human primary breast cancer[7], and NF-κB, which is activated in most breast cancer samples investigated to date[8]. Further research is needed to elucidate the upstream components of the pathway (or pathways) that activate cyclin D1 and NF-κB in mammary cancer.

Although RANKL is believed to be involved in physiological mammary gland proliferation, it does not appear to be an important player in breast cancer—its expression was not detectable in human primary mammary tumors[9]. Experiments with tumor cell lines[10] and transgenic mice[7] indicate that receptor tyrosine kinases (epidermal growth factor receptor, *Neu*/HER2) and the *ras* oncogene are important for the activation of cyclin D1 and/or NF-κB. Such studies have suggested that inhibitors of cyclin D1 (ref. 7) or IKK (ref. 10) might provide drugs for breast cancer therapy. If the finding that cyclin D1 expression is blocked by selective inhibition of IKK-α proves to be relevant to human breast cancer, IKK-α will provide a promising therapeutic target.

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From CSD to headache: A long and winding road

Migraine sufferers experience pounding headaches, which sometimes are preceded by a visual aura. Now Bolay *et al.* show that cortical-spreading depression, the cause of the aura, activates trigeminal afferents, which act to cause inflammation of the pain-sensitive meninges, generating the headache. (pages 136–142)

COSTANTINO IADECOLA

Migraine is a debilitating illness that afflicts 10–15% of men and women worldwide. Patients experience a pounding headache associated with nausea, vomiting and intolerance to bright light and loud noises[1,2]. In 15–20% of migraine sufferers, the headache is preceded by an 'aura', which typically consists of flashing lights or shiny angular shapes that slowly drift across the visual field[2], and lasts for about 20–30 minutes. Toward the end of the aura, the flashing lights become transient blind spots. There is increasing evidence that the aura is the result of cortical spreading depression (CSD), a wave of neuronal depolarization that spreads across the cerebral cortex[3]. Although it has long been hypothesized that the aura is causally related to the headache, evidence for a mechanism linking the two has been lacking[3]. Now, on page 136–142, Bolay *et al.*[4] provide evidence that CSD activates trigeminal afferents that, through central and peripheral reflex mechanisms, cause inflammatory changes in the pain-sensitive meninges to generate the headache. These findings finally provide a link between CSD and the meningeal alterations underlying the headache.

In animals, CSD occurs in response to a localized painful stimulus to the cerebral cortex, such as a pinprick, and is characterized by a band of neuronal and glial depolarization that propagates on the cortical surface at a speed of 2–3 mm/min, followed by a long-lasting suppression of neural activity[3]. The depolarization phase is associated with an increase in cerebral blood flow, whereas the phase of reduced neural activity is associated with a reduction in flow[3]. The idea that the migraine aura was related to CSD, introduced by A. Leão in 1944, was supported by earlier descriptions that the visual symptoms of the aura migrate across the visual field at a speed consistent with CSD propagation[3]. The positive phenomena of the visual aura (flashing lights), were thought to be related to the depolarization phase of CSD, whereas the negative phenomena (blind spots), were thought to reflect the phase of reduced neural activity. The relevance of CSD to the migraine aura was questioned because CSD had not been observed in the human brain, for example, during neurosurgical manipulations known to elicit CSD in other species[2].

There has been increasing evidence to indicate that the aura arises from CSD and the migraine headache from trigeminal-induced meningeal inflammation. Migraine is associated with a propagating reduction in cortical blood flow that resembles the cerebrovascular changes produced by CSD (ref. 3). More recently, functional brain imaging and magnetoencephalography in patients experiencing the aura have clearly shown that the cerebrovascular and magnetic field correlates of CSD occur in the cortex of migraineurs[5,6]. Advances have also occurred in the understanding of the neurobiology of the headache that follows the aura[7]. Unlike the brain parenchyma, meninges and cerebral blood vessels are pain-sensitive structures, and are heavily innervated by the trigeminal nerve. Activation of trigeminal fibers causes an inflammatory reaction in the meninges, which is initiated by the release of inflammatory mediators from trigeminal nerve endings and mast cells. Drugs with anti-inflammatory properties, such as steroids and non-steroidal anti-inflammatory agents, alleviate the headache; and classical anti-migraine drugs, such as sumatriptan, attenuate meningeal inflamma-

