

Neurochemicals Involved in Medullary Control of Common Carotid Blood Flow

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Abstract: The common carotid artery (CCA) supplies intra- and extra-cranial vascular beds. An area in the medulla controlling CCA blood flow is defined as the dorsal facial area (DFA) by Kuo *et al.* in 1987. In the DFA, presynaptic nitrergic and/or glutamatergic fibers innervate preganglionic nitrergic and/or cholinergic neurons which give rise to the preganglionic fibers of the parasympathetic 7th and 9th cranial nerves. Released glutamate from presynaptic nitrergic and/or glutamatergic fibers can activate N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors on preganglionic nitrergic and/or cholinergic neurons. By modulating this glutamate release, several neurochemicals including serotonin, arginine, nitric oxide, nicotine, choline and ATP in the DFA regulate CCA blood flow. Understanding the neurochemical regulatory mechanisms can provide important insights of the physiological roles of the DFA, and may help develop therapeutic strategies for diseases involving CCA blood flow, such as migraine, hypertensive disease, Alzheimer's disease and cerebral ischemic stroke.

Keywords: Carotid artery, Cerebral blood flow, Medulla, Parasympathetic nucleus, Vascular regulation, Neurotransmitter

INTRODUCTION

Common carotid artery (CCA) is an important artery supplying both intra- and extra-cranial tissues of the head, but the brain regulation of CCA blood flow has been rarely addressed. Vasodilatations in extra- and intra-cranial tissues have long been known to be induced by stimulating parasympathetic 5th [1, 2], 7th and 9th cranial nerves or by stimulating the locus coeruleus or nucleus raphe dorsalis [3, 4]. In addition, CCA blood flow increase that involves in the defense reaction can be induced by stimulations of the periaqueductal gray of the midbrain [5, 6]. However, a preganglionic nucleus controlling CCA blood flow had not been investigated until Kuo *et al.* reported that dorsal portion of the lateral tegmental field in cat medulla controlled mainly an ipsilateral increase in CCA blood flow without significant changes in other cardiovascular parameters [7]. Thence the authors defined this area as the dorsal facial area (DFA) because it is located dorsally to the facial nucleus in cats. Similar to the location of DFA, an area located dorsolaterally to the facial nucleus in rats was defined by Nakai *et al.* [8] as a parasympathetic cerebrovasodilator center.

In the last 25 years, we have established anatomical organizations and several neurochemical regulatory

mechanisms of the DFA, as summarized in Figs. (1 and 2). Understanding the anatomical organizations and neurochemical regulatory mechanisms may provide important information for understanding the physiological functions of the DFA and developing therapeutic strategy for diseases involving CCA blood flow, such as migraine, hypertensive disease, Alzheimer's disease, and cerebral ischemic stroke.

DFA IS A PARASYMPATHETIC PREGANGLIONIC NUCLEUS

DFA-induced increase in CCA blood flow is abolished by ipsilateral sectioning of both parasympathetic 7th and 9th cranial nerves, but is not abolished by ipsilateral cervical sympathectomy [7]. It is partially blocked by intravenous administrations of high dose of atropine, a parasympathetic blocking agent [9]. In addition, ChAT-reactive neurons (cholinergic neurons) are present in the DFA where preganglionic neurons give rise to the parasympathetic 7th and 9th cranial nerves and they seem to be colocalized in the inferior and superior salivary nuclei [10]. The DFA of the cat may be functionally and anatomically equivalent to an area located dorsolaterally to the facial nucleus in rats as demonstrated by Nakai *et al.* [8] who defined the area as a parasympathetic cerebrovasodilator center. Based on the anatomical location, both areas in cats and rats are likely the rostral extension of the dorsal motor nucleus of the vagus nerve. The DFA is therefore a parasympathetic preganglionic

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nucleus that gives rise to both parasympathetic 7th and 9th cranial nerves (Fig. (1)).

