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BRIEF COMMUNICATION

Study of the 5-HT_2 Antagonist Ritanserin on Sleep-Waking Cycle in the Rat

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SILHOL, S., L. GLIN AND C. GOTTESMANN. *Study of the 5-HT_2 antagonist ritanserin on sleep-waking cycle in the rat.* PHARMACOL BIOCHEM BEHAV **41**(1) 241–243, 1992.—Ritanserin, a 5-HT_2 receptor antagonist, was injected intraperitoneally to rats at light onset. It was found that 0.63 mg/kg decreased waking, increased the slow waves characteristic of the first stage of sleep, and decreased paradoxical sleep (PS) during the first four hours. Active waking was further decreased and slow wave stage increased during the following four hours. The number of synchronized and paradoxical sleep phases decreased whereas their duration increased during the first four hours. Ritanserin at 2.5 mg/kg decreased active waking and PS, whereas quiet waking and slow wave stage were increased during the first four hours. Quiet waking was increased during the following four hours. It is concluded that serotonin acting on 5 HT_2 receptors is actively involved in sleep-waking regulation.

Sleep Waking Serotonin Ritanserin 5-HT_2 antagonist Rat

NUMEROUS studies related to the function of serotonin (5-HT) on sleep-waking cycle have been performed since the pioneer work of Jouvet's (8) and Koella's (10) groups.

In the few past years researchers have investigated the function of 5-HT receptor subtypes (4). Recent investigations have shown that compounds modulating 5-HT_{1A} (11) and 5-HT_2 (2) receptors influence the sleep-waking cycle. Ritanserin, a 5-HT_2 antagonist, was the most intensively studied. It increases slow wave sleep (SWS) in man (7) and in the rat decreases waking, increases deep SWS and decreases paradoxical sleep (PS) (2). At the transition of deep SWS and PS there is an intermediate stage of sleep (IS) characterized by an unusual association of high amplitude cortical spindles, which are an index of deep SWS, and low frequency theta rhythm in the dorsal hippocampus, indicating central activation (5). This short-lasting stage corresponds to a "cerveau isolé"-like preparation, i.e., to a transient disconnection of the forebrain from the brain stem (6). This stage is substantially increased at the expense of PS by several psychotropic drugs (5,9) which inhibit the release of PS specific processes. We wanted to study whether the increase of deep SWS and the decrease of PS described in rats under ritanserin is related to an increase of IS.

METHOD

Seven male Wistar rats weighing between 250 and 300 g were anesthetized with sodium penthiobarbital (55 to 60 mg/kg IP according to time of day). They were implanted with electrodes for sleep-waking recordings and housed in individual cages with 12:12 light-dark regime (lights on at 0900). The temperature was maintained at 23°C.

During the one-week recovery the animals were habituated to recording cables. The electrophysiological recordings were calibrated to be scored by an automatic sleep-waking scoring system (3). Seven stages were identified: 1) waking with theta activity in the dorsal hippocampus, which corresponds to attentive and/or psychomotor active waking; 2) waking without theta, corresponding above all to quiet waking; 3) cortical slow waves, characteristic of first sleep stage; 4) frontal spindles which appear interspersed with slow waves as sleep deepens; 5) intermediate stage. The last three stages constitute synchronized sleep; 6) PS without eye movements; 7) eye movement periods of PS.

During the first day of experimentation, the animals received saline injection. The following day they were given 1 mM tartric acid solution and the third day 0.63 mg/kg ritanserin in tartric acid solution (2). Four days later, after the same experimental steps, 2.5 mg/kg ritanserin, were administered. All injections were done at 0845.

Sleep-waking parameters were quantified for each of the six successive 4-h periods and compared to baseline (vehicle injection). They were expressed as percentages of recording time. Visual scoring was done blind. Statistical significance of the data was assessed by means of the nonparametric two-tailed Wilcoxon test.

