13. J. A. Martinez, R. Parrilla, and M. S. Aguso, Biochim. Biophys. Acta, 760, 175 (1983).

ROLE OF THE ENDOTHELIUM IN THE DEVELOPMENT OF REACTIVE HYPEREMIA

V. F. Sagach and M. N. Tkachenko

UDC 616.005.2].3-092:616.13].14-018.74

KEY WORDS: reactive hyperemia; endothelium.

The development of reactive hyperemia is a fundamental phenomenon found in various vascular regions of the circulation [1]. An important role in the development of reactive hyperemia is ascribed to metabolic agents secreted during previous ischemia. An important role in the formation of vascular reactions has recently been discovered for the endothelium, which is involved in the mechanism of action of various agents on blood vessels through the secretion of endothelial relaxation factors [3, 4], which has recently been identified as an endogenous nitrate [8, 10]. The endothelium has been shown to take part in the development of vasomotor reactions to a change in the velocity of the blood flow [2]. It very probably has a role also in the development of postocclusive vasodilatation.

The aim of this investigation was to study whether the endothelium is involved in the development of reactive hyperemia.

EXPERIMENTAL METHOD

Experiments were carried out on 22 dogs weighing 15-20 kg, anesthetized with chloralose and urethane (0.05 and 0.5 g/kg, respectively, intravenously). The following series of experiments were carried out: I) to study the effect of chemical removal of the endothelium, II) to study the effect of blocking biosynthesis of derivatives of the cyclooxygenase pathway of arachidonic acid metabolism, III) to study the effect of lipoxygenase blockade, and IV) the effect of guanylate cyclase blockade on reactive hyperemia in the system of the femoral artery. The reaction was reproduced after circulatory arrest (compression of artery and vein) for a period of between 5 and 120 sec. Changes in blood flow were determined with the aid of an RK-2 electromagnetic blood flowmeter and recorded on a multichannel automatic writer. The reaction was recorded in the initial state, after which the endothelium was removed or blockade carried out, and reactive hyperemia was again induced. The endothelium was removed by injecting a solution of saponin (1 mg/ml) into the system of the femoral arteries and arresting the circulation for 5 min, thereby destroying the endothelial cells and selectively inhibiting endothelium-dependent relaxation [14]. Cyclo-oxygenase was blocked by intravenous injection of a solution of indomethacin (3 mg/kg), lipoxygenase by intravenous injection of quercetin solution (10 mg/kg), and guanylate cyclase by intravenous injection of a solution of methylene blue (4 mg/kg). Possible changes in reactivity of the vascular smooth muscles under the influence of the above chemical agents were monitored on the basis of the response to injection of papaverine (4 mg in 1 ml physiological saline). The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

The postocclusive increase in blood flow depended strictly on the duration of preceding occlusion. This reaction was considerably reduced after chemical removal of the endothelium with saponin (Fig. 1). For instance, whereas in the animals before treatment with saponin the peak blood flow after its arrest varied from 24.4 ± 2.9% after occlusion for 5 sec to 167.4 ± 25.8% of the initial blood flow after occlusion for 2 min, after treatment

Department of Physiology of the Circulation, A. A. Bogomolets Institute of Physiology, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR B. I. Tkachenko.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 108, No. 10, pp. 421-423, October, 1989. Original article submitted May 3, 1988.

 0007-4888/89/0010-1404 $12.50 © 1990 Plenum Publishing Corporation

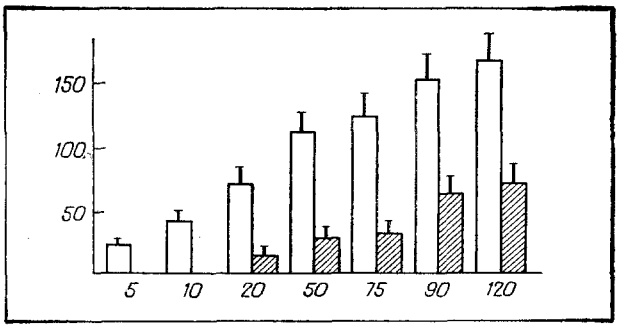

Fig. 1. Effect of chemical removal of endothelium from femoral artery on development of reactive hyperemia after occlusion of varied duration (5-120 sec). Abscissa, time (in sec); ordinate, peak blood flow (in per cent of initial blood flow). Unshaded columns – reactive hyperemia of intact vessel, shaded columns – reactive hyperemia of de-endothelized vessel.

with saponin reactive hyperemia was absent after transient circulatory arrest (5 and 10 sec), and after occlusion for 20-75 sec it was 3-4 times less than initially. Differences between the magnitude of the responses before and after removal of the endothelium were significant in all cases ($p < 0.01$). The reaction of an increase in the blood flow to injection of 20-40 μg acetylcholine (in 1 ml physiological saline) into the femoral artery (acetylcholine, as is well known, realizes its dilator action on blood vessels through the intermediary of endothelial relaxation factor) was reduced by the same degree after treatment with saponin. Meanwhile the reaction of an increase of blood flow to papaverine, which realizes its action on vessels without the participation of the endothelium, was virtually unchanged after treatment with saponin. Consequently, treatment of the inner vascular surface with saponin largely inhibits the physiological activity of the endothelial cells and has little effect of function of the smooth-muscle cells of the vascular wall. Thus significant inhibition of reactive hyperemia after chemical de-endothelization is evidence that the endothelium plays an important role in the development of this vascular reaction.