DIFFERENTIAL CONTROL OF INTRA- AND EXTRA-CRANIAL BLOOD FLOWS

Since CCA supplies blood flows to intra- and extra-cranial tissues, while the DFA-induced CCA blood flow increase can only be partially blocked by intravenous administration of atropine, indicating different mechanisms for increasing blood flows in intra- and extra-cranial vessels [9]. The increase in extra-cranial tissues is completely abolished by atropine but further increased by physostigmine (an acetylcholine-esterase inhibitor acting as a muscarinic agonist), demonstrating that the increase in extra-cranial

tissues is completely mediated by muscarinic action of acetylcholine. The increase in intra-cranial tissues, in contrast, is enhanced by atropine but inhibited by physostigmine, suggesting that the increase in intra-cranial tissues is mediated by a non-muscarinic vasodilator mechanism (Fig. (1)); nevertheless, this finding also indicates that a muscarinic mechanism can inhibit the increase in intra-cranial tissues. The non-muscarinic vasodilator for the increase in intra-cranial tissues, possibly a co-transmitter nitric oxide, can be released in association with the release of acetylcholine to the brain vessel from the post-ganglionic fibers of parasympathetic 7th and 9th cranial nerves and be modulated by the axo-axonal mechanism for cerebral vasomotor regulations [11, 12]. The muscarinic inhibition to the increase in intra-cranial tissues may be attributed to an inhibition by muscarinic action of acetylcholine to the vasodilator effect of adrenergic β -receptor on the parasympathetic terminal. This mechanism explains the notion that muscarinic action can inhibit the increase in the intra-cranial blood flow induced by DFA stimulation [9].

In conclusion, DFA stimulation promotes release of acetylcholine to CCA vascular beds, the vessels of the intra- and extra-cranial tissues. The muscarinic receptor is responsible for increasing blood flow in the extra-cranial tissues, but for inhibiting the increase of blood flow in the intra-cranial tissues. For increasing intra-cranial blood flow, the non-cholinergic vasodilator is most likely nitric oxide co-released with acetylcholine from the parasympathetic terminals, which are modulated by the axo-axonal mechanism.

GLUTAMATERGIC ACTION, RELEASE AND RECEPTORS

An increase in CCA blood flow evoked by glutamate stimulation of the DFA was first reported by Kuo *et al.* [7]. This response is independent by supra-collicular decerebration [10]. Activation of the DFA with glutamate, N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) induces dose-dependent increases in CCA blood flow, and the order of potencies is AMPA > NMDA > glutamate [13]. Pretreatment with either non-competitive NMDA receptor antagonist or AMPA/kainate receptor antagonist attenuates the glutamate-induced increase in CCA blood flow in a dose-dependent manner [13]. These findings demonstrate that NMDA and AMPA receptors on neurons in the DFA are responsible for the increase in CCA blood flow.

The microdialysis hyphenated with high performance lipid chromatography (HPLC) [14] is a useful technique for measuring glutamate and serotonin (5-HT) released in the brain [15-17]. Perfusion with KCl, a neuronal depolarizing agent, through a microdialysis probe in the DFA increases dose-dependently glutamate concentration in the dialysate, suggesting an endogenous release of glutamate in the DFA [15]. Perfusion with 5-HT or alaproclate (a serotonin reuptake inhibitor) decreases glutamate concentration accompanied with a parallel decrease in CCA blood flow, suggesting a tonic glutamate release is inhibited by a tonic 5-HT release in the DFA [15]. The latter proposal was confirmed later [13, 16]. Neuronal release of glutamate is

