PIAL ARTERIOLAR RESPONSE TO ACETYLCHOLINE IN GLOBAL CEREBRAL ISCHAEMIA/REPERFUSION IN CATS

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SUMMARY:

The effects of 10' global cerebral ischaemia on cortical arteriolar responses to Acetylcholine (ACh) application was examined in anaesthetized cats by closed cranial window technique. The physiological response of the pial arterioles to topical ACh (10-7 M) was endothelium- dependent vasodilation. Following 10 min global ischaemia pial vessel diameters were measured during minutes 30, 45, 60, 75, 90, and 120 of the reperfusion period before and after ACh application. During the reperfusion period the pial arteriolar response to ACh was reversed and sustained up to 75 minutes of reperfusion and then vasodilatory response to ACh recovered. However, vasodilatory responses to ACh at 120 min of reperfusion were still less than the baseline values.

We concluded that; 1) Endothelium-dependent vasodilation with ACh is present in pial arterioles. 2) 10 min global cerebral ischaemia temporarily reverses the endothelium- dependent pial arteriolar responses to ACh. 3) Since the production of free oxygen radicals following 10 min global ischaemia has been demonstrated, inhibition of endothelium- dependent vasodilation of the pial arterioles during the reperfusion period, depends on inactivation of the endothelium-dependent relaxing factor(s) (EDRFs) by free oxygen radicals. 4) Since the vasodilatory responses of the pial arterioles recovered in the late reperfusion period we concluded that EDRF(s) were still produced and the endothelial cells were intact or minimally damaged. These findings suggest that elimination of endothelium-dependent vasodilation is the result of relatively minor injury rather than complete destruction of the pial arteriolar endothelial cells. 5) Vasoconstriction of the cerebral arterioles after ACh application during the early reperfusion period was the unmasked vasoconstrictory effect of ACh on the vascular smooth muscle, because of EDRF inactivation by free oxygen radicals.

KEY WORDS:

Cerebral ischaemia, endothelium-dependent relaxing factors (EDRFs), acetylcholine, vasodilation, free oxygen radicals,

INTRODUCTION:

Cerebral ischaemia/reperfusion, results in damage of the cerebral microvasculature, and may be followed by the Blood-Brain Barrier (BBB) disruption and brain aedema (2).

Disruption of the BBB, due to free oxygen radical mediated damage occurs the during reperfusion (8). Endothelial cells of the cerebral microvasculature are vulnerable to free oxygen radical mediated damage (8).

Endothelial cells release some substances such as EDRF that affect the vascular tone and blood fluidity (8). EDRF stimulates guanylate cyclase of vascular smooth muscle and increas-

ed cyclic guanosine monophosphate (cGMP) relaxes vascular smooth muscle. EDRF also inhibits platelet aggregation and their adhesion to vessel wall (4). EDRF is a very labile non-prostanoid compound, rapidly inactivated by miscellaneous chemicals such as oxyhaemoglobin and superoxide anion (12).

Recent studies with mice and cats, have shown that cerebral arteriolar response to topical application of ACh is endothelium-dependent (13). Several vasodilatory agents relax vascular smooth muscle by releasing EDRFs, which diffuse to the vascular smooth muscle and activate guanylate cyclase thereby inducing relaxation (6).

Removal of endothelium inhibits the endothelium-dependent vasodilatory response of ACh (4). Moreover several other techniques such as topical arachidonate application (9), ultra-violet radiation in the presence of photosensitizing dye (15), ischaemia/reperfusion injury (8,14), and hypertension (18) cause endothelial damage and impair the vasodilatory response of the cerebral arterioles to ACh.

In this study we have shown that reperfusion following 10 min of global cerebral ischaemia, temporarily reversed the vasodilatory responses of the cerebral arterioles to ACh. Inhibition of endothelium-dependent relaxation, is due to inactivation of EDRFs with free oxygen radicals, which are produced during the reperfusion period.

MATERIAL AND METHODS:

Adult mongrel cats (n=7) of either sex, and weighting 2.9-4.3 kg. were anaesthesized with ketamine hydrochloride (25 mg/kg i.m.), and halothane (2%). Following endotracheal intubation and mechanical ventilation (Harvard Apparatus, South Natwick, MA), anaesthesia was maintained with penthobarbital sodium (30 mg/kg), and gallamine triethiodite (10-15 mg/kg/h). Animals were ventilated with room air and normocapnia (pCO_2=30 mm/Hg). Both femoral arteries and veins are cannulated for BP monitoring, drug and saline infusions and blood withdrawal. End tidal CO_2 (Accucap, Datascope Corp, Paramus, NJ) and arterial blood pressure (Grass Instruments, Quincy, MA) were continuously monitored. Body temperature was maintained at 37-38 Co (Homothermic Blanket Control Unit, Harvard Apparatus, MA). Frequent blood gas determinations were made (178 pH Blood Gas Analyzer, Corning, Medfield, MA).

