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Review article

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LABILE PRODUCTS OF VASCULAR ENDOTHELIUM AS MEDIATORS AND MODULATORS OF THE FUNCTIONS OF THE CENTRAL NERVOUS SYSTEM

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A rapid development of the knowledge about vascular endothelial cell function as an "endocrine gland" releasing the labile, highly biologically active products, caused a major reapprisal of our concepts concerning the pathophysiology of our body. The publication summarizes the present understanding of the involvement of nitric oxide (NO), endothelins (ETs) and arachidonic acid products in the mechanisms underlying the regulation of the tonus of vessels supplying blood to the CNS, their known modulatory and mediatory role in CNS functions such as a development and memory, peripheral nonadrenergic noncholinergic, or sensory neurotransmission. The regulation intracellular Ca^{++} ion levels as a proposed mechanism for the neuroprotective, as well as the neurotoxic effect of the described endothelial products is presented. The supposed therapeutical usefullness of compounds which can modulate their biosynthesis, substitute their activity, or modify its degradation are also summarized.

K e y w o r d s: *endothelins, prostanoids, nitric oxide, central and peripheral nervous system*

INTRODUCTION

The rapidly developing knowledge of the function of the vascular endothelial cells in maintaining the integrity of the vascular wall, patency of vessels, and fluidity of blood, completely changed the concept of their role in physiology and pathology of our body. Endothelial cells function is related to their capacity to synthetize a vast number of substances such as: prostacyclin (PGI_2) and the other arachidonic acid metabolites (1—3), "endothelium derived relaxing factor" (EDRF) (4), proved to be nitric oxide (NO) (5),

endothelins (6), activators (t-PA) (7), and inhibitors (PAI) (8) of the plasma fibrinolytic activity, cytokines (9), adhesion and chemotaxis promoting selectins and integrins (10–12), growth promoting factors (13), a platelet activating factor (PAF) (14), a wide range of antioxidant enzymes (15) and others. Most of these substances released continuously, support the blood/vessel wall homeostasis and the sufficient blood supply to the tissues.

Thus, hyperoxia, hypoxia or anoxia of tissues (4, 15), tissue damage related to inflammation, such as oedema and cytotoxicity due to the presence of activated phagocytic cells (6, 17) are strongly influenced by the biological efficiency of the endothelium.

The endothelial regulatory system is still not fully understood. Three components of this system will be reviewed here: EDRF/NO, endothelins, and some metabolites of arachidonic acid in the aspect of blood supply and the function of the central nervous system.

EDRF/NO

EDRF identified as nitric oxide (NO) (5), or rather as its free radical ($NO^{\cdot}$) (17) is biosynthesized from L-arginine (L-Arg) by oxidative desimination (18, 19) or perhaps from peptides which contain L-Arg (20). The co-product of this reaction, citrulline, is further metabolised back to arginine, forming "a half-urea cycle" (21). (*Fig. 1*). This enzymic process takes place not only in vascular

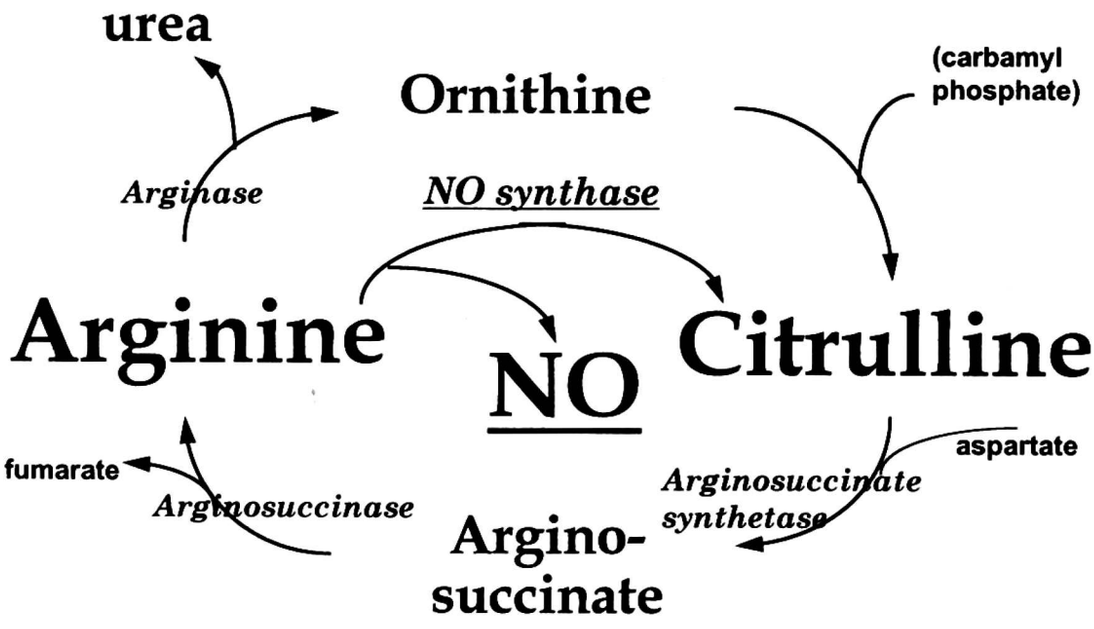

Fig. 1. Metabolism of arginine in the body; "the urea cycle". NO-synthase (NOS) is forming NO in the "part-urea cycle".

endothelium (4, 19), but also in macrophages (22), neutrophils, blood monocytes (23), mastocytes, hepatocytes, Kupfer cells (24, 25), non-adrenergic non-cholinergic (NANC) nerve endings (26), and neurons (27, 28).