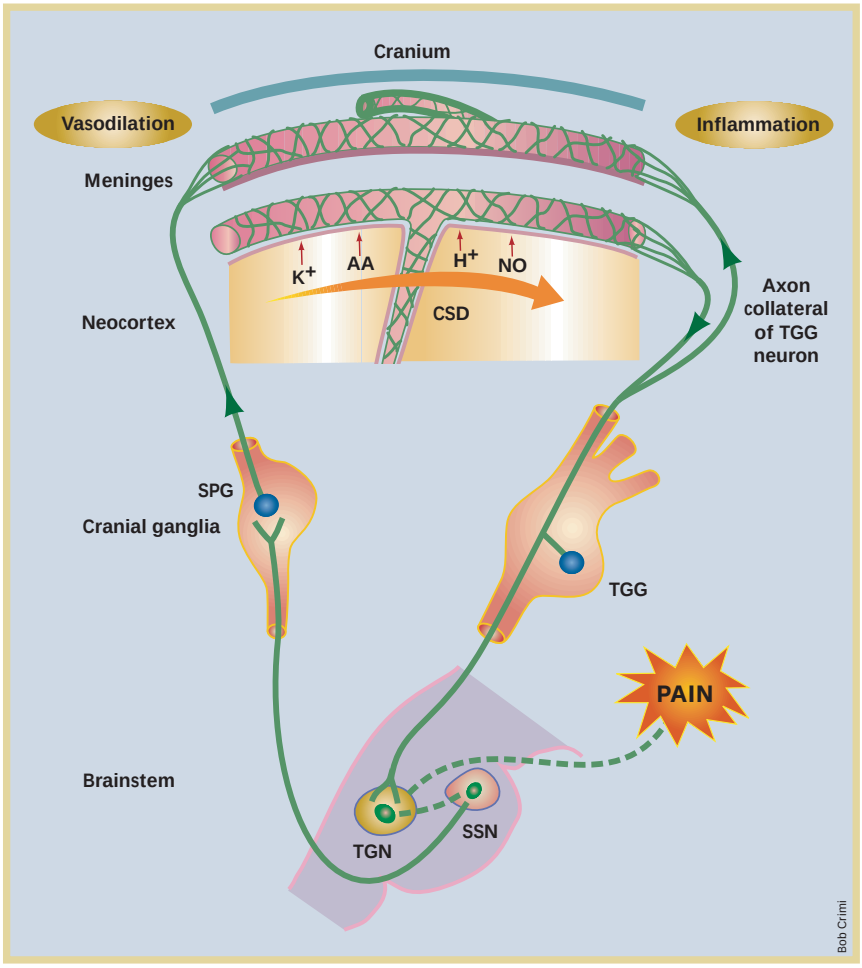

Fig. 1 The relationship between CSD and headache in migraine with aura. CSD releases H^+, K^+ and other agents, including arachidonic acid (AA) and nitric oxide (NO), in the extracellular space of the neocortex. These agents diffuse toward local blood vessels and depolarize perivascular trigeminal terminals that, in turn, causes activation of the caudal portion of the trigeminal nucleus (TGN) in the brainstem. At the same time, collateral axons of activated neurons in the trigeminal ganglion (TGG) release pro-inflammatory peptides in the meninges and their vessels, leading to a local inflammatory reaction. The activation of TGN caused by CSD produces vasodilation of meningeal vessels through a pathway originating from the SSN and reaching meningeal blood vessels via the sphenopalatine ganglion (SPG). The perception of pain is mediated by higher-order projections from the TGN. The dashed lines between TGN, SSN and regions generating the pain indicate that these connections are either unknown or have not been depicted.

tion in animal models and release of inflammatory peptides in migraine patients[7,8].

Despite these advances in the understanding of the mechanisms of the aura and the headache, the relationship between the two remained unclear[3]. It was unknown how the local neurophysiological perturbations produced by CSD could evoke the delayed meningeal inflammation mediating the headache. Bolay *et al.*[4] begin to fill this gap by demonstrating that CSD, evoked in the rat cerebral cortex by a pinprick or local electrical stimulation, activates trigeminal nerve terminals on cerebral blood vessels and increases neural activity in the ipsilateral trigeminal nucleus (Fig. 1). Importantly, they find that the trigeminal activation produced by CSD causes inflammation in the meninges that occurs after the CSD has subsided. Lesion experiments indicated that the inflammatory changes (vasodilation, edema and protein extravasation) are mediated through reflex mechanisms integrated both within and outside the brain[4]. The vasodilation was caused by release of vasoactive agents from neurovascular projections originating from the superior salivatory nucleus, whereas the protein extravasation was mediated through release of pro-inflammatory peptides from axon collaterals of trigeminal ganglion neurons that innervate the meninges (Fig. 1). Administration of a neurokinin-1 receptor inhibitor attenuated the meningeal changes, suggesting that these receptors mediate inflammation. Moreover, meningeal vasodilatation did not occur if CSD was prevented by treating the injured cortex with a glutamate receptor antagonist[4]. This indicates that CSD, and not cortical injury, causes trigeminal activation, vasodilation and, presumably, inflammation. These data, together with earlier work, point to CSD as a critical event in the mechanisms of migraine with aura, and support the emerging notion that CSD is a valid therapeutic target [9].

Several major issues remain to be resolved. First, what triggers CSD? It has been suggested that alterations in the excitability of the visual cortex could induce CSD and initiate the migraine cascade[3]. Although this seems conceivable in migraines precipitated by visual stimuli as the stimulus-induced increase in extracellular K^+ in the visual cortex could facilitate CSD, in other cases, the mechanisms triggering CSD remain obscure. Studies investigating whether there is a genetic basis for the aura may provide useful insights[1]. Second, in the majority of patients the headache is not preceded by an aura, and it is unclear whether the aura is an absolute requirement for the headache. Although it is possible that in most patients the aura is clinically silent, there is also experimental evidence that, in contrast to the findings of Bolay *et al.*[4], the aura (CSD) and the headache (meningeal inflammation) are unrelated[10]. Third, how accurately does a rodent model mimic the human disease, and can this model be used to develop new therapies? In addition to the validity of the model, species differences in the action of pain medicines that antagonize the neurokinin-1 receptor[11] may also confound the issue. Although there are many questions that await answers, the work of Bolay *et al.*[4] represents a step forward in our understanding of the link between aura and migraine headache. It suggests a mechanism by which painless neural events initiated within the substance of the brain can produce pain through central and peripheral reflexive actions resulting in meningeal inflammation.

