Effect of Nimodipine on Cell Calcium Concentration and Platelet Aggregation in Patients with Ischemic Stroke

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The rate of spontaneous platelet aggregation is 1.5-2-fold increased in patients with ischemic stroke in comparison with control values. Monotherapy with nimodipine lowers parameters of spontaneous platelet aggregation virtually to normal values. Nimodipine inhibits ADP-induced aggregation but does not affect the ADP affinity of platelet receptors. Experiments with a Fura 2-AM fluorescent probe show that the basal calcium level in platelets from patients with ischemic stroke reliably surpassed that in healthy donors. Nimodipine inhibits the ADP-induced rise of the cell calcium level.

Key Words: *calcium channel blockers; ischemic stroke; platelets*

Calcium channel blockers - 1,4-dihydropyridine derivatives such as nifedipine, nicardipine, and nimodipine are promising drugs for the treatment of disturbed cerebral circulation. Clinical trials have demonstrated that the therapeutic effect of nimodipine (ND) is due to its ability to diminish neurological deficiency in ischemic brain disease of varying severity. The use of ND in the treatment of vascular pathology of the brain is a new trend in neuropharmacology.

Platelets represent a traditional cell model in studies of the mechanisms of action of calcium channel blockers, since receptor-mediated regulation of calcium metabolism in these cells is very similar to that in smooth muscle cells [1].

Some authorities have postulated the independent role of bloodflow disturbances and hemostatic activation, which manifests itself in hyperreactivity of platelets, as risk factors for ischemic disturbances of the cerebral circulation. Previous experimental data suggest the possibility of using hemostatic parameters as diagnostic criteria for evaluating the severity of cerebrovascular disorders and the probability of thrombosis and thrombemboli [2,5].

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The objectives of the present study were to explore spontaneous and induced platelet aggregation and to determine the cytoplasmic concentration of free calcium ions in platelets from patients with ischemic stroke against the background of ND pharmacotherapy.

MATERIALS AND METHODS

A total of 18 patients with ischemic stroke (against the background of essential hypertension) and 35 healthy donors were examined.

Blood from the ulnar vein was collected in a plastic tube containing 0.5 ml preservative (5 mM glucose, 85 mM sodium citrate, 65 mM citric acid, pH 6.55). The blood was used for the preparation of platelet-rich and platelet-depleted plasma. A suspension of washed platelets was obtained as described elsewhere [12].

Spontaneous and induced platelet aggregation were assessed after Born and O'Brien on a Biola aggregometer (Moscow) using adenosine diphosphate (ADP, Sigma) in final concentrations of 0.5-5 μM as the inductor.

The level of free calcium ions in the platelet cytoplasm was determined using a FURA 2-AM fluorescent probe (Calbiochem) on a Hitachi MPF-3 spectrofluorimeter as described elsewhere [13].

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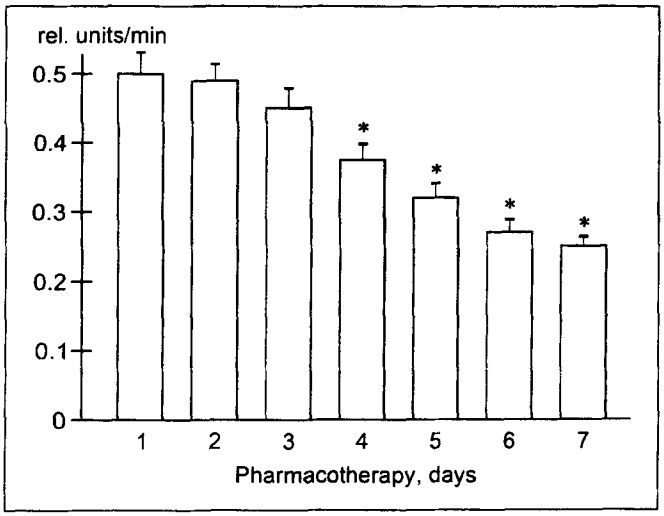

Fig. 1. Dynamics of the rate of spontaneous platelet aggregation in patients with ischemic stroke in the course of nimodipine therapy. *Reliable differences in comparison with the initial level.

