# POTENTIATION OF DAMAGING ACTION OF ANGIOTENSIN II ON EPITHELIUM OF RABBIT AORTA BY THE β-ADRENORECEPTOR AGONIST ISOPROTERENOL

O. I. Stenina, T. A. Voino-Yasenetskaya, G. B. Men'shikov, and V. S. Repin

UDC 615.357:577.175.852].015.2:615.217.24].015.4:616.132-018.74].076.9

KEY WORDS: angiotensin; β-adrenoreceptors; damage to endothelium; permeability of vascular wall; low-density lipoproteins.

Intravenous injection of angiotensin II into experimental animals is accompanied by contraction of the endothelial cells, expansion of intercellular junctions, and a change in permeability of the vascular wall for plasma macromolecules [11], which facilitates the onset of changes leading to an atherosclerotic lesion. The action of angiotensin II is mediated by receptors on the cell surface, coupled with stimulation of phosphoinositide metabolism [12]. Previously the writers described the potentiating action of adenylate cyclase stimulators on the damaging action of stimulators of phosphoinositide metabolism in the perfused rabbit aorta [2].

The aim of this investigation was to discover whether potentiation of the action of angiotensin II on the morphology of the endothelium of the rabbit aorta by the β-adrenoreceptor agonist angiotensin II takes place and how the morphological changes discovered are linked with a change in permeability of the aortic wall for low-density lipoproteins (LDL) and albumin.

## EXPERIMENTAL METHOD

Experiments were carried out on 52 male chinchilla rabbits weighing 2.5-3 kg. Connecting tubes and cannulas for perfusion were obtained from "LKB Producter AB" (Sweden), medium 199 and embryonic calf serum were obtained from "Gibco Europe" (Great Britain), angiotensin II and isoproterenol were from "Sigma" (USA), and human serum albumin from "Reanal" (Hungary). The thoracic aorta was separated under intravenous hexobarbital anesthesia (80 mg/ml) with adequate artificial ventilation of the lungs, all its side branches were ligated, and it was divided into two segments of equal length (1-2.5 cm) as described previously [4, 10]. Perfusion took place under recirculation conditions for 1 h at 37°C with a hydrostatic pressure of 100 mm Hg and a flow rate of 10 ml/min. The perfusion fluid consisted of medium 199 with 10% lipoprotein-deficient calf serum; the volume of perfusion fluid did not exceed 6 ml. For oxygenation, a mixture of $O_2$ and $CO_2$ was passed through the perfusion medium. After perfusion the vessels were irrigated with 100 ml of medium 199. The vessels were prepared for scanning electron microscopy (SEM) as described previously [4]. Intercellular boundaries of the endothelium were revealed by impregnation with 0.1% silver nitrate. Specimens were examined on the PSEM-500 scanning electron microscope. Damage to the endothelium and intercellular boundaries was assessed quantitatively by the method described previously [4]. The index of cell shape (the ratio of the greatest width to the greatest length of the cell) was determined by means of an MOP-3 semiautomatic image analyzer ("Kontron-München"). The quantity of silver incorporated into the intercellular spaces was measured by means of a "Kevex" x-ray Spectral Analyzer. LDL (1.019-1.065) were isolated from plasma from healthy donor animals by preparative ultracentrifugation [7]. Albumin and LDL were iodinated by the iodine monochloride method in Bilheimer's modification [3]. $^{125}$I-LDL and $^{125}$I-albumin with radioactivity of 20-160 cpm/ng protein were used. $^{125}$I-LDL were used in a concentration of 5 μg/ml and $^{125}$I-albumin in a concentration of 50 μg/ml. The protein concentration was determined by Lowry's method [8]. Total incorporation of $^{125}$I-LDL and $^{125}$I-albumin into the aortic wall and their distribution through the thickness of the wall were determined. For this purpose, sections 20 μ thick were cut parallel to the luminal surface on a "Reichert" cryotome and the radioactivity of each section was determined.

Institute of Experimental Cardiology, All-Union Cardiologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Smirnov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 433-436, October, 1988. Original article submitted December 18, 1987.

