Non-invasive selective transcranial electrostimulation of the brain endorphinergic structures activates regeneration of hepatocytes and pancreatic beta-cells

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Abstract

It was earlier shown that the selective transcranial electrostimulation (TES) of endorphinergic brain structures, which we have developed, activated reparative regeneration of a number of damaged tissues including gastric and duodenal mucosa. In the present work, this action was shown to also activate efficiently the reparative regeneration of enterocyte derivatives — hepatocytes and pancreatic β-cells on experimental models in rats. The data obtained in the experiments were completely confirmed in clinical practice.

1. INTRODUCTION

Earlier it was shown that the selective trans-cranial electrostimulation (TES) of endorphinergic brain structures developed by us [1, 2] activated reparative regeneration of several damaged tissues including cells of gastric and duodenal mucosa [3] that are derivative of enterocytes — the primary gut epithelium.

The goal of the present work was to study a possibility that TES also could activate reparative regeneration of other enterocyte derivatives — hepatocytes and pancreatic β-cells, as these have common regeneration factors [4] and abundant opioid receptors [5].

2. METHODS

The experiments were carried out on Wistar male rats weighing 200-240 g. Acute lesion of hepatocytes was produced by a threefold daily peroral administration of 2 ml/kg 50% oil solution of dichloroethane (C₂H₄Cl₂) or carbon tetrachloride (CCl₄), with amounts approximately LD₅₀. For chronic poisoning, dichloroethane was administered per os 2-3 times a week for 4 weeks at a dose of 0.1 mg/kg in 50% oil solution.

TES was performed by the apparatus TRANSAIR manufacturing by TES Center and in the regime that we adapted for rats [6]. For the acute damaged rats, 3 daily TES sessions were performed 72 hr after the last poisoning day. In the rats with chronic poisoning, TES was performed 3 times a week by alternating with days of poisoning. The substances necessary for analysis of the mechanisms of the TES effect were injected intraperitoneally (naloxon – 2 mg/kg, D-leucine – 350 mg/kg, “Essentiale” – 50 mg/kg). Blood and liver samples were taken after euthanasia with Pentobarbital sodium (70 mg/kg). The main part of biochemical parameters was determined using an FP-901M analyzer. For histological control of adipose degeneration after poisoning, light microscopy sections were stained with sudan, the degree of growth interlobular connective tissue after chronic poisoning was evaluated with van Gieson staining. To determine activity of mitotic process after a partial hepatectomy, historadiography with methyl-3H-thymidine was used. Effect of TES on excess of malignization was determined by action on the growth of implanted hepatoma-27.

Models of diabetes were produced in rats by a single ip administration of alloxan (100 and 150 mg/kg) and streptozotocin (40 and 50 mg/kg). Glucose level was measured by glucose oxidase method in the blood taken from tail vein. Blood insulin level was determined by immunoenzyme method (Rat Insulin Elisa, Germany). TES sessions were performed 3 times daily, the other day after administration of the diabetogenic substances. Other substances were administered intraperitoneally (naloxon – 2 mg/kg) or the sugar-lowering substances per os (methforminum – 50 mg/kg).

Morphological changes of β-cells in Langerhans’ islands were determined in sections of pancreas stained with hematoxylin-eosin, while restoration of their insulin production — sections stained with paraldehyde fuchsin and with Gomori’s mixture.

In all experiments, control was the complete imitation of experiment with TES, but without turning on the current.

3. RESULTS

1.1. Hepatocytes and TES

In the rats survived after acute poisoning, 17 parameters of liver function were studied. They could be combined in three type.
degree of hepatocyte cytolysis was evaluated from levels of alanine and aspartate aminotransferases, alkaline and acid phosphatases, lactate dehydrogenases, and ceruloplasmin. To estimate synthetic function, the total protein, total lipids, cholesterol, liver glycogen, blood glucose, cholesterol, and blood bilirubin were determined. The detoxicational liver function was determined from the rate of change of liver of introduced bromosulphaleine, time of the duration of Hexobarbital sodium sleep, and thymol test. Besides, the glutathione and of cytochrome P_{450} and B_{5} level were measured in liver tissue.

In experiments with acute poisoning, as soon as at the 10th day after its end and three TES sessions, an obvious, statistically significant normalization of all three types of biochemical parameters were revealed. For instance, the alanine aminotransferase level in control amounted to 0.2 ± 0.03 µkat/l, in animals after poisoning – 1.62 ± 0.11 (an increase by more than 800%), while in the TES-treated animals at the same time period, to 0.32 ± 0.06 (an increase only about 160%). The aspartate aminotransferase level even descended below the norm, while the levels of alkaline and acid phosphatases, lactate dehydrogenase, and ceruloplasmin became normal.

In sudan-stained liver tissue histological sections of the non-treated animals, pronounced adipose degeneration (the presence of large fat droplets) was revealed in all hepatocytes. At the same time, after the TES course, practically no signs of adipose degeneration were seen in hepatocytes. A comparison has shown that restoration of hepatocyte function was more pronounced after effect of TES than under action of “Essentiale” – the standard hepatoprotective agent.