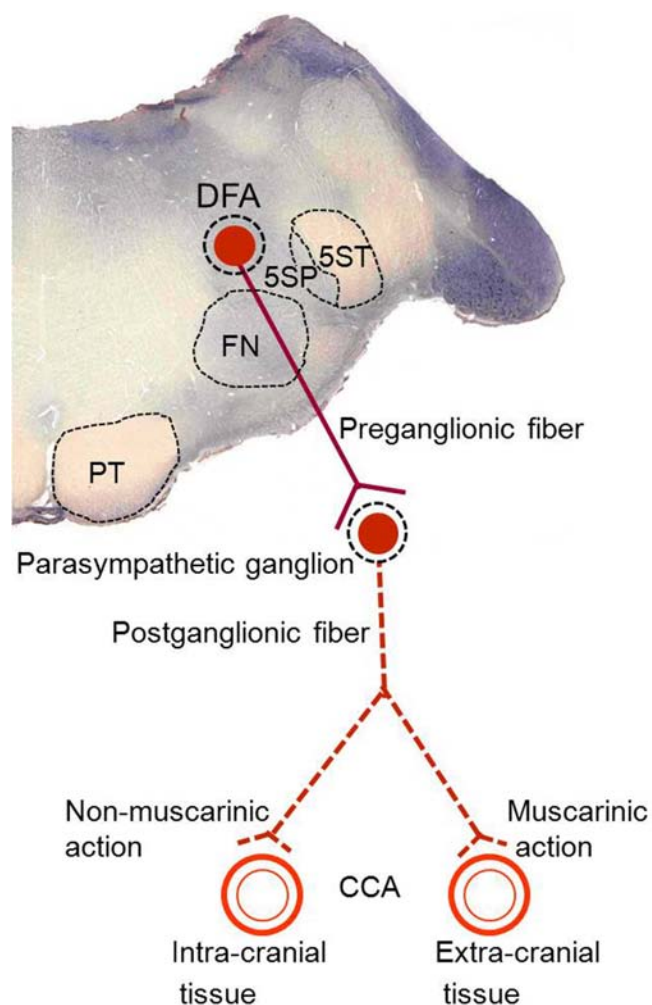


Fig. (1). The DFA and its related connections. The DFA is located at the medulla dorsally to the facial nucleus. The preganglionic neurons in the DFA give rise to the preganglionic and postganglionic fibers of the parasympathetic 7th and 9th cranial nerves. The CCA vascular beds that supply intra- and extra-cranial tissues are innervated by the postganglionic fiber that releases acetylcholine. Muscarinic and non-muscarinic action of acetylcholine is responsible for blood flow increase in the extra-cranial and intra-cranial tissues, respectively. Abbreviations: CCA, common carotid artery; DFA, dorsal facial area; FN, facial nucleus; 5SP, spinal trigeminal nucleus; 5ST, spinal trigeminal tract; PT, pyramidal tract.

further confirmed by the findings that CCA blood flow increase induced by intra-DFA administrations of several neurochemicals, such as arginine (NO precursor), S-nitroso-N-acetylpenicillamine (NO donor) [18], ATP (P2 receptor agonist) [19], and choline ($\alpha 7$ -nAChR agonist) or nicotine (non-specific nAChR agonist), can be inhibited by glutamatergic receptor antagonists. Furthermore, endogenously released [18] and exogenously administered glutamate [13] are similarly blocked by the NMDA or AMPA receptor antagonist; both receptors have therefore been proposed to be present on preganglionic neurons, nitroergic and/or cholinergic neurons which give rise to the parasympathetic 7th and 9th preganglionic nerves [18].

In conclusion, the glutamatergic fiber releasing glutamate in a tonic manner is present in the DFA. The released glutamate stimulates both AMPA and NMDA receptors on the preganglionic neurons in the DFA to induce an increase of CCA blood flow. Various neurochemicals may activate their respective receptors on the glutamatergic fiber to modulate the glutamate release in the DFA.

SEROTONERGIC RELEASE, ACTION, AND RECEPTOR

The 5-HT nerves and their receptors exist widely in the brain including the medulla oblongata, playing important roles in cardiovascular or cardiopulmonary regulations. For example, the 5-HT₂ receptors in the nucleus ambiguus mediates GABAergic activity [20]; the 5-HT_{2A} receptor in the nucleus tractus solitarius inhibits sympathetic activity [21] and the 5-HT₃ and 5-HT₄ receptors in the nucleus tractus solitarius participate in the cardiopulmonary reflex [22]. Furthermore, activation of various brain regions with selective agonists for 5-HT_{1D}, 5-HT₂, and 5-HT_{2A} receptors or with 5-HT reuptake inhibitors can inhibit glutamate release [15, 23-25]. Nevertheless, Li *et al.* [15] first reported 5-HT inhibition of glutamate release in the DFA.

5-HT-reactive nerves in the DFA have been identified by immunohistochemical staining of tyrosine hydroxylase [10, 26]. These anatomical findings are functionally substantiated by a finding of neuronal release of 5-HT in tonic [9]. Furthermore, perfusion in the DFA with 5-HT₂ receptor antagonist increases while that with 5-HT₂ receptor agonist decreases glutamate release in the DFA, accompanied with an increase and a decrease of CCA blood flow [15], respectively. Perfusion with 5-HT₁ agonist or antagonist, however, does not affect glutamate release in the DFA [16]. 5-HT₂ but not 5-HT₁ action in the DFA is further substantiated by pharmacological interactions of various agonists and antagonists for 5-HT₁ and 5-HT₂ in the DFA [13].

