PHYSIOLOGY

Effect of External pH on Initial Tone of Rat Basilar Artery and Its Reactions to Serotonin

O. E. Fadyukova, T. P. Storozhevykh, V. G. Pinelis, and V. B. Koshelev

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Extracellular acidosis and alkalosis induce constriction of perfused *a.basilaris* isolated from rats. Acidosis suppresses, while alkalosis has no effect on serotonin-induced vasoconstriction.

Key Words: *constant flow perfusion; vasoconstriction; vasodilation*

Secondary ischemia is characterized by changes in neurogenic regulation of cerebral vessels [12] and their reaction to vasoactive agents [1]. It has been supposed that these changes underlie delayed vascular spasm in progressive brain ischemia [1,12]. Ischemia is usually accompanied by a decrease in intra- and extracellular pH (pH_i and pH_o, respectively) and accumulation of lactate [10]. Postishcemic reperfusion is usually accompanied by alkalosis [5]. These shifts in pH_o can considerably affect the reactivity of vascular smooth muscle cells (SMC) through modulating ion currents and cell metabolism [11]. In light of this, the aim of the present study was to analyze the effect of pH_o shifts on the initial tone and reactivity of *a.basialris* from rat brain.

MATERIALS AND METHODS

Experiments were carried out on segments of *a. basilaris* isolated from rat brain. Vascular segments were obtained from 39 rats sacrificed under ether narcosis. The brain was removed and a 4-5-mm-long fragment of the basilar artery above the vertebral artery junction was separated. A plastic cannula was inserted into the proximal end of the vessel segment, lateral branches were ligated, while the distal end was free. In some control experiments, a 400 mg load was attached to the distal end to create initial myogenic tone. The preparation was placed into a thermocontrolled flow (6 ml/min) chamber and perfused with Krebs solution at a constant flow conditions (1-1.2 ml/min) using a Rabbit peristaltic pump. Under these conditions, changes in the perfusion pressure (PP) corresponded to changes in vessel resistance. The recorded PP is a superposition of constant resistance of the catheter and vessel resistance. The inflow PP was recorded with Statham P23Db transducers (Gould Inc.) connected to a Beckman polygraph. The vessel and the chamber were perfused with Krebs solution (95% O_2+5% CO_2, 37°C, pH 7.4) containing (in mM): 130.0 NaCl, 4.7 KCl, 14.9 $NaHCO_3$, 1.5 $CaCl_2$, 1.2 $MgSO_4$, 1.18 KH_2PO_4, and 6.0 D-glucose. In some experiments, the segment was perfused with a Ca-free solution containing 0.1 mM EGTA ($CaCl_2$ was equimolarly replaced with NaCl). pH_o was increased to 8.3-8.35 with NaOH (no more than 2 mM) and to 6.55-6.6 with HCl (~4.5 mM). The effects of pH_o shifts on the initial and potassium-induced (115 mM) tone and on serotonin-induced contraction (10^{-6} M) were studied after a 30-40 min stabilizing perfusion with a control solution. The test solutions were simultaneously delivered to the bath and vessel, except for experiments

Department of Human and Animal Physiology, Biological Faculty of M. V. Lomonosov Moscow State University, Laboratory of Membranology, Institute of Pediatrics, Russian Academy of Medical Sciences, Moscow

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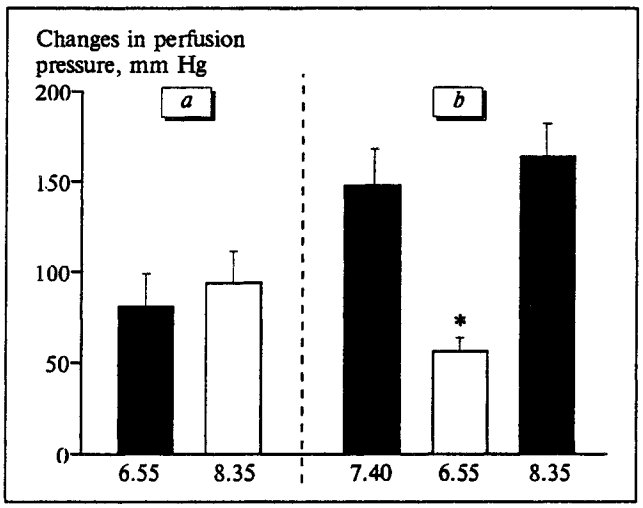