 0007-4888/88/0010-1422$12.50 ©1989 Plenum Publishing Corporation

TABLE 1. IDC (number of damaged cells in 1 $mm^2$), IDB (number of cells with damaged cell boundaries in 1 $mm^2$), and ICS (ratio of greatest width of cell to its greatest length) (n = 4)

| Substance | IDC | IDB | ICS |
|---|---|---|---|
| Control | 4,4±0,4 | 58,0±10,1 | 0,21±0,01* |
| Isoproterenol $10^{-5}$ M | 3,0±0,6 | 50,0±9,0 | 0,20±0,007* |
| Angiotensin $10^{-10}$ M | 3,0±0,5* | 35,5±10,0 | 0,22±0,009* |
| Angiotensin $10^{-10}$ M+ isoproterenol $10^{-5}$ M | 3,0±0,4* | 70,0±10,0* | 0,21±0,007* |
| Angiotensin $10^{-9}$ M | 2,7±0,77* | 45,6±10,9* | 0,3±0,008 |
| Angiotensin $10^{-9}$ M+ isoproterenol $10^{-5}$ M | 14,2±1,1 | 92,0±22,6 | 0,33±0,009 |
| Angiotensin $10^{-8}$ M | 4,62±1,1* | 222,7±32,0 | 0,32±0,007 |
| Angiotensin $10^{-8}$ M+isoproterenol $10^{-5}$ M | 24,1±13,4 | 315,0±34,0 | 0,41±0,001 |
| Angiotensin $10^{-7}$ M | 16,2±9,0 | 181,5±20,0 | 0,29±0,01 |
| Angiotensin $10^{-7}$ M+isoproterenol $10^{-5}$ M | 57,6±16,5 | 340,7±34,3 | 0,32±0,009 |
| Angiotensin $10^{-6}$ M | 30,2±15,8 | 225,0±30,1 | 0,31±0,009 |
| Angiotensin $10^{-6}$ M+ isoproterenol $10^{-5}$ M | 48,9±10,7 | 360,8±48,0 | 0,28±0,007 |

Legend. Here and in Table 2, *$p > 0.05$; in all other cases $p < 0.05$.

## EXPERIMENTAL RESULTS

Angiotensin II ($10^{-9}$ M) and isoproterenol ($10^{-5}$ M) did not damage the endothelial cells or intercellular junctions and did not change the shape of the endotheliocytes (Table 1). During simultaneous perfusion of the rabbit aorta with $10^{-9}$ M angiotensin II and isoproterenol the index of damage to the endothelial cells (IDC) increased up to 3.2 times its value for the control segments, whereas the index of damage to cell boundaries (IDB) increased by 1.6 times, and the index of cell shape (ICS) also increased significantly (Table 1, Fig. 1). With all concentrations used, potentiation of the damaging action of angiotensin II on the endothelium of the rabbit aorta by isoproterenol was observed (Table 1). The change in shape of the endothelial cells is one of the earliest signs of damage to the endothelial cells, accompanied by increased permeability of the vascular wall for high-molecular-weight compounds. Correlation was found previously between the change in shape of the endotheliocytes on contraction, the presence of morphological injuries to the endothelium, and increased permeability of the aortic wall for high-molecular-weight compounds [5, 6, 14]. Under the influence of angiotensin II, alone or in combination with isoproterenol, a change in the shape of the cells was also the first sign of changes in the endothelial monolayer (Table 1).

The increase in incorporation of silver into the intercellular junctions was connected with their expansion and the increase in paracellular permeability for high-molecular-weight compounds. Isoproterenol potentiated widening of the intercellular spaces and the increase in permeability of the endothelial barrier caused by the action of angiotensin II. In the case of simultaneous action of angiotensin II and isoproterenol, incorporation of silver into the intercellular junctions was increased compared with the control with angiotensin in a concentration as low as $10^{-9}$ M (an increase of 1.5 times), whereas incorporation of $^{125}$I-LDL into

