Effect of Anti-migraine 5-HT\textsubscript{1B/1D/1F} Receptor Agonists on \textit{In Vivo} Serotonin Synthesis Rates in Rat and Human Brain.

Colin Dobson

Department of Neurology and Neurosurgery

McGill University, Montreal

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ABSTRACT

PURPOSE. This work investigates the effect of anti-migraine serotonin (5-HT) receptor agonists (the triptans) on \textit{in vivo} 5-HT synthesis rates in the brain. In rats, rates are examined after acute and chronic drug administration. In humans, rates are measured in different migrainous conditions, namely, during a headache, after sumatriptan, and between attacks. METHODS. Synthesis rates are determined using the $\alpha$-[\textsuperscript{14}C or \textsuperscript{11}C]methyl-L-tryptophan tracer kinetic model, combined in rats with autoradiography and in humans with positron emission tomography. RESULTS & CONCLUSIONS. Acute triptan administration reduces 5-HT synthesis rates in rat brain while chronic delivery increases rates at serotonergic projection areas. Preliminary results in human migraine sufferers suggest a trend, though not significant, towards an acute decrease in central rates after sumatriptan injection and higher rates during migraine pain than between attacks. Such central effects might or might not help alleviate pain but would have repercussions for chronic triptan users.
RÉSUMÉ

OBJECTIF. Ce travail examine l'effet d'agonistes des récepteurs à la sérotonine (5-HT) utilisés comme anti-migraineux (les triptans) sur les taux de synthèse de 5-HT dans le cerveau in vivo. Les taux sont mesurés après l'administration aiguë ou chronique des agonistes chez le rat et, chez l'homme, à différentes étapes d'un épisode migraineux, notamment pendant la céphalée, après l'administration de sumatriptan et en période de rémission. MÉTHODE. Les taux de synthèse sont déterminés avec l’α-[14C ou 11C] méthyl-L-tryptophan, combiné à une détection autoradiographique chez le rat et à la tomographie par émission de positons chez l'homme. RÉSULTATS & CONCLUSIONS. Chez le rat, l'administration aiguë de triptan réduit significativement le taux de synthèse de 5-HT dans plusieurs régions du cerveau, alors qu'en chronique une augmentation significative est observée dans les aires de projection des neurones 5-HT. Les résultats préliminaires chez les migraineux suggèrent une diminution dans la synthèse de la 5-HT après l'administration de sumatriptan, et des taux plus élevés pendant la crise qu'en période de rémission. Ces résultats montrent que les triptans affectent la neurotransmission sérotoninergique, un effet possiblement relié à leur efficacité clinique et qui pourrait avoir des conséquences pour les utilisateurs chroniques de triptans.
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1.1. INTRODUCTION

Sumatriptan, the first of a whole family of triptans, was undoubtedly a significant advance in the symptomatic treatment of migraine attacks (1, 2). The members of the family, which also includes such compounds as zolmitriptan, naratriptan, rizatriptan, eletriptan, almotriptan, and frovatriptan, differ primarily in their relative affinity for the different serotonin receptors and relative ability to cross the blood brain barrier (BBB). Although they are known to bind at the 5-HT_{1B}, 5-HT_{1D} and 5-HT_{1F} receptors and, to a lesser, but not insignificant, extent to the 5-HT_{1A} and even 5-HT_{1E} receptors (Table 1), the exact mode of action of these drugs is not yet fully elucidated. The triptans are proposed to have three possible mechanisms of action in migraine (reviewed in 1): (1) constriction of blood vessels on the surface of the brain thought to be dilated during the pain phase of a migraine attack, (2) reduction of inflammatory neuropeptide release from peripheral trigeminal nerve endings surrounding the meningeal and cerebral blood vessels, and (3) inhibition of impulse transmission centrally in the trigeminal nucleus caudalis (Figure 1).

Sumatriptan’s reported minimal BBB penetrance has thus far reduced the certainty over whether it also exerts actions on central serotonergic receptors, let alone whether these might play any role in its ability to abate migraine symptoms (pain, phono- and/or photophobia, vomiting, etc.). The latter notion might be bolstered, however, by the finding of a significant inverse relationship between the lipophilicity of triptans and their clinically effective absolute dose (absolute dose = administered dose (mg) x absolute bioavailability (%)) (3).

The initial preclinical studies done by the manufacturer showed a systemic
Table 1. pKᵢ values of Sumatriptan and Zolmitriptan at select 5-HT receptors (23)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Sumatriptan</th>
<th>Zolmitriptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT 1A</td>
<td>6.90, 6.43</td>
<td>9.20</td>
</tr>
<tr>
<td>5-HT 1B</td>
<td>7.82</td>
<td>8.30</td>
</tr>
<tr>
<td>5-HT 1D</td>
<td>8.46</td>
<td>9.20</td>
</tr>
<tr>
<td>5-HT 1E</td>
<td>5.8</td>
<td>7.95</td>
</tr>
<tr>
<td>5-HT 1F</td>
<td>7.86</td>
<td>7.20</td>
</tr>
<tr>
<td>5-HT 2A</td>
<td>&lt; 5.0</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>5-HT 2B</td>
<td>n.a.</td>
<td>7.19</td>
</tr>
<tr>
<td>5-HT 2C</td>
<td>&lt; 5.0 (pig)</td>
<td>4.10 (guinea pig)</td>
</tr>
<tr>
<td>5-HT 3</td>
<td>&lt; 5.0 (mouse)</td>
<td>&lt; 5.5 (mouse)</td>
</tr>
<tr>
<td>5-HT 4</td>
<td>&lt; 5.0 (guinea pig)</td>
<td>&lt; 5.5 (guinea pig)</td>
</tr>
<tr>
<td>5-HT 5A</td>
<td>5.50</td>
<td>6.40</td>
</tr>
<tr>
<td>5-HT 6</td>
<td>5.31</td>
<td>&lt; 5.5</td>
</tr>
<tr>
<td>5-HT 7</td>
<td>6.51</td>
<td>7.02</td>
</tr>
</tbody>
</table>

All values refer to the human receptor, except when stated otherwise. Multiple entries are the results of different studies.
Figure 1. (Adapted from 1) Schematic representation of the proposed sites of action for the triptan compounds in the symptomatic treatment of migraine headache. 1. Activation of postsynaptic 5-HT<sub>1B</sub> receptors on vascular smooth muscle will result in contraction of blood vessels on the surface of the brain, which are putatively distended during migraine. These blood vessels are not yet surrounded by the blood brain barrier. 2. Activation of presynaptic 5-HT<sub>1D</sub> or 5-HT<sub>1F</sub> receptors on trigeminovascular afferents will block the release of inflammatory peptides (for example: substance P and calcitonin gene-related peptide (CGRP)) and hence the generation of neurogenic inflammation, which would block the generation and propagation of pain. 3. In the trigeminalis nucleus caudalis (TNC), presynaptic and postsynaptic 5-HT<sub>1</sub> receptors have been identified and could interrupt the transmission of pain centrally. This would particularly be true for the BBB penetrant triptans. 4. As described in the text, serotonin receptors exist in the raphe nuclei postsynaptically as well as presynaptically on the end of projections from the raphe nuclei. The agonism of these receptors, particularly by the more brain penetrant triptans, might or might not be part of the process of pain relief but might represent an additional pharmacological effect for these compounds.
distribution expected of a weakly basic drug: about 0.05% of a 1mg/kg intravenous dose reached the rat brain (5). Further, whole body autoradiographic analysis showed that drug and drug-related material is widely distributed throughout the body at higher concentrations than blood levels in all tissues except the central nervous system 10 minutes after intravenous administration of radiolabeled \[^{14}\text{C} \]sumatriptan (6).

Recently, however, sumatriptan was found in significant concentrations in the spinal fluid of healthy volunteers, peaking in concentration 5 hours after administration of a therapeutic dose (100mg, p.o.) (7). The average peak concentration at the 5 hour mark was 3.3 ng/mL in the spinal fluid, which is about 6% of the maximal plasma concentration of this dose typically found after pill ingestion (\(C_{\text{max}}=54\text{ng/mL}, T_{\text{max}}=1.5\text{ hrs}\)) (8). The authors of this same study note that 9 hours after drug injection spinal fluid levels of sumatriptan were higher than plasma levels. These findings are suggestive of a greater passage across the BBB than previously thought.

1.1.1. Study Rationales and Objectives.

Greater passage across the BBB also seemed more likely upon inspection, as described below, of the preliminary results of our study on central 5-HT synthesis rates obtained in migraine sufferers treated with sumatriptan during their migraine attacks. The results suggested that, regardless of the extent to which it crosses the BBB, sumatriptan might be able to bring about changes or effects on 5-HT synthesis rates, evaluated by positron emission tomography (PET) technology within the central nervous system (CNS) at the commonly used clinical dose of 6 mg (s.c.). This study has as objective the measurement of \textit{in vivo} 5-HT synthesis rates in three conditions for each participant: 1) at
some point during the first 6 hours of a migraine pain (the ictal measurement), 2) after the injection of sumatriptan (immediately following the ictal measurement), and 3) during a migraine free period (the interictal measurement). Given the migraine generator theory (see below) and the presumed minimal central action of sumatriptan, it was hypothesized that rates would be higher in the first two measurements and lower in the third. This would be the case in almost all areas of the brain as serotonergic neurons project diffusely throughout the CNS from their brainstem nuclei (9). However, preliminary results raised the possibility that the drug could lower 5-HT synthesis rates within the brain as tracer uptake rates in the second condition were consistently the lowest of all three conditions. However, interpretation of the initial \textit{in vivo} findings was complicated by the possible breach of the BBB during a migraine attack (reviewed in 10).

Thus a complementary course of study arose to investigate whether subcutaneously administered sumatriptan could elicit effects on central serotonin synthesis in healthy rats with intact BBBS. The same model for measurement of 5-HT synthesis rates is employed. The effects of sumatriptan are not only compared to saline controls but are also compared with those of zolmitriptan - a triptan known to more readily cross the BBB and access central receptor sites (11). A similar pattern of effects on central synthesis rates between the two drugs would indicate that a new explanation as to their exact mode of action is necessary. A different pattern of effects, particularly one with zolmitriptan lowering synthesis rates and sumatriptan having no effect, would increase the evidence in favour of a breach of the BBB during migraine.

As a clinically effective anti-migraine agent, sumatriptan is being used by an
increasing number of people worldwide a subset of which is over-consuming the drug, which has been shown to worsen migraine symptoms (12). Therefore it was also of interest to investigate the effect of chronic administration of sumatriptan on the central rates of 5-HT synthesis using the same method. Osmotic mini-pumps, containing either sumatriptan or saline vehicle, were inserted under the skin of rat necks. These pumps, which assure a constant rate of flow of solution into the rat, were inserted 21 days before the synthesis rate measurement procedure.

2. REVIEW OF THE LITERATURE

2.1 Serotonin: anatomy, function and metabolism

2.1.1. Serotonin: location in the body and CNS.

Serotonin (5-Hydroxytryptamine) is a signalling molecule that elicits a variety of effects throughout the body (13). These include, but are not limited to, actions in sleep, pain, appetite, and sexual behaviour (14). About 95% of serotonin is found in the gastrointestinal tract, the rest is located in the CNS, blood platelets, pineal gland and mast cells.

In the CNS, serotonergic cell bodies are restricted mostly to the raphe nuclei of the brainstem (15-17) (Figure 2). Their projections, however, innervate nearly every brain region, and extensively so: serotonergic terminals, for example, comprise about one in two hundred of all axon terminals present in the cerebral cortex (18). This pattern of distribution appears to be stable across phylogeny (19).
Figure 2. Schematic drawing of major serotonergic nuclei along with their ascending and descending projections (9).

Of the raphe nuclei, the dorsal and median raphe nuclei contain the largest number of 5-HT cell bodies and the broadest arrays of projection fibers (16). The dorsal raphe nucleus, situated in the ventral part of the periaqueductal gray matter of the midbrain and extending into the periventricular gray matter of the pons, contains 40-60\% of 5-HT neurons in the CNS, depending on the species. Serotonergic cells comprise only one third of its cells however: other neurotransmitters, such as GABA, and substance P, are released from raphe neurons both from non-serotonergic cells, and from serotonergic cells as well (20).

Raphe nuclei projections are divided into ascending and descending pathways (21).
The former projects extensively to the cerebral cortex, basal ganglia, limbic system and diencephalon. The latter project to the locus coeruleus, cerebellum, and to the substantia gelatinosa and ventral horn of the spinal cord. Most serotonergic fibers are unmyelinated (22).

