Participation of Endogenous Dilation Factor of the Adrenals in Hypotensive Effect of Indapamide

N. A. Medvedeva, M. A. Nesterova, and O. S. Medvedev*

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 126, No. 11, pp. 365-368, November, 1998
Original article submitted June 30, 1997

The effect of indapamide on neurogenic constrictor responses was examined in an isolated segment of rat tail artery perfused by blood from intact rats and rats with surgically removed adrenal medulla. It was found that adrenal demedullation abolishes reduction in neurogenic constrictor responses of the arterial segment observed under the effect of indapamide.

Key Words: *tail artery; blood perfusion; electrical stimulation; adrenals*

The antihypertensive drug indapamide (IP) is a lipophilic indolyl derivative of chlorosulfonamide. In low doses (2.5-3 mg/kg) it displays weak diuretic and strong vasodilatory activities.

Indapamide reduces the pressor response to angiotensin II, norepinephrine, and phenylephrine in hypertensive patients [7,15] and decreases peripheral vascular resistance [16]. The *in vitro* studies have shown that vasodilatory activity of IP is associated with its direct effect on vascular smooth muscle cells manifesting itself in decreased Ca^{2+} entry [13] and Ca^{2+} mobilization from the sarcoplasmic reticulum [12]. Indapamide stimulates the synthesis of prostacyclin, inhibits thromboxane A production [6], and reduces the release of norepinephrine from the sympathetic endings [4].

Vascular tone is determined predominantly by sympathetic nervous system and humoral factors produced by the kidneys and adrenals. Clinical studies have shown that the hypotensive effect of IP is preserved in hypertensive patients after renalectomy [10]. There is considerable evidence that IP produces no significant effect the plasma levels of norepinephrine and epinephrine [3]. However, secretory granules of adrenal medulla contain not only these hormones but also some other substances, including enkephalins [5] and the depressor peptide adrenomedullin [9]. Systemic administration of adrenomedullin reduces the total peripheral vascular resistance [14], while enkephalins inhibit transmitter secretion from sympathetic endings in the vascular wall [8].

Proceeding from these data, we attempted to find out whether some vasodilatory effects of low IP doses are associated with adrenal medulla.

MATERIALS AND METHODS

An isolated segment of rat tail artery was perfused by the blood of alert rats-donors (*ex vivo*) [1]. The segment was obtained from rats euthanized by ether overdosage. It was perfused at a constant rate (2 ml/min, Stathan peristaltic pump) in a thermostat-controlled chamber filled with a modified Krebs-Henseleit solution. Electrical stimulation (50 V, 0.1-0.5 A, pulse duration 0.08-0.1 msec, 2-16 Hz) was performed via two electrodes. The segment was mounted on one of them (metal cannula) and the other (14-karat gold) was applied to it from the outside (Fig. 1).

Male Wistar rats (body weight 250-350 g, age 4-6 months) were used. Three polyethylene catheters: in the carotid artery, femoral vein, and femoral artery were implanted into donors rats under Nembutal anesthesia (40 mg/kg, intraperitoneally) one day prior the experiment. Blood for perfusion was pumped out from the carotid artery and, after being heated

Department of Human and Animal Physiology, Biological Faculty; *Department of Pharmacology, Faculty of Fundamental Medicine, M. V. Lomonosov Moscow State University

0007-4888/98/0011-1144$20.00

to the body temperature, pumped in the femoral vein. Indapamide (3 mg/kg) and agonists of α-adrenoreceptors (AR) were added to venous blood. Arterial pressure (AP) and heart rate were recorded trough the catheter inserted into the femoral artery. The donor rats were alert throughout the entire perfusion session.

Two series of experiments were performed. In the first series the arterial segment was perfused by blood of donor rats given a bolus injection of IP (3 mg/kg). Electrical stimulation of the segment (4 pulses at a 4-min interval) was performed before and 30 min after administration of IP. The agonists of α_1- and α_2-AR were injected intravenously according to this regimen. The responses of the rats to an equal volume of the vehicle (ethanol) were studied in a control series.

In the second series, the segment was perfused by blood from rats without adrenal medulla. Demedullation was performed by the standard method [11]. The catheters were inserted 5 days after the surgery. The segment was obtained from intact rats. Electrical stimulation of the vascular wall sympathetic fibers was performed before and 30 min after administration of IP to donor rats.

The following compounds were used: IP (3 mg/kg, Servier), the α_1-AR agonist phenylephrine (0.007-0.02 mg/kg, Sigma), the α_2-AR agonist (-)3,4-dihydrooxynorephedrine (0.002-0.01 mg/kg, Sigma). Indapamide was dissolved in 96% ethanol (stock solution 10 g/ml). Modified Krebs-Henseleit solution contained (mmol/liter): NaCl 122.2, KCl 6.7, $CaCl_2$ 2.1, $MgSO_4$ 1.3, $KHPO_4$ 1.1, glucose 14.9, carbogen 96% O_2 and 4% CO_2 (20 min).

