Mechanisms Involved in the Cerebrovascular Dilator Effects of N-methyl-D-aspartate in Cerebral Cortex

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Abstract

Glutamate and its synthetic analogues N-methyl-D-aspartate (NMDA), kainate, and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) are potent dilator agents in the cerebral circulation. The close linkage between neural activity-based release and actions of glutamate on neurons and the related decrease in cerebral vascular resistance is a classic example in support of the concept of tight coupling between increased neural activity and cerebral blood flow. However, mechanisms involved in promoting cerebral vasodilator responses to glutamatergic agents are controversial. Here we review the development and current status of this important field of research especially in respect to cerebrovascular responses to NMDA receptor activation.

Keywords

Glutamate; cerebral circulation; cerebral arteries; nitric oxide; neurons; ischemia; NMDA receptors; rats; rabbits; piglets

1. Introduction

Glutamate is one of the most prevalent neurotransmitters in the brain (Bonvento *et al*., 2002; Kang *et al*., 2005; Fellin *et al*., 2006) and it can activate a number of ionotropic receptors on neurons and astroglia (Garthwaite, 1991; Aoki *et al*., 1997; Guerguerian *et al*., 2002). The three types of ionotropic glutamatergic receptors are characterized by the names of their synthetic analogues: N-methyl-D-aspartate (NMDA), kainate, and alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA) (McDonald et *al*., 1990; Garthwaite *et al*., 1991; Lerma *et al.*, 1997). Activation of ionotropic glutamatergic receptors on neurons leads to calcium entry, membrane depolarization, activation of intracellular signaling pathways, and the subsequent production and release of vasoactive agents which can diffuse to vascular smooth muscle and dilate cerebral arteries (Garthwaite, 1991; Domoki et al., 2002). Under some circumstances, additional neurons or astroglia may be involved in transmitting or modifying the initial signal from the target neurons to the resistance blood vessels in brain

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(Filosa *et al*., 2004; Fellin *et al*., 2006; Filosa *et al*., 2006). Recent evidence suggests that activation of the metabotropic glutamate receptors situated on the astrocytes also produce rapid vasodilation of nearby arterioles (Zonta et al., 2003).

The linkage between neural activity-based release and actions of glutamate on parenchymal cells, and the associated decrease in cerebral vascular resistance, provide for the tight coupling between cerebral metabolism and blood flow. However, the chemical nature of the dilator agents and the relative roles of neurons, astroglia, and endothelium in promoting dilator responses have become controversial (Domoki *et al*., 2002; Fiumana *et al*., 2003; Zonta *et al*., 2003; Simandle *et* al., 2005; Ayata & Moskowitz, 2006; Leffler *et al*., 2006 a,b). The purpose of this review is to critically examine the literature concerning the factors mediating glutamatergic-mediated dilation in the circulation of the cerebral cortex.

2. Defining the "Playing Field" for responses to glutamatergic agents

The most comprehensive approach for examining the mechanisms involved in glutamatergic effects on the cerebral circulation is to consider the cerebral blood vessels, astroglia, and neurons as functionally inter-related components of the "neuronal-vascular axis," as originally introduced in 1998 (Veltkamp *et al*., 1998; Domoki *et al*., 1999a), or as the more recently designated "neurovascular unit" (Hawkins and Davis, 2005) (Figure 1). The tight coupling of brain metabolism and blood flow is a well established concept (Roy and Sherrington, 1890) and provides the functional basis for the neurovascular unit (Busija and Leffler, 1987a; Busija, *et al*., 1988). However, the precise inter-relationships among the components of the neurovascular unit, which account for metabolism/blood flow coupling, is only now being defined for various stimuli. In particular, the potential for a prominent role of astroglia (Filosa *et al*., 2004; 2006; Mulligan and MacVicar, 2004; Zonta, *et al*., 2003; Koehler *et al*., 2006; Lalo *et al*., 2006) and interneurons (Cauli et al., 2004; Vaucher *et al*., 2000; Rancillac *et al*., 2006) in conveying signals between neurons and blood vessels has been described recently using several novel approaches. Interneurons have access to parenchymal arterioles but apparently not to arterioles and arteries proximal to the appearance of the Virchow-Robin space (Abadia-Fenoll, 1969; Busija 1993; Lovick *et al*., 1999). These interneurons may be involved in vascular signaling from either other local neurons or from projections from distant neurons. Virtually the entire parenchymal segment of the cerebral vasculature is surrounded by astroglial end feet, and this anatomical arrangement is one component of the blood-brain barrier (Hawkins and Davis, 2005; Abbott et al., 2006). While the surface of pial cerebral arteries and arterioles are not usually thought to be invested with astroglia (Mercier and Hatton, 2002), they are positioned over the *glia limitans* (Niermann et al., 2001) and thus can be influenced by glial-derived factors released into the cerebrospinal fluid (CSF) which is in contact with the blood vessels. A recent report indicates that astroglia, detected with immunostaining against glial fibrillary acidic protein (GFAP), are present in surface arterioles and small arteries from the cerebral circulation of piglets (Leffler et al., 2006a). However, additional studies using other approaches are needed to support this unusual finding. The ubiquitous positioning of the astroglia among cerebral blood vessels and neurons and the well known intercellular interactions involving these cells, appear optimal for rapid and integrated control of vascular tone in order to meet metabolic demands (Figure 1). Cortically-derived astroglial cells are avid producers of prostaglandins and thromboxane (Nam *et* al, 1996; Thore *et al*., 1994; 1996) and cerebral arteries normally located close to cortical astroglia are responsive to even small concentrations of dilator and constrictor prostanoids (Busija and Leffler, 1987b; Leffler and Busija, 1985; Wagerle and Busija, 1990). In particular, the profile of prostanoids produced by cerebral arteries varies considerably from that produced by cortical astrogial of the same species (Busija, 1997; 2002) and thus the CSF bathing the cerebral arteries contains substantial amounts of vasoactive prostanoids arising from the brain parenchyma. Other studies have shown that astroglia and neurons can produce and release a wide range of vasodilator

