# Age-Related Changes in Electrical Reactions of Rat Aorta Endothelium Elicited by Acetylcholine and ATP

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Age-related changes in some electrophysiological parameters of the endothelium in isolated rat aorta were studied. In comparison with young rats (6 months), resting potential of the endothelium in aged rats (24-26 months) was significantly higher and varied in a wider range. The electrical responses of intact endothelium of isolated aorta to acetylcholine were typical and regular in young rats, while in aged rats these responses were irregular and atypical. It is hypothesized that atypical electrical responses of the endothelium to acetylcholine and ATP in aged animals result from functional clustering associated with disturbances in electrical contacts between endotheliocytes. We hypothesize that the age-related decrease in NO production is related to disturbances of electrical properties of endothelial membrane.

**Key Words:** *aging; endothelium dysfunction; membrane resting potential; nitric oxide*

Aging induces irreversible changes in the cardiovascular system, and first of all, these changes affect the endothelium-dependent regulation of the vascular tone [2,11]. It is well known that acetylcholine-induced endothelium-dependent dilation, which is mediated via synthesis and release of endothelium-derived vasoactive substances NO and endothelium-derived hyperpolarizing factor (EDHF), significantly decreases during aging [12]. The age-related inhibition of endothelium-dependent relaxation induced by acetylcholine is characteristic of capacitance [6,7] and resistance [10] vessels. Similar inhibition of endothelium-dependent relaxation in response to ATP and histamine during aging was also reported.

NO is produced by endothelial NO-synthase, a calcium-dependent enzyme, which is activated by a rise in intracellular concentration of calcium ions after the action of acetylcholine, ATP, histamine, or bradykinin [1]. Generally, the rise of $Ca^{2+}$ concentration induced by the above agonists is a result of a chain of events including agonist binding to the corresponding receptor, generation of second messengers transmitting the signal from endotheliocyte plasma membrane to intracellular depots, rapid release of $Ca^{2+}$ from these depots, and activation of Ca-dependent potassium channels leading to hyperpolarization of endothelial membrane and long-term increase of $Ca^{2+}$ inward current through nonselective cation channels into endotheliocytes [11]. The mechanisms of depression of endothelium-dependent relaxation during aging are still not clear. It is assumed that this phenomenon can result from attenuation of NO release [3] and/or moderation of NO-synthase activity [4,5]. It cannot be excluded that inhibition of NO production and release results from changes or disturbances in electrophysiological properties of endothelial membrane, which can be reflected in changes in $Ca^{2+}$ concentration induced by acetylcholine, ATP, histamine, and bradykinin.

Our aim was to study possible changes in some electrical properties of endothelium in isolated rat aorta during aging.

## MATERIALS AND METHODS

Experiments were carried out on male Wistar—Kyoto rats aged 6 (control group) and 24-26 months (aged).

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The measurements were performed on intact endothelium of thoracic subdivision of rat aorta.

The aorta was isolated and freed from fat and connective tissue, cut into segments (3-4 mm), and kept in modified Krebs solution containing (in mM): 118.3 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 $Na_2HPO_4$, 2.5 $CaCl_2$, 1.2 $MgSO_4$,×$7H_2O$, 11.1 glucose, and 50 μg/ml gentamicin, and saturated with 95% $O_2$ and 5% $CO_2$ mixture. Before the experiment, the segment was cut longitudinally, fixed in a 100-μl chamber, and perfused at the rate of 0.6 ml/min.

Membrane potential of intact endothelial layer of rat aorta was measured using the patch-clamp technique [8,9]. Under these conditions endothelial cells retain the properties of electrical syncytium and myoendothelial connections are preserved. The recording pipettes were filled with a solution containing (in mM): 149.0 KCl, 10.0 NaCl, 10.0 HEPES-KOH (pH 7.3). Nistatin (100 μg/ml) was added as a perforating agent.

Experiments were carried out at room temperature (20-24°C). All reagents were from Sigma.

