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NOVEL THERAPEUTIC INVESTIGATIONS IN MIGRAINE PAIN

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Abstract

It has to be premised that migraine can be, according to our present knowledge, primary or direct, an invalidating disturbance largely spread (>12%) in the world population, having the form of an autonomous disease, as well secondary or indirect (2-3%), in this last case being only a pathologic symptom of different etiologies. We will concentrate our attention on migraine pathogenesis, taking in consideration involved afferent and efferent functions of primary and secondary neurons, with relative *neurotransmitters.* Furthermore, this review is concerned with the nature, type and proposed mechanisms of migraine pain as well as recent clinical investigations.

Keywords: Migraine, Neurotransmitters, Recent clinical investigations, Pain

Introduction

The pathophysiology of migraine is not completely understood and continues to be investigated. Neuronal components are relevant in migraine pathophysiology: there could be a generalized interictal abnormal excitability of the cerebral cortex in migraine, possibly favoring the occurrence of spreading depression with consequent activation of the trigeminal system. The complexity of interactions taking place in the sensory neuronal network with the mediation of all different neurotransmitters involved gives the measure of the extreme difficulty connected with the knowledge of migraine pathogenesis and in particular of its cardinal sign, namely the pain, in spite of the very significant scientific contributions of these last years, from which the problem has received an enormous broadening and enlightenment. Many theories have been formulated in these last sixty years about the pathogenesis of migraine and other forms of primary headache, but the problem is still far to be fully clarified (1). this review is concerned with the nature, type and proposed mechanisms of migraine pain as well as novel therapeutic applications.

The somatic sensitivity, which contributes to the preservation of physical integrity and *homeostatic* balance, depends upon the activation of sensitive receptors and relative nervous endings which are showing a different structural complexity and threshold and are distributed into cutaneous and visceral tissues as muscles, vessels and *splanchnic* organs. They convert physical and chemical stimuli into a painful afferent message, transmitted under the form of an action potential.

Primary sensory neurons, whose cellular bodies are located in the ganglia of dorsal spinal roots, forward to the central nervous system the action potential produced at the receptor site and relative nervous endings, being able to establish *synaptic* connections with second order neurons and, through them, with higher nervous centres, like *thalamus* and *cerebral cortex.*

The action potential conduction varies considerably in dependence of the afferent fibres dimension and *myelinization.* Teguments and joints contain *myelinated* sensory afferent fibres of middle diameter able to transport the stimulus with a relative rapidity (A-beta fibres), while free afferent endings and those coming from receptors activated by heat and other physical or chemical stimuli reach the spinal cord with thin fibres, poor of *myelin (A-delta fibres)* or without *myelin (C fibres,* defined *polymodal).*

The distribution of *nociceptors* is not uniform in the human body. They are present in large number at superficial levels (skin, cornea, tympanum) and in other areas, as *dura mater, leptomeninges* and vascular walls. From *Gasser ganglion* take origin *nociceptive* fibres directed to *dura mater's vessels* which play a prominent role in migraine pathogenesis. Actually their electrical stimulation produces, in animal experimental models, the release of *neuropeptides* as *CGRP* and *substance P,* which seem to be strongly involved in the induction of migraine pain; in the man , *neurokinin A* can be considered the equivalent of *substance P* in the rat.

The main function of *nociceptors* is to evidence the presence of noxious stimuli which, when applied for a sufficient time, are able to injure tissues by them innervated. *Nociceptors* do not show any spontaneous activity and in basal conditions possess an elevated threshold toward heat and mechanical stimuli.

A-delta and *C fibres* increase significantly their sensitivity when are chemically sensitized by the endogenous substances released during the inflammatory process.

The sensitization is a peculiar function of *nociceptors,* consisting in a reduced threshold and an increased response to repeated or prolonged stimulations, as it happens for traumatic or inflammatory events. This phenomenon is due to chemical agents released at the tissue level following a cellular injury produced by noxious agents (**Table 1**). *Hydrogen (H+) or potassium (K+) ions, bradykinin, histamine and serotonin*, liberated by inflamed tissues, activate *nociceptors* with different mechanisms. The *serotonin* receptors stimulation provokes the opening of specific ionic channels. *Bradykinin*, through its receptors B2, induces the synthesis, mediated by the enzyme *phospholipase C ,* of the intracellular *second messenger inositoltriphosphate (IP3)* and of *diacilgycerol (DG). Proteinkinase C,* activated by *DG,* increases the conductance for *Na+* and *Ca++*, so eliciting the genesis of the action potential and the consequential release of *neuropeptides CGRP, substance P* and

neurokinin A. Peripheral endings of *sensory nociceptive neurons* can exert also efferent functions. A subgroup of them, sensitive to the excitatory and desensitizing activities of *capsaicin,* which are able to synthesize *neuropeptides* in their cellular bodies, then transported to central and peripheral axonal endings. This phenomenon is produced at the peripheral endings level also by the low pH present in the inflamed tissues because of the high content of *protons,* with consequent *antidromic* reflexes and release of *neuropeptides CGRP* and *substance P,* able to induce vasodilatation and increase vascular permeability in the proximity of the same endings, as well as *passage* in the interstitial spaces of *kininogen,* the precursor of *bradykinin.* The following synthesis of *bradykinin* brings to a further amplification of this vicious circle contributing in this way to raise the activation of *nociceptors* either directly or indirectly, through the stimulation of the synthesis and release of *histamine, serotonin* and *prostaglandins,* specially *PGE2.* While the first two substances excite directly *polymodal nociceptors, PGE2*, produced by the enzymatic activity of *cyclooxygenase* on its substrate *arachidonic acid* in mast cells, macrophages and other inflammatory cells at the level of injured tissues, induces *hyperalgesia,* mostly through a *nociceptor sensitization.* The result of this sequence of events is the induction of vasodilatation associated to increased vascular permeability and moreover to the stimulation of afferent endings of sensory capsaicin-sensitive neurons, with increase of intracellular AMPc levels and consequent activation of *proteinkinase A*, *enzyme* able to *phosphorilate* both *voltage-dependent Ca++ channels* and *tetradotoxin-resistant Na+ channels.*

These last two events, in turn, decrease the action potential threshold facilitating the activation of *nociceptive fibres.* The mechanism of action of *aspirin* and other *NSAIDs,* employed also in the therapy of migraine pain, is based on the inhibition of *COX-1* and *COX-2* and consecutive reduction of *prostaglandin* synthesis.

GLUTAMATE AND CHRONIC PAIN

Afferent fibres of *nociceptive* primary neurons are ending in the superficial part of the ipsilateral spinal horn after having contracted *synaptic* connections with the second order excitatory *glutamatergic* neurons. *Glutamate* is *able* to activate different types of receptors, *ionotropic* and *metabotropic,* located centrally as well in the periphery. *AMPA* and *kainate receptors, ionotropic,* provoke excitatory *post-synaptic potentials (EPSPs)* having a rapid onset and a short duration (few ms). *NMDA receptors,* also *ionotropic,* induce slower *EPSPs,* lasting about 100 ms. *Metabotropic glutamate receptors,* called *mGluR,* play an important modulating role on the *hyperalgesic* response; for some of them, like *mGlu 4* and *mGlu 6-8* , positive, for others (*mGlu1* and *mGlu 5*) *negative.* They activate, through *G-proteins,* intracellular metabolic chains. The *phosphorilation* of intracellular substrates like *ionic channels* is able to modulate different phases of the *synaptic transmission* and the activation of *mGlu 5* can increase the sensitivity of *ionotropic receptors* also for the *glutamate* itself. All different *glutamate receptors* seem to be involved in the phenomenon of *hyperalgesia (lowering of nociceptors threshold and intensity),* bringing to the elevation of pain induced by noxious stimuli and chronic pain mechanisms. Central sensitization phenomena to which the *hyperalgesia* is connected seem to depend significantly from the *NMDA and mGluR receptors* activation operated by *glutamate* (2).

