# CONNECTION BETWEEN WITHDRAWAL ANALGESIA AND DEVELOPMENT OF HEART DISTURBANCES IN RATS WITH AN ETHANOL WITHDRAWAL SYNDROME

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**Key Words:** ethanol withdrawal syndrome; analgesia; heart damage.

The ethanol withdrawal syndrome is accompanied by activation of the sympathicoadrenal system [7, 11], by lowering of sensitivity to pain [2, 4], and by the development of heart damage [5]. The leading role of catecholamines in the pathogenesis of heart damage during withdrawal [6] and in the mechanism of release of opioid peptides, inducing the development of analgesia [8, 10], suggests the existence of correlation between withdrawal analgesia (WA) and heart damage associated with the ethanol withdrawal syndrome.

The aim of this investigation was to look for correlation between withdrawal analgesia and the development of heart damage as part of the ethanol withdrawal syndrome in rats.

## EXPERIMENTAL METHOD

Experiments were carried out on 42 male Wistar rats aged 3 months. Ethanol, as a 25% solution, was injected into the stomach twice a day (at 9 a.m. and 9 p.m.) in a daily dose of 7-10 g/kg body weight for 5.5 days. Before alcohol administration and 20-24 h after the last dose of ethanol (the period of maximal severity of the withdrawal syndrome) the latent period of pain was determined using the "tail flick" (TF) and the "hot plate" (HP) methods. The temperature of the plate was 55 ± 1°C. The WA index was calculated by the equation:

where $T_a$ denotes the latent period of pain after withdrawal of ethanol (in sec); $T_b$ the latent period of pain initially (in sec), and 10 or 210 the maximal duration (in sec) of thermal stimulation of the tail or paws, respectively. The intensity of the withdrawal syndrome was estimated visually, using a weighted total of points. The presence or absence of individual manifestations of the withdrawal syndrome was determined and, depending on their severity, they were combined into three groups, with four symptoms in each group. The presence of one symptom in groups 1, 2, and 3 was assessed as 1, 2, and 3 points, respectively [1]. The rats were anaesthetized with urethane 3 days after the last injection of ethanol, heparin was injected, and the heart was removed and perfused through the aorta with Krebs–Henseleit solutions containing 1% gelatinal [5], under a pressure of 70 mm Hg. The pH of the solution when saturated with carbogen (95% $O_2$ and 5% $CO_2$) and at a temperature of 37°C was 7.4. After 10 min of continuous perfusion the heart was switched over to reperfusion, with a volume of 35 ml of recirculating solution, and this continued for 30 min. The pressure in the left ventricle was recorded by means of an electromanometer ("Statham," USA) on a "Biomedica" polygraph. The peak systolic pressure (PSP), the rate of contraction (RCH) and relaxation of the heart (RRH), designated $dp/dt_{syst}$ and $dp/dt_{diast}$, respectively, and the tension time index (TTI) of the myocardium, by the equation PSP × HR × $T$/1000, where $T$ denotes the time of the period of tension of the left ventricle, were estimated. Creatine phosphokinase (CPK) activity was determined in the perfusion fluid [3]. Instead of ethanol, control animals received water. The results were subjected to statistical analysis by Student's test and by Kendall's rank correlation method on a CM-1600 computer.

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TABLE 1. Parameters of Contractile Function and Rate of Flow of CPK from Isolated Heart of Rats after Ethanol Withdrawal Syndrome

| Group of animals | PSP, mm Hg | TTI (mm Hg × sec/min × 1000) | RCH, mm Hg/sec | RRH, mm·Hg/sec | Rate of flow of CPK, units/g dry weight of tissue/30 min |
|---|---|---|---|---|---|
| Control | 155±2 | 2,64±0,06 | 2120±43 | 1299±32 | 0,772±0,102 |
| Experimental | 131±2* | 2,03±0,06* | 1714±39* | 1035±22* | 3,110±0,382* |

**Legend.** *$p < 0.05$ compared with control. Here and in Table 2: PSP) Peak systolic pressure, TTI) Tension time index, RCH) Rate of contraction of heart, RRH) Rate of relaxation of heart, CPK) Creatine phosphokinase.

