Effect of Calcium Channel Blocker Verapamil on DNA Synthesis and Activity of Nucleolar Organizer in Cardiomyocytes of Newborn Albino Rats

N. P. Mel'nikova and S. S. Timoshin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 127, No. 6, pp. 658-660, June, 1999
Original article submitted June 21, 1998

Fivefold administration of L-type Ca^{2+} channel blocker verapamil in a dose of 1.5 mg/kg to newborn rats increased the parameters of DNA-synthetic activity in the myocardium: labeling index and intensity increased 1.24-fold on average. Body weight decreased with simultaneous increase in the absolute and relative heart weight. Morphometric parameters of the nucleus and nucleoli were practically unaffected. Our findings suggest that the Ca^{2+} channel blocker verapamil administered at the early postnatal stages can modulate morphogenesis of the heart.

Key Words: *Ca^{2+} channels; DNA synthesis; myocardium; nucleolar organizer; ontogeny*

Influences on the heart in the early postnatal ontogeny considerably modulate its sensitivity to damaging factors in adults [3]. L-type voltage-dependent Ca^{2+} channels appear in the early postnatal period, which allows to use verapamil in pediatrics [10,11]. Ca^{2+} channel blockers improve heart recovery after myocardial infarction and stimulate fibroblast proliferation in cell culture [7,12]. At the same time verapamil inhibits proliferation of smooth muscle cells in the intima of blood vessels during atherosclerosis development [4] and proliferation of B cells in mice in response to T-dependent antigen [9]. In light of this, of particular interest is to study the effect of verapamil on DNA synthesis in the myocardium of newborn rats.

MATERIALS AND METHODS

Experiments were carried out on 43 rat pups. Verapamil (in 2-ml ampoules, 50 mg per ampoule, Knoll) was administered intraperitoneally in a dose of 1.5 mg/kg on days 2 through 6 between 11.0 and 13.00. Control animals received equivalent volume of isotonic NaCl. The animals were decapitated one day after the last injection. ^{3}H-thymidine (specific activity 1530 TBq/mol) was injected intraperitoneally in a dose of 1 μCi/g body weight 1 h before sacrifice.

Parameters of DNA synthesis: labeling index (LI, %) and label intensity (mean number of silver grains over nucleus) were assessed on histotopographic sections in 10 myocardial areas: subendocardial, intramural, and subepicardial layers of the left and right ventricles, subendocardial and intramural layers of the interventricular septum, and in the left and right atria. The dynamics of the body weight and the absolute and relative weight of the heart (heart weight/body weight ratio at sacrifice) were evaluated.

The data were processed statistically using the Student *t* test.

RESULTS

Five intraperitoneal injections of verapamil in a dose of 1.5 mg/kg significantly increased LI in 6 myocardial areas (Table 1). The maximum increase was observed in subepicardial layer of the left ventricle (by 27.34%).

In parallel with the increase in LI, the intensity of label increased in all heart areas (by on average 24%). This parameter maximally increased in areas exhibiting no LI rise: intramural myocardial layer of the

Central Research Laboratory, Far-East State Medical University, Khabarovsk

0007-4888/99/0006-0600$22.00

TABLE 1. Effect of 5-Fold Verapamil Administration on DNA-Synthetic Activity in Cardiomyocytes of Newborn Rats (*M±m*)

Area	LI, %		Label intensity	
	control	verapamil	control	verapamil
Atrium				
left	8.02±0.78	8.01±0.35	11.13±0.72	17.07±0.58*
right	8.64±1.01	8.3±0.44	12.56±0.77	16.32±0.44*
Left ventricle				
subendocardial	9.29±0.43	11.87±0.26*	12.6±0.69	15.92±0.61*
intramural	7.09±0.45	8.86±0.43*	11.15±0.63	13.71±0.43*
subepicardial	5.91±0.47	7.35±0.33*	11.32±0.58	13.87±0.43*
Interventricular septum				
subendocardial	9.31±0.42	11.77±0.34*	12.88±0.63	15.62±0.62*
intramural	7.86±0.34	9.63±0.43*	11.61±0.55	14.3±0.24*
Right ventricle				
subendocardial	7.63±0.49	9.04±0.46*	12.09±0.68	14.39±0.48*
intramural	6.4±0.48	7.42±0.52	10.43±0.49	13.6±0.29*
subepicardial	5.21±0.51	6.38±0.3	10.6±0.55	12.1±0.36*

Note. *$p<0.05$ compared with the control.

right ventricle (by 30.39%) and in the left and right atria (by 30.21 and 29.94%, respectively).

