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Editor-in-Chief: ***JAMES A. BRISTOL***

PARKE-DAVIS PHARMACEUTICAL RESEARCH
DIVISION OF WARNER-LAMBERT COMPANY
ANN ARBOR, MICHIGAN

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CONTENTS

VII. TRENDS AND PERSPECTIVES

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PREFACE

Annual Reports in Medicinal Chemistry continues to strive to provide timely and critical reviews of important topics in medicinal chemistry together with an emphasis on emerging topics in the biological sciences which are expected to provide the basis for entirely new future therapies.

Volume 32 retains the familiar format of previous volumes, this year with 31 chapters. Sections I - IV are disease oriented and generally report on specific medicinal agents with updates from Volume 31 on antithrombotics, endothelin, neurokinin antagonists, cell cycle regulation, and obesity. As in past volumes, annual updates have been limited to only the most active areas of research in favor of specifically focussed and mechanistically oriented chapters, where the objective is to provide the reader with the most important new results in a particular field. To this end, chapters on topics not reported in at least five years include: migraine therapy, Alzheimer's disease, melatonin, bacterial resistance, bacterial genomics, antiretroviral resistance antifungals, angiogenesis, T lymphocyte potassium channel blockers, male contraception, psoriasis, selective PGHS2 inhibitors, and growth hormone secretagogues.

Sections V and VI continue to emphasize important topics in medicinal chemistry, biology, and drug design as well as the critical interfaces among these disciplines. Included in Section V, Topics in Biology, are chapters on regulation of gene expression, blockade of TNF-α, and nuclear orphan receptors. Each of these areas is likely to lead to novel medicinal agents in the future. Chapters in Section VI, Topics in Drug Design and Discovery, reflect the current focus on mechanism-directed drug discovery and newer technologies. These include chapters on combinatorial mixtures as discovery tools, mass spectrometry of non-covalent adducts, nonpeptide agonists of peptide receptors, natural products, and cytochrome P-450.

Volume 32 concludes with To Market, To Market - a chapter on NCE introductions worldwide in 1996. In addition to the chapter reviews, a comprehensive set of indices has been included to enable the reader to easily locate topics in volumes 1-32 of this series.

Over the past year, it has been my pleasure to work with 6 highly professional section editors and 70 authors, whose critical contributions comprise this volume.

James A. Bristol
Ann Arbor, Michigan
May 1997

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SECTION I. CENTRAL NERVOUS SYSTEM DISEASES

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Chapter 1. Recent Advances in Migraine Therapy

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Introduction - The search for safe and effective treatments for migraine pain has accelerated in the pharmaceutical industry since the introduction of sumatriptan (1) in the marketplace. This chapter will highlight the proposed mechanisms of action of this acute therapy, the various approaches to improve both efficacy and safety, and future areas of study.

Mechanisms and Models of Migraine - The etiology of migraine is not certain although there are a set of hypotheses concerning various stages of the process of migraine. Migraine is thought to be initiated by a "trigger" of unknown origin. Environmental and physiological factors impinge upon this trigger to set in action the events leading to the migraine episode. It may be that migraineurs have an underlying "neural instability" which facilitates setting off the trigger (1). Once initiated, there are several components. The earliest phase may be a premonitory symptom the evening before a migraine headache. The next may be "aura" consisting of visual field disturbances which may be related to changes in cerebral blood flow; these blood flow changes may alter cortical perfusion. Ultimately, the headache appears. Headache pain has been attributed to several factors including dilation of the cerebral blood vessels (2), cerebral edema (3), neurogenic inflammation (4), and central transmission of pain in the brain itself (5).

The early animals models of migraine were based on the study of vascular changes in response to test compounds. The contraction of isolated blood vessels, such as the dog saphenous vein, was used to discover the activity of sumatriptan (6). Other vascular responses such as blood shunting through the arterial-venous anastomoses of the intact pig using radiolabeled microspheres have also been employed (7). More recently a model based on a "neurogenic" hypothesis has been widely explored (8,9). In this model, the trigeminal ganglion is stimulated electrically, which leads to release of neurotransmitters from the peripheral nerve terminals surrounding blood vessels in the dura. This leads to an increased permeability of the vessels which can be monitored by use of a tracer.

