

Antimigraine Drug Interactions with Serotonin Receptor Subtypes in Human Brain

Stephen J. Peroutka, MD, PhD

The interactions of antimigraine agents with serotonin (5-hydroxytryptamine, 5-HT) receptor subtypes were analyzed in human frontal cortex membranes. The drugs studied included 5-HT antagonists, beta-adrenergic antagonists, and calcium channel blockers. At 5-HT_{1A} sites labeled by ³H-8-hydroxy-2-(N,N-dipropylamino)-tetralin, (-)pindolol, alprenolol, (-)propranolol, methysergide, cyproheptadine, and pizotifen are similar in that they display affinities of approximately 100 nM for this receptor. By contrast, only methysergide displays relatively high affinity (120 ± 60 nM), whereas all other drugs have affinities greater than 1,000 nM for non-5-HT_{1A} sites labeled by ³H-5-HT in human cortex. Finally, at 5-HT₂ receptors labeled by ³H-spiperone, cyproheptadine, methysergide, and pizotifen are extremely potent agents (affinity constants of 1 to 10 nM), whereas amitriptyline (23 ± 4 nM), verapamil (140 ± 50 nM), and nifedipine (320 ± 80 nM) are moderately potent. All other drugs are inactive at concentrations below 1,000 nM. These data demonstrate that most antimigraine drugs display high affinity for the 5-HT_{1A} and/or 5-HT₂ receptor subtypes in human brain. However, antimigraine efficacy cannot be explained by drug interactions with a single 5-HT receptor subtype.

Peroutka SJ. Antimigraine drug interactions with serotonin receptor subtypes in human brain. *Ann Neurol* 1988;23:500-504

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated in the pathogenesis of migraine [1-4]. Depending on the vascular tone, 5-HT may cause either vasodilation or vasoconstriction of intracranial vasculature [5, 6]. 5-HT is also an important neuromodulator of pain pathways, as evidenced by its ability to lower pain thresholds in inflamed tissues [7]. Moreover, 5-HT terminals have been identified in both cerebral blood vessels [8, 9] and central pain pathways [10-12]. Indeed, the first major class of prophylactic antimigraine agents were 5-HT antagonists such as methysergide [13], cyproheptadine, and pizotifen [14].

However, antimigraine agents do not appear to share a common mechanism of action [2, 3, 6, 15]. Thus, 5-HT antagonists, beta-adrenergic antagonists, and calcium channel blockers have all been reported to be effective in the prophylactic treatment of migraine [15, 16]. It has recently been reported that (-)propranolol and several 5-HT antagonists share a similar affinity for the 5-HT_{1A} subtype of 5-HT receptor in the central nervous system of the rat [17]. These data suggested that interactions with 5-HT_{1A} receptors may be an important action of migraine prophylactic agents. In the present study we therefore analyzed a variety of migraine prophylactic agents at the 3 known 5-HT receptor subtypes in human frontal cortex.

Materials and Methods

Radioligand binding studies were performed as described previously [18, 19]. Briefly, human frontal cortex samples from 4 patients dying of nonneurological causes (32- and 70-year-old women; 52- and 58-year-old men) were obtained post mortem from the Department of Pathology, Stanford University Medical Center. Tissue samples were obtained within 8 to 11 hours of death and rapidly frozen and stored at -70°C until needed. On the day of study, the samples were thawed in TRIS-HCl buffer. Tissues were homogenized in 20 volumes of TRIS-HCl buffer (pH 7.7 at 25°C) using a Brinkmann Polytron and then centrifuged in an IEC B20A centrifuge at 45,000 g for 10 minutes. The supernatant was discarded and the pellet was resuspended in the same volume of TRIS-HCl buffer and incubated at 37°C for 10 minutes before a second centrifugation at 49,000 g for 10 minutes. The final pellet was resuspended in 80 volumes of TRIS-HCl buffer containing 10 µM pargyline, 4 mM calcium chloride, and 0.1% ascorbic acid. The suspensions were immediately used in the binding assay. Radioligand binding studies consisted of 0.1 ml ³H-radioligand (0.4 nM ³H-8-hydroxy-2-[N,N-dipropylamino]-tetralin [OH-DPAT]; 2 nM ³H-5-HT; 0.7 nM ³H-spiperone), 0.1 ml buffer or displacing drug, and 0.8 ml tissue suspension. After incubation at 25°C for 30 minutes, the samples were rapidly filtered under vacuum through no. 32 glass fiber filters (Schleicher and Schuell, Keene, NH) with two 5-ml washes using 50 mM TRIS-HCl buffer. Radioactivity was measured by liquid