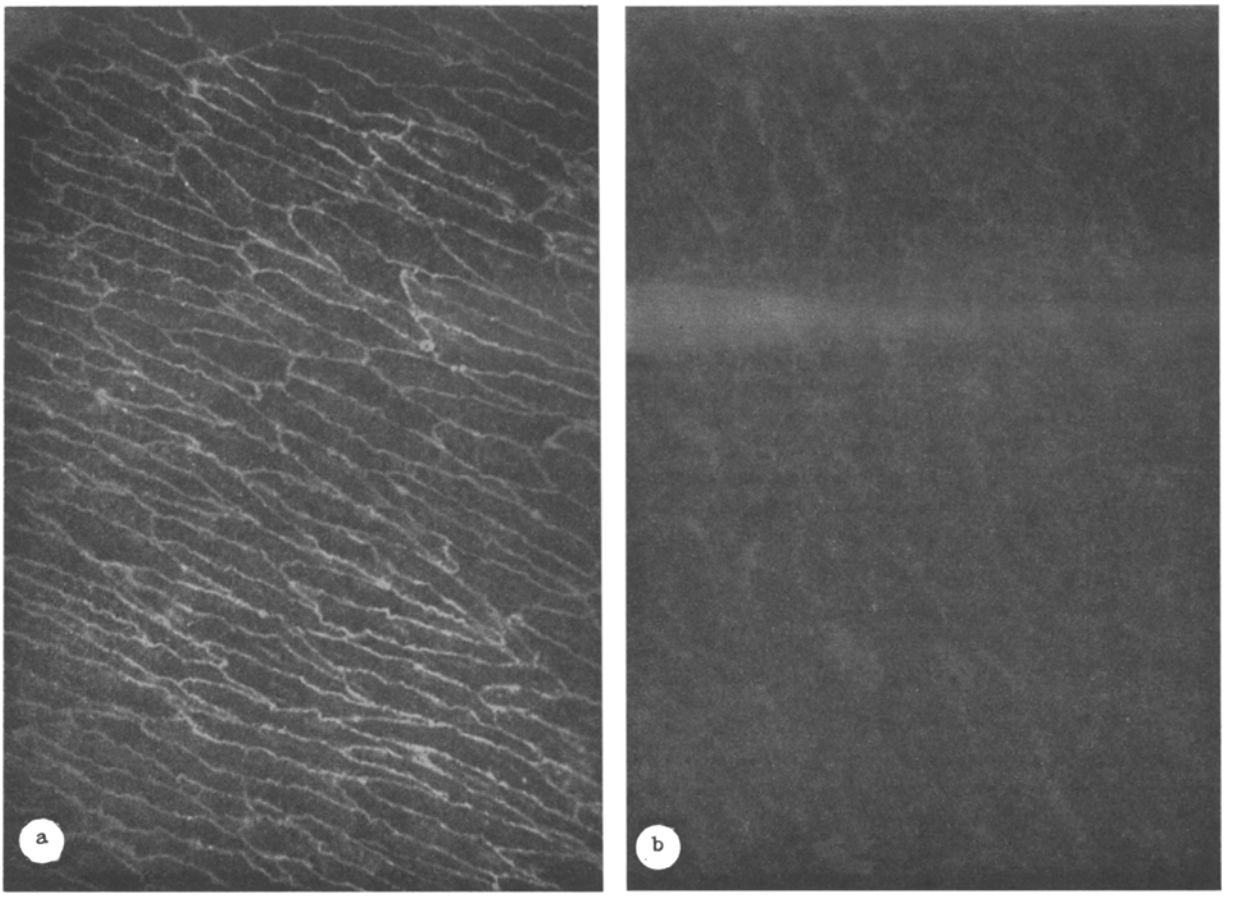


Fig. 1. Changes in shape of cells under the influence of $10^{-8}$ M angiotensin II + $10^{-5}$ M isoproterenol. a) Endothelial monolayer after perfusion with medium No. 199, b) endothelial monolayer after perfusion with $10^{-8}$ M angiotensin II + $10^{-5}$ M isoproterenol. SEM. 640×. Impregnation with 0.1% silver nitrate.

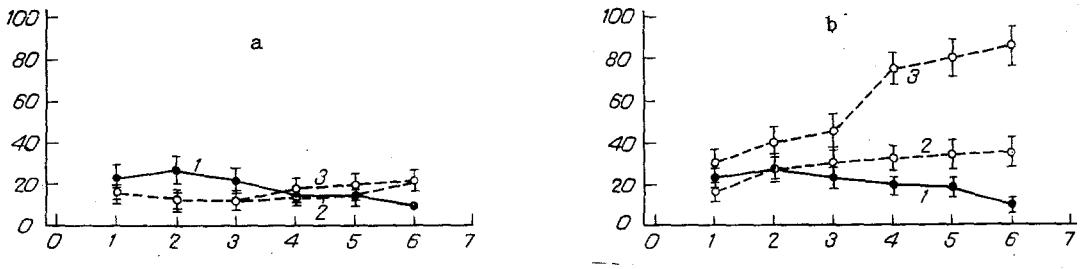


Fig. 2. Distribution of $^{125}$I-LDL through thickness of wall after perfusion for 1 h with $^{125}$I-LDL (5 μg/ml). a) After action of $10^{-9}$ M angiotensin II + $10^{-5}$ M isoproterenol and $10^{-9}$ M angiotensin II; b) after action of $10^{-8}$ M angiotensin II + $10^{-5}$ M isoproterenol and $10^{-8}$ M angiotensin II. Abscissa, No. of section (40 μ) in order of distance from luminal surface of vessel; ordinate, cpm of section × 1000/cpm of perfusion fluid × weight of section (in mg). 1) Control segment (perfusion with medium No. 199); 2) perfusion with angiotensin II; 3) perfusion with angiotensin II + isoproterenol.