Global Cerebral Ischemia:

Via left lateral thoracotomy left subclavian artery is ligated and brachiocephalic truncus is dissected and prepared for induction of cerebral ischaemia. Ischemia is induced both occlusion of brachiocephalic truncus and combined hypotension [Systolic Blood Pressure (SBP)<50mm/Hg] via arterial blood withdrawal and continuous i.v. Adenosine triphosphate (ATP) infusion (Harvard Infusion Pump, Cambridge, MA). The reason for continuous ATP infusion during global cerebral ischemia was to drop the SBP without withdrawing the considerable amount of blood. The ATP infusion was discontinued immediately before reperfusion. A total of 120-140 mg of ATP was infused in each animal. Global cerebral ischemia was confirmed by microscopic observation of the pial circulation visualized through a cranial window (see cranial window technique) installed overlying the parietal cortex alternately on the left or right in the consecutive animals. Following ischemia the withdrawn blood returned and the string around the brachiocephalic truncus was released. Reperfusion was also visualized from the cranial window. At the conclusion of the experiment animals were killed with overdose phenobarbital. With cat model cerebral postocclusive hyperemia are longer and more abundant than the other species. Moreover global cerebral ischemia could only be created in cats by the clippage of brachiocephalic truncus, because of their vascular anatomy (in cats left vertebral artery originates from the left subclavian artery; however, both common carotid and the right vertebral arteries originate from the brachiocephalic truncus).

Cranial Window Technique:

The head was fixed in a stereotaxic frame (Kopf, Tjunga, CA). The cranial window technique was described previously in details (11).

In brief, a stainless steel window with a glass cover slip was implanted into the right parietal region with dental acrylic cement, overlying the ectosylvian and suprasylvian gyri. The window was equipped with inlet and outlet portals. The space under the glass was filled with artificial cerebrospinal fluid (CSF) at pH 7.4 (Ames Reagent, Sigma, St Louis, MO), kept at 37 Co. The pial arteriolar diameter was measured with a Vickers A.E.1 image splitting device attached to a Wild (Heerbrugh) microscope. The diameter of pial arterioles was measured under basal conditions ($PaCO_2$=30 mm/Hg), and at intervals for 2 hours after reperfusion.

Acetylcholine (ACh):

ACh (ACh; Sigma, St Louis, MO) was reconstituted with artificial CSF (pH 7.4, 37 Co) at a concentration of 10-7M. Approximately 2 ml was instilled under the window before ischaemia, and at intervals after reperfusion. The vessel response was measured 5-10 minutes later, and measurements were repeated after washing with artificial CSF.

RESULTS:

Measurements were obtained from up to 8 large (r> 100 um) and small (r< 100 um) pial arterioles in each animal (range 7-8). During global cerebral ischaemia, no flow was observed in the pial circulation. Following reperfusion, marked hyperaemia was observed in all cats. The temporal profile of the vessel diameter was remarkably similar for large and small pial arterioles, and is shown in Figure 1. Vessel diameters at 30 min of reperfusion were 179% of the control diameter. Vessels constricted progressively thereafter, but remained dilated in appearance for the duration of the study (Figure 1).

A response to ACh 10-7M was observed in 6 out of 7 animals. Although the seventh animal responded to a concentration of 10-6M, this animal was not included in the data. The response of large and small arterioles was almost identical (Table 1 & Figure 2). Under resting conditions, all vessels dilated to a mean of 117% of control diameter. However up to

