as no significant changes in enzyme activity were found.

On the other hand, in a concentration of $10^{-3}$ M hydrocortisone induced an increase in the potential for enzymatic degradation of substrates (both proteins and carbohydrates) in the derma of females and males. In the epidermis an increase in both the free and total activity of cathepsin D, though without a change in the fraction of the latter, was also registered.

The unequivocal effect of pharmacological doses of hormone (respectively, 1 mg per 100 g body weight *in vivo* and $10^{-3}$ M *in vitro*) on the lysosomes, regardless of the sex of rats, seems to evidence a direct interaction of hydrocortisone with the lysosomal membrane [2]. Differences in hormone-induced enzymatic changes in derma and epidermis may result from: a) differences in the functional organization of the lysosomal membranes in these skin compartments (the presence or absence of hormone receptors, their specificity, etc.) and b) differences in the composition and/or hormone-dependent "transitions" of the lysosomal membrane phospholipids, which are known to effect the function of membranes and cells [6]. Validation of one or another hypothesis and, consequently, the gathering of new information about the mechanisms underlying the side effects of endocrine therapy call for further detailed study.

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# Role of Nicotinic Acid and Its Analogs in the Regulation of Hemostasis

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According to numerous data in the literature, nicotinic acid (NA) and its precursor pyridine produce a complex effect on hemostasis [6,8,12]. They have been shown to be able to increase the fibrinolytic and thrombolytic activity of the blood [3], to reduce platelet aggregation [1,8], and to suppress thromboxane synthesis [9].

Despite NA belonging to the thrombolytics, its wide application in hemostasiology is restricted, because it has no effect on the activity of a number of the most important enzymes regulating platelet formation, such as cyclooxygenase and prostaglandin

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TABLE 1. Effect of NA, Nicomorpholine, and Asethylnicotinate on Platelet Aggregation Induced by ADP ($M \pm m$, $n=6$)

| Substance | Dose, mg/kg | Changes of platelet aggregation, % decrease of optical density | | |
|---|---|---|---|---|
| | | control | 60 min postinjection | 4 h postinjection |
| NA | 20 | 62.9±1.8 | 45.1±1.1* | 60.6±1.4 |
| | 50 | 66.1±1.4 | 46.1±2.0* | 64.2±1.8 |
| Nicomorpholine | 10 | 65.2±2.1 | 35.3±0.9* | 49.3±1.1* |
| | 20 | 66.3±1.2 | 27.8±0.6* | 41.1±1.2* |
| Asethylnicotinate | 10 | 64.7±1.1 | 39.3±1.2* | 53.8±1.2* |
| | 20 | 63.9±1.8 | 31.4±0.7* | 57.8±1.5 |

**Note:** Here and in Tables 2–4: *) $p<0.05$.

synthetase. The effect of NA on the prostacyclin-generating function of the vessel wall is of great importance. The possible stimulation of the synthesis of prostacyclinlike substances justifies the search for new antiaggregants, among the pyridine derivatives in particular.

The aim of the present study was to discover active inhibitors of platelet aggregation and prostacyclin inducers synthesized by the method of construction of the pyridine molecule [2]. The ability of novel agents to suppress platelet aggregation and thromboxane $A_2$ formation was assessed by measuring the level of malonic dialdehyde (MDA), the cAMP and cGMP content, as well as the level of free and membrane-bound calcium in the platelets.

## MATERIALS AND METHODS

NA and its novel derivatives nicomorpholine and asethylnicotinate, representing ethyl esters of pyridinediacetic acid with substitutes in the β- and γ-positions were tested. The experiments were carried out on rabbits weighing 2.5-3 kg and on rats weighing 160-220 g. Platelet aggregation induced by the test substances was determined as described [10], the anti-aggregating activity of the vessel wall was studied by MacIntyre's method [14] in a modification [4], the MDA concentration in platelets was determined according to [15], the cAMP and cGMP level was measured by Smith's method [11], and the concentration of free and membrane-bound calcium was measured according to [13]. NA and its derivatives were injected intravenously in equimolar doses (10-50 mg/kg) by the routine scheme applied under clinical conditions.

