The influence of nimodipine on cerebral and general hemodynamics in rats under condition of hypokinesia

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The aim of the study was to reveal the influence of nimodipine on cerebral and general hemodynamics at different periods of hypokinesia (HK). The gauging of parameters was carried out under general anesthesia before and at the third minute after intravenous injection of three doses of nimodipine (1, 3 and 10 μg/kg). The local cerebral blood flow (lCBF) was measured with laser-Doppler flowmetry probe placed on the parietal cortex. In the 15-day HK indices did not differ from the control group, only mean the arterial blood pressure (MABP) and resistance of cerebral vessels (CVR) were smaller when the highest dose was used. In the 30- and 45-day HK a statistically significant increase of lCBF and a decrease of CVR and MABP was observed. These changes were more expressed in the 45-day HK, and in the 60-day HK some stabilization of these parameters was noted. Most vividly expressed augmentation of HR, in comparison with the control is revealed at the dose of 10 μg/kg on the 30th day of HK. The ΔHR/ΔMABP ratio was decreased with augmentation of term of the HK, achieving its minimum by the 45th day. However, at the dose of 10 μg/kg in 60-day HK this ratio decreased even more.

It has been concluded that HK was able to change the sensitivity to the cerebrovasoselective calcium channel blocker – nimodipine.

Keywords: nimodipine, hypokinesia, cerebral blood flow, calcium antagonist, rat

At the beginning of the new millenium the development of society is characterized by the restriction of movement activity, which is considered as a serious stress and risk factor in the development of cardiovascular diseases (32). Hypokinesia leads to significant morphological disorders of the brain (45, 46), to negative dynamics of the fluid-coagulant equilibrium of the blood (17), and electrolyte and gas composition of
the blood (4). Experimental and clinical data indicate that hypokinesia should be regarded as a serious risk factor stimulating pathological alterations in the visceral organs.

Hypokinesia has one more aspect – such as a space medicine problem. It has been known for many years that weightlessness induces changes in numerous physiological systems: the cardiovascular system declines in both aerobic capacity and orthostatic tolerance; there is a reduction in fluid and electrolyte balance, hematocrit, and certain immune parameters; bone and muscle mass and strength are reduced; various neurological responses include space motion sickness and posture and gate alterations. These responses are caused by the hypokinesia of weightlessness, the cephalic fluid shift, the unloading of the vestibular system, stress, and the altered temporal environment (41).

Numerous investigators are using laboratory rats to study the influence of hypokinesia on mammalian organism, because modeling of hypokinesia on this species is easy to perform (17, 29). Definitely, results obtained in rats cannot correlate fully with expected results in humans, but they could show preliminary expected shifts in humans.

Proceeding from this assumption, investigations along the line should be persevered in order to contribute to the general theory of key reactions of organism to hypokinesia, and search for preventive means and methods to be applied both to space medicine and public health (33). Our department has been studying this problem during the recent years. A special attention was devoted to the study of shifts of cerebral blood flow and metabolism in hypokinesia. Particularly ischemic alterations of the brain were observed. We have been making attempts to find out ways to prevent and treat pathological shifts occurring in hypokinesia using GABA-ergic substances (1, 19–21). Our investigations showed the intensification of contractile responses of rat isolated vessels to norepinephrine and dopamine and involvement of the endothelium in these processes (3). From this point of view, we think, it is important to determine whether hypokinesia changes sensitivity to cerebrovasoselective calcium channel blocker of 1,4-dihydropyridine group – nimodipine as it is widely used under pathological conditions in human to prevent and treat the delayed vasospasm due to subarachnoid hemorrhage, migraine attacks or ischemic stroke (26, 36).

The aim of the study was to reveal the influence of 1,4-dihydropyridine derivant – nimodipine on cerebral and general hemodynamics at different periods of hypokinesia in rats. There are at least two reasons for this study: first, restriction of movement activity (hypokinesia) is a very common concomitant factor in angioneurological practice, and, second, hypokinesia itself results in ischemic shifts which, perhaps, need to be corrected with cerebrovasoselective vasodilators.
Materials and Methods

Substances

The materials used in the experiments were of the following origin: sodium pentobarbital (Clin-Midy, France), α-chloralose (Fluka, Switzerland), lidocaine hydrochloride (Sopharma, Bulgaria), Heparine (Leo, France), pipecuronium bromide (Arduan, Gedeon Richter, Hungary), nimodipine (Nimotop, Bayer AG, Germany).