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A new piece in the diabetes puzzle

Increased levels of circulating triglyceride and free fatty acids are common features of diabetic dyslipidemia. Positional cloning has led to the identification of a liver-derived protein, angiopoietin-like protein 3, that is largely responsible for diabetic dyslipidemia in an animal model of type 2 diabetes mellitus.

LUCIANO ROSSETTI[1] & IRA J. GOLDBERG[2]

Alterations in circulating lipoproteins have long been recognized as a major determinant of atherosclerotic cardiovascular disease (ASCVD) in humans. Individuals with type 2 diabetes mellitus (DM2) have a markedly enhanced risk for ASCVD. Despite improved therapeutic intervention in lipid abnormalities, the consequences of accelerated ASCVD in DM2 remain devastating and largely account for increased mortality in this population. Thus, a better understanding of the molecular and biochemical determinants of diabetic dyslipidemia, which would result in possible novel approaches to treatment, is sorely needed. A study that appears in the February issue of *Nature Genetics*[1] provides a step in this direction by describing a gene (*Angptl3*) that plays a central role in regulating lipid metabolism in mice.

The typical alteration in lipoprotein profiles in DM2 is an elevation in very-low-density lipoprotein (VLDL, a liver-derived triglyceride-rich lipoprotein), accompanied by a decrease in high-density lipoprotein (HDL, often referred to as 'good cholesterol'). These DM2-associated lipid profiles partly account for the increased prevalence of ASCVD in the DM2 population[3]. The other main source of triglycerides in the circulation is the chylomicron population. These particles carry dietary triglyceride and circulate primarily during the postprandial period. Triglycerides within VLDL and chylomicrons undergo lipolysis—a step required for liberating free fatty acids (FFA) that are then taken up by tissues (Fig. 1*a*). The resultant lipoproteins—low-density lipoproteins (LDL) and chylomicron remnants—are removed from the circulation primarily via receptors in the liver.

The KK obese mouse is a model of DM2 which also features hyperlipidemia primarily due to an increase in VLDL. Using positional cloning, Koishi *et al.*[1] have identified a recessive mutation in a mutant strain of KK mice (KK/San) that is resistant to hyperlipidemia. The authors demonstrate that the hepatic protein encoded by the gene, previously named angiopoietin-like protein 3 (Angptl3), regulates lipoprotein metabolism in diabetic mice. Adenoviral expression of Angptl3 and infusion of recombinant protein into the non-hyperlipidemic KK/San strain of mice confirms that the mutation in *Angptl3* can account for their resistance to hyperlipidemia. The conclusion from the elegant genetic analysis and *in vivo* reconstitution experiments is that Angptl3 is required for the development of diabetic dyslipidemia in KK mice.

A pivotal role of Agptl3 in modulating lipoprotein metabolism in mice is strongly supported by these findings. However, the mechanism by which Angptl3 increases the levels of circulating triglycerides and FFA—generated by lipolysis of adipose tissue triglyceride by hormone sensitive lipase—and of circulating triglycerides is open to speculation.

Based on the results presented by Koishi *et al.*, the hypolipidemic effect of the *Angptl3* mutation in the KK/San mice results from a relatively minor (15%) decrease in triglyceride production by the liver (Fig. 1*a*) and a more robust (85%) increase in triglyceride clearance. A small portion of the removal of triglyceride from the circulation involves the uptake of intact lipoproteins mediated by the LDL receptor and the LDL receptor-related protein (LRP). However, it should be pointed out that inhibition of both of these receptors, and not just one, is necessary to produce commensurate elevations of triglyceride and cholesterol[4].

The rapid increase primarily in plasma triglyceride levels in KK/San mice injected with recombinant Angptl3 suggests that this protein blocks lipolysis of triglyceride-rich lipoproteins. Angptl3 may induce an endogenous inhibitor of lipolysis, such as the apolipoproteins CI and CIII or ApoE (refs. 5,6), but the rapidity of the effect makes this scenario unlikely. A more likely possibility is that Angptl3 itself is a lipolysis inhibitor, either by attaching to the lipoprotein and preventing its lipolysis or by directly inhibiting the rate-limiting enzyme for the lipolysis of triglyceride-rich lipoproteins, LpL (Fig. 1*b*)[7].

LpL is synthesized in adipocytes and myocytes but must transfer to the endothelial cell and then be positioned on the luminal surface to interact with circulating lipoproteins. Similar to the mutation in *Angptl3* in the KK/San mice, overexpression of LpL reverses