2.1.2. Serotonin receptor types, locations and functions.

Response by targets to 5-HT release is mediated by seven distinct receptor families (5-HT₁ through 5-HT₇) (23). All receptors are G-protein coupled, except for the 5-HT₃ ligand-gated channel, and contain seven transmembrane-spanning domains and three intracellular loops.

Of importance to this study are the location and action of the 5-HT₁ receptor family because the anti-migraine drugs under study are 5-HT₁ receptor agonists (Table 1) (1). Receptors of this family are linked to the inhibition of adenylyl cyclase.

5-HT₁A receptors, although widespread throughout the brain, are found to be particularly abundant in the hippocampus, entorhinal cortex, septum, and the dorsal and median raphe nuclei (24, 25). In the dorsal raphe nucleus, they are predominantly located on the neuron cell bodies and dendrites and are thought to inhibit neuronal firing (26).

5-HT₁B receptors are found in highest abundance at the basal ganglia and, at lower levels, in the hippocampus, cerebellum and raphe nuclei (27,28). They have been classically described as being the presynaptic inhibitory autoreceptors in the regulation of 5-HT release (29). They also inhibit the release of other neurotransmitters upon their stimulation at the presynaptic terminals of those neurons. 5-HT₁B receptors are also located on cerebral and peripheral blood vessels and mediate contraction and possibly
dilation by altering functioning of vascular smooth muscle (30).

5-HT_{1D} receptors are frequently co-distributed with 5-HT_{1B} receptors, but often in lower amounts (28). There is evidence that these receptors play an inhibitory somatodendritic role apart from the 5-HT_{1A} receptors in rats (31). But generally they are thought to play the same role as the 5-HT_{1B} receptors at the nerve terminals.

5-HT_{1F} receptors are thought to act as another autoreceptor and are most concentrated in the dorsal raphe nucleus, striatum, hippocampus and layers V and VI of the cerebral cortex (32).

As is mentioned above, it is pertinent to note, as it relates to migraine and the drugs used in the experiments below, that the nucleus trigeminalis caudalis (the spinal nucleus that receives input from the trigeminal nerve) is populated with low levels of all of the 1B, 1D and 1F receptors (35).

5-HT_{7} receptors can be found in the hypothalamus, cerebellum, superior colliculus, raphe nuclei, intestine and heart of rats and mice (33). Recently, 5-HT_{7} receptor mRNA has also been found in human trigeminal ganglia (34).

2.1.3. Serotonin action on neurons, cerebral blood vessels and the BBB

Centrally, the majority of 5-HT is released by volume transmission (36). That is, it is released from nerve terminals and reaches targets by diffusion through extracellular space. The effect of 5-HT release is thus generally slow, long-lasting, diffuse and modulatory. However, classic junctions do occur on neurons.

These modulatory effects can be either inhibitory or excitatory depending on receptor type and location. For example, in the hippocampus, 5-HT_{1A} receptors abound and are partially responsible for pyramidal cell (CA1) membrane hyperpolarization upon
their stimulation by 5-HT (37). However, the same 5-HT$_{1A}$ receptors can enhance neurotransmitter release of norepinephrine neurons in the some veins (38). Stimulation of 5-HT$_2$ receptors by serotonin on facial motoneurons has been shown to cause a slow depolarization, and increased excitability (39). Even within a given cell type, differential receptor type can create for different effects. For example, 5-HT can both depolarize and hyperpolarize cortical neurons depending on the relative concentration of 5-HT$_2$ versus 5-HT$_1$ receptors, respectively (40). 5-HT$_3$ receptors appear to modulate fast excitations, both in the periphery and in the brain (for example in the hippocampus and striatum) (41). Many excitatory responses previously believed to be mediated by the 5-HT$_{1A}$ receptor perhaps actually correspond to activation of the 5-HT$_7$ receptor (42).

Central 5-HT release can also affect blood vessels. 5-HT neurons from the raphe nuclei send direct projections to intracerebral blood vessels (43) and their 5-HT release may ultimately also affect extracerebral blood vessels despite the fact that they do not directly appose on the latter vessels (44). Both intra- and extracerebral blood vessels have the capacity to take up 5-HT from the circulation (42,45). 5-HT release, regardless of the exact source, generally constricts arteries and arteriovenous anastomoses but can, in certain conditions, dilate arterioles (46-48).

Serotonin release has even been shown to increase BBB permeability in rats put under stressful conditions such as heat and forced swimming exercise (49,50). Evidence for such a role under regular conditions is contradictory (51,52).

2.1.4. Serotonin: cerebral uptake and synthetic pathway.

As 5-HT is unable to pass through the BBB, central neurons must synthesize it from the essential amino acid, L-tryptophan (reviewed in 53) (Fig. 3). L-tryptophan crosses the BBB by way of the L-amino acid transporter on both the luminal and
abluminal sides of the capillary endothelial cells, and enters serotonergic neurons once inside the brain, through this same transporter (54). Once inside the serotonergic cells, L-tryptophan is hydroxylated to form 5-hydroxytryptophan via the enzyme tryptophan hydroxylase (TPH: E.C. 1.14.16.4). This is considered to be the rate limiting step of synthesis once L-tryptophan is in the cell (55). 5-hydroxytryptophan is then rapidly decarboxylated to 5-HT by the enzyme aromatic L-alpha amino acid decarboxylase (AAAD: EC 4.1.1.28).

Figure 3. The metabolic pathways for the synthesis and degradation of 5-HT. In the blood, tryptophan binds to proteins, in the brain, it is incorporated into them. See text for details. (Trp = tryptophan, TPOH = tryptophan hydroxylase, 5-HTrp = 5-hydroxytryptophan, AAAD = amino acid decarboxylase, 5-HIAA = 5-hydroxyindoleacetic acid, MAO = monoamine oxidase).

5-HT synthesis is affected by the concentration and functioning of tryptophan hydroxylase (56). Tryptophan hydroxylase requires molecular oxygen and a reduced pterin cofactor and a high extracellular calcium levels for optimal functioning (57). 5-HT synthesis is also affected by neuronal activity (58) and, most influentially, by the
availability of L-tryptophan in the blood plasma (59-61). The latter is affected by its absence or presence in the diet and concentration relative to other amino acids that compete for the same BBB transporter.

2.2. Serotonin and Migraine Pathogenesis

In some respects, the understanding of migraine pathophysiology can still be considered to be in its early stages. It has been hampered chiefly by technological and clinical limitations. That is, PET and other scanners are still mostly available in large centres only and have been so for at most the past 30 years. Moreover, pain assessment is subjective and many signs and symptoms of other conditions present themselves in tandem with migraine. Thus, the theories and data presented below are the tentative collection of what is still a fledgling body of information.

2.2.1 The serotonin hypothesis.

Several lines of evidence point to serotonin as playing a potentially pivotal role in the pathogenesis of migraine headaches (reviewed in 62-64). One suggested hypothesis is that migraine sufferers have chronically low serotonin functioning which brings about sensitization or up-regulation of serotonin receptors, possibly the 5-HT$_{2B}$ receptor. A sudden increase in 5-HT levels, as the result of an external trigger, to such a genetically predisposed brain would precipitate or help precipitate the chain of events collectively known as a migraine attack (Fig. 4). The evidence for this hypothesis is mostly indirect and circumstantial.
Figure 4. Hypothetical serotonergically-based mechanism of migraine pathogenesis. Endothelial cells, highly sensitized to 5-HT possibly through their $5\text{-HT}_{2B}$ receptors, would potentially release NO in response to high levels of 5-HT that have arisen from an increase in raphe nuclei firing rate. This both vasodilates cranial blood vessels and activates trigeminovascular afferents leading to migraine pain sensation.

First, there is evidence for altered 5-HT turnover during attacks. Blood plasma 5-HT content increases during migraine attacks concurrent with decreases in 5-hydroxyindoleacetic acid (5-HIAA) have consistently been found in both major forms of migraine (with and without aura) (65). Increased excretion of 5-HIAA in urine has been found during migraine attacks (66), although this has not been consistently reproduced (67). 5-HIAA levels are reportedly increased in migraine patients in the cerebrospinal fluid (CSF) as well (63). These changes are thought primarily to reflect changes in the brain. However, platelet 5-HT content has also been shown to decrease during migraine attacks (68).

Further, there is evidence that migraineurs have low 5-HT tone compared to non-sufferers. The amplitude of auditory evoked potentials has been shown to be inversely related to central serotonergic neurotransmission (69). A marked increase in the
amplitude of these potentials was seen in migraine patients between attacks, supporting low 5-HT transmission and suggesting abnormal cortical processing of sensory information (70). Also, after administration of brain penetrant $5\text{-HT}_{1B/1D}$ receptor agonist anti-migraine drugs, which would activate inhibitory presynaptic receptors and thus reduce 5-HT release, increases in the amplitude of these potentials in both migraine and non-migraine sufferers occur (71). In line with these findings are migraine’s comorbidity with depression and anxiety (72), disorders also thought to be a result of constitutively low serotonin levels, and an anecdotical finding of a patient with a 5-HT-releasing tumour who’s migraines decreased in frequency and intensity until they ultimately stopped as the tumour grew. Removal of the tumour saw the recommencement of headaches (73).

Support of the serotonin hypothesis is also bolstered by the findings that migraine or migraine-like headaches are more easily elicited by serotonin releasing agents in sufferers than non-sufferers. Several drugs that release 5-HT from neurons and blood platelets (fenfluramine and reserpine) and some 5-HT reuptake inhibitors are all able to precipitate migraine attacks more frequently in migraine patients than controls (63,64,74). Similarly, the 5-HT receptor agonist $m$-chlorophenylpiperazine (m-CPP) can induce severe attacks, indistinguishable from spontaneous migraines, in sufferers and those with a family history of migraine (75). While some of the prophylactic medications used for migraine may act at the 5-HT$_{2B}$ receptor, not all serotonin releasing agents generate migraines nor do all 5-HT$_2$ receptor antagonists effectively prevent migraines (76,77).
2.2.2. *A migraine generator?*

Raphe nuclei involvement in the surge of 5-HT responsible for spontaneous attacks is a distinct possibility. In 1995, a positron emission tomography (PET) study was performed measuring cerebral blood flow in migraine sufferers (78). During the attacks, increased blood flow was found in the cerebral hemispheres in cingulate, auditory and visual association cortices and in the brain stem. However, only the brain stem activation persisted after the injection of sumatriptan had induced complete relief from headache and phono- and photophobia. The resolution of the PET scanner was such that isolating the activity of the raphe nuclei was not possible. However, the active region encompasses the raphe nuclei and the locus coeruleus (78). This year another PET study induced migraine without aura in an individual who also suffered from cluster headache. Upon induction of the pain, an area in the dorsal rostral brainstem was activated as measured by the scanner - an area that did not light up in the cluster headaches (79). In further support of raphe involvement in migraine, brain serotonin synthesis capacity has been seen to increase after prophylactic treatment in migrainous women, as measured by PET and the alpha-methyl-L-tryptophan method described below (80).

The 'migraine generator' theory is a very enticing possibility, as this could tie together a number of headache-related phenomena. Raphe nuclei are involved in regulation of many functions including, but not limited to, stress, pain, appetite, mood and sleep (81) all of which are affected during migraine. The nuclei have been shown to increase their firing pattern in response to stressful stimuli (82), which is consistent with the observation that stress can induce migraine headaches. Raphe firing patterns also
change with position in the sleep-wake cycle: their activity is highest during wakefulness and is absent during rapid eye-movement sleep (83). This may explain why the most commonly used and intuitive remedy for migraines is sleep.

2.3. Measuring In Vivo Serotonin Synthesis

Given the possible importance of serotonin in the pathogenesis of migraine, a method to measure its synthesis in vivo was clearly needed. The method used in many of the experiments below is elaborate enough to warrant a detailed review. This is a method that has been developed in the past decade that purports to measure in vivo serotonin synthesis, as opposed to release, using PET technology in humans and other larger animals and radioautography in rats and other smaller animals (84,85).

2.3.1. Previous methods of synthesis measurement.

Prior to the inception of this method, two approaches were used to measure in vivo 5-HT synthesis (86,87). The rate of serotonin synthesis has been estimated on the basis of changes in the concentration of tryptophan metabolites after pharmacological manipulations. This is problematic because feedback in the synthetic pathway is eliminated by the drug used giving misleading results. The other approach has been to radioactively label L-tryptophan. This approach also lacks appeal as it 1) necessitates chemical separation of metabolites and removal of tracer incorporated into proteins, and 2) is hindered by the very fast removal of 5-HIAA from the brain.