TABLE 2. Correlation between Parameters of Withdrawal Analgesia and Parameters of Activity of Isolated Heart of Rats Having Experienced the Alcohol Withdrawal Syndrome (Kendall's Rank correlation coefficient — $\tau$)

| Index of withdrawal analgesia | Parameter of cardiac activity | | | | |
|---|---|---|---|---|---|
| | PSP | TTI | RCH | RRH | CPK |
| TF («tail flick») | —0,272** | —0,262** | —0,367** | —0,321** | —0,410** |
| HP («hot plate») | — | — | —0,207* | —0,293** | —0,208* |

**Legend.** Asterisks indicate significance of differences: *$p < 0.05$, **$p < 0.01$.

## EXPERIMENTAL RESULTS

The withdrawal syndrome reached maximal intensity in the rats 20-24 h after the last injection of ethanol: disturbance of behavior, hyperexcitability, muscular hyperreactivity (general estimate 6.4 ± 0.5). During this period the lowering of sensitivity to pain was manifested as an increase in the latent period of TF and of the response to pain by the HP method. In the control the latent period of the response to pain by the TF and HP methods was 26 ± 0.1 and 43.1 ± 3.2 sec, respectively. During development of the withdrawal syndrome the latent period of TF increased to 3.5 ± 0.2 sec (WA index 0.124 ± 0.032). The latent period of the response to pain in the HP test exceeded the maximal duration of stimulation (210 sec) in 18 of the 42 rats, and in the rest it amounted to 96.1 ± 8.1 sec (WA index 0.251 ± 0.039). Correlation between the WA indices, determined by the TF and HP methods, could not be found. The pain reflex, tested by the HP method, unlike the TF method, involved the participation of the higher levels of the CNS, and its inhibition may have been connected with the multicomponent effect of the neurotransmitter systems tested. The possibility likewise cannot be ruled out that the marked analgesia discovered by means of the HP test could be explained by the presence of muscular rigidity in the animals and by difficulty of coordination during the performance of a motor act as complex as licking the hind limbs.

Comparison of the severity of the withdrawal syndrome and WA revealed positive correlation between the severity of the animals' condition and the WA index, estimated by the HP method ($\tau = +0.343$, $p < 0.01$). No correlation was found with the WA index estimated by the TF method.

During perfusion of the heart rats which had experienced a withdrawal syndrome, a marked decrease of PSP, RCH, RRH, and TTI and an increase in the rate of flow of CPK were observed (Table 1). The results of correlation analysis (Table 2) showed the presence of negative correlation between the WA index, estimated by the TF method, and all parameters of disturbance of cardiac activity. Correlation between the WA index, estimated by the HP method, and parameters of cardiac activity was weaker.

The results of this investigation confirmed the view that correlation exists between WA and heart damage developing in the ethanol withdrawal syndrome. In animals with the most marked WA heart damage was minimal, and vice versa. Consequently, the parameter WA can be used as a prognostic test of the probability and degree of damage to heart muscle in the ethanol withdrawal syndrome.

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# MORPHOLOGICAL ANALYSIS OF CHANGES INDUCED IN THE LUNGS AND KIDNEYS BY ACETYLGLYCERYL PHOSPHORYLCHOLINE ESTER AND LIMITED BY VERAPAMIL


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**Key Words:** platelet activation factor; thrombosis; lungs; kidneys; verapamil.


The acetylglyceryl ester of phosphorylcholine (1-alkyl-2-acetyl-sn-glycero-3-phosphocholine), known in the literature as platelet activation factor (PAF), plays an important role in inflammation, shock, and disturbances of hemostasis. Although discovered initially as a mediator of IgE-dependent anaphylaxis, it is probably a universal signal for activation of cells and an endogenous mediator of damage in various pathological processes, proceeding on an immune basis [1, 2]. Investigations in vitro have shown that the mechanism of cellular activation by PAF is connected with the rapid opening of calcium channels and a change in the intracellular calcium homeostasis, followed by the release of secondary mediators [4]. PAF has a varied systemic and local action, for it can induce hypotension, and can increase vascular permeability and edema, intravascular aggregation of platelets and neutrophils, chemotaxis, and tissue infiltration by monocytes [8].

In experiments with a single intravenous injection of purified PAF, characteristic shock reactions developed after doses of between 100 and 2000 ng/kg, and their intensity depends on the dose, species, and age of the experimental animals [5, 6]. Morphological and functional changes in the myocardium, lungs, and kidneys in response to small and average doses of PAF are reversible. The blood level of secondary mediators of inflammation and of clotting factors returns to normal under these circumstances; platelets and neutrophils, aggregated and sequestered in the microcirculatory bed, become taken up in the systemic blood flow. Large doses of this mediator lead to death of the animals from severe circulatory disorders [5]. Because of these properties of PAF, changes within target organs in response to long-term exposure to PAF in vivo still remain unexplained. The involvement of calcium channels in the realization of this action likewise has not been adequately studied.


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