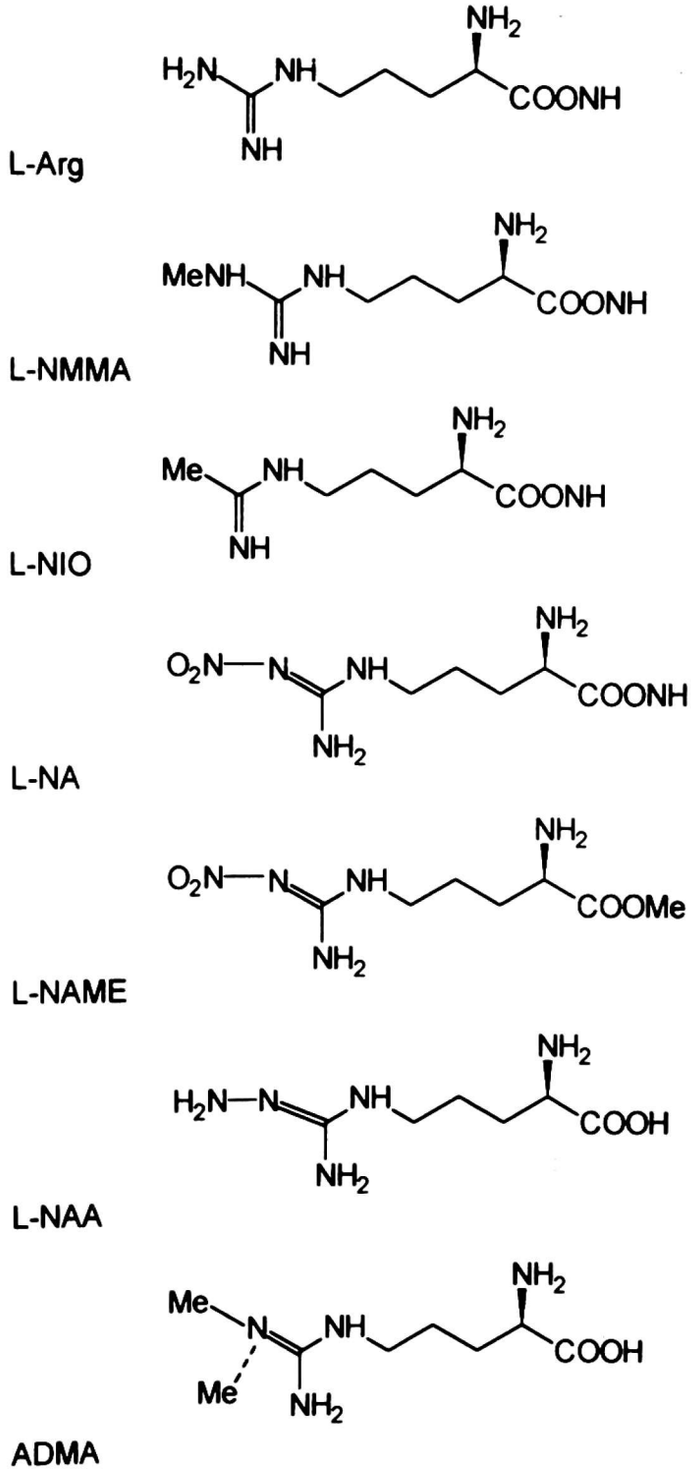

Fig. 2. Structural formulae of L-arginine (L-Arg), and some of NOS inhibitors: N^{ω}-monomethyl-L-arginine (L-NMMA); N-iminoethyl-L-ornithine (L-NIO); N^{ω}-nitro-L-arginine (L-NA); N^{ω}-nitro-L-arginine methyl ester: (L-NAME); N^{ω}-amino-L-arginine (L-NAA); asymmetrical N^{ω}-dimethyl-L-arginine (ADMA).

Some structural analogs of L-Arg (*Fig. 2*) (22, 23) have been used as the NO-synthase (NOS) antagonist to study the biochemistry and physiology of the NOS-NO pathway.

Recently it has become apparent, that there are at least two types of NOS. One is constitutive, membrane bound (in endothelium) (29), or cytosolic (in brain), Ca^{++}/calmodulin dependent, and releases NO in smaller amounts for short periods of time in response to receptor or physical stimulation (25, 30, 31). NO released by this enzyme acts as the transductive responses mechanism,

mediating several physiological responses through stimulation of soluble cytosolic guanylate cyclase, and elevating the cellular c-GMP levels (32, 33). Acetylcholine, bradykinin, substance P, thrombin, adenosine diphosphate, and 5-hydroxytryptamine, are the best known activators of the NO release, by constitutive NOS from endothelial cells, elevating intracellular Ca^{++}via receptor mediated increase of inositol 1,4,5-triphosphate and diacylglycerol levels (25, 30, 31). NO released in this way, plays an important role in maintaining the basal tonus of the vessel, counteracting the activity of all endogenous and exogenous vasoconstrictors (25, 30, 34, 35). It has been recently postulated that the endogenously formed L-Arg metabolite ADMA (*Fig. 2*) is responsible for the hypertension observed in patients with severe kidney insufficiency (36) or eclampsia (37).

Intercellular liberation of NO constitutes the common principle of pharmacological action for glyceryl trinitrate and other organic nitrates recognized recently as the "NO-donors" (38). In contrast to endogenous, the generation of NO from exogenous NO-donors is a reductive process that requires cysteine, glutathione and special enzymes, and takes place in some cells, for example in the vascular smooth muscle, but not in platelets (39). Another NO-donor, SIN-1A, the metabolite of molsidomine, releases NO in an aqueous solution in a non-enzymatic-, pH-, temperature- and time-dependent manner, causing the relaxation of the vasculature without any signs of tachyphylaxis (40).

Another biologically potent activity of the NO formed by the constitutive NOS in endothelium is the inhibition of platelet aggregability, release reaction, and its adhesion to endothelium and polymorphonuclear leukocytes (PMNs) (41—44). In the antiplatelet activity EDRF/NO synergises with PGI_2 on the basis of accumulation of the intracellular c-GMP and cAMP levels (43, 45). No synergistic activity in vasodilatory properties of both autacoids has been demonstrated (46).

NO molecule is short living and decomposes in the aqueous solution to nitrite and nitrate ions, deprived of the biological activity of nitric oxide. NO is instantly destroyed by superoxide anions ($O_2^{\cdot}$) (47). Oxyhaemoglobin is a powerful scavenger of NO, methylene blue while inhibiting guanylate cyclase hinders biological effect of EDRF/NO (48). Superoxide dismutase (SOD) and inhibitors of c-GMP phosphodiesterase potentiate the biological effects of NO (47, 48).

Another izoform of NOS is induced after activation of macrophages, monocytes, neutrophils, fibroblast, endothelial and a number of other cells by cytokines (TNF-α, IL-1, IF-γ or LPS), and once expressed, sythesizes the larger (comparing to inducible NOS) amounts of NO for long periods of time (25, 30, 31, 49, 50, 51). Furthermore, this enzyme which is cytosolic, Ca^{++}/calmodulin independent, requires tetrahydrobiopterin as a cofactor and its induction is

inhibited by corticosteroids and some cytokines (IL-8, IL-10) (52, 53). So far, the only clearly established role of this NO is as a cytotoxic molecule for invading microorganisms and tumor cells, thus mediating the so called nonspecific immunity of our body (25, 54–57). The release of NO *via* this enzyme is also included in pathological vasodilatation, observed in septic shock (58, 59) and an endothelium and tissue damage accompanying the inflammatory/immune reactions (16, 17). The existence of a human inducible NOS (iNOS) is strongly suggested by the elevation of nitrate in the plasma and urine of individuals who have received cytokines (61, 62), or become septic (63).

The cytotoxicity of NO released by iNOS is linked to several processes. NO binds to all Fe-S enzymes, which results in inhibition of many cell oxydoreductases and *cis*-aconitase (64).

NO disruption of ferritin may acount for the Fe release from target cells incubated with activated macrophages (64). The free Fe may promote lipid peroxidation (65).