2.3.2. The tracer: alpha-methyl-L-tryptophan.

The method uses a labelled analogue of L-tryptophan called alpha-Methyl-L-Tryptophan (α-MTrp). α-MTrp follows the metabolic pathway of tryptophan and is
ultimately converted into alpha-Methyl serotonin (α-M5HT) (88). Unlike 5-HT, α-M5HT is not a substrate for the enzyme monoamine oxidase (MAO: E.C. 1.4.3.4) and therefore accumulates in brain tissue, staying there for a time period longer than the experimental procedure (88,89). α-M5HT has also been shown to replace serotonin functionally, at least in some situations (89).

2.3.3. The three-compartment mathematical model.

Ultimately, the rate of uptake and conversion of the tracer into α-M5HT is calculated, based on a 3 compartment mathematical model, and this is used to calculate the rate of uptake of L-tryptophan and its conversion into 5-HT. This model is based on the same principles as the 2-deoxyglucose model of Sokoloff et al (90).

![Figure 5. Schematic of the mathematical, three-compartment model as proposed by Diksic et al (84). The asterix represents movements of tracer. Lack of an asterix represents movement of tracee. Cp = concentration of tracee/er in blood plasma, C_e = concentration of tracee/er in metabolic pool, C_M = concentration of tracee/er in metabolised or irreversible pool. See text for further details.](image-url)
The biological model can be represented as shown in Figure 5 (84,85). Tracer movement between different compartments is described by the first-order rate constants. Transfer from the plasma to brain is represented with $k_1^* \text{ (min}^{-1})$ and back with $k_2^* \text{ (min}^{-1})$, and the conversion of $\alpha$-MTrp into $\alpha$-M5HT is represented by $k_3^* \text{ (min}^{-1})$. In the derivation of the operational equation, it is assumed that hydroxylation or the process described by $k_3^*$ is the rate-limiting step in the conversion of $\alpha$-MTrp into $\alpha$-M5HT. This is supported by the fact that very little alpha-Methyl-5-Hydroxytryptophan is found in the brain tissue following administration of $\alpha$-MTrp. However, $k_3^*$ can also represent any step after which $\alpha$-MTrp cannot return to the precursor pool. Tracer movement between different compartments can be described with a set of differential equations:

\[ \frac{dC_E^*}{dt} = K_1^* \cdot f_F^* \cdot C_p^*(t) - (k_2^* + k_3^*) \cdot C_E^* (t) \quad (1) \]

\[ \frac{dC_M^*}{dt} = k_3^* \cdot C_E^* (t) \quad (2) \]

Equation 1 describes the change in the amount of tracer (in nCi g\(^{-1}\) min\(^{-1}\)) in the brain supply compartment dC\(_E^*\)/dt (assumed to be the brain amino acid pool drawn on for serotonin synthesis), and dC\(_M^*\)/dt (Eq. 2) describes the change in the amount of tracer in the brain metabolic compartment with time. The constant $K_1^*$ (in mL g\(^{-1}\) min\(^{-1}\)) is the first-order rate constant $k_1^*$ multiplied by the plasma volume in a unit weight of the brain (mL/g). The term $f_F^*$ represents the fraction of free tracer present in the plasma (a part of the tracer, just like the tracee, is bound to the plasma proteins and is not considered to
influence the serotonin synthesis rate). The terms $C_p^*(t)$, $C_E^*(t)$ represent the plasma and precursor pool concentrations of tracer as a function of time, respectively. Similarly, $C_M^*(t)$ represents that of the 'irreversible' pool.

By using the initial conditions $C_E^*(0) = 0$ and $C_M^*(0) = 0$, a solution to the differential equations can be attained that describes the activity in each brain compartment as a function of time. A nonlinear least-squared fitting of measured brain tissue tracer radioactivity after different circulation times (various times after tracer injection in PET studies), using the plasma tracer concentration as an input function, yields values for the transfer coefficients. However, since the individual rate constants are estimated with quite large errors, and the fraction of the tracer in the precursor pool is large, the use of the full operational equations is not practical unless the experiment is of long duration and regional rate constants are known.

An alternate approach here takes advantage of the fact that after a steady state has been reached the tissue volume of distribution of tracer is a linear function of the exposure time ($\Theta(T) = \int_0^T C_p^*(t) \cdot dt / C_p^*(T)$ such that each unit of $\Theta$ time exposes the brain to equal amounts of tracer).

\[
\frac{C_T^*(T)}{C_p^*(T)} = K^* \Theta(T) + V_{app} \tag{3}
\]

Here $K^*$ is a constant for the net unidirectional trapping (transfer into the metabolic compartment; $K^* = f_p^* \times (K_1^* \times k_3^*)/(k_2^* + k_3^*)$) and $V_{app}$ is an apparent volume of the tracer distribution. This method of analysis prevents the estimation of $k_3^*$, which
might be related to in vivo enzyme activity. Because the objective of this model is the measurement of the rate of serotonin synthesis rather than the rate constant of tryptophan hydroxylase, the parameter \( K^* \) obtained from Eq. 3 is all that is needed. \( K^* \) is related to serotonin synthesis by Eq 4.

\[
R = \frac{C_p}{LC} \cdot \frac{K_1^* k_3^*}{k_2^* + K_3^*} = \frac{C_p}{LC} \cdot K^* \tag{4}
\]

Here \( R \) is the rate of 5-HT synthesis, \( C_p \) is the plasma free tryptophan concentration and the lumped constant \( (LC) \) is the ratio between the unidirectional uptake constant of the tracer over that of the tracee. It can also be defined as the ratio between the tryptophan hydroxylase Michaelis-Menten constants for tracer \( (K_m^* \text{ and } V_{\text{max}}^*) \) and tracee \( (K_m \text{ and } V_{\text{max}}) \) and their volumes of distribution \( (LC = (V_{\text{D}}^*/V_D) \times (K_m/V_{\text{max}})/(K_m^*/V_{\text{max}}^*).) \)

2.3.4. Controversy over the method's validity.

The evidence supporting the validity of the method is substantial, but not uncontested (91-103). The single biggest debate surrounds the issue of the time line of conversion of the tracer into \( \alpha \)-M5HT, particularly in the PET studies. In the founding article, HPLC analysis showed that 31 and 45% of tracer was converted into \( \alpha \)-M5HT in rat dorsal raphe nucleus after 60 and 150 minutes, respectively (84). The percent conversion in the pineal gland was even higher in this autoradiographic experiment over the same time period. In PET studies, an apparent steady state, represented by the straight line portion in the plot of Eq. 3, has been shown to occur between around 20 to
60 minutes into scanning in all brain areas in more than one laboratory (93,95). However, in at least one cortical region, regression line calculations found another possible steady state from 60 to 90 minutes in monkey scans (99). These same investigators claim that post-mortem sampling of brain regions (of monkeys scanned for 180 minutes) with HPLC showed undetectable levels of α-M5HT (100).

The finding of undetectable levels of α-M5HT is very difficult to reconcile with the rest of the literature. There are a number of findings pointing to conversion of α-MTrp into α-M5HT within experimental time lines in addition to the conversion finding mentioned above. For example, a number of drugs that alter 5-HT neurotransmission without changing L-tryptophan concentrations alter the uptake of α-MTrp (97). In addition, differences in plasma L-tryptophan concentrations between males and females did not linearly reflect differences in serotonin synthesis values (94). That is, more than just α-MTrp uptake is likely being measured.

One measure that might be used to determine if the method is measuring what it purports to, might be to measure the concentrations of the chief metabolite of 5-HT, namely 5-HIAA, in the cerebrospinal fluid. However, it is not clear just how indicative a measure this is with regards to 5-HT synthesis. One study found that whole-brain 5-HT synthesis rates, as calculated by the α-MTrp method, where not significantly correlated with CSF 5-HTIAA levels of monkeys in a steady state (98). Advocates of the method reply that this might be expected given the number of other influences on CSF levels of 5-HIAA, and that CSF 5-HIAA changes slowly in response to changes in the rate of 5-HT synthesis because of the inherently slow rate of turnover of the pool of 5-HIAA (97).
Detractors claim that the turnover of this pool is sufficiently rapid to have been able to
detect changes in synthesis rates that occur within the experimental time line (99).

While some believe that no conversion takes place within experimental time lines,
others argue about the extent of conversion. As such, there is some debate over the exact
value of the lumped constant (101,102). Different assertions of its value often reflect
different methodologies or conceptions of how it should be calculated. For example, as
described above, the free fractions of tracer and tryptophan are used in the original model.
Some feel that the use of total tracer/tryptophan concentrations is more valid (102). It is
interesting to note however, that most who disagree with the value originally proposed
use either in vitro or ex vivo methods to support their claims for a different value. As
there are so many interdependent factors influencing the value of the lumped constant, the
in vivo estimation is the only measure with biological significance. Ultimately, this line of
debate does not question the model, simply the values of 5-HT synthesis estimated by it.

Another concern occurs over the universal use of one LC as opposed to different
LC's for each brain structure. Again, the above issues surface, but variability seen by all
investigators has been shown to be within the range of measurement error probability
(101). Again, should strong arguments come forward for the use of different LC's for
each structure, this would simply change the synthesis value estimations and not result in a
total rejection of the method.

Finally, critics of the method have claimed that the method is essentially simply
measuring tryptophan uptake and trapping given their finding of small levels of conversion
and a high correlation of R values with levels of free tryptophan in the plasma (98,99).
Recently, however, uptake and trapping constants of tryptophan and tracer have been shown to not be correlated with BBB permeability (as shown be the permeability surface area products) of tryptophan or tracer, respectively (103). Nor are uptake and trapping constants for tryptophan significantly correlated with protein synthesis rates (recall, α-MTrp is not incorporated significantly into proteins). The line of reasoning of the method’s proponents is thus presumably that the correlation of R values with plasma free tryptophan levels is a reflection of increased serotonin synthesis (and not protein synthesis) that takes place given a greater availability of the precursor.

3. EXPERIMENTAL PROCEDURES AND RESULTS

3.1. Study 1: Imaging the rate of serotonin synthesis by Positron Emission Tomography (PET) in human brain during migraine attack.

Hypothesis. The CBF increase in the brainstem of migraine patients (78) reflects an activation of serotonin neurons within the dorsal raphe nucleus that can be visualized in vivo by PET as an increase in the rate of 5-HT synthesis in brainstem and its projection areas.

Objectives. 1. To evaluate the rate of 5-HT synthesis in brain of migraine patients during migraine attacks and during migraine-free episodes. 2. To evaluate if sumatriptan (Imitrex; 6mg, s.c.) is able to affect 5-HT synthesis in brain, and if this occurs in the brainstem raphe nuclei as well.

3.1.1. Procedure

A cohort of 6 migraineurs with attacks without aura (selected according to the International Headache Classification Committee), known to be responsive to sumatriptan
has participated in the study thus far. Further patients will be recruited from the outpatient population at the Neurology Clinic of the Montreal Neurological Institute.

Suitability for the study is determined by Dr. Michel Aubé, neurologist and headache specialist at the Montreal Neurological Institute. Migrainous volunteers: 1. are male or female, between the ages of 18 to 65, 2. have a history of migraine without aura for at least 12 months prior to the study, 3. are able to distinguish migraines from other types of headaches they might have, and 4. are responsive to sumatriptan. They must not: 1. have a history of cardiovascular problems (ex. angina, atherosclerotic disease, arrhythmias, hypertension), 2. be pregnant or likely to become so during the study, 3. have certain concurrent illnesses or medications (ex. use of SSRI’s, or migraine prophylactics, have epilepsy, etc), 4. currently abuse non-medical drugs, 5. have a known hypersensitivity or intolerance of sumatriptan, 6. have previous radiation doses received within the past year (over 5mSv), and 7. have a pacemaker, aneurism chip, prostatic valve or metal prosthesis.

The subjects enrolled in the study are scanned (i) within 6 hours after the onset of migraine symptoms, (ii) after significant symptom relief with sumatriptan, and (iii) during headache-free intervals. The first 2 scans take place in succession on one occasion. The last scan takes place after the subject has been free of any migraine for at least 5 days.

Migraine severity is evaluated on a 3 point scale (3 = severe pain, 0 = no pain) before the treatment is administered and at 15, 30, 45, 60, 90 and 120 minutes post-dose or until the patients records Mild or Absent. Should sumatriptan fail to relieve migraine symptoms in the administration of codeine, as a rescue analgesic, will result and the patient will be withdrawn from the study.