substances such as nitric oxide (NO) carbon monoxide (CO), adenosine, hydrogen ion, potassium ion, cyclooxygenase, lipoxygenase, and p-450 monooxygenase products (Bhardwaj *et al.*, 2002; Zonta *et al.*, 2003; Filosa *et al.*, 2006; Koehler *et al.*, 2006; Leffler *et al.*, 2006a; Murphy et al., 1994; Ohata *et al.*, 2006). Neuronal- and astroglial-derived factors can act directly on vascular smooth muscle (Domoki *et al.*, 2002; Filosa *et al.*, 2006) or can act indirectly via endothelium-dependent dilator agents (Murphy *et al.*, 1994). In some brain preparations such as cerebellar slices, astrocytes can also release vasoconstrictor agents (Mulligan and MacVicar, 2004; Rancillac *et al.*, 2006). Recent studies in retina indicate that glial cells can produce both constrictor and dilator agents through omega-hydroxylase and cytochrome P450 epoxygenase pathways, repectively (Metea and Newman, 2006; Metea *et al.*, 2007).

3. Studies of glutamatergic agonists in intact brain

3.1. Responses during normal conditions

Busija and Leffler (1989) were the first to demonstrate that glutamate and its synthetic analogue, *N*-methyl-D-aspartate (NMDA) are dilator agents in the intact cerebral circulation (Table 1). Glutamate and NMDA, when applied to the exposed surface of the piglet cerebral cortex, dilated overlying pial arterioles in a dose-dependent fashion and these responses were unaffected by blockade of prostaglandin synthesis. The control mechanisms of the cerebral circulation and stage of cortical neuronal development of the neonatal pig appear to be similar to the human neonate (Busija, 2002). Dilator responses to glutamate and NMDA were repeatable several times in the same preparation following removal of these agents by flushing with artificial cerebrospinal fluid (aCSF) (Busija and Leffler, 1989; Busija *et al.*, 1996; Philip and Armstead, 2003) and even prolonged application to the cortical surface did not appear to damage the cortex *in vivo* or affect pial arteriolar responses to other, non-glutamatergic dilator stimuli (Busija and Leffler, 1989). These findings concerning repeatable dilator responses to glutamate and NMDA, which imply little or no damage to arterioles or cortex are in contrast to studies on cultured cerebral endothelium (Sharp et al., 2005; Parfenova *et al.*, 2006) or neurons (Nagy *et al.*, 2004) where glutamate or NMDA can be toxic. Numerous studies by several laboratories have repeated the essential features of these response to glutamate and NMDA in piglets (Armstead et al., 2001; Philip and Armstead, 2003; Leffler et al., 2006a). In addition, all of the original studies in piglets with glutamate and NMDA have been replicated numerous times in other species such as the rat (Bhardwaj *et al.*, 2000, Iliff e*t al.*, 2003, Pelligrino *et al.*, 1995; 1996; Sun *et al.*, 2005) and rabbit (Faraci and Breese, 1993). Thus, the fundamental finding that glutamate and NMDA elicit cerebrovascular dilation *in vivo* appears to be a universal finding across species. In contrast to these studies, Huang *et al.* (1994) has reported that NMDA and glutamate constrict cortical arteries in the rat. The reason for this unusual finding by Huang *et al.* (1994) is not clear.

Faraci and Breese (1993) first demonstrated that dilation to NMDA was eliminated or reduced by treatment with the general NOS inhibitor, N^{ω}-nitro-L-arginine (L-NAME) in rabbits. These results were later been confirmed in the piglet (Meng *et al.*, 1995; Bari *et al.*, 1996) and in the rat (Pelligrino *et al.*, 1995; 1996). Subsequent studies published in the same year for piglet and rabbit showed that administration of 7-nitroindazole (7-NI), the selective inhibitor of neuronal NOS (nNOS), abolished or reduced dilation to NMDA or glutamate (Faraci and Brian, 1995; Meng *et al.*, 1995). Thus, another similar finding in all species studied is that NOS inhibition reduces or eliminates cerebrovascular dilation to NMDA and glutamate. Support for these pharmacological studies was the finding that NMDA application to the cortex resulted in accumulation of NO degradation products in the CSF surrounding the arteries (Domoki *et al.*, 2002). The presence of an underlying vein under the pial arteriole under study reduced its ability to dilate to NMDA compared to an arteriole directly on the cortical surface (Domoki *et al.*, 2002), thereby further reinforcing the concept that diffusion of a vasoactive, possibly

gaseous substance from the cortical surface reaching the arteriole caused the dilation. Robinson *et al*., (2002) have reported that 1-aminocyclopropanecarboxylic acid (ACPD), which they characterized as a NMDA receptor agonist, dilates piglet pial arterioles. However, depending upon the exact configuration of this compound (*trans-/cis-/*racemic), which was not specified in the paper, it could also act as a glycine receptor agonist and/or a glutamate-site antagonist at NMDA receptors (Nahum-Levy *et al*., 1999; Peterson *et al*., 2004). We are unaware of any other *in vivo* cerebral circulatory studies in any other species using this compound.