Fig. 1. Effect of changes in extracellular pH on initial resistance (*a*) and serotonin-induced contraction (*b*) of rat basilar artery (n=5). Initial perfusion pressure is taken as zero. *p<0.05 compared with control reaction at pH 7.4. Abscissa: pH_o

with potassium-depolarized vessels, when the test solutions were delivered to only the vessel. Serotonin (5-hydroxytryptamine creatinine sulfate, Sigma) was infused into the lumen in a dose of 10^{-6} M using a Secura perfusor. The preparation was washed with the control solution after each experiment. Experiments with Ca-free or nifedipine-containing (5×10^{-6} and 10^{-5} M) solutions were preceded by 10-min preincubation of the segment in these solutions. The data were processed statistically using the Wilcoxon paired test.

RESULTS

Both acidification (pH_o 6.55-6.6) and alkalinization (pH_o 8.3-8.35) induced a rise in PP (Fig. 1, *a*) and, consequently, in vascular resistance of the basilar artery from rat brain. This rise was not caused by minor changes in osmolarity associated with alkalinization and acidification of the perfusion solution, since an osmolar shift induced by addition of 10 mM NaCl had practically no effect on the vascular tone. Air-stream denudation (1 min) had virtually no effect on vascular responses to pH_o shifts, whereas endothelium-dependent reactions of K^+-precontracted vessel (60 mM) to acetylcholine (10^{-6} M) were considerably suppressed. This suggests that pH shifts evoke immediate reactions of vascular smooth muscles. Removal of Ca^{2+} the perfusate virtually abolished contractile response of the basilar artery to alkalinization and acidification. Nifedipine, an L-type voltage-dependent calcium channel blocker, reduced the contractile response to acidification and alkalinization by 56±6 and 62±6%, respectively. Against the background of K^+-induced depolarization (115 mM), the drop of pH_o induced a weak reversible relaxation of the vessel by 7±1%, while its rise enhanced vascular resistance to 39±8%. Acidification of bathing solution diminished the serotonin-induced contraction by 60±5%, while alkalinization had no effect on this process (Fig. 1, *b*).

Thus, both the decrease and increase in pH_o (to 6.55 and 8.35, respectively) induced similar constrictive responses of the basilar artery from rat brain. These responses strictly depended on the presence of Ca^{2+} in bathing solution. At least 50% of these Ca^{2+} ions entered SMC via L-type voltage-dependent calcium channels, while the remainder portion of Ca participating in SMC contraction entered the cell due to activation of Na^+,Ca^{2+}-exchange and via receptor-operated ion channels. In our experiments alkalinization and acidification of the prefusate induced similar contractile responses of isolated artery.

Elevation of pH_o can induce contraction of the basilar artery from rat brain through different mechanisms. It has been previously shown that alkalinization of bathing medium improve conductivity of L-type voltage-dependent Ca^{2+} channels in guinea pig basilar artery [13]. Depolarization of SMC plasma membrane in the middle cerebral artery [9] and cerebral arterioles [7] accompanied alkalosis in rats. This probably results in a rise of cell Ca^{2+} concentration and induces vasoconstriction. Moreover, alkalosis inhibits Ca^{2+} removal from the cytoplasm [8] through blocking $Ca^{2+},2H^+$-ATPase of SMC membrane [6].

Inhibition of ion conductance of L-type voltage-dependent calcium channels [13] and hyperpolarization of SMC membrane [7] induced by acidification of bathing medium were previously described by others. These phenomena can explain minor dilatation of K^+-precontracted rat basilar artery in response to pH_o decrease, but not the contractile effects of extracellular acidification observed by us. It was shown that decrease in pH_o leads to intracellular acidosis [2,3]. Selective acidification of the cytoplasm mobilizes Ca^{2+} from intracellular Ca^{2+} stores [4], which probably triggers Ca^{2+} entry from extracellular medium, inhibits its outward current, and induces vascular contraction. This can explain contraction of the basilar artery in response to pH_o drop and inhibition of serotonin-induced contraction under these conditions, since replacement of Ca^{2+} by H^+ in intracellular acidosis can block cell signal transduction pathways from serotonin receptors. Inhibition of vascular constriction induced by various agonists at low pH_o was described by others [11].

Thus, extracellular acidosis and alkalosis induce contraction of the basilar artery from rat brain. Shifts in pH_o under conditions of brain ischemia and post-

ischemic reperfusion may underlie the development of secondary vasospasm.

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