The results were subjected to statistical analysis using Student's $t$ test.

TABLE 2. Effect of NA, Nicomorpholine, and Asethylnicotinate on Vessel Wall Anti–Aggregating Activity Reduced by Adrenalin ($M \pm m$, $n=10$)

| Substance | Dose, mg/kg | Anti–aggregating activity of aorta wall, % |
|---|---|---|
| Control | | 71.8±1.2 |
| Adrenalin | 0.02 | 32.2±3.3 |
| Adrenalin + NA | 50 | 63.4±1.1* |
| Adrenalin + nicomorpholine | 20 | 68.0±1.7* |
| Adrenalin + asethylnicotinate | 20 | 70.2±0.7* |

## RESULTS

NA and its derivatives significantly reduced the optical density of platelet-rich plasma, this being evidence of the anti-aggregating properties of the test substances. One hour postadministration, NA itself (50 mg/kg) induced a decrease of the optical density percentage in the plasma (from 66 to 46%). Within the same period its analogs nicomorpholine and asethylnicotinate (20 mg/kg) reduced the optical density to 27 and 31%, respectively. The dynamics of aggregation is given in Table 1. The effect of NA was observed somewhat earlier but was short-term, while the effect of the other preparations was more significant and prolonged (lasting four hours and longer).

No effect of the test substances on the anti-aggregatory properties of the normal vessel wall was discovered in the second series of experiments, carried out on intact animals. However, the new preparations potentiated the functional recovery of vascular endothelium of the aorta wall during simulation of adrenalin-induced disturbances of its anti-aggregating activity (Table 2). The ability of asethylnicotinate to restore the activity of the vessel wall exceeded that of the other two preparations.

When used in the doses inhibiting platelet aggregation, NA and its analogs studied in the thrombotest were shown to inhibit thromboxane $A_2$ synthesis. At the same time, the MDA concentration compared to the basal level fell by one half. The nicomorpholine-induced decrease of thromboxane $B_2$ percentage was more significant than that of the other preparations.

TABLE 3. Effect of Nicomorpholine and Asethylnicotinate on Thromboxane $B_2$ and Cyclic Nucleotide Levels ($M \pm m$, $n=6$)

| Substance | Dose, mg/kg | Thromboxane $B_2$ level, mg/3×10⁸ cells | Nucleotide level, nmole/3×10⁸ cells | |
|---|---|---|---|---|
| | | | cAMP | cGMP |
| Initial level | | 720±26 | 16±2.3 | 1.4±0.05 |
| Nicomorpholine | 1.0 | 240±19 | 60±2.9 | 5.8±0.4 |
| Asethylnicotinate | 1.0 | 480±23 | 29.2±4.0 | 3.6±0.28 |

The function and mechanism of the decrease of the thromboxane balance were assessed by studying the changes of cyclic nucleotide levels. The results showed that the anti-aggregating activity of the test substances corresponded to the increase of the cAMP and cGMP concentration. Nicomorpholine and asethylnicotinate were shown to increase the platelet nucleotide levels 2.5 times on the average (Table 3).

Changes of the platelet free and membrane-bound calcium levels were also observed. On the average, nicomorpholine and asethylnicotinate, when injected in a dose of 20-50 mg/kg, reduced the calcium content from 3.6 to 0.12 and from 3.8 to 0.24 mmole/3×10⁸ cells, respectively (Table 4). The level of membrane-bound calcium, determined according to the variation of chlorotetracycline fluorescence, under the influence of nicomorpholine and asethylnicotinate fell from 100% in the control to 17.4 and 75.8%, respectively, ($p<0.001$), i.e., the substances demonstrated an antagonism in relation to both free and membrane-bound calcium.