The *spreading depression (SD),* characterized by a massive failure of ion homeostasis associated to a transient cessation of neuronal function, and believed to be involved in migraine pathogenesis, requires the release of *glutamate; NMDA receptors* play a crucial role in the propagation of this process. In isolated layers of murine brain enthorinal cortex slices containing the *NMDA –receptor-mediated component* of *extracellularly recorded field EPSPs,* the electrical stimulation performed in order to elicit spontaneous *SD* , in the presence of *ifenprodil* ,a selective *NMDA receptor* antagonist, was nearly unable to produce the occurrence of *SD* (3).

In anaesthetised cats, recordings of the trigemino-cervical complex activity evoked by electrical stimulation of *superior sagittal sinus,* in cells activated by *L-glutamate,* showed that *CGRP receptor* antagonists *alpha-CGRP* and *BIBN4096BS,* given by microiontophoresis onto neurons in the trigemimovascular complex or intravenously, significantly inhibit the evoked activity. Data obtained suggest

that there are non-presynaptic *CGRP receptors* in the trigeminocervical complex that can be inhibited by *CGRP receptor* blockade and therefore that a *CGRP receptor* antagonist could be effective in the acute treatment of migraine and cluster headache (4). An *in vitro* study in human embryonic kidney has shown that a selective and non-competitive *GLU-K5* antagonist is able to inhibit *l-glutamate* and *domoate- evoked currents* blocking the *kainate* subtype of *glutamatergic receptors*(5). *Nociceptive spinal* neurons present a high degree of plasticity revealed by the increased intensity and duration of neuronal discharges after repeated injuries and prolonged activation of C fibres. *Neuronal plasticity* and *Long Term Potentiation (LTP)* processes could be responsible for chronic pain manifestations which are at the basis of *chronic migraine.* In the *LTP* genesis is fundamental the *NMDA receptor* activation which induces a raise of intracellular *Ca++* concentrations stimulating *NO-synthase (NOS)* , a *Ca++ - calmodulin – dependent* enzyme, able to provoke an increased *NO* formation. This last compound is active at the level where it is produced as well as in the extracellular space, reached by diffusion, and in this place stimulates the *guanilato-cyclase* of adjacent neuronal endings, provoking a further release of *glutamate).*

The repeated and intense stimulation of *NMDA receptors* occurring during migraine attacks and the consequent onset of sensitization could explain the progressive worsening leading from a form of headache showing many free intervals to the *daily inter-critical chronic headache* which obliges the patient to daily administrations of analgesics under the risk of abuse.

NMDA antagonists like ketamine, given subcutaneously or intravenously by daily continuous infusions, have been assayed in order to avoid the chronic evolution and the addiction toward analgesics, frequently observed in these conditions (6).

A rational perspective for the future is represented by antagonists and modulators of *ionotropic or metabotropic glutamate receptors* wich could be therapeutically efficacious in the treatment of chronic forms of migraine and other types of primary headache. It has been recently shown that the *glutamatergig system* is implicated in *cortical spreading depression, trigemino-vascular activation* and *central sensitization* in preclinical models of migraine, and in the clinic (7).

OPIOID PEPTIDES AND PAIN PATHWAYS

The transmission of *nociceptive* messages from afferent primary fibres as well as the activity of spinal *nociceptive* neurons are modulated by *presynaptic* and *postsynaptic* mechanisms, mediated through different subtypes of spinal *interneurons (gabaergic, glycinergic and enkephalinergic).*

The first control station of painful afferences is the *gelatinous substance (GS)* located in the *spinal grey posterior horn. GS* is rich of *opioid peptides (enkephalins* and *dynorphins)* and, of course, of *opioid receptors mu* and *k* as well as of theirs *interneurons,* which selectively inhibit *neurotransmitters* release from *A-delta and C efferent fibres*, so making an *access gate* for painful afferences. *Opioid peptides* exert a prevalent inhibiting effect on neuronal activity due to the opening of *K+ membrane channels*, operated by *mu* and *delta receptors*, or to the closing of *Ca++ channels (k receptors).*

Spinal *nociceptive circuits* and especially *enkephalinergic neurons* are under the control of neuronal systems descending from the *brainstem* able to exert facilitating or inhibiting effects and therefore playing an important role in the regulation of *nociceptive reflexes* and pain perception.

The *grey periaqueductal substance (PAG),* the *raphe magnum nucleus (NRM), reticular paragigantocellular (NRPG)* and *magnocellular (NRMC) nuclei* form all together the *inhibitory descending system. PAG neurons* project upon *NRM*, where arise descending *serotoninergic fibres and NRPG as well as NRMC*, and from this last structures take origin *noradrenergic descending fibres,* directed, as the *serotoninergic fibres,* to the *spinal cord. Serotoninergic* and *noradrenergic pathways* activate *spinal enkephalinergic interneurons* , negatively modulating, through a *presynaptic* inhibition, the transmission of the *nociceptive* message. In this way *opioid agonists,* like *morphine, fentanyl, pentazocine , buprenorphine* and others,exert their *analgesic* effect.

Though the *opioid* system has received not a major consideration in the study of migraine pathophysiology , its involvement can be postulated. As far as *morphine* is concerned,

according to several observations, it seems that this drug could worsen rather than attenuate the migraine pain.

In United States only *butorphanol* has been employed by intranasal route in the therapy of very severe migraine attacks. It has been observed the absence of interaction between *butorphanol* and *sumatriptan* also when the administration of these two drugs is practised closely in time (8).

Recently it has been found that the gene codifying for *mu receptors* is polymorphous and this peculiarity could explain the individual variability to stimuli generating pain. Since migraine is very likely a disorder characterized by a variable threshold and a multiform pathogenesis, both characters being tied to different genes, it is not possible to exclude that the *mu opioid receptor* could represent one the factors contributing to the determination of migraine threshold and on this basis it will be rational to address in such direction future studies on migraine genetic aspects, in order to evaluate precisely the eventual involvement of all sub-types of *opioid receptors*.

Opioid analgesics appear to be generally inefficacious in the management of chronic daily headache. The study of pathophysiological models of *opioidergic pathways* for migraine and chronic daily headache, as well as for neural plasticity in the context of neuropathic pain states, have allowed the concept of chronic daily headache as a neuropathic pain syndrome (9).

SEROTONINERGIC RECEPTORS AND TRIGEMINO-VASCULAR SYSTEM

The experimental research in animal models has given in these last years the opportunity to obtain a large amount of significant data on the role of *trigeminal receptors* in the pathogenesis of headache and headache pain, specially for migraine and cluster headache. Although the knowledge of complex pathogenetic factors involved at the level of the central nervous system in the production of the migraine attack is still full of gaps, we dispose of a considerable information on peripheral factors presumably responsible of pain mechanisms in migraine and other types of primary headache.

The brain is not provided with pain sensitive endings, while meninges are rich of *nociceptors.* The ophthalmic branch of trigeminal nerve reaches extra-cerebral vessels at the level of meninges (*dura mater, arachnoid and pia mater*) which receive peripheral fibres coming from *pseudo-unipolar neurons* present in the *trigeminal ganglion.* These fibres form the neuroanatomical circuit known as *trigemino-vascular system,* forwarding stimuli received from afferent peripheral endings to the *trigeminal caudalis nucleus* and to other brainstem nuclei as *superior salivatorius nucleus.* According to Edvinsson and Goadsby (10), through this last nucleus could be activated an afferent *cholinergic* pathway of the seventh cranial nerve, with the release at the meningeal level of *Vasoactive Intestinal Peptide (VIP),* able to provoke further vasodilatation and hyperactivation of trigeminal fibres An important role is attributed to *parasympathetic pathways* in migraine pathogenesis specially in order to explain some symptoms like tearing, conjunctival blood injection and rhinorrhea observed frequently in patients suffering from cluster headache.