Data analysis is performed on the 70 minute scans under all 3 conditions. An
anatomical magnetic resonance imaging scan is performed (on the same occasion as the 3rd PET scan). This is used to overlap (co-register) with the PET images so as to more accurately identify activity in regions of interest. The arterial input function is actually derived from veinous blood sampling in combination with saggital sinus activity measurements in a method previously described (103.5). Activity versus time curves and distribution volume versus θ time plots (also called Patlak plots) are completed for K* determination. Brain images from all subjects are transformed onto the Talairach space in order to perform statistical comparisons. The latter is done using the Statistical Parametric Mapping (SPM) program at the brain imaging centre. With the aid of SPM, regions of significant difference are identified between and across the three conditions. An a priori identified set of regions of interest is also be statistically compared. These consist of gross circumscriptions of the Frontal, Parietal, Occipital, and Temporal cortices, the Caudate, Putamen, Globus Pallidus, Thalamus, Amygdala, Hippocampus, Cerebellum and an attempt to encircle a brainstem region that encompases the Dorsal Raphe nucleus (called here the Greater Dorsal Raphe region).

3.1.2. Preliminary Results

Table 2 gives some of the key characteristics and physiological parameters for each subject. Table 2B shows the averaged free, total and free fraction of tryptophan (=free tryptophan/ total tryptophan) values for the 6 subjects for all 3 conditions. No significant difference was found between free tryptophan levels (One-way repeated measures ANOVA, F(2,5)=0.0745, p=0.49). This was also the case for the total tryptophan and free fraction of tryptophan (p=0.47 and 0.99 respectively). The first subject scanned is male, the remaining subjects are female.
Figure 6 shows the global or whole brain $K^*$ values measured in all three investigated states for the 6 participants studied thus far. These values are derived from the slope of the graph of the distribution volume versus theta time (see theory) between 20 and 70 minutes. Global $K^*$ values are measured based on the whole brain activity of approximately 12 slices (from slices surrounding the basal ganglia and 6 from slices near the top of the ventricles).

Figure 7 shows the global $R$ values in all 3 conditions. One-way Anova analyses comparing any given region of interest across the 3 conditions show no significant differences at the $p=0.05$ level. Figure 8 shows the $R$ values, in pmol/g/min for each of the 6 participants averaged across the 12 regions of interest.

### Table 2. Key characteristics and physiological parameters for each subject to date.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>BP</th>
<th>Pulse</th>
<th>HA Frequency</th>
<th>HA</th>
<th>Suma</th>
<th>Scanned at</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>54</td>
<td>1.73</td>
<td>79.4</td>
<td>120/75</td>
<td>65</td>
<td>18-20/month</td>
<td>3</td>
<td>3</td>
<td>5 hr 20 min</td>
</tr>
<tr>
<td>2</td>
<td>Female</td>
<td>21</td>
<td>1.73</td>
<td>65.8</td>
<td>110/70</td>
<td>72</td>
<td>5/year</td>
<td>3</td>
<td>1</td>
<td>14 hours</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>26</td>
<td>1.7</td>
<td>63.5</td>
<td>130/80</td>
<td>82</td>
<td>4-6/month</td>
<td>2.7</td>
<td>0</td>
<td>4 hr 50 min</td>
</tr>
<tr>
<td>4</td>
<td>Female</td>
<td>48</td>
<td>1.65</td>
<td>63.5</td>
<td>115/80</td>
<td>78</td>
<td>7/month</td>
<td>2</td>
<td>0</td>
<td>6 hr 40 min</td>
</tr>
<tr>
<td>5</td>
<td>Female</td>
<td>36</td>
<td>1.57</td>
<td>61.2</td>
<td>120/84</td>
<td>76</td>
<td>2-3/month</td>
<td>2.8</td>
<td>1.5</td>
<td>6 hours</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>46</td>
<td>1.49</td>
<td>43.1</td>
<td>120/80</td>
<td>72</td>
<td>6-7/month</td>
<td>3</td>
<td>0</td>
<td>5 hr 15 min</td>
</tr>
</tbody>
</table>

Average 38.5 1.645 62.8 120/80 74.2 6.7/month 2.75 0.92 7 hrs

Pain relief reflects the patients' subjective reporting of the degree of pain experienced on a three point scale: HA= pain reported during headache just prior to the first scan, Suma= lowest pain level reported after sumatriptan injection. The 'Scanned at' column lists the time between reported headache onset and injection of tracer. 'HA Frequency' in the frequency that participants typically experienced their migraines. Height is listed in metres, weight in kilograms, pulse in beats per minute and age is listed in years. Pulse and blood pressure (BP) are measured on the day of the migraine and sumatriptan scans.
Table 2B. Average plasma tryptophan levels in nmol/mL in the 3 conditions investigated.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Free 1</th>
<th>Free 2</th>
<th>Free 3</th>
<th>Total 1</th>
<th>Total 2</th>
<th>Total 3</th>
<th>Fraction 1</th>
<th>Fraction 2</th>
<th>Fraction 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7.13</td>
<td>7.80</td>
<td>12.78</td>
<td>32.10</td>
<td>63.50</td>
<td>44.30</td>
<td>0.22</td>
<td>0.12</td>
<td>0.29</td>
</tr>
<tr>
<td>2</td>
<td>7.28</td>
<td>7.69</td>
<td>5.10</td>
<td>43.80</td>
<td>42.70</td>
<td>58.40</td>
<td>0.17</td>
<td>0.18</td>
<td>0.09</td>
</tr>
<tr>
<td>3</td>
<td>3.30</td>
<td>6.74</td>
<td>3.99</td>
<td>44.90</td>
<td>51.90</td>
<td>55.10</td>
<td>0.07</td>
<td>0.13</td>
<td>0.07</td>
</tr>
<tr>
<td>4</td>
<td>6.82</td>
<td>8.67</td>
<td>7.60</td>
<td>51.30</td>
<td>56.40</td>
<td>30.40</td>
<td>0.13</td>
<td>0.15</td>
<td>0.25</td>
</tr>
<tr>
<td>5</td>
<td>9.28</td>
<td>8.83</td>
<td>8.42</td>
<td>23.99</td>
<td>24.33</td>
<td>23.57</td>
<td>0.39</td>
<td>0.36</td>
<td>0.36</td>
</tr>
<tr>
<td>6</td>
<td>9.03</td>
<td>10.60</td>
<td>6.55</td>
<td>40.35</td>
<td>39.88</td>
<td>41.53</td>
<td>0.22</td>
<td>0.27</td>
<td>0.16</td>
</tr>
<tr>
<td>Average</td>
<td>7.14</td>
<td>8.39</td>
<td>7.41</td>
<td>39.41</td>
<td>46.45</td>
<td>42.22</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
</tr>
<tr>
<td>SD</td>
<td>2.15</td>
<td>1.32</td>
<td>3.09</td>
<td>9.83</td>
<td>13.90</td>
<td>13.56</td>
<td>0.11</td>
<td>0.09</td>
<td>0.11</td>
</tr>
</tbody>
</table>

1 = Migraine, 2 = After Sumatriptan injection, 3 = Migraine Free. Free tryptophan indicates all tryptophan that is not bound to plasma proteins, total includes both that tryptophan bound and unbound and the free fraction of tryptophan ('Fraction' in table) is the ratio of the free to the total tryptophan levels. Subject number 1 is male, the remaining subjects are female. The numbers are derived from the average of 3 measurements in the case of the free tryptophan and the average of 2 measurements in the case of the total tryptophan, all taken immediately prior to or at selected intervals during the scans.
Figure 6. Global or whole brain K* values in mL/g min in each of the 3 Migrainous states.

Figure 7. Global in vivo 5-HT synthesis values (R values) Measured to date.
Average R Value For Each Region of Interest

![Graph showing R values for different regions of interest]

<table>
<thead>
<tr>
<th>Region of Interest (ROI)</th>
<th>Frontal Cx</th>
<th>Parietal Cx</th>
<th>Occip Cx</th>
<th>Temp Cx</th>
<th>Caudate</th>
<th>Putamen</th>
<th>Glob Pall</th>
<th>Thalamus</th>
<th>Amyg</th>
<th>Hipp</th>
<th>Chlm</th>
<th>Greater Dura</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migraine</td>
<td>103.618</td>
<td>94.6103</td>
<td>82.6418</td>
<td>101.791</td>
<td>100.549</td>
<td>116.469</td>
<td>100.663</td>
<td>106.347</td>
<td>93.7453</td>
<td>104.25</td>
<td>117.572</td>
<td>72.4458</td>
</tr>
<tr>
<td>Sumatriptan</td>
<td>82.8826</td>
<td>71.92</td>
<td>60.5423</td>
<td>80.3878</td>
<td>76.6887</td>
<td>93.1688</td>
<td>80.3934</td>
<td>84.2269</td>
<td>74.9143</td>
<td>85.9512</td>
<td>84.2583</td>
<td>62.3043</td>
</tr>
<tr>
<td>No Migraine</td>
<td>97.2394</td>
<td>88.953</td>
<td>78.087</td>
<td>92.9139</td>
<td>97.8591</td>
<td>105.292</td>
<td>90.9959</td>
<td>96.2527</td>
<td>91.6534</td>
<td>87.8783</td>
<td>109.546</td>
<td>66.5465</td>
</tr>
</tbody>
</table>

Figure 8. R values, in pmol/g/min, for each region of interest (ROI), averaged 6 subjects, in the 3 Migrainous conditions examined. See text for description of the regions of interest.

A correlation analysis found no significant relation between 5-HT synthesis level change ($\Delta R = R_{\text{after Sumatriptan injection}} - R_{\text{during Migraine}}$) and reported pain change levels on the 3 point scale ($\Delta \text{Pain} = \text{Pain}_{\text{after Sumatriptan injection}} - \text{Pain}_{\text{during Migraine}}$) ($r = -0.19, t = -0.41, p = 0.85$).

Statistical Parametric Mapping (SPM) analysis on the first 5 subjects yielded the following results (Table 3). Clearly, these results, based on such a small sample size, are
not conclusive. However, this is an example of the form of the results that will be seen upon completion of the study.

Table 3. SPM results based on the first 5 subjects.

<table>
<thead>
<tr>
<th></th>
<th>Scan 1 (Migraine)</th>
<th>Scan 2 (Sumatriptan)</th>
<th>Scan 3 (Interictal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scan 1</td>
<td></td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
<tr>
<td>(Migraine)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scan 2</td>
<td></td>
<td>Cuneus.</td>
<td>n.s.</td>
</tr>
<tr>
<td>(Sumatriptan)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scan 3</td>
<td>n.s.</td>
<td>Left Precuneate and</td>
<td></td>
</tr>
<tr>
<td>(Interictal)</td>
<td></td>
<td>Cingulate Gyri,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right Cingulate Gyrus</td>
<td></td>
</tr>
</tbody>
</table>

Columns have significantly higher rates of 5-HT synthesis than rows. For example, in column 2, row 3 one reads that the Cingulate Gyrus was found by the program to exhibit significantly higher 5-HT synthesis rates during the sumatriptan scan than during the interictal (between attacks) scan.

3.1.3. Discussion

Perhaps the most surprising finding that can be acted upon until further volunteers are available for scanning is the possible ability of sumatriptan to influence 5-HT synthesis in central brain structures. Sumatriptan has commonly been thought to be poorly BBB penetrant (1), despite the fact that there are clinical reports of fatigue and mood changes following its administration (104). Should this be true then a number of possibilities emerge.

There is always the possibility that, during migraine, there is a breach of the BBB which lets the drug through centrally. To the extent that cerebrospinal fluid content levels
are indicative of a BBB breach this is not likely as no change in CSF pressure, proteins or cell count has been found during migraine except in life-threatening migraine (reviewed in 105). Sumatriptan could also be accessing the brain through the few known areas of BBB absence, such as around the hypothalamus. Certainly in rats, there is evidence, using at least one other method of measurement of 5-HT turnover that the levels in the hypothalamus and striatum are affected (turnover increased) by sumatriptan (106).

Sumatriptan could also be BBB permeable, but only over a longer period of time than previously examined in humans or other animals. A recent study, investigating the transport of various triptans across an in vitro culture of a particular cell line over-expressing P-glycoprotein (Pgp, an efflux transporter at the BBB), showed that all triptans did cross the cell line (107). While some of the more brain-penetrant triptans were a substrate for Pgp (ex. eletriptan), sumatriptan was not. Perhaps then, sumatriptan’s slow penetration might be offset by a lack of rapid exit through the efflux transporter. In support of the BBB penetrance possibility is the recent finding that sumatriptan can be found in the spinal fluid of healthy volunteers, peaking in concentration 5 hours after injection (108). Also consistent with this is the finding of a lack of correlation between lipophilicity of triptans and the frequency of CNS adverse events attributable to each triptan (3). Should the drug more readily cross the BBB than previously thought, the findings are consistent with the ability of 5-HT₁B/D autoreceptors to inhibit serotonin release and synthesis. Discussion of this possibility is elaborated upon in the rat studies.

