NITRIC OXIDE-INDUCED NEURONAL ACTIVATION IN THE CENTRAL NERVOUS SYSTEM AS AN ANIMAL MODEL OF MIGRAINE: MECHANISMS AND MEDIATORS

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INTRODUCTION

Understanding of the pathophysiological mechanisms of migraine is currently poor. Most ideas focus on calcium channel mutations and serotonin neurotransmission. An increasing body of evidence also suggests a pivotal role for nitric oxide (NO) in migraine headache.

NO is the most powerful endogenous vasodilator identified to date, and a non-conventional neurotransmitter, active at various sites in the central nervous system (1-5). Several NO effects are theoretically implicated in the neurovascular mechanisms mediating migraine attacks. First of all, NO causes endothelium-dependent vasodilatation, which is important in vascular regulation. In addition, neurogenic vasodilatation may be mediated via perivascular nerves, which operate through NO (6-7). Moreover, NO contributes to the control of platelets (8). On the neuronal side, NO is an important modulator of neuronal activity at peripheral, spinal and supraspinal levels. In particular, NO plays an important part in the processing of nociceptive information (9-11). Neuronal nitric oxide synthase (NOS) has been located in the superficial dorsal horn and intermediolateral cell column, which suggests that NO might regulate...
autonomic tone and sensory transduction at spinal cord level (12,13). Several reports have suggested that NO is strongly involved in the development and maintenance of hyperalgesia (14-20), which is likely to take place following the activation of Ca\(^{2+}\)-dependent NOS. Additionally, NO is considered a retrograde transmitter in peripheral nociceptive stimulation (21).

Most physiological actions of NO are mediated via the activation of soluble guanylate cyclase and a consequent increase in cGMP, ultimately leading to a decrease in intracellular calcium in target cells (22,23).

NITRIC OXIDE AS A TRIGGER OF MIGRAINE ATTACKS

Nitric oxide donors, such as nitrates, have been known for decades to induce migraine headaches in migraineurs. Several decades ago, Wolff showed that oral nitrites induce headache in migraineurs (24) and, later on, Sicuteri described a specific headache response to nitroglycerin (NTG) administration in a small group of migraine patients (25). This headache response was characterized by a delayed onset and spontaneous-like features. Subsequent studies confirmed that NTG administration is associated with headache more often in migraineurs than in controls and that the delayed headache developed by most migraineurs is identical to spontaneous migraine attacks (26,27). NTG-induced headache is very likely to be related to NTG-derived NO. According to Olesen, the nitroglycerin-induced symptoms depend on a vascular supersensitivity to NO in migraine sufferers (28). Whether the sensitivity of migraineurs to NO is purely vascular or also encompasses the neuronal compartment is still matter of debate. However, while NTG-induced vasodilatation seems a reasonable explanation for the immediate response observed in both healthy and migraine subjects, the delayed headache observed in migraine patients cannot be simply ascribed to vasodilatation, even in the context of supersensitivity to NO. In fact, NTG disappears from the blood compartment within minutes of its systemic administration, and the half-life of its metabolites does not exceed 40 minutes (29). In addition, because of its lipophilicity, NTG may deliver NO to several tissues, including the brain and meninges. Previous experience has shown that administration of L-Arginine, a precursor of NO via the activation of NO synthase, induces the opposite effect to NTG on the cerebral blood velocity of healthy subjects (30). In addition, it has been demonstrated that L-Arginine induces analgesia following intravenous administration in humans (31). This finding strengthens the idea that acute NO increase at peripheral and/or central sites is not able per se to induce migraine headache, and suggests the intervention of more complex mechanisms.

STUDIES ON NITROGLYCERIN-INDUCED NEURONAL ACTIVATION IN THE RAT BRAIN

In order to further elucidate the role of NO in the initiation and propagation of the migraine attack, the biological effects of NTG on the central nervous system were investigated by means of the study of Fos expression in the rat brain. According to the pioneering work of Morgan and Curran, neuronal Fos results from the expression of immediate-early gene c-Fos in response to a specific stimulus. For this reason, the protein has been widely used as a marker of neuronal activation (32-35). As regards the time course, Fos expression following a single, acute stimulus reaches a peak at one hour; then it starts declining rapidly, until it disappears by the fourth hour after the stimulus application.

The mediators involved in the NTG-induced neuronal activation were identified and characterized by combining the study of Fos expression and the immunocytochemical or histochemical detection of other neuronal markers.
Neuropharmacological probing was used for identification of possible mechanisms, while in vivo tract-tracing was adopted to establish the pathways involved (36-39).