TABLE 2. Incorporation of $^{125}$I-LDL into Aortic Wall, cpm of Vessel × 1000/cpm of Perfusion Medium × Weight of Vessel (in mg), and Incorporation of Silver into Intercellular Spaces, in % of Control cpm (n = 4)

| Substance | $^{125}$I-LDL | Ag |
|---|---|---|
| Control | 170,0±55,6 | 100,0±19,5 |
| Isoproterenol $10^{-5}$ M | 185,2±46,0* | 97,5±15* |
| Angiotensin $10^{-10}$ M | 176,6±51,7* | 105,4±17,6* |
| Angiotensin $10^{-10}$ M + isoproterenol $10^{-5}$ M | 181,3±43,6 | 110,5±27,3 |
| Angiotensin $10^{-9}$ M | 127,3±65,0* | 132,4±21,5 |
| Angiotensin $10^{-9}$ M + isoproterenol $10^{-5}$ | 172,2±54,9* | 150,0±18,1 |
| Angiotensin $10^{-8}$ M | 304,5±10,4 | 119,3±32,7 |
| Angiotensin $10^{-8}$ M + isoproterenol $10^{-5}$ | 596,1±86,2 | 228,4±21,3 |
| Angiotensin $10^{-7}$ M | 245,4±62,5 | 134,7±20,5 |
| Angiotensin $10^{-7}$ M + isoproterenol $10^{-5}$ M | 350,3±67,0 | 268,0±12,8 |
| Angiotensin $10^{-6}$ M | 345,5±64,3 | 520,0±9,7 |
| Angiotensin $10^{-6}$ M + isoproterenol $10^{-5}$ M | 376,2±61,8 | 706,8±9,2 |

the wall was doubled under the influence of $10^{-8}$ M angiotensin II and $10^{-5}$ M isoproterenol compared with the action of angiotensin II alone in the same concentration (Table 2). In the intact arterial wall the endothelium and inner elastic membrane are known to act as a barrier for LDL, which accumulate only in the intima [13]. With damage to the endothelium and inner elastic membrane the amount of LDL penetrating into the media and adventitia is increased by 25 times [13]. The increase in the quantity of $^{125}$I-LDL in the wall under the influence of angiotensin II and isoproterenol takes place through accumulation of $^{125}$I-LDL in the outer layers of the wall (Fig. 2). Incorporation of $^{125}$I-albumin and its distribution in the wall were unchanged by the action of angiotensin II and isoproterenol compared with the control segments, and this may be explained by the free passage of molecules of that size through the intercellular spaces of the endothelium in the control [1, 9]. Differences in the accumulation and distribution of LDL and albumin in the aortic wall may perhaps be explained by selective accumulation of LDL in the aortic wall associated with damage to the endothelial barrier.

Potentiation of the action of angiotensin II on the aortic endothelium by isoproterenol may indicate that even with a very small increase in the concentration of angiotensin II and a simultaneous increase in the concentration of agents activating β-adrenoreceptors, damage to the cells and intercellular junctions and disturbance of the barrier function of the endothelium may take place.

## LITERATURE CITED


1. V. Kh. Anestiadi and V. A. Nagornev, Morphogenesis of Atherosclerosis [in Russian], Kishinev (1982).
2. T. A. Voino-Yasenetskaya, I. K. Kondakov, V. A. Tkachuk, et al., Dokl. Akad. Nauk SSSR, 290, No. 5, 1238 (1986).
3. D. W. Bilheimer, S. Eisenberg, and K. I. Levy, Biochim. Biophys. Acta, 260, 212 (1972).
4. V. V. Dolgov, O. E. Zaikina, M. F. Bondarenko, and V. S. Repin, Diabetologia, 22, 338 (1982).
5. B. Haraldsson and B. Rippe, Acta Physiol. Scand., 124, Suppl. 542, 97 (1985).
6. R. R. Johansson, Acta Physiol. Scand., 124, Suppl. 542, 93 (1985).
7. F. F. Lingren, Analysis of Lipids and Lipoproteins, ed. by E. G. Perkin, New York (1975), pp. 9 and 204.
8. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, J. Biol. Chem., 193, 265 (1951).
9. T. Okuda and T. Yamamoto, Cell Tissue Res., 231, 117 (1983).
10. S. N. Preobrazhensky, V. V. Dolgov, H. G. Flegel, et al., Atherosclerosis, 48, 147 (1983).
11. A. L. Robertson and P. A. Khairallah, Circulat. Rev., 31, 923 (1972).