Alternatively, the drug could be acting peripherally in such a manner that central 5-HT synthesis is affected. For example, sumatriptan could possibly act on the amino acid
transporter competing for or inhibiting amino acid transport (and therefore α-MTrp and tryptophan) into the brain. However, this possibility seems unlikely as the drug would have to attain levels in the plasma that approximates the $K_m$ of the large neutral amino acid transporter at the BBB ($K_m$ = amino acid concentration at which the speed of transport is half that of its maximum speed). For sumatriptan, the plasma drug level would have to be greater than 1 µM (Pardridge, WM, 2000, personal communication). A subcutaneous dose of 6 mg of sumatriptan leads to an average plasma concentration of 0.17 µM in a human having the average 5.6 L of blood ($M_{W_{sumatriptan}} = 413.5$ g/mol) (108).

Another alternative peripheral action of sumatriptan which could indirectly alter synthesis levels might arise from its effect on the hypothalamic-pituitary-adrenal (HPA) axis. The HPA axis and the serotonergic systems interact extensively in animals (109, 110). Investigations into the effect of sumatriptan on the HPA axis have yielded mixed results: blood cortisol concentration (the chief end-product of the axis, see Figure 9) has been reported to increase (111), decrease (112) and not change at all (114) after drug administration in humans. The time course after drug administration and the method of administration are critical here however. Namely, all of the three studies report an increase in blood cortisol concentration during the first two hours, it is simply the first that finds the increase significant within this time period. The other two studies claim the decrease and lack of change on time intervals of twenty-four and two hours, respectively. Also, the decrease finding was after oral administration whereas the no change and increase findings were after the drug was administered by subcutaneous injection.

Certainly anatomy makes an indirect effect on synthesis through the HPA axis a
possibility as direct stimulatory serotonergic innervation of the hypothalamus (particularly around the paraventricular nucleus) and pituitary are known to exist \((112, 114)\). Of course, the BBB is more permeable and non-existent in these two areas, respectively. As it pertains to our findings, it is therefore possible that \(5\text{-HT}_{1B/1D}\) autoreceptor binding on serotonergic nerve terminals would inhibit serotonin release onto these two key components of the axis and therefore subsequent release of vasopressin (AVP) and/or corticotrophin releasing factor (CRF) from the hypothalamus and/or adrenocorticotropic releasing hormone (ACTH) from the pituitary which would in turn reduce cortisol release from the adrenal gland. This could then result in lowered \(5\text{-HT}\) synthesis as cortisol blood levels is known to be positively correlated with \(5\text{-HT}\) synthesis \((115, 116, 117)\). In addition, \(5\text{-HT}_{7}\) receptors are known to exist on the adrenal gland \((118)\) and, as seen in Table 3, sumatriptan has some affinity for these receptors. Again, as data is mixed on the effect of sumatriptan on the HPA axis this is still speculative.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure9.png}
\caption{Schematic of some of the connections between the HPA axis and the serotonergic systems. A positive sign represents a stimulatory effect; a negative sign represents an inhibitory effect. The Hippocampus is shown as an example of a central structure that is affected by blood cortisol levels. (AVP - vasopressin, CRH - corticotropin, ACTH - adrenocorticotropic)}
\end{figure}
It is important to address the issue of the drug's ability to constrict blood vessels. It might be tempting to speculate that vasoconstriction of the cerebral microvasculature would result in reduction of free tryptophan available to the brain and therefore and reduction in the synthetic rate of 5-HT. However, evidence points to this possibility not being likely. Treatment of migraine attacks without aura with 6 mg of subcutaneous sumatriptan are not associated with detectable focal changes of CBF (119). Also, brain imaging studies on migraine without aura find no difference in blood flow between ictal and interictal states, regionally or globally (reviewed in 120). In healthy human volunteers, a 2 mg (i.v.) dose of sumatriptan had no detectable effect on regional cerebral perfusion, pulse, blood pressure or electrocardiogram indices (121).

In terms of the other amino acids competing for the transporter, most participants did not eat as a result of the headache (which is common in migraine) and one ate the same food on both days of her inter and interictal scans. Only one participant (#3) ate between headache and drug scans which is likely the reason for noticeable difference between free tryptophan levels in the two conditions. Both the levels of interictal free and total tryptophan compare with those published (94), offering tentative support thus far to the notion that should there be a systemic alteration in serotonin metabolism in those with migraine, it does not seem to affect, nor likely be a result of, the levels of plasma free or total tryptophan.

The interictal K* values of migraine sufferers are somewhat lower than those published in the only other study to use this method in migraine (80). In this study, by Chugani et al, values are based on the Patlak plot between 20 and 60 minutes as opposed
to between 20 and 70 minutes as was done here. $K^*$ values in our participants did rise when such a portion of the curve was used. Chugani et al did not report R values. Interictal R values, however, are not higher than reported before in healthy volunteers using this same method (94). These values, however, might or might not be directly comparable as our participants were not required to fast prior to scanning.

Interestingly, the correlation between pain and serotonin synthesis becomes positive, though still not significant, after elimination of the lone male participant. In addition to being the lone male, his headache has been transformed from the overuse of sumatriptan (Dr. M. Aubé, personal communication), he reported no pain decrease upon administration of the drug (the only participant to do so), and a mild (1 on the 3 point scale) headache started but then vanished just prior to the interictal scan. In general, however, pain is not necessarily expected to correlate with serotonin synthesis given the other inputs (psychological, vascular, and myofascial) deemed necessary for its sensation (121i).

While the overall trend as seen thus far is consistent with the migraine generator theory (namely, higher 5-HT production ictally than interictally) individual variability is significant. One possible explanation is that values measured here are ‘snapshots’ in time of the overall temporal pattern of production during and between attacks. For example, it is possible that measurements taken at later time points after onset will be less than those closer to attack onset. Thus the desire to capture spontaneous and not induced attacks introduces practical time limits to the study. Chief amongst these are additional time introduced in participant transport, tracer production, and making the scanner available.
3.2. Study 2: Effect of anti-migraine 5-HT₁ receptor agonists on the rate of serotonin synthesis in rat brain

The preliminary results of the first study raised the possibility of a central effect of sumatriptan on central synthesis patterns. In order to help determine the mechanism of action of sumatriptan, it was decided to mimic the PET project as best as possible in healthy rats, using autoradiography, after injection of sumatriptan or zolmitriptan (85). The latter triptan is known to be brain penetrant (11). Should the pattern of 5-HT synthesis of the two drugs be identical this would support the claim that sumatriptan is actually BBB penetrant over the time course of our study, including the PET study in migraine sufferers. Should sumatriptan not have central effects in the healthy rats, other mechanisms of action of the drug will be more likely (such as a breach of the BBB during migraine or passage through the areas of BBB absence).

Sumatriptan was administered at 2 doses: 1mg/kg and 0.3mg/kg. The first dose is used in animal models of neurogenic inflammation, the second is more clinically relevant (see discussion below).

3.2.1. Procedure

Male Sprague-Daly rats weighing 200-250 g were used in the experiments. All animals underwent the surgical preparation as described (84). Two hours after surgery, and 20 minutes before tracer injection, ~0.45 mL of either saline, zolmitriptan (100μg/kg) or sumatriptan (1000μg/kg) was injected subcutaneously (beneath the loose skin on the rat's neck). α-[¹⁴C]MTrp, ~30 μCi in 0.5 ml of normal saline, was injected intravenously over 2 min at a constant rate using an infusion pump (model 355; Sage Instruments). To obtain a time-radioactivity curve for plasma, 12-14 arterial blood samples were collected
at increasing time intervals up to the killing time. The blood samples were rapidly centrifuged and the plasma was treated as described previously (84). $^{14}$C radioactivity in the deproteinized plasma samples was measured with a scintillation counter (1212 Dackbeta Liquid Scintillation Counter; Wallac by Turke, Finland). At the beginning and at selected time points throughout each experiment, further 50 µl volume of plasma were pipetted into microcentrifuge tubes containing 25 µl of 20% trichloroacetic acid. After centrifugation, 50 µl of supernatant was used for the plasma tryptophan determination. Supernatants were stored at - 83°C until HPLC analysis was done.

Animals were decapitated at two different times, 60 and 150 min after the beginning of tracer injection for each of the three conditions (saline, sumatriptan and zolmitriptan). All animals were killed by guillotine between 1:30 and 3:30 pm to avoid the effect of circadian rhythm.

Tissue slices were digitized using a microcomputer-based image analysis system (image Calculator; Soquelec Ltd., Montreal) consisting of a video camera, a frame grabber, an IBM compatible computer, and appropriate software. Optical densities were converted into tissue tracer concentration (nanocuries per gram) based upon a standard calibration curve made up with $^{14}$C- standards.

A non-linear regression (double exponential decay) was performed on the plasma input curves from their peaks onwards in order to fit the curves and thus determine the best Cp*(T) values for each of the curves. $K^*$ and R values were calculated for both control and drug conditions as described in the theory and compared statistically using a standard regression analysis (see Appendix).
3.2.2. Results - Part A.

The average values for physiological parameters of rats used in the experiments described here are given in Table 4. It can be seen that there is no significant difference in any of the basic physiological values between rats in any of the three conditions. Body weights do vary significantly between control and migraine drugs (Mann Whitney, p<0.05) but not between migraine drugs.

Table 4. Physiological parameters of rats used in Part A experiments.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Saline</th>
<th>Sumatriptan</th>
<th>Zolmitriptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.45 ± 0.03</td>
<td>7.44 ± 0.03</td>
<td>7.44 ± 0.02</td>
</tr>
<tr>
<td>PCO₂ (mm Hg)</td>
<td>37.79 ± 4.7</td>
<td>39.63 ± 2.9</td>
<td>38.75 ± 3.1</td>
</tr>
<tr>
<td>PO₂ (mm Hg)</td>
<td>97.04 ± 12.6</td>
<td>92.89 ± 8.7</td>
<td>90.49 ± 8.2</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47.9 ± 2.2</td>
<td>48.4 ± 3.6</td>
<td>46.1 ± 3.6</td>
</tr>
<tr>
<td>Plasma total tryptophan (nmol/mL)</td>
<td>67.0 ± 19.6</td>
<td>72.0 ± 18.6</td>
<td>70.4 ± 13.9</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>206 ± 11</td>
<td>222 ± 15</td>
<td>232 ± 19</td>
</tr>
</tbody>
</table>

All values are given as mean ± standard deviation.

Representative autoradiograms obtained from saline and sumatriptan treated rats are shown in Fig 10. Tracer concentrations as shown on the figure do not transform directly into volumes of distribution because small differences in the plasma curves (input functions) and plasma tracer concentrations preclude direct quantitative comparison. Despite what is indicated in the figure, structures where measured and averaged bilaterally as no significant lateralization of serotonin synthesis is found, as in other studies using this
As outlined in the theory, plasma input functions are determined and fitted for each rat in all conditions. A representative curve, in this case from a sumatriptan-treated rat sacrificed after 1 hour, is shown in Figure 11. The divisor of the tissue activity by the plasma activity at sacrifice time, ie. the distribution volume, is plotted versus Theta time for each brain structure. The example shown here is for the Dorsal Raphe nucleus (Figure 12). The slope of the regression line through these graphs gives the unidirectional uptake constants (\( K^* \) values) which are not shown.

Figure 10. Representative autoradiograms obtained for brain slices from rats killed 2.5 hours after the beginning of tracer injection (30 \( \mu \)Ci of \( \alpha-[\text{C}] \)-methyl-L-tryptophan). The sections are at the level of the hippocampus and thalamus, both of which are circled as they were for measurement. The left-most slides are from saline treated rats and the right most slides are taken from sumatriptan treated rats.
Figure 11. A representative plasma input function obtained in a rat injected with 30 μCi of α-[14C]methyl-L-tryptophan. Experimental or actual values are compared with those obtained from a non-linear regression exponential decay fit to the portion of the curve from the peak onwards.

Figure 12. Plot of the Dorsal Raphe distribution volume as a function of Theta in all 3 conditions.
Serotonin synthesis rates (R values) after sumatriptan injection are shown in Table 5. All 5-HT synthesis values are significantly lower than controls except for those of the caudate-putamen and the hippocampus. Synthesis rates after zolmitriptan injection are shown in Table 6. Again, all values are significantly lower than controls with the exception, in this instance, of the hypothalamus. A similar table contrasting the rates of the two drugs shows no significant difference in synthetic rates in all brain structures with the exception of the hypothalamus, where the average synthesis value is significantly lower in sumatripan treated rats ($t=3.65$, $p=0.001$).

