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Benzodiazepines Promote the Intermediate Stage at the Expense of Paradoxical Sleep in the Rat

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GANDOLFO, G., R. SCHERSCHLICHT AND C. GOTTESMANN. *Benzodiazepines promote the intermediate stage at the expense of paradoxical sleep in the rat.* PHARMACOL BIOCHEM BEHAV **49**(4) 921–927, 1994.—The effects of diazepam, a long half-life benzodiazepine, midazolam and triazolam, two with short half-life, on the transitional stage between deep slow wave sleep and paradoxical sleep were studied in Wistar and WAG/Rij rats. This intermediate stage is characterized by the unusual association of cortical spindles and low frequency hippocampal theta rhythm. The main result was extension of the intermediate stage at the expense of paradoxical sleep by diazepam and triazolam by influencing only the duration of the intermediate stage and both the onset and maintenance of paradoxical sleep. Midazolam increased both intermediate stage and paradoxical sleep. Several differences in the qualitative modulation of the stage characteristics and between rat strains were found. In regard to the possible peculiar physiological significance of the intermediate stage, we conclude that benzodiazepines promote a transient pharmacological cerveau isolé-like stage during sleep in rats.

Benzodiazepines Sleep Intermediate stage Paradoxical sleep Rat

PARADOXICAL sleep (PS) in the rat (4,5,11,38), cat (14), and mouse (10) is preceded and sometimes followed by a short-lasting electrophysiological stage characterized by high-amplitude spindles in the frontal cortex (sign of advanced slow wave sleep) and low-frequency hippocampal theta rhythm (sign of central activation because it occurs during psychomotor active and/or attentive waking and PS). This intermediate stage (IS) in the rat occurs in 75% of cases prior to PS and in 15% of cases just after it and in the cat in 27% and 4% of cases, respectively (13). Previous studies by evoked potentials showed that it is characterized by the lowest thalamic transmission level of all sleep–waking stages in the rat (7) and the lowest thalamocortical responsiveness in the cat (14). These two processes are under the control of brain stem-activating influences (32). Moreover, the GABAergic thalamic reticular nucleus neurons that project peculiarly onto the thalamic relay nuclei are the more activated during the transition from slow wave sleep to PS (26). This fact could explain the very low thalamic transmission level and the occurrence of the high-amplitude cortical spindles during IS because the thalamic reticular nucleus is the spindles pacemaker (36).

In other respects, acute intercollicular transections in the rat (16) and cat (2,14) induce high-amplitude cortical spindles. In the same way, the hippocampal theta rhythm of IS is of low frequency as compared to PS in the rat and cat (13) and intercollicular transections also induce high amounts of low-frequency theta rhythm in these two species (14,16). Consequently, it was hypothetized that IS corresponds to a transient intracerebral deafferentation inducing a disconnection of forebrain structures from brain stem and major peripheral influences (13). This could be the consequence of the lowering and brief disappearance of ascending activating influences of waking that already decrease during slow wave sleep (27,36) and the transient absence or very low level of brain stem ascending influences of PS.

In the rat (11) and cat (14), IS is massively extended at the expense of PS by medium doses (15–25 mg/kg and 10 mg/kg, respectively) of pentobarbital. The general aim of this study

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was to analyze the quantitative and qualitative effects of benzodiazepines (BDZ) on this transitional stage and the following PS to know whether BDZ have similar effects than barbiturates. This study was undertaken in normal Wistar rats and in the WAG/Rij strain, which frequently produces electric patterns resembling absence epilepsy (37) and shows an unusual high amount of IS (8). Indeed, it was interesting to know if BDZ are able to extend an already pronounced IS or whether this behavioral stage is specifically limited in duration because of its peculiar physiological significance (13). To perform this neuropharmacological study on IS and PS in both rat strains, we chose diazepam, the prototype of BDZ, with a vast therapeutic spectrum and a narrow dose range (9) and two other classical BDZ with shorter half-life, midazolam and triazolam.

