Nanotechnological Approach to Evaluation of Mechanical Properties of Cell Surfaces during Stimulation and Blockade of Adrenoceptors

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We used a new nanotechnological approach for evaluation of functional activity of adrenoceptors during treatment of cell surfaces with various drugs. Local elasticity maps by nanoindentation points were constructed, which show the targets for drugs on transporter cells under natural conditions. The applied approach allowed identification of structural transformations in the membrane leading to changes in its elasticity, which can be used in cell physiology studies for controlling the processes of cell signaling.

Key Words: *atomic force spectroscopy; coefficient of elasticity; cell surface; mechanical properties*

Due to intensive introduction of atomic force microscopy (AFM) methods into biological researches, new approaches can now be used for the evaluation of mechanical properties of cell surface [9,10]. One of these methods is atomic force spectroscopy (AFS) allowing measurements of elasticity coefficient under near-natural conditions, *e.g.* under the effect of various drugs. In most cases, elements of cell membrane (glycocalyx, β-adrenoceptors, *etc.*) are the immediate targets of drug action irrespective of their pharmacological effects [1].

Here we studied mechanical properties of erythrocyte surface by AFS under conditions of adrenoceptor activation and blockade.

MATERIALS AND METHODS

Experiments were carried out on blood erythrocytes from *Rana ridibunda pall* frogs. Adrenoceptors were stimulated and blocked with epinephrine and propranolol in concentrations of 10^{-3}, 10^{-6}, and 10^{-9} mmol/liter. The blood (diluted 1:10 with incubation medium) was treated with the test drugs for 30 min at room temperature physiologically adequate for frog blood. Blood samples diluted 1:10 with physiological saline and incubated under the same conditions served as the control.

After the end of incubation, a suspension specimen from each blood sample was prepared on a clean degreased glass slide and placed into a humid chamber for preserving native properties of the membrane. Mechanical properties of cell surface were studied using an INTEGRA Vita atomic force microscope (configuration on the basis of Olympus IX-71 inverted microscope). Scanning of native cells was performed in a tapping mode (scan frequency 0.6-0.8 Hz) using a NSG03 cantilever with a tip radius of 10 nm and spring constant of 1.1 N/m. Mechanical properties of cell surfaces were studied in AFS mode by applying force in 25 local points of the cell surface (Fig. 1).

The method is based on recording of force curves (DFL, Z) from the cell surface reflecting deflection of flexible cantilever of AFS probe when it comes close to the sample in each nanoindention point [2]. Deflection of light beam is detected with a four-section photodiode and mismatch between the upper and lower sections of the photodiode produces a mismatch

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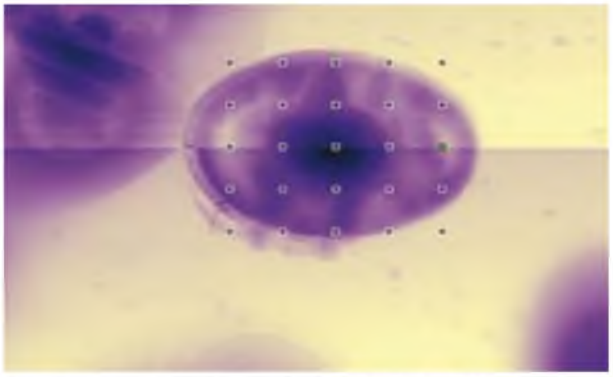

Fig. 1. Points of nanoindentation on the cell surface.

current. The local Young module was calculated as described elsewhere [10,11] using Sneddon's modification of the Hertzian model for the elastic indentation of a flat soft sample by a stiff cone [4,6]. We assumed the cell is an elastic isotropic medium with Poisson coefficient of ν=0.5 and AFS tip is a solid cone.

The obtained force curves were processed using Ef3 software (NT-MDT). Analysis of sample deformation as a function of applied force allows us to calculate the elasticity coefficient and compare this parameter at different sites of the cell surface. AFS measurements of Young's module were performed on 30 cells in each sample series.

Significance of differences was evaluated using Student t test.

RESULTS

Differences in integral values of Young's module under conditions of stimulation and blockade of β-adrenoceptors were revealed (Table 1).

Treatment of cell surfaces with epinephrine (10^{-9} mmol/liter) and propranolol (10^{-3} mmol/liter) increased their elasticity coefficient by 33.97 and 37.8%, respectively, compared to the control (p<0.05). The increase in elasticity modulus under conditions of β_2-agonist treatment can be explained by the phenomenon of up-regulation of adrenoceptor number upon long-term exposure to β-adrenoblockers [7]. Active center of the receptor interacting with β_2-agonists is located at $^1/_3$ distance (15 Å) to the middle of the receptor [3]. It interaction with catecholamines is determined by the presence of certain functional groups of adrenoceptor molecule. Various physicochemical factors reversibly change the properties of protein thiol groups of adrenergic receptors. Some authors admit the possibility of mutual conversion of α- and β-adrenoceptors regulated by phosphorylation and internalization upon changes in environmental conditions [5].

