Pergamon

0028-3908(93)E0039-G

Neuropharmacology Vol. 33, No. 3/4, pp. 467–471, 1994

0028-3908/94 $7.00 + 0.00

Slow Wave Sleep in Humans: Role of 5-HT_{2A} and 5-HT_{2C} Receptors

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(Accepted 8 December 1993)

Summary—We studied the effects of the 5-HT_2 receptor antagonists, ritanserin and ketanserin, on the sleep of healthy volunteers in order to clarify the role of 5-HT_{2A} and 5-HT_{2C} receptors in the regulation of slow wave sleep (SWS) in humans. Ritanserin, 5 mg, produced a substantially larger increase in SWS (51.4%) than either ketanserin, 20 mg (17.2%) or ketanserin, 40 mg (24.4%). Ritanserin has a significantly higher affinity than ketanserin for 5-HT_{2C} receptor binding sites in the human brain and, based on estimates of per cent occupancy by the two compounds at brain 5-HT_{2A} and 5-HT_{2C} receptors, we conclude that SWS in humans is primarily regulated by 5-HT_{2C} receptors.

Keywords—Slow wave sleep, 5-HT_{2A} receptor, 5-HT_{2C} receptor.

Studies in both humans and animals indicate that 5-HT_2 receptors are involved in the regulation of slow wave sleep (SWS) (Sharpley *et al.*, 1990; Dugovic and Waquier, 1987). For example, in rodents, SWS is increased by a variety of selective 5-HT_2 receptor antagonists (Dugovic and Waquier, 1987; Stutzmann *et al.*, 1990), while drugs with 5-HT_2 receptor agonist properties such as 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) and *meta*-chlorophenylpiperazine (*m*CPP) produce the opposite effect (Stutzmann *et al.*, 1990; Dugovic and Van den Broeck, 1991). In healthy human subjects the selective 5-HT_2 receptor antagonists, ritanserin and ICI 169,369, produce dose-related increases in SWS (Sharpley *et al.*, 1990). In contrast, *m*CPP administration appears to lower SWS in a dose-dependent manner (Katsuda *et al.*, 1993).

On the basis of ligand binding, molecular biological and functional studies, the 5-HT_2 receptor family has recently been reclassified and is currently subdivided into 3 specific subtypes, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, though it is not yet known whether 5-HT_{2B} receptors are present in the human brain (Humphrey *et al.*, 1993). It is important to determine whether a particular 5-HT_2 receptor subtype may play a critical role in SWS regulation in humans, but the current lack of selective ligands makes this a difficult issue to resolve. For example, the 5-HT_2 receptor antagonists, ritanserin and ICI 169,369, bind with a high affinity to both 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer, 1988; Blackburn *et al.*, 1987). Similarly, the 5-HT receptor agonist, *m*CPP, has a significant affinity for both these 5-HT receptor subtypes (Maj and Lewandowska, 1980; Hamik and Peroutka, 1989). However, studies on the effects of *m*CPP on 5-HT_2 receptor-mediated phosphinositide (PI) hydrolysis, suggested that this ligand acts as an agonist at 5-HT_{2C} receptors but as an antagonist at 5-HT_{2A} receptors (Conn and Sanders-Bush, 1987). These data suggest that *m*CPP may lower SWS through activation of 5-HT_{2C} receptors. The aim of the present study was to examine further the role of 5-HT_{2A} and 5-HT_{2C} receptors in SWS in humans by comparing the effects on SWS of two 5-HT_2 receptor antagonists, ritanserin and ketanserin. Ligand binding studies in rodents have shown that ritanserin and ketanserin have equivalent affinities for the 5-HT_{2A} receptor but that the affinity of ritanserin for the 5-HT_{2C} receptor is significantly higher than of ketanserin (Hoyer, 1988). We have now confirmed these findings in human brain. Based on the hypothesis that SWS is primarily controlled by 5-HT_{2C} receptors we predicted that compared to ritanserin, ketanserin would have only a modest effect on SWS.

METHODS

Subjects and drug administration

The study, approved by the local psychiatric ethics committee, was carried out in normal healthy volunteers who had no history of psychiatric disorder, sleep disturbance or drug abuse and were not taking any medication. Prior to entry into the study all subjects received

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a physical examination and routine haematology and biochemistry screening tests. The study medication was administered orally 90 min before retiring in a double blind, balanced order design.