TES not only restored function of damaged hepatocytes, but also stimulated their regeneration. This was established in experiments with a partial hepatectomy from the value of mitotic index determined in historadiograms with methyl-3H-thymidine and acceleration of restoration of liver mass.

Since the excess of mitotic division can be dangerous due to subsequent dysplasia, metaplasia or even carcinogenesis, effect of TES on implantation and growth of hepatoma-27 was studied. In the most cases, no implantation has been established to occur. In the cases of implantation the hepatoma mass accounted only for 15% of the tumor mass in control animals at the moment of their hepatoma-produced death. Whereas in the tumor sections in control, obvious signs of adenocarcinoma were seen, in the experimental group the main part of the tumor mass was replaced by connective tissue.

It is important to note that at the chronic dichloroethan poisoning, it was possible not only to maintain, using TES, normal functional activity of hepatocytes, but also not to increase expression of the interlobular connective tissue, i.e. the use of TES did not produce a danger of development of cirrhosis.

All the revealed positive TES effects with respect to liver function in rats after poisoning with chlororganic substances were eliminated on the background and after block of µ-opioid receptors with naloxon and increased markedly after administration of D-leucine, an inhibitor of enkephalinase. These facts indicate participation of endorphinergic mechanisms of the TES reparative effects.

### 1.2. Beta-cells and TES

In the cases of experimental diabetes produced by alloxan or streptozotocin, after three TES séances, a fast decrease of blood sugar to the initial norm began, but without signs of subsequent hypoglycemia. The TES effect was eliminated by naloxon, which indicates its endorphinogenic nature.

Changes of morphological structure of Langerhans’ islands composed predominantly of insulin-producing β-cells (up to 75%, according to [7]) showed obvious signs of degeneration after administration of alloxan and streptozotocin. The island structure in animals after three TES sessions at the 15th observation day, on the background of the complete normalization of glucose level, practically did not differ from norm. Besides, formation of new islands of small diameter was noticed, which indicates their formation de novo from cells-progenitors.

The Gomori staining revealed in β-cells of the experimental group animals a statistically significant restoration of granularity – the sign of insulin production. Thus, the intensive and moderate granularities were observed in 65.0 ± 3.5% and 33 ± 4.1% of normal β-cells (i.e. about 98%), respectively, whereas after the diabetogenic drugs - 0.6 ± 0.1% and 15.0 ± 2.0% (about 15.6%), respectively. In the TES-treated animals these values were much higher and amounted to 24 ± 1.5% and 58 ± 4.1 (about 82%), respectively, and approached norm.

Use of immunoenzyme method allowed determination of blood insulin level in parallel with blood glucose. With equal initial glucose level (3.6–3.7 mmol/l), at the 15th day after
administration of alloxan the glucose level in control amounted to 12.2 ± 0.71 mmol/l, while in the TES-treated rats, to 4.0 ± 0.16 mmol/l. With equal initial blood insulin level (about 3.6 µg/l), it reached in untreated animals about 0.16 ± 0.014 µg/l, while after TES – 0.27 ± 0.02 µg/l (p < 0.05). The glucose and insulin levels correlated negatively (in control r = −0.987, in experiments r = −0.912).

The antidiabetic TES effect enhancing insulin production was potentiated by metmorphinum, whose action consists in acceleration of beta-cell function and activates processes of reparative regeneration of these ones. TES has been proved to activate these processes namely with participation of endorphin, although a concrete mechanism of this effect has not yet been known so far.

It is important to note that this effect, as we have shown earlier, also promotes reparative regeneration of skin epithelium, myocardium scar tissue after acute myocardial infarct, afferent and efferent nerve fibers. From this, it follows that the endorphinergic mechanism stimulating reparation is sufficiently universal [3, 8].

Data of the present experiments begin to be applied in clinical practice. As a way of monotherapy, TES was used with a good result for treatment of adipose hepatoses, chronic hepatitis, and liver cirrhosis. The best positive results being achieved at treatment of alcoholic cirrhosis. Like in experiments, it was shown that after TES therapy (10 daily sessions) there was a marked decrease of cytolysis and lipid peroxidation, an increase of parameters of antioxidant protection and improved of liver blood circulation.

The TES effects begin to be studied in patients with diabetes I and II. At any rate, it was established that TES increased insulin production on volunteers. The first observations have shown that TES also can be effectively used in complications of diabetes – neuropathy and ulcerative processes.

In conclusion, it is to be emphasized that TES, due to its endorphinergic action, produces a complex and systemic homeostatic effect by increasing the quality of life parameters in treating of patients with various pathologies. The several models of TRANSAIR’s adapted to home, outpatient clinics and hospitals are manufacturing by TES Center of the Pavlov Institute of Physiology in St.-Petersburg and broadly used in Russia [9].

References

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