Table 5. 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl) or sumatriptan (1000 µg/kg).

<table>
<thead>
<tr>
<th>Rat Brain Structure</th>
<th>Control SD</th>
<th>Sumatriptan SD</th>
<th>t</th>
<th>p</th>
<th>Ratio(S/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n=9</td>
<td>n=13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fr</td>
<td>43.40</td>
<td>34.34</td>
<td>2.32</td>
<td>0.031</td>
<td>0.791</td>
</tr>
<tr>
<td>Cg</td>
<td>47.88</td>
<td>35.78</td>
<td>3.25</td>
<td>0.004</td>
<td>0.747</td>
</tr>
<tr>
<td>Par</td>
<td>41.93</td>
<td>27.76</td>
<td>3.78</td>
<td>0.001</td>
<td>0.662</td>
</tr>
<tr>
<td>Cpu</td>
<td>55.16</td>
<td>48.61</td>
<td>1.73</td>
<td>0.099</td>
<td>0.881</td>
</tr>
<tr>
<td>GP</td>
<td>63.12</td>
<td>41.69</td>
<td>7.90</td>
<td>0.000</td>
<td>0.660</td>
</tr>
<tr>
<td>Th</td>
<td>51.42</td>
<td>33.94</td>
<td>5.60</td>
<td>0.000</td>
<td>0.660</td>
</tr>
<tr>
<td>Hipp</td>
<td>59.37</td>
<td>53.16</td>
<td>1.74</td>
<td>0.097</td>
<td>0.895</td>
</tr>
<tr>
<td>Hy</td>
<td>49.56</td>
<td>33.72</td>
<td>3.12</td>
<td>0.005</td>
<td>0.680</td>
</tr>
<tr>
<td>Amyg</td>
<td>68.89</td>
<td>51.64</td>
<td>3.72</td>
<td>0.001</td>
<td>0.750</td>
</tr>
<tr>
<td>Oc</td>
<td>48.34</td>
<td>33.99</td>
<td>3.16</td>
<td>0.005</td>
<td>0.703</td>
</tr>
<tr>
<td>Dra</td>
<td>184.97</td>
<td>156.57</td>
<td>3.22</td>
<td>0.004</td>
<td>0.846</td>
</tr>
<tr>
<td>Cbl</td>
<td>40.60</td>
<td>21.10</td>
<td>6.00</td>
<td>0.000</td>
<td>0.52</td>
</tr>
</tbody>
</table>

Rates are expressed in pmol/g/min. Standard deviations (SD) are given as well as the t-values, p-values. The last column displays the ratio of the R values of Sumatriptan treated rats to Control rats. (CODE: Fr - frontal cortex, Cg - cingulate cortex, Par - parietal cortex, Cpu - caudate-putamen, GP - globus pallidus, Th - thalamus, Hipp - hippocampus, Hy - hypothalamus, Amyg - amygdala, Oc - occipital cortex, Dra - dorsal raphe nucleus, Cbl - cerebellum)
**Table 6.** 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl) and the acute migraine treatment drug zolmitriptan (100 µg/kg).

<table>
<thead>
<tr>
<th>Rat Brain Structure</th>
<th>Control SD</th>
<th>Zolmitriptan SD</th>
<th>t</th>
<th>p</th>
<th>Ratio(Z/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr</td>
<td>43.40</td>
<td>6.27</td>
<td>29.64</td>
<td>14.02</td>
<td>2.81</td>
</tr>
<tr>
<td>Cg</td>
<td>47.88</td>
<td>5.91</td>
<td>30.33</td>
<td>12.61</td>
<td>3.96</td>
</tr>
<tr>
<td>Par</td>
<td>41.93</td>
<td>6.38</td>
<td>29.65</td>
<td>13.92</td>
<td>2.52</td>
</tr>
<tr>
<td>Cpu</td>
<td>55.16</td>
<td>4.25</td>
<td>41.84</td>
<td>13.46</td>
<td>2.88</td>
</tr>
<tr>
<td>GP</td>
<td>63.12</td>
<td>3.21</td>
<td>38.50</td>
<td>12.14</td>
<td>5.94</td>
</tr>
<tr>
<td>Th</td>
<td>51.42</td>
<td>4.30</td>
<td>32.42</td>
<td>11.62</td>
<td>4.73</td>
</tr>
<tr>
<td>Hipp</td>
<td>59.37</td>
<td>5.76</td>
<td>48.48</td>
<td>12.13</td>
<td>2.55</td>
</tr>
<tr>
<td>Hy</td>
<td>49.56</td>
<td>5.64</td>
<td>51.52</td>
<td>13.97</td>
<td>-0.40</td>
</tr>
<tr>
<td>Amyg</td>
<td>68.89</td>
<td>4.37</td>
<td>57.30</td>
<td>15.50</td>
<td>2.19</td>
</tr>
<tr>
<td>Oc</td>
<td>48.34</td>
<td>10.22</td>
<td>28.78</td>
<td>13.27</td>
<td>3.93</td>
</tr>
<tr>
<td>Dra</td>
<td>184.97</td>
<td>25.11</td>
<td>164.54</td>
<td>24.51</td>
<td>2.08</td>
</tr>
<tr>
<td>Cbl</td>
<td>40.60</td>
<td>7.41</td>
<td>21.35</td>
<td>13.39</td>
<td>4.03</td>
</tr>
</tbody>
</table>

Rates are expressed in pmol/g/min. Standard deviations (SD) are given as well as the t-values, p-values. The last column displays the ratio of the R values of Zolmitriptan treated rats to Control rats.

3.2.3. Results - Part B.

Table 7 shows the physiological parameters and tryptophan concentrations of rats measured during the second part of the experiment. Table 8 lists R values for the various rat brain structures after subcutaneous injection of either vehicle (0.9% NaCl) or sumatriptan (300 µg/kg). Significant decreases can be seen in the Hippocampus, Hypothalamus and Amygdala.
Table 7. Physiological parameters and tryptophan concentrations of rats in the autoradiographic determination of 5-HT synthesis rates, using the α-MTrp method, after subcutaneous injection of saline controls or the anti-migraine agent sumatriptan.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sumatriptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (g)</td>
<td>233 ± 14</td>
<td>240 ± 22</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48 ± 6</td>
<td>47 ± 3</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.43 ± 0.03</td>
<td>7.40 ± 0.04</td>
</tr>
<tr>
<td>Arterial PCO₂ (mmHg)</td>
<td>40 ± 5</td>
<td>39.1 ± 3</td>
</tr>
<tr>
<td>Arterial PO₂ (mmHg)</td>
<td>97.4 ± 8</td>
<td>96.5 ± 6</td>
</tr>
<tr>
<td>Total Tryptophan (nmol/mL)</td>
<td>55.0 ± 9.9</td>
<td>56.3 ± 6</td>
</tr>
<tr>
<td>Free Tryptophan (nmol/mL)</td>
<td>10.4 ± 5.1</td>
<td>10.1 ± 6.2</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. Body weights were taken just prior to subcutaneous drug or vehicle injection. See text for definition of Free vs. Total tryptophan arterial plasma concentrations. Parameters between the two conditions are not significantly different. Control = 0.9% NaCl, s.c. Sumatriptan = 300μg/kg, s.c.

Table 8. 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl, s.c.) or sumatriptan (300 μg/kg, s.c.).

<table>
<thead>
<tr>
<th>Rat Brain Structure</th>
<th>Control SD</th>
<th>Sumatriptan SD</th>
<th>p</th>
<th>Ratio (S/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr</td>
<td>46.91</td>
<td>11.52</td>
<td>38.15</td>
<td>10.18</td>
</tr>
<tr>
<td>Cg</td>
<td>47.96</td>
<td>11.71</td>
<td>40.61</td>
<td>12.59</td>
</tr>
<tr>
<td>Par</td>
<td>40.84</td>
<td>12.82</td>
<td>34.49</td>
<td>10.61</td>
</tr>
<tr>
<td>Cpu</td>
<td>54.87</td>
<td>12.63</td>
<td>45.80</td>
<td>10.32</td>
</tr>
<tr>
<td>GP</td>
<td>53.57</td>
<td>12.54</td>
<td>43.14</td>
<td>11.38</td>
</tr>
<tr>
<td>Th</td>
<td>44.46</td>
<td>10.41</td>
<td>35.05</td>
<td>12.84</td>
</tr>
<tr>
<td>Hipp</td>
<td>61.57</td>
<td>9.66</td>
<td>44.73</td>
<td>10.75</td>
</tr>
<tr>
<td>Hy</td>
<td>62.75</td>
<td>9.26</td>
<td>38.89</td>
<td>11.68</td>
</tr>
<tr>
<td>Amyg</td>
<td>67.21</td>
<td>9.79</td>
<td>46.82</td>
<td>12.99</td>
</tr>
<tr>
<td>Oc</td>
<td>43.56</td>
<td>11.22</td>
<td>37.06</td>
<td>9.30</td>
</tr>
<tr>
<td>Dra</td>
<td>226.76</td>
<td>33.01</td>
<td>206.93</td>
<td>13.57</td>
</tr>
<tr>
<td>Cbl</td>
<td>36.37</td>
<td>12.28</td>
<td>31.59</td>
<td>10.66</td>
</tr>
</tbody>
</table>

Rates are expressed in pmol/g/min. Standard deviations (SD) are given as well as the p-values. The last column displays the ratio of the R values of Sumatriptan treated rats to Control rats.
3.2.4. Discussion

A generally similar pattern of decrease in 5-HT synthesis values can be seen in both drugs investigated here despite the known difference in the ability of the drugs to access the brain. A decrease in 5-HT synthesis values across most central rat brain structures measured is consistent with the interpretation of the brain penetrance of sumatriptan, but other mechanisms of drug action cannot be ruled out.

As also mentioned in the human study, another route of entrance into the brain is around the hypothalamus as areas there lack the BBB. Significant decreases in synthesis found in the hypothalamus after sumatriptan injection but not after zolmitriptan injection could be interpreted as consistent with this putative mode of action, given BBB penetrance and concentration differences. This possibility is also supported by at least one other study measuring serotonin turnover. After 30 minutes of exposure to sumatriptan, Wistar rats were seen to have decreased serotonin concentration, as measured by HPLC, in the hypothalamus after 0.6mg/kg (s.c.) and 0.9mg/kg (s.c.) but not after a 0.3 mg/kg (s.c.) injection (106). An increased 5-HIAA/5-HT ratio at a dose of 0.6 mg/kg (s.c.) but not at 0.3 mg/kg (s.c.) or 0.9 mg/kg (s.c.) doses was also found in the hypothalamus and in the striatum. These investigators also examined the frontal cortex and hippocampus but found no change in serotonin levels or its metabolites in these other structures at any dose of sumatriptan. Of course, zolmitriptan should also affect hypothalamic 5-HT synthesis levels, BBB penetrance or not as there are raphe projections and thus 1B and 1D receptors in this location (109,110). Further interpretation of this finding is found below.

Control R values determined here do not differ significantly from those of other
studies employing this method on male Sprague-Daley rats of this mass, with the exception of the dorsal raphe where they are slightly lower than found elsewhere (122,123). Serotonin synthesis value decreases are mostly comparable to those found, using this method on the same type of rats, after acute injection of the nonselective 5-HT<sub>1B/2C</sub> receptor agonist m-trifluoromethyl- phenylpiperazine (TFMPP) (10mg/kg, i.p.) (Tohyama, Y., personal communication. Dr. Tohyama was a research fellow in the laboratory of Dr. Diksic). Interestingly, there is a relative similarity of percent decrease (approximately 20%) despite the large difference in size of dose. This 5-HT<sub>1B/2C</sub> receptor agonist also reduced synthesis at the raphe nuclei. Tohyama also investigated the effect of 5-HT<sub>1A/1B</sub> agonist, CGS12066B (5mg/kg, i.p.) and found it to decrease 5-HT synthesis primarily in the raphe nuclei, but did find a significant decrease at the medial caudate nucleus.