Eight subjects (5 female; 3 male), mean age 30 yr (range 24–36 yr), mean weight 67.7 kg (range 59–79 kg) were studied on 4 nights, each separated by 1–2 weeks. Each subject received on separate nights, placebo, ketanserin 20 mg, ketanserin 40 mg and ritanserin 5 mg.

Sleep recordings

Sleep recordings were made in the subjects' own homes using the Medilog 9000-II cassette monitoring system. On each of the study nights, subjects attended the Research Unit at approx. 1700 hr, to have the sleep montage electrodes applied [two electroencephalogram (EEG) channels: C_4–A_1, C_3–A_2, two electro-oculogram (EOG) channels from the outer canthus of each eye referred to the mastoid and submental electro-myogram (EMG)]. Subjects were instructed to abstain from alcohol on each of the study nights and the night preceding each study night, but normal caffeine intake was allowed. Subjects retired and rose at their usual time, and this was kept constant for all study nights and all preceding nights. They started the recorder as they settled down to sleep. The records were analysed using the Oxford Medilog sleep stager (9200) and also visually inspected and edited. The sleep data were analysed by a two way repeated measures analysis of variance (ANOVA). Significant effects were further assessed using post hoc paired t-tests.

Radioligand binding studies

Human brain tissue ($n = 3$) was dissected at post mortem from non-neurological cases within 24–48 hr of death and stored for up to 4 weeks at −70°C. After thawing, frontal cortex (Brodman area 11) was homogenized in 10 vol 5 mM Tris/EDTA and then centrifuged at 1000 ***g***. Membranes from this supernatant were washed by centrifugation at 30,000 ***g*** at 4°C and finally resuspended in incubation buffer (50 mM Tris, 5 mM $MgCl_2$, 1 mM EGTA, pH 7.4) at a final tissue density of approx. 1 mg/ml of protein. Membranes of choroid plexus, taken from the lining of the lateral ventricles, were prepared in a similar fashion and resuspended in incubation medium at approx. 0.3 mg/ml of protein. Binding with [^{3}H]ketanserin or [^{3}H]mesulergine was carried out for 60 min at 37°C and terminated by filtration through Whatman GF-C filters. Non-specific binding was defined by the addition of 1 μM mianserin for both radioligands.

Data were analysed by non-linear regression analysis using the commercial programme EBDA/Ligand (Biosoft). The affinities of ketanserin and ritanserin at the 5-HT_{2A} receptor were determined as the IC_{50} values for inhibition of [^{3}H]ketanserin binding in frontal cortex and at the 5-HT_{2C} receptor by inhibition of [^{3}H]mesulergine binding in choroid plexus. K_i values were estimated from IC_{50} assuming competitive inhibition kinetics, according to the Cheng–Prusoff relationship (Cheng and Prusoff, 1973). Receptor occupancy by ketanserin or ritanserin, following correction for protein binding, was estimated using the derived binding affinity values assuming simple

Table 1. The effect of ketanserin (20 and 40mg) and ritanserin (5mg) on sleep in 8 healthy volunteers

Sleep parameter (min)	Placebo	Ketanserin 20mg	Ketanserin 40mg	Ritanserin 5mg
Sleep continuity				
Total sleep period	428.2 ± 10.8	427.8 ± 10.0	424.1 ± 11.0	425.3 ± 13.9
Actual sleep time	400.8 ± 10.7	410.5 ± 10.1	405.2 ± 10.7	404.6 ± 16.5
Sleep efficiency %	91.3 ± 1.2	93.4 ± 1.3	92.2 ± 1.3	92.0 ± 1.6
Sleep onset latency	10.8 ± 2.8	11.0 ± 4.0	14.2 ± 3.9	13.1 ± 3.6
Wake after sleep onset	21.9 ± 3.2	12.4 ± 2.3[i]	14.2 ± 2.7[g]	16.7 ± 3.6
No awakenings >120sec	4.5 ± 0.7	1.6 ± 0.5[j]	2.3 ± 0.4[g]	2.5 ± 0.8[c]
No awakenings <120sec	8.8 ± 1.9	8.6 ± 2.3	5.9 ± 1.3	6.9 ± 1.3
Total movement time	5.6 ± 0.8	4.9 ± 1.1	4.8 ± 0.9	3.9 ± 0.9
Non REM measures				
Stage 2 latency	6.4 ± 2.4	9.8 ± 2.6	6.3 ± 2.5	7.8 ± 2.1
SWS latency	23.1 ± 4.6	21.3 ± 4.0	29.8 ± 13.7	26.5 ± 6.3
Stage 1	34.3 ± 3.9	35.0 ± 2.5	34.2 ± 4.6	32.3 ± 4.1
Stage 2	160.3 ± 17.4	141.2 ± 15.1	135.1 ± 17.8[h]	128.2 ± 17.2[b]
SWS	112.5 ± 21.9	131.8 ± 19.9	139.9 ± 23.0[g]	170.3 ± 25.4[a,d,f]
Non REM	312.7 ± 9.1	312.9 ± 10.8	313.9 ± 14.6	334.7 ± 15.4[c,e]
REM measures				
REM Sleep	93.6 ± 5.3	102.6 ± 5.6	96.0 ± 10.1	73.9 ± 4.1[a,d]
REM Latency	74.1 ± 8.5	79.1 ± 14.4	74.7 ± 10.3	98.3 ± 20.5
REM Periods	4.4 ± 0.2	4.1 ± 0.2	3.9 ± 0.2	3.6 ± 0.3
Subjective measures				
Vigilance	4.2 ± 0.6	5.0 ± 0.6	4.9 ± 0.7	5.8 ± 0.5
Sleep quality	4.8 ± 0.6	5.1 ± 0.8	5.1 ± 0.6	4.1 ± 0.5