12. E. B. Smith and E. M. Staples, Atherosclerosis, 37, 579 (1980).
13. J. B. Smith, L. Smith, E. R. Brown, et al., Proc. Natl. Acad. Sci. USA, 81, 7812 (1984).
14. E. Svensjo, Acta Physiol. Scand., 124, Suppl. 542, 98 (1985).

# MECHANISM OF ACTION OF BACLOFEN ON MYOCARDIAL AND VASCULAR CONTRACTILITY

Yu. A. Darinskii, V. Ya. Egorov,
T. A. Smirnova, Yu. D. Ignatov,
and A. V. Dmitriev

UDC 615.217:547.466.3:001.6

KEY WORDS: baclofen; myocardium; vein; artery.

In its effective dose range the γ-aminobutyric acid derivative baclofen (Lioresal), with a muscle-relaxing and analgesic action, has a marked effect on the cardiovascular system [9]. Changes in activity of the cardiovascular system are considered to be due mainly to the central action of the drug, leading to intensification of the flow of sympathetic impulses to adrenoreceptors of the heart and vessels [1, 3-6, 9]. Meanwhile the problem of whether baclofen may have a direct peripheral action on autonomic functions of the body remains open.

The aim of this investigation was to study the effect of baclofen on contractility of cardiomyocytes and smooth-muscle cells (SMC) of blood vessels in vitro.

## EXPERIMENTAL METHOD

Experiments were carried out on isolated segments of arteries and veins and the papillary muscle of the left ventricle of the heart of a Wistar albino rat. After decapitation of the animal the papillary muscle, circular segments of the abdominal aorta and iliac artery 2 mm wide, and a longitudinal segment of the portal vein 7 mm long were dissected. The finished preparations were placed in a thermostatted working chamber, perfused with oxygenated Krebs' solution at 32°C, and fixed for recording isometric contractions by means of a 6MKh1S mechanical to electrical transducer. Contractions were recorded on an N-338-2 automatic writer. Contractility of the portal vein (tone, amplitude, and frequency of spontaneous contractions), the abdominal aorta, and iliac artery (tone and amplitude of contractions evoked by electrical stimulation), and the papillary muscle (amplitude, velocity of contraction and relaxation in response to electrical stimulation) were recorded. For electrical stimulation square pulses with a duration of 0.5 msec, frequency of 0.5 Hz, and amplitude of twice the threshold were used for electrical stimulation. Baclofen was added to the perfusion solution after incubation of the preparations in the working chamber for 30 min.

## EXPERIMENTAL RESULTS

In low concentrations (up to $10^{-6}$ M) baclofen caused no significant changes in the parameters recorded. Addition of baclofen to the control solution in a concentration of $10^{-5}$ M led to an increase in tone of the abdominal aorta and iliac artery of the rats (by 21.7 ± 1.2 and 18.7 ± 1.3% respectively; $n = 27$, $p < 0.05$) and a decrease in amplitude of the phasic contractions (by 15.7 ± 1.4 and 11.9 ± 0.7%; Fig. 1a). The ionotropic effect of the drug lasted 10 min. Baclofen gave none of the effects described above if preceded by α-adrenoreceptor blockade by dihydroergotoxin ($10^{-5}$ M).

Baclofen increased the amplitude of spontaneous phasic contractions of a segment of the rat portal vein (Fig. 1b). Maximal changes in the parameter were recorded after 1 min of the action of baclofen and the inotropic effect lasted 10 min. The frequency of contractions and tone of the vessel showed no significant change. The positive inotropic effect of the drug was preserved after β-adrenoreceptor blockage by propranolol ($10^{-5}$ M), in hypercalcium solution

Department of Anatomy and Physiology of Man and Animals, A. I. Gertsen Leningrad Pedagogic Institute. Department of Pharmacology, Academician I. P. Pavlov First Leningrad Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR A. V. Val'dman.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 106, No. 10, pp. 436-438, October, 1988. Original article submitted February 28, 1988.

 0007-4888/88/0010-1426$12.50 © 1989 Plenum Publishing Corporation