Although glutamate has the potential to exert vasoactive influences via activation of any of the three ionotropic glutamate receptors, topical application to the surface of the cerebral cortex appears to predominantly activate the NMDA receptors. Thus, MK-801, a selective NMDA receptor antagonist, blocks most if not all of the cerebral dilator response to glutamate as well as NMDA in rabbits, piglets, and rats (Faraci and Breese, 1993; Meng *et al*., 1995; Pelligrino *et al*., 1995;1996). The reason for this preference for the NMDA receptors in the cortex is not known, but may be due to their location in the cerebral cortex or greater affinity for glutamate than kainate or AMPA receptors. Administration of the sodium channel blocker tetrodotoxin, an agent which does not usually affect the endothelium (Emerson and Segal, 2001), completely eliminates NMDA-induced dilation *in vivo* in piglet (Leffler *et al*., 2006a), rabbit (Faraci and Breese, 1993), and rat (Pelligrino *et al*., 1996) cerebral arterioles and arteries. However, dilator responses of cerebral resistance vessels to glutamate were reported to be intact in piglets despite the presence of tetrodotoxin (Leffler *et al*., 2006a).

Despite several recent reports (Fiumana *et al*., 2003; Parfenova *et al*., 2001; 2003), it is questionable that the primary effects of glutamate and NMDA receptor agonists represent direct, endothelium-dependent effects on arteries and arterioles. Isolated cerebral arteries in a number of species have not been found to dilate in response to application of glutamate or NMDA (Faraci and Breese, 1993; Simandle *et al*., 2005) and endothelial damage *in vivo* failed to affect NMDA-induced dilation while responses to other endothelium-dependent dilator stimuli were blocked (Domoki *et al*., 2002). Furthermore, the absence of functional NMDA and AMPA receptors in rat and human cerebromicrovascular endothelial cells has also been demonstrated (Morley *et al*., 1998).

Consistent with a direct role on parenchyma, glutamate application increased cortical metabolic rate in proportion to calculated changes in cerebral vascular resistance (Armstead *et al*., 1992). NMDA-induced dilation also apparently does not involve activation of perivascular nerves, since blockade of muscarinic and CGRP receptors does not affect the cerebral vascular dilator response to topical NMDA in rabbits (Faraci and Breese, 1994).

Application of kainate and AMPA receptor agonists also results in dilation of cerebral arteries in all species studied when applied to the cerebral cortex, but these agents have not been as extensively studied in this context as have glutamate and NMDA. Kainate leads to prolonged dilator responses in piglets that are partially blocked equally by indomethacin and L-NAME, but not by adenosine or NMDA receptor antagonists (Bari *et al*., 1997). Similarly, kainate-induced dilation of cerebral arterioles is reduced by NOS inhibition in rabbits (Faraci *et al*., 1994). Garthwaite *et al.* (1989) have reported that kainate leads to production of NO by brain. It also appears that antagonists or blockers of heme oxygenase also reduce dilation to kainate (Ohata *et al*., 2006) or to the kainate receptor agonist, (*RS*)-2-amino-3-(3-hydroxy-5-*t*-butylisoxazol-4-yl)propionic acid (ATPA) (Robinson *et al*., 2002). AMPA application leads to cerebrovascular dilation which reportedly is reduced with inhibitors of heme oxygenase (Ohata et al., 2006; Robinson *et al*., 2002) and adenosine receptor blockade (Ohata et al., 2006). Bhardwaj *et al*. (1997) has reported that AMPA application leads to cortical production of NO. Thus, activation of all three types of glutamatergic ionotropic receptors in cerebral cortex leads to activation of nNOS and production of NO.

Activation of glutamatergic receptors on neurons and astroglia in the cerebellum also promotes vascular dilation. The relative contribution of the AMPA and NMDA receptors to vasodilation appears to be different than in the cerebral cortex probably due to the regional distribution of these receptor subtypes. Effects of glutamatergic stimuli on the cerebellar arteries and arterioles are discussed in detail in several relevant papers (Akgoren *et al*., 1997; Rancillac *et al*., 2006; Yang and Iadecola, 1996; Yang *et al*., 1999). For example, both electrical stimulation of parallel fibers in the cerebellum, which causes release of glutamate, and the microinjection of glutamate to this region, increases blood flow in part via activation of AMPA receptors and subsequent production and actions of neuronally-derived NO (Yang and Iadecola, 1996). In contrast, activation of stellate cells or application of NMDA dilates cerebellar arteries via production and actions of NO (Rancillac *et al*., 2006).

3.2 Altered responses during pathological conditions

Maintenance of metabolism/blood flow in the brain may be disrupted by pathological conditions (Table 1). Several groups have found that cerebrovascular dilator responses to glutamatergic agonists can be changed by a variety of experimental conditions in all species studied. For example, cerebrovascular dilation to NMDA is reduced or eliminated in various species following asphyxia (Busija and Wei, 1993), alcohol administration (Mayhan and Didion, 1995), hyperglycemia (Mayhan and Patel, 1995), ischemia/reperfusion (Busija *et al*., 1996), hypoxia/reoxygenation (Bari *et al*., 1996;1998), and traumatic brain injury (Armstead, 2000) via mechanisms largely dependent upon production and actions of reactive oxygen species (ROS) such as superoxide anion (Bari *et al*., 1996;Philip and Armstead, 2003;2004). The primary site of action of the ROS appears to be at the level of the NMDA receptor (Kim *et al*., 1999;Choi and Lipton, 2000;Guerguerian *et al*., 2002) since dilator responses of pial arterioles are still intact to exogenous NO after ischemic stress (Busija *et al*., 1996) (Figure 1). Attenuation of NMDA-induced dilator responses can be prevented by pre-treatment with activators of plasma membrane or mitochondrial K_{ATP} channels (Veltkamp *et al*., 1998;Domoki *et al*., 1999), cyclooxygenase inhibitors (Busija et al., 1996;Domoki et al., 2001), protein synthesis inhibitors (Veltkamp *et al*., 1999), and superoxide dismutase (Bari *et al*., 1996). In contrast to these findings with NMDA, kainate-induced cerebral arteriolar dilation is resistant to ischemic stress (Bari *et al*., 1997). This finding may indicate that kainate receptors are present on ischemia-resistant neurons and astroglia or that kainate receptors are not readily affected by actions of ROS. We are unaware of studies examining effects of ROS-mediated stresses on AMPA-induced dilation.