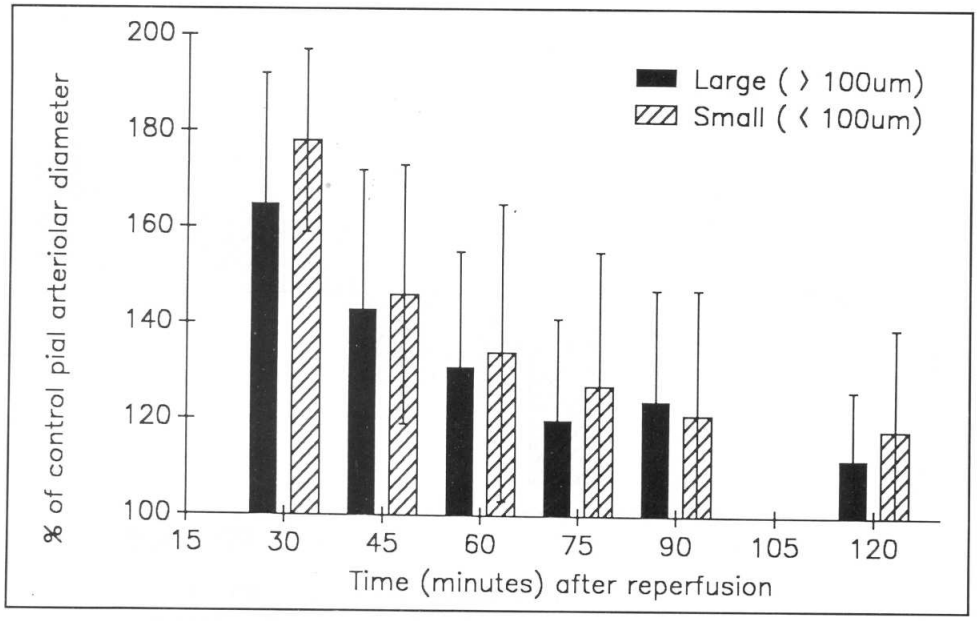

Fig. 1 : Sustained pial arteriolar dilation during reperfusion period. Large (r>100um) and small (r<100um) pial arterioles remain dilated during 120 minutes of reperfusion following 10 minutes of global cerebral ischaemia (n=6 cats).

Table 1. Cerebral Arteriolar Response to ACh (10-7M = 0.1uM)
[All values are % of control diameter (n=6)]

		Reperfusion (Minutes)					
Small	Control	30	45	60	75	90	120
Mean	115%	86%	93%	93%	105%	108%	111%
SD	7	1	9	8	3	5	4
SE	2	0.3	5	2	1	2	1
n=	18	3	3	12	6	9	12
Large							
Mean	120%	89%	96%	96%	106%	107%	108%
SD	10	2	1	8	4	4	5
SE	3	1	1	3	2	2	2
n=	14	3	3	8	6	5	8
TOTAL							
Mean	117%	87%	95%	94%	105%	108%	109%
SD	8	2	6	8	4	5	4
SE	1	1	2	2	1	1	1
n=	32	6	6	20	12	14	20

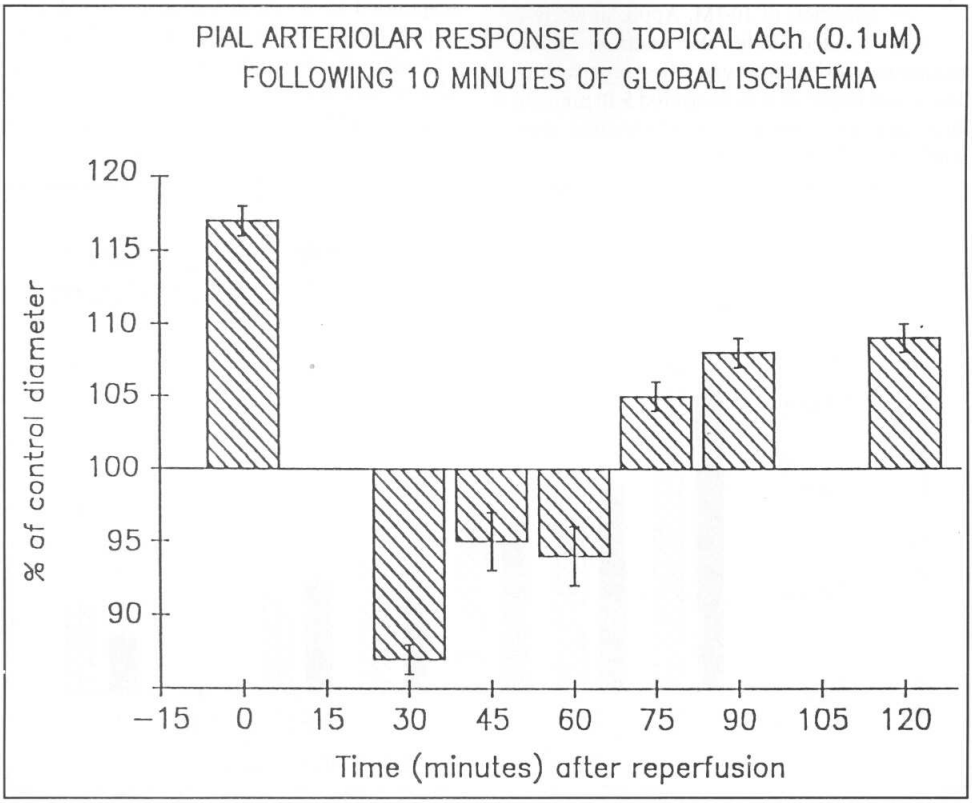

Fig. 2 : Pial arteriolar response to topical ACh (10-7M = 0.1uM) following 10 minutes global cerebral ischaemia (n=6 cats). Since both large (r>100 um) and small (r<100um) pial arteriolar response to ACh are almost identical, data were analyzed and given together (all).