A prominent action of macrophage derived NO on tumor cells is the inhibition of their synthesis of DNA by inhibition of ribonucleotide reductase (64). Superoxide anion may be another important target of the toxic activity of NO. Reaction of $O_2^{\cdot}$ with NO results in formation of peroxynitrite, which decays to nitrogen dioxide and hydroxyl radical, considered as the strongest oxidant in biological systems (17).

The interaction of NO with sulfhydryls has brought the hypothesis that S-nitrosothiols could be long-living reservoirs of bioactive NO (66). On the other hand, S-nitrosylation inactivates SH-dependent bacterial dehydrogenases and nitrosylation of proteins leads to the formation of N-nitrosoamines with cancerogenic properties (67).

cNOS and iNOS differ also in the susceptibility to inhibition by various L-Arg analogs, most evident in the relative selectivity of L-NA for cNOS (68). Also compounds that bind calmodulin, such as calcineurin or trifluoroperazine, are the only selective inhibitors on cNOS activity known so far (69). The above mentioned inhibition of the iNOS induction by corticosteroids (52), may add one more mechanism to the understanding of an anti-inflammatory activity of these compounds.

In the central nervous system the biological activity of NO is not restricted to the vessels, but the discoveries of the last five years have demonstrated the function of NO as a messenger and a synaptic plasticity modulator in CNS and in peripheral NANC and sensory neurons (21, 25, 28, 31). The participation of NO in neurotoxicity and opioid dependence has also been suggested (70, 71).

Neurotransmission by agents such as ACh, glutamate, and glycine has long been known to be associated with calcium requiring elevation of c-GMP levels in the brain, and particularly in cerebellum (72). In 1982 L-Arg was identified as the endogenous activator of the soluble guanylate cyclase in neuroblastoma

cells (73). These observations together with the discovery of the L-Arg/NO pathway in vascular endothelium led to the concept of the existence of such a pathway in the central nervous system. Knowles et al., demonstrated that addition of L-Arg to rat synaptosomal cytosol in the presence of NADPH, resulted in the formation of NO and cytrulline and was accompanied by the stimulation of soluble guanylate cyclase (74). This enzyme, calcium/calmodullin dependent, is inactive in a resting (80 nM) concentrations of Ca^{++} in synaptosomes (25) whereas it was fully active at Ca^{++} concentrations of 400 nM (74). Interestingly enough, physiological Ca^{++} levels, which are essential for the action of the brain NOS, were found to inhibit the brain soluble guanylate cyclase (74). This could represent a control mechanism whereby guanylate cyclase is not activated in those CNS cells stimulated to produce NO, but only in the effector cells (74). The brain, but not endothelial cNOS contains flavins (FAD and FMN), and thus may act as a cytochrome P-450 reductase (75). The brain NOS is competitively inhibited by L-MNNA, L-NA and L-NIO, but not by L-canavinine (76). Histoimmunochemical studies using antibodies to the cNOS (28) followed by the measurement of the NOS activity in the cytosolic fraction of different rat brain regions (77) have showed that the highest concentration of NOS was present in the cerebellum, followed by the hypothalamus and mildbrain, striatum, and hipocampus, with the lowest activity found in the medulla oblongata. The granule cells have been suggested to be the principal neurons in the cerebellum, which release NO in response to exogenous excitatory aminoacid (NMDA) receptors (28, 78). An increased activity in the excitatory pathways has long been known to cause increased levels of cGMP particularly in cerebellar cortex (21, 78).

The neurotransmitter released in all of the main excitatory synapses in the cerebellum acts through excitatory aminoacid receptors, and is probably glutamate (21). There are two categories of recognised excitatory aminoacid receptors: the ones connected with ion channel (ionotropic receptors), and those coupled to G proteins (metabotropic receptors). The ionotropic receptors are subdivided into three types, according to their selective agonists: NMDA, AMPA (quisqualate), or kainate. These types of ionotropic receptors are habituary termed NMDA and others are referred to as: non-NMDA receptors (21). In developing rat cerebellum NMDA receptors mediate most, if not all of the cGMP response to glutamate (79). In adult mice "basal" cerebellar, as well as after pharmacological intervention increased cGMP levels are reduced by the selective NMDA agonists (80). Up to now it has not been proved that either exogenous glutamate or the endogenous neurotransmitter elicits cGMP accumulation through non-NMDA receptors. In fact, glutamate has been shown to be a potent inhibitor of the elevations in cGMP that are induced by the exogenous non-NMDA agonist — kainate (81). This observation, with the evidence that the accumulation of cGMP levels is observed not in neurones

that are stimulated by the glutamate receptor agonist, but in surrounding cells only, raised the concept of existence of the mobile, permeable molecule mediating the intracellular communication (79, 81, 82). Nitric oxide with its lipophylic properties, and the short-lasting activity was a good candidate for such an action. The activation of NMDA receptors raises the cytosolic Ca^{++} levels due to the receptor-operated ion channels. The same Ca^{++} influx is believed to mediate the brain cNOS activity and to initiate many physiological and pathological effects of NMDA receptor activation (21).

Brain NOS is inhibited by the L-Arg metabolites. This effect can be reversed in vitro by the supplementation of the exogenous L-Arg (21). Interestingly enough, in the immature cerebellum, the inhibition curve for L-NA (but not L-NMMA) shows two components (83). One component is evident for very low (IC_{50} = 6nM) concentration, but with maturation, this component is lost, leaving the other component (IC_{50} = 600nM) observable in the adult. This suggests the existence of two NO-synthases, which are differently sensitive to L-NA, (but not to L-NMMA), and that one of them exists only during the development period (21,83).

Cerebellum is that part of the brain where the NO synthase is concentrated. Cerebellum contains two main neurone types: the large Purkinje cells, and small but numerous granule cells. The granule cells are a major site of NMDA receptor-mediated NO formation, and of cGMP accumulation in the developing tissue (84, 77, 78). NO generated presynaptically in granule cells appears to have at least two potential targets located in postsynaptic membranes. One is in astrocytes, and the other one in the Bergman glial cell bodies (21). The regional distribution of NOS does not entirely match that of NMDA receptors, and for example, neurones in the deep cerebellar nuclei express many NMDA receptors, but NMDA is unable to induce measurable increases in cGMP levels there (82). Cells in these nuclei do however, respond to the exogenous NO-donors (85), suggesting a different stimulus for NO formation, or the inhibition of guanylate cyclase by accumulating intracellular Ca^{++}, as mentioned above (74).