4. Mechanisms of dilation

The combined results from many investigators in different species have lead to the proposal that NMDA receptor stimulation in cortical neurons results in calcium influx, membrane depolarization, activation of intracellular nNOS, and the subsequent production of NO, which diffuses to cerebral arteries and arterioles and causes relaxation of vascular smooth muscle without the involvement of astroglia or endothelium (Bari *et al*., 1998; Domoki *et al*., 1999; Faraci and Breese, 1993; Faraci and Brian, 1995; Meng *et al*., 1995; Pelligrino *et al*.,1996; Veltkamp *et al*., 1998) (Figure 1).

Immunohistochemistry of the piglet brain revealed a particularly distinct band of NMDA receptors (NMDAR 2A/B) containing neurons in cortical layer II/III interspersed with large nNOS-positive neurons (Bari *et al*., 1998). NMDAR 2A/B-nNOS double-immunopositive neurons usually were not found, or at least were not prominent at the light microscopy level. Unlike cortical neurons in culture, a situation in which almost all the mature neurons contain NMDA receptors and nNOS (Kim *et al*., 1999), NMDA receptors are restricted to more limited populations of neurons in cerebral cortex and may or may not be co-localized with nNOS depending upon anatomical location, developmental stage, or species studied (Aoki *et al.*,

1997; Bari *et al.*, 1998; Gracy and Pickel, 1997). In particular, Aoki *et al.*, (1997) detailed the difficulty and uncertainty of fully evaluating dual NMDA receptor and nNOS localization in neurons in the sensory cortex at the light and electron microscopic levels. Thus, it seems likely that additional neurons, or possibly astroglia, are involved in the transmission of the dilator signaling from the activated neurons possessing NMDA receptors to blood vessels. This view is reinforced by pharmacological results from rats (Bhardwaj et al., 1997) indicating that NMDA-induced brain production of NO involved not only neurons with NMDA-receptors but also those with AMPA-receptors. We are unaware of any additional studies which have examined the relationship between NMDA-receptor and nNOS containing neurons in the cerebral cortex. Based upon well known age- and species- differences in cerebrovascular control mechanisms, it is not surprising that part of the dilator responses to NMDA in rats may be due to production and release of other substances such as adenosine (Iliff et al., 2003) or P-450 monooxygenase metabolites (Bhardwaj *et al.*, 2002) (Figure 1). However, we could find no evidence that adenosine (Bari et al., 1998), prostaglandins (Busija and Leffler, 1989), or P-450 monooxygenase products (Domoki *et al.*, 2002) contribute substantially to NMDA-induced cerebral arterial dilation in piglets. Nonetheless, the common finding is that one or more dilator agents, primarily arising from cortical neurons, mediate the dilator responses to NMDA, which is consistent with the probable involvement of multiple neurons or sequential signaling pathways.

The lack of principal involvement of astroglia as initiators of NMDA-induced cerebral dilation is indicated by the ability of tetrodotoxin to totally block the response to topically applied NMDA (Farci and Breese, 1993; Pelligrino *et al.*, 1995; Leffler *et al.*, 2006a) and by the persistence of the dilator response to NMDA despite destruction of the *glia limitans* (Leffler, CW, personal communication). Tetrodotoxin does not normally impair astroglia-initiated responses or endothelium-dependent dilation (Emerson and Segal, 2001). The ability of astroglia to produce large amounts of vasoactive prostaglandins (Busija 1997; Busija 2003; Nam *et* al, 1996; Thore *et al.*, 1994; 1996) and the lack of effect of indomethacin on NMDA- or glutamate-induced cerebral arteriolar dilation in piglets (Busija and Leffler, 1989), provides additional evidence that astroglia do not play a role in conveying or modifying signaling between NMDA receptor containing neurons and vascular smooth muscle. Several studies in diverse species (rat, cat, piglet, cow, man) fail to show significant dilator effects of NMDA and glutamate on isolated cerebral arteries (Faraci and Breese, 1993; Hardebo *et al.*, 1989; Simandle *et al.*, 2005; Takayasu and Dacey, 1989; Wendling *et al.*, 1996). Additionally, impairment of endothelial function does not affect pial arterial and arteriolar dilation *in vivo* to topical application of NMDA (Domoki *et al.*, 2002). Again, the lack of direct effects of NMDA or glutamate in cerebral resistance vessels appears to be a common finding among the species studied.

The magnitude of the increase in NO production upon NMDA application probably is underestimated by measurements of degradation products in perivascular CSF (Domoki *et al.*, 2002) because a high percentage of the NO generated by neurons is prevented from reaching the pial vessels by reactions with iron containing substances such as hemoglobin and by other cellular components. This assumption is supported by the finding that arteriolar segments being "shielded" from the parenchyma by an underlying large vein show significantly smaller dilatory responses to NMDA (Domoki et al., 2002). Nonetheless, it appears that NO is able to diffuse considerable distances in healthy cortical tissue to exert vascular effects.