From the Departments of Neurology and Pharmacology, Stanford University Medical Center, Stanford, CA 94305.

Received May 11, 1987, and in revised form Sep 1. Accepted for publication Nov 27, 1987.

500 Copyright © 1988 by the American Neurological Association

Drug	Receptor Affinity (K _i , nM)		
	5-HT _{1A}	Non-5-HT _{1A}	5-HT ₂
5-HT Antagonists			
Methysergide	59 ± 10	120 ± 60	5.7 ± 3
Cyproheptadine	99 ± 3	5,600 ± 1,000	2.2 ± 0.6
Pizotifen	200 ± 40	2,200 ± 700	3.6 ± 0.5
Amitriptyline	1,800 ± 300	4,300 ± 800	23 ± 4
Beta-adrenergic antagonists			
(-)-Pindolol	4.5 ± 0.4	13,000 ± 3,000	22,000 ± 3,000
Alprenolol	93 ± 7	2,800 ± 5,000	5,100 ± 700
(-)-Propranolol	160 ± 60	29,000 ± 7,000	4,100 ± 700
(+)-Pindolol	1,100 ± 400	> 100,000	38,000 ± 7,000
(+)-Propranolol	1,900 ± 300	80,000 ± 6,000	9,000 ± 500
Timolol	2,500 ± 700	> 100,000	> 100,000
Atenolol	> 100,000	> 100,000	> 100,000
Calcium channel antagonists			
Verapamil	2,100 ± 300	41,000 ± 5,000	140 ± 50
Nifedipine	> 100,000	12,000 ± 4,000	320 ± 80
Diltiazem	> 100,000	> 100,000	2,400 ± 600

^aRadioligand studies were performed as described in Materials and Methods. Data shown are the mean ± SE of 3 or 4 experiments, each performed in triplicate on individual brain samples.

5-HT = serotonin.

scintillation spectroscopy in 5 ml of 3a70 Counting Cocktail (Research Products International, Mt Prospect, IL) at 54% efficiency. Specific binding was defined as the excess over blanks taken in the presence of 10⁻⁵ M 5-HT for 5-HT_{1A} sites labeled by ³H-8-OH-DPAT, 10⁻⁵ M 5-HT for non-5-HT_{1A} sites labeled by ³H-5-HT + 100 nM 8-OH-DPAT, and 10⁻⁶ M cinanserin for 5-HT₂ sites labeled by ³H-spiperone.

All drugs were diluted and dissolved in assay buffer with the exception of calcium channel blockers, which were dissolved in ethanol at 10⁻³ M and then diluted in assay buffer. Drug sources were as follows: ³H-radioligands (Dupont–New England Nuclear, Boston, MA); alprenolol, amitriptyline, cyproheptadine, 5-HT, methysergide (Sigma, St Louis, MO); cinanserin (Organon, West Orange, NJ); (-)-pindolol, (+)-pindolol, pizotifen (Sandoz, East Hanover, NJ); (-)-propranolol, (+)-propranolol (Ayerst, New York, NY); timolol (Merck, Sharp and Dohme, Rahway, NJ); atenolol (Stuart, Wilmington, DE); verapamil (Knoll, Whippany, NJ); nifedipine (Pfizer, Brooklyn, NY); diltiazem (Marion, Kansas City, MO); 8-OH-DPAT (Research Biochemicals, Waltham, MA).