60 minutes of reperfusion following 10 min of global cerebral ischaemia, ACh in the same concentration, elicited a vasoconstrictory response (Table 1 & Figure 2). Thereafter, vessels responded by vasodilation, but even 120 min after reperfusion, the response was less than that was observed prior to ischaemia (109% vs 117%, $p < 0.05$). Results are shown in figures 1, 2 and table 1. Physiological parameters are given in table 2.

cAMP and cGMP both seem to mediate relaxation of vascular smooth muscle (5). It is thought that EDRF relaxes vascular smooth muscle via activation of guanylate cyclase (3). EDRFs are short-lived (16) and their chemical structure resembles nitrosothiols (12).

The presence of endothelium-dependent vasodilation to ACh in the pial arterioles is impaired or reversed by free radical generating

Table 2 : PHYSIOLOGICAL PARAMETERS (n=6 cats)

		Time (minutes) after reperfusion					
	Control	30	45	60	75	90	120
MABP	100±10	110±8	90±9	88±9	85±9	95±15	105±15
pCO2	31±2	39±5	34±6	33±3	32±4	32±4	35±5
pO2	98±18	95±15	96±22	100±18	95±12	100±8	105±10
pH	7.33	7.13	7.20	7.21	7.27	7.27	7.30
	±0.03	±0.1	±0.15	±0.11	±0.1	±0.09	±0.07

Values are expressed as mean+sd

DISCUSSION:

The data of this study demonstrate the following: 1) following 10 min of global cerebral ischaemia endothelium-dependent vasodilatory response to ACh was temporarily reversed in all animals, 2) free oxygen radical production during the reperfusion period caused to impaired endothelium-dependent relaxation since EDRF was inactivated 3) recovery of endothelium-dependent responses during the late reperfusion period (75 min) showed that endothelium was still intact or minimally damaged. However the respond to ACh were less than baseline values at 120 min of reperfusion. 4) pial arteriolar vasoconstriction to ACh during the early reperfusion period, showed that inactivation of EDRFs unmasked the direct vasoconstrictory effect of ACh on the vascular smooth muscle.

Large and small cerebral arterioles dilate in response to ACh both in vivo and in vitro (7). Activation of muscarinic receptors on the surface of endothelial cells triggers the release of EDRFs that reach the vascular smooth muscle fibres by diffusion and cause relaxation (3).

techniques (12). Free radical is a powerful oxidizing agent, and inactivates EDRF by direct chemical oxidation (12).

We examined the reactivity of cerebral arterioles to ACh during reperfusion following 10 min global cerebral ischaemia. In our study, following ischemia up to 60 min of reperfusion, the pial arteriolar response to ACh was vasoconstriction (Table 1 & Figure 2). Inactivation of EDRFs occurred during the reperfusion period by way of generated free oxygen radicals. Inhibition of the endothelium-dependent relaxation unmasked the direct vasoconstrictory effect of ACh on the vascular smooth muscle. ACh in physiological conditions stimulates vascular smooth and causes contraction activating muscarinic receptors and secreting prostaglandins (17). Low doses of ACh modulate adrenergic nerve terminals that also cause vasoconstriction (1). However EDRF is more potent that those vasoconstrictory substances, so that only vasodilation takes place. During reperfusion, since EDRF is destroyed by free oxygen radicals, vasoconstrictory mechanisms overwhelm.

However in anaesthetized newborn piglets 20 min global cerebral ischaemia caused arteriolar dilation after ACh application (10-3M) up to 24 hours of reperfusion (10).

Before ischaemia topical application of ACh caused vasoconstriction and following 20 min global cerebral ischaemia ACh responses reversed to dilation up to 24 hours of reperfusion (10). ACh induced vasoconstriction of newborn piglets was mediated by prostanoids and the authors showed that ACh induced prostanoid synthesis was markedly attenuated after ischaemia. They concluded that the cholinergic mechanism in the cerebral circulation of newborn piglets is immature (17). Similar studies in adults of other species have clearly demonstrated that ACh in concentrations of 10-7M to 10-7M cause vasodilation of the cerebral arterioles (12).

In our study at 30 min of reperfusion, vasoconstrictory responses of the pial arterioles were maximum (Figure 2). However during 45 and 60 min of reperfusion vasoconstrictory responses of pial arterioles declined. We speculated that the amount of free oxygen radicals produced reaches the highest level in the first 30 min of reperfusion which is also the peak of hyperaemic period. After 75 min of reperfusion the dilatory responses of the pial arterioles to ACh returned, however they were less than the baseline values. This suggests that the endothelium is still intact or minimally damaged and can still produce EDRFs.

In conclusion; we demonstrated the temporary selective inhibition of endothelium-dependent dilation of the pial arterioles in all of our animals. However endothelium-dependent responses returned after 75 min of reperfusion. We suggest that the recovered endothelium-dependent dilatory responses after ischaemia, makes the endothelium less likely to be the causative factor of postischaemic hypoperfusion.

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