THE ROLE OF SEROTONIN-2 (5-HT₂) RECEPTORS IN THE REGULATION OF ANXIETY AND SLEEP

Ph.D. theses

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1 INTRODUCTION

Neuronal serotonin plays an essential role in various physiological functions including feeding, sex, aggressive behaviour, thermoregulation, endocrine regulation, motor activity pain modulation, learning and memory, mood, anxiety and sleep regulation (Kahn and Wetzler, 1991; Barnes and Sharp, 1999). In addition, dysfunction of the serotonergic system has been documented in numerous CNS disorders including depression, generalized anxiety, obsessive-compulsive disorder, panic disorder, migraine, feeding disorders and schizophrenia (Murphy et al, 1989). 5-HT produces its manifold effects through more than 14 receptors with different structures, anatomic distributions and functions (Barnes and Sharp, 1999). The analysis of the role of the receptors in various physiologic and pathologic processes has been difficult due to the lack of the receptor subtype-selective drugs (Bagdy, 1999).

2 OBJECTIVES

The present work is based on physiological and pre-clinical experiments related to the serotonergic system performed in rats. From the several different 5-HT receptors these studies focused on the 5-HT2 receptor family (5-HT2A, 5-HT2B and 5-HT2C). The following questions were raised:

1) Which subtype of the 5-HT2 receptor family mediates anxiety?
   a) What kind of altered anxiety-related behaviours can be observed in FH animals with impaired central serotonergic functions?
   b) Is there any difference between FH rats and other strains in the level of anxiety mediated by 5-HT2 receptor activation?

2) Which subtype of the 5-HT2 receptor family (5-HT2A, 5-HT2B or 5-HT2C) mediates the tonic deep sleep inhibiting effect of 5-HT?
   a) What are the effects of the 5-HT2 receptor antagonist ritanserin on the EEG power spectra with slow wave sleep increasing properties and which 5-HT2 receptor subtype mediates these effects?

3) Can the selective blockade of the 5-HT2 receptor subtypes be anxiolytic and if so, is this the consequence of the sedative-hypnotic effect of the compound?
3 MATERIALS AND METHODS

Male Sprague-Dawley (SD) Wistar (W) and Fawn-Hooded (FH) rats were used in this study. The animals were kept under standard conditions (12:12 h light-dark cycle, temperature: 21±1°C) with standard food and water freely available.

3.1 Study of anxiety

To study anxiety we used the social interaction test. The following behaviours were included in total social interaction: sniffing partner, anogenital sniffing, peaceful following, grooming partner, under crawling, over climbing, chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing, and wrestling. Chasing, aggressive grooming, dominant posture, submissive posture, biting, boxing, kicking, pushing, and wrestling were summarized in the aggressive behaviour. Rearing, crossing of lines, and self-grooming were also scored.

3.2 Sleep studies and quantitative EEG

Animals were chronically equipped with (epidural, fronto-parietal) EEG and electromyogram (EMG, in the muscles of the neck) electrodes. EEG, EMG and motor activity were recorded. The vigilance states were scored based on the polygraph recordings. EEG power spectra were computed by Fast Fourier transformation.

3.3 Statistical analysis

The data were analysed using one- or two-way analysis of variance (ANOVA) and Kruskal-Wallis non-parametrical test followed by Tukey-Kramer and Mann-Whitney post-hoc tests.

4 RESULTS

The results of these experimental studies related to the serotonergic system can be summarized as follows:
- anxiety induced by m-CPP (5-HT\textsubscript{2} agonist), acute SSRI (fluoxetine and sertraline) treatment or anxiogenic environment can be reversed by pre-treatment with the selective 5-HT\textsubscript{2C} receptor antagonist (SB-242084) but not the 5-HT\textsubscript{1A} receptor antagonist (WAY-1000635); therefore, anxiety during increased 5-HT release is mediated by 5-HT\textsubscript{2C} receptors.