Serotonin Receptor Subtypes - Serotonin (5-HT) receptors have been divided into seven classes based upon pharmacological, structural and signaling properties (10). They are: five 5-HT1 receptor subtypes, three 5-HT2 receptor subtypes, one 5-HT3 receptor, one 5-HT4 receptor, two 5-HT5 receptor subtypes, one 5-HT6 receptor and

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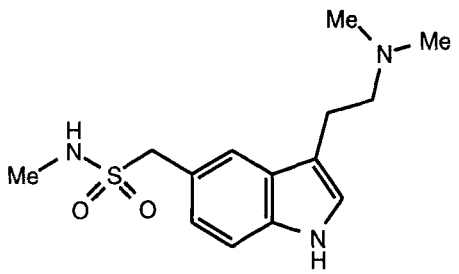
one 5-HT7 receptor. Splice variants or additional subtypes are likely for the 5-HT2C (11) and 5-HT2A (12) and have already been demonstrated for the 5-HT3 (13), 5-HT4 (14), and 5-HT7 receptors (15). The 5-HT3 receptor is a member of the ligand-gated ion channel superfamily (16). All other cloned 5-HT receptors are members of the G-protein coupled receptor superfamily. The pharmacological properties of these receptors have been extensively reviewed elsewhere (10,17). Only the 5-HT1 family has been implicated in the acute treatment of migraine. The five 5-HT1 receptors are termed 5-HT1A, 5-HT1B (formerly called 5-HT1Dβ), 5-HT1D (formerly called 5-HT1Dα), 5-HT1E and 5-HT1F (10,17) and they each contain comparable amino acid chain lengths (421, 377, 390, 365 and 366 respectively for the human sequences). These proteins all share a "homology cluster" displaying mutual conservation of greater than half of the amino acid residues in their transmembrane spanning regions which are likely to contain the ligand binding pocket (18,19). In contrast, the extracellular amino termini and loop regions and carboxyl termini lack significant homology that would enable prediction of their common intracellular coupling to second messengers. All 5-HT1 receptors couple to G-proteins which leads to inhibition of adenylate cyclase activity (10). The 5-HT1A (20), 5-HT1B (21), 5-HT1D (21), and 5-HT1F (22) subtypes also modulate intracellular Ca^{++} through PTX-sensitive G-proteins. In addition to these pathways, each of the 5-HT1 receptors, except 5-HT1E (23), has been shown to increase the turnover of inositol phosphates (21,22,24,25). Physiologically, these receptors may act through G-protein-gated channels such as Girk1 (26). The 5-HT1B, 5-HT1D and 5-HT1F all are thought to inhibit the release of neurotransmitters.

Role of 5-HT1B/1D/1-like Receptors - Sumatriptan (1) was developed using classical pharmacological methodology employing the contraction of the dog saphenous vein preparation (27). As modern receptor binding and classical pharmacological methods began to be reconciled, it appeared there was a relationship between the "5-HT1-like" receptor of the dog saphenous vein and the 5-HT1D receptor described in binding studies (28). Sumatriptan became a selective tool to study 5-HT1D receptors. The advent of molecular cloning further complicated interpretation of these data because two separate genes encoding 5-HT1D receptors were discovered (29), each with high affinity for sumatriptan. Further, in the rat and mouse, one the these receptors, 5-HT1Dβ, had a very different pharmacological profile and was known as a separate subtype, 5-HT1B. Molecular cloning demonstrated that the 5-HT1Dβ and 5-HT1B gene were species homologs (30,31). Their deduced amino acid sequences were 93% identical over the entire sequence and 96% identical in the transmembrane domains. In fact, a single amino acid difference pinpointed to TM VII (T355N) appears to be responsible for the disparity in the pharmacological profiles of these human and rat 5-HT receptor subtypes (32). The complications in the receptor nomenclature have led to a recent revision (32) and the 5-HT1Dβ receptor is now known as 5-HT1B. Both 5-HT1B and 5-HT1D receptors are distributed in the brain, where they appear to have primarily presynaptic localization (34). In several preparations, these receptors inhibit the release of serotonin from either terminal or somatodendritic processes (35). They also act as heteroreceptors, inhibiting the release of glutamate, acetylcholine and other neurotransmitters (36,37).



