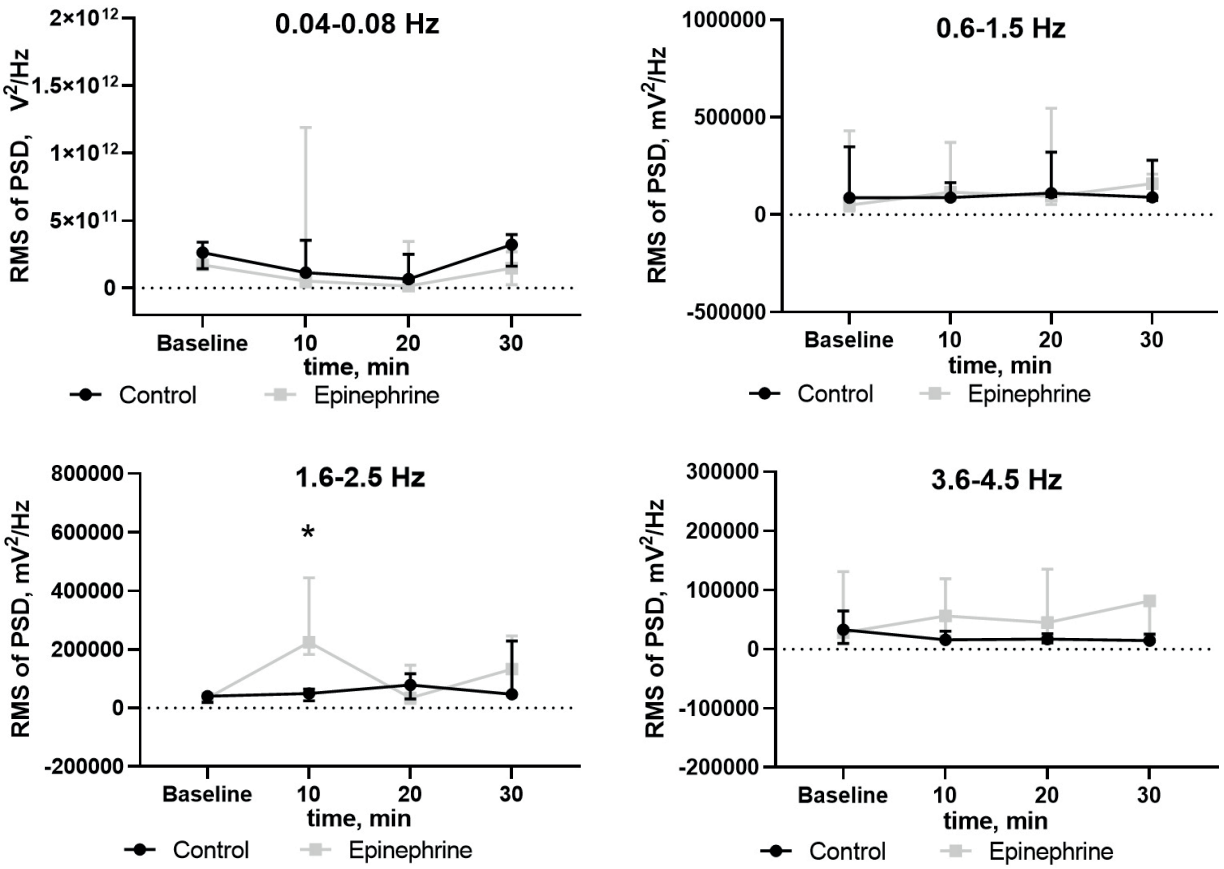

Fig. 3. Root mean square of power spectral density for different frequency ranges. (Median and interquartile range. *P<0.05 compared to control group)

higher ranges there were no significant changes of RMS of PSD between two groups but there was a trend to increasing PSD after epinephrine administration (Fig. 3). Our data suggest that liver electrical activity measured from its surface could indicate changes in functional state of the organ.