Both the localisation of NOS as well as the effector cells reactivity and its relation to physio- and pathological meaning need further investigations. As it is presently understood, NO is the one of the supposed candidates for mediating (via NMDA receptor) the processes of memory and learning (21). It could be involved in triggering the long-lasting changes in synaptic strength on which certain forms of learning are believed to depend (21). The other mechanism the NMDA-activity dependent reorganisation of the afferent fibers, with respect to the target neurones during brain maturation and development, is also suggested to be mediated by NO (86). The astrocytes activation has been demonstrated to influence the plasticity of neuronal synaptic connections (87) which may be related to the changes in the membrane ion channels, which are

dependent on the cGMP level. This effect is also supposed to be mediated by NO in CNS (21). Moreover, the endogenously formed inhibitors of NOS such as LNMMA, ADMA and D'MA have been recently isolated from the bovine brain (88).

The L-Arg/NO pathway may also play a role in the pathology of the central nervous system. It has been demonstrated that an excessive NMDA receptor activation, with the consequent increase in intracellular Ca^{++} accumulation, contributes to glutamate neurotoxicity by an enhanced production of NO (89, 90). Thus, it appears, that the biological responses to NO could be biphasic, as it is observed in glutamate and related excitatory amino acids: that is, physiological or pathological effects may occur. An activation of NMDA receptor can influence neuronal development, differentiation and plasticity (21, 91–93) via NO-stimulated cGMP accumulation, as it has been suggested for rodent cerebellar tissue (21, 78, 79, 94). The observations that NMDA and kainate enhance the viability of cerebellar granule neurons grown under low potassium conditions (90) confirm the trophic effect of excitatory amino acids on cerebellar granular cells (95, 96). However, Boje and Skolnick demonstrated that the exogenous NO-donor, SNAP (S-nitroso-N-acetylpenicillamine) was toxic for the cultured cerebellar granule neurons (90). This toxicity was enhanced by SOD, which protects NO, and abolished in the presence of oxyhemoglobin, which scavenges NO, pointing to toxic effect on nitric oxide itself. The NO-mediated neurotoxicity after glutamate was soon after that (97). It was also suggested that the stimulation of NMDA receptors induced by ischaemic insult, could raise cytosolic calcium followed by the overproduction of NO (21). However, NMDA antagonists exert few, if any, protective effects of stratial infarctus induced by occlusion of middle cerebral artery (98), the inhibition of NO biosynthesis by L-NAME, or L-NA which protected the rat brain against the focal cerebral ischaemia (99, 100). These observations may suggest that the NO production by ischaemic insult may be at least in part unrelated to the NMDA receptor overstimulation. They also suggest that the inhibition of the CNS NOS is beneficial for the treatment of the cerebral ischaemia.

Within the discrete pattern of NO-synthesizing neurons in brain, NOS was found to colocalize with the cholinergic brain stem-thalamic system, which is thought to regulate the state-dependent activity of the thalamocortical circuit (28). It was demonstrated that the release of NO onto thalamocortical neurons results in an alteration in voltage dependence of the hyperpolarization-activated cation conductance, probably mediated via cGMP (101). The administration of L-Arg into the lateral cerebral ventricle in rats resulted in behavioural stimulation, electrocortical desynchronization with occasional isolated high voltage spikes, but not motor seizures (102). The simultaneous administration of low doses of NMDA resulted in behavioural and

electrocortical seizures, and these effects were prevented by pretreatment of the rats with L-NA (102). Thus, the L-Arg/NO pathway may also participate in the pathomechanism of epileptic seizures associated with brain insult. Zhang and co-workers (103) demonstrated that hyperoxia induced convulsions in rats which are associated with the decrease in cerebral norepinephrine and GABA content, and accumulation of L-Arg. Pretreatment of rats with pargyline (an MAO inhibitor), or L-NA, completely protected from seizures, inhibited the accumulation of L-Arg, and depletion of epinephrine, but not of GABA. Moreover, Oury and co-workers even suggested, that the superoxide anion formed during O_2 toxicity may have some protective activity *via* inactivation of NO overproduced in brain during hyperoxia (104). Comparing to normal mice, they demonstrated that, the increased mortality during the 25 minut exposure of the transgenic animal, overexpressing human extracellular SOD, to hyperbaric oxygen can be prevented by the pretreatment with L-NA (104). Thus, the oxygen toxicity may be also related to the overproduction of NO in CNS.

The central and peripheral neurotoxicity may be also mediated by biologically active substances released not only by cells constitutively present in the neural tissue, but also by the migrating, phagocytizing cells. Hartung et al. (105) have recently reviewed the role of nitric oxide, oxygen radicals, arachidonic acid metabolites, proteases in inflammatory demyelination of neurons, mediated by cytokines (IL-1, IF N-γ TNF-α), as well as complement activated astrocytes, microglial cells and macrophages. Microglial cells transformation to phagocytosing brain macrophages takes place in neuronal and/or terminal degeneration after the nerve lesion (106), and the induction of NOS by phagocytosis, and the above mentioned cytokines, has recently been demonstrated in cloned murine microglial cells (107).

In the peripheral nervous system the postulated role of NO as a neurotransmitter released by "non-adrenergic, non-cholinergic" (NANC) nerves (108 – 110) has been confirmed by the reaction of anti-cNOS antibody with neurons in the myenteric plexus in the intestine and medulla of the adrenal glands (111). The activation of these neurons leads to the inhibition of the tonus of the smooth muscle in the gastrointestinal tract (esophagus, stomach, duodenum, ileocolonic junction) where it mediates adaptive relaxation (108 – 110), penile corpus cavernosum, (108 – 111), airways (109, 112), pulmonary arteries (113) mediated by the release of NO, and the accumulation of cGMP in effector smooth muscle cells. However extensively studied, there is no clear-cut mechanism explaining how the initiation of NANC activation is induced. Electrical stimulation of the vagal NANC nerves leads to the profund relaxation of the esophagus, stomach fundus and corpus promoting the propulsion of food (108, 109). The rise in intragastric pressure leads to a sudden relaxation of the stomach fundus, which prevents the further

increase in the lumen pressure, arguing for the local inhibitory reflex activated by the extension of the stomach (110). Ganglionic nicotinic transmission leading to the NO-mediated relaxation of the stomach and guinea-pig trachea for the local sensory reflex was postulated (110, 112).

Interestingly enough, all the known activators of NO release from endothelium such as Substance P, VIP, CGRP, 5-HT and neuropeptide-Y containing neurons have been immunolocalized in the myenteric plexus (114), that suggests the participation of these mediators in the local reflexes with the final activation of NO release from neuronal or from the effector tissue. The resistance of pressure induced gastric relaxation (110), or the nicotinic receptor activated relaxation of guinea-pig trachea (112) to hexametonium can also be explained by an axon reflex (115), mediated by NO released from sensory nerves. In rabbits, a nociceptive effect of acetylcholine has been shown to be mediated through nicotinic receptors present on perivascular sensory nerve endings (116).