IC₅₀ values were determined by log-logit analysis of drug competition studies. K_i values were determined by the equation K_i = IC₅₀/(1 + [I]/K_D), where K_D was 1.0 nM for ³H-8-OH-DPAT; 3.2 nM for ³H-5-HT, and 0.71 nM for ³H-spiperone. Each experiment was performed in triplicate and repeated 3 or 4 times.

Results

Drug Interactions with ³H-8-OH-DPAT Binding to 5-HT_{1A} Receptors

The majority of antimigraine drugs analyzed in the present study display high to moderate affinity for 5-HT_{1A} receptors labeled by ³H-8-OH-DPAT in human frontal cortex (Table). As previously reported in

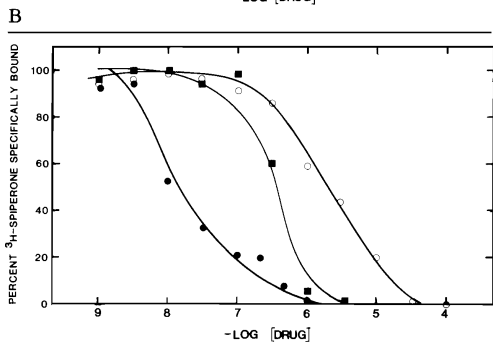
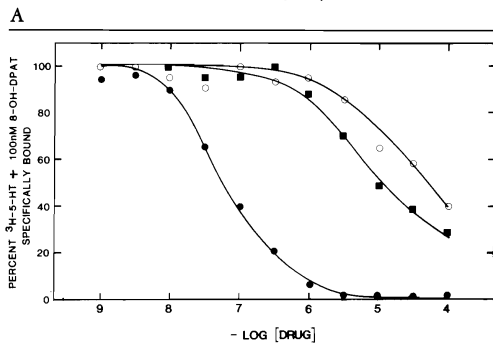
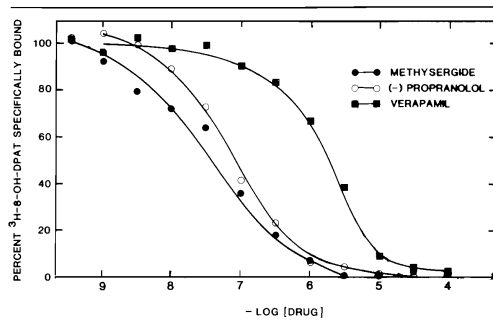
rat brain membranes, methysergide, cyproheptadine, and pizotifen are essentially equipotent at 5-HT_{1A} receptor sites (K_i values = 59 to 200 nM). Amitriptyline displays moderate to low potency for this site (1,800 ± 300 nM).

Putative beta-adrenergic agents vary widely in their affinity for the 5-HT_{1A} receptor. (-)-Pindolol is the most potent drug studied, with a K_i value of 4.5 ± 0.4 nM. Alprenolol and (-)-propranolol are 21- and 36-fold, respectively, less potent than (-)-pindolol at the 5-HT_{1A} receptor. (+)-Propranolol, (+)-pindolol, and timolol display similar, moderate affinity for the 5-HT_{1A} site. By contrast, atenolol is inactive at concentrations below 10⁻⁴ M. Verapamil (K_i = 2,100 ± 300 nM) is the only calcium channel blocker that is active at the 5-HT_{1A} site at concentrations below 10⁻⁴ M.

Drug competition data for methysergide, (-)-propranolol, and verapamil are shown in the Figure (A). Methysergide is the most potent agent; displacement of specific ³H-8-OH-DPAT to the 5-HT_{1A} site is first noted at concentrations above 10⁻⁹ M. (-)-Propranolol is slightly less potent, with the majority of competition occurring between concentrations of 10⁻⁸ M and 10⁻⁵ M. Verapamil is the least potent of the three agents and competes for specific ³H-8-OH-DPAT to 5-HT_{1A} sites at micromolar concentrations.

