

5-Hydroxytryptamine-2 antagonist increases human slow wave sleep

C. IDZIKOWSKI, F.J. MILLS and R. GLENNARD

Clinical Pharmacology Unit, Janssen Pharmaceutical Ltd., Grove, Wantage, Oxon (U.K.)

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Ritanserin, a specific 5-HT₂ antagonist, was given to volunteers in a double-blind placebo controlled sleep study. Slow wave sleep doubled in duration at the expense of stage 2. The finding that a serotonin antagonist changed the architecture of sleep without producing insomnia is of fundamental importance and calls for a re-examination of traditional theories of sleep control which assign a facilitatory role to serotonin.

Many studies on humans have shown that high doses of L-tryptophan lead to increased sleep, particularly slow wave sleep (SWS)^{2,4,5,21,23}. This effect has been attributed to the conversion of L-tryptophan to serotonin¹. The rate-limiting enzyme controlling the conversion of tryptophan to serotonin is tryptophan hydroxylase which is inhibited by *p*-chlorophenylalanine (PCPA). PCPA leads to a reduction in rapid eye movement sleep (REM) but because PCPA is toxic only relatively small doses have been given to man²². A number of serotonin subtypes with varying functional roles have now been identified in animals³. Examination of human volunteer data revealed that serotonin antagonists of mixed or undefined receptor specificity generally produce decreases in the duration of REM sleep and mixed effects on SWS. For example, methysergide produces a large decrease in REM sleep time with no overall change in SWS although stage 3 increases and stage 4 decreases¹². Mianserin leads to decreases in REM sleep time and in high doses some increase in SWS^{14,20}. Sandoz's FU 29-245, a mixed serotonin/dopamine antagonist increased the time spent in SWS¹⁶. The synthesis of ritanserin, a highly specific 5-HT₂ antagonist which is devoid of any 5-HT₁, dopaminergic or adrenergic binding^{8,11} in vitro and in vivo animal systems provides an opportunity to investigate serotonergic mechanisms in the control of human sleep.

The experiment consisted of four conditions: pla-

cebo, 10 mg ritanserin given in the evening (RitPM), or in the morning (RitAM) and 5 mg nitrazepam (Nz), a benzodiazepine used as a positive control with a similar half-life to ritanserin.

Nine fit male volunteers (mean age 33.3 years, range 24–43) were used and any concomitant medication was excluded for a period of one week prior to the study and during the study. In order to prevent withdrawal effects, subjects were allowed to maintain their normal intake of caffeine-containing drinks but alcohol was not allowed for 24 h prior to sleeping in the laboratory.

After completing an adaptation night before the main study, subjects slept at the laboratory for 12 nights divided into four periods of three consecutive nights each. These triplets of nights were separated by at least one week and the sequence consisted of adaptation, baseline and test nights. Capsules were given on the morning (08.00 h) and evening (22.30 h) of the test day.

Lights were turned off at 23.00 h and turned on again at 07.00 h, at which time each subject got out of bed to prevent him from returning to sleep (which could influence the next night's reading). A standard breakfast was provided.

Silver/silver chloride electrodes for measuring sleep were placed in a standard configuration¹⁷ and connected to a Nihon-Kohden 4221 electroencephalograph.

Correspondence: C. Idzikowski, Clinical Pharmacology Unit, Janssen Pharmaceutical Ltd., Grove, Wantage, Oxon OX12 0DQ, U.K.

TABLE I

Baseline and drug means of morning VAS (standard deviations in parentheses)

| | Sleep quality (mm) | Morning vigilance (mm) | Alertness (mm) | Tranquility (mm) |
|----------------|--------------------|------------------------|----------------|------------------|
| <i>Placebo</i> | | | | |
| Baseline | 52.94 (3.42) | 45.00 (2.36) | 37.08 (4.56) | 27.31 (3.47) |
| Test | 61.61 (6.61) | 48.11 (6.85) | 28.98 (4.60) | 23.61 (2.42) |
| <i>RitAM</i> | | | | |
| Baseline | 58.27 (8.45) | 51.94 (7.05) | 32.25 (3.81) | 23.04 (2.34) |
| Test | 73.24 (4.17) | 38.50 (6.74) | 32.53 (3.84) | 24.01 (2.65) |
| <i>RitPM</i> | | | | |
| Baseline | 53.94 (5.29) | 48.55 (7.72) | 33.29 (3.81) | 23.26 (3.02) |
| Test | 62.56 (6.21) | 51.61 (6.00) | 33.72 (4.25) | 23.24 (2.32) |
| <i>Nz</i> | | | | |
| Baseline | 54.67 (5.84) | 48.44 (6.04) | 34.09 (3.53) | 25.44 (2.42) |
| Test | 68.67 (6.56) | 44.67 (6.56) | 32.74 (4.31) | 24.91 (2.74) |

Visual analogue scales (VAS) measuring sleep quality, morning vigilance¹⁵, alertness and tranquillity⁶ were filled in at 22.30 h on the evening of the test night's sleep and at 08.00 h the next morning. Control VAS data were obtained by asking the subjects to complete VAS at the corresponding time for the baseline night. The electroencephalographic, electro-oculographic and electromyographic records were scored blind according to the criteria set out by Rechtschaffen and Kales¹⁷. Onset of stage 2 was used to mark sleep onset. Computerized analysis of the raw scores provided data on the amounts and distri-

bution of stages and events which were used in the final analysis of results.