**Structures**

Systemic (i.p.) administration nitroglycerin – at a dose (10 mg/kg) known to induce a moderate decrease in blood pressure for about 70 minutes – caused marked Fos expression in a variegated group of brain nuclei that may be schematically subdivided into structures involved in the control of:

- nociception (periaqueductal gray, nucleus trigeminalis caudalis);
- regulation of baroreception (nucleus tractus solitarius, ventrolateral medulla);
- neuroendocrine function (paraventricular and supraoptic nuclei of the hypothalamus);
- integration of autonomic, nociceptive and behavioral responses (locus coeruleus);
- integration of autonomic and behavioral responses (central nucleus of the amygdala, parabrachial nucleus).

Interestingly, Fos reached its maximal expression after 2 hours in the nuclei more directly involved in the control of blood pressure (e.g., ventrolateral medulla, nucleus tractus solitarius, parabrachial nucleus). Conversely, in the nuclei with nociceptive or integrative functions (e.g., nucleus trigeminalis caudalis, periaqueductal gray, paraventricular and supraoptic nuclei of the hypothalamus, central nucleus of the amygdala and locus coeruleus) the maximal Fos expression was reached 4 hours after the administration of NTG.

This bi-phasic time course of Fos expression, together with the physiological function of activated nuclei, suggests that nitroglycerin is likely to elicit a dual response in central structures: an initial response (elicited by the drug-induced vasodilatation), followed by a more complex, possibly multi-mediated, recruitment of a widespread set of central structures.

**Mediators**

Noradrenergic structures in the brainstem were massively activated following NTG administration. This activation involved the noradrenergic neurons of the locus coeruleus and subcoeruleus, caudal ventrolateral medulla, A2 and C2 groups.

Serotonergic fibers were consistently found in close proximity to activated neurons in the caudal ventrolateral medulla, spinal trigeminal nucleus caudalis and lateral aspects of the parabrachial nucleus. The principal site of origin of these fibers is not clear at the present time; however, it is tempting to hypothesize that these fibers arise from the serotonin- and Fos-positive neurons in the ventrolateral periaqueductal gray, which are known to represent an important relay station in the regulation of blood pressure and nociception.

In the hypothalamus, NTG-activated neurons in the paraventricular nucleus contained vasopressin, oxytocin and corticotropin releasing hormone. Most of the activated neurons in the supraoptic nucleus were vasopressinergic or oxytocinergic.

Nitroglycerin-induced Fos expression was strong in some areas known to contain NOS. Our studies confirm that a subpopulation of NOS-containing neurons in the paraventricular and supraoptic nuclei of the hypothalamus express Fos protein following systemic administration of NTG. In addition, Fos-immunoreactive neurons were shown to receive close appositions from NOS-positive fibers in the locus coeruleus, parabrachial nucleus, nucleus tractus solitarius and spinal trigeminal nucleus caudalis.

Preliminary findings suggest that the final messenger responsible, at least partly, for the neuronal activation induced by systemic nitroglycerin may be cGMP (Tassorelli et al., unpublished data). Indeed, the administration of the drug causes a dramatic increase in the immunocytochemically-detectable amount of this intracellular messenger in neuronal processes located...
in specific medullary areas that are involved in the transmission of nociceptive impulses to higher centers.

Mechanisms

The synthesis of endogenous NO both at the endothelial and neuronal levels emerged as a necessary component of NTG-induced neuronal activation of nociceptive and non-nociceptive structures. In order to take place, the neuronal response to NTG required the integrity of sensory fibers, and the functional activity of cyclooxygenase and it occurred in several areas also independently of hypotension. An additional mechanism involved was represented by NTG-induced changes in monoaminergic transmitters in selected diencephalic and brainstem structures (Tassorelli et al., unpublished observations).

Pathways

To investigate afferent and efferent projections of the nuclei activated by NTG we injected combinations of retrograde and anterograde tracers in the areas that express Fos in response to the drug (40). The findings obtained demonstrate the existence of a functional tri-nuclear (locus coeruleus, parabrachial nucleus and paraventricular nucleus of the hypothalamus) complex that responds to the systemic injection of NTG and that has major reciprocal connections, plus unidirectional connections with other nuclei involved in NTG-induced neuronal activation.