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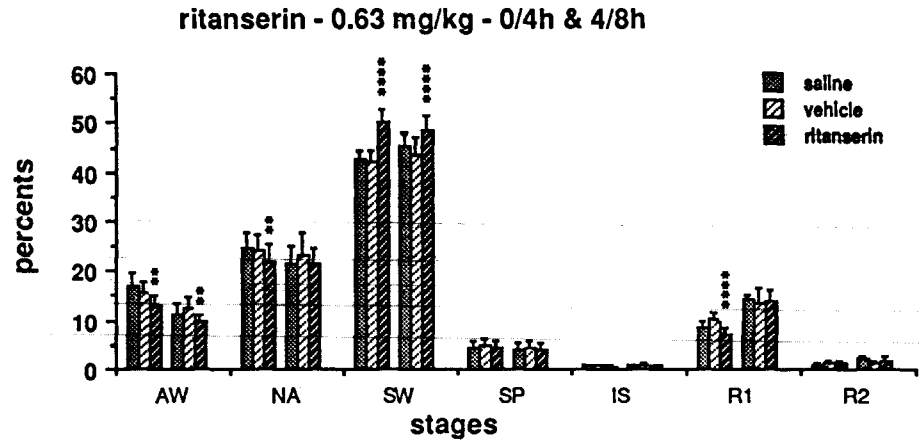

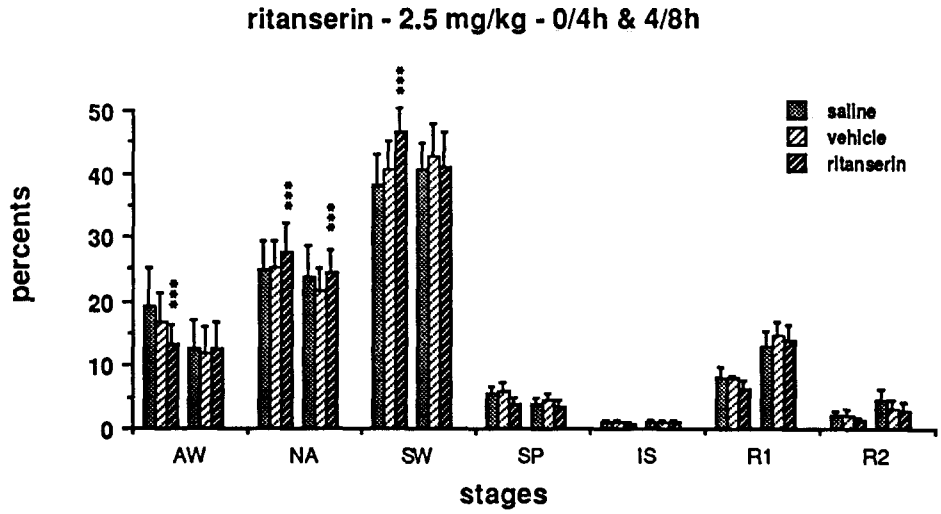

FIG. 1. Percentage of sleep-waking stages for the first four hours of recording and the four following ones after saline, vehicle and ritanserin at 0.63 and 2.5 mg/kg. The values (mean and S.E.M.) are expressed as percentage of the recording period. AW: waking with theta activity in the dorsal hippocampus; NA: waking without theta activity; SW: cortical slow waves; SP: spindles; IS: intermediate stage; R1: paradoxical sleep without eye movements; R2: eye movement periods of paradoxical sleep.*$p<0.05$; **$p<0.04$; ***$p<0.03$; ****$p<0.02$.

RESULTS

The vehicle induced no changes other than an increase of total PS during the first four hours ($8.9 \pm 1.2\%$ vs. $11.5 \pm 1.3\%$, $p<0.05$) in the first study step (low dose), probably related to the stress of the first injection.

At 0.63 mg/kg, waking with theta activity decreased from $15.8 \pm 2.6\%$ (S.E.M.) of the sleep-waking stages to $13 \pm 2.2\%$ ($p<0.04$) during the first four hours and from 12.7 ± 2.2 to $9.7 \pm 1.5\%$ ($p<0.04$) during the following four hours (Fig. 1). Waking without theta rhythm was decreased, from 24.5 ± 3.2 to $22 \pm 3.3\%$ ($p<0.04$) during the first four hours. The slow wave stage increased from 42 ± 2.5 to $50.1 \pm 2.8\%$ ($p<0.02$) during the first four hours and from 43.5 ± 3.3 to $48.3 \pm 3.1\%$ ($p<0.02$) during the following four hours. Spindle stage and IS were unchanged. PS without eye movements was decreased from

10.2 ± 1.3 to $7.4 \pm 1.1\%$ ($p<0.02$), whereas the eye movement periods of PS were unchanged during the same period. Total PS decreased from 11.5 ± 1.3 to $8.9 \pm 1\%$ ($p<0.02$) during the same four-hour period. PS without eye movements increased from 2.3 ± 0.5 to $3.8 \pm 1.1\%$ ($p<0.04$) between the 12th and 16th hour.