In conclusion, 5-HT reactive nerves are present in the DFA; they release 5-HT in tonic, which activates 5-HT₂ receptors on the presynaptic nitroergic and/or glutamatergic fibers to cause an inhibition of glutamate release in the DFA, leading to the reduction in CCA blood flow.

NICOTINIC ACTIONS AND RECEPTORS

Wide cholinergic innervations and diverse muscarinic and nicotinic actions are present in the brain and medulla for

cardiovascular regulations or transmitter releases. For instance, activation of cholinergic nerves projecting to the rostral ventrolateral medulla [27], the dorsal motor nucleus and solitary nucleus of the vagus nerve [28] can regulate various cardiovascular functions. The $\alpha 7$ -nAChR on the striatal glutamatergic terminals promotes glutamate release [29]. The $\alpha 3\beta 2$ - and $\alpha 4\beta 2$ -nAChRs on dopaminergic terminals in the striatum promote dopamine releases [29]. Nevertheless, Gong *et al.* [30] demonstrated for the first time that activation of $\alpha 7$ -, $\alpha 4\beta 2$ -, and $\alpha 3\beta 4$ -nAChRs in the DFA increase CCA blood flow, and Kuo *et al.* [31] further demonstrated that activation of $\alpha 7$ -nAChR on glutamatergic terminals in the DFA leads to glutamate release, as evidenced by the following experiments.

Nicotine (a non-selective nAChR agonist) or choline (a selective $\alpha 7$ -nAChR agonist) stimulation of the DFA results in dose-dependent increases in CCA blood flow [30]. The nicotine-induced increase is dose-dependently inhibited by $\alpha 7$ -nAChR antagonists (α -bungarotoxin and methyllycaconitine), a relatively selective $\alpha 4\beta 2$ -nAChR antagonist (dihydro- β -erythroidine), and a relatively selective $\alpha 3\beta 4$ -nAChRs antagonist (mecamylamine), while the choline-induced increase is inhibited in a large extent by $\alpha 7$ - and, in a lesser extent, by $\alpha 3\beta 4$ nAChR antagonist, but not by $\alpha 4\beta 2$ -nAChRs antagonist, indicating $\alpha 7$ -nAChR plays the most important role [30]. Furthermore, microinjections of muscarinic receptor agonists (muscarine and methacholine) and antagonist (atropine) do not affect the basal CCA blood flow; muscarinic receptors if any in the DFA, therefore, are not likely to regulate CCA blood flow. These findings suggest the presence of $\alpha 7$ (the major subtypes), $\alpha 4\beta 2$, and $\alpha 3\beta 4$ subunits of nAChRs in the DFA and their involvement in regulation of CCA blood flow.

In another experiment, the increase of CCA blood flow induced by stimulation of the DFA with nicotine or choline is abolished by pretreatment with either $\alpha 7$ -nACh receptor antagonist (α -bungarotoxin) or glutamate antagonist (such as MK-801, an NMDA antagonist, or glutamate diethylester, an AMPA antagonist), suggesting glutamate release in the DFA upon activation of $\alpha 7$ -nACh receptor by nicotine or choline [31]. On the other hand, the flow increase induced by intra-DFA administration of glutamate or KCl, which can induce releases of various transmitters including glutamate and acetylcholine, is greatly inhibited by MK-801 and glutamate diethylester, but not by α -bungarotoxin [31]. These findings suggest that activation by nicotine or choline of nACh receptors, primarily $\alpha 7$ -nACh receptors, causes a release of glutamate, but does not cause a release of cholinergic substance that stimulates $\alpha 7$ -nACh receptors in the DFA. The $\alpha 7$ -nAChR and other nAChRs may be present on the nitroergic and/or glutamatergic fiber which innervates and releases glutamate to stimulate preganglionic neurons of the parasympathetic 7th and 9th cranial nerves, leading to an increase in CCA blood flow [18].