In men, iontophoresed ACh can stimulate peripheral nociceptive C-fibres to produce flare (neurogenic vasodilatation) which is abolished by an anaesthetic, and is absent in denervated skin (117, 118). Neurogenic inflammation involves vasodilatation, plasma protein extravasation and oedema. It can be also elicited by antidromic stimulation of the unmyelinated C-fibres of sensory nerves by topical application of chemical irritants, such as capsaicin, mustard oil, xylene (119, 120). Pretreatment of rats with high doses of capsaicin, which selectively destroys C-fibres (121), completely prevents the ability to elicit neurogenic inflammation by electrical stimulation (122). The capsaicin-sensitive somatic neurones include the polymodal nociceptors, which are sensitive to a variety of noxious chemical, thermal and mechanical stimuli, mediating autonomic reflexes (123, 124). Lembeck was the first who suggested that Substance P is the primary afferent neurotransmitter of inflammation (120). Subsequent studies demonstrated the accuracy of this proposal. Substance P along with neurokinin A, and CGRP (125 – 127) was found in C-fibres sensory nerves, indicating that it may play a role in neurogenic inflammation. Both symptoms: vasodilatation and oedema, of neurogenic inflammation, are attenuated by pretreatment with L-NA and L-NMMA, but not by their D-stereoisomers (127, 128). These results may add to the understanding of the hypothesis concerning the participation of NO in the control of basal tonus of vasculature (25, 31, 35, 36). It well may be that NO controlling the tonus is released by activation of the local sensory reflex arches. The afferent sensory neurones containing the above tachykinins as well as bradykinin, 5-HT, histamine, all known mediators of NO release from endothelium, were found to control vascular tonus and permeability in numerous tissues other than the skin, such as coronary vasculature (129), hepatic (130), airway and nasal mucosa (131, 132), meninges (133), eyes (134), joints (135 – 137), urinary bladder (135, 136), gastrointestinal tract (137, 138). It well can be that functionary

hyperaemia (139), as well as reactive hyperaemia (140) observed in working tissues or in tissues during reperfusion following short, seconds-lasting ischaemia, could be mediated by NO released from sensory nerves or from endothelium activated by tachykines, released from these nerves on the way of the local reflex. The local biochemical (pH, pO_2) and physical (pressure, stretch) properties, are good candidates for the initiation of such a reflex.

It is interesting that the neurogenic inflammation can be inhibited at the presynaptic level with opioid receptor agonists (141). Ferreira and co-workers (142–144) demonstrated that the local analgesic effect of substances such as ACh, or morphine is mediated *via* stimulation of the L-Arg/NO/c-GMP pathway. They also showed, that NO-donors, such as NaNP, or Sin-1 antagonized carrageenin and PGE_2 hyperalgesia in rats, and applied locally, caused analgesia and the reduction of the arm volume in patients with thrombophlebitis (144). The potentiation of the antinociceptive effect of β-endorphin in mice by L-Arg was also reported (145).

On the other hand, the pretreatment of animals with the NOS inhibitor: L-NMMA, reduced the carageenin-induced oedema in rat paw (146), and the L-NA administered intrathecally enhanced morphine nociception in the rat spinal cord (147) (*Fig. 3*). Thus, the results concerning the role of NO in

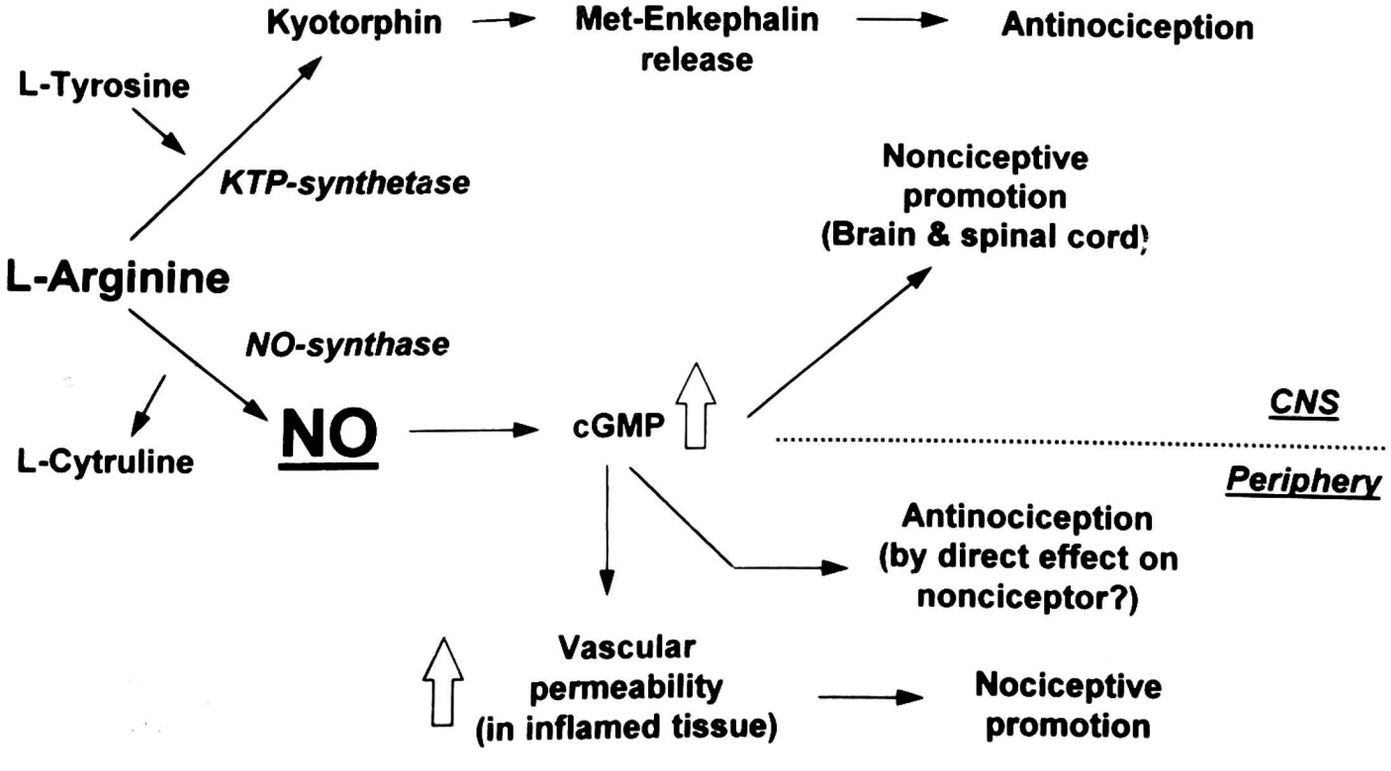

Fig. 3. The suggested participation of L-Arg and NO in central and peripheral antinociception. (according to A. Kawabata et al. 1993).

peripherally mediated nociception are not consistent. A histochemical study has suggested that NO may act as a messenger from sensory neurons in dorsal root ganglia to their satellite cells, where c-GMP levels increase in response to NO (148). Thus, it has been suggested that L-Arg may act as a nociceptive

promoter through the enhancement in NO production at spinal and supraspinal levels (147–149). Thus, it is obvious that NO-pathway may be differently involved in nociceptive processes at the level of the pheripheral and central nervous system.