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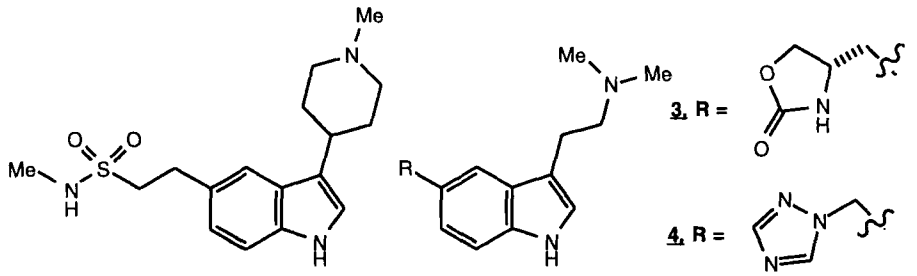
How do these receptors and the activity of sumatriptan and other 5-HT1 agonists described below relate to the models and mechanisms of migraine headache? It is clear that all 5-HT1 agonists that have been reported to be useful in the treatment of migraine, including naratriptan (2), zolmitriptan (311-C90, 3), and rizatriptan (MK 462, 4) have substantial affinity for both the human 5-HT1D and 5-HT1B receptor subtypes. It is possible that both activities are therapeutic in alleviation of the migraine episode. Several lines of evidence suggest that the 5-HT1B receptor may be the subtype most closely related to the dog saphenous vein 5-HT1-like receptor (34). Therefore, these agonists may act on 5-HT1B receptor on the smooth muscle of human cerebral blood vessels to cause vasoconstriction (similar to their activity in the dog saphenous vein). In addition, the 5-HT1-like agonists may act on 5-HT1D receptors on the terminals of the trigeminovascular system to inhibit neurotransmitter release and to thus stop the neurogenic inflammation (34,38). The clinical utility of compounds acting at only one of these two sites has not yet been reported although it has been suggested that a 5-HT1D-selective compound may be therapeutic and may reduce some of the cardiovascular liabilities of sumatriptan (38, *vide infra*).

Table 1. Affinities of Antimigraine Compounds at Cloned 5-HT1D and 5-HT1B Receptors.

Compound	pK_i 5-HT1D	pK_i 5-HT1B	Reference
Sumatriptan (1)	8.5	8.1	39
Naratriptan (2)	8.3	8.7	39
Zolmitriptan (3)	9.2	8.3	40
Rizatriptan (4)	7.7	7.3	39
CP 122,288 (5)	8.1	7.5	39
Avitriptan (8)	8.3	7.7	41
Alniditan (9)	9.0	8.7	42



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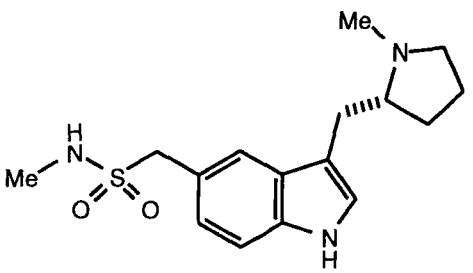
Designing an Improved Sumatriptan - While sumatriptan is effective as a migraine abortive agent, about 15% of patients fail to respond to treatment and up to 40% may suffer from recurrence of headache within 24 hours (43,44). Similar rates of headache recurrence have been observed with other antimigraine treatments. Further, the finding of decreased latency of onset of headache relief after subcutaneous *versus*

oral sumatriptan suggests a possible relationship between plasma drug concentrations and headache relief. A number of approaches for designing improvements beyond sumatriptan in migraine abortive treatment have resulted. These approaches include improvements in pharmacokinetics (45), central penetration, and binding profile for 5HT1D (46) and other receptors such as the 5HT1F (47,48).

Rizatriptan (4) has been demonstrated in preclinical studies to show both increased oral bioavailability and more rapid absorption compared to oral sumatriptan (49). These findings, supported by preliminary clinical pharmacokinetic studies, suggest that this agent might offer greater consistency of effect due to the increased bioavailability. Improvements in onset of efficacy might also occur as a consequence of the more rapid absorption (49).

