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ACh- and VIP-induced vasorelaxation in rabbit facial artery after carotid artery occlusion

Jelena Roganović [a], *Miroslav Radenković* [b], *Dragica Stojić* [a,*]

[a] *Department of Pharmacology, Faculty of Stomatology, University of Belgrade, Belgrade, Serbia*
[b] *Department of Clinical Pharmacology, Pharmacology and Toxicology, Medical Faculty, University of Belgrade, Belgrade, Serbia*

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ABSTRACT

Objectives: The influence of carotid artery occlusion (10, 30 and 60 min) on regulatory mechanisms implicated in the vasorelaxant responses of isolated glandular branch of rabbit facial artery to acetylcholine (ACh) and vasoactive intestinal polypeptide (VIP) was examined.

Design: In organ bath studies with arterial rings precontracted with phenylephrine (1 μM), before and after carotid artery occlusion, changes in isometric tension were recorded.

Results: Endothelium-dependent vasorelaxation by ACh and endothelium-independent vasorelaxation by VIP were significantly reduced, started from 30 and 10 min of carotid occlusion, respectively. Inhibitory effect of indomethacin on ACh vasorelaxation was enhanced whilst effect of N^G-nitro-L-arginine reduced, started from 30 min of carotid occlusion. Sodium nitroprusside-induced vasorelaxation was not changed after carotid occlusion. Inhibition of VIP vasorelaxation by L-N^{ω}-nitroarginine-2,4-L-diaminobutyric-amide, was reduced, started from 30 min of carotid occlusion. Forskolin enhanced VIP-induced vasorelaxation in control rings but this effect was reduced started from 30 min of occlusion. In the presence of VIP, vasorelaxant effect of ACh was increased; the increase was reduced, started from 10 min of carotid occlusion.

Conclusions: The present investigation provides evidence for the decreased responsiveness to both, ACh-endothelium-dependent and VIP-endothelium-independent vasorelaxation in rabbit facial artery after carotid occlusion. In addition, the data suggest that ischaemia alters contribution of endothelial nitric oxide (eNO) and prostaglandin to ACh, and vascular smooth muscle's cAMP and neuronal NO to VIP vasorelaxant effects.

1. Introduction

Every cell in the human body is dependent upon adequate oxygen supply to maintain physiological cellular functions. Hypoxic state, one of the consequences of ischaemia of the human vascular tissue, belongs to the one of the most frequent events and plays critical role in development and progression of ischaemic disorders.[1] Stenosis or occlusion of the common carotid artery is one of the aetiological factors in ischaemic disorders of orofacial tissues. Under experimental conditions, Vág et al.[2] showed that unilateral carotid artery occlusion resulted in an immediate decrease of blood flow in the rat submandibular gland and suggested that the synthesis and liberation of nitric oxide in blood vessels within the gland decreases during carotid ligation.

* *Corresponding author at*: Department of Pharmacology, Faculty of Stomatology, University of Belgrade, Dr Subotića 1, 11000 Belgrade, Serbia. Tel.: +381 112682373; fax: +381 112685361.
E-mail address: dragica.stojic@stomf.bg.ac.rs (D. Stojić).

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It is noteworthy to point out that atherosclerotic-occlusive changes could be observed not only in common carotid artery, but also in human facial, maxillary and lingual arteries which maintain local blood flow in salivary glands.[3] Vascular dysfunction observed in hypoxia is related to the impairment of endothelium-dependent vasorelaxation, mainly due to hypoxia-induced decrease in production and release of endothelial factors, as well as to increased vascular tone due to alterations in arterial smooth muscle cells.[4–8] It is of relevance, also, that the observed alterations in vascular responsiveness under hypoxia depend not only on the vascular bed and the species studied, but also on the degree of hypoxia.[5,9]

Our previous study, in isolated glandular branch of rabbit facial artery (feeding artery for submandibular gland), revealed that whilst acetylcholine (ACh) provoked endothelium-dependent vasorelaxation, mediated by nitric oxide (NO) and prostacyclin, vasoactive intestinal polypeptide (VIP) induced endothelium-independent vasorelaxant effect, mediated by cyclic adenosine 3′,5′-monophosphate (cAMP) from vascular smooth muscle and by neural NO (nNO) .[10]