In conclusion, functional $\alpha 7$, $\alpha 4\beta 2$, and $\alpha 3\beta 4$ subunits of nAChRs, with the most prominent one being the $\alpha 7$ subunit, appear to be present in the DFA on the nitroergic and/or glutamatergic fiber which innervates and releases glutamate to stimulate the preganglionic neurons of the parasympathetic

7th and 9th cranial nerves for increasing CCA blood flow. Muscarinic receptors if any in the DFA are not likely to regulate CCA blood flow.

PURINERGIC AGONISTS AND RECEPTORS

Although adenosine (a P1 purinergic receptor agonist) is generated by enzymatic degradation of the neurotransmitter ATP, adenosine is exocytotically released [32, 33] in the brain affecting cardiovascular function. Adenosine modulates neuron activities in the rostral ventrolateral medulla [34] and baroreceptor activities in the area postrema [35] and the nucleus tractus solitarius [36]. On the other hand, ATP triggers physiological functions *via* activating P2 purinoceptors that are divided into two main families: P2X and P2Y purinoceptor families [37]. ATP in the medulla regulates cardiovascular functions [38] through modulating neuron activities *via* purinergic actions [39, 40]. Furthermore, ATP can cause release of glutamate through stimulating purinergic receptors in the brain stem autonomic network [41].

An assertion that adenosine and ATP can mediate release of glutamate through stimulating purinergic receptors in the DFA to increase CCA blood flow has been demonstrated by Kuo *et al* [19]. Microinjection into the DFA with adenosine, a P1 receptor agonist, results in only mild and poorly reproducible increase, while stimulation with ATP or α,β -MeATP, a P2 purinergic receptor agonist, results in a markedly dose-dependent increase in CCA blood flow. P2 receptor-induced increase in CCA blood flow is dose-dependently attenuated by pretreatment with either P1 receptor antagonist (dipropyl-8-p-sulfophenylxanthine, DPSPX) or P2 receptor antagonist (pyridoxalphosphate-6-azophenyl-2', 4'-disulfonic acid, PPADS). The effect of ATP or α,β -MeATP, a P2 receptor agonist, is also inhibited by P1 receptor antagonist, suggesting a degradation of ATP to adenosine, a P1 receptor agonist. The increase of CCA blood flow caused by purinergic agonists (ATP and α,β -MeATP) or glutamate is dose-dependently attenuated by pretreatment with MK-801 (a non-competitive NMDA receptor antagonist) or glutamate diethyl ester (GDEE, a competitive AMPA/kainite receptor antagonist), indicating that the purinergic activation mediates a release of glutamate that stimulates AMPA and NMDA receptors in the DFA to induce the increase in CCA blood flow. The above findings suggest that P2 and P1 purinergic receptors are present in the DFA, with P2 receptors being the majority; activation of these two receptors result in release of glutamate, which increases CCA blood flow.

In conclusion, purinergic receptors, predominantly P2 with a lesser extent P1, appear to be present in the DFA; activations of these receptors result in release of glutamate that activates the NMDA and AMPA receptors in the DFA, leading to the increase in CCA blood flow.

PRESYNAPTIC NITRERGIC AND/OR GLUTAMATERGIC FIBER

Nitric oxide synthases (NOSs), which in the presence of Ca^{2+} -calmodulin catalyze L-arginine (a NO precursor) to synthesize NO in cells, include neuronal NOS (nNOS), endothelial NOS (eNOS), and inducible NOS (iNOS). The

nitrergic cells/neurons (the NOS-containing neurons) releasing NO as a neurotransmitter are widely distributed in the brainstem [42, 43], including the nucleus tractus solitarius [44, 45], hypothalamus [46], and rostral ventrolateral medulla [47]. The nNOS, nevertheless, is co-localized in the glutamatergic neurons/fibers in the rostral ventrolateral medulla [48]. This suggests that NO, synthesized by nNOS in these neurons/fibers, may be released to act as a neurotransmitter itself, or alternatively may stimulate guanylyl cyclase to convert cyclic guanosine triphosphate (cGTP) to cyclic guanosine monophosphate (cGMP) that may then stimulate release of glutamate from the glutamatergic neurons/fibers. The latter notion is seldom addressed except for a report in which presynaptic nitrergic and/or glutamatergic fibers have been proposed to be present in the solitary nucleus, releasing NO and glutamate in the regulation of sympathetic out flow [49]. Similar presynaptic nitrergic and/or glutamatergic fibers have been proposed to be present in the DFA in the regulation of CCA blood flow [18, 50] according to the following findings.