Epinephrine also caused changes in bile acid concentration. To analyze secretory possibility of liver conjugation ratio and hydroxylation ratio were calculated. There was a trend to decreasing conjugation ratio after epinephrine administration (Fig. 4). It suggests that there are individual peculiarities of epinephrine effects on conjugation process in hepatocytes. In contrast hydroxylation ratio significantly decreased after epinephrine administration (Fig. 4). Decreasing of hydroxylation ratio indices on activation of alternative pathway of bile acid synthesis which is initiated by mitochondrial sterol 27-hydroxylase (CYP27A1) [12]. It indirectly indicates increasing in hepatocytes mitochondria activity what could be caused by both direct and indirect (through blood vessels tone) effects of epinephrine. Accordingly increasing in PSD at 1.6-2.5 Hz range could be related with blood vessels muscles activity.

There was no correlation between RMS of PSD and conjugation and hydroxylation ratios after epinephrine administration. Correlation between RMS of PSD and conjugated bile acids concentration appeared at ranges 0.6-1.5, 2.6-3.5, 3.6-4.5 Hz ($r = 0.8857$, $P = 0.0333$, $r = 0.7658$, $P = 0.0443$, $r = 0.8763$, $P = 0.0291$, respectively). Also negative correlation between RMS of PSD and free bile acids concentra-

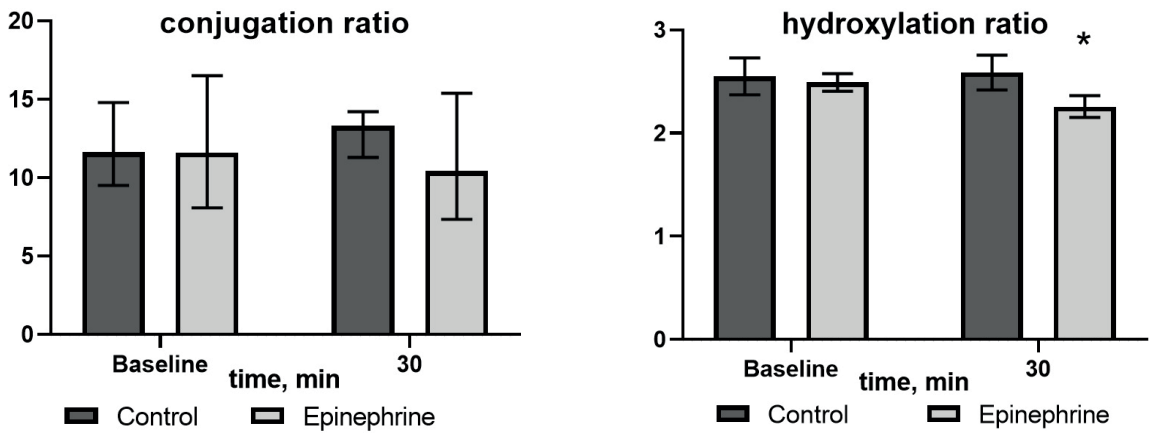

Fig. 4. Influence of epinephrine on conjugation and hydroxylation ratio. (Median and interquartile range, *P<0.05 compared to control group)

tion was shown at the same frequency ranges (r = -0.8986, P = 0.0278, r = -0.7858, P = 0.0333, r = 0.8963, P = 0.0365, respectively). It indirectly indicates influence of epinephrine on bile acid conjugation processes. Moreover, correlation between deoxycholates and trioxycholates concentration and RMS of PSD at frequency ranges 0.6-1.5, 2.6-3.5, 3.6-4.5 Hz was also shown (Table 2).

Therefore liver electrical activity at these frequency ranges is likely linked with bile acid concentration after epinephrine administration. A trend to increasing RMS of PSD at these ranges could indicate to intensification of hepatocytes electrical activity. Thus, electrical activity at ranges near 1 Hz and higher than 3 Hz could reflect secretory processes in the liver.

Blood glucose concentration significantly increased in 10 and 20 min after epinephrine administration (Fig. 5). Dynamics of glucose level was similar to RMS of PSD at 1.6-2.5 Hz frequency range (Fig. 3).

The correlation between the RMS of PSD and the blood glucose level observed in control disappeared upon epinephrine administration (the highest r value was 0.27 among all frequency ranges compared to ones in control (Table 1) and the correlation was not statistically significant in all ranges).