It has been suggested that endothelium-derived NO plays a permissive role in carbon monoxide (CO)-mediated dilator responses to glutamate and NMDA receptor agonists in the piglet cerebral circulation (Barkoudah *et al.*, 2004; Leffler *et al.*, 2006b). Therefore, L-NAME may not only block NO-dependent responses but those involving CO as well. In our studies using 7-NI, which blocked both glutamate and NMDA-induced dilator responses in arterioles (Meng

et al., 1995), endothelial NOS would still be functionally intact and capable of generating substantial amounts of vascular NO from resistance and capacitance vessels as well as capillaries and thus provide the necessary background levels of NO for CO production. If NO is necessary to allow the expression of CO-mediated dilation to glutamatergic receptor agonists, then is unclear why 7-NI abolishes cerebral vascular dilation to glutamate and NMDA. In contrast to the results in piglets, CO has been shown to be a tonic suppressor of NO-dependent dilation in the rat cerebral circulation via inhibition of NOS activity (Ishikawa *et al*., 2005). Ishikawa *et al*. (2005) also found a similar mechanism operating in cultured porcine endothelial cells.

5. Controversies

Two recent publications have challenged the concept that glutamate- or NMDA-mediated dilator responses involve sequential stimulation of neuronal NMDA receptors, activation of nNOS, and direct relaxation of vascular smooth muscle by NO. First, Ayata and Moskowitz (2006) have reported that NMDA application elicits cortical spreading depression (CSD) which complicates the elucidation of mechanism involved in NMDA-induced cerebral vasodilation. Second, Fiumana *et al*., (2003) have reported that glutamate and a NMDA receptor agonist are able to dilate isolated arteries via production and actions of CO.

5.1. Contribution of cortical spreading depression to NMDA-mediated responses

Ayata and Moskowitz (2006) have suggested an alternative mechanism concerning NMDA-induced cerebrovascular responses in mice. Based upon electrophysiological recordings, they propose that the cerebrovascular responses to topical NMDA represents a complex response due to overlapping vasoactive influences associated with CSD-related effects and direct effects of NMDA on individual neurons (Ayata and Moskowitz, 2006). Such a mechanism is plausible since it was shown earlier by other authors (Lauritzen and Hansen, 1992; Lauritzen, 1994) that propagation of CSD is promoted by neuronal release of glutamate and subsequent activation of NMDA receptors on neighboring neurons. Whether a similar complex interaction between CSD and direct effects on neurons by NMDA also occurs in other species with lissencephalic brains such as rats and rabbits in unclear. L-NAME treatment in rats fails to affect cerebrovascular responses to CSD (Fabricius *et* al., 1995; Shimizu *et al*., 2002) while dilator responses to topical NMDA are reduced or eliminated following acute NOS blockade in this species (Pelligrino *et al*., 1996). Similarly, in rabbits, nNOS-derived NO and calcitonin gene-related peptide released from perivascular nerves contribute equally to CSD-induced dilation of pial arterioles (Colonna *et al*., 1993; 1994; 1997) while topical application of 100 μM NMDA is completely abolished by inhibition of NOS (Faraci and Breese, 1993).

CSD probably does not occur in piglet brain or in other gyrencephalic brains from other species in response to glutamate or NMDA for several reasons. Cortical spreading depression is not easily invoked in piglet cortex or in the neonatal brain in general and the pattern of dilation to NMDA is not consistent with that seen with CSD (Busija and Leffler, 1989; Faraci and Breese, 1993; Shibata *et al*., 1990). Additionally, the application of NMDA to the large area of the cortex present under the cranial window as used in piglets (and rabbits), probably affected all of the exposed neurons simultaneously and therefore would not be expected to initiate a CSD-like episode that would progress across the cortical surface under observation. While it is possible that widespread cortical depolarization, rather than selective depolarization of NMDA-receptor containing neurons, occurs under the cranial window with glutamate or NMDA application, at least in the piglet, the arteriolar effects of cortical depolarization (Domoki *et al*., 1999b) are completely different than observed with application of glutamatergic agonists in this species (Busija and Leffler, 1989; Meng et al., 1995). Additionally, while blockade of K_{ATP} channels with glibenclamide enhances dilator effects to CSD in rats (Shimizu *et al*., 2000), glibenclamide attenuates cerebral vascular responses to

NMDA (Philip and Armstead, 2004). Thus, it appears that in mice, but probably not in other species under normal conditions, NMDA decreases cerebrovascular resistance via mechanisms that are not related to initiation or propagation of CSD, but rather involve the selective, direct activation of NMDA-containing neurons.

5.2. Direct effects of glutamate and NMDA receptor agonists on cerebral arteries

Fiumana et al., (2003) reported that both glutamate and 1-aminocyclopentane-*cis*-1,3-dicarboxylic acid (*cis*-ACPD), the latter a putative NMDA receptor agonist, dilated isolated arteries up to 15–20% via generation and actions of CO. Thus, cell types specific to the vasculature rather than neurons were the apparent targets of glutamate and *cis*-ACPD and the dilator agents were produced solely within the vascular wall. This study contrasts with all other studies done in isolated cerebral arteries from a variety of species. Additionally, these authors indicated an essential role of the endothelium since glutamate-induced dilation of isolated piglet arteries was reversed to constriction following endothelial denudation. Whether *cis*-ACPD continued to dilate these arteries following endothelium denudation was not reported. Unfortunately, our laboratory has been unable to replicate the results for glutamate or NMDA on isolated piglet arteries and arterioles, not only at physiological doses, but at pharmacological doses as well (Simandle *et al.*, 2005). Furthermore, we have shown that NMDA-induced dilation *in vivo* was still intact following impairment of endothelial function (Domoki *et al.*, 2002). Other authors similarly have shown that NMDA does not dilate isolated cerebral arteries from rabbit (Faraci and Breese, 1993), rat (Hardebo *et al.*, 1989; Takayasu and Dacey, 1989), cat (Hardebo *et al.*, 1989), cow (Wendling *et al.*, 1996) and man (Hardebo *et al.*, 1989) (Table 1). In addition, cerebral vascular dilator responses to glutamate, when present, may not be specific for the R- and L- isomers of glutamate (Wendling *et al.*, 1996), thereby indicating a lack of specific action of this amino acid against NMDA or other glutamatergic receptors. The reasons for these differences could involve use of different NMDA-receptor agonists or other aspects of the experimental approach. While *cis*-ACPD has been reported to be a NMDA receptor agonist, this agent apparently has not been used by other groups and its potency and specificity for NMDA receptors has not been examined in the cerebral circulation.