When analyzing the mechanisms of stimulation of DNA synthesis one should take into account the fact that the decrease of Ca^{2+} concentration in the medium stimulates cell proliferation, which is associated with a decrease in cell adhesion, enlargement of intercellular spaces, and disappearance of desmosomes [8]. Verapamil interacts with calmodulin involved in tubulin polymerization and formation of mitotic spindle microtubules [1].

Treatment with verapamil increased the content of not only Ca^{2+}, but also other second messengers. There are experimental data on accumulation of cGMP and a decrease in the content of isoproterenol-induced cAMP in guinea pig heart [2,5]. These shifts in the concentration of second messengers are typical of stimulation of DNA synthesis.

On postnatal day 2, rat pups of the control and experimental groups did not differ in body weight (8.2±0.18 and 7.91±0.3 g, respectively). Verapamil treatment significantly decreased the body weight on postnatal day 7 (13.23±0.3 *vs.* 14.55±0.46 g in the control).

Verapamil increased both the absolute (90.44± 2.45 *vs.* 76.31±3.06 mg in the control) and relative weight of the heart (6.78 *vs.* 5.32±0.24 mg/g in the control).

The state of protein-synthesizing apparatus in cardiomyocytes under these conditions was assessed by morphometric parameters of the nucleolar organizer determined by analyzing histological sections stained with $AgNO_3$ on a MEKOS-Ts image analysis system [6]. The area and number of nucleoli per nucleus and the number of NOR granules (nucleolar organizer region-associated proteins) and their area in the nucleolus were determined (Table 2). Additionally, derivative parameters were calculated, which most reliably characterized functional activity of nucleoli: total area of nucleoli (number of nucleoli×area of nucleolus) and NOR granules in the nucleolus (number of granules×area of granule). According to published data, these parameters characterize activity of ribosome genes and indirectly reflect the intensity of protein synthesis in cardiomyocytes. The area and perimeter of cardiomyocyte nuclei were also measured [6].

TABLE 2. Effect of 5-Fold Verapamil Administration on Morphometric Parameters of Nucleus and Nucleoli in Cardiomyocytes of Newborn Rats

Parameter	Control	Verapamil
Perimeter of nucleus, μ	23.77	22.81
Area of nucleus, μ^2	38.25	36.14
Number of nucleoli	1.98	2.07
Area of nucleoli, μ^2	0.85	0.81
Number of granules	4.53	4.28
Total area, μ^2		
of nucleoli	1.65	1.67
of granules	0.17	0.15

The number of nucleoli was the same in the experimental and control groups (1.98±0.07 and 2.07± 0.06, respectively, Table 2), which agrees with published data on the presence of 1-2 nucleoli in cardiomyocyte nuclei.

The absence of significant changes in morphometric parameters indirectly suggests that the increase in heart weight is not associated with activation of protein synthesis. The most probable explanation is enhanced blood supply of the microcirculaty bed [1]. However, other mechanisms cannot be excluded.

REFERENCES

1. P. V. Avdonin and V. A. Tkachuk, *Receptors and Cell Calcium* [in Russian], Moscow (1994).
2. I. B. Bardamova and S. A Afanas'ev, *Vopr. Med. Khimii*, No. 3, 47-50 (1993).
3. V. Ya. Brodskii, *Byull. Eksp. Biol. Med.*, **119**, No. 5, 454-459 (1995).
4. L. V. Glushko, *Kardiologiya*, No. 4, 45-46 (1991).
5. B. I. Laptev, I. B. Bardamova, S. A Afanas'ev, and E. Yu. Talaeva, *Byull. Eksp. Biol. Med.*, **110**, No. 11, 455-457 (1990).
6. N. N. Mamaev, A. Ya. Gudkova, Kh. K. Amineva, *et al.*, *Arkh. Anat.*, No. 5, 69-72 (1985).
7. V. N. Sokrut, N. I. Yabluchanskii, and L. A. Pavlenko, *Eksp. Klin. Farmakol.*, No. 2, 20-22 (1992).
8. V. P. Tumanov, R. P. Gasanov, G. G. Serov, *et al.*, *Byull. Eksp. Biol. Med.*, **106**, No. 8, 231-233 (1988).
9. V. S. Yampol'skii and V. I. Ratnikov, *Farmakol. Toksikol.*, No. 5, 43-45 (1987).
10. M. P. Davies, R. N. An, P. Doevendans, *et al.*, *Circ. Res.*, No. 1, 15-25 (1996).
11. V. Ramesh, M. J. Kresch, A. M. Katz, D. H. Kim, *Am. J. Physiol.*, No. 3, Pt. 2, H778-H782 (1995).
12. B. Willershausen-Zonnchen, Ch. Lemmen, B. Zonnchen, *et al.*, *Biol. Chem.*, No. 5, 299-303 (1994).