Thus, intensification of glucose secretion induced by epinephrine is likely to be a reason for vanishing the correlation.

Ergo, the liver electrical activity significantly changed due to action of epinephrine at range 1.6-2.5 Hz only and these changes were similar to blood glucose concentration dynamics. Yet there was no correlation between these two indexes. In other frequency ranges there was trend to increasing PSD after epinephrine administration which correlated with bile acids concentration unlike to control group. Herewith increasing PSD at frequency range 1.6-2.5 Hz

Table 2. Correlational matrix between bile acids concentration and PSD of liver electrical activity

Bile acids	Frequency range	Spearman (r)	P
Deoxycholates	0.6-1.5 Hz	0.8967	0.0385
	2.6-3.5 Hz	0.9276	0.0167
	3.6-4.5 Hz	0.8354	0.0213
Trioxycholates	0.6-1.5 Hz	0.8857	0.0333
	2.6-3.5 Hz	0.9125	0.0236
	3.6-4.5 Hz	0.7998	0.0417

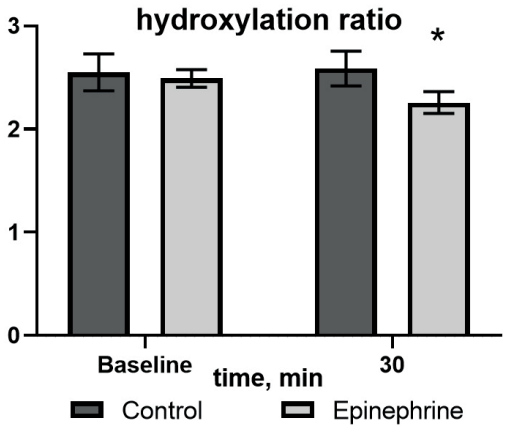

Fig. 5. Influence of epinephrine on blood glucose concentration. (Median and interquartile range. *P<0.05 compared to control group)

was short – 10 min only – and increasing PSD at other frequency ranges lasted a 30 min. A possible explanation for this phenomenon can be existence of a link between electrical activity at 1.6-2.5 Hz and liver blood flow. It was shown that liver parenchymal cells resting potential is dependent on oxidative metabolism [13]. Also liver electrical activity measured transcutaneously from gland's surface changed due to clamping of vena porta and arteria hepatica; those changes were not generated neither by smooth muscles of blood vessels or bile ducts nor by respiratory muscles [2]. Decreasing of hydroxylation ratio due to action of epinephrine which connected with hepatocytes mitochondria activation, could suggest changes in liver blood flow too. So, liver electrical activity could be dependent on its blood flow but that does not mean it is generated by very blood vessels. Therewith duration of electrical activity changes at other frequency ranges along correlation between electrical activity and bile acids concentration allow us to suppose a relationship between secretory process in hepatocytes and liver electrical activity. The latter one could suggest that the liver electrical activity at frequency ranges 3-10 Hz is generated due to changes in the membrane potential of hepatocytes. Our results are consistent with the data of other authors [3, 14]. However, a complete understanding of the sources of liver electrical activity requires further investigations.

CONCLUSION

1.Epinephrine causes increasing in liver electrical activity at frequency range 1.6-2.5 Hz.

2. There is correlation between the level of glucose concentration and the liver electrical activity in control but not during epinephrine administration.

3. Liver electrical activity could be dependent on its blood flow.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

П.К. Цапенко[1], Т.П. Лященко[2], С.П. Весельський[3]

ЕЛЕКТРИЧНА АКТИВНІСТЬ ПЕЧІНКИ ПІД ДІЄЮ АДРЕНАЛІНУ

[1]Bogomoletz Institute of Physiology of NAS of Ukraine, Kyiv; [2]Educational and Scientific Center "Institute of Biology and Medicine" Taras Shevchenko National University of Kyiv; [3]Chemical faculty of Taras Shevchenko National University of Kyiv; e-mail: p.tsapenko@biph.kiev.ua