It is also possible that some aspects of our experimental protocol prevented the detection of significant NMDA or glutamate dilator responses in isolated arteries of piglets. For example, Leffler *et al*. (2006a) have reported the presence of positive GFAP-immunostaining of piglet pial arterioles and arteries (diameters up to ~200 μM), and suggested that the presence of functionally intact astroglia associated with the vessel wall was necessary for the dilator responses to glutamate in pressurized, isolated cerebral vessels. Thus, based upon this publication (Leffler *et al.*, 2006a) and an earlier one (Fiumana et al., 2003) in which it was reported that endothelium damage prevented dilation to glutamate, functionally intact cerebral vascular astroglia and endothelium apparently are both required for glutamate-induced dilation of isolated arteries. This finding concerning the presence of astroglia in surface cerebral vessels is in contrast to previous reports which limit projections of the astroglia to the *glia limitans*, which apparently does not penetrate the pia and arachnoid layers of the meninges where the pial blood vessels are located, and to the exterior of parenchymal blood vessels distal to the Virchow-Robins space (Mercier and Hatton, 2002; Niermann et al., 2001). The surface resistance vessels of the cerebral cortex are tightly bound by the pia to the underlying glia limitans and it is possible that positive GFAP immunostaining is due to extravascular contamination by attached cortical astroglia during removal of the arteries and arterioles. However, it seems unlikely that astroglia pulled off the cortical surface along with the arteries and arterioles could be functionally intact following procedures and conditions involved in the preparation and study of pressurized cerebral arteries *in vitro*. Nonetheless, this interesting finding concerning the presence of functionally intact astroglia in surface cerebral arteries and arterioles should be confirmed by another laboratory.

A possible role of CO in conveying or modifying the initial NO signal from NMDA receptor containing neurons in the cortex to vascular smooth muscle cannot be excluded especially when glutamate is administered. Histological examination of the cortical areas underlying pial arterioles indicate that NMDA receptors and brain NOS are located on different neurons (Bari *et al*., 1998) and thus a multi-cellular sequence of events involving adjacent neurons and perhaps astroglia is likely required for the generation of dilator agents responsible for the vascular response. Glutamate in particular can have effects on multiple cell types in brain either via actions on metabotropic and inotropic receptors, or as a consequence of uptake and metabolism by astroglia (Schousboe and Waagepetersen, 2005). Further evidence for a multi-cellular mechanism also is suggested by studies by Phililp and Armstead (2004b), where blockade of ATP-sensitve potassium (K_{ATP}) channels or calcium-activated potassium (K_{Ca}) channels both reduce dilation to NMDA application, although the contribution of K_{ATP} channels is much greater. There are other examples of multiple cell types mediating cerebrovascular dilation to physiological stimuli. For example, we have shown that in CSD-induced cerebrovascular dilation in rabbits, cortically derived NO, perivascular nerve derived calcitonin gene-related peptide, and prostaglandins from parenchyma and/or blood vessels all contribute to the final blood flow response (Colonna *et al*., 1993; 1994; 1997).

Interpretation of data from recent studies in piglet by Leffler and colleagues (Leffler *et al*., 2005; 2006a) leads to the suggestion that nitric oxide availability and related intracellular actions represent mandatory components linking activation of glutamatergic receptors to CO-mediated cerebral vascular dilation. In support of this theory are findings that indicate that the NO donor, sodium nitroprusside, increases CO levels in piglet cerebral microvessels to the same extent as glutamate (Leffler et al., 2005) and that CO apparently dilates piglet pial arterioles via activation of smooth muscle K_{Ca} channels (Barkoudah *et al*., 2004). On the other hand, Armstead (1997) has reported that the K_{Ca} channel inhibitor, iberiotoxin, which should block CO-mediated smooth muscle responses, does not affect pial arteriolar dilation following sodium nitroprusside administration in piglets. Production of CO also appears to occur following activation of AMPA receptors in rat and piglet cerebral cortex (Ohata *et al*., 2006; Robinson *et al*., 2002). On the other hand, there are contrasting findings in which it was shown that CO inhibits NOS activity in rat cerebral arteries and cultured porcine endothelial cells (Ishikawa *et al*., 2005). Additionally, a role of CO in control of the adult cerebral circulation, especially in the rat, has been discounted by recent experiments (Andressen *et al*., 2006). Thus, this interesting area needs additional research in order to more precisely define the possible role of CO in glutamatergic receptor-induced vasodilation in the cerebral circulation.

6. Conclusions

Activation of all three types of ionotropic receptors on neurons by glutamatergic agonists leads to dilation of cerebral resistance vessels and provides the foundation for tight coupling between metabolic demand and blood flow in brain. Glutamate appears to preferentially activate NMDA receptors in cortex, and the preponderance of evidence indicates that NMDA receptor activation leads to depolarization of cortical neurons and enhanced production of NO from those or adjacent neurons. The NO produced by neurons then diffuses relatively long distances to cerebral arterioles and arteries and relaxes vascular smooth muscle apparently without the contribution of vasoactive substances from endothelium, perivascular nerves, or astroglia. While NO appears to be the predominant, perhaps initiating component of the cascade of events leading to cerebrovascular dilator responses following NMDA-receptor activation of cortical neurons, it appears likely that additional vasoactive agents such as adenosine and cytochrome p-450 monooxygenase products also make important contributions, especially in rats. However, the potential contribution of astroglial- or endothelial-derived CO especially in the neonate in response to glutamate deserves further consideration.