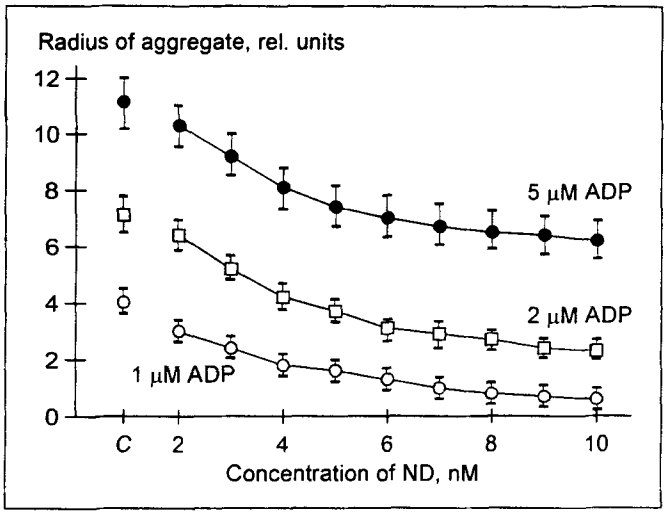

Fig. 2. Effect of nimodipine (ND) on radius of platelet aggregates in ADP-induced aggregation. *C*: control (no drug therapy).

Confidence intervals of the experimental values and reliability of the differences were evaluated using the Student *t* test at $p=0.05$ and standard mathematics software.

RESULTS

In the first series the effect of ND on spontaneous platelet aggregation was assessed. The final concentration of ND was chosen on the basis of the fact that the mean therapeutic blood concentration of the drug is about 4 nM.

Curves of spontaneous aggregation were analyzed using the aggregation rate parameter, i.e., the maximal rate of changes in the mean radius of aggregates and light transmission for a particular curve.

The rate of platelet aggregation in patients with ischemic stroke was reliably increased (1.5-2 fold) in comparison with the control values. This observation is in conformity with previous data which demonstrated platelet hyperreactivity in patients with cardiovascular pathology (hypertension, ischemic heart disease, and ischemic stroke) [3,4,6,7].

The dynamics of the rate of spontaneous platelet aggregation in patients with ischemic stroke during the course of ND pharmacotherapy (15 μg/kg, i.v. drip) is presented in Fig. 1. A reliable drop of the aggregation rate (by 15-20%) was observed starting from day 4 of treatment and on day 6-7 it attained 50%. Thus, ND lowers the parameters of spontaneous platelet aggregation in patients with ischemic stroke. These data allowed us to assume that ND modulates the sensitivity of platelets to activating agents.

For elucidation of the molecular mechanism of action of ND we studied its effect on ADP-induced platelet aggregation in the patients with ischemic stroke. Figure 2 depicts the mean radius of aggregates as a function of the concentration of ND (2-10 nM). ND inhibited the ADP-induced platelet aggregation in a dose-dependent manner (4-8 nM) at all three concentrations of ADP studied (1, 2, and 5 μM).

The data of inhibitory analysis were presented in Lainweaver-Burke coordinates (Fig. 3, *a*). The bottom line depicts the aggregation rate as a function of the concentration of inductor; the two upper lines characterize the combined effect of ADP and ND in concentrations of 4 and 8 nM. The lines intersect at one point on the abscissa axis, signifying noncompetitive inhibition. For calculation of the inhibition constant (K_i) the experimental data were also presented in Eisenthal

TABLE 1. Effect of Nimodipine (ND, 4 nM) on Basal and ADP-Induced (1 μM) Intracellular Calcium Concentration in Platelets (*M±m*)

Group	Concentration of Ca^{2+}, nM			
	basal	ND	ADP-stimulated	ND+ADP
Healthy donors (*n*=12)	93±10	87±4	397±66	123±16
Patients with ischemic stroke:				
before treatment (*n*=18)	121±9	81±8	581±68	318±40
after treatment (*n*=18)	130±11	-	437±41	-

Note. A dash indicates that the parameter was not studied.