Drug Interactions with ³H-5-HT Binding to Non-5-HT_{1A} Receptors

In human frontal cortex, non-5-HT_{1A} binding has been defined using ³H-5-HT in the presence of 100 nM



C
Drug competition studies of methysergide, (-)propranolol, and verapamil with serotonin (5-HT) receptor subtypes in human brain. Drug competition studies were performed as described in Materials and Methods. Data given are the results from a single experiment, performed in triplicate. Each experiment was repeated 3 to 5 times. (A) Drug competition with specific ³H-8-hydroxy-2-(N,N-dipropylamino)-tetralin binding to 5-HT_{1A} receptors. (B) Drug competition with specific ³H-5-HT + 100 nM 8-OH-DPAT binding to non-5-HT_{1A} receptors. (C) Drug competition studies with specific ³H-spiperone binding to 5-HT₂ receptors.

8-OH-DPAT. Non-5-HT_{1A} sites in human frontal cortex are composed almost exclusively of the 5-HT_{1D} binding site subtype (Peroutka S. J., unpublished observations). With the exception of nifedipine, each of the antimigraine agents is one to two orders of magnitude less potent at non-5-HT_{1A} binding site subtypes than at 5-HT_{1A} sites. (+)Pindolol, timolol, atenolol, and diltiazem are totally inactive at the non-5-HT_{1A} site at concentrations below 10⁻⁴ M.

Methysergide is the only agent that competes for specific binding to the non-5-HT_{1A} site at concentrations below 10⁻⁶ M. As shown in the Figure (B), methysergide competition for non-5-HT_{1A} binding begins at a concentration of 10⁻⁸ M. At approximately 10⁻⁷ M methysergide, 50% of specific ³H-5-HT binding is displaced. Essentially all specific binding to the non-5-HT_{1A} site is eliminated by methysergide concentrations above 10⁻⁵ M. By contrast, both (-)propranolol and verapamil are considerably less potent in competing for non-5-HT_{1A} binding. Drug concentrations greater than 10⁻⁵ M are needed to compete for 50% of specific ³H-5-HT binding.

Drug Interactions with ³H-Spiperone Binding to 5-HT₂ Receptors

A number of antimigraine agents interact potently with 5-HT₂ receptors labeled by ³H-spiperone in human brain membranes (see Table). For example, methysergide, cyproheptadine, and pizotifen display nanomolar affinity for this 5-HT receptor subtype. Amitriptyline is only slightly less potent (K_i = 23 ± 4 nM), whereas verapamil and nifedipine display apparent affinities of 140 ± 50 and 320 ± 80 nM, respectively. Diltiazem and the majority of beta-adrenergic agents display micromolar affinity for the 5-HT₂ site. Only timolol and atenolol are inactive at this receptor below a concentration of 10⁻⁴ M.

Drug competition studies with the 5-HT₂ binding site are shown in the Figure (C). Methysergide begins to displace specific ³H-spiperone binding at concentrations above 10⁻⁹ M. All specific ³H-spiperone binding is eliminated by methysergide concentrations above 10⁻⁶ M. Verapamil is approximately two orders of magnitude less potent than methysergide, with displacement of specific ³H-spiperone binding occurring between 10⁻⁸ and 3 × 10⁻⁵ M verapamil. (-)Propranolol is the least potent of the three agents analyzed by drug competition studies, with approximately 50% displacement of the radioligand observed at a drug concentration of 2 × 10⁻⁶ M.

Discussion

The major finding of the present study is that the majority of prophylactic antimigraine agents displays a relatively high affinity for 5-HT_{1A} and/or 5-HT₂ receptors in human brain. Since a satisfactory animal model

for migraine does not exist, attempts to determine a common mechanism of action for effective antimigraine agents may be of benefit in elucidating the pathogenesis of this neurological syndrome. However, the present study demonstrates that a single 5-HT receptor subtype in brain membranes is unlikely to mediate antimigraine drug efficacy.