The trigemino-vascular system, therefore, with its different components, represents one of the most important anatomic and functional areas for the study of headache pathophysiology.

The characterization of *5-HT receptors* within trigeminal sensory neurons has deeply improved the knowledge about the pathogenesis and treatment of migraine; among them *5-HT1B* are located in the smooth muscle of meningeal extracranial vessels, *5-HT1D* in trigeminal sensory endings (11) and *5-HT1F* at the level of *trigeminal nucleus caudalis,* as well as peripherally in trigeminal gangliar endings (12). The stimulation of first two sub-types of *5-HT receptors* inhibits the release of vasoactive peptides like *CGRP* and pain transmitter peptides like *substance P* and *neurokinin A* (13). The peptides are involved in the dural plasma extravasation provoked by the neurogenic inflammation due, in experimental animal models, to the depolarization following the electric stimulation of peripheral trigeminal fibres. In these models the release of *CGRP* caused by the activation of trigeminal fibres leads to a further dilatation of meningeal vessels, wich, in turn, brings about the *hyperactivation* of central and peripheral trigeminal endings responsible of pain

(**Figure 1**). These pathophysiological mechanisms,. discovered by Moskowitz (14), could be, in the rat, at the basis of migraine pain, but this hypothesis has been not yet definitely validated in the man, though it has received a strong support from the significant therapeutical activity exerted by *triptans* toward the primary headache pain. Actually it is well known that *sumatriptan* inhibits the *CGRP* gene transcription in trigeminal sensory neurons, but it has not been clarified yet whether this action is mediated only by *5-HT1D* or also by *5-HT1B receptors*, the last having been isolated, as the relative *RNAm,* in human trigeminal gangliar neurons, in absence of an histochemical demonstration of this receptor transportation at the level of central and peripheral endings, as it happens for *5-HT1D* (12), which is present also at the level of the *solitary tract nucleus.* In this site, believed to be involved in the pathogenesis of some migraine symptoms like nausea and vomiting for its connections with the autonomous nervous system, arrive numerous trigeminal afferent fibres and, therefore, the therapeutic activity of *triptans* on the above mentioned symptoms, associated to the analgesic effect, could be exerted either directly on the *solitary tract nucleus* or indirectly, as a consequence of the reduced activation of the trigeminal *nociceptive system*.

Another indirect proof of the possible application to humans of the *neurogenic inflammation theory of Moskowitz* seems to reside in some observations made by Goadsby et al (15), according to them *CGRP* levels increase in the external jugular vein blood during migraine attacks.

On the other hand, the same Authors have evidenced the correlation existing between the decrease of blood CGRP levels and therapeutic efficacy of *triptans*. Through their agonist activity on *5-HT1 B-D-F receptors* these drugs could inhibit at the *presynaptic* level the release of neuropeptides able to provoke either intracranial vasodilatation or pain characteristic of migraine and related to the stimulation of primary trigeminal *nociceptive neurons,* which represent the first step in the pain transmission. These receptors, in turn, activate, through their central endings, second order *nociceptive* neurons at the level of brainstem, in the *trigeminal nucleus caudalis* where also *5-HT1 F receptors* are located as well as at peripheral endings of gangliar trigeminal neurons in which they could have a function analogous to that of *5-HT1 D receptors* (12).

Antagonists of *5-HT1 F* like *LY 3334370* inhibit selectively, in anesthetized rats, the activation produced by the electrical stimulation of *dura mater* and of second order neurons in *trigeminal nucleus caudalis*, in this way blocking the transmission of *nociceptive* inputs to central areas (16).

Since many *triptans* show to be provided with a *5-HT1 F* agonist activity it is possible to hypothesize that their therapeutic efficacy be partially due to the ability by them possessed to activate this sub-type of *5-HT1* receptors either at peripheral levels or, perhaps more likely, centrally, in correspondence of the *trigeminal nucleus caudalis* (16,17).

Preclinical investigations in rats have shown that anti-migraine *triptan* drugs exert their effect not only by blocking the neuropeptide neurotransmitter release from sensory nerve terminals and directly constricting blood vessel smooth muscles, because also the modulation of *glutamate* and *NO* release by them operated can contribute to their activity (18,19) . The majority of *5-HT1B, 5-HT1D and 5-HT1F* are co-located with *glutamate* receptors being *glutamate positive* ,as it is possible to demonstrate using techniques of immunohistochemical staining (20). According to Levy et al (21), *triptans* analgesic action is exerted through presynaptic *5-HT1B-D* receptors in the dorsal horn by blocking the synaptic transmission between axon terminals of the trigeminovascular neurons and cell bodies of their central counterparts. On this basis the analgesic action of *triptans* can be attained specifically in presence and not in the absence of central sensitization. The early treatment with triptan drugs provides a powerful means of preventing the initiation of central sensitization triggered by chemical stimulation of menigeal *nociceptors* (22) An important site of action for *triptan* anti-migraine drugs is the nucleus tractus solitarius where trigeminovascular activation triggers nausea and vomiting, frequently associated with pain in the migraine attack(23).

THE ROLE OF GABAERGIC RECEPTOR

Among receptors with a prevailing inhibitory activity an important role is played by *GABA receptors*, specially in the neurogenic inflammation process, as it has been proven in animal experimental models using *valproate* (24), an anticonvulsant drug currently used in the preventative treatment of migraine, also assayed as a symptomatic of second order in the therapy of cluster headache (25). Good results have given in the treatment of primary headache also more recent anticonvulsant like *gabapentin*, *lamotrigine*, *tiagabine*, *topiramate* and others(26, 27).

GABA-A receptors are located in trigeminal sensory neurons. *Valproate* is provided with two mechanisms of action, being able to inhibit *GABA-transaminase*, which causes *GABA* degradation and, at the same time, to activate *glutamic - decarboxylase,* that induces *GABA* synthesis. Therefore, following its administration, *GABA* extracellular concentrations in the proximity of *GABAergic endings* are increased (28).

Valproate, in animal experimental models, blocks *dural plasmatic extravasation* induced by neurogenic inflammation due either to the electric stimulation of trigeminal fibres or to the intravenous administration of *substance P.* For this aspect preclinical data have been obtained lather than clinical evidences, when *the use* of *valproate* in migraine therapy was already consolidated., but anyhow they bring a strong support to the hypothesis that the activation of trigeminal sensory neurons is an initial step in the neurogenic inflammation process and in the pathogenesis of primary headache pain.

About *GABA-B receptors* they have also been implicated with migraine pathogenetic mechanisms. The coupling between *GABA* and *GABA-B receptors* induces the activation of a specific *Gi-protein,* exerting an inhibitory effect on the enzyme *adenylate-cyclase* with the reduction of intracellular *cAMP* levels and decreased phosphorylation: Consequently it is produced a functional block of *voltage-dependent Ca++ channels* involved in the neurotransmitter release. *GABA-B agonists, as baclofen,* then, may negatively modulate, trough these mechanisams, the release of many neurotransmitters, *GABA* included. *Baclofen* indeed has shown to inhibit the activation of trigemino-vascular system at the level of the *spinal trigeminal caudatus nucleus* and therefore to be furnished of a distinguished *anti-nociceptive* activity, observed in many clinical trials on patients suffering from migrasine or cluster headache(29,30).