Animals and care

The experiments were performed on 53 mongrel albino rats of either sexes weighting 190–210 g. The control group of rats (n=14) was placed under ordinary vivarium conditions (37) with a 12:12 light-dark cycle. Food and water were given *ad libitum*. The experimental groups were kept in individual narrow (450 cm³) plexiglas cages for simulation of hypokinesia during 15 (n=12), 30 (n=11), 45 (n=9) and 60 (n=7) days. Principles of laboratory animal care were followed according to Sharp and La Regina (37).

Surgical preparation and experimental protocol

Rats were anesthetized with sodium pentobarbital (40 mg/kg i.p.). Anesthesia was maintained by hourly injections of α-chloralose (75 mg/kg i.v.). Rectal temperature was maintained at 37–38 °C throughout the experiment with a thermostatically controlled blanket. All skin incisions were infiltrated with 2% lidocaine hydrochloride. First, a polyethylene catheter (ID 0.38 mm; OD 0.76 mm) was advanced into the abdominal aorta from the site of cannulation in the femoral artery. This catheter was used for MABP recordings and blood gas analysis (PaO₂, PaCO₂ and pHa). Than a femoral vein was cannulated (ID, 0.58 mm; OD 0.96 mm) to perform intravenous injections. Heparine was given intavenously (1000 IU/kg) and subsequently at hourly intervals (1000 IU/kg) to ensure patency of the two catheters. The rats were then tracheotomized and artificially ventilated, the respiratory pump (tidal volume and ventilation rate) was adjusted to maintain PaO₂ and PaCO₂ within physiological ranges. For the paralization we used pipecuronium bromide (0.05 mg/kg /hour, iv.). The rats were positioned in a stereotaxic frame, parietal trepanation was performed and a bone area (3 mm in diameter) was removed without damaging the dura mater so that the pial vessels were visible. Since the laser-Doppler flowmetry readings represent the flow in the superficial cortex, in dura mater and the pial vasculature, care was taken not to place the laser-Doppler flowmetry probe above large pial vessels. The probe of type N (tip diameter of 0.8 mm with 3 optical fibers, 1 light emitter, and 2 collectors, interaxis distance of 0.5 mm) of the laser-Doppler flowmeter (BLF 21, Transonic Systems Inc., USA) was carefully positioned to avoid major cerebral vessels and fixed by using special frame. The electrodes were fixed to the rats’ pads to measure heart rate by R–R intervals of electrocardiogram (ECG).
After stabilization of ICBF and MABP, an arterial blood gas analysis was performed. The reactivity of cerebral arterioles investigated by the laser-Doppler flowmeter was then tested by making the animals breath an $O_2/N_2$ mixture (45%/45%) enriched with 10% $CO_2$.

Cerebrovascular resistance (CVR) was calculated from mean arterial blood pressure (MABP) and from ICBF it and represents the quotient of MABP/ICBF.

Hematocrit was measured before and 10 minutes after nimodipine injections. Hematocrit was not modified by nimodipine injections.

We used three most common doses of nimodipine (1 $\mu$g/kg, 3 $\mu$g/kg and 10 $\mu$g/kg) in our experiments (14). Single injections of nimodipine were used rather than continuous infusions because this type of delivery allowed us to check the reversibility of the effects, since $t_{1/2}$ of nimodipine is approximately 10 min (22, 26). This choice also allowed us to study several doses in the same animal. The injection were given at intervals of thirty minutes. The effects on ICBF, MABP, HR were assessed before, and 3, 10, 20, 30 min after the end of each injection and compared with the control group of rats treated in the same way.

Mean values of the physiological variables before injections were: pH=7.40±0.03; $PaO_2$=108.2±13.2 mm Hg; $PaCO_2$=34.5±5.2 mm Hg.

After the in vivo study animals were killed with barbiturate injection. Thoracic and abdominal parts of aorta were dissected, cut in 2 mm. rings, connected by platinum wires and mounted in an isolated organ bath (“Isotonische Messeinrichtung”) for measuring the amplitude of isotonic contractions (5). As a perfusion solution Krebs-Henseleit buffer solution of following composition was used: NaCl 6.89 g, KCl 335 mg, CaCl$_2$ 227 mg, MgSO$_4$×7H$_2$O 246 mg, KH$_2$PO$_4$ 136 mg, NaHCO$_3$ 2.1 g, glucose×H$_2$O 1.08 g (per liter), temperature was maintained at 37–38 °C and pH=7.40±0.04. Perfusion solution was bubbled with air/CO$_2$ mixture (95%/5%). Aortal rings were contracted by increasing K$^+$ concentration in Krebs-Henseleit buffer solution up to $5\times10^{-2}$ M by adding necessary quantity of KCl solution, after that nimodipine ($10^{-7}$ M) was added to relax the rings.