Since the demonstration in 1959 [13] that methysergide was an effective migraine prophylactic agent, a number of serotonergic agents have been shown effective in the treatment of migraine [14]. Recently, the observation that (-)propranolol, methysergide, cyproheptadine, and pizotifen were equipotent agents at the 5-HT_{1A} receptor site in rat brain led to the suggestion that this receptor may play a role in the pathogenesis of migraine [17]. The 5-HT_{1A} site can be radiolabeled with ³H-8-OH-DPAT [18, 20] and has a distinct regional localization in human brain [21]. 5-HT_{1A} sites are most dense in the raphe nuclei and hippocampus [21].

The 5-HT_{1A} receptor has been shown to mediate 5-HT-induced contractions of the canine basilar artery [22, 23]. Perhaps more important, 5-HT_{1A} selective agonists inhibit intrinsic raphe cell firing. The inhibitory effects of 5-HT_{1A} agonists such as 8-OH-DPAT can be blocked by (-)propranolol [24]. As shown in the present study, methysergide, cyproheptadine, pizotifen, and amitriptyline all interact with 5-HT_{1A} receptors in human brain.

In addition, a number of effective beta-adrenergic antimigraine agents such as (-)propranolol and timolol display moderate affinity for the 5-HT_{1A} site [15, 25]. On the other hand, (-)pindolol is the most potent beta-adrenergic agent at the 5-HT_{1A} receptor with a K_i value of 4.5 ± 0.4 nM. Clinical studies of pindolol in the prophylactic treatment of migraine have produced conflicting results. In three published reports, the drug was found to be effective in one clinical trial [26], possibly effective in a small subgroup of patients in a second study [27], and ineffective in a third study [28]. In addition, atenolol is totally inactive at all three 5-HT receptor subtypes yet has been reported to be effective in two independent clinical trials [29, 30]. As a result, the antimigraine efficacy of certain beta-adrenergic agents cannot derive solely from antagonism of the 5-HT_{1A} receptor.

In comparison to their 5-HT_{1A} and non-5-HT_{1A} potencies, methysergide, cyproheptadine, pizotifen, and amitriptyline are even more potent antagonists of the 5-HT₂ receptor in human brain. Indeed, it has been hypothesized that the 5-HT₂ receptor, which is most densely present in Layer IV of the cerebral cortex, might play a key role in one of the biochemical events of migraine [3, 15]. The 5-HT₂ receptor has been shown to mediate contraction of smooth muscle in many vascular beds [31]. In addition, Coughlin and

colleagues [32, 33] have demonstrated that 5-HT can stimulate production of prostacyclin and other products of arachidonic acid metabolism in smooth muscle cells *in vitro* [32, 33]. This action of 5-HT appears to be mediated by 5-HT₂ receptors, since methysergide, cyproheptadine, and pizotifen potently prevent this effect. The importance of this finding is that modulation of prostacyclin and arachidonic acid metabolism may have important effects on vascular tone [32] and/or local inflammation [15].

Calcium channel blockers are the newest class of antimigraine prophylactic agents. Initial studies have demonstrated that a variety of calcium channel blockers are effective in migraine prophylaxis. Previously, their antimigraine efficacy had been attributed to their protective effects during anoxia [34] and/or their ability to block intracranial vasoconstriction, irrespective of the constricting agent [35]. It is surprising that the present study demonstrated that calcium channel blockers display moderate affinity for the 5-HT₂ receptor. Previously, verapamil and D600 were the only two calcium channel blockers that had been reported to affect 5-HT₂ sites [36, 37]. Their ability to block 5-HT₂ receptors in human brain must now also be considered as a possible therapeutic effect of these drugs.