METHOD

Surgery

Under penthiobarbital anaesthesia (55 mg/kg IP), the animals (adult male Wistar and WAG/Rij rats weighing 250–320 g) were permanently implanted bilaterally with silver ball (1 mm diameter) electrodes to record the frontal corticogram from Krieg's area 10 (22). To record hippocampal theta rhythm, a bipolar electrode made up of coated (except at the tip) stainless steel wires (10/100 mm) was stereotaxically (30) placed in the CA1 area (A: 5.4; L: 2.6; D: +7.4). A silver ball (1 mm diameter) was placed on each side of one orbit to record eye movements. Two twisted stainless steel wires (25/100 mm) were inserted bilaterally in the dorsal neck muscles to record the electromyogram. A ground electrode was screwed in front of the olfactory bulb in the middle plane. All the recording electrodes were soldered to a connector (Connectral, France) and secured to the skull of the animal with dental cement (Texton, England). At the end of surgery, for prophylactic antibiotic therapy, each rat received an IM injection (50,000 u) of benzylpenicilline (Specia, France). Postoperative recovery with cables for habituation lasted at least one week in natural day–night lighting, the animals being individually housed in the recording room with free access to commercial rat chow and water. The ambient temperature was maintained constant at 23°C.

Experimental Procedure

Four recording channels were used to characterize IS and PS stages: a) interhemispheric bipolar recording of the frontal cortex; b) a bipolar recording of the right dorsal hippocampus (CA1 area) for theta rhythm; c) a bipolar electrooculogram from the right eye orbit; d) a bipolar electromyogram from the dorsal neck muscles. Groups of six rats of each strain were recorded on the EEG recorder (Alvar, France) at a speed of 10 mm/s during 6 h in the daylight period (from 0830–1430 h) after an injection of vehicle solution for control. On the following day, they were administered with one of the three BDZ at two chosen doses: either 1 or 3 mg/kg of diazepam and midazolam and either 0.3 or 1 mg/kg of triazolam. All injections were made intraperitoneally. Diazepam and triazolam were suspended in distilled water with 3% Tween 80, the maleate salt of midazolam was dissolved in distilled water. The animals were injected in a randomized order of administration and allowed to recover for at least 2 weeks between two experimental sessions. A total of 12 Wistar and 15 WAG/Rij rats were used.

Scoring of the Data

BDZ could modulate IS and PS stages both quantitatively and qualitatively (by changing their characteristics). Thus, several parameters were measured by visual scoring of every IS and PS episodes:

Quantitative parameters. The total amount per hour of IS and PS reflects globally the quantitative change, but it is not sufficient in regard to the neuropharmacological processes underlying this modulation. Indeed, a change in the amount could be due to a change either in the number of stage episodes or in the duration of each episode or both. These two parameters reflect two different neurophysiological mechanisms: the onset and the maintenance of the behavioral stage, respectively. For the sake of clarity in quantification of PS stage, the attempt episodes with a duration less than 15 s were eliminated.

Although IS and PS are distinct behavioral stages, they are not totally independent because IS always heralds PS. Thus, quantitative changes in each stage must be also regarded as a linkage (or not) between IS and PS. In this aim, the ratio number of IS episodes/number of PS episodes allows to know whether the occurrence of IS prior to PS is systematic or not and is able to be modified by BDZ. The ratio number of PS episodes/number of IS episodes reflects the facility to enter PS from IS.

Finally, the latency of occurrence of the first IS and PS episode after BDZ injection was measured.

Qualitative parameters. The IS stage being defined by the simultaneous occurrence of cortical spindles and hippocampal theta rhythm, we had to consider whether these two characteristics are able to be qualitatively modified by BDZ. Thus, we quantified the number of fully developed cortical spindles per IS episode, their amplitude, intrinsic frequency and duration and, for hippocampal theta rhythm, its amplitude and frequency.

The PS stage is qualitatively dependent on the steadiness of the hippocampal theta rhythm (thus, of its amplitude and frequency) and of the number of eye movements, which must be dissociated in single isolated (smooth) movements and in bursts (saccadic) because they correspond to two different neuropsychological behaviors during PS (34), and are reliable to BDZ pharmacodynamic measures (32). Moreover, it is well known that during a PS episode the level of central activation can fluctuate and that the cortical desynchronization can be shortly disrupted by the irruption of cortical spindles although hippocampal theta rhythm continues being present. Thus, if such IS episodes (by definition) occurred during a PS episode, they reflect its instability: the quantification of this parameter was yielded by the percentage of PS episodes during which one or several IS episodes occurred (at least 30 s after the PS episode started).

Finally, the heart rate was taken into account as an index of the neurovegetative effects of BDZ (28): it was measured by averaging EKG pulses in five periods of 10 s during muscular atonia (IS and/or PS).

Except for the latency of occurrence of the first IS and PS episode after BDZ injection and the mean number of eye movements (single and in burst) per PS episode (averaged only during the first three hours of recording), all other parameters were quantified for each hour of recording to estimate the kinetic effects of each BDZ in relation to its half-life. All quantifications of amplitude and frequency of spindles and theta rhythm were averaged from five samples of 1 s (for IS) or 5 s (for PS), for each IS and PS episodes.