Results expressed as mean ± SEM.

Results of post-hoc paired t-test: Ritanserin compared to placebo, [a] $P < 0.005$, [b] $P < 0.01$, [c] $P < 0.05$; Ritanserin compared to ketanserin 20 mg, [d] $P < 0.01$, [e] $P < 0.05$; Ritanserin compared to ketanserin 40 mg, [f] $P < 0.01$; Ketanserin 40 mg compared to placebo, [g] $P < 0.05$, [h] $P < 0.005$; Ketanserin 20 mg compared to placebo, [i] $P < 0.005$, [j] $P < 0.0005$.

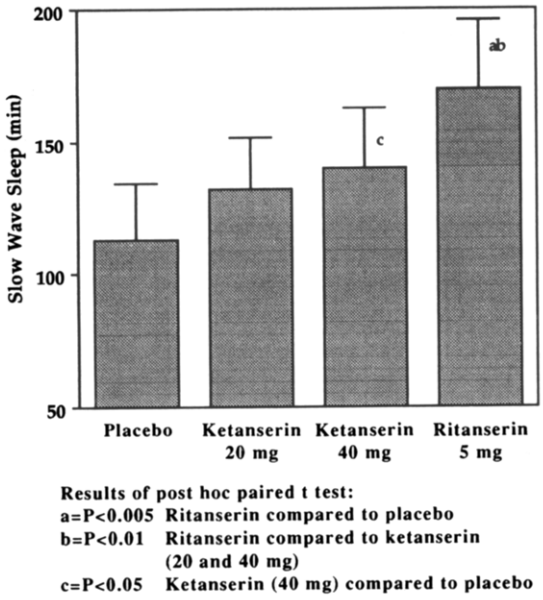

Fig. 1. Mean (±SEM) slow wave sleep (SWS) minutes in eight healthy volunteers who were tested on four separate nights with the following treatments: (1) placebo; (2) ketanserin (20 mg orally); (3) ketanserin (40 mg orally); (4) ritanserin (5 mg orally). The following comparisons were significant (post-hoc paired t-test); (a) ritanserin vs placebo ($P < 0.005$); (b) ritanserin vs ketanserin (20 mg and 40 mg) ($P < 0.01$); (c) ketanserin (40 mg) vs placebo ($P < 0.05$).

binding to a single site according to the equation $B = F/K_i + F$) where B is fractional receptor occupancy, F is free concentration of drug and K_i is the receptor binding affinity.

RESULTS

Sleep studies

The ANOVA showed significant changes in SWS (min) ($F = 10.88_{3,21}$, $P < 0.001$), stage 2 sleep (min) ($F = 5.06_{3,21}$, $P < 0.01$), non REM sleep (min) ($F = 3.41_{3,21}$, $P < 0.05$), REM sleep (min) ($F = 5.21_{3,21}$, $P < 0.01$), wake after sleep onset (WASO) (min) ($F = 3.12_{3,21}$, $P < 0.05$) and number of awakenings >120 sec ($F = 6.55_{3,21}$, $P < 0.01$).