Profuse nitrergic fibers surrounding nitrergic neurons in the DFA have been identified with nicotinamide adenine dinucleotide phosphate diaphorase (NADPH-diaphorase), a marker for the nitrergic neurons [50]. The increased CCA blood flow induced by intra-DFA administration of L-arginine is markedly inhibited not only by pretreatment with nNOS inhibitor but also by glutamate receptor antagonists, suggesting that nNOS-containing neurons/fibers may release glutamate in the DFA [50]. In microdialysis and HPLC studies, perfusion with a NO donor into the DFA increases, but co-perfusion of a NO donor with a guanylyl cyclase (GC) inhibitor blocks release of glutamate, further suggesting that glutamate is released from the nNOS/GC/cGMP-containing neuron/fiber in the DFA and glutamate release is triggered by cGMP [18]. The above findings suggest that the presynaptic glutamatergic fiber in the DFA can release glutamate through activation of the nNOS/GC/cGMP pathway in the fiber, and is defined as presynaptic nitrergic and/or glutamatergic fibers. The released glutamate thus activates post-synaptically on the preganglionic neurons of parasympathetic 7th and 9th cranial nerves (Fig. (2)).

In conclusion, the presynaptic nitrergic and/or glutamatergic fibers that can release glutamate through activation of the nNOS/GC/cGMP in the fiber exist in the DFA for regulation of CCA blood flow [18].

PREGANGLIONIC NITRERGIC AND/OR CHOLINERGIC NEURON

Both cholinergic neurons [10, 26] and nitrergic neurons [50] are present in the DFA. Preganglionic neurons projecting to the parasympathetic sphenopalatine ganglia of the facial nerve reside also in the DFA [10]. All these neurons are similar in morphology and location in the DFA, indicating that the preganglionic neurons are nitrergic and cholinergic in nature. Thus they are named preganglionic nitrergic and/or cholinergic neuron [18]. Other studies demonstrate that glutamate exogenously administered [13], or endogenously released from the presynaptic nitrergic and/or glutamatergic fibers [18] in the DFA causes the increase in CCA blood flow, which can be markedly