CANNABINOID, VANILLOID AND PAR RECEPTORS RELATED DRUGS AND MIGRAINE PAIN

At the level of peripheral and central endings of primary sensory neurons are present also *cannabinoid, vanilloid* and *PAR receptors*, characterized only few years ago (**Table 2**), which seem to play an important role in pain pathophysiology *Endocannabinoids (anandamide* and *2-acyl-glycerol ,* having a *lipidic* structure, derive, like *eicosanoids* and *Platelet Activating Factor (PAF),* from membrane phospholipids and their pharmacodynamic profile is similar to that of the *Cannabis* active principle delta-9-tetrahydrocannabinol and other natural and synthetic *cannabinoi*ds. The synthesis of *anandamide* is regulated by the *phospholipase D,* while another intracellular enzyme, the *Fatty Acid Amide Hydrolase (FAAH)* is responsible of its degradation, followed by the reassumption of its constituents in phospholipids (31). Differently from *neurotransmitters and neuropeptides* which are continuously synthesized and then stored in special deposit sites, from where they are released in consequence of the excitation of nerve endings, *endogenous cannabinoids* are produced from endothelial cells, macrophages and peripheral cells only when their *holocrine-paracrine* function has to be developed, during the inflammation process and in all conditions of cellular injury (32, 33).

Once released in the extracellular space, anandamide activates, at nanomolar concentrations,its own receptors defined *cannabinergic(CB)* and generally distinguished in *CB-1 and CB-2,* while, at micromolar concentrations (34), stimulates *vanilloid receptors(VR-1).*

CB receptors of both types *are coupled,* through a *Gi- protein*, with the *adenilate cyclase* (35). *CB-1s* mediate analgesic effects of *anandamide* either peripherally or centrally; *CB-2s,* present in *lymphocytes B* and *natural killer,* seem able to produce, when activated, an anti-inflammatory action.

Anandamide (AEA) thus exerts centrally and peripherally an inhibitory effect on the

pain, due to the activation not only of encephalic and spinal(10th lamina) *CB receptors,* as in the *PAG* (36), but also of peripheral sensory neurons (37), in particular at the level of central *peptidergic capsaicin-sensitive* endings, containing *CGRP* and *substance P,* where their release is reduced(38).

Hohmann and Herkenham (39) have demonstrated, by means of histochemical techniques, the presence of *CB-1* also in the peripheral endings. Calignano et al (40) have observed that *CB-1 agonists,* like *WIN552122,* when applied on the skin, are able to reduce the *nociceptive* activity of *formalin.*

After a protracted and intense noxious stimulation, sufficient to provoke an inflammatory reaction, central and peripheral neuronal excitability is significantly increased until the onset of *hyperalgesia* and pain production by normally innocuous events (*allodynia*). *Cannabinoids*, in animal experimental models, antagonize either *hyperalgesia* or *allodynia* (41, 42, 43). On the other hand, *CB-1 antagonists,* like *SR141716A,* decrease the activation threshold, increasing the response to mechanic and thermic stimuli.

Therefore, it may happen that, following repeated or protracted noxious stimulations, as after a traumatic injury or in course of inflammation, *endocannibanoid* levels appear increased either peripherally or centrally, for example in the *PAG*, while at the same time a sensitization of *CB-1 receptors* is observed. Thus experimental data seem to demonstrate the central role of *endocannibanoids* in pain pathways, disclosing the perspective of a possible interaction on these new targets in the treatment of various clinical forms of pain.

*Anandamide,*therefore, activates, respectively at *nanomolar and micromolar concentrations, CBs and VR-1 receptors,* these last capsaicin-sensitive (34), with opposite effects on pain, toward which it exerts a dual activity, stimulatory or inhibitory, so assuming in the regulation of this function, a balancing role. *Vr-1 receptor,* discovered in 1977, when stimulated, provokes the inflow of extracellular Ca++ in response to the loss of intracellular Ca++ (44).

Vanilloid receptor VR-1 is a non selective *receptor-channel,* which can be activated by protons as well as by *capsaicin-like substances* with a *simil-lipidic structure,* producing the release of inflammation mediators with pain; moreover it is able to transduce also heath stimuli in *nociceptive*.sensations. *Capsaicin-sensitive neurons* have a small diameter (*A-delta and C fibres*) and are *peptidergic*, releasing *CGRP* and *substance P* through a *Ca++ - dependent mechanism,* not only at the peripheral level but also centrally, in spinal endings of primary sensory neurons (45).Therefore they can exert either peripherally or centrally both the analgesic or algesic effects on *nociception,* sustained by opposite modifications of *Ca++* intracellular content (46).Ethanol potentiates *VR-1 nociceptive* response (47).

It is not yet possible to argue whether such a mechanism could or not be involved in the neurogenic inflammation process, validating in the first case Moskowitz's theory (14)

Another class of receptors, named *PAR (Protease Activated Receptors)* has been isolated in the rat, by means of immunohistochemical techniques (48), in *capsaicin-sensitive* primary sensory neurons, at the level of trigeminal and spinal dorsal root ganglia, where they are colocated with *VR-1 receptors*, being probably involved also in primary headache pathogenetic mechanisms.

The *PAR-2 receptor* (49) is selectively activated by *SLIGRL* and *SLIGKV peptides, trypsin* and *tryptase, proteases* abundantly contained in mast cells and released by them in course of the inflammatory process; in many tissues mast cells are in close contact with the endings of capsaicin-sensitive primary sensory neurons containing neuropeptides. The activation of *PAR-2* provokes *Ca++* mobilization and *CGRP* and *substance P* release in slices of rat isolated neurons or vascular intracranial structures cultured *in vitro,* while *in vivo* the swelling *CGRP*-induced of the rat paw is observed.

Therefore *CB, VR* and *PAR receptors* with their ligands seem worthy of attention in the study of pain pathophysiology and therapeutical problems related to the primary headache (50,51, 52,53).

RECENT CLINICAL INVESTIGATIONS IN MIGRAINE PAIN

Lamey et al [54] report their study relates to a novel biochemical marker associated with migraine and a method of predicting forthcoming migraine attacks. The study also relates to a novel vasodilatory agent and to

the use of a peptide to develop an antimigraine therapy.

In a first aspect the present The study aims to provide a product to predict the onset of migraine. The study provides a method of predicting the onset of migraine attack, through the detection of elevated levels of salivary peptide Cystatin SN. Following the establishment of a patient's normal Cystatin levels, variations of this level can serve as an indication of an impending attack of migraine. According to the present study there is provided a method for predicting potential migraine attacks, the method comprising the steps of establishing a normal level of Cystatin SN for an individual and subsequently testing for variations thereof wherein elevated levels of Cystatin SN indicate the on set of a migraine attack. Elevated levels of Cystatin SN will preferably be at least three time the normal levels. Prediction of a migraine attack will enable an individual to commence treatment of the attack before onset therefore minimising any ill-effects or to plan ahead accordingly [54].

The study comprises the use of the amino acid sequence of Cystatin SN in the elucidation of the or a nucleic acid sequence in the development of a test for elevated expression of Cystatin SN. The The study provides such a test for detection of levels of expression of the Cystatin SN gene [54].

Lamey et al [54] have found that levels of Cystatin SN are about ten times higher in migraineurs than non-migraineurs and levels rose markedly in the 24 hours before a migraine attack. As this molecule is intimately linked with migraine attack, it can be concluded that its release as a result of tooth clenching may be the main factor responsible for attacks of migraine.