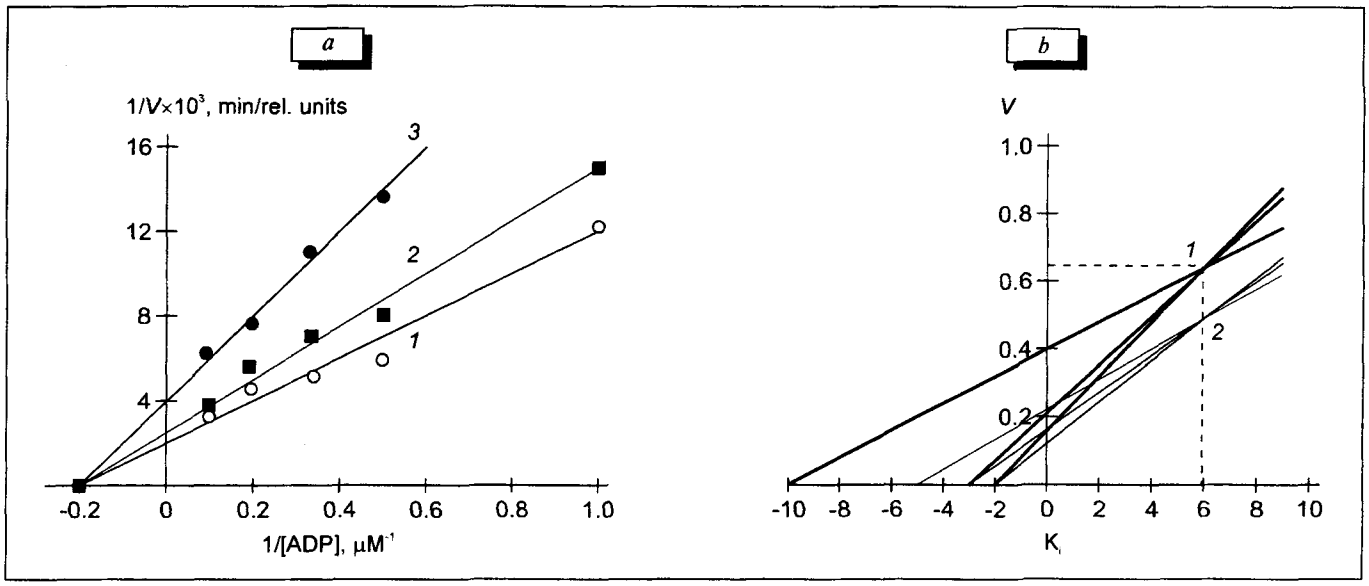

Fig. 3. Rate of platelet aggregation (*V*) as a function of ADP concentration in the absence (*1*) and presence of 4 nM (*2*) and 8 nM (*3*) nimodipine in Lainweaver-Burke (*a*) and Eisenthal and Cornish-Bowden (*b*) coordinates.

and in Cornish-Bowden coordinates (Fig. 3, *b*). K_i for ND was 6.1 nM. ND was found not to alter the affinity of ADP receptors on platelets but to modulate some later steps of transmembrane signal transduction.

A rise of the intracellular calcium concentration is known to be essential for the ADP-induced activation and degranulation of platelets [8,9].

In light of this, in the next experiments we studied the effect of ND on the basal and ADP-stimulated calcium level in platelets in health and ischemic stroke. These data are summarized in Table 1.

The basal calcium level in platelets from healthy donors measured using FURA 2-AM fluorescent dye was 93±10 nM (n=12). Addition of ADP (1 μM) raised it to 397±66 nM (n=12). This increase was observed immediately after the addition of the inductor and attained the maximum after 1-2 min, after which during the following 10 min fluorescence gradually dropped to a new level that was still considerably higher than the initial.

ND (4 nM) does not reliably change the basal calcium level but inhibits the ADP-stimulated increase of the calcium concentration by 180% on average. The calcium-blocking effect of ND appears without a latency.

In platelets from the patients with ischemic stroke the basal calcium level before treatment was higher than in the control (121±9 nM, n=18) and the Ca^{2+} response to ADP was much more pronounced (581± ±68 nM). ND (4 nM) inhibited the ADP-induced rise of the intracellular calcium level by 85% on average.

After a 7-day course of ND treatment the basal calcium level remained elevated (130±10 nM) but the ADP-stimulated Ca^{2+} response was markedly lowered in comparison with the respective values before treatment. A similar effect of dihydropyridines has been previously described in patients with cardiovascular disease [1].

Our data suggest that in patients with ischemic stroke ND reduces the sensitivity of platelets to both endogenous (experiments with spontaneous aggregation) and exogenous activators. Calcium antagonists, 1,4-dihydropyridine derivatives, modulate calcium metabolism by blocking calcium entry into platelets through receptor-operated [10] and voltage-dependent [11] calcium channels.

Platelets can thus be used for *in vitro* preliminary testing of individual sensitivity to ND.

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