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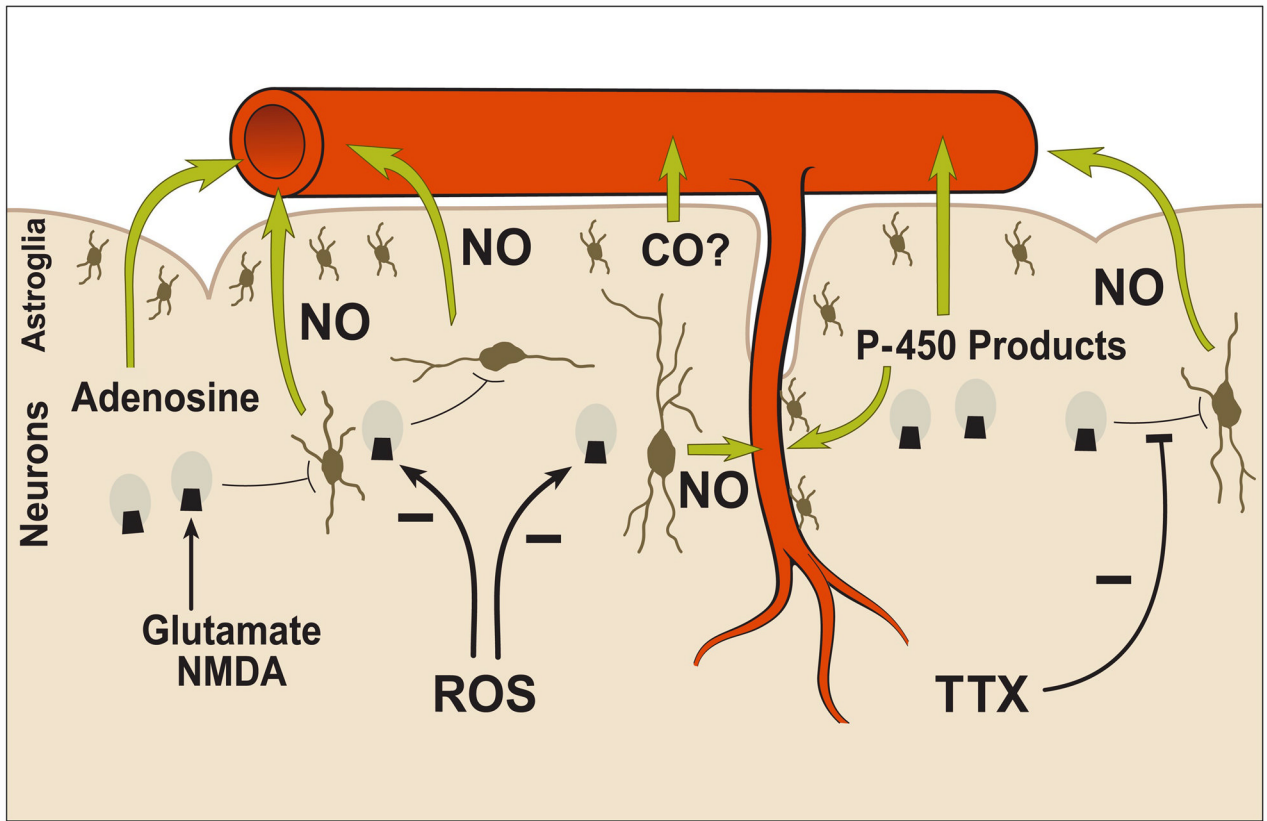
Figure 1.
Schematic illustration of likely mechanisms linking activation of neuronal NMDA receptors in the cerebral cortex to dilation of cerebral arteries and arterioles. NMDA or glutamate activates NMDA receptors on neurons, and following intercellular signaling to nNOS containing neurons, nitric oxide (NO) NO is produced which diffuses to arteries and arterioles and causes vasodilation. This intercellular transmission is blocked by tetrodotoxin (TTX), and the NMDA rceptors can be impaired by actions of reactive oxygen species (ROS). It seems likely that other dilator agents in addition to NO such as adenosine and P-450 monooxygenase products also are involved especially in some species. The role of carbon monoxide (CO) derived from astroglia remains controversial.