Therefore, the present study provides a novel therapy for use in migraine management or prevention considering that the substance Cystatin SN is intimately linked to attacks of migraine [54]. This offers the opportunity to investigate the mechanism by which Cystatin SN causes attacks of migraine and therefor offers new drug possibilities as an agent for developing a drug against Cystatin SN or its receptor or increasing drug metabolism or excretion or allowing current drug therapies, some of which are not presently indicated for use in the treatment of migraine, to be given at an earlier stage when they perhaps would be effective. As there are no current reliable biochemical markers for migraine and there is some doubt as to the mechanism by which even acute migraine drug therapy is effective and as such the present invention in conjunction with the identification of the Cystatin SN molecule offers a different mechanism to prevent migraine attack [54]. The present study thus provides the use of a Cystatin molecule in developing a treatment for migraine wherein the treatment is based on an antagonist of cystatin.

Chissoe study [55] is related to the identification of genes that are associated with migraine and to screening methods to identify chemical compounds that act on those targets for the treatment of migraine or its associated pathologies. The purpose of the present study was to identify genes coding for tractable targets that are associated with migraine, to develop screening methods to identify compounds that act upon such targets, and to develop such compounds as medicines to treat migraine and its associated pathologies. Family and twin studies indicate that genetic factors are involved in the aetiology of migraine.

A first aspect of the present study [55] is a method for screening small molecule compounds for use in treating migraine, by screening a test compound against a target selected from the group consisting of gene products of the genes APOE, GNAL, NEDD4L, PDIP, TPCN1, TRPM8, ADRA1B, P2RX4, TAAR2, TAAR3, USP 11, CHRNA5, RAB5A, DPP8, F2RL1, FZD5, PTGER1, SPI, ALOX5, CMTM8, DCBLD2, DPYS, IKBKB, OVCH1, PDE4DIP, PPM1G, PYY2, RYR1, BRD2, CAD, F2RL2, NCOA3, ADORA2B, BMX, CHRNA3/CHRNB4, F2R, GRIK5, ITGB4, MAPK10, NPEPL1, PTGIS, UCN2, and WASF1. Activity against said target indicates the test compound has potential use in treating migraine.

The present study tested genes that encode for potential tractable targets to identify genes that are associated with the occurrence of migraine and to provide methods for screening to identify compounds with potential therapeutic effects in migraine [55].

An aspect of the present study is a method for screening small molecule compounds for use in treating migraine, by screening a test compound against a target selected from the group consisting of proteins encoded by the

genes APOE, GNAL, NEDD4L, PDIP, TPCN1, TRPM8, ADRA1B, P2RX4, TAAR2, TAAR3, USP1, CHRNA5, RAB5A, DPP8, F2RL1, FZD5, PTGER1, SPI, ALOX5, CMTM8, DCBLD2, DPYS, IKBKB, OVCH1, PDE4DIP, PPM1G, PYY2, RYR1, BRD2, CAD, F2RL2, NCOA3, ADORA2B, BMX, CHRNA3/CHRNB4, F2R, GRIK5, ITGB4, MAPK10, NPEPL1, PTGIS, UCN2, and WASF1. Activity against said target indicates the test compound has potential use in treating migraine. Activity may be enhancing (increasing) the biological activity of the gene product, or diminishing (decreasing) the biological activity of the gene product [55].

Frants et al [56] report that family, twin and population-based studies suggest that genetic factors are involved in migraine, most likely as part of a multifactorial mechanism. The complex genetics has hampered identification of candidate genes for migraine. Familial Hemiplegic migraine (FHM) is a rare, autosomal dominant, subtype of migraine with aura, associated with ictal hemiparesis and, in some families cerebellar atrophy. Otherwise, the symptoms of the headache and aura phase of FHM and "normal" migraine attacks are very similar and both types of attacks may alternate within subject and co-occur within families. FHM is thus part of the migraine spectrum and can be used as a model to study the complex genetics of the more common-forms of migraine [56]. Since FHM is part of the migraine spectrum, thus be used to study the genetic factors and biological mechanisms that are related to various episodic neurological disorders such as FHM, EA-2, common migraine and others such as epilepsy.

The present study [56] provides access to and methods to study the genetic factors and biological mechanisms that are related to various episodic neurological disorders such as FHM leading to migraine. The invention provides cells or animals in which changes such as deletions or mutations in said gene have been introduced by recombinant nucleic acid techniques. All such cells or animals provided by the invention can be used to study the pathophysiology of FHM, EA-2, migraine or other neurological disorders associated with cation channel dysfunction, for example to test or develop specific medication for the treatment of said disorders [56].

The study also provides proteins or peptides encoded by said genes, or fragments thereof, related with cation channel dysfunction, and detection of such proteins or peptides by antibodies directed against said proteins or peptides. Such antibodies can be of natural or synthetic origin, and can be produced by methods known in the art. Such proteins and antibodies and detection methods can be used to further in vitro or in vivo studies towards the pathophysiology of FHM, EA-2, migraine or other neurological disorders associated with cation channel dysfunction, in addition such proteins, antibodies and detection methods can also be used to diagnose or identify such disorders in patients or in experimental animals [56].

Lesniewski et al. study [57] relates to diagnostic assays useful with endothelin receptor antagonist therapy, and in particular relates to measurement of certain biomarkers that allow identification of patients eligible to receive endothelin receptor antagonist therapy and that permit monitoring of patient response to such therapy.

The study has significant capability to provide improved selection of patients for Endothelin Receptor Antagonist therapy. The assessment of these biomarkers with the invention also allows tracking of individual patient response to the therapy. The inventive assays have utility with any ETRA therapy, including treatment of cancer, coronary angina, cerebral vasospasm, acute and chronic renal failure, gastric ulceration, cyclosporin-induced nephrotoxocity, endotoxin-induced toxicity, asthma, LPL-related lipoprotein disorders, other proliferative diseases, acute or chronic pulmonary hypertension, platelet aggregation, thrombosis, IL-2 mediated cardiotoxicity, colitis, vascular permeability disorders, ischemia-reperfusion injury, Raynaud's disease and migraine [57].

Sudilovsky et al, study [58], provided for inhibiting onset of or treating migraine headaches wherein a therapeutically effective amount of an angiotensin converting enzyme inhibitor alone or in combination with a calcium channel blocker is systemically, such as orally or parenterally, administered over a prolonged period, whereby frequency and intensity of migraine headaches are significantly reduced. In

carrying out the method of the present invention, the angiotensin converting enzyme inhibitor alone or in combination with the calcium channel blocker may be administered to mammalian species, such as monkeys, dogs, cats, rats and humans, and as such may be incorporated in a conventional systemic dosage form, such as a tablet, capsule, elixir or injectable. The above dosage forms will also include the necessary carrier material, excipient, lubricant, buffer, antibacterial, bulking agent (such as mannitol), anti-oxidants (ascorbic acid of sodium bisulfite) or the like. Oral dosage forms are preferred, although parenteral forms such as intramuscular, intraperitoneal, or intravenous are quite satisfactory as well [58].

The dose administered must be carefully adjusted according to age, weight and condition of the patient, as well as the route of administration, dosage form and regimen and the desired result. The formulations as described above will be administered for a prolonged period, that is, for as long as the potential for onset of a migraine headache remains or the symptoms of a migraine headache continue. Sustained release forms of such formulations which may provide such amounts biweekly, weekly, monthly and the like may also be employed. A dosing period of at least two weeks and preferably at least 4 to 6 weeks are required to achieve minimal benefit [58].