OTHER FINDINGS RELEVANT TO MIGRAINE OBTAINED WITH NITROGLYCERIN IN THE ANIMAL

Nitroglycerin infusion into rats caused increased beadings of NOS-immunoreactive fibers in the supratentorial cerebral dura mater, and an apparent increase in the number of NOS-reactive NO-ergic fibers in the dura at one hour after its administration (41). This structural alteration of fibers innervating dural blood vessels may be the result of NO-mediated arrest of axoplasmic transport at the level of the peripheral receptors. Alternatively, NO can alter the immunohistochemical appearance of dural nerve fibers by interfering with their metabolic processes (42). In another study it was demonstrated that following NTG, intracranial arteries are more sensitive to serotonin-induced contraction and to CGRP-mediated vasodilatation (43). Reuter et al. showed that systemic NTG administration activated induced a delayed inflammatory response in rat dura mater, which is likely to be mediated by an increase in inducible NOS (44).

Systemic NTG produced a significant increase in neuronal NOS and Fos-immunoreactive neurons in the cervical portion of the nucleus trigeminalis caudalis 4 hours after its administration (45). Neither neuronal activation nor Fos expression was observed in the thoracic dorsal horn. Lambert et al. (46) showed that i.v. infusion of NTG markedly increased the basal discharge of second-order neurons in the nucleus trigeminalis caudalis of cats. In addition, they showed that repeated infusion of NTG caused a progressive increase in the discharge rate of neurons located in the nucleus trigeminalis caudalis both during the resting phase and in response to superior sagittal sinus stimulation. These findings suggest the occurrence of a sort of long-term “potentiation” of NTG effect on trigeminal neurons. More recently, Martin and Martin failed to observe any Fos accumulation in nucleus trigeminalis caudalis following NTG administration (47). Unfortunately, the authors used a very low dose of nitroglycerin (100 µg/kg i.v.) when compared to the studies available from the literature (dose ranging from 1.5 to 10 mg/kg) (36-39,45,46). It is noteworthy that the low dose adopted by Martin and Martin did not induce cardiovascular changes in the rats studied. If a dose that is not vasoactive does not activate neurons in the nucleus trigeminalis caudalis, while a vasoactive dose does activate them, it follows that NTG vasoactivity is a necessary
Nitric oxide and migraine: an animal model

SIGNIFICANCE OF FINDINGS AND RELEVANCE TO MIGRAINE

The combination of our findings with data from the literature suggests that the study of the effects of systemic NTG on animal cerebral tissue and blood vessels provide interesting information on the pathophysiological mechanisms underlying migraine. Nitroglycerin is able to induce specific changes in a double target, blood vessels and neurons. These effects may be direct and/or indirect (48). The direct effects are likely to result from the local (at the vascular and neuronal site) formation of NO and cGMP. NTG in-

step for trigeminal activation, possibly via the activation of craniovascular sensory endings.

Fig. 1 - Putative effects of nitric oxide donors (nitroglycerin) on neuronal and vascular structures in the rat brain with a proposed hypothesis for the relevance of these mechanisms in migraine understanding.

Upper panel: data from the literature obtained with animal studies demonstrate that systemic administration of nitroglycerin induces, over several hours, specific changes in the asset of neurotransmitters (biogenic amines, neuropeptides) and neuromodulators (i.e., exogenous and endogenous NO). Specific functional and anatomic changes are also observed in the cerebrovascular compartment (see text for further details). The observed changes might lead to trigeminal sensitization via the activation of brain nuclei which are directly or indirectly connected to the nucleus trigeminalis caudalis and/or via the induction of meningeal vasodilatation and inflammation. Sensitized trigeminal neurons facilitate the transmission of pain signal to central structures.

Lower panel: administration of NO donors to normal subjects simply evokes a short-lasting, mild pain which seems related to the short vasodilatatory effect of these substances. According to the most recent experimental advances, migraine can be viewed as a complex disorder resulting from altered neuronal and vascular functioning. In this setting, NO-releasing drugs might trigger painful attacks via mechanisms that are analogous to those outlined in upper panel for the animal.
The direct effects seem more complex and include the interaction with the synthetic apparatus of endogenous NO, the induction of inflammation and the activation of the trigeminovascular system.

Most of the experimental evidence gathered to date show consistently that NTG effects appear after a delay of some hours (36-39, 44-46). This observation, along with preliminary findings from a behavioral nociceptive study conducted in our laboratory (Tassorelli et al., personal observations), suggest that, through the mechanisms outlined in the above paragraph, and probably additional ones, NTG is able to induce a state of hyperalgesia. These observations disclose interesting pathogenetic aspects of migraine and, at the same time, suggest new potential approaches to the pharmacological treatment of headache (Fig. 1).

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