Moreover, to know whether BDZ extend IS at the expense of PS, a comparison of their variation percentages of duration from vehicle was performed during the first 3 h of recording.

For statistical analysis Duncan's test was used when the F test (MANOVA) was significant. Duncan's test was chosen

because it compares each data point to the general variance, i.e., it allows a study of kinetic effects of the drugs. Student's *t*-test was used only for the occurrence latency of the first IS and PS episode, the number of eye movements per PS episode, and to compare the spindle characteristics between diazepam-induced spindles and extended IS.

RESULTS

As illustrated by Fig. 1 with midazolam, the BDZ generally extended the duration of IS at the expense of PS in both rat strains: IS appeared periodically and was long lasting, i.e., its duration was similar to that of an usual PS episode. These spindles of the extended IS were quite different from the well-known (1,3,6) BDZ-increased spindling activity, as shown by Fig. 2. For example, after diazepam in Wistar rats, the BDZ-induced spindles during slow wave sleep had different characteristics when compared to those of the extended IS: they were of lower amplitude (0.37 ± 0.03 mV vs. 0.54 ± 0.03 mV, respectively, $p < 0.01$), higher frequency (15.58 ± 0.03 c/s vs. 10.45 ± 0.02 c/s, $p < 0.005$) and shorter duration (0.75 ± 0.03 s vs. 1.43 ± 0.02 s, $p < 0.001$).

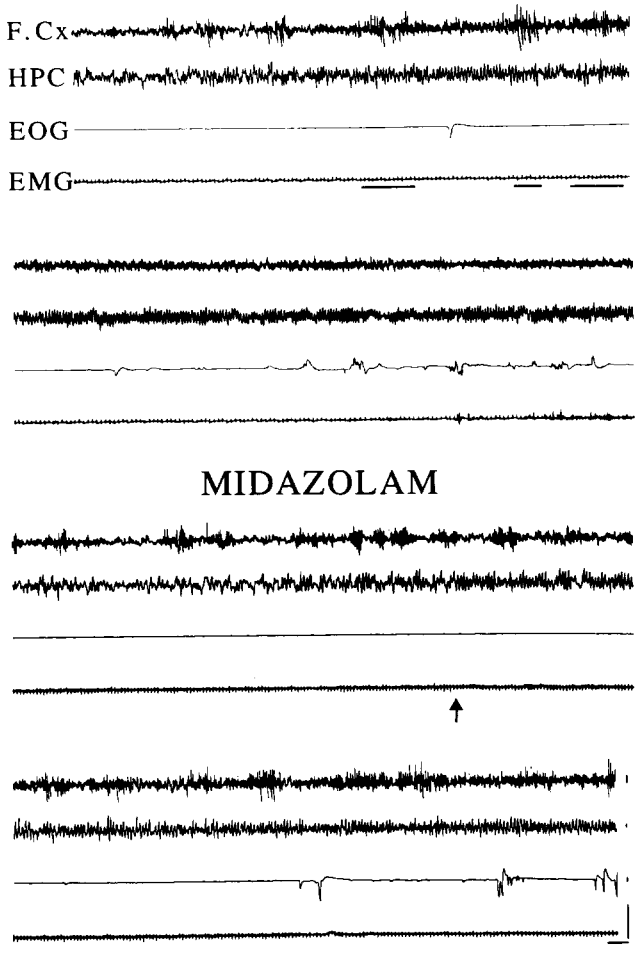

FIG. 1. The benzodiazepine midazolam extends the intermediate stage. Top: control recording. The animal (WAG/Rij rat) entered into paradoxical sleep after an episode of intermediate stage characterized by the unusual association of cortical spindles and hippocampal theta rhythm (underlined). The two tracings are a continuous recording. Bottom: 65 min after an IP injection of midazolam (3 mg/kg), the same rat showed a prolonged intermediate stage with: a) an absence of cortical desynchronization, b) more numerous cortical spindles of higher frequency and lower amplitude, c) a continuous hippocampal theta rhythm of lower frequency (from the arrow), d) an increase of heart rate, e) a presence of eye movement bursts as in paradoxical sleep. The two tracings are a continuous recording. Abbreviations: F.Cx: Frontal cortex (interhemispheric bipolar recording); HPC: dorsal hippocampus (CA1 area bipolar recording); EOG: electrooculogram; EMG: electromyogram from the dorsal neck muscles. Calibration: 1 s, 200 μV.