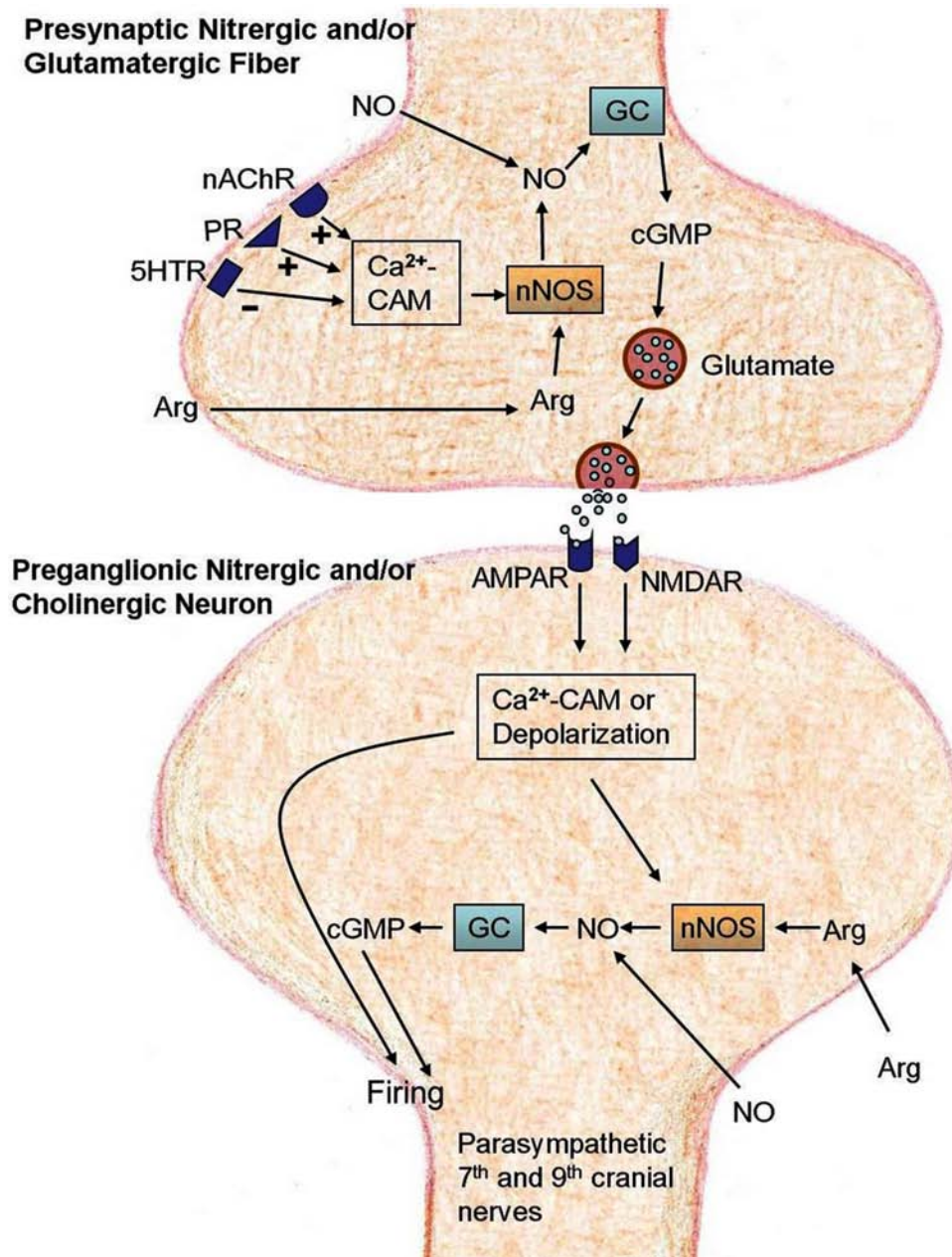


Fig. (2). Neurochemical modulations in the DFA. Preganglionic nitroergic and/or glutamatergic fiber and preganglionic nitroergic and/or cholinergic neurons contain the nNOS/GC/cGMP system. Nitric oxide (NO) and arginine may presynaptically activate the nNOS/GC/cGMP system in the preganglionic nitroergic and/or glutamatergic fiber to induce glutamate release, or postsynaptically activate that in the preganglionic nitroergic and/or cholinergic neurons to induce neuron firing (excitation). Other neurochemicals have their receptors on the preganglionic nitroergic and/or glutamatergic fiber but not on the preganglionic nitroergic and/or cholinergic neurons. Choline and nicotine may presynaptically activate nAChRs, while ATP and adenosine activate PR on the preganglionic nitroergic and/or glutamatergic fiber to enhance the nNOS/GC/cGMP activities in the fiber, resulting in glutamate release; nicotinic or choline action is mediated primarily *via* $\alpha 7$ -nAChR, and purine/ATP action primarily *via* P2 receptor. However, 5-HT stimulation of 5HTR on the presynaptic nitroergic and/or glutamatergic fibers is mediated primarily by 5-HT₂ receptor, probably through an inhibition of the nNOS/GC/cGMP activities leading to a reduction of glutamate release. The released glutamate in turn postsynaptically activates NMDA and AMPA receptors on the preganglionic nitroergic and/or cholinergic neurons. This activation may directly depolarize these neurons *via* NMDA or AMPA channels and/or indirectly depolarize preganglionic nitroergic and/or cholinergic neurons *via* activating their nNOS/GC/cGMP system. Consequently excitation impulses of the preganglionic nitroergic and/or cholinergic neurons are conducted through the pre- and post-ganglionic fibers of the parasympathetic 7th and 9th cranial nerves to CCA vascular beds for intra- and extra-cranial tissues, causing increase of blood flow in these tissues. Abbreviations: AMPAR, receptor for α -amino-3-hydroxy-5- methylisoxazole-4-propionic acid; Arg, L-arginine; CAM, calmodulin; cGMP, cyclic guanosine monophosphate; 5HTR, serotonin receptor; GC, guanylyl cyclase; nAChR, nicotinic acetylcholine receptor; NMDAR, receptor for N-methyl-D-aspartate; NO, nitric oxide; nNOS, neuronal NO synthase; PR, purinergic receptor.

inhibited by nNOS or guanylyl cyclase (GC) inhibitors as well as glutamatergic (NMDA and AMPA) receptor inhibitors. The above findings together suggest that the glutamate released from the presynaptic nitrergic and/or glutamatergic fibers may stimulate the NMDA and AMPA receptors on the preganglionic nitrergic and/or cholinergic neuron.