The data were analyzed statistically using ORIGIN 6.0 software.

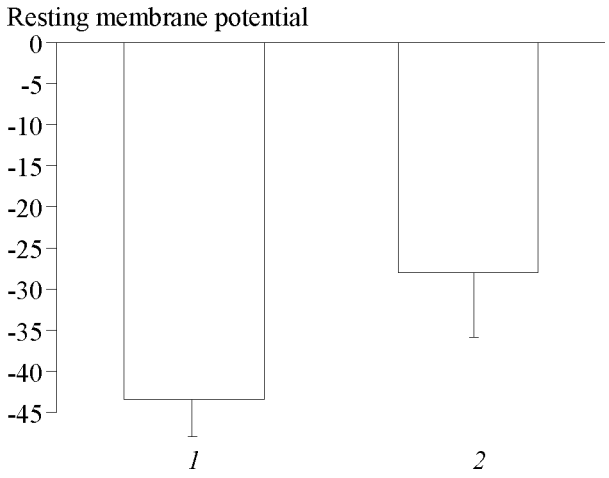


**Fig. 1**. Mean resting membrane potential in control (*1*) and aged (*2*) rats.

## RESULTS

First, we measured resting potentials of endotheliocytes in aortas isolated from control and aged rats. In control rats, the membrane potential varied from -39.5 to -48.1 mV (mean -43.6±3.0 mV, *n*=13). The diffe-