Statistics

Results are expressed as mean ± SE. The significance of differences between the corresponding mean values within and between groups was studied by Student’s t-test with two-tailed distribution (paired-within group and with two-sample unequal variance between groups) using Microsoft Excel 2000 with additional programs.

Results

In vivo study

Measurements of changes in ICBF made by LDF correlate well with measurements made by established techniques (9, 38). Laser-Doppler flowmetry allows rapid,
instantaneous measurements of blood flow variations by measuring red cell flux. Since results can vary with hematocrit changes, in the pilot study we monitored the absence of any significant short-term effect of nimodipine injection on hematocrit. Because ICBF estimated with laser-Doppler flowmetry correlates better with relative changes in ICBF rather than with absolute values (9), changes were calculated as percentages of baseline ICBF (ICBF₀) – the mean flow obtained during the minute before the injection of nimodipine.

CVR was calculated as the ratio of MABP to concomitant ICBF. It should be kept in mind that laser-Doppler flowmetry does not measure actual volumetric blood flows and thus does not allow the calculation of absolute CVR. Nevertheless, we have used throughout this report the classic terms of ICBF and CVR to mean relative changes in ICBF and CVR, respectively. The ΔHR/ΔMABP ratio expresses the balance between the influence on the heart and on the mean arterial blood pressure. ΔHR and ΔMABP represent the difference between corresponding parameters on the 3rd minute after nimodipine injection and those before injection.

The absolute values of MABP, HR and Ht gauged under general anesthesia before nimodipine injections are shown in the table. In our experiments we noticed the tendency of increasing of MABP in 15- and 30-day hypokinesia, in 45- and 60-day HK MABP was authentically increased (p<0.05). HK results in significant augmentation of HR: even in the early period of restriction of movement activity (15-day HK) HR was raised (p<0.05), compared with control, and later (30, 45, 60 days of HK), it was more increased. The same shifts we found measuring hematocrit (Table I).

Table I

<table>
<thead>
<tr>
<th></th>
<th>Control (n=12)</th>
<th>15 days (n=10)</th>
<th>30 days (n=8)</th>
<th>45 days (n=7)</th>
<th>60 days (n=7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg)</td>
<td>100.0 ± 2.2</td>
<td>102.5 ± 4.0</td>
<td>104.1 ± 4.3</td>
<td>106.6 ± 5.2*</td>
<td>107.0 ± 5.3*</td>
</tr>
<tr>
<td>HR (b./m.)</td>
<td>356.0 ± 4.3</td>
<td>369.4 ± 9.5*</td>
<td>381.3 ± 11.8#</td>
<td>397.2 ± 20.5#</td>
<td>400.9 ± 12.9#</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>40.8 ± 1.7</td>
<td>44.1 ± 2.7*</td>
<td>45.5 ± 3.4*</td>
<td>47.2 ± 3.8*</td>
<td>47.2 ± 3.7*</td>
</tr>
</tbody>
</table>

*p<0.05 and # p<0.01 compared with the control group.
In our experiments nimodipine increased ICBF, HR, and lowered MABP and CVR in all used doses. The effect of nimodipine on HR consists of two components: actual influence on sinoatrial node (blockade of calcium channel of type L) and reflector action via lowering MABP (reflector tachycardia). In case of nimodipine we observed tachycardia in all experimental groups.

The time-courses of ICBF and MABP after single injections of nimodipine in control animals are shown in Figure 1. Most of the indices were changed only in the third minute comparing with those before injections, so we compared the difference of values between the third minute and that before the injection in all groups. The results are presented in Figure 2.

![Figure 1](image)

**Fig. 1.** Time-courses of ICBF and MABP after single bolus injections of nimodipine (1 μg/kg (A); 3 μg/kg (B); 10 μg/kg (C)) in the rats of control group. (The symbols are full if $p<0.05$, compared with the same values before injection)
In 15-day HK indices did not differ from the control group, only MABP and resistance of cerebral vessels (CVR) were smaller when the highest dose was used. In 30- and 45-day HK significant increase of ICBF and decrease of CVR and MABP was observed. These changes were more expressed in 45-day HK, and in 60-day HK some stabilization of these parameters was marked. Most vividly expressed augmentation of HR, in comparison with the control is revealed at the dose of 10 µg/kg at 30-day HK. We observed the recession of ΔHR/ΔMABP ratio. The ΔHR/ΔMABP ratio was decreased with augmentation of the term of a hypokinesia, achieving a minimum by the 45th day. However, at the dose of 10 µg/kg in 60-day HK this ratio decreased even more which could be the manifestation of decreased sensitivity of the heart compared with vessels.