A recent study also found a decrease in 5-HT synthesis in cortical structures of male Wistar rats after acute TFMPP injection (1.5 and 3 mg/kg) but not in midbrain structures (124). As this study employed a method of synthesis measurement that involved measuring accumulation of 5-Hydroxytryptophan (5-HTP), the discrepancy in midbrain synthesis findings compared to those of Dr. Tohyama could be a result not only strain differences but also of methodology. In the same study (124), a variant of the Wistar rat line (the Wistar Kyoto line) continued to show lowered synthesis values by TFMPP despite pre-injection of antagonists of 5-HT<sub>1A</sub> ((+)-WAY 100135; 3 mg/kg) and 5-HT<sub>1B/1D</sub> receptors (GR 127935, 1.5 and 3 mg/kg), or reserpine (5 mg/kg), ketanserin (1 mg/kg) mianserin (2 mg/kg) nor idazoxan (1 mg/kg) ruling out a role for monoamine
release, 5-HT2 receptors and alpha2-adrenoceptors. These investigators concluded that TFMPP therefore reduced synthesis through nonserotonergic mechanisms in this strain and suggested that the reduction in 5-HTP accumulation might be a result of the drug’s known ability to block alpha1-adrenoceptors (125).

These last results re-open the search for peripheral mechanisms that might prove to be relevant here. As discussed before, it is not clear if sumatriptan has either a stimulatory or inhibitory effect on the HPA axis (111, 112). However the HPA axis has clearly been shown to have effects on the central 5-HT system. Most studies indicate that rat corticosterone levels are proportional to 5-HT synthesis and mostly inversely proportional to the density of specific subtypes of 5-HT receptors: 1) Serotonin turnover is decreased 2 hours after adrenalectomy (ADX) in rats (115), 2) ADX leads to an increase in rat 5-HT1A and 1B receptors in the Hippocampus (126,127), 3) high doses of cortisol decrease 5-HT1B receptor numbers throughout the cortex in rats after ADX (127), and 4) ADX has no effect on somatodendritic 5-HT1A receptors or mRNA levels in the dorsal raphe nucleus (128). Given findings such as these sumatriptan would thus have to inhibit rat corticosterone production in this study or its stimulatory actions of the HPA would have to not be enough to counteract its apparent inhibitory actions on central synthesis as found here. Of course there are many hormones released by the adrenal gland and/or the HPA axis. It is therefore still a possibility that sumatriptan could have an effect on these other hormones which would subsequently reduce serotonin synthesis levels.

Where would sumatriptan act at on the HPA axis? Action at the hypothalamus
could explain the difference in synthesis reduction of the two drugs at this structure found in this study. Interestingly, oral zolmitriptan was found to not have a significant effect of cortisol blood levels in healthy humans up to 180 minutes after ingestion (110). The drug could act at inhibitory $5\text{-HT}_{1B/1D}$ receptors on descending projections to the hypothalamus and/or pituitary. Alternatively, or perhaps additionally, sumatriptan could work at the adrenal gland. Certainly another $5\text{-HT}_{1B/1D}$ agonist (TFMPP) acts on adrenoreceptors there (125). $5\text{-HT}_7$ receptors are found to exist on the adrenal gland (118) and sumatriptan has low, but non-negligible binding at these receptors (1).

As with the human study one might wonder if sumatriptan constricts the cerebral microcirculation. In rats, cerebral blood flow reduction was not found to occur after intracarotid injection of 0.6 or 6 $\mu$g/kg in the parietal cortex, using Laser-Doppler flowmetry, but the 6 $\mu$g/kg dose did reduce flow in the caudoputamen, as measured by the hydrogen clearance method (129). Systemically, sumatriptan is actually a vasodilator in rats and this is seen at doses as low as 2.5 $\mu$g/kg (i.v.) (130). However, it does not to alter the rat CBF autoregulation blood pressure plateau in the rat microvasculature at blood levels below or up to 3 times the dose used here (40 - 300 $\mu$g/kg, i.v.) (131). Thus, vasoconstriction is unlikely here as an explanation for the reduced R values as the only structure found to have reduced blood flow by other investigators at a somewhat comparable dose is one of the 2 structures found not to have significantly reduced $5\text{-HT}$ synthesis levels in this study.

While it is clear that sumatriptan can exert central effects in the presence of a healthy blood brain barrier, it is of course important to see if the effect can be seen at
more clinically relevant concentrations. The dose of 1000 μg/kg (s.c.) was chosen because it was 10 times the very commonly used dose of 100 μg/kg (i.v.) in different rat brain migraine models of neurogenic inflammation (132). Ten times is the general rule of conversion between subcutaneous and intravenous administration. However, a clinical dose of 6 mg (s.c.) translates to a lesser blood concentration than that of a 100 μg/kg (i.v.) injection (108). Therefore an acute sumatriptan injection study was performed at the new dose of 300 μg/kg (s.c.), using the α-MTrp radioautographic method. New controls were necessary in order to avoid possible seasonal changes in synthesis rates, as the experiment was done at a later point in the year.

The second part of the study shows that sumatriptan can have an effect on 5-HT synthesis inside the rat brain when there is a healthy, intact blood brain barrier, even at the lower and more clinically relevant dose. The mechanism(s) behind sumatriptan’s actions are only slightly clearer however. Perhaps the areas of BBB absence are key to the drug’s effects either directly, through diffusion, or indirectly, through an action on the HPA axis.

Perhaps the limited amount of entry into the brain is sufficient to activate receptors centrally. Sumatriptan does have nanomolar affinity for these receptors (1). If this is the case, then the reduction in synthesis rates would predominantly be the result of the agonism of 5-HT1B/1D receptors on serotonergic nerve terminals. These are known to inhibit release of 5-HT from neuron terminals in both animals and humans (reviewed in 133). They have also been found to inhibit 5-HT synthesis in serotonergic terminals, independent of nerve impulse changes, as measured by the 5-HTP accumulation and 5-
HT/5-HIAA turnover ratio methods (134-137). Should sumatripan be able to agonise the 1A autoreceptor to any extent, this would also decrease 5-HT neuron firing rate with subsequent attenuation of both 5-HT release and synthesis (138-141).

The minimal BBB crossing yet sufficient receptor stimulation notion is also consistent with the fact that the lower concentration elicited no change in synthesis rates in the dorsal raphe whereas the higher concentration lowered synthesis rates significantly. That is, a lower concentration of sumatriptan might not be able to agonise the predominantly 1A receptors or smaller number of 1B receptors located on serotonergic cell bodies, due to the drug's lower affinity for this receptor subtype, whereas the higher concentration might be able to do so.

3.3. Study 3: Central effects of chronic sumatriptan administration on serotonin synthesis rates in the rat

Upon the determination of a drug's acute effect on 5-HT synthesis, it is often standard fare to perform a chronic administration study (see for example 142,143). However, in this instance it is particularly pertinent for at least two more reasons. First, inappropriate use and overuse of the triptans does occur (144, 12). Secondly, the fact that the central serotonergic system has been implicated in serious psychiatric and neurological conditions makes its potential alteration more dangerous clinically. Finally, serotonergic receptors have been shown to up and down regulate in response to chronic drug administration (145).
3.3.1. Procedure

Over the course of 21 days, 30 male Sprague-Daley rats were inserted with a subcutaneous osmotic mini-pump (Model 2004, Alzet Corp, Mountain View, CA) beneath the skin of their backs. The pumps administered sumatriptan or vehicle (0.9% NaCl) for 21 days. Rat weights were chosen such that, over the course of the 21 days, they were to receive an average dose of 300 μg/kg/day. The actual average amounted to 333 μg/kg/day due to slightly lesser weight gain than anticipated. On the 22nd day, with the pumps still in their backs, the rats underwent the procedure as outlined in Study 2 above.

3.3.2. Results

Table 9 lists the physiological parameters and tryptophan concentrations of rats measured during the experiment. No significant differences occur between the two conditions.

Table 10 lists 5-HT synthesis rate values (R values) in various rat brain structures after 21 days of sumatriptan (333μg/kg/day) or control (0.9% NaCl) administration by subcutaneous osmotic mini-pump. Significant increases occur in the frontal and cingulate cortices, caudate-putamen, globus pallidus, thalamus, amygdala and cerebellum. No differences are found in the other structures measured.

Figure 13 shows the regression lines through the data for the frontal cortex for both saline and sumatriptan. The slope of the sumatriptan line (or K* value) is significantly higher than that of control.

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Table 9. Physiological parameters and tryptophan concentrations of rats measured during the α-MTrp autoradiographic study of serotonin synthesis rates after chronic Sumatriptan administration by subcutaneous osmotic pumps.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Sumatriptan</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body Weight (before; g)</td>
<td>204 ± 16</td>
<td>205 ± 18</td>
</tr>
<tr>
<td>Body Weight (after; g)</td>
<td>337 ± 21</td>
<td>330 ± 22</td>
</tr>
<tr>
<td>Gain in Body Weight (g)</td>
<td>134 ± 23</td>
<td>125 ± 16</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>47 ± 2</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.48 ± 0.03</td>
<td>7.46 ± 0.03</td>
</tr>
<tr>
<td>Arterial PCO$_2$ (mmHg)</td>
<td>41.8 ± 5.1</td>
<td>42.1 ± 3.4</td>
</tr>
<tr>
<td>Arterial PO$_2$ (mmHg)</td>
<td>95.4 ± 12.8</td>
<td>91.5 ± 6.3</td>
</tr>
<tr>
<td>Total Tryptophan (nmol/mL)</td>
<td>131.0 ± 39.9</td>
<td>116.3 ± 35.6</td>
</tr>
<tr>
<td>Free Tryptophan (nmol/mL)</td>
<td>12.2 ± 5.1</td>
<td>11.9 ± 4.3</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± standard deviation. Body weights were taken just prior to subcutaneous osmotic mini-pump injection (before) and just prior to the cannulation procedure (after), 21 days later. See text for definition of Free vs. Total tryptophan arterial plasma concentrations. Parameters are not significantly different. Control = 0.9% NaCl, s.c. Sumatriptan = 333 μg/kg/day, s.c.

Frontal Cortex

Figure 13. Patlak plot (regression) analysis in the frontal cortex of rats after either chronic saline (straight) or sumatriptan (dashed) administration. See text for details.
Table 10. 5-HT synthesis rate values (R values) in various rat brain structures after 21 days of sumatriptan (333μg/kg/day) or control (0.9% NaCl) administration by subcutaneous osmotic mini-pump.

<table>
<thead>
<tr>
<th>Control (n = 16)</th>
<th>Sumatriptan (n = 14)</th>
<th>SD</th>
<th>SD</th>
<th>p</th>
<th>Ratio(S/C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fr</td>
<td>32.6</td>
<td>8.0</td>
<td>46.0</td>
<td>11.8</td>
<td>0.001</td>
</tr>
<tr>
<td>Cg</td>
<td>35.0</td>
<td>8.7</td>
<td>47.4</td>
<td>11.8</td>
<td>0.003</td>
</tr>
<tr>
<td>Par</td>
<td>32.4</td>
<td>7.6</td>
<td>35.1</td>
<td>11.1</td>
<td>0.434</td>
</tr>
<tr>
<td>Cpu</td>
<td>42.5</td>
<td>7.5</td>
<td>52.3</td>
<td>11.3</td>
<td>0.008</td>
</tr>
<tr>
<td>GP</td>
<td>42.1</td>
<td>5.6</td>
<td>54.3</td>
<td>12.8</td>
<td>0.002</td>
</tr>
<tr>
<td>Th</td>
<td>47.0</td>
<td>8.1</td>
<td>62.8</td>
<td>13.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Hipp</td>
<td>32.9</td>
<td>7.4</td>
<td>39.9</td>
<td>13.8</td>
<td>0.087</td>
</tr>
<tr>
<td>Hy</td>
<td>56.9</td>
<td>8.0</td>
<td>61.1</td>
<td>13.3</td>
<td>0.299</td>
</tr>
<tr>
<td>Amyg</td>
<td>51.0</td>
<td>6.5</td>
<td>59.8</td>
<td>13.1</td>
<td>0.024</td>
</tr>
<tr>
<td>Oc</td>
<td>37.8</td>
<td>9.1</td>
<td>43.9</td>
<td>14.9</td>
<td>0.180</td>
</tr>
<tr>
<td>Dra</td>
<td>241.5</td>
<td>12.6</td>
<td>236.3</td>
<td>26.5</td>
<td>0.488</td>
</tr>
<tr>
<td>Cbl</td>
<td>25.9</td>
<td>7.7</td>
<td>35.1</td>
<td>12.3</td>
<td>0.019</td>
</tr>
</tbody>
</table>

Rates are expressed in pmol/g/min. Standard deviations (SD) are given as well as the p-values. The last column displays the ratio of the R values of Sumatriptan treated rats to Control rats.

