EFFECT OF TUMOR NECROSIS FACTOR ON CEREBRAL ARTERIAL TONE

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Vascular tone and the structural organization of the vascular bed are closely dependent on the level of function of the various tissue elements, and the functions of the organ receiving blood are dependent on metabolic activity [5]. It can accordingly be postulated that the mechanisms of the structure of the vascular system, like the physiological mechanisms of regulation of the blood flow, must be in close correlation, and must evidently be coordinated by common humoral regulatory signals. The list of factors stimulating angiogenesis includes tumor necrosis factor (TNF), produced by macrophages [4, 6, 7]. TNF production in vivo is stimulated by injection of *Corynebacterium parvum* endotoxin [8], but to obtain this factor in vitro, bioengineering methods involving cloning and recombination of DNA of *Escherichia coli* have been developed [9]. In its chemical structure TNF is a glycoprotein (mol.wt. 70,000) [3], homologous in amino-acid composition with lymphotoxin and lymphokinin. We were struck by the fact that the TNF in vivo causes the development of a capillary network, but in vitro it stimulates chemotaxis of a culture of endotheliocytes, with the formation of capillary-like structures [4, 6, 7]. This effect suggests that TNF is involved both in the structural organization of the vascular bed and also in mechanisms of regulation of vascular tone.

The aim of this investigation was to determine the direct vasoactive action of TNF in the system regulating the cerebral blood flow and to demonstrate that the mechanisms of this action are dependent on the original state of the endotheliocytes.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature rabbits of both sexes weighing 2-3 kg and anesthetized with hexobarbital and urethane. The intensity of the local cerebral blood flow (LCBF) was determined by the hydrogen clearance method, using electrodes implanted into the parietal cortex [2]. The heart rate and systemic blood pressure were recorded at the same time. In some experiments the diameter of the pial arteries was measured by intravital photography through a burr-hole, followed by quantitative analysis of the data. TNF (produced at the M. M. Shemyakin Institute of Bioorganic Chemistry, Academy of Sciences of the USSR, mol.wt. 17,000) was injected into the animals by means of a catheter through the carotid artery. The direct effect of TNF on smooth muscle tone was studied on isolated ring segments of the bovine middle cerebral artery and rabbit carotid artery under isometric conditions. To study the role of the endothelium, experiments were carried out simultaneously on isolated intact and de-endothelized preparations, using two mechanotron systems with identical kinematic and electrical characteristics. Functional integrity of the endothelium was confirmed by the method of stimulating endothelium-dependent relaxation of smooth muscles by acetylcholine [1].

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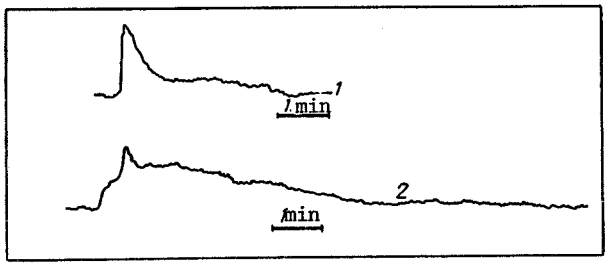

Fig. 1. Intensity of LCBF under normal conditions (1) and after injection of TNF (2).