It is also suggested, that L-Arg itself plays a dual role in the nociceptive processing in brain, being nociceptive via the NO/cGMP pathway, and antinociceptive via the kyotorphin-Met-enkephalin pathway (149, 150) (*Fig. 3*). Kyotorphin (L-tyrosyl-L-arginine), an endogenous peptide isolated from bovine brain, produces naloxone-reversible antinociception by enhancing Met-enkephalin release, and is localized in synaptosomes of CNS (151). It is suggested that L-Arg acts as a precursor for the biosynthesis of kyotorphin, that results in antinociception (147, 148). The therapeutical significance of L-Arg is also supported by clinical findings, presenting that intravenous infusion of L-Arg, produces potent analgesia in the naloxone-reversible manner in patients with various types of chronic pain (152, 153).

The participation of NO in specificity of the regulation of the cerebrovascular tonus was also reported (154). On the contrary to the coronary, and mesentery vascular beds, the relaxation caused by transmural neural stimulation of basilar, middle cerebral and posterior cerebral arteries of dogs were suppressed by L-NA. This suppresion was reversed in the presence of exogenous L-Arg (154, 155). Thus, NO may play a crucial role in the genesis of neurally induced vasodilatation of cerebral arteries, such as migraine.

Hypoxia increased the c-GMP level in main rabbit cerebral arteries, and increased the cerebral blood flow (156). This effect is prevented in the presence of methylene blue, the known inhibitor of guanylate cyclase. Exogenous L-Arg dilates rat pial arterioles by NO-dependent mechanisms, and increases blood flow during focal cerebral ischaemia (157). Thus, L-Arg or NO-donors were suggested to be useful for the increase of cerebral blood flow during ischaemic stroke in men (157).

Endothelins and the nervous system

In March 1988 Yanagisawa and co-workers (6) described an endothelium derived vasoconstrictor peptide, endothelin, with the regional homologies to a group of neurotoxins, and suggested its action as an endogenous modulator of voltage-dependent ion channels. Subsequent studies resulted in the identification of several endothelin (ET) peptide isoforms and the genes that encode them. The ETs (*Fig. 4*) include three isopeptides ET-1, ET-2 and ET-3. They all contain 21 aminoacids and two disulfide bonds. ET-1 differs from ET-2 by two and from ET-3 by six amino acids, and each peptide is derived from a seperate gene (158). In humans, ET-1 m-RNA codes for a 212-amino acid precursor (prepro-ET-1), which undergoes proteolytic cleavage to

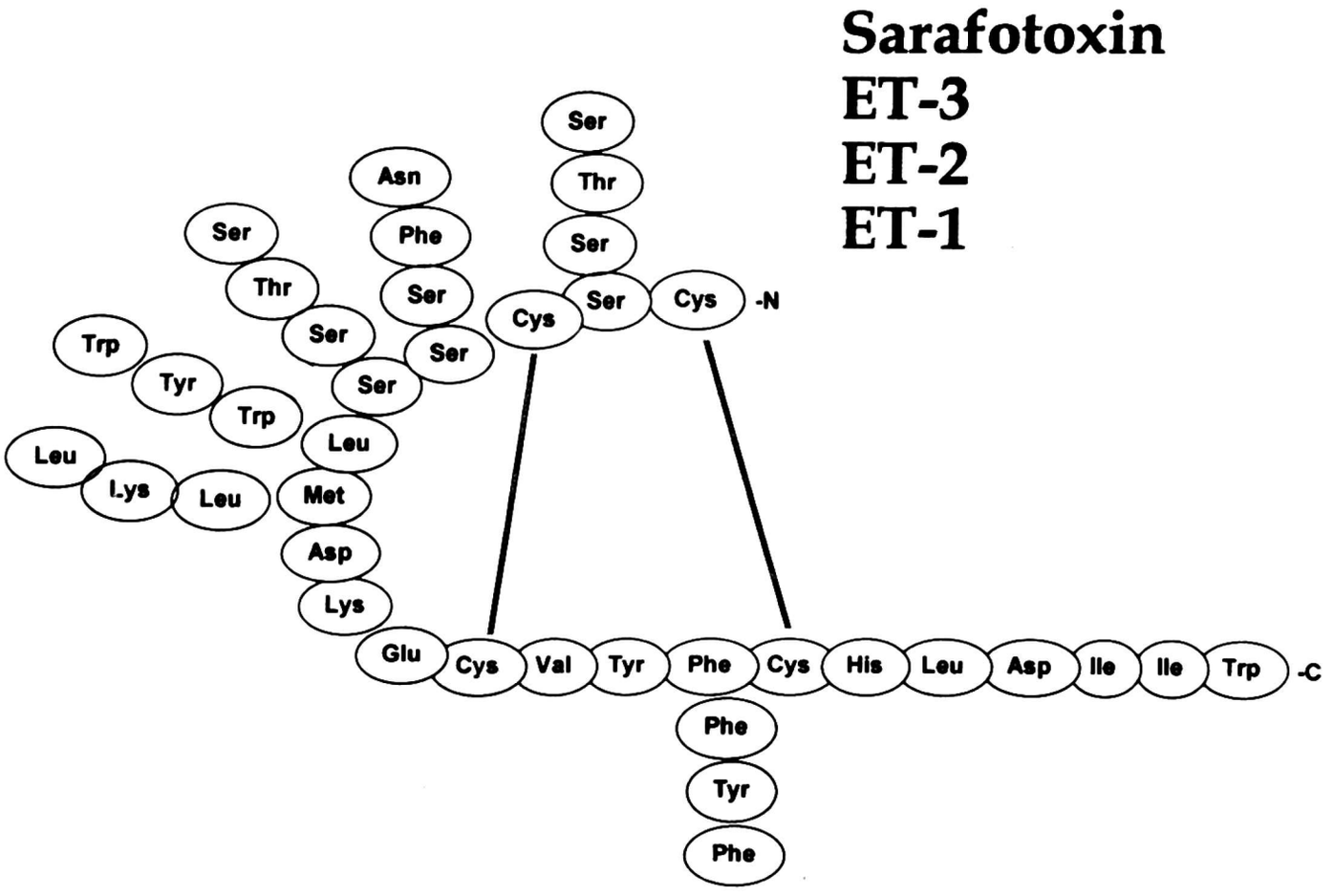

Fig. 4. Amino acid sequences of endothelins and sarafotoxin. The main sequence ET-1 is presented, when the differences in substituing amino acids are consecutively presented for ET-2, ET-3 and sarafotoxin.

a 38-aminoacid residue (pro- or "big" ET-1), and subsequently, through the action of a metalloprotease (ET-converting enzyme) to ET-1 (159). The ETs are structurally and functionally homologous to another mammalian peptides: vasoactive intestinal contractor (160) and to sarafotoxins found in the venom of the snake and burrowing asp, *Atractaspis engaddensis* (161). ET-1 is the only ET produced by human and porcine endothelial cells, while both ET-1 and ET-3 have been identified in neural tissue such as brain, spinal cord and dorsal root ganglia (162, 163). Ischaemia, thrombin, transforming growth factor-β, norepinephrine, phorbol esters and calcium ionophore A23187, were reported to activate release of ETs from endothelium (164, 165). In experimental animals ET-1 has a greater vasoconstrictor potency than any other known vasoactive hormone causing the long-lasting contraction of the vascular smooth muscle (164, 166, 167).

