Leukocyte Involvement in Vasomotor Reactivity of the Cerebral Vasculature

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Activation of leukocytes may play a part in the complex pathophysiological cascade leading to vasomotor reaction in cerebral arteries. In this issue of Stroke, Akopov et al report changes in endothelium-dependent relaxation by intravascular chemical leukocyte activation involving the cerebral circulation. This interesting study highlights a potential role for leukocytes in the modulation of vasomotor reactivity of large cerebral arteries. Large cerebral arteries, including the middle cerebral artery (MCA), are already well known for their marked reactivity, even hyperreactivity, which is best demonstrated in the vasospasm associated with subarachnoid hemorrhage. The potential effects of leukocyte-vascular interactions on vasoregulation can be seen in the larger context of cerebral ischemia and its initiation of the mechanisms of so-called reperfusion injury, the "no-reflow" phenomenon, endothelial cell damage (initiated by oxygen free radicals, polymorphonuclear [PMN] leukocyte adherence, transmigration, and phagocytosis), and vascular smooth muscle responses.

Two strong activators of leukocytes, phorbol 12-myristate 13-acetate (PMA) and N-formylmethionyl-leucylphenylalanine (fMLP), were infused into the cerebral circulation of rabbits in the present experiments. Vasodilator stimuli (acetylcholine, ADP, and sodium nitroprusside) and platelet aggregates were applied to MCA segments studied in vitro following in vivo infusion of fMLP or PMA. The main result was increased vasomotor reactivity of the isolated MCA segments by platelets (after leukocyte activation) and reduced endothelium-dependent vasodilation. Non-endothelium-dependent relaxation caused by sodium nitroprusside was not affected. The control animals (leukocyte-depleted rabbits) in this study suggest that leukocyte activation was the principal intermediary. The authors assert that leukocytes could contribute to regulation of cerebral vascular tone, in accordance with the work of Faraci et al.

Recent work has focused on the regulatory mechanisms of vasomotor reactivity by endothelial cells in large vessels. Endothelium-dependent relaxation is mainly provided by two different pathways, the prostacyclin (prostaglandin [PG]I2) and the nitric oxide (NO), although less-well-understood factors such as endothelium-dependent hyperpolarizing factor (EDHF) may provide another pathway. Different mediators result in vascular smooth muscle relaxation (eg, 5-hydroxytryptamine [5-HT] or serotonin [in microvessels], acetylcholine, ADP, bradykinin, and thrombin). The molecular mechanisms of NO or PGH2 release involve both Ca2+-dependent and Ca2+-independent pathways. Elevation of endothelial cell Ca2+ concentration causes endothelium-dependent relaxing factor (EDRF)-related vasodilation, whereas increases in smooth muscle Ca2+ content produce constriction (see the Figure). Factors that initiate vasoconstriction are superoxide anions, thromboxane A2 (from platelets) or PGH2, and endothelin-1. Endothelin-1 expression can be induced by PMN leukocytes, which offers one possible explanation for the reported results.

Where do the present experiments lead us?

1. Could the observed increased tendency to vasoconstriction and reduced dilatation be caused by endothelial cell injury? It is known that PMN leukocytes may contribute to endothelial damage. Vanhoutte and colleagues have shown that mechanical injury of the endothelium may alter vascular relaxation by means of several identified mediators, including 5-HT. Whereas 5-HT promotes vasodilatation in normal noncerebral arteries, its effect in the presence of injured endothelium is a vasocostrictive one. 5-HT is released from activated platelets and may be important in leukocyte-endothelial cell interactions by leukocyte-platelet coactivation. Atherosclerosis also potentiates vasoconstrictor responses to some stimuli (eg, 5-HT). It may even be postulated that the atherosclerotic vessel changes may be important in transient ischemic events as a source for vasoconstrictive mediators. The leukocytes and monocytes that invade atherosclerotic lesions are also potential contributors to this reaction. Therefore, leukocyte-related endothelial damage, in addition to the release of vasoactive mediators, may be important.