The observation that sumatriptan fails to prevent headache development upon subcutaneous administration in the aura phase but shows effect after headache, possibly due to diminished integrity of the blood brain barrier during migraine episode, suggests that central penetration might be an important attribute of an effective antimigraine therapeutic (50). Zolmitriptan (3) is less hydrophilic than sumatriptan and shows both peripheral and central activities, with activity as a vasoconstrictor (peripherally mediated), as an inhibitor of trigeminally mediated neuropeptide release, and on the central components of the trigemino-vascular system within the nucleus caudalis (51). Preliminary studies suggest that oral zolmitriptan may be useful in preventing migraine when taken during aura (52), supporting the importance of central penetration.



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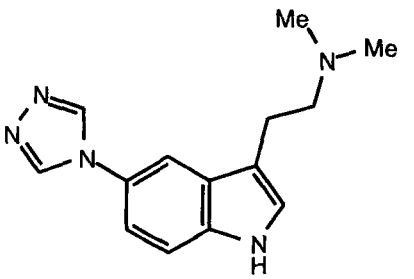

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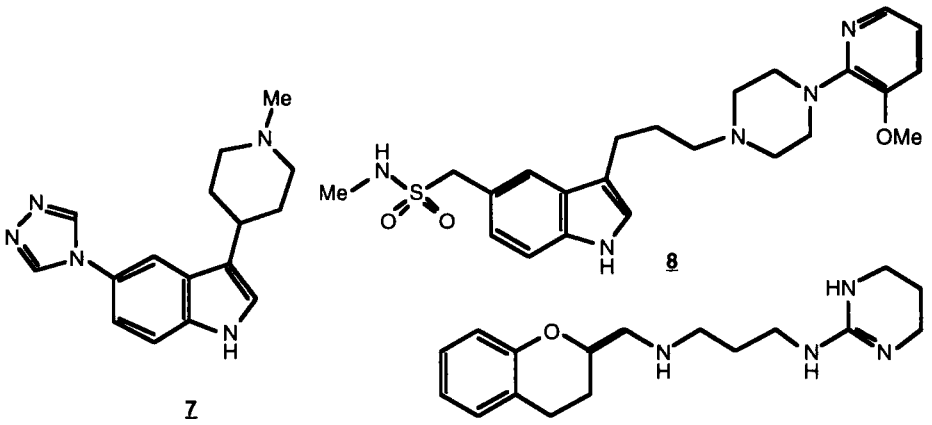
A conformationally restricted analog of sumatriptan, 5 (CP-122,288) selectively inhibits neurogenic inflammation in intracranial tissues at doses which lack vasoconstrictor effects in animal studies (53). With affinity comparable to sumatriptan for 5HT1D and 5HT1B receptors, 5 is 40,000 times more potent in blocking rat dural neurogenic plasma protein extravasation, while similarly potent to sumatriptan as a vasoconstrictor. The clinical experience with this agent should help to elucidate the relevance of each of these activities in migraine intervention. High oral availability is also found with the 5HT1D selective agonist 6 (L-741,604), which also shows reduced plasma clearance compared to either sumatriptan or rizatriptan in preclinical studies (54). The corresponding indolylpiperidine 7 (L-741,519) additionally has an increased *in vivo* half-life relative to the tryptamine analogs. This attribute might prove valuable in reducing the incidence of headache recurrence. Developed as a 5HT1B/1D agonist, avitriptan (BMS-180048, 8) selectively decreases corotid blood flow due to a reduction in arteriovenous anastomotic blood flow. This agent also potently constricts isolated human coronary artery with effects comparable to those of sumatriptan (55). Chemically unique among the serotonergic antimigraine agents, alniditan (9) is a non-

indole with high affinity for 5HT1B, 5HT1D, and 5HT1A receptors, and lacking significant affinity for the 5HT1F (*vide infra*) receptor (56).