Since the number and functional activity of adrenoceptors on the cell surface are not constant, an attempt was undertaken to construct maps of local elasticity of the cell surface by nanoindentation points under conditions of adrenoceptor stimulation (epineph-

TABLE 1. Young's Module of Erythrocyte Surface (Pa; $M \pm m$)

Drug concentration, mmol/liter	Control	Epinephrine	Propranolol
10^{-3}	13.49±1.79	17.55±1.55	21.69±3.61*
10^{-6}		17.83±1.63	11.39±2.13
10^{-9}		20.43±1.89*	8.62±1.20

Note. *p<0.05 compared to the control.

1	**2**	**3** 2.29±0.01 29.82±0.9	**4**	**5**
10 24.30±1.01 16.56±0.70	**9** 13.59±0.40 10.21±0.30	**8**	**7**	**6** 39.27±0.20 2.83±0.04
11 13.53±0.90 12.33±0.40	**12**	**13**	**14**	**15**
20	**19**	**18** 2.09±0.05 10.34±0.20	**17** 21.07±0.70 12.81±0.90	**16**
21	**22**	**23**	**24** 10.24±0.60 7.07±0.90	**25** 18.90±0.50 7.26±0.80

1 2 3 4

Fig. 2. A map of local elasticity (Pa) of erythrocyte cell surface under the effect of epinephrine. Here and on Fig. 3: figures in bold: site number. Upper row: experimental samples; bottom row: controls. *1*) sites of insignificant elevation of local rigidity; *2*) sites of minimal rigidity; *3*) no differences in rigidity between the control and experimental samples; 4) sites with maximum rigidity of the cell surface.

1	**2**	**3** 7.85±0.04 29.82±0.90	**4**	**5**
10 31.04±1.30 16.56±0.70	**9** 10.45±0.60 10.21±0.30	**8**	**7**	**6** 17.01±1.20 2.83±0.04
11 14.74±0.60 12.33±0.40	**12**	**13**	**14**	**15**
20	**19**	**18** 6.04±0.80 10.34±0.20	**17** 17.82±0.20 12.81±0.90	**16**
21	**22**	**23**	**24** 11.82±0.70 7.07±0.90	**25** 3.34±0.05 7.26±0.80

1 2 3 4

Fig. 3. A map of local elasticity (Pa) of erythrocyte cell surface under the effect of propranolol.

rine, 10^{-9} mmol/liter) and blockade (propranolol, 10^{-3} mmol/liter). We detected a decrease in Young's module by 74.14% ($p<0.05$) in nanoindentation point 3 (surface glycocalyx) and its increase by 92.7% ($p<0.05$) compared to the control in the area of submembrane cytoskeleton structures (nanoindentation point 6) under the effect of epinephrine (Fig. 2). Insignificant increase in local elasticity of the cell surface was also found in nanoindentation points 10 (cell periphery) and 17 (area of nuclear membrane). In the area of concentration of genetic apparatus, Young's module decreased by 79.78% ($p<0.05$) compared to the control. In nanoindentation points 9, 11, and 24 on the cell surface corresponding to the area of perinuclear space and hyaloplasma, the elasticity modulus did not appreciable differ from the control. Under conditions of β-adrenoceptor blockade, Young's module in nanoindentation point 3 (surface glycocalyx) decreased by 73.67% ($p<0.05$) compared to the control (Fig. 3), while in points 6 (submembrane structures of the cytoskeleton) and 10 (cell periphery) it increased by 83.36 and 46.65%, respectively ($p<0.05$). In the zone of concentration of cell chromatin (point 18) and in the area of contact between the glycocalyx and the neighboring cell (point 25), Young's modulus decreased by 41.58 and 53.99%, respectively ($p<0.05$).

Propranolol in different concentrations produces opposite effects on cell surface elasticity. Being a nonselective β-adrenoceptor blocker, it affects both β_1- and β_2-adrenoceptors [1]. Published data suggest that frog erythrocytes carry atypical β-adrenoceptors similar to both β_1- and β_2-adrenoceptors of higher animals [3]. The blocker (in a concentration of 10^{-9} mmol/liter) binds to β-adrenoceptors and suppresses physiological cycle of their activation, thus reducing elasticity of the cell surface. This agrees with the results of previous studies demonstrating that blockade of β-adrenoceptors increases membrane permeability for monovalent ions and changes membrane elasticity [5].

Thus, the use of a new nanotechnological approach to the evaluation of mechanical properties of the cell surface allowed us to detect "local targets" for the applied drugs on transporting cells (erythrocytes) irrespective of their pharmacological effects. AFS analysis revealed glycocalyx sites with increased and reduced functional activity of adrenoceptors under the action of the applied drugs. This approach is a promising tool for further studies aimed at the purposeful modulation of structural and functional state of cell membranes.

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