Post hoc testing showed increased levels of SWS with ritanserin compared to placebo ($P < 0.005$) and ketanserin (20 and 40 mg) ($P < 0.01$) (Table 1). SWS was also increased with ketanserin 40 mg compared to placebo ($P < 0.05$) (Fig. 1). Stage 2 sleep (min) was significantly decreased with ritanserin ($P < 0.01$) and ketanserin (40 mg) ($P < 0.005$) compared to placebo. Non REM sleep (min) was significantly increased with ritanserin compared to placebo ($P < 0.05$) and ketanserin (20 mg) ($P < 0.05$). REM sleep (min) was significantly decreased on ritanserin compared to placebo ($P < 0.005$) and ketanserin (20 mg) ($P < 0.01$). WASO (min) was significantly decreased with ketanserin, both 20 mg ($P < 0.05$) and 40 mg ($P < 0.05$) compared to placebo. Finally, the actual number of awakenings which lasted >120 sec was significantly decreased with ritanserin ($P < 0.05$), and ketanserin 20 mg ($P < 0.0005$) and 40 mg ($P < 0.05$) compared to placebo. No other significant differences were noted. We also studied the effect of ritanserin and ketanserin on SWS in the first 2 hr of sleep because during this time plasma levels of both drugs would be expected to reach their maximum value (see below). Mean ± SEM SWS minutes in the first 2 hr of sleep for ritanserin (59.5 ± 6.8 min) were significantly greater than placebo (44.6 ± 6.2 min; $P = 0.003$), while neither dose of ketanserin significantly increased SWS minutes above placebo values (ketanserin 20 mg, mean ± SEM = 53.1 ± 7.0 min; ketanserin 40 mg, mean ± SEM = 47.5 ± 9.2 min; $P > 0.05$).

Radioligand binding studies

Radioligand binding studies in human post mortem brain demonstrated that both ketanserin (pK_i 8.67 ± 0.10) and ritanserin (pK_i 8.30 ± 0.13) showed similar high affinity for the 5-HT_{2A} receptor. In contrast, ritanserin (pK_i 9.06 ± 0.14) showed a significantly higher affinity for the 5-HT_{2C} receptor than ketanserin (pK_i 7.24 ± 0.09; $P < 0.01$, Student's unpaired t-test.

DISCUSSION

We have previously demonstrated that the use of home ambulatory sleep EEG recording with automatic staging analysis provides a reliable and valid means of detecting the effects of 5-HT_2 receptor antagonists on SWS (Solomon *et al.*, 1989). This has been confirmed by the present investigation where the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, produced a substantial (51.4%) and highly significant increase in SWS in healthy subjects. The magnitude of the increase in SWS is similar to

Table 2. Comparison of the effects of ritanserin and ketanserin on slow-wave sleep and estimations of 5-HT_{2A} and 5-HT_{2C} receptor occupancy

	Plasma conc. (μmol/l)	Estimated % 5-HT_{2A}	Receptor occupancy 5-HT_{2C}	% Increase SWS
Ketanserin 20 mg	0.15	78	11	17.2
Ketanserin 40 mg	0.42	91	26	24.4
Ritanserin 5 mg	0.14	36	77	51.4

Receptor occupancy was estimated from the free drug concentration (assuming 95% protein binding for ketanserin and 98% for ritanserin) and the receptor binding affinities (identified in human post-mortem brain tissue) according to simple mass-action equilibrium kinetics, as described in the Methods.

that seen in our previous studies with ritanserin and is comparable to results reported from sleep laboratory investigations (Sharpley *et al.*, 1990; Idzikowski *et al.*, 1991).

As far as we are aware, there are no previous reports on the effect of ketanserin on the sleep EEG in humans. In keeping with our hypothesis, however, ketanserin had only a modest effect on SWS, producing a 24.4% increase with the higher dose of 40 mg. From the peak plasma concentrations achieved in healthy volunteers by single doses of 5 mg of ritanserin and 20 and 40 mg of ketanserin, and allowing 98% and 95% respectively of drug to be bound to plasma proteins (Brogden and Sorkin, 1990; Janssen Pharmaceuticals, data on file), it is possible to estimate the percent receptor occupancy by both drugs at 5-HT_{2A} and 5-HT_{2C} receptors, using the pK_i values for human brain tissue estimated from the radioligand binding studies (Table 2). At a dose of 40 mg, ketanserin occupies about one third of the proportion of 5-HT_{2C} receptors occupied by ritanserin 5 mg. In contrast, the occupancy of 5-HT_{2A} receptors is higher for both doses of ketanserin than for ritanserin. These values can obviously only be treated as estimates because a simple one compartment kinetic model is assumed. They are, however, compatible with the proposal that the regulation of SWS in humans is critically dependent on the activity of 5-HT_{2C} receptors. Before this conclusion is accepted, however, a number of methodological issues must be addressed.