Analysis of variance (ANOVA) with drug conditions as a factor was used to test for differences between conditions on baseline nights. If no differences were found ANOVA was then performed on drug minus baseline differences thus adjusting for individual's baseline values. Significant differences between treatment means were further explored using least significant differences (LSD).

Tables I–IV show mean VAS and sleep measures for baseline and test nights. No significant differ-

TABLE II

Baseline and drug means of evening VAS (standard deviations in parentheses)

| | Alertness (mm) | Tranquility (mm) |
|----------------|----------------|------------------|
| <i>Placebo</i> | | |
| Baseline | 37.08 (4.56) | 27.31 (3.47) |
| Drug | 28.98 (4.60) | 23.61 (2.42) |
| <i>RitAM</i> | | |
| Baseline | 32.25 (3.81) | 23.04 (2.34) |
| Drug | 32.53 (3.84) | 24.01 (2.65) |
| <i>RitPM</i> | | |
| Baseline | 33.29 (3.81) | 23.26 (3.02) |
| Drug | 33.72 (4.25) | 23.24 (2.32) |
| <i>Nz</i> | | |
| Baseline | 34.09 (3.53) | 25.44 (2.42) |
| Drug | 32.74 (4.31) | 24.91 (2.74) |

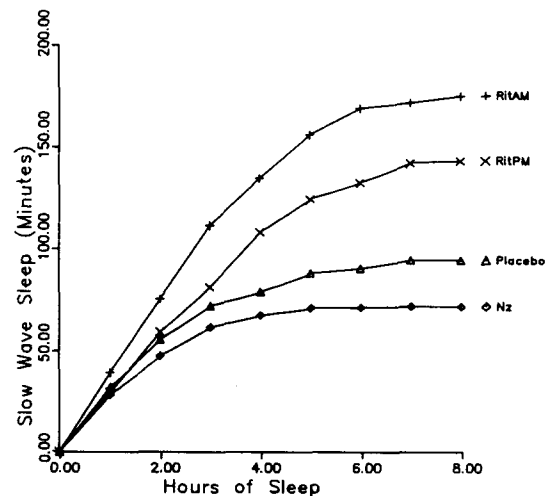


Fig. 1. Cumulative slow wave sleep (min).

TABLE III

Summary sleep data means (standard deviations in parentheses)

| | Total sleep time (min) | Sleep onset latency (min) | SWS latency (min) | REM latency (min) | Number of REM periods |
|----------------|------------------------|---------------------------|-------------------|-------------------|-----------------------|
| <i>Placebo</i> | | | | | |
| Baseline | 438.0 (17.5) | 19.08 (9.1) | 20.3 (17.0) | 89.7 (28.9) | 4.56 (0.07) |
| Test | 442.4 (33.4) | 17.46 (11.6) | 13.9 (6.3) | 96.1 (48.7) | 4.89 (1.05) |
| <i>RitAM</i> | | | | | |
| Baseline | 440.9 (29.4) | 18.10 (10.7) | 17.3 (8.4) | 71.6 (10.8) | 4.68 (0.05) |
| Test | 451.8 (18.7) | 13.84 (9.0) | 15.1 (6.5) | 102.9 (39.9) | 3.56 (0.07) |
| <i>RitPM</i> | | | | | |
| Baseline | 453.4 (13.5) | 18.76 (14.0) | 16.1 (7.6) | 86.4 (49.4) | 4.78 (1.3) |
| Test | 451.7 (14.0) | 21.47 (12.9) | 13.9 (3.1) | 107.6 (55.3) | 3.78 (0.07) |
| <i>Nz</i> | | | | | |
| Baseline | 441.4 (38.0) | 15.17 (13.0) | 14.2 (6.8) | 78.9 (36.2) | 4.44 (0.08) |
| Test | 450.4 (33.6) | 17.16 (10.2) | 30.3 (31.3) | 121.8 (45.05) | 4.33 (0.07) |