Barlow et al study [59] is related to methods for treating diseases and conditions of the central and peripheral nervous system by stimulating or increasing neurogenesis via modulation of angiotensin activity. The study includes methods based on the application of an agent which modulates angiotensin action to stimulate or activate the formation of new nerve cells.

Disclosed herein are methods for the prophylaxis and treatment of diseases, conditions and injuries of the central and peripheral nervous systems by stimulating or increasing neurogenesis. Aspects of the invention include increasing neurogenesis in cases of a disease, disorder, or condition of the nervous system. Embodiments of the invention include methods of treating a neurodegenerative disorder, neurological trauma including brain or central nervous system trauma and/or recovery therefrom, depression, anxiety, psychosis, learning and memory disorders, and ischemia of the central and/or peripheral nervous systems [59] .

Also disclosed are methods for preparing a population of neural stem cells suitable for transplantation, comprising culturing a population of neural stem cells (NSCs) in vitro, and contacting the cultured neural stem cells with at least one modulator of angiotensin activity. In some embodiments, the stem cells are prepared and then transferred to a recipient host animal or human. Non-limiting examples of preparation include 1) contact with a modulator until the cells have undergone neurogenesis, such as that which is detectable by visual inspection, marker, or cell counting, or 2) contact with a modulator until the cells have been sufficiently stimulated or induced toward or into neurogenesis. The cells prepared in such a non-limiting manner may be transplanted to a subject, optionally with simultaneous, nearly simultaneous, or subsequent administration of a neurogenic agent, or a modulator of angiotensin activity to the subject. While the neural stem cells may be in the form of an in vitro culture or cell line, in other embodiments, the cells may be part of a tissue which is subsequently transplanted into a subject, based upon the cell morphology [59].

In yet another aspect, the study includes methods of stimulating or increasing neurogenesis in a subject by administering a modulator of angiotensin activity. In some embodiments, the neurogenesis occurs in combination with the stimulation of angiogenesis which provides new cells with access to the circulatory system [59].

CONCLUSIONS

The complexity of interactions taking place in the sensory neuronal network with the mediation of all different neurotransmitters involved gives the measure of the extreme difficulty connected with the knowledge of primary headache pathogenesis and in particular of its cardinal sign, namely the pain, in spite of the very significant scientific contributions of these last years, from which the problem has received an enormous broadening and enlightenment.

Many theories have been formulated in these last sixty years about the pathogenesis of migraine and other forms of primary

headache, but the problem is still far to be fully clarified.

The first theory, conceived by Wolff in 1948 (60), is build up on a vascular basis, considering three main aspects: a) during the migraine attack extra-cranial vessels dilate and are throbbing in a large percent of patients; b) the stimulation of intra-cranial vessels provokes an ipsilateral headache; c) vasoconstrictor drugs, like ergot derivatives, show a curative effect, while vasodilators, like nitrates, may induce an attack. On this observations he hypothesized that an intra-cranial vasoconstriction could be responsible of the migraine aura and of the following hyperemic reaction associated to local vasodilatation, with consecutive activation of perivascular nociceptive endings. Ten years later Heyck (61) completed the theory adding the concept that during the attack arterio-venous anastomoses were closed, explaining in this way the decreased oxygen extraction observed in the symptomatic side.

Successive theories were underlining the importance of vasoactive and neuroactive substances (as plasmatic kinins, endorphins, serotonin, histamine, fatty acids, adenosine, prostaglandins, NO and endothelin-1), released in perivascular areas, to which could be attributed the responsibility of the neurogenic inflammation and its symptoms (62, 63, 64, 65).

The already mentioned theory of Moskowitz (14) was synthesizing in an appreciable way all theories till now exposed, with the idea of the neurogenic inflammation in the trigemino-vascular area and the integration in the cerebral cortex of the painful information.

Another possibility to interpret the headache pain is proposed on the basis of a depression of the cortical electrical activity similar to the *spreading depression* observed in animal experimental models by Leao longtime ago (66) and consisting in the production of cortical hyperexcitation waves followed by the electrical activity suppression after chemical or mechanic stimuli, this last progressing by contiguity within the cerebral structures in posterior-anterior direction, at a speed of 2-5 mm/min (67). The *spreading depression* can be considered, according to Olesen (68), the pathophysiological substrate of the migraine *aura,* during which it has been observed with the technique of *xenon 133* a decreased cerebral blood perfusion specially in the occipital area, lasting about an hour. These data have been validated by means of magneto-EEG, SPECT, MRI and PET studies (69), specially for the *migraine with aura* and, at least partially, also for the *migraine without aura.* Mitochondrial alterations and a decrease in Mg++ blood levels

(64) have also been associated to migraine in neuroimaging investigations (70).

Lance (71), Diener and May (72), and Welch (73) have proposed to consider the participation in the migraine pathogenesis of orbital, frontal and limbic cortical areas and brainstem structures specialized in controlling pain mechanisms , like *trigeminal nucleus caudalis, locus coeruleus, raphe nuccleus dorsalis,, PAG* and *area postrema,* interacting with several *neurotransmitters* as noradrenaline, serotonin, dopamine and others, which could be "migraine generators".

About the cluster headache , among other theories, it seems worthy of interest the immunological theory, dealing mostly with *interleukins and cytokines receptors* and involving the complex relationships between neuro-psychic, endocrine and immunological systems (74), as it is demonstrated by the reduced response of cortisol to meta-chlorophenyl-piperazine (75).During the attack there is the activation of the hypothalamic grey ipsilateral to the side affected (76).

As far as tension headache episodic and chronic, is concerned, it is not clear at all whether its pathogenesis is similar or different, and in what extent, in respect to that of migraine, but many resemblances have been recognised. The influence of stress and of protracted muscular contraction has been largely investigated as well as immunological implications, specially in the autoimmune sense (77).

It is necessary to assess the importance of genetic factors in the pathogenesis of headache and headache pain (78,79).

Finally, it can not be omitted that the knowledge of headache pain pathophysiology has tremendously improved because of the advances realised by clinical pharmacology studies, from which further and ever more important insights in the field likely are going to be reached in a next future.