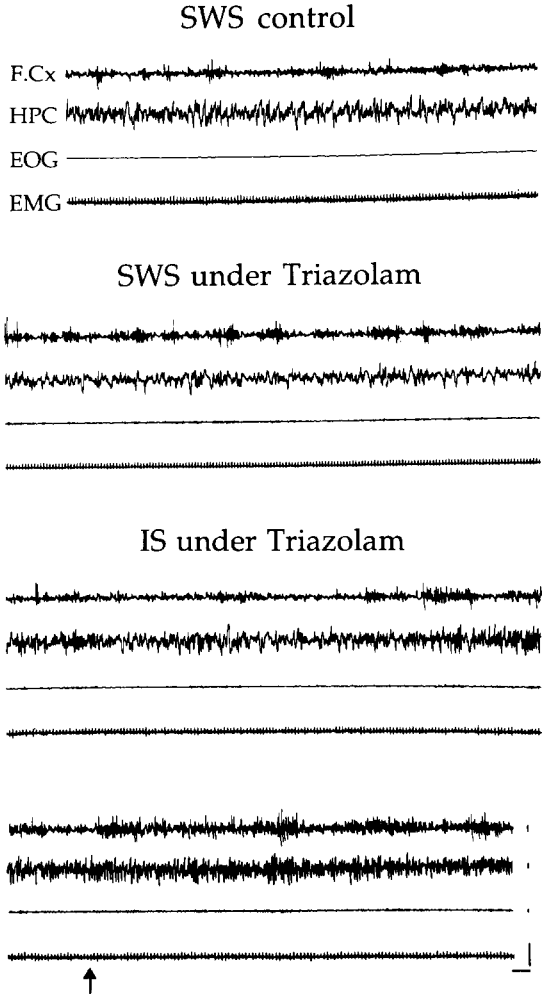

FIG. 2. Difference between triazolam-induced EEG spindling during slow wave sleep and extended intermediate stage. Top: control recording. The animal (Wistar rat) showed a slow-wave sleep (SWS) episode recorded 35 min after vehicle injection. This stage is classically characterized by cortical spindles and slow waves on frontal cortex and in dorsal hippocampus. Middle: slow-wave sleep (SWS) episode recorded in the same rat 20 min after an IP injection of triazolam (1 mg/kg). The spindling activity on the frontal cortex was increased. The spindles were of higher frequency than the control spindles. Bottom: extended intermediate stage (IS) recorded 110 min after the same IP injection of triazolam in the same rat. From a slow-wave sleep episode, the frontal cortex showed a long-lasting burst of spindles of higher amplitude and lower frequency (when compared to middle recording). These spindles were accompanied by theta activity in the dorsal hippocampus (from the arrow). The two tracings are a continuous recording. Abbreviations: see Fig. 1. Calibration: 1 s, 200 μV.

For the sake of clarity, all the specific effects of each BDZ on IS and PS characteristics in the two rat strains are not detailed in the text. Quantitative data are given in Table 1 for the occurrence latencies of each stage and Table 2 for the percentages of variation from vehicle. Table 3 shows the effect of BDZ on the eye movements of PS and Table 4, by giving the parallel evolution of IS and PS durations from vehicle, allows to know whether IS is extended or not at the expense of PS.

The results are summarized in terms of common effects of the three BDZ on the two rat strains and of differences between the rat strains and between the BDZ.

Common Effects of the Three Benzodiazepines in Both Rat Strains

The latency of occurrence of the first IS episode was reduced after injection of BDZ (Table 1). The increase in IS total amount during the 2 or 3 first hours was mainly due to an increase in the mean duration of IS episodes and in the number of fully developed cortical spindles. A secondary, less marked, late effect (during the fifth or sixth hour after injection) was generally observed (Table 2).

BDZ induced both quantitative and qualitative modifications of IS: during the first hours IS is increased but with cortical spindles of increased frequency and decreased amplitude (Table 2), whereas during the following hours these spindles of high and low amplitude are mixed up with midvoltage fast synchronized activity (not shown). This fact explains that the enhancement of the number of fully developed high-voltage cortical spindles seems undervalued when compared to the increase of IS duration and amount (Table 2).