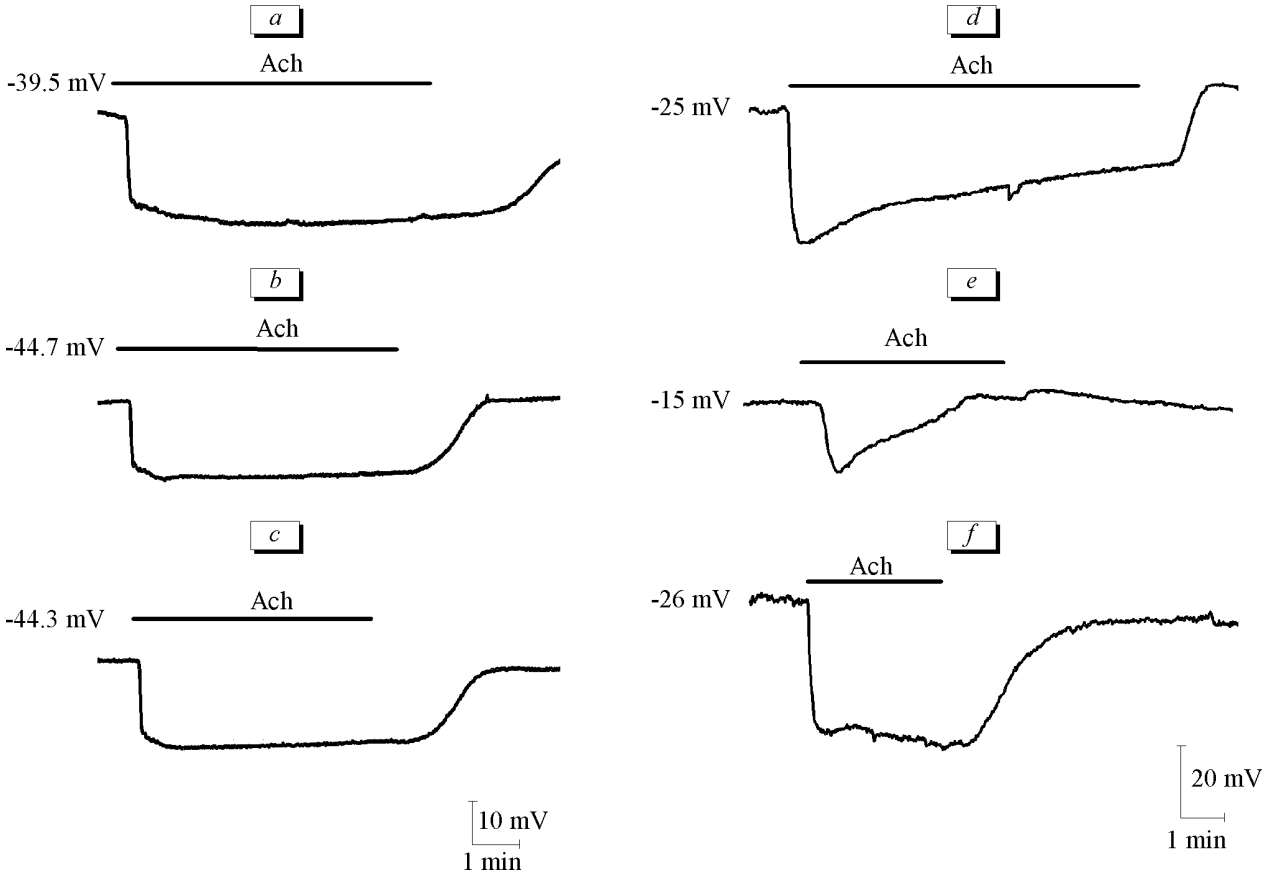


**Fig. 2**. Response of membrane potential in intact rat aorta to acetylcholine (Ach). The bar marks application of Ach. Here and in Fig. 3: The records were made on a single vascular preparation from young (control, *a-c*) and aged (*d-f*) rat.