3.3.3. Discussion

Chronic subcutaneous administration of 0.333 mg/kg/day of sumatriptan over a 21 day time period resulted in an increase in 5-HT synthetic rates in serotonergic projection areas but no change in rates in the dorsal raphe nucleus (Table 10). No significant differences in physiological parameters can be found between the two conditions.

Many of the issues with respect to mode of action of sumatriptan raised in the acute studies are relevant here. Chief amongst them is likely the question of whether the drug acts directly on the central serotonergic system or whether an indirect mechanism must be invoked in order to explain the observed effects.

Should the drug have a greater ability to cross the BBB than traditionally thought,
the results can be interpreted in traditional agonist terms. That is, it seems likely that the 
5-HT_{1B/1D} receptors have become desensitized and/or down-regulated over time as a result 
of chronic drug exposure. This might result in diminished negative feedback signalling 
and subsequent increase in 5-HT production, presumably through an intracellular pathway 
between presynaptic autoreceptors and the tryptophan hydroxylase (TPH) enzyme.

Several pathways have been suggested (146-152) but their reaction and interactions in 
different drug administration regimes have not been fully delineated. Of course, 5-HT_{1B} 
and 5-HT_{1D} receptors exist as heteroreceptors on non-serotonergic neurons as well (133) 
and feedback through these neurons could conceivably play a role in bringing about the 
newly altered synthetic rates.

Evidence in the literature of direct desensitization or down regulation of 5-HT_{1B/1D} 
receptors by chronic agonist administration is difficult to find. However, chronic 
administration of selective serotonin re-uptake inhibitor or tricyclic anti-depressant drugs, 
which ultimately increases the amount of 5-HT in synaptic clefts, has been shown in 
several studies to bring about a down-regulation and desensitization of the terminal 5-
HT_{1B} autoreceptor (reviewed in 133).

Using the α-MTrp autoradiographic method with rats an unpublished study found 
significantly reduced 5-HT synthesis rates after 7 days of administration of the 5-HT_{1B/2C} 
agonist TFMPP (10 mg/kg, i.p.) (Tohyama, Y., personal communication). Tohyama also 
found significant rate decreases using the 5-HT_{1A/1B} agonist CGS 12066B (5 mg/kg, i.p.) 
for the same length of time. For both drugs, this was the case in all structures measured 
except the Dorsal Raphe nucleus, where no significant differences were found between
drug and control conditions. Given this data, it seems likely that the increase in rates found in our study is either peculiar to the drug or took effect after the 7\textsuperscript{th} day of drug administration.

Contrary findings within the scope of the serotonergic system are not unprecedented, however. For example, it is not always the case that agonism of the terminal receptors results in decrease and antagonism in an increase in terminal serotonin release. In fact, acute administration of the 5-HT\textsubscript{1B/1D} receptor antagonist GR 127935 has been found to decrease extracellular levels of 5-HT in the frontal cortex and striatum, and SB-224289, another 5-HT\textsubscript{1B} receptor antagonist, had no effect on 5-HT release (154). However, authors of this study explain this by stating that antagonism of inhibitory 5-HT\textsubscript{1B/1D} receptors on raphe cell bodies might lead to a local increase in 5-HT, which would then stimulate 5-HT\textsubscript{1A} receptors to decrease cell firing, and therefore 5-HT release from terminals. The lack of effect of SB-224289 is reported by these authors as due to either lesser 5-HT\textsubscript{1B} receptor antagonism compared to when both 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors are blocked at the level of the dorsal raphe nucleus or other projections with 5-HT\textsubscript{1D} receptors of the dorsal raphe nucleus to the frontal cortex, mediating inhibition of terminal 5-HT release. This kind of connectivity is plausible, and taken together with the relatively recent discovery that 5-HT\textsubscript{1B} and 5-HT\textsubscript{1D} receptors can dimerize (155), help illustrate the complexity of the system.

In addition to the effects of chronic drug administration on the serotonergic system, there are the concerns of effects on other systems. As it might pertain to serotonin synthesis and migraine however, likely the chief amongst these are the effects on
the HPA axis. Sumatriptan has been shown to significantly affect levels of cortisol, growth hormone, and prolactin, to name just a few products of the axis (111,156,157). The complex interaction between the serotonergic system and the HPA axis merits a lengthy review but it is worthwhile to restate that there are many products of this axis and many of these can affect central 5-HT neuron function both acutely and chronically (109,111,115,118,125-128).

As zolmitriptan is known to effectively cross the BBB, the manufacturer insists that it not be used in an excessively frequent or chronic manner clinically and would therefore neither provide a sample nor endorse the investigation of potential chronic effects on central synthesis rates. It was therefore not investigated in this course of study despite the value that might have for comparative purposes with the acute results.

It is interesting to speculate how increased rates of 5-HT synthesis after chronic administration of sumatriptan might relate to migraine. As mentioned, overuse or misuse of sumatriptan does occur (12,158,159). Approximately 4% of patients take the drug daily or more than 10 times each week while approximately 20% of patients use sumatriptan more than 10 times each month (158). As elaborated upon above, while 5-HT is clearly involved in migraine pathogenesis its exact role is still unclear. Should the migraine generator theory hold however, in at least a certain fraction or type of migraineur, one might predict that an increase in synthesis rates as a result of chronic sumatriptan agonism might have a prophylactic effect. That is, putatively low baseline levels of serotonin production might be normalized and stressors might no longer be as readily able to elicit attacks. In at least one study, naratriptan, used once daily, has been
found to reduce to frequency and severity of daily headache (160). However, chronic use of the triptans can also make headaches worse (12). Seemingly contradictory findings are not new to migraine research.

4. **SUMMARY AND CONCLUSIONS**

4.1. **Preliminary results on the rates of serotonin synthesis in different migraine conditions using the α-MTrp method and PET.**

Preliminary results in migraine sufferers suggest a trend, though not significant, towards an acute decrease in central rates after sumatriptan injection (6 mg, s.c.) administered to alleviate the pain and associated symptoms of a migraine attack. Rates of 5-HT synthesis during the migraine pain phase thus far tend to be higher than during the interictal or headache free phase.

4.2. **Effect of the acute administration of sumatriptan and zolmitriptan on 5-HT synthesis rates in the rat brain using the α-MTrp method and autoradiography.**

Acute subcutaneous administration of both triptans significantly reduces 5-HT synthesis rates in the rat brain to similar extents. A lower, and more clinically relevant dose of sumatriptan can also significantly reduce 5-HT synthesis rates, but to a lessor extent and in fewer structures. This occurs despite a known difference in the ability of drugs to cross the BBB. The results are consistent with the known ability of 5-HT_{1B/1D} agonists to inhibit 5-HT release and synthesis but other mechanisms of action can not be ruled out as pertinent to 5-HT synthesis rates.
4.3. Effect of the chronic administration of sumatriptan 5-HT synthesis rates in the rat brain using the α-MTrp method and autoradiography.

Chronic administration of a clinically relevant dose of sumatriptan using subcutaneously implanted osmotic mini-pumps significantly increases 5-HT synthetic rates at serotonergic projection areas. However, no change in synthesis rates occurred in the Dorsal Raphe nucleus.

This is the first report showing central effects of the drug sumatriptan on in vivo serotonin synthesis rates in rats or humans. Such central effects might or might not help alleviate pain but may have repercussions for chronic triptan users.
5.1. LIST OF FIGURES

Figure 1. (Adapted from 4) Schematic representation of the proposed sites of action for the triptan compounds in the symptomatic treatment of migraine headache.

Figure 2. Schematic drawing of major serotonergic nuclei along with their ascending and descending projections (9).

Figure 3. The metabolic pathways for the synthesis and degradation of 5-HT.

Figure 4. Hypothetical serotonergically-based mechanism of migraine pathogenesis.

Figure 5. Schematic of the mathematical, three-compartment model as proposed by Diksic et al (84).

Figure 6. Global or whole brain $K^*$ values in mL/g min in each of the 3 Migrainous states.

Figure 7. Global or whole brain R values, in pmol/g/min measured in three conditions.

Figure 8. R values, in pmol/g/min, for each region of interest (ROI), averaged 6 subjects, in the 3 Migrainous conditions examined.

Figure 9. Schematic of some of the connections between the HPA axis and the serotonergic systems.

Figure 10. Representative autoradiograms obtained for brain slices from rats killed 2.5 hours after the beginning of tracer injection (30 μCi of α-[14C]methyl-L-tryptophan).

Figure 11. A representative plasma input function obtained in a rat injected with 30 μCi of α-[14C]methyl-L-tryptophan.

Figure 12. Plot of the Dorsal Raphe distribution volume as a function of Theta in all 3 conditions.

Figure 13. Patlak plot (regression) analysis in the frontal cortex of rats after either chronic saline (straight) or sumatriptan (dashed) administration.
5.2. **List of Tables**

**Table 1.** pKᵢ values of Sumatriptan and Zolmitriptan at select 5-HT receptors (1)

**Table 2.** Key measures in the 6 participants to date in the human PET study.

**Table 2B.** Average plasma tryptophan levels in nmol/mL in the 3 conditions investigated.

**Table 3.** SPM results based on the first 5 subjects.

**Table 4.** Physiological parameters of rats used in Part A experiments.

**Table 5.** 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl) or sumatriptan (1000 μg/kg).

**Table 6.** 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl) and the acute migraine treatment drug zolmitriptan (100 μg/kg).

**Table 7.** Physiological parameters in part B autoradiographic experiments.

**Table 8.** 5-HT synthesis rate values (R values) in rats injected with saline (0.9% NaCl, s.c.) or sumatriptan (300 μg/kg, s.c.).

**Table 9.** Physiological parameters and tryptophan concentrations of rats measured during the α-MTrp autoradiographic study of serotonin synthesis rates after chronic Sumatriptan administration by subcutaneous osmotic pumps.

**Table 10.** 5-HT synthesis rate values (R values) in various rat brain structures after 21 days of sumatriptan (333μg/kg/day) or control (0.9% NaCl) administration by subcutaneous osmotic mini-pump.
6. LIST OF ABBREVIATIONS

5-HIAA = 5-hydroxyindolacetic acid
5-HT = Serotonin, 5-Hydroxytryptamine
AAAD = amino acid decarboxylase enzyme
Amyg = amygdala
BBB = blood brain barrier
CBF = cerebral blood flow
Cbl = cerebellum
Cg = cingulate cortex
Cmax = maximal plasma drug concentration reached after drug administration
CNS = central nervous system
Cpu = caudate-putamen
Dra = dorsal raphe nucleus
Fr = frontal cortex
GABA = gamma amino butyric acid
GP = globus pallidus
Hipp = hippocampus
HPA = hypothalamic pituitary adrenal (axis)
Hy = hypothalamus
i.v. = intravenous administration
in vivo = process taking place in the organism while it is alive/ functioning
K* = unidirectional uptake or trapping constant for the tracer α-MTrp
LC = lumped constant for the conversion of the rate of uptake of tracer to the rate of uptake of tracee
Oc = occipital cortex
p.o. = oral administration
Par = parietal cortex
PET = positron emission tomography
R = 5-HT synthesis rate
s.c. = subcutaneous administration
Th = thalamus
Tmax = time that Cmax is reached
TPH = tryptophan hydroxylase enzyme
α-M5HT = alpha-methyl-serotonin
α-MTrp = alpha-methyl-L-tryptophan
7. APPENDIX

Linear Regression

Linear regression is used to analyze the relationship between two variables, typically labeled X and Y. For each subject, both X and Y are known and the best straight line through the data is desired. There are two reasons to do this:

- Because the slope and/or intercept have a scientific meaning (e.g., $K^*$ values).
- To create a standard curve to find new values of X from Y, or Y from X.

The linear regression model is based on the following assumptions:

- The relationship between X and Y can be graphed as a straight line.
- The variability of values around the line follows a Gaussian distribution.
- The variability is the same everywhere (i.e., that the SD is homoscedastic).
- X is known exactly.
- Each XY data pair was randomly sampled from a large population.
- Each XY data pair was selected independently.

Linear regression finds the line that best predicts Y from X by finding the line that minimizes the sum of the squares of the vertical distances of the points from the line. Why minimize the square of the distances?

- Distances are squared because it is better to have two points sort of close to the line (say 5 units each) than to have one very close (1 unit) and one further (9 units). If the scatter of points around the line is Gaussian, the former is far more likely than the latter.
- A more rigorous answer (for those who have studied statistics intensively) is that minimizing the sum-of-squares results in the same line that would be given by maximum likelihood calculations.

Note that linear regression does not test whether data are linear (except for the runs test). It assumes that data are linear, and finds the slope and intercept that make a straight line best fit of the data.
8. REFERENCES


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