Therefore, the present study demonstrates that a large number of migraine prophylactic agents share an ability to interact with 5-HT receptor subtypes in human brain. More specifically, antimigraine drugs display high or moderate affinity for 5-HT_{1A} and/or 5-HT₂ receptors in human brain membranes. However, we could not identify a single site of action for all antimigraine agents.

At the same time, these data do offer a novel approach to the analysis of antimigraine agents. Drugs could be selected for use in clinical migraine studies based on their selectivity for a specific 5-HT receptor subtype. For example, an agent that displays both high affinity and selectivity for 5-HT_{1A} receptors could be clinically evaluated. Its effectiveness, or lack thereof, would indicate the importance of the specific 5-HT receptor site in the pathogenesis of migraine.

Supported in part by the John A. and George L. Hartford Foundation, the Alfred P. Sloan Foundation, the National Headache Foundation, and NIH grants NS 12151-12 and NS 23560-01.

I thank Christina M. Demopoulos and Anne Hamik for excellent technical assistance and Faith H. Smith for manuscript preparation.

References

1. Dalessio DJ. On migraine headache: serotonin and serotonin antagonism. *JAMA* 1962;181:318-321
2. Raskin NH. Pharmacology of migraine. *Ann Rev Pharmacol Toxicol* 1981;21:463-478
3. Fozard JR. Basic mechanisms of antimigraine drugs. *Adv Neurol* 1982;33:295-307