TABLE 4. Effect of Nicomorpholine and Asethylnicotinate on Total Platelet Calcium Level ($M \pm m$, $n=8$)

| Substance | Dose, mg/kg | Platelet calcium level, mmole/3×10⁸ cells | |
|---|---|---|---|
| | | initial level | 30 min postinjection |
| Nicomorpholine | 10 | 3.57±0.03 | 0.42±0.03* |
| | 20 | 3.60±0.03 | 0.12±0.02* |
| Asethylnicotinate | 10 | 3.87±0.04 | 0.66±0.02* |
| | 20 | 3.72±0.04 | 0.24±0.02* |

Analysis of the results suggests that NA and, to a greater degree, its analogs nicomorpholine and asethylnicotinate are able to suppress platelet aggregation and to inhibit thromboxane $A_2$ synthesis, this being manifested in the fall of both the MDA concentration and the content of stable thromboxane $A_2$-thromboxane $B_2$. It is known that during the induced aggregation the activation of "arachidonic acid cascades" occurs together with the formation of prostaglandin endoperoxides [5,6,9]. In turn, the latter and the transformed prostacyclin potentiate membrane stabilization and suppress the biosynthesis of thromboxane $A_2$ (a proaggregator and a powerful vasoconstrictor). Thus, prostacyclin and thromboxane are antagonists of the same process, this attesting to the presence of a "balance" between these two metabolites [7,8]. The investigated preparations caused a shift of this "balance" in the direction necessary for an antithrombotic effect. The increase of the level of both cyclic nucleotides under the influence of nicomorpholine and asethylnicotinate is, in our opinion and according to other data [1,8], evidence that these preparations stimulate prostacyclin-generating function of the vessel wall and thereby its anti-aggregating activity. The substances were shown to induce a decrease of both intracellular and membrane-bound calcium. This effect can be achieved in diverse ways, for example by intensifying the passage of $Ca^{2+}$ from the cytoplasm into the platelet organelles. An effect of the same kind is typical of prostaglandins, dipyridamole, cAMP, etc. [12]. Another way is platelet aggregation blocking by such calcium antagonists as verapamil, nifedipine, etc. [7]. Here, a decrease of the calcium concentration in the cytoplasm leads to inhibition of thrombostenin contractility and reduced synthesis of thromboxane and some proteases. All these reactions constitute the essence of the process of platelet pro- and anti-aggregation.

Thus, highly active inhibitors of thromboxane formation were found among the nicotinic acid derivatives capable of enhancing the anti-aggregating properties of the blood and vessel wall owing to prostacyclin generation. This phenomenon is accompanied by a decrease of the intracellular calcium content and an increase of the cyclic nucleotide content. The indicated effects of nicomorpholine and asethylnicotinate suggest their possible application for the prevention and treatment of thromboembolic diseases and for the improvement of the microcirculation in the blood vessels including the cerebrovascular circulation.

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# Cardioprotective Effects of Adaptogens of Plant Origin

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Adaptogenic preparations of plant origin (henceforth termed adaptogens) are finding increasingly wide application in the systematic treatment of various diseases [1,5]. It is known that the preadaptation of experimental animals (their exposure to certain physical effects) can to a considerable extent prevent stress-induced and ischemic damage to the heart [3,4]. There are grounds for assuming that adaptogens of plant origin can effectively protect the heart from stress-induced damage [1,5]. This subject, however, has been little studied.

In the present investigation the effect of plant adaptogens (extracts of *Rhodiola rosea, Eleuterococcus,* and *Leuzea carthamoides,* and p-hydroxyphenethyl alcohol) was studied on animals subjected to stress. Since stress-induced damage to the heart is adrenergic in nature, we studied the effect of the above-mentioned preparations with respect to cyclic nucleotides (CN) as markers for determining the state of the autonomic innervation of the heart.

## MATERIALS AND METHODS

Experiments were carried out on 200 male rats weighing 180-200 g. The cardioprotective effect of the above-mentioned preparations, which were given to the experimental animals before they were subjected to stress, was investigated with the aid of an emotional-painful stress model (EPS) [8]. Officinal extracts of *Rhodiola rosea, Eleuterococcus,* and *Leuzea carthamoides* were administered through a gastric tube (course of treatment 8 days; a single dose of 1 ml/kg) [1,5]. p-hydroxyphenethyl alcohol (a biologically active substance isolated from *R. rosea*) was injected intramuscularly (a single dose of 20 mg/kg) [5]. For purposes of comparison we used a widely known adaptation method whereby the animals were subjected to brief periods of immobilization according to the following scheme [3]: the animals were made to

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