The number and duration of synchronized sleep and PS phases were studied in six rats by visual scoring. The number of synchronized sleep periods during the first four hours was decreased from mean 78.7 ± 6.5 to 59.5 ± 10.3 ($p<0.03$), whereas their duration was increased from 109.4 ± 8.3 to 189.4 ± 29.9 s ($p<0.03$). In addition, PS phases decreased from 24.3 ± 2.8 to 14.5 ± 1.6 ($p<0.05$), whereas their duration increased from 63.7 ± 2.9 to 88.9 ± 6.6 s ($p<0.04$).

At the dose of 2.5 mg/kg studied in six rats, waking with theta decreased from 16.9 ± 4.2 to $13.2 \pm 3.3\%$ ($p<0.03$) during the first four hours, whereas waking without theta increased from 25 ± 4.2 to $27.5 \pm 4.5\%$ ($p<0.03$). The slow waves increased from 40.7 ± 4.5 to $46.4 \pm 5.1\%$ ($p<0.03$), whereas spindles and IS were unchanged. PS decreased from 10.3 ± 1.1 to $7.7 \pm 1.5\%$ ($p<0.03$). During the following 4-hour period waking without theta increased from 21.6 ± 3.4 to $24.4 \pm 3.5\%$ ($p<0.03$). PS was increased ($p<0.03$) from 8th to 12th hour (12.7 ± 2.2 vs. $14.2 \pm 2\%$).

On six rats the number and duration of synchronized sleep and PS phases were studied during the first four hours. The number of synchronized sleep phases was unchanged (72.3 ± 4.3 vs. 61 ± 9.2, NS) their duration increasing from 115.7 ± 9.4 to 172.8 ± 27.4 s ($p<0.03$). The PS phases decreased from 28.8 ± 2.3 to 14 ± 1.7 ($p<0.03$) during the same period but their duration increased from 61.9 ± 8.4 to 85.5 ± 6.1 s ($p<0.03$).

DISCUSSION

Ritanserin, a 5-HT_2 antagonist, also has a low affinity for histaminergic H_1, dopaminergic D_2, noradrenergic α_1 and α_2 (12) and 5-HT_{1C} (4) receptors. However, at 0.63 mg/kg, the influence on these receptors is negligible (12). Consequently, the results obtained in our experiment at low dose appear to be related to a lowering of serotonin acting at 5-HT_2 receptor level.

Ritanserin decreases waking and increases the slow waves characteristic of light sleep, whereas the spindles, indices of deeper synchronized sleep, are unchanged. Consequently, "deep slow wave sleep" (SWS2), as described by Dugovic et al. (2), i.e., slow waves without interspersed desynchronized waking activity, is certainly increased under ritanserin. However, this stage definition corresponds to steady slow wave sleep rather than to deep synchronized sleep following more precise electrophysiological criteria. Indeed, in the rat the spindles, interspersed with slow waves, increase in number, duration and amplitude as synchronized sleep deepens, and they are maximal during IS (13). But, under ritanserin, the spindle level and IS are unchanged. Consequently, the results suggest that the deepest stages of synchronized sleep are not increased.

As described by Dugovic et al. (2) and Borbely et al. (1), ritanserin decreases PS in the rat. Since IS is not correlatively increased, the decrease suggests that PS neurochemical processes are not disturbed by the drug but that the animal has some difficulty entering PS. This hypothesis appears to be reinforced by the significant lower number of PS phases and the increase in their duration.

With 2.5 mg/kg, the specificity for 5-HT_2 receptors is less pronounced and other sites are probably implicated. Our results are in agreement with Borbely's et al. (1) data. Indeed they are similar to those induced by the low dose but waking without theta is significantly increased during 8 hours, whereas waking with theta is diminished only during the first four hours. It is difficult to speculate on the neurochemical basis of this increase of waking without theta since several neurotransmitters could be involved.

To conclude, this research provides further support for the function of serotonin on the sleep-waking cycle. The 5-HT_2 receptors appear to be implicated in the process. Ritanserin at doses known to act specifically on 5-HT_2 receptors increases one stage of sleep but does interfer with the normal sequence of sleep stages.

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