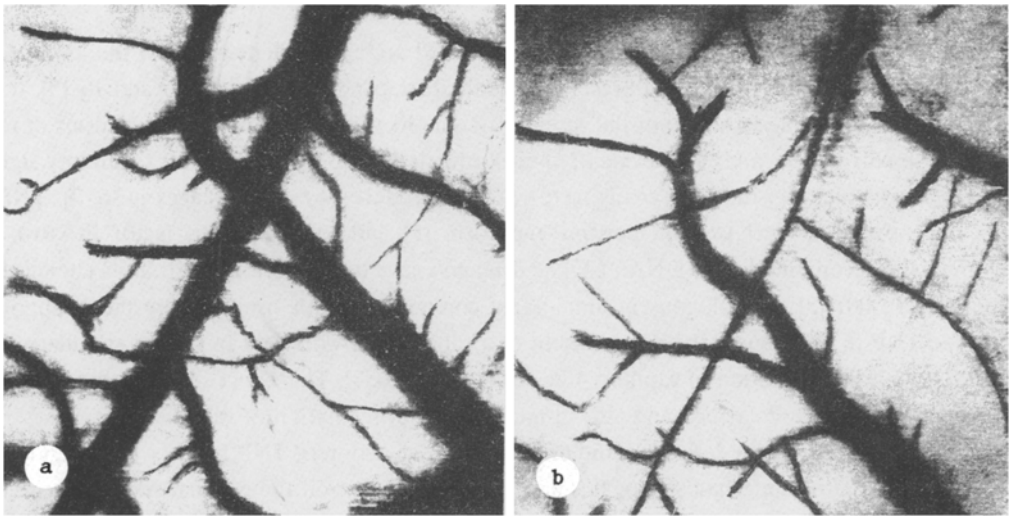

Fig. 2. Changes in diameter of pial arteries in response to injection of TNF: a) normal conditions, b) 20 min after injection of TNF.

EXPERIMENTAL RESULTS

The experiments showed that in response to intracarotid injection of TNF, in a dose of 6 μg/kg into the rabbits a statistically significant decrease in the intensity of LCBF developed, on average by 45.6% ($n = 6$, $p < 0.05$). The dynamic characteristics of one typical vascular response are shown in Fig. 1.

During development of the effect, no changes took place in the heart rate or systemic blood pressure.

Intravital photography followed by measurement of the diameter of the superficial arteries of the cortex (initial diameter between 20 and 140 μm) in response to injection of TNF revealed marked narrowing of the lumen of the pial vessels, by 39.6% on average ($p < 0.05$, Fig. 2).

Considering that the vascular effects develop without any change affecting the central hemodynamics, we postulated a role of the angiogenic factor in the mechanism of regulation of LCBF.

The next task was to discover if TNF affects the cerebral arteries by conducting experiments on isolated vessels. Experiments on segments of the middle cerebral artery gave the following results: in standard Krebs' medium, i.e., without the addition of any biologically active substances, smooth muscle tone was unchanged by injection of TNF (3.6×10^{-8} M); in intact vessels the angiogenic factor increased the amplitude of phasic rhythmic contractions evoked by histamine (10^{-6} M); in intact vessels the angiogenic factor increased the amplitude of phasic rhythmic contractions evoked by histamine (10^{-6} M) on average by 34%. In de-endothelized vascular preparations the effect of potentiation of rhythmic contractions was weakened (Fig. 3). The statistical testing of the results of the measurements is shown in Table 1.

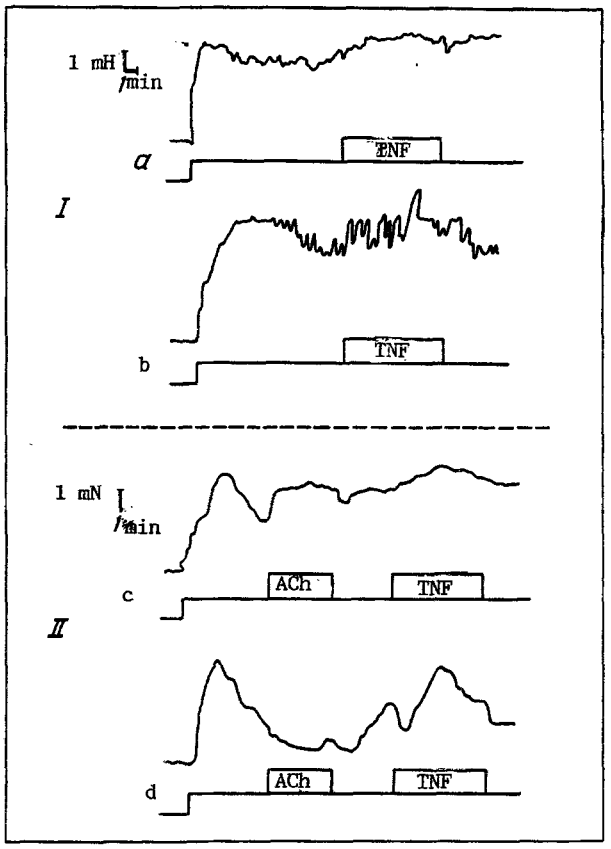

Fig. 3. Changes in smooth muscle tone of isolated segments of cerebral vessels in response to TNF application preceded by exposure to monoamines and depending on structural and functional integrity of the endothelium: a, c) endothelium removed; b, d) endothelium intact. I) Histamine, II) Noradrenalin.