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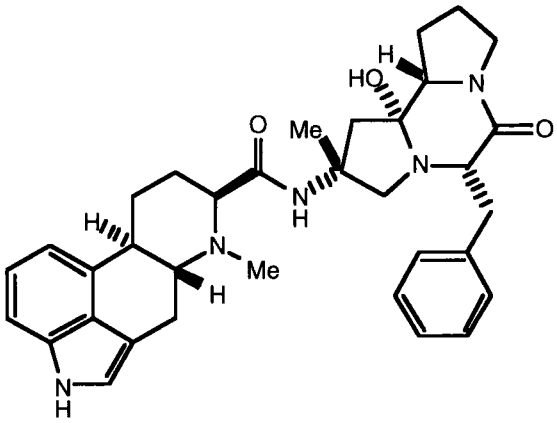
Novel Presynaptic Receptor Targets: 5-HT1F - In addition to the 5-HT1B and 5-HT1D receptors, a novel or "orphan" 5-HT receptor called 5-HT1F was cloned and characterized (57). This receptor showed many similarities to 5-HT1B and 5-HT1D receptors with respect to receptor binding properties, coupling to the inhibition of adenylate cyclase activity, and general distribution in the brain, although its abundance as reflected by the mRNA level for this receptors was relatively low. The pharmacological profile of this receptor was not easily related to any of the known pharmacological subtypes measured in native tissues. For example, the 5-HT1F receptor was characterized by the following rank order of agonist potencies: 5-HT> sumatriptan >> 5-CT, forming a unique pharmacological profile. The surprisingly high affinity and potency of sumatriptan for the 5-HT1F receptor subtype lead to speculation that sumatriptan might exert its anti-migraine action through this "orphan" subtype (57). Supporting this notion, the mRNA for this receptor was detected in the nucleus caudalis as well as in other central pain-processing nuclei (58) and was also demonstrated in the trigeminal ganglion (59).



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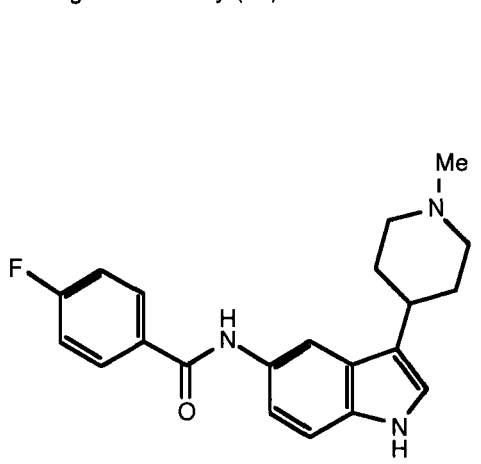
Further support for the 5-HT1F presynaptic mechanism came from a comparison of the activity of "5-HT1D/1B " agonists such as naratriptan, rizatriptan, zolmitriptan, and dihydroergotamine (DHE, 10) in human migraine and in migraine models with their affinities at the cloned human 5-HT1D, 5-HT1B, and 5-HT1F receptors (60,61). As

shown in Table 2, each compound had substantial activity at the 5-HT1F receptor in addition to affinity at the 5-HT1D and 5-HT1B sites.

Table 2. Affinities of Compounds at Cloned Human 5-HT1F Receptor (60,61).

Compound	**pK_i 5HT1F**
LY334370 (11)	8.8
LY302148 (12)	8.6
Naratriptan (2)	8.4
Zolmitriptan (3)	7.6
Sumatriptan (1)	7.6
DHE (10)	6.6
Rizatriptan (4)	6.6

Furthermore, comparison of these affinities to the potencies of the same compounds to inhibit plasma extravasation in the guinea pig neurogenic model indicated the best correlation was with the 5-HT1F receptor (60,61). Finally, selective 5-HT1F agonists were designed and evaluated in the cloned receptors as well as in the plasma extravasation model. The activity *in vivo* most closely matched predictions based on their 5-HT1F activity (47,48). Moreover, these 5-HT1F selective agonists were not potent stimulators of contraction of the rabbit saphenous vein, another of the preparations used to hunt for "5-HT1D/1B agonists" (60,61). The 5-HT1F agonist LY 334370 (11) is presently under clinical investigation and determine whether a serotonergic presynaptic receptor agonist devoid of vasoconstrictor properties has antimigraine efficacy (60).