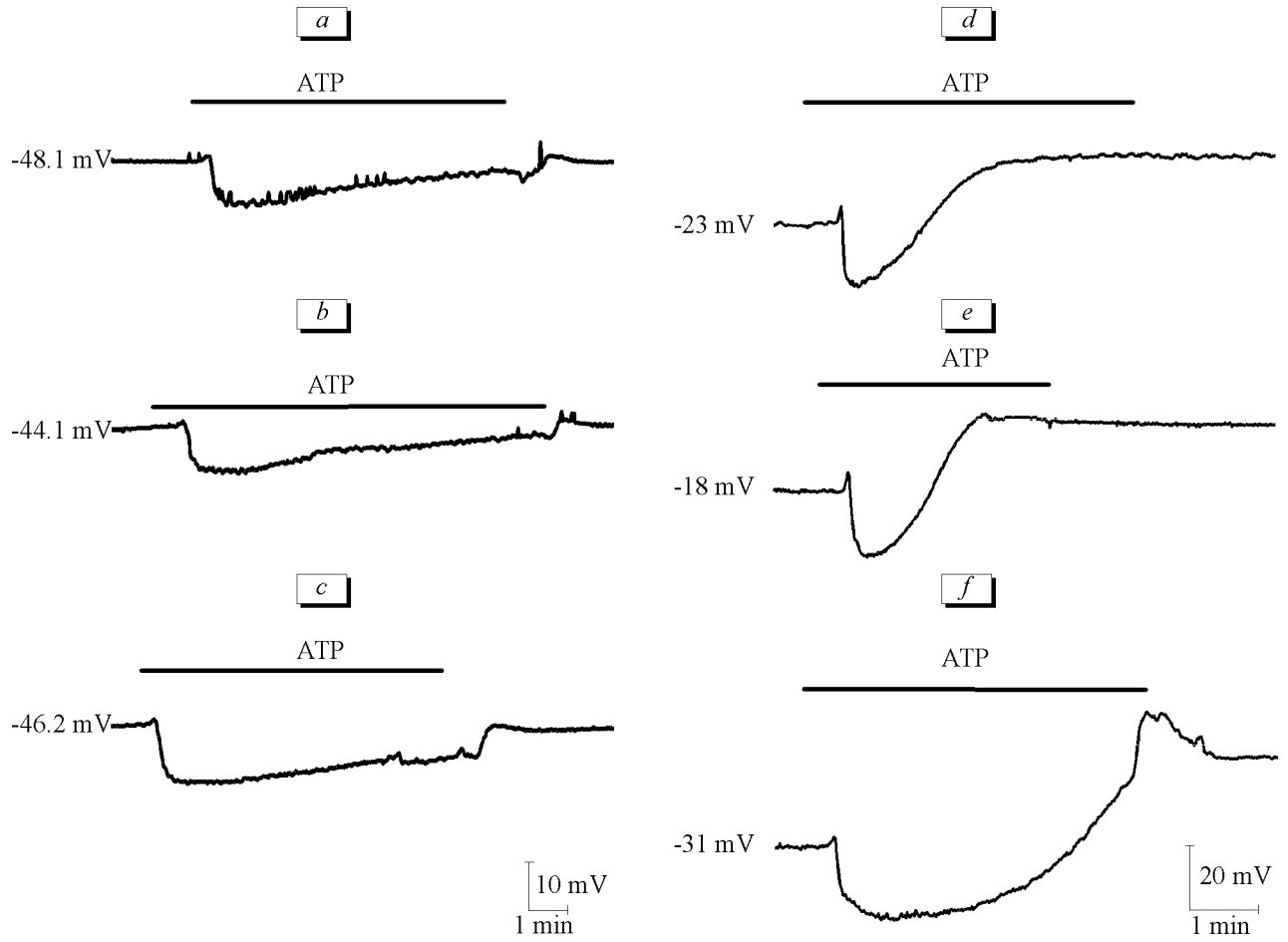


**Fig. 3**. Response of membrane potential in intact rat aorta to ATP. The bar marks ATP application.

rences between membrane potentials recorded repeatedly from the same preparation were less than 4 mV. For aged rats the corresponding values were -46 to -15 mV, -28±9 mV ($n$=23), and 10 mV. These data suggest that endothelial membrane potential in aged rats is higher (more positive) than in controls (Fig. 1). Therefore, in aged rats the intracellular endothelial concentration of calcium ions should be lower than that in control rats. As a result, basal production of NO should be also lower in aged rats in comparison with controls.

Then we examined electrical responses of intact endothelium to Ach (1 μM) and ATP (100 μM). In the control group, Ach always induced a biphasic reaction with initial rapid hyperpolarization by 22.0±3.2 mV ($n$=5) followed by a hyperpolarization plateau (Fig. 2). In aged rats, Ach also induced a biphasic response with initial rapid hyperpolarization of 27±9 mV ($n$=12). However, the second phase was either hyperpolarization plateau ($n$=6) or gradual depolarization ($n$=6). It should be emphasized that different changes in resting potential during the second phase were observed during repeated recordings from the same preparation.

ATP also produced a complex response with the first phase of rapid hyperpolarization by 9.9±1.9 mV ($n$=5) in control group and by 22.6±5.6 mV in aged rats followed by depolarization during the second phase in both groups. However, in contrast to control group, the depolarization phase in aged rats markedly differed in kinetics and amplitude even when it was repeatedly recorded from the same preparation.

In aged rats, the responses to Ach and ATP had a common feature: stable first phase (rapid hyperpolarization) and variable second phase. By contrast, in young rats the responses to Ach and ATP were typical and reproducible [8,9]. It is known that intact vascular epithelium in young animals is characterized by spatiotemporal synchronization of calcium and electrical signalization [13]. First of all, this peculiarity is explained by endothelium-endothelium contacts. Endothelium is a continuous monolayer working as a single organ and responding by a typical reaction to various stimuli. Our findings suggest that aging disturbs the endothelium-endothelium contacts. This hypothesis is corroborated by the fact that resting membrane potential of isolated (not connected) endotheliocytes is

higher than that in connected endotheliocytes [11]. This feature explains higher values of resting membrane potential in aged rats in comparison with those in young controls. Therefore, the endothelial cells form functional clusters, which in our experiments demonstrated their individual features in responses to Ach and ATP.

Thus, our findings suggest that aging promotes functional clustering of the endothelium, which contributes to depression of endothelium-dependent relaxation normally produced by Ach and ATP.

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