TABLE 1. Characteristics of Effect of TNF on Contractility (in mN) of Intact and De-Endothelized Vascular Preparations ($M \pm m$)

Process recorded	Intact vascular prepn.		De-Endothelized vascular preparation	
	control	action of TNF	control	action of TNF
Amplitude of rhythmic contractions	3,59±1,33	5,45±1,39 p <0,05	0,80±0,47	0,93±0,53 >0,05
Response to histamine	3,68±0,72	6,62±0,96 p <0,05	3,97±0,65	6,86±1,32 <0,05

Experiments on segments of the carotid artery showed that TNF in intact and de-endothelized vessels potentiated contractile responses to histamine (10^{-6} M) on average by 44% ($n = 5$). However, no significant difference could be found between the effects of potentiation of the responses between intact and de-endothelized preparations (Table 1). After preliminary activation of the vessel wall by noradrenalin (10^{-6} M) TNF induced a biphasic response of changes in tone (Fig. 3, II). No initial relaxation took place in the de-endothelized vessels, and the typical response to TNF was the development of contraction ($n = 8, p < 0.05$).

The results are evidence that the angiogenic factor has a direct action on the mechanisms regulating contractility of arterial smooth muscles. However, this action is manifested only in the presence of a simultaneous regulatory influence of monoamines on the vessel wall. Dependence of the effects on integrity of the endothelium will be noted. This dependence is confirmed by calculations of parameters of rhythmic activity induced by histamine and the results of experiments conducted after

preliminary activation of the adrenoreceptors by noradrenalin. Dependence on the endothelium and correlation between the action of TNF and activation of aminergic receptors indirectly points to the localization of these receptors in endothelial cells, by means of which this correlation between the action of TNF and aminergic mechanisms perhaps takes place.

It can be postulated on the basis of these results that a humoral system, regulating angiogenesis and vascular tone in a coordinated manner, exists in the body. In our opinion, TNF is involved in the working of this system. The physiological role of this normally exogenous substance is best regarded as the role of a factor simultaneously regulating morphogenesis and tone of the muscle wall, depending on the metabolic activity of the tissues supplied with blood. It can also be postulated that under normal conditions natural production of TNF is responsible for protecting the body against the development of tumors.

LITERATURE CITED

1. A. L. Azin and A. I. Kuz'min, Fiziol. Zh. SSSR, **75**, No. 2, 206 (1989).
2. V. B. Grechin (ed.), Methods of Clinical Neurophysiology [in Russian], Leningrad (1977), p. 356.
3. A. L. Lobashevskii and Z. N. Kochemasova, Usp. Sovrem. Biol., **105**, No. 2, 217 (1988).
4. L. D. Old, V Mire Nauki, No. 7, 22 (1988).
5. V. V. Kupriyanov, I. I. Bobrik, and Ya. L. Karaganova (eds.), The Vascular Endothelium [in Russian], Kiev (1986), p. 248.
6. M. Frater-Schroeder, W. Risan, R. Hallmann, et al., Proc. Natl. Acad. Sci. USA, **84**, No. 15, 5277 (1987).
7. S. J. Leibovich, P. J. Polverini, H. M. Separd, et al., Nature, **329**, 630 (1987).
8. D. Pennica, G. E. Nedwin, J. S. Hayflick, et al., Nature, **312**, 724 (1985).
9. K. Suyama, J. Goldstein, and S. Green, Exp. Cell Biol., **53**, No. 2, 85 (1985).