The spindle characteristics were gradually modulated by BDZ: their intrinsic frequency was increased already after the lower dose, their amplitude was decreased only after the higher one (Table 2) and their mean duration was not modified whatever the dose was used (not shown). In the same way, BDZ did not affect the amplitude of hippocampal theta rhythm (not shown), but decreased its frequency during IS (except in Wistar rats) as during PS (Table 2).

In reverse, the latency of occurrence of the first PS episode was increased (except for midazolam: see Table 1) and the PS total amount showed during the 2 or 3 first hours a decrease that was mainly due to a decrease in the mean duration of PS episodes and generally related to a decrease in the facility to enter PS. This was followed by a late increase during the fifth and/or the sixth hour after injection. Finally, an increase in heart rate was observed during extended IS and/or recovering PS (Table 2).

Differences Between the Two Rat Strains

The increase in IS total amount, mean duration of IS episodes and number of cortical spindles was longer lasting in Wistar than in WAG/Rij rats (5 h vs. 2 or 3 h), in which the spindle amplitude was in contrast more affected by BDZ (1 or 2 h vs. 3 or 4 h). Two other differences were observed: a) the frequency of the hippocampal theta rhythm was decreased only in WAG/Rij rats (Table 2); b) the occurrence level of IS prior to PS was increased only in Wistar rats (Table 2).

In contrast to IS, the decrease in PS total amount was longer lasting in WAG/Rij than in Wistar rats (4 h vs. 2 h). The instability of PS episodes as PS recovered was decreased in Wistar, but increased in WAG/Rij rats (Table 2). The increase in heart rate lasted two times longer in WAG/Rij than in Wistar rats (except for triazolam). The decrease in the mean number of eye movements per PS episode was observed already at the low dose of diazepam and triazolam in WAG/Rij rats, but only at the high dose in Wistar rats (Table 3). Finally, the substitution of PS by the BDZ-extended IS was not always complete in WAG/Rij rats (Table 4).

Thus, the main interstrain difference revealed by this study with BDZ was found in the recovery of PS: although less sensitive to the effect of BDZ on IS, the WAG/Rij strain showed a longer duration for PS recovery with more residual IS during PS episodes, whereas Wistar rats showed a faster PS recovery with more stable episodes.

Differences Between the Three Benzodiazepines

Diazepam had effects on IS in both rat strains only after a high dose (3 mg/kg), whereas midazolam and triazolam had

TABLE 1

LATENCIES OF OCCURRENCE OF THE FIRST IS AND PS EPISODES

BDZ	Doses mg/kg IP	Wistar		WAG/Rij	
		IS	PS	IS	PS
Diazepam	0.0	56 ± 3	81 ± 3	57 ± 3	59 ± 4
	1.0	47 ± 4	102 ± 3*	39 ± 8*	63 ± 8
	3.0	25 ± 3*	62 ± 4*	36 ± 7*	105 ± 5†
Midazolam	0.0	69 ± 7	79 ± 5	80 ± 5	97 ± 6
	1.0	18 ± 4†	35 ± 7*	28 ± 4†	57 ± 6*
	3.0	34 ± 10*	82 ± 10	17 ± 3†	96 ± 8
Triazolam	0.0	51 ± 4	69 ± 6	62 ± 3	78 ± 3
	0.3	35 ± 7*	102 ± 10*	35 ± 2*	65 ± 9
	1.0	26 ± 7*	100 ± 4*	21 ± 3†	177 ± 12†

The three benzodiazepines decreased the occurrence latency of the first IS episode in the two rat strains. They are different effects on the occurrence latency of the first PS episode, i.e., as the recovery of cortical desynchronization started: it was increased after diazepam (except a decrease at the higher dose in Wistar rats) and triazolam, but it was decreased after midazolam (at lower dose only). (mean ± SEM in min).

*$p < 0.05$; †$p < 0.01$ (Student's t-test).

IS: intermediate stage of sleep; PS: paradoxical sleep.