The response of the segment to electrical stimulation and α-AR agonists was estimated by changes in the perfusion pressure relative to the initial level which was taken as 100%.

The results were analyzed by the Student *t* test.

RESULTS

A decrease in the neurogenic response of an isolated segment of rat tail artery perfused with blood from a rat injected with 3 mg/kg IP was observed in the first series of experiments (Fig. 2). Before administration of IP the perfusion pressure of the segment in response to electrical stimulation increased by 77.5±18.9% in comparison with the initial value, while 30 min after administration of IP it increased by 37.0±13.7% ($p<0.05$, $n=6$). In the control series, administration of an equal volume of the vehicle had no effect on the constrictor response of the segment.

The response of the segment to the α-AR agonists did not change against the background of IP.

Thus, perfusion of an isolated segment of rat tail artery by blood from an alert donor rat injected with IP (3 mg/kg, intravenously) reduces the constrictor response of the segment. Since the reactivity of the segment in response to α-AR agonists against the background of IP remained unchanged, we have suggested that IP affects the sympathetic endings in

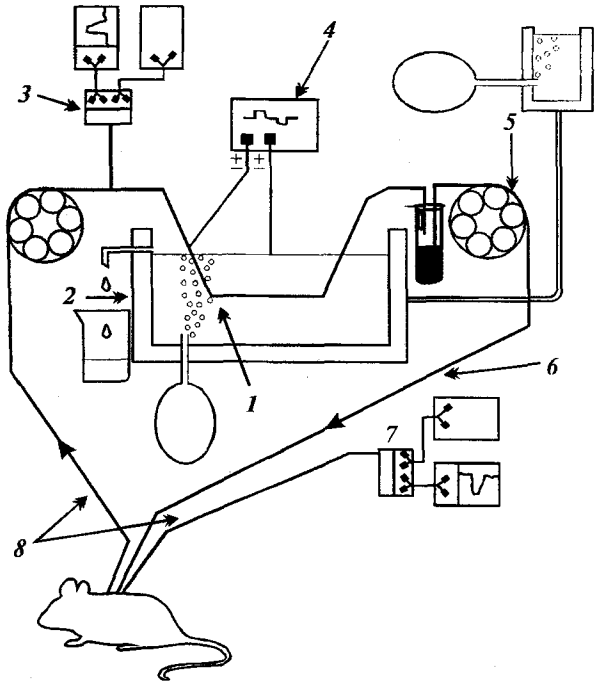

Fig. 1. A scheme of experiment. *1)* isolated blood vessel; *2)* thermostat-controlled chamber, *3)* perfusion pressure sensor, *4)* electrical stimulator, *5)* pump, *6)* venous catheter, *7)* arterial pressure sensor, *8)* arterial catheters.

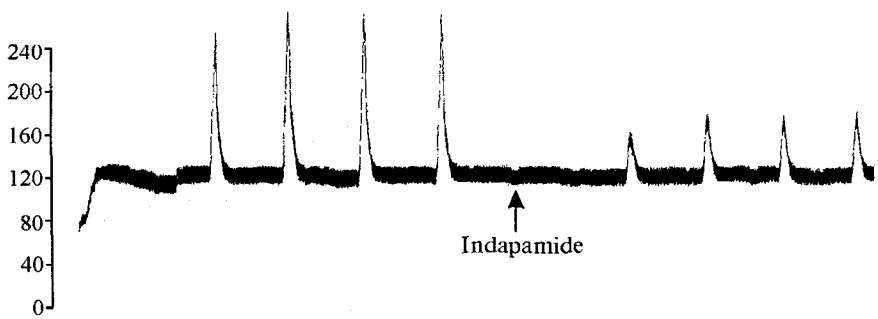

Fig. 2. Original record of changes in perfusion pressure from isolated arterial segment in response to electrical stimulation of the vascular wall sympathetic fibers before and 30 min after administration of indapamide. Ordinates: perfusion pressure increase caused by electrical stimulation of sympathetic fibers of vascular wall, mm Hg.

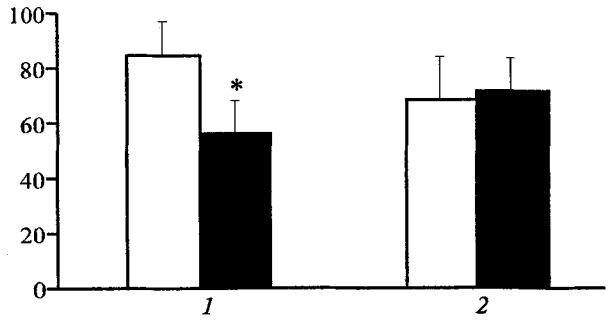

Fig. 3. Changes in neurogenic constrictor responses of isolated segment of tail artery upon perfusion by blood from intact *(1)* and demedullated *(2)* rats before administration of indapamide (n=5). Ordinate: perfusion pressure increase in response to electrical stimulation of the vascular wall sympathetic fibers, % of basal perfusion pressure. Light bars: changes in perfusion pressure before administration of indapamide; dark bars: the same parameter 30 min after administration of indapamide. *p<0.05 compared with the control.

the vascular wall but not the smooth muscle cells. This suggestion is consistent with the observation that IP reduces the neurotransmitter release from sympathetic endings [4].