References

1. Headache Classification Subcommittee of the International Headache Society. (Cephalalgia, 24 - S 1) 2nd. Oxford: Blackwell Publishing; 2004. The international classification of headache disorders.
2. Waeber C, Moskowitz MA. Therapeutic implications of central and peripheral neurologic mechanisms in migraine. Neurology. 2003;61:S9–20.
3. Faria LC, Mody I. Protective effect of Ifenprodil against spreading depression in the mouse entorhinal cortex. J Neurophysiol. 2004;92:2610–4.
4. Storer RJ, Akerman S, Goadsby PJ. Calcitonin gene-related peptide (CGRP) modulates nociceptive trigeminovascular transmission in the cat. Br J Pharmacol. 2004;142:1171–81.
5. Christensen JK, Varming T, Ahring PK, Jorgensen TD, Nielsen EO. *In vitro* characterization of 5-carboxyl-2,4-di-benzamidobenzoic acid (NS3763), a noncompetitive antagonist of GLUK5 receptors. J Pharmacol Exp Ther. 2004;309:1003–10.
6. Nicolodi M, Sicuteri F. Negative modultors of excitatory amino acids in episodic and chronic migraine: preventing and reverting chronic migraine. Special lecture 7th INWIN Congress. J Clin Pharmacol Res. 1998;18:93–100.
7. Welch KM, D'Andrea G, Tepley N, Barkley G, Ramadan NM. The concept of migraine as a state of central neuronal hyperexcitability. Neurol Clin. 1990;8:817–28
8. Vachharajani NN, Shyu WC, Nichola PS, Boulton DW. A pharmacokinetic interaction study between butorphanol and sumatriptan nasal sprays in healthy subjects: importance of the timing of butorphanol administration. Cephalalgia. 2002;22:282–7.
9. Biondi DM. Opioid resistance in chronic daily headache: a synthesis of ideas from the bench and bedside. Curr Pain Headache Rep. 2003;7:67–75.
10. Edvinsson L, Goadsby PJ. Neuropeptides in headache. Eur J Neurol. 1998;5:329–41.
11. Longmore J, Shaw D, Smith D, et al. Differential distribution of 5HT1D- and 5HT1B-immunoreactivity within the human trigemino-cerebrovascular system: implications for the discovery of new antimigraine drugs. Cephalalgia. 1997;17:833–42.
12. Bouchelet I, Cohen Z, Case B, Seguela P, Hamel E. Differential expression of sumatriptan-sensitive 5-hydroxytryptamine receptors in human trigeminal ganglia and cerebral blood vessels. Mol Pharmacol. 1996;50:219–23.
13. Shepheard SI, Williamson DJ, Beer MS, Hill RG, Heargreaves RG. Differential effects of 5-HT1B/1D receptor agonists on neurogenic dural plasma extravasation and vasodilation in anaesthetized rats. Neuropharmacology. 1997;36:525–33.
14. Moskowitz MA. Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol Sci. 1992;13:307–11.
15. Goadsby PJ, Edvinsson L, Ekman R. Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann Neurol. 1990;38:183–6.
16. Shepheard SI, Edvinsson L, Cumberbatch M, et al. Possible antimigraine mechanisms of action of the $5HT_{1F}$ receptor agonist LY334370. Cephalalgia. 1999;19:851–8.
17. Goldstein DJ, Roon KI, Offen WW, et al. Selective seratonin 1F ($5\text{-}HT_{1F}$) receptor agonist LY334370 for acute migraine: a randomised controlled trial. Lancet. 2001;358:1230–4.
18. Ramadan MM, Skljarevski V, Phebus IA, Johnson KW. $5\text{-}HT_{1F}$ receptor agonists in acute migraine treatment: a hypothesis. Cephalalgia. 2003;23:776–85
19. Lambert GA, Hoskin KL, Zagami AS. Nitrergic and glutamatergic neuronal mechanisms at the trigeminovascular first-order synapse. Neuropharmacology. 2004;47:92–105.
20. Ma QP. Co-localization of $5\text{-}HT_{1B/1D/1F}$ receptors and glutamate in trigeminal ganglia in rats. Neuroreport. 2001;12:1589–91.
21. Levy D, Jakubowski M, Burstein R. Disruption of communication between peripheral and central trigeminovascular neurons mediates the antimigraine action of $5HT_{1B/1D}$ receptor agonists. Proc Natl Acad Sci USA. 2004;101:4274–9.
22. Burstein R, Jakubowski M. Analgesic triptan action in an animal model of intracranial pain: a race against the development of central sensitization. Ann Neurol. 2004;55:27–36.
23. Hoskin KL, Lambert GA, Donaldson C, Zagami AS. The $5\text{-hydroxytryptamine}_{1B/1D/1F}$ receptor agonists eletriptan and naratriptan

inhibit trigeminovascular input to the nucleus tractus solitarius in the cat. Brain Res. 2004;998:91–9.
24. Lee WS, Limmroth V, Ayata C. In: Toward migraine. Ross FC, et al., editors. Amsterdam: Elsevier; 1996. pp. 289–93.
25. Freitag FG, Collins SD, Carlson HA, et al. Migraine Study Group. A randomized trial of divalproex sodium extended-release tablets in migraine prophylaxis. J Neurol. 2002;58:1652–9.
26. Freitag FG. Preventative treatment for migraine and tension-type headaches: do drugs having effects on muscle spasm and tone have a role? CNS Drugs. 2003;17:373–81.
27. Knight YE, Bartsch T, Goadsby PJ. Trigeminal antinociception induced by bicuculline in the periaqueductal gray (PAG) is not affected by PAG P/Q-type calcium channel blockade in rat. Neurosci Lett. 2003;16:113–6.
28. Kondo E, Kiyama H, Araki T, Shida T, Ueda Y, Tohyama M. Coexpression of GABAA receptor gamma 1 and gamma 2 subunits in the rat trigeminal ganglion. Brain Res Mol Brain Res. 1994;21:363–7.
29. Hering-Hanit R. Baclofen for prevention of migraine. Cephalalgia. 1999;19:58–929.
30. Hering-Hanit R, Gadoth N. Baclofen in cluster headache. Headache. 2000;40:48–51.
31. Piomelli D, Beltramo M, Giuffrida A, Stella N. Endogenous cannabinoid signaling. Neurobiol Dis. 1998;5:462–73.
32. Wagner JA, arga K, Ellis EF, Rzigalinski BA, Martin BR, Kunos G. Activation of peripheral CB_1 cannabinoid receptors in haemorrhagic shock. Nature. 1997;390:518–21
33. Varga K, Wagner JA, Bridgen DT, Kunos G. Platelet- and macrophage-derived endogenous cannabinoids are involved in endotoxin-induced hypotension. FASEB J. 1998;12:1035–44.
34. Zygmunt PM, Petersson J, Andersson DA, et al. Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature. 1999;400:452–7.
35. Pertwee RG. Pharmacology of cannabinoid CB_1 and CB_2 receptors. Pharmacol Ther. 1997;74:129–80.
36. Maione S, Oliva P, Marabese I, et al. Periaqueductal gray matter metabotropic glutamate receptors modulate formalin-induced nociception. Pain. 2000;85:183.
37. Martin WJ, Coffin PO, Attias E, Balinsky M, Tsou K, Walker JM. Anatomical basis for cannabinoid-induced antinociception as revealed by intracerebral microinjections. Brain Res. 1999;822:237–42.
38. Ahluwalia J, Urban L, Capogna M, Bevan S, Nagy I. Cannabinoid 1 receptors are expressed in nociceptive primary sensory neurons. Neuroscience. 2000;100:685–8.
39. Hohmann AG, Herkenham M. Cannabinoid receptors undergo axonal flow in sensory nerves. Neuroscience. 1999;92:1171–5.
40. Calignano A, La Rana G, Giuffrida A, Piomelli D. Control of pain initiation by endogenous cannabinoids. Nature. 1998;394:277–88.
41. Richardson JD, Kilo S, Hargreaves KM. Cannabinoids reduce hyperalgesia and inflammation via interaction with peripheral CB1 receptors. Pain. 1998;75:111–9.
42. Li J, Daughters RS, Bullis C, Bengiamin R, Stucky MW, Simone DA. The cannabinoid receptor agonist WIN 55,212-2 mesylate blocks the development of hyperalgesia produced by capsaicin in rats. Pain. 1999;81:25–33.
43. Martin WJ, Loo CM, Basbaum AI. Spinal cannabinoids are anti-allodynic in rats with persistent inflammation. Pain. 1999;82:199–205.
44. Caterina MJ, Schumacher MA, Tominaga M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature. 1997;389:816–24.
45. Tognetto M, Amadesi S, Harrison S, et al. Anandamide excites central terminals of dorsal root ganglion neurons *via* vanilloid receptor-1 (VR-1) activation. J Neurosci. 2001;15:1104–9.
46. Tsuchiya M, Sakakibara A, Yamamoto M. A tachykinin NK_1 receptor antagonist attenuates the 4ß-phorbol-12-myristate-13-acetate-induced nociceptive behaviour in the rat. Eur J Pharmacol. 2005;507:29–34
47. Trevisani M, Smart D, Gunthorpe MJ, et al. Ethanol elicits and potentiates nociceptor responses via the vanilloid receptor-1. Nat Neurosci. 2002;5:546–51.
48. Steinhoff M, Buddenkotte J, Shpacovitch V, et al. Proteinase-activated receptors: transducers of proteinase-mediated signaling in inflammation and immune response. Endocr Rev. 2005;26:1–43.
49. Dai Y, Moriyama T, Higashi T, et al. Proteinase-activated receptor 2-mediated potentiation of transient receptor potential vanilloid subfamily 1 activity reveals a mechanism for proteinase-induced