- increased anxiety and decreased aggression were found in the FH strain compared to the control animals (SD, W) in the social interaction test

- the elevated response produced by m-CPP (increased anxiety and the total lack of baseline aggression) shows an abnormal serotonergic neurotransmission in FH rats

- the deep sleep and delta activity increasing effect of the 5-HT\textsubscript{2} antagonist ritanserin was not reproduced either by the 5-HT\textsubscript{2B} (SB-215505) or by the 5-HT\textsubscript{2C} (SB-242084) receptor-selective antagonists; and this led us to the conclusion that 5-HT produces tonic inhibition of slow wave sleep through blockade of 5-HT\textsubscript{2A} receptors.

- In summary, we can conclude that 1) the anxiolytic effect of the selective 5-HT\textsubscript{2C} receptor antagonists is not the result of the sedative-hypnotic effect, 2) because blockade of different 5-HT\textsubscript{2} receptor subtypes mediates anxiolytic (5-HT\textsubscript{2C}) and deep sleep-inducing (5-HT\textsubscript{2A}) effects.

5 SUMMARY

I studied the role of the 5-HT\textsubscript{2} receptor family (5-HT\textsubscript{2A}, 5-HT\textsubscript{2B} and 5-HT\textsubscript{2C}) in the regulation of anxiety and sleep/wakefulness in experimental studies to get information about the possible physiological functions of the serotonergic system in humans.

In Sprague-Dawley rats, the subtype-selective 5-HT\textsubscript{2C} receptor antagonist SB-242084 very efficiently reversed the anxiety induced by the 5-HT\textsubscript{2} receptor agonist m-CPP and acute SSRI (fluoxetine and sertraline) treatment, moreover, in higher doses it reversed the effects of anxiogenic environment, whereas the 5-HT\textsubscript{1A} antagonist WAY-100635 was ineffective. These data led to the conclusion that increased 5-HT release and activation of the 5-HT\textsubscript{2C} receptors play an important role in the generation of anxiety.
The Fawn-Hooded (FH) rat strain with an inborn serotonin metabolism disorder showed increased anxiety, decreased aggression and altered 5-HT receptor functions, relative to controls. The differences can be explained by the dysfunction of the serotonergic neurotransmission, characteristic in human anxiety diseases as well. Taken together, the present data suggest that the FH rat strain may be a genetic animal model of the human anxiety disorders, especially of social phobia.

The 5-HT₂ antagonist ritanserin increased deep sleep and delta activity as described previously in the literature. Based on our quantitative-EEG studies and sleep results we conclude that the effect of ritanserin is mediated by the blockade of 5-HT₂A receptors since the 5-HT₂B and 5-HT₂C receptor antagonists did not produce an effect similar to the one produced by ritanserin.

We conclude that anxiety and wakefulness caused by serotonin are mediated by the activation of different 5-HT₂ receptor subtypes and different mechanisms. Our results provide evidence that 5-HT₂C receptors are involved in the mediation of anxiety, and 5-HT₂A receptors are involved in the regulation of vigilance level.

6 BIBLIOGRAPHY


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8 The dissertation is based on the following publications:

a. Journal articles:


Bagdy, G., Graf, M., Anheuer, Z.E., Modosne, A.E., Kantor, S. Anxiety-like effects induced by acute fluoxetine, sertraline or m-CPP treatment are reversed by pretreatment with the 5-HT2C receptor antagonist SB-242084 but not the 5-HT1A receptor antagonist WAY-100635. Int. J. Neuropsychopharmacol. 4:399-408; 2001.


b. Citable Abstracts:


9 OTHER PUBLICATIONS, JOURNAL ARTICLES, ORAL AND POSTER PRESENTATIONS


Bagdy, G., Graf, M., Kantor, S. Acute anxiety induced by SSRI antidepressants or m-CPP are prevented by 5-HT_{2C} but not 5-HT_{1A} receptor antagonists. Joint meeting of SFP and Pharmacological Societies of Brazil and Portugal, Rennes, France. Book of Abstracts: p.6. O25; 2002.