In conclusion, glutamate-releasing presynaptic nitrergic and/or glutamatergic fibers innervate preganglionic nitrergic and/or cholinergic neurons, which bear NMDA and AMPA receptors for glutamate [18] (Fig. (2)).

NEUROCHEMICAL MODULATIONS OF PRE-SYNAPTIC NITRERGIC AND/OR GLUTAMATERGIC FIBERS BY nNOS/GC/cGMP SYSTEM

Glutamate release by the presynaptic nitrergic and/or glutamatergic fibers in the DFA depends on activation of the nNOS/GC/cGMP system in the presynaptic nitrergic and/or glutamatergic fibers [18, 50, 51]. These findings suggest that neurochemical modulations through their respective receptors on the presynaptic nitrergic and/or glutamatergic fibers to release glutamate can be mediated *via* the activations of the nNOS/GC/cGMP system. This hypothesis has been supported by the following findings.

Intra-DFA administrations of arginine (NO precursor) or S-nitroso-N-acetylpenicillamine (NO donor) [18, 50], choline, nicotine [31, 51] or ATP [19] in the DFA induce an increase of CCA blood flow. The increased CCA blood flow can be blocked by the antagonist for glutamate or the nNOS/GC/cGMP system, suggesting glutamate release *via* activation of the nNOS/GC/cGMP system in the presynaptic nitrergic and/or glutamatergic fibers. The increased CCA blood flow can also be blocked by the respective receptor antagonists for the above mentioned neurochemicals, suggesting a neurochemically presynaptic activation of the presynaptic nitrergic and/or glutamatergic fibers to release glutamate is mediated *via* activation of the nNOS/GC/cGMP system in the presynaptic nitrergic and/or glutamatergic fibers.

In conclusion, neurochemical agonists may presynaptically act on their respective receptors on the presynaptic nitrergic and/or glutamatergic fibers to activate the nNOS/GC/cGMP system and subsequently modulate glutamate release (Refer to Fig. (2)).

CONCLUDING REMARKS

Both presynaptic nitrergic and/or glutamatergic fibers and preganglionic nitrergic and/or cholinergic neurons are activated through the nNOS/GC/cGMP system in the DFA. The released glutamate from presynaptic nitrergic and/or glutamatergic fibers can activate the preganglionic nitrergic and/or cholinergic neurons which bear the NMDA and AMPA receptors and give rise to the preganglionic fiber of the parasympathetic 7th and 9th cranial nerves controlling CCA blood flow. The release of glutamate from presynaptic nitrergic and/or glutamatergic fibers can be modulated by many neurochemicals, neurotransmitters, and neuromodulators, such as arginine (NO precursor) or S-nitroso-N-acetylpenicillamine (NO donor), serotonin, choline, nicotine,

ATP, or even more to be addressed in the future. All these mechanisms integrate to play important roles in regulation of CCA blood flow that supplies the intra- and extra-cranial tissues. Presence of various receptors of neurochemicals, neurotransmitters, and neuromodulators in the DFA may produce physiological or pathological effects that regulate intra- and extra-cranial circulation.

So far as we know, "the role of the DFA in pathophysiology associated with altered carotid blood flow" has not been investigated. It is interesting and possible that many factors such as smoking (effects of nicotine), glutamate in food, neural inflammation, neural degeneration in aging, agonists and antagonists of serotonin, and purine, may involve in the pathophysiology of the DFA. Although the pathological role of the DFA is yet unknown, interventions to improve CCA blood flow is possible to use the above mentioned neurochemicals to activate the DFA. Accumulation of knowledge from these and future investigations may provide insight into understanding the DFA mechanisms and developing therapeutic strategy in management of diseases involving CCA blood flow, such as migraine, Alzheimer's disease, hypertensive disease and cerebral ischemia.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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