inflammatory pain. J Neurosci. 2004;5:4293–9.
50. Russo EB. Clinical endocannabinoid deficiency (CECD): can this concept explain therapeutic benefits of cannabis in migraine, fibromyalgia, irritable bowel syndrome and other treatment-resistant conditions? Neuroendocrinol Lett. 2004;25:31–9. 51. Amenta V, Pitari GM, Caff M, et al. In: Headache patogenesis, monoamines, neuropeptides, purines and nitric oxide. Olesen J, Edvinsson L, editors. Vol. 7. Philadelphia: Lippincott-Raven Press; 1997. p. 107.
52. Bianchi A, Tognetto M, Salomone S, et al. The Vanilloid Receptors: Research of new agonists. Recent Res Dev Life Sci. 2004;2:99–109.
53. Cichewitz DL. Synergistic interactions between cannabinoid and opioid analgesics. Life Sci. 2004;74:1317–24.
54. Lamey, Philip-john, Lundy, Fionnuala Teresa, Shaw, Christopher, Migraine and vasodilation treatment, *United States Patent 2008, 7396649*
55. Chissoe, Stephanie, 2008, Genes Associated With Migraine, *United States Patent Application 2008, 0108078*
56. Frants, Rune Robert Isak Erik, Ferrari, Michel Dominique, Terwindt, Gisela Marie, Ophoff, Roel Andre. Gene related to migraine in man, *United States Patent Application 2005, 0090658*
57. Lesniewski, Richard R., Semizarov, Dimitri, Van Sant, Charles L. Companion diagnostic assays for endothelin receptor antagonists *United States Patent Application 2008, 0102451*
58. Sudilovsky, Abraham Method for inhibiting onset of or treating migraine headaches employing an ACE inhibitor *United States Patent H000734 1990*
59. Barlow, Carolee, Carter, Todd A., Treuner, Kai, Lorrain, Kym I. Neurogenesis By Modulating Angiotensin, *United States Patent Application 2008, 0167291*
60. Wolff HG. Headache and other head pain. New York: Oxford University Press; 1993.
61. Ninck B. Migraine and epilepsy. Eur Neurol. 1970;3:168–78
62. Sicuteri F, Fanciullacci M, Anselmi B. Mast cells and their active substances: their role in the pathogenesis of migraine. Int Arch Allergy. 1963;7:88–92.
63. Sicuteri F. Headache as possible expression of deficiency of brain 5-hydroxytryptamine (Central Denervation Supersensitivity) Headache. 1972;12:69–72.
64. Sicuteri F. Migraine: a central biochemical dysnociception. Headache. 1976;16:145–9.
65. Hoskin KL, Bulmer DC, Goadsby PJ. Fos expression in the trigeminocervical complex of the cat after stimulation of the superior sagittal sinus is reduced by L-NAME. Neurosci Lett. 1999;266:173–6
66. Leao AA. Spreading depression of activity in the cerebral cortex. Arch Neurol Psychiatry. 1941;46:333–9.
67. Lauritzen M. Pathophysiology of the migraine aura the spreading depression theory. Brain. 1994;117:199–210.
68. Olesen J, Friberg L, Olsen TS, et al. Timing and topography of cerebral blood flow, aura, and headache during migraine attacks. Ann Neurol. 1990;28:791–8.
69. Cutrer FM, Sorensen AG, Weisskoff RM, Ostegaard L, Sanchez del Rio M, Lee EJ. Perfusion-weighted imaging defects during spontaneous migrainous aura. Ann Neurol. 1998;43:25–31.
70. Lodi R, Iotti S, Cortelli P, et al. Deficient energy metabolism is associated with low free magnesium in the brains of patients with migraine and cluster headache. Brain Res Bull. 2001;54:437–41.
71. Gallai V, Sarchielli P, Coata G, Firenze C, Morucci P, Abritti G. Serum and Salivary magnesium levels in migraine: results in a group of juvenile patients. Headache. 1992;32:132–5.]
72. Lance JW. The pathophysiology of migraine: a tentative synthesis. Pathol Biol. 1992;40:355–60.
73. Diener HC, May A. New aspects of migraine patho-. physiology: lessons learned from positron emission tomography. Curr Opin Neurol. 1996;9:199–201.
74. Welch KMA, Cao Y, Aurora S, Wiggings G, Vikingstad EM. MRI of the occipital cortex, red nucleus and substantia nigra during a aura of migraine. Neurology. 1998;51:1465–9.
75. Giacovazzo M, Martelletti P, Valeri M, Piazza A, Casciani CU. A new immunological aspect of cluster headache: the increase of monocyte and NK cell populations. Headache. 1986;26:134–6.
76. Waldenlind E, Bussone G. In: The headaches. 2nd. Olesen J, Tfelt-Hansen P, Goadsby PJ, editors. Philadelphia: Lippincott-Williams and Wilkins; 2000. pp. 687–95.
77. Leone M, Franzini A, Bussone G. Stereotactic stimulation of posterior

hypothalamic gray matter in a patient with intractable cluster, headache. N Engl J Med. 2001;345:1428–9.
78. Colucci d'Amato C, Alfano V, Giordano E, Marmolo T, Pizza V. Le Cefalee. 2nd. Napoli: Idelson; 1997.
79. Montagna P. Molecular genetics of migraine headaches: a review. Cephalalgia. 2000;20:3–14.

TABLE 1. Substances released during inflammation able to activate or sensitize nociceptors

SUBSTANCE	SOURCE	ACTIVITY ON PRIMARY AFFERENT FIBRES
K+ or H+	Injured cells	Activation
Serotonin	Platelets	Activation
Bradykinin	Plasmatic kyninogen	Activation
Histamine	Mast cells	Activation
Prostaglandins	Injured cells, Macrophages	Sensitization
Substance P or Neurokinin A	Primary afferent fibres	Sensitization

TABLE 2. Receptors present at the level of sensory primary neurons and possible effects on the release of neuropeptides involved in neurogenic inflammation and migraine pain.

RECEPTORS	LIGANDS	ACTIVITY ON THE SENSORY PRIMARY NEURON
5-HT1-D	SEROTONIN	DECREASED RELEASE OF CGRP AND SUBSTANCE P
GABA-A	GABA	DECREASED RELEASE OF CGRP AND SUBSTANCE P
CB-1	ANANDAMIDE*	DECREASED RELEASE OF CGRP AND SUBSTANCE P
VR-1	CAPSAICIN OR ANANDAMIDE**	INCREASED RELEASE OF CGRP AND SUBSTANCE P
PAR-2	PEPTIDIC CHAINS SLIGRL AND SLIGKV	INCREASED RELEASE OF CGRP AND SUBSTANCE P

FIGURE 1. Role Of The Trigeminal Neuron In The Migraine Attack

Attack eliciting factors at the sub-cortical level provoke dilatation of cranial vessels with the activation of trigeminal sensory afferent endings and a consequent neurogenic inflammation process, due to the release of CGRP and SP or NK-A..

BK: bradykinin; NO: nitric oxide; CGRP: calcitonin gene-related peptide; SP: substance P;

NK-A: neurokinin A.