Електричнй потенціал є інтегральним показником функціонального стану будь-якого органа, крім цього, його коливання відображають зміни функціонального стану при дії регуляторних чинників. Однією з переваг вимірювання електричної активності є неінвазивність (або мала інвазивність) цих методів. Нині природа електричної активності печінки та її залежність від систем нервової та гуморальної регуляції залишається нез'ясованою. Тож метою нашої роботи стало дослідити вплив адреналіну на електричну активність печінки та показники її функціонального стану: концентрацію жовчних кислот та глюкози в крові. Показано, що дія адреналіну спричиняла короткочасне зростання спектральної щільності електричного потенціалу в діапазоні 1,6–2,5 Гц, концентрації глюкози в крові та зниження коефіцієнта гідроксилювання. Зниження останнього вказує на активацію альтернативного (кислого) шляху утворення жовчних кислот і може бути пов'язане зі зростанням активності мітохондрій печінки. У зв'язку з цим, можливо,

що електричні коливання в діапазоні 1,6–2,5 Гц пов'язані зі змінами тонусу кровоносних судин печінки. Водночас спостерігалася тенденція до тривалого зростання спектральної щільності у діапазонах 0,6–1,5 та 2,6–10 Гц та зниження коефіцієнта кон'югації. Під дією адреналіну зникала кореляція між електричною активністю печінки та вмістом глюкози, що була встановлена для контрольної групи. На противагу цьому, під дією гормону посилювалася кореляція між електричною активністю та концентрацією основних фракцій жовчних кислот. Це вказує на чутливість електричної активності печінки до регуляторних впливів на секреторні процеси залози. Таким чином, електрична активність печінки відображає секреторні процеси в цій залозі. Визначення типу клітин, які роблять ключовий внесок у створення коливань електричного потенціалу, потребує подальших досліджень.

Ключові слова: печінка; електрична активність; спектральна щільність; жовчні кислоти; адреналін; глюкоза.

REFERENCES

1. Becker RO, Selden G The Body electric. New York: Morrow, 1985. 79-102.
2. Reinhart PH, Taylor WM, Bygrave FL. The mechanism of alpha-adrenergic agonist action in liver. Biol Rev Camb Philos Soc. 1984 Nov;59(4):511-57.
3. Lelou E, Corlu A, Nesseler N, Rauch C, Mallédant Y, Seguin P, Aninat C. The role of catecholamines in pathophysiological liver processes. Cells. 2022 Mar 17;11(6):1021.
4. Ianchuk PI, Vynohradova OO, Pasichnichenko OM, Kostenko SS Participation of eicosanoids in the mechanisms of contractile reactions of the portal vein to adrenaline and noradrenaline. Fiziol Zh. 2013; 59(6): 43-8.
5. Cheung PY, Barrington KJ. The effects of dopamine and epinephrine on hemodynamics and oxygen metabolism in hypoxic anesthetized piglets. Crit Care. 2001;5(3):158-66.
6. Goldstone TP, Crompton M. Evidence for beta-adrenergic activation of Na^+-dependent efflux of Ca^{2+} from isolated liver mitochondria. Biochem J. 1982 Apr 15;204(1):369-71.
7. David IG, Popa DE, Buleandra M. Pencil graphite electrodes: A versatile tool in electroanalysis. J Anal Method Chem. 2017;2017:1905968.
8. Chernuha IS, Reshetnik YM, Liashevych AM, Veselsky SP, Makarchuk MY. Bile acids from bile of rats of different sexes under testosterone. Regul Mech Biosyst. [Internet]. 2018Nov.4;9(3):396-00.
9. Chiang JY. Bile acid metabolism and signaling. Compr Physiol. 2013 Jul;3(3):1191-212.
10. Petersen OH. Electrophysiology of mammalian gland cells. Physiol Rev. 1976 Jul;56(3):535-77.
11. Shafik A. Study of the electric activity of the liver with identification of a normal 'electrohepatogram' in a canine model. Eur J Gastroenterol Hepatol. 1999 Nov;11(11):1239-43.
12. Valiathan MS, Topaz SR, Ballinger WF 2nd. Electrical activity of the canine liver. J Surg Res. 1967 Apr;7(4):186-7.
13. Shafik A. Study of the electric activity of the cirrhotic liver with identification of an electrohepatogram. Front Biosci. 1999 May 15;4:B5-8.

Received 16.01.2024