Peroutka: Migraine and 5-HT Receptors 503

4. Fozard JR. 5-Hydroxytryptamine in the pathophysiology of migraine. In: Bevan JA, Godfrand T, Maxwell RA, et al, eds. *Vascular neuroeffector mechanisms*. Amsterdam: Elsevier, 1985:321-327
5. Edvinsson L, Hardebo JE, MacKenzie ET, Stewart M. Dual action of serotonin on pial arterioles in situ and the effect of propranolol on the response. *Blood Vessels* 1977;14:366-371
6. Edvinsson L, Hardebo JE, Owman C. Pharmacological analysis of 5-hydroxytryptamine receptors in isolated intracranial and extracranial vessels of cat and man. *Circ Res* 1977;42:143-151
7. Moskowitz MA. The neurobiology of vascular head pain. *Ann Neurol* 1984;16:157-168
8. Reinhard JF, Liebmann JE, Schlosberg AJ, Moskowitz MA. Serotonin neurons project to small blood vessels in the brain. *Science* 1979;206:85-87
9. Edvinsson L, Degueurce A, Duverger D, et al. Central serotonergic nerves project to the pial vessels of the brain. *Nature* 1983;306:55-57
10. Basbaum AI, Fields HL. Endogenous pain control mechanisms: review and hypothesis. *Ann Neurol* 1978;4:451-462
11. Schaffar N, Jean A, Calas A. Radioautographic study of serotonergic axon terminals in the rat trigeminal motor nucleus. *Neurosci Lett* 1984;44:31-36
12. Cropper EC, Eisenman JS, Azmitia EC. 5-HT-Immunoreactive fibers in the trigeminal nuclear complex of the rat. *Exp Brain Res* 1984;55:515-522
13. Sicuteri F. Prophylactic and therapeutic properties of 1-methyl-lysergic acid butanolamide in migraine. *Int Arch Allergy* 1959;15:300-307
14. Lance JW, Anthony M, Somerville B. Comparative trial of serotonin antagonists in the management of migraine. *Br Med J* 1970;2:327-330
15. Peatfield RC, Fozard JR, Rose FC. Drug treatment of migraine. In: Vinken PJ, Bruyn GW, Klawans HL, eds. *Handbook of Clinical Neurology*, Vol 4. Amsterdam: Elsevier, 1986:173-216
16. Daroff RB, Whitney CM. Treatment of vascular headaches. *Headache* 1986;26:470-472
17. Hiner BC, Roth HL, Peroutka SJ. Antimigraine drug interactions with 5-hydroxytryptamine_{1A} receptors. *Ann Neurol* 1986;19:511-513
18. Peroutka SJ. Pharmacological differentiation and characterization of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{1C} binding sites in rat frontal cortex. *J Neurochem* 1986;47:529-540
19. Heuring RE, Peroutka SJ. Characterization of a novel ³H-5-hydroxytryptamine binding site subtype in bovine brain membranes. *J Neurosci* 1987;7:894-903
20. Gozlan H, El Mestikawy S, Pichat L, et al. Identification of presynaptic serotonin autoreceptors using a new ligand: ³H-PAT. *Nature* 1983;305:140-142
21. Hoyer D, Pazos A, Probst A, Palacios JM. Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT_{1A} recognition sites. Apparent absence of 5-HT_{1B} recognition sites. *Brain Res* 1986;376:85-96
22. Taylor EW, Duckles SP, Nelson DL. Dissociation constants of serotonin agonists in the canine basilar artery correlate to K_i values at the 5-HT_{1A} binding site. *J Pharmacol Exp Ther* 1985;236:118-125
23. Peroutka SJ, Huang S, Allen GS. Canine basilar artery contractions mediated by 5-hydroxytryptamine_{1A} receptors. *J Pharmacol Exp Ther* 1986;237:901-906
24. Sprouse JS, Aghajanian GK. (-)Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT_{1A} selective agonists. *Eur J Pharmacol* 1986;128:295-298
25. Weerasuriya K, Patel L, Turner P. B-adrenoceptor blockade and migraine. *Cephalalgia* 1982;2:33-45
26. Anthony M, Lance JW, Somerville B. A comparative trial of prindolol, clonidine and carbamazepine in the internal therapy of migraine. *Med J Aust* 1972;1:1343-1346
27. Sjaastad O, Stensrud P. Clinical trial of a beta-receptor blocking agent (LB 46) in migraine prophylaxis. *Acta Neurol Scand* 1972;48:124-128
28. Ekblom K, Lundberg PO. Clinical trial of LB-46 (d, 1-(4-(2-hydroxy-3-isopropylaminopropoxy)indol. An adrenergic beta-receptor blocking agent in migraine prophylaxis. *Headache* 1972;12:15-17
29. Stensrud P, Sjaastad O. Comparative trial of Tenormin (atenolol) and Inderal (propranolol) in migraine. *Headache* 1980;20:204-207
30. Forssman B, Lindblad CJ, Zbornikova V. Atenolol for migraine prophylaxis. *Headache* 1983;23:188-190
31. Peroutka SJ. Vascular serotonin receptors. Correlation with 5-HT₁ and 5-HT₂ binding sites. *Biochem Pharmacol* 1984;33:2349-2353
32. Coughlin SR, Moskowitz M, Antoniades HN, Levine L. Serotonin receptor-mediated stimulation of bovine smooth muscle cell prostacyclin synthesis and its modulation of platelet-derived growth factor. *Proc Natl Acad Sci USA* 1981;78:7134-7138
33. Coughlin SR, Moskowitz M, Levine L. Identification of a serotonin type 2 receptor linked to prostacyclin synthesis in vascular smooth muscle cells. *Biochem Pharmacol* 1984;33:692-695
34. Amery WK. Brain hypoxia: the turning-point in the genesis of the migraine attack? *Cephalalgia* 1982;2:83-109
35. Peroutka SJ. The pharmacology of calcium channel antagonists: a novel class of anti-migraine agents? *Headache* 1983;23:278-283
36. Taylor JE, Defeudis FV. Inhibition of [³H]spiperone binding to 5-HT₂ receptors of rat cerebral cortex by the calcium antagonists verapamil and D600. *Eur J Pharmacol* 1985;106:215-216
37. Affolter H, Burkard WP, Pletscher A. Verapamil, an antagonist at 5-hydroxytryptamine receptors of human blood platelets. *Eur J Pharmacol* 1985;108:157-162