The data showing a marked increase in release of cyclo-oxygenase derivatives by cells of the vascular wall during reperfusion [6] suggested that prostaglandins may be involved in the mechanism of reactive hyperemia. However, the facts obtained in this respect previously were contradictory [5]. It follows from our results that blocking prostaglandin synthesis by injection of indomethacin potentiated the increase in blood flow after circulatory arrest for a varied duration (Table 1). This effect of indomethacin increased with an increase in the duration of preceding occlusion. Hyperemia was most marked after occlusion for 2 min, i.e., when the role of metabolic agents in the development of the response may be more important. In this case indomethacin increased the peak blood flow by 53 ± 10% compared with the initial response ($p < 0.01$). These data are in agreement with the increase in postanoxic hyperemia after cyclooxygenase blockade in blood vessels of the brain [11] and skeletal muscles [12], and the increase in endothelium-dependent relaxation of the vessels by acetylcholine [7], which realizes its action on the vessels through secretion of endothelial relaxation factor from endothelial cells. Thus indomethacin, stimulating endothelium-dependent relaxation, increases reactive hyperemia in the system of femoral vessels.

Secretion of relaxing factor by the endothelial cells and endothelium-dependent relaxation, as shown previously, are inhibited by lipoxygenase blockers [4]. In the present experiments injection of the lipoxygenase blocker, quercetin, significantly inhibited the development of reactive hyperemia in the femoral vessels of a dog by 60-90% of its initial value (Table 1). Thus lipoxygenase blockade, inhibiting the ability of the endothelium to produce relaxation factor, considerably weakens the development of reactive hyperemia.

The action of endothelium relaxation factor on smooth muscles is known to be realized through activation of guanylate cyclase, elevation of the intracellular cGMP level [13], and it is inhibited by guanylate cyclase blockade [9]. In the present experiments this type of blockade, carried out with methylene blue, led to disappearance of the response

TABLE 1. Effect of Blockade of Cyclo-Oxygenase, Lipoxygenase, and Guanylate Cyclase on Development of Reactive Hyperemia

Experimental conditions	Peak blood flow (in per cent of initial blood fow) after stopping perfusion of the limb						
	time after stopping perfusion, sec						
	5	10	20	50	75	90	120
Initial response	+34±14	+42±5,7	+68,8±10	+95,8±15	+100±11	+136±13	+155±18
Injection of indomethacin	+64±10**	+74±7*	+103±15**	+167,4±27*	+185,8±23*	+204±18*	+229,2±29*
Initial response	+42,6±7,3	+46,4±7,8	+71,5±7,8	+110,6±12,8	+118,8±10,8	+126,6±11,3	+130±15,2
Injection of quercetin	+3,5±1,5*	+5,4±1,1*	+17,4±4,4*	+26,2±7,1*	+43±10,6*	+45,1±13,8*	+48,7±13,3*
Initial response	+35±8,6	+59±5,1	+74,5±12	+132,5±17,5	+162±25	+165,5±28,5	+183±29
Injection of methylene blue	No change	−1,3±0,7*	−4,8±1,9*	−8,8±2*	−6,6±1,6*	−6,3±1,4*	−7,75±0,8*

Legend. *) Difference from initial response is significant, **) calculation by the difference method.

and even to failure of the blood flow to be restored during reperfusion (Table 1). Meanwhile the reaction to papaverine showed no significant change after injection of methylene blue, just as after the action of indomethacin and quercetin.

Diminution of reactive hyperemia after chemical de-endothelization, or after blockade of the vasodilator activity of the endothelium by the lipoxygenase blocker quercetin or blockade of guanylate cyclase by methylene blue, and the increase in postocclusive hyperemia by indomethacin lead to the conclusion that an essential role in the development of reactive hyperemia is played by the endothelium, whose involvement in the development of postocclusive hyperemia is realized through the secretion of endothelial relaxation factor. Postocclusive vascular reactions can be considerably modulated by pharmacologic action on biosynthesis of biologically active substances by the endothelium.

LITERATURE CITED

1. B. I. Tkachenko (ed.), Physiology of the Circulation: Physiology of the Vascular System [in Russian], Leningrad (1984), pp. 337-351.
2. V. M. Khayutin, Vest. Akad. Med. Nauk SSSR, No. 6, 89 (1987).
3. R. Busse, G. Trogisch, and E. Bassenge, Basic Res. Cardiol., 80, 475 (1985).
4. R. Furchgott, Circulat. Res., 53, 557 (1983).
5. M. Karmazyn and N. D. Dhalla, Can. J. Physiol. Pharmacol., 61, 1207 (1983).
6. S. Lelcuk, F. Alexander, C. R. Valeri, et al., Ann. Surg., 202, 642 (1985).
7. T. F. Luscher, J. C. Romero, and P. M. Vanhoutte, J. Hypertens., 4, Suppl. 6, s81 (1986).
8. W. Martin, C. M. Villani, D. Jothianandan, and R. F. Furchgott, J. Pharmacol. Exp. Ther., 232, 708 (1985).
9. S. Moncada, A. G. Herman, and P. M. Vanhoutte, Trends. Pharmacol. Sci., 8, 365 (1987).
10. R. M. J. Palmer, A. G. Ferrige, and S. Moncada, Nature, 327, 524 (1987).
11. J. W. Phillis, R. E. Delong, and J. K. Towner, Eur. J. Pharmacol., 124, 85 (1986).
12. R. S. Rankin and R. E. Klabande, Life Sci., 36, 161 (1985).
13. R. M. Rapoport and F. Murad, Circulat. Res., 52, 352 (1983).
14. K. Samata, T. Kimbura, S. Satoh, and H. Watanabe, Eur. J. Pharmacol., 128, 85 (1986).