2. What is the mechanism of the PMN leukocyte-arterial interaction in these experiments? fMLP is a relatively specific leukocyte activator, whereas PMA has more general properties, stimulating endothelial cells, platelets, and smooth muscle cells. The lack of a significant difference between both activators in the study of Akopov et al suggests an important role for leukocyte activation. Activation of platelets by granulocyte-elaborated platelet-activating factor (PAF) in the vicinity of
activated leukocytes may lead to platelet release of potential vasoconstrictor substances (e.g., thromboxane $A_2$ and 5-HT). A further direct vasoconstricting mechanism could be provided by the production of PGE$_2$ by endothelial cells.$^3$ Inhibition of the release of EDRF or NO is another indirect path for vasoconstriction, secondary to reduced vasodilatation, which may be a product of evolving endothelial cell dysfunction.$^{15,16}$

"Priming," or partial constriction of MCA segments in this experimental setting, may also effectively reduce absolute vasodilatation evoked by the stimuli.

3. The very early occurrence of the observed vasoconstrictive effect is remarkable. There is an apparent discrepancy between the observations that PMN leukocyte-vessel wall interaction in acute inflammation result in extensive dilatation of blood vessels with loss of endothelial integrity and plasma extravasation$^{17}$ and the present data that underscore reports of vasoconstrictive responses following leukocyte-vessel wall interactions.$^5$

The inflammatory leukocyte-endothelial interaction producing vasodilatation occurs mostly in the postcapillary venules.$^{18}$ In contrast, the vascular effects described by Akopov et al$^1$ were shown in large cerebral vessels. One may speculate that the very early interaction of granulocytes with the MCA wall may contribute to endothelial dysfunction (e.g., reduced secretion of EDRF), which in the presence of platelet-derived 5-HT may contribute to arterial constriction. Later vascular structural changes (associated with inflammation) may follow, which may lead to loss of the endothelial cell-dependent mechanisms and may even reduce medial smooth muscle vasoconstrictive properties directly.

4. Can PMN leukocyte-endothelium interactions promote or alter vasomotor responses of small arteries, arterioles, and vessels of less than 100 $\mu$m? One recent focus on leukocyte involvement in cerebrovascular processes has been the microvascular responses to ischemia and reperfusion.$^{3,18}$ The binding of leukocytes to cerebrovascular endothelium has a molecular basis in the complementary interactions of intercellular adhesion molecules and integrins.$^{20-22}$ Although granulocyte-fibrin-(degranulated) platelet aggregates may occlude portions of the ischemic microvasculature,$^{3}$ there is little information about the vasomotor consequences of these aggregates or isolated cells on the deep cerebral microvasculature. Direct cortical observations have shown the pial arteriolar response to ischemia to be one of significant vasodilatation.$^{24}$ In separate experiments, impaired pial vasodilatation responses but preserved vasoconstriction and reduced cortical flow have been observed after ischemia/reperfusion.$^{3}$ Preischemic depletion of neutrophils attenuates hypoperfusion significantly, which may be caused by reduced vasoconstriction.$^{26}$ Although the described findings in larger cerebral vessels may affect flow events in the microvasculature, there is a paucity of information about whether and how PMN leukocytes might accomplish vasomotor changes in the microvasculature.

5. What is the clinical relevance of the described findings? The authors suggest that endothelial dysfunction may be a part of postischemic reperfusion injury. Reperfusion injuries are thought to be important in capillaries and postcapillary venules.$^{27}$ There is little clinical evidence that vasoconstriction or reduced vasodilatation result from occlusion and reperfusion of large intracranial arteries in humans (in the absence of vascular manipulation), and little is known about whether ischemic stroke causes angiospastic reactions, particularly in deep vascular beds. Other experimental studies in coronary arteries$^{28}$ and intestinal vessels$^{29}$ have shown reduced endothelium-dependent relaxation after reperfusion injury. In the mesenteric artery, activated leukocytes cause vasodilatation in the absence of ischemia, but vasoconstriction occurs after ischemia.$^{29}$ Compared with those findings, the isolated MCA may be different in its vasomotor reactivity, and/or the
injection of activated leukocytes may simultaneously cause endothelial injury and vasocostriction or decreased vasodilation to specific agonists. In the coronary arteries vasocostriction follows ischemia/reperfusion through mechanisms involving reduced EDRF release and increased constricting prostaglandin synthesis (ie, PGH₂). Vasocostriction of the coronary arteries is seen as a major contribution to impaired coronary blood flow and further myocardial damage. The future challenge will be to see whether these observations may be generalized to other cerebral vessels and to examine PMN leukocyte-platelet-associated vasomotor events at the site of cerebral ischemic injury.

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