Given the critical role of the vascular endothelium and smooth muscle cells in the production of vasoactive molecules that regulate blood flow and the deleterious effects of ischaemic events on endothelial cell function, we hypothesized that acute ischaemia affects vascular responses of ACh and VIP, as the major vasodilators in salivary gland, in isolated glandular branch of rabbit facial artery. In order to test this hypothesis, we investigated the impact of 10, 30 and 60 min of common carotid artery occlusion on vasorelaxation induced by ACh or VIP, as well as on the contribution of endothelial and non-endothelial signalling molecules, NO, prostanoids and cAMP, involved in these vascular responses. The selected times of ischaemia correspond with reported impact of ischaemia duration on the reactivity of different peripheral arteries.[5,11,12] Our approach could give insight into the mechanisms underlying salivary gland diseases related to ischaemic circulatory disorders.

2. Method

2.1. *Vascular preparations*

The Ethical committee of Faculty of Stomatology at the University of Belgrade approved the study design.

In the present study, animals (80 Chincilla rabbits, 70 males and 10 females, three months old, weighing 2.5–3.0 kg) were anaesthetized with urethane (1 g kg^{-1}).

Ischaemia was induced *in vivo* by carotid artery occlusion, in order to mimic orofacial ischaemia usually encountered in clinical situations and considering the fact that *in vivo* regional ischaemia, but not *in vitro* hypoxia, leads to endothelial dysfunction, measured by endothelium-dependent vasorelaxation responses.[13] Responsiveness of the glandular branch of the facial artery was examined *in vitro* in order to gain insight into pathophysiological mechanisms of ischaemic vascular injury.

In experiments, left or right common carotid artery was occluded for 10, 30 or 60 min by cords. After this period, the segments of the ipsilateral glandular branch of facial artery as well as the segments of the contralateral side, which served as controls, were carefully dissected free from surrounding connective tissue and cut into 3-mm long circular rings. In some rings the endothelium was mechanically removed by gently rubbing the intimal surface with a stainless-steel wire. The absence of endothelium was checked by testing the absence of a relaxant response to ACh.

2.2. *Isometric force measurements*

Ring preparations were mounted between two stainless-steel triangles in an organ bath containing 10 ml Krebs–Ringer bicarbonate solution (37 °C, pH 7.4), aerated with 95% O_2 and 5% CO_2. Isometric tension was recorded on a Hugo Sachs model MC 6621 recorder. The preparations were allowed to equilibrate for 60 min. A pretension of 1.0 g was applied and artery segments were allowed to stabilize for 30 min before experimentation.

2.3. *Experimental procedure*

At the beginning of the experiment, endothelium functional integrity was examined by precontraction of the isolated glandular branch of rabbit facial artery with submaximal concentration (EC_{80}) of phenylephrine (1 μM), followed by addition of acetylcholine (10 μM). Arterial rings showing relaxation to ACh of more than 70% were counted as endothelium intact. Since separate experiments with facial artery rings demonstrated that the first and the second concentration–response curves (obtained after 60 min) for ACh, bradykinin and VIP were not significantly different, a multiple curve experimental design was applied: first cumulative concentration–response curves obtained with agonists, ACh (0.1–30 μM), bradykinin (0.01–3 μM) or VIP (3–300 nM) on precontracted facial artery were established; thereafter, nonselective inhibitor of NO synthase, N^G-nitro-L-arginine (L-NOARG, 10 μM), or nonselective inhibitor of cyclooxygenase, indomethacin (10 μM), or a selective inhibitor of neural NO synthase, L-N^{ω}-nitroarginine-2,4-L-diaminobutyric-amide (N^{ω}, 10 μM), or a stimulator of adenylate cyclase, forskolin (0.1 μM) was added to the organ baths and allowed in contact with preparations for 20–45 min before second cumulative concentration–response curves for agonists were established. In another group of experiments, VIP (0.1 μM) was added to the organ baths before repeating acetylcholine cumulative concentration–response curve. In separate experiments, relaxing effects to an exogenous donor of NO, sodium nitroprusside (SNP, 0.03–10 μM) was investigated.

2.4. *Treatment of data and statistics*

The relaxation induced by each concentration of agonists was expressed as a per cent relaxation of the phenylephrine-induced maximal precontraction. For each dose–response curve, the maximum effect (E_{max}), as a measure of responsiveness to an agonist, and the concentration of the agonist which produced half of E_{max} ($\log EC_{50}$), were calculated using