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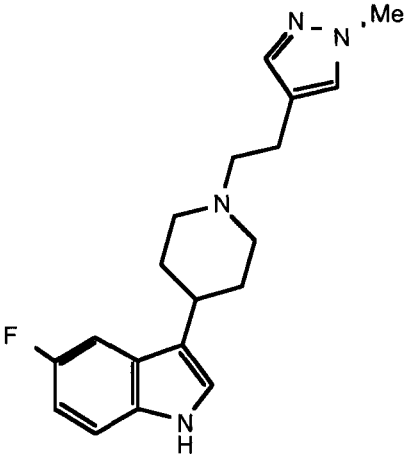

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Beyond Serotonin - Several additional pharmacological approaches to the treatment of migraine are in various stages of testing including substance-P antagonists, CGRP antagonists, somatostatin agonists, GABA A antagonists, and others (62). Although the clinical data accumulating on the tachykinin antagonists has not been promising

thusfar, additional clinical data are required to evaluate the potential of these other receptor-based therapies.

However, new insights into possible molecular neurobiological substrates which may underlie migraine pathology have been provided from the study of human genetics of a rare form of migraine, known as familial hemiplegic migraine (FHM). This condition is an autosomal dominant subtype of migraine with aura which is characterized by the occurrence of hemiplegia, or hemiparesis, during the period of aura (63,64). Due to the strong genetic basis for this disease, investigators have sought to find the possibly mutated FHM genes which may be involved in its pathophysiology. The frequency of migraine in patients suffering from another autosomal dominant neurological condition, CADISIL (Cerebral Autosomal Dominant Artereopathy with Subcritical Infarcts and Leukoencephalopathy), is extremely high (65), and this observation led to the hypothesis that the CADISIL gene might be responsible for FHM. Since the CADISIL gene had been mapped to chromosome 19, it was possible that FHM also mapped to this chromosome. Using genetic linkage analysis (66), it was demonstrated that the FHM gene resided within a chromosomal interval containing the CADISIL locus on chromosome 19. This set the stage for cloning efforts which have already yielded two interesting discoveries, a Notch protein and a calcium channel (*vide infra*).

In a recent report, a candidate gene from the critical region of the CADISIL locus was identified (67). This gene was shown to be a member of the Notch gene family, highly homologous to the murine Notch-3. It maps to human chromosome 19p 13.2-13.2. Ten different missense mutations occur in the sequence from over a dozen separate CADISIL families. The mutations are expected to result in an altered folding of the Notch protein because they are additions or mutations of cysteine residues. The functional consequences for the mutant Notch proteins remain to be elucidated. Interestingly, Notch signaling in *Caenorhabditis elegans* is affected by an interacting protein related to the mammalian presenilin genes, PS-1 and PS-2 which may be the keys to early onset familial Alzheimer's dementia (68). Coupled with the role of Notch proteins in Drosophlia development, these finding suggest that the mutations in the Notch-3 gene in CADISIL patients may be somehow responsible for the "adult-onset" aspect of this neurological condition thus as some kind of "clock". It is also interesting to note that nearly half of the CADISIL patients develop migraine as a first syndrome at about age 20, relatively late compared with the onset of symptoms in common migraine. The more severe aspects of the CADISIL syndrome often take another 20 years to develop (69).

Simultaneous with the discovery of the human Notch-3 gene in CADISIL another gene was isolated from the same chromosomal region, 19p 13. This gene encoded an alpha1 subunit of a type P/Q calcium channel (CACNL1A4, 70) which was mutated in FHM patients. This gene also maps to human chromosome 19p 13.1-p13.2. Four distinct missense mutations in five unrelated FHM families were found. One mutation may affect the voltage sensor (R129Q), a second may affect the ion selectivity filter (T666M), and two others are in S6 segments (V714A; I1811L). The role of these residues is as yet unknown although their absolute conservation in all calcium channel alpha1 subunits suggests a key role. Interesting, mutations at other loci in the same gene are observed in patients displaying episodic ataxia type 2 (EA-2) which is another episodic, autosomal dominant disorder with migraine as part of the syndrome. The mutations in these patients lead to truncated proteins which are unlikely to form functional calcium channels (70).