Five and ten minutes after administration of IP, AP decreased, respectively, by 8.8% (p<0.05) and 8% (p<0.01) of the initial level (100.2±9.1 mm Hg) and normalized by the 30th min. The vehicle had no significant effect on AP. Five minutes after administration of IP, heart rate increased by 3.7% (p<0.05) of the initial level (354.5±12.2 beats/min) and normalized by the 30th minute.

In the second series, demedullation abolished the inhibitory effect of IP on neurogenic constrictor responses of the segment. The perfusion pressure in response of IP to electrical stimulation before and 30 min after administration was 79.4±15.6 and 78.8±10.7 mm Hg, respectively (58.6 and 55.9% relative to the initial perfusion pressure, n=5). Thus, we observed no reduction in neurogenic constrictor responses of isolated segment of rat tail artery perfused by blood from rats without adrenal medulla (Fig. 3). It can be suggested that IP induces the release of a humoral factor from the adrenal medulla cells and this factor inhibits the neurogenic constrictors responses of the segment (for example, by decreasing the neurotransmitter release from sympathetic endings in the vascular wall) or participates in the cascade of reactions leading to inhibition of neurogenic constrictor responses. Enkephalins and adrenomedullin are potential candidates for these factors.

Presumably, this mechanism of the antihypertensive effect of IP is part of the general endogenous mechanisms of AP regulation by humoral factors of the adrenal medulla. This mechanism operates in spontaneously hypertensive rats in which humoral factor(s) reduce neurogenic constrictor responses of an isolated segment of rat tail artery by inhibiting the release of neurotransmitter from the sympathetic fibers of the vascular wall [2]. Demedullation abolishes this effect.

The results obtained in the present study indicate that 1) perfusion of an isolated arterial segment with blood from an IP-treated rat reduces the neurogenic constrictor response of the segment and 2) perfusion of this segment with blood from rats with surgically removed adrenal medulla abolishes reduction in the neurogenic response of the segments under the effect of IP.

REFERENCES

1. S. Yu. Mashina, N. A. Medvedeva, and O. S. Medvedev, *Fiziol. Zh. SSSR*, **74**, No. 9, 1341-1344.
2. N. A. Medvedeva, M. A. Zharkova, A. A. Chuiko, and O. S. Medvedev, *Byull. Eksper. Biol. Med.*, **121**, No. 4, 380-383 (1996).
3. B. D. Campbell and F. Brackman, *Am. J. Cardiol.*, **2**, 11-27 (1990).
4. R. Carretta, B. Fabris, F. Fischetti, *et al.*, *Am. J. Med.*, **84**, Suppl. 1B, 31-35 (1988).
5. R. Fischer-Colbrie, A. Iacangelo, and L. E. Eiden, *Neurobiology*, **85**, 3240-3244 (1988).
6. F. M. Gbeassor, J. H. Grose, and M. Le Bel, *Clin. Invest. Med.*, **5**, 26B (1982).
7. M. Grimm, P. Weidman, A. Meier, *et al.*, *Br. Heart J.*, **46**, 404-409 (1981).
8. P. Illes, D. Ramme, and K. Starcke, *Eur. J. Pharmacol.*, **107**, 397-398 (1985).
9. F. Kato, K. Kitamura, Y. Yamamoto, *et al.*, *J. Hypertens. Suppl.*, **12**, No. 3, 238-244 (1994).
10. F. Leenen, *Am. J. Med.*, **84**, Suppl. 1B, 76-85 (1988).
11. P. I. Majewski and M. J. Rand, *Clin. Exp. Pharmacol. Physiol.*, **13**, 283-288 (1986).
12. J. Mironneau, *Am. J. Med.*, **84**, 10-14 (1988).
13. J. Mironneau and Y. M. Gargouil, *Eur. J. Pharmacol.*, **57**, 57-67 (1979).
14. S. Nakamura, K. Kitamura, Y. Ishiyama, *et al.*, *J. Hypertens. Suppl.*, **12**, No. 3, 198-246 (1994).
15. R. J. Noveck, A. Quiroz, T. Jiles, *et al.*, *Clin. Pharmacol. Ther.*, **31**, 257-260 (1982).
16. H. Villarreal, E. Nuner-Poissot, M. C. Anguas, *et al.*, *Curr. Med. Res. Opin.*, **8**, Suppl. 3, 135-139 (1983).