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In vitro study

The ability of nimodipine (10^{-7}M) to decrease the amplitude of isotonic contractions of isolated aortic rings, previously contracted by the increase of K^+ concentration in Krebs-Henseleit buffer solution up to 5\times10^{-2}M, has been estimated. Nimodipine reduced the isotonic contractions' amplitude of isolated aortic rings, precontracted by KCl in control group by 76.71\pm3.32%; in the 15-day HK group 82.77\pm5.46%; in the 30-day HK group 83.70\pm5.32% (P<0.05); in the 45-day HK group 87.83\pm7.31% (P<0.05); and 83.46\pm4.76% (P<0.05). Thus, authentic augmentation of sensitivity of isolated aortic rings to nimodipine in the 30-, 45- and 60-day hypokinesia, which achieved the maximal value in 45 days in comparison with the control group has been observed, therefore the in vitro study results are in concord with those in vivo.

Discussion

We think that the increase of MABP, HR and Ht indicates considerable shifts in the heart-vessels-blood system and shows its detrained status. Our findings resemble those obtained in human (16). Prolonged hypokinesia causes an imbalance in an organism's control systems, specifically, depressor reactions are distorted. It provokes disturbances of the heartbeat and hypertensive reactions in human (16). We think chronic activation of sympathetic-adrenomedullary system is one of the main factors responsible for these shifts (10, 18, 28).

The influence of nimodipine on ICBF and general hemodynamics have been studied previously in the rat (15, 42), in the baboon (22, 31), in the rabbit (14) and in the cat (43), from the point of autoregulation, when MABP is decreased, but the results have been contradictory. Bonvento et al. (14) suggest that the absence of effects of intravenous nimodipine in some species may be due to poor passage of nimodipine across the blood-brain barrier. Therefore we think this factor – changes of permeability of blood-brain barrier – may be, partly, the cause of changes of sensitivity of rats’ cerebral vessels in hypokinesia in our experiments, for perhaps, through the activation of brain mast cells even a short-time immobilization stress is able to increase blood-brain barrier permeability (11, 12). However it is still a debatable question (35).

We think, the observed shifts of \Delta HR/\Delta MABP ratio could be explained partly by a more expressed augmentation of the sensitivity of arterial vessels in comparison with sinoatrial node of the heart, partly the changed baroreflex control in hypokinesia (34, 39), since, the baroreflexes are important mediators of cardiovascular adjustments to both orthostatic stress and dynamic exercise, and in our model of hypokinesia rats almost cannot move and are absolutely unable to get an orthostatic posture (in usual cages they move freely and from time to time get an orthostatic posture), which could result in attenuated baroreflexes.

Nimodipine has membrane stabilizing properties via inhibiting the lipid peroxidation and subsequently restoring the activity some membrane bound and lipid dependent enzymes such as Na^+-K^+/Mg^{2+} ATPase (24). It is an additional reason for
using nimodipine in hypokinesia, since previous studies of our colleagues (2, 30) have shown the intensification of lipid peroxidation processes in central nervous system and blood in condition of hypokinesia. Farkas et al. (13) have described that chronic treatment of aging hypertensive stroke-prone rats with nimodipine or nifedipine could preserve microvascular integrity in the cerebral cortex. At the same time nimodipine has been demonstrated to have neuroprotective properties (25).

As it has been shown by numerous publications, in hypokinesia conditions miscellaneous endocrine shifts occur, partly similar to those described in space flight conditions (29, 41).

The restriction of movement activity is a typical example of chronic stress, so hypothalamic-pituitary-adrenal axis is activated and content of glucocorticoids is elevated (8, 27, 28). Glucocorticoids increase Ca\(^{2+}\) uptake possibly mediated by an increase in the number of dihydropyridine-sensitive Ca\(^{2+}\) channels (23), they increase the number of dihydropyridine binding sites, so that voltage-dependent L-type Ca\(^{2+}\) channel expression is regulated by glucocorticoids (6). Yamada et al. (44) has observed an increased in vivo binding affinity for nimodipine in chronic ischemic brain of rat.

**Conclusion**

Thus, the compelled hypokinesia results in the augmentation of sensitivity to the cerebrovasoselective calcium channel blocker – nimodipine which is widely used in angioneurological practice, where the hypokinesia is a frequently observed concomitant state and a risk factor at the same time (7, 40). Although the molecular mechanisms of this process are unclear, it is important to take our results into account, and to continue research in this field with a subsequent formation of clinical recommendations.

**REFERENCES**

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