Although three peptides induce the potent vasoconstriction *in vitro*, the transient depressor response followed by a sustained pressor response *in vivo* is observed. ET-1 is the most active vasoconstrictor, while ET-3 is the most potent vasodilator (158, 168, 169). In addition to that, endothelins have been reported to produce a wide spectrum of biological effects, such as: stimulation of proliferation of vascular smooth muscle cells and fibroblasts (170),

contraction of human airway and intestinal smooth muscle (171, 172), positive inotropic and chronotropic effects on the myocardium (173), release of eicosanoids from vascular beds (172), stimulation of atrial natriuretic peptide (174). They stimulate the vasopressin and oxitocine release from hypothalamus (175) and modulate norepinephrine release from sympathetic terminals (176). ET-1 is equipotent with ET-3 in releasing EDRF/NO from vascular endothelium (172, 177, 178), and the anti-platelet and fibrinolytic properties of ET-3 *in vivo*, may be partially related to this effect (179). Radioimmunoassay has demonstrated low plasma levels of ET-1 (ca 1 pg/ml for healthy volunteers). It increased (up to 20 pg/ml) in acute stages of renal failure (180), endotoxic shock (181), myocardial infarction, pulmonary hypertension (182, 183) and uraemia (184). During acute asthmatic episodes, ET-1 levels rise in bronchial exudates (185).

ETs affect target cells by binding to receptors on the cell surface. Genes encoding multiple ET receptors have been cloned and expressed, revealing both ET-1 preferring (ET_A) and isopeptide-nonselective (ET_B) receptor subtypes, and the corresponding mRNAs have been detected also in mammalian brain (186–188). Activation of receptors by ETs in smooth muscle as well as in cultured astrocytes neublastoma and C6 glioma cells, stimulates Ca^{++} influx and intracellular Ca^{++} mobilisation, activates phospholipases A_2 and C, protein kinase C, activates Na^+-H^+ exchange, induces transcription of the *c-fos* protooncogene, and inhibits Na^+-K^+-ATP-ase (189–192). In contrast, ET-1 does not stimulate Ca^{++} influx or alter $[Ca^{++}]_i$ levels in rat brain synaptosomes (193), indicating, that neuronal ET receptors involved in Ca^{++} signalling may be localized preferentially with postsynaptic elements. It is suggested that in neuroblastoma x glioma NG108-15 cells responses to ET-1 involve receptor-mediated Ca^{++} influx and mobilisation of Ca^{++} from inositol phosphate-sensitive intracellular stores, while plateau responses result from Ca^{++} influx through dihydropyridine-sensitive voltage-gated channels (175, 194).

ETs were found to modify some of neuronal functions. ET-1 applied locally into area postrema of rats caused an increase, followed by a decrease in mean arterial blood pressure (195). ET-1 inhibits the release of norepinephrine from sympathetic neurons (196), and stimulates the release of acetylcholine from parasympathetic neurons (197), vasopressin from hypothalamus (198) and aspartate from cerebellar granule cells (199).

ETs seem to play an important role in the pathology of CNS connected with the functional effects on cerebrovascular and neural tissues. ETs induce longlasting constrictions of mammalian cerebral arteries and arterioles (200–202), including human vertebral, basilar, and middle cerebral arteries (203) as it was shown *in vitro*, and pial arterioles and basilar artery *in vivo* (204). It was demonstrated that ETs being large molecules, did not cross the

blood/brain barrier, and thus, they may act from the adventital-side of cerebral blood vessels (204, 204). This suggestion is confirmed by observations, that intercisternal, but not inraarterial application of ET-1 decreases the blood flow in canine basilar arteries (204), and argues for the action of the locally biosynthesized ETs in the CNS pathology.

Ischaemia was primarily found to activate ETs production by porcine aortic endothelial cells (6), coronary artery endothelium in culture (206), isolated mesenteric arteries (207), and the increase of the plasma ETs-levels were reported during myocardial infarction (208) and hypertension (209). Antibodies to ET-1 have been reported to attenuate the experimental myocardial (210), and renal (211) ischaemia. On the other hand, carbon dioxide induced hypoxia did not activate ETs release from cerebral vessel endothelial cells in culture (212), and unlike the patients with subarachnoid haemorrhage, the ETs-like radioimmunoactive material was not detected in cerebrospinal fluid (CSF) (213, 214). Even the clinical significance of the reported elevations of plasma (215, 216), and CSF (215, 216) ETs-like material following subarachnoid haemorrhage is unclear, since no correlation between the ETs level in CSF and the incidence of vasospasm was found (216). Vasospasm and ischaemic deficits in hemorrhage are commonly related to the subarachnoid blood (217). ETs might promote ischaemic neuronal injury by the high input of Ca^{++} ions, and indirectly, by stimulating the release of excitotoxic amino acids (199), while vasospasm may also be related to the presence of hemoglobin, which scavenges, and thus prevents the vasodilatory action of EDRF/NO (25). The isolated cerebral arteries of rats with subarachnoid haemorrhage were found to be more sensitive to vasoconstrictory properties of ETs (218). Alternatively ETs were reported to activate NO release and cGMP accumulation not only in the different vascular beds (172, 177, 178), but also in neuroblastoma x rat glioma hybrid cells (219). Thus, the observed vascular and the delayed ischemic deficit results from the complicated paralelly running processes.

It seems that ETs are mostly involved in pathological events in our body. They may mediate fatal contractions of our microvasculature and hypertension. They may promote the tumor growth and atherosclerosis development by mitogenic activity. They promote the cell death by overloading with Ca^{++} ions. And the activation of vasodilatory EDRF/NO or PGI_2 or PGE_2 (220) may not counteract its pathogenicity.