TABLE IV

Summary stage data means (standard deviations in parentheses)

| | Stage W (min) | Stage 1 (min) | Stage 2 (min) | Stage 3 (min) | Stage 4 (min) | REM total (min) |
|----------------|---------------|---------------|---------------|---------------|---------------|-----------------|
| <i>Placebo</i> | | | | | | |
| Baseline | 9.26 (10.4) | 40.1 (41.9) | 217.7 (43.1) | 35.3 (13.1) | 57.2 (22.8) | 80.9 (19.8) |
| Test | 4.25 (4.2) | 25.8 (10.9) | 220.1 (38.2) | 33.6 (7.2) | 62.0 (35.2) | 94.2 (19.3) |
| <i>RitAM</i> | | | | | | |
| Baseline | 8.71 (7.7) | 42.0 (18.3) | 227.3 (31.6) | 30.2 (11.0) | 51.4 (28.1) | 83.6 (18.2) |
| Test | 4.37 (8.3) | 23.3 (6.3) | 161.0 (45.8) | 69.5 (20.2) | 105.9 (60.8) | 86.5 (16.8) |
| <i>RitPM</i> | | | | | | |
| Baseline | 7.57 (7.3) | 34.5 (17.4) | 207.1 (43.0) | 33.1 (18.6) | 58.4 (31.4) | 111.5 (40.8) |
| Test | 4.89 (3.3) | 27.0 (15.0) | 190.4 (59.1) | 63.9 (17.2) | 79.6 (63.6) | 85.3 (19.7) |
| <i>Nz</i> | | | | | | |
| Baseline | 14.99 (17.8) | 27.4 (9.6) | 213.4 (29.5) | 38.4 (18.5) | 62.5 (22.2) | 93.0 (15.6) |
| Test | 12.22 (28.1) | 16.4 (12.1) | 273.8 (44.8) | 34.1 (8.4) | 37.8 (22.5) | 83.2 (26.1) |

ences were found on baseline nights. Examining differences between drug and baseline nights revealed no significant changes in VAS scores (Tables I and II), sleep onset and other stage latencies, within-sleep wakefulness and the time spent in stage 1 (Table III). On the other hand, significant differences between conditions were found in the amount of time spent in stage 2 ($F = 18.25$, $df = 3,24$, $P < 0.01$, $LSD = 35.75$), stage 3 ($F = 14.12$, $df = 3,24$, $P < 0.01$, $LSD = 17.31$) stage 4 ($F = 10.67$, $df = 3,24$, $P < 0.01$, $LSD = 29.52$), stage REM ($F = 4.10$, $df = 3,24$, $P < 0.05$, $LSD = 24.54$) and the number of

REM periods ($F = 4.79$, $df = 3,24$, $P < 0.01$, $LSD = 0.94$, Table IV). The positive control nitrazepam reduced the duration of stages 4 and REM and increased the time spent in stage 2. In contrast, placebo had little effect, whereas ritanserin caused a massive increase in time spent in stages 3 and 4 with concomitant decreases in stages 2 (RitAM) and REM (RitPM). A cumulative plot of the SWS data (Fig. 1) shows clearly the increase in duration caused by ritanserin and the decrease caused by nitrazepam. Ritanserin also reduced the number of REM periods.

The decrease in the number of REM periods asso-

ciated with ritanserin probably reflects the slight increase in cycle length caused by the increase in SWS. The decrease in the duration of REM in the RitPM condition may also indicate a displacement of REM by SWS. However, the baseline values for REM in this condition are suspiciously high.

The increase in SWS is larger in the RitAM condition than in the RitPM condition presumably because of ritanserin's slow penetration of the blood-brain barrier. It is interesting to note that the observation of a large increase in SWS in the RitAM condition and no detectable differences in VAS scores has been repeated recently in a chronic study in this laboratory⁷. The lack of sedation in association with a large effect on sleep suggests that ritanserin is exerting a specific action on those mechanisms that control SWS and not those that control sleep initiation.

Animal work indicates strongly that serotonin levels are correlated positively with SWS. Manipulations that increase brain serotonin such as injection of serotonin or its precursors L-tryptophan or 5-hydroxytryptophan, administration of monoamine oxidase inhibitors, stimulation of the raphe nuclei, all lead to increased SWS whereas serotonin levels lowered through electrolytic lesions, lesions caused by 5,6- or 5,7-dihydroxytryptamine and administration of PCPA all lead to decreased SWS and insomnia⁹. It

would therefore appear that the finding that a serotonin antagonist increased SWS is paradoxical. As serotonin normally activates both 5-HT₁ and 5-HT₂ receptors we postulate that SWS is under the control of inhibitory 5-HT₂ and facilitatory 5-HT₁ systems. Mixed serotonin antagonists mentioned earlier give rise to varying effects on SWS, clinically 5-HT_{1B} blockers such as propranolol may cause insomnia¹⁸ and ritanserin through its 5-HT₂ antagonistic properties promotes SWS.

Alternatively, in order to explain the potentiation of the inhibitory effects of serotonin by ketanserin in rat prefrontal cortex Lakoski and Aghajanian¹⁰ suggested that 5-HT₂ sites modulate 5-HT₁ responses. Similarly, Goodwin and Green³ in trying to interpret the various behavioural changes produced in rats by serotonin propose that between a 5-HT₂ initiated behavior and the behaviour itself there exists a 5-HT_{1A} link.

The large increase in SWS caused by ritanserin may reflect either an effect on the final generating mechanisms of EEG slow waves or an effect on the fundamental mechanisms that control sleep. Clearly more work needs to be conducted with specific serotonin antagonists to re-evaluate the role of serotonergic mechanisms in sleep.

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