Table 1

Glutamate agonists and cerebral vascular tone

Year	Authors	Preparation	Species	Agent	Application	Effect	Mechanism
1989	Busija & Leffler	Cranial Window	Piglet	Glutamate	Topical	Dilation	Non-prostanoid
				NMDA	Topical	Dilation	Non-prostanoid
1989	Hardebo et al.	Isolated arteries	Rat, cow, man	Glutamate	Topical	No response	NA
1992	Armstead et al.	Cranial Window	Piglet	Glutamate	Topical	Increased metabolism	Not examined
1993	Faraci & Breese	Cranial Window	Rabbit	NMDA	Topical	Dilation	NO mediated
		Isolated arteries	Rabbit	NMDA	Topical	No response	Tetrodotoxin-sensitive
1993	Busija & Wei	Cranial Window	Piglet	NMDA	Topical	Dilation	NA
1994	Huang et al.	Cranial Window	Rat	Glutamate	Topical	Constriction	Anoxia-sensitive
				NMDA	Topical	Constriction	Non-prostanoid
1994	Nakai & Maeda	Microspheres	Rat	NMDA	Injection	Increased CBF	Not examined
1994	Faraci et al.	Cranial Window	Rabbit	Kainate	Topical	Dilation	NO-mediated
1994	Faraci & Breese	Cranial Window	Rabbit	NMDA	Topical	Dilation	Non-trigeminal Non-cholinergic
1994	Faraci & Heistad	Cranial Window	Rat	NMDA	Topical	Dilation	Unaffected by aging
1995	Mayhan & Didion	Cranial Window	Rat	NMDA	Topical	Dilation	Reduced by alcohol
1995	Meng et al.	Cranial Window	Piglet	Glutamate	Topical	Dilation	NMDA receptor mediated
1995	Faraci & Brian	Cranial Window	Rabbits	NMDA	Topical	Dilation	Neuronal NO mediated
1995	Northington et al.	Microspheres/ H Clearance	Sheep	NMDA	Microdialysis	Dilation	Neuronal NO mediated
1995	Kaiser & During	Laser Doppler	Rat	NMDA	Microdialysis	Increased CBF	NO mediated
1995	Pelligrino et al.	Cranial Window	Rat	NMDA	Topical	Dilation	NO-/adenosine mediated
1995	Faraci & Brian	Cranial Window	Rabbit	NMDA	Topical	Dilation	Tetrodotoxin-sensitive
1995	Mayhan & Patel	Cranial Window	Rat	NMDA	Topical	Dilation	Neuronal NO mediated
				NMDA	Topical1	Dilation	Reduced by hyperglycemia
1996	Bari et al.	Cranial Window	Piglet	NMDA	Injection	Increased CBF	Inhibited by superoxide anion
1996	Nakai & Maeda	Laser Doppler/ Microspheres	rats	NMDA	Injection	Increased CBF	Activated central pathway
1996	Pelligrino et al.	Laser Doppler	Rat	NMDA	Intravenous	Increased CBF	NO-mediated
1996	Wendling et al.	Arterial rings	Bovine	Gltuamate	Topical	No response	NA
				NMDA	Topical	No response	NA
1996	Weiss et al.	14C-iodoantipyrine	Rat	NMDA	Topical	Increased CBF	Increased O2 consumption
1996	Hara et al.	Laser Doppler	Rat	NMDA	ICV	Increased CBF	Increased brain PO2
1997	Fergus & Lee	Hippocampus slice	Rat	Glutamate	Topical	Dilation	NA
				AMPA	Topical	No response	Neuronal NO mediated
				Kainate	Topical	Dilation	Tetrodotoxin-sensitive
							NA
							NA
1997	Lu et al.	14C-iodoantipyrine	Rat	Glutamate	Topical	Increased CBF	Increased O2 consumption
1997	Bari et al.	Cranial Window	Piglet	Kainate	Topical	Dilation	NO and prostaglandin mediated
1998	Yang & Chang	Laser Doppler	Rat	NMDA	Topical	Increased CBF	Neuronal NO mediated
1998	Bari et al.	Cranial Window	Piglet	NMDA	Topical	Dilation	nNOS and NMDA receptors located on different neurons
1998	Taylor et al.	Doppler Ultrasound	Piglet	NMDA	Injection	Increased CBF	Not examined
1999	Lovick et al.	Hippocampus slice	Rat	NMDA	Topical	Dilation	Neuronal NO mediated
							Blocked by Hb
							NOS neurons close to arterioles
1999	Domoki et al.	Cranial Window	Piglet	NMDA	Topical	Dilated	Resistant to cortical depolarization
2000	Bhardwaj et al.	H Clearance	Rat	NMDA	Microdialysis	Increased CBF	NO-and P-450 monooxygenase mediated
2002	Domoki et al.	Cranial Window	Piglet	NMDA	Topical	Dilation	Endothelium Independent NO metabolite in CSF
2002	Chi et al.	Cranial Window	Rat	NMDA	Topical	Increased CBF	Neuronal NO dependent

Year	Authors	Preparation	Species	Agent	Application	Effect	Mechanism
2003	Parfenova *et al.*	Endothelium	Piglet	Glutamate *cis*-ACPD	Topical Topical	CO production CO production	Heme oxygenase dependent Heme oxygenase dependent
2003	Fuimana *et al.*	Isolated arteries	Piglet	Glutamate *cis*-ACPD	Topical Topical	Dilation Dilation	CO-Mediated CO-Mediated
2003	Iliff *et al.*	Cranial Window	Rat	Glutamate	Topical	Dilation	Adenosine-mediated
2004	Philip & Armstead	Cranial Window	Piglet	NMDA	Topical	Dilation	Inhibited by superoxide anion
2005	Simandle *et al.*	Isolated arteries	Piglet	NMDA Glutamate	Topical Topical	No dilation No dilation	NA NA
2006	Ayata & Moskowitz	Cranial Window Laser Doppler	Mouse	NMDA	Topical	Increased CBF	CSD-mediated NO-mediated
2006a	Leffler *et al.*	Cranial Window	Piglet	Glutamate	Topical	Dilation	Astrocyte dependent CO mediated Tetrodotoxin independent
2006	Bari *et al.*	Cranial Window	Piglet	NMDA	Topical	Dilation	Reduced by kynurenic acid
2006	Ohata *et al.*	Cranial Window	Rat	AMPA	Topical	Dilation	Adenosine/CO coupled

Abbreviations: NMDA, N-methyl-D-aspartate; AMPA, alpha-amino-3-hydroxy-5-methylisoxazole-4-propionate; NO, nitric oxide; CO, carbon monoxide, 1-aminocyclopropanecarboxylic acid (ACPD), ACPD, 1-aminocyclopropanecarboxylic acid; Hb, hemoglobin; NA, not applicable.