Arachidonic acid metabolites

Eicosanoids are biological mediators derived from arachidonic acid (AA), an essential 20-carbon fatty acid of membrane phospholipids (221 – 223). AA may be metabolized by either cyclooxygenases (COX) forming prostacyclin (PGI_2), prostaglandins (PGs) or thromboxane (TXA_2), or by lipoxygenases

(LOX), leading to formation of leukotrienes (LTs) and dihydroxy fatty acids (HETEs). Lipoxygenases with specificity for the 5-, 12-, or 15- position have been characterized (222), and lipoxins (LXs) are compounds resulting from AA metabolism by two different LOX (221). LXs are mostly involved in the immune/inflammatory processes in our body (221).

The levels of prostanoids in brain tissue and cerebrospinal fluid are rather low (222, 223), however, the injury to a neural tissue such as trauma, ischaemia, hypoxia, reperfusion or subarachnoid haemorrhage causes a considerable increase in amounts of free arachidonic acid and its metabolites in brain (224, 225) and CSF (225, 226).

There are marked species differences in the ability of AA conversion to the different eicosanoids. In the brain of gebril and rabbits the main PGs are as follows (in the decreasing order): PGD_2, $PGF_{2\alpha}$, $PGE_2 > 6\text{-keto}PGF_{1\alpha}$, TXB_2 (227, 228), while in canine brain the low concentrations of PGD_2, with the relatively high 6-keto $F_{1\alpha}$ were found. In human brain $PGF_{2\alpha}$ is the predominating PGs followed by PGE_2 and low concentrations of 6-keto $PGF_{1\alpha}$ (229). A relatively high level of 6-keto $PGF_{1\alpha}$ in the CSF argues for the biosynthesis of PGI_2 by the choroid plexus and pial vessels (224). The ability of a rabbit, gebril and human brain to generate LTs has been recently demonstrated (230, 231, 232). In contrary to other prostanoids, sulfidopeptide-LTs (LTC_4, LTE_4, LTD_4) level is relatively low. However, when synthesized, they participate in the development of the brain injuries, such as vasoconstriction, oedema, and seizure activity (232, 233, 234).

The postischaemic production of prostanoids was extensively studied in gebril brain, because of the specificity of brain blood redistribution (the carotid arteries are the only way of supply). PGD_2 predominated in cortex and hypothalamus, PGE_2 and $6\text{-keto}PGF_{1\alpha}$ in hippocampus. The pathophysiological meaning of this event is not clear. The increase of $PGF_{2\alpha}$ was observed in the development of cytotoxicity whereas the late accumulation of PGE_2 in cerebral tissue was coupled with vasogenic brain oedema (235, 236). In patients with stroke (237, 238), or with aneurysmal subarachnoid haemorrhage (239, 240), an imbalance between vasoconstrictory PGD_2, $PGF_{2\alpha}$, and TXA_2, and vasodilatatory PGI_2 levels in cerebrospinal fluid is suggested. It may be responsible for the development of vasoconstriction (241), since only PGI_2 from among the other prostanoids dilates the vessels of CNS (242).

Cerebral ischaemia is associated with the generation of oxygen free radicals, which accelerate during the reperfusion phase. Superoxide anion, hydroxyl radical, and singlet oxygen destroy endothelium and its products such as PGI_2 and EDRF/NO. Since both autacoids act synergistically in inhibiting platelet activity, the local aggregation of plateles, accompanied by the decreased plasma fibrinolytic activity, promotes local thromboembolic complications

(243). The metabolic and functional protection of the ischaemic brain of the spontaneously hypersensitive rats was observed during pretreatment with the PGI_2-analogue or TXA_2 synthase inhibitor OKY-046 or trapidil (244). So far, a selective pharmacological inhibition of TXA_2 synthase has been shown to be beneficial in only a few experimental models of cerebral ischaemia. The insufficient amounts of endogenous PGI_2 biosynthesized during the brain insult may be more important in brain injury pathology. In dogs with subarachnoid haemorrhage, ibuprofen (a COX inhibitor), prevented cerebral vasospasm (245), whilst indomethacin decreased basal cerebral blood flow, probably due to the inhibition not only of TXA_2, but also PGI_2 formation (246). In the treatment of experimental brain ischaemia in dogs, the best therapeutical results were obtained when PGI_2 was simultaneously administered with indomethacin and additionally with heparin (247–249). The beneficial effects of PGI_2 in brain injury are supposed to be related to vasodilatatory, antiplatelet/fibrinolytic and "cytoprotective" properties of this eicosanoid. On the basis of experimental work, it is stressed, that the beneficial effect of PGI_2 administration is observed only when it is administered before, or during the initial period of brain injury (250, 251). The intensive research in these fields is in progress and the other combinations (for example, with calcium channel blockers) have recently been proposed (252).

As the brain cannot repair itself by increasing the number of neurons, the early preventive therapeutical intervention is necessary in case of any signs of cerebellar insufficient blood supply. The surgery with carotid endarterectomy is the common intervention with recommended antiplatelet and fibrinolytic therapy (253–255).

Aspirin alone, or combined with dipyridamole, sulphinpyrazone, warfarin, heparin, streptokinase or ticlopidine is commonly used in secondary stroke prevention (256, 257). The latest meta-analysis of seven randomized, controlled trials, in which the effectiveness of aspirin in the treatment of 6409 patients with TIA and minor strokes was examined, demonstrated the significant risk reduction for total death, total strokes and cardiovascular death in patients receiving this compound (257). These trials have proved that aspirin is preventive in patients with TIA and minor strokes.

The preliminary trials of the treatment of ischaemic stroke with PGI_2 pointed at a significant alleviation of neurological deficit which occurred at 6 and 54 hour after the treatment with PGI_2, however, this improvement in two weeks after the treatment was no more statistically different from the group of patients receiving the conventional therapy (258). The placebo-controlled, randomized trials (259, 260) demonstrated that between the period of 2 weeks and 18 months there is no evidence for a therapeutic benefit from prostacyclin given even in the maximally tolerated dosage, either intermittently or continuously for 30–64 hours to patients with an acute,

completed stroke. Thus, prostacyclin alone seems to be too weak to prevent all signs of ischaemic insult in completed stroke, but it does not exclude its usefulness (also in combined therapy with TXA_2, or LOX inhibitors) in patients with TIA.

CONCLUSIONS

Discovery of the neuronal source and the functions of the labile substances, found previously to originate from endothelial cells, opened the large possibility for the research, and new ways to understanding the physiology and pathology of our nervous system. These substances affect neurons, glia, endothelial cells, vascular smooth muscle and platelets by elevating cyclic nucleotide levels, modifying $[Ca^{++}]_i$ levels, and the release of neurotransmitters. Their disturbed biosynthesis contributes to the development of atherosclerosis, as well as to ischaemic neuronal injury associated with stroke and subarachnoid haemorrhage. For most of these substances (maybe, excluding PGI_2, and TXA_2), in spite of intensive study, it is too early for the final statement concerning the therapeutical implications of drugs modifying their biosynthesis.

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