Some previous reports on ketanserin have suggested that, in contrast to ritanserin, its 5-HT_2 receptor blocking properties might be confined to peripheral sites (Shenker *et al.*, 1985; Barone *et al.*, 1986). However, a variety of *in vivo* animal experimental studies have indicated that this is not the case (see Cohen *et al.*, 1989). For example, both ketanserin and ritanserin were equally effective in antagonizing the increase in plasma corticosterone produced by quipazine in the rat, a response mediated by central 5-HT_2 receptors (Cohen *et al.*, 1989). In this method the ED_{50} value for ketanserin to inhibit quipazine-induced corticosterone release was 0.5 mg/kg while that of ritanserin was 0.9 mg/kg. This study suggests that ketaneserin is at least as effective as ritanserin in antagonizing responses mediated by brain 5-HT_{2A} receptors *in vivo*.

It is also possible that pharmacokinetic differences between ritanserin and ketanserin may account for their differential effects on SWS *in vivo*. However, clinical pharmacokinetic studies indicate that this is unlikely to be the case. Ketanserin and ritanserin are well absorbed with peak plasma levels occurring within 2 hr of ingestion for both compounds (Brogden and Sorkin, 1990; Janssen Pharmaceutical, data on file). The elimination of half-life of ritanserin is significantly longer than that of ketanserin (40 vs 14 hr) but this difference is unlikely to account for our findings because the majority of SWS occurs in the early part of the night. In addition measuring changes in SWS for the first 2 hr of sleep, when both drugs would be expected to be present in plasma at maximum concentration, showed that only ritanserin produced a significant increase in SWS over this time period.

A further issue that needs to be addressed is the fact that ketanserin also possesses significant antagonist activity at brain α_1-adrenoceptors and histamine H_1 receptors (Brogden and Sorkin, 1990), However, previous studies with more selective α-adrenoceptor antagonists, have suggested that α_1-adrenoceptors do not play a significant role in the regulation of SWS, and it therefore appears unlikely that α_1-adrenoceptor blockade produced by ketanserin could have offset a potential increase in SWS (Gaillard, 1979). Similarly, it seems unlikely that the histamine H_1 receptor blocking effects of ketanserin could have attenuated a potential increase in SWS because cyproheptadine, a 5-$HT_{2A/2C}$ receptor antagonist with potent anti-histaminic properties, produces reliable increases in SWS in humans (Solomon *et al.*, 1989). Interestingly, ketanserin (40 and 20 mg) significantly decreased the amount of wake time experienced by subjects, an effect consistent with the known sedating properties of α_1-adrenoceptor and histamine H_1 receptor antagonists (Peroutka and Snyder, 1981).

In the present study, ritanserin also decreased REM sleep, a finding which has been reported in some but not all previous investigations (Idzikowski *et al.*, 1987). The reason for the inconsistent effect of ritanserin on REM sleep is not clear at present. It is possible that the decrease in REM sleep sometimes seen with ritanserin represents a compensatory change obtained at the expense of the large facilitation of SWS, and is therefore not a primary consequence of 5-HT_2 receptor blockade.

In conclusion, the findings from the present study are consistent with the proposal that SWS in humans is primarily regulated by brain 5-HT_{2C} receptors. However, this hypothesis will need to be tested further with more selective 5-HT_{2A} and 5-HT_{2C} receptor ligands as these become available for clinical study. In addition it remains possible that 5-HT_{2B} receptors will, in future, be identified in human brain. Ritanserin is an effective antagonist at 5-HT_{2B} receptors in the rat fundic strip model (Baez *et al.*, 1990), and thus a possible role for 5-HT_{2B} receptors in the regulation of SWS cannot presently be excluded. It is also likely that other 5-HT receptor subtypes play a role in the regulation of the sleep wake cycle and it is conceivable that differing affinities of ketanserin and ritanserin at other, recently described, 5-HT receptor subtypes could explain their differing effects on SWS in humans.

Acknowledgements—We thank the Janssen Research Foundation for supplies of ketanserin and ritanserin and for financial assistance. ALS is a Janssen Research Fellow.

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