TABLE 2

MAIN SIGNIFICANT EFFECTS GIVEN IN PERCENTAGES OF VARIATION FROM VEHICLE

	Diazepam				Midazolam				Triazolam			
	Wistar		WAG/Rij		Wistar		WAG/Rij		Wistar		WAG/Rij	
BDZ-Induced Effects	1.0	3.0	1.0	3.0	1.0	3.0	1.0	3.0	0.3	1.0	0.3	1.0
IS												
Initial (2 or 3 first h) increase in the total amount	NS	932	NS	242	144	922	191	1,622	301	1,238	230	351
Late (5th or 6th h) increase in the total amount	NS	277	NS	82	NS	186	NS	70	431	355	78	NS
Increase in the mean duration per episode	NS	1,292	NS	296	NS	1,348	NS	695	269	903	161	145
Increase in the number of cortical spindles	NS	263	NS	167	NS	202	52	139	NS	223	NS	172
Increase in the frequency of cortical spindles	24	45	NS	53	11	54	17	31	40	51	39	51
Decrease in the amplitude of cortical spindles	NS	51	NS	NS	NS	34	NS	46	NS	28	NS	61
Decrease in the frequency of hippocampal theta rhythm	NS	NS	NS	20	NS	NS	11	17	NS	NS	NS	19
Increase in the occurrence level of IS prior to PS	NS	9	NS	NS	24	24	NS	NS	21	23	NS	NS
PS												
Initial (2 or 3 first h) decrease in the total amount (*except increase with midazolam)	30	65	53	61	67*	50*	107*	53*	66	63	34	75
Late (5th or 6th h) increase in the total amount	NS	31	NS	24	119	83	53	39	133	NS	NS	NS
Decrease in the mean duration per episode	21	51	43	56	NS	36	NS	83	37	60	51	41
Decrease in the frequency of hippocampal theta rhythm	10	15	6	20	9	23	12	31	14	24	11	13
Decrease in the facility to enter PS	54	NS	70	100	NS	NS	NS	NS	51	100	40	100
Decrease (−) or increase (+) in the instability of recovering PS episodes	NS	−70	+116	+100	NS	−100	NS	NS	−60	−67	+296	NS
Increase in the heart rate	NS	18	NS	28	NS	17	6	7	13	26	19	22

Values are significant at least at $p > 0.05$ with Duncan's test. NS: non significant. The doses are in mg/kg (IP). IS: intermediate stage; PS: paradoxical sleep.

effects already at 1 and 0.3 mg/kg, respectively. Some specific effects of one of the three BDZ were observed: a) diazepam was without any effect on spindle amplitude in WAG/Rij rat (Table 2), whereas midazolam had the longest-lasting effect (3 h) on the decrease of theta rhythm frequency, followed by an increase during the fourth hour after injection; b) triazolam had the longest-lasting effect (4 and 3 h in Wistar and WAG/Rij rats, respectively) on the increase of spindle frequency and

TABLE 3

MEAN NUMBER OF EYE MOVEMENTS (SINGLE AND IN BURSTS) PER EPISODE OF PARADOXICAL SLEEP

		Wistar		WAG/Rij	
BDZ	Doses mg/kg IP	Single EM	EM bursts	Single EM	EM bursts
Diazepam	0.0	5.4 ± 1.9	5.6 ± 2.7	7.5 ± 3.7	6.0 ± 0.9
	1.0	4.5 ± 0.7	3.5 ± 2.1	3.0 ± 2.7	1.7 ± 2.1*
	3.0	0.8 ± 0.8*	1.2 ± 1.1*	4.1 ± 1.9	1.1 ± 0.6*
Midazolam	0.0	6.3 ± 1.3	6.5 ± 3.9	7.4 ± 1.3	7.7 ± 1.9
	1.0	7.0 ± 2.0	4.3 ± 1.5	7.7 ± 3.7	5.3 ± 1.9
	3.0	5.0 ± 2.0	7.0 ± 2.5	6.0 ± 0.9	6.5 ± 0.4
Triazolam	0.0	7.4 ± 1.5	6.4 ± 1.5	6.6 ± 0.4	5.6 ± 1.3
	0.3	6.3 ± 2.5	4.3 ± 1.5	3.3 ± 1.8*	2.5 ± 1.9*
	1.0	3.7 ± 1.2*	1.7 ± 1.1*	2.7 ± 1.1*	3.3 ± 0.4*

Diazepam and triazolam at higher dose decreased both single eye movements (EM) and EM bursts in Wistar rats. The WAG/Rij strain was more sensitive because this decrease was observed already at lower dose, excepted after diazepam for single EM, which must be, thus, dissociated from EM bursts. Midazolam was without any effect whatever the dose and the rat strain had been used (mean ± SEM, average during the first 3 h of recordings).

*$P < 0.05$ (Student's t-test).

TABLE 4

PERCENTAGES OF VARIATION OF IS AND PS DURATION FROM VEHICLE DURING THE FIRST 3 H OF RECORDING AFTER BENZODIAZEPINE ADMINISTRATION IN THE TWO RAT STRAINS

BDZ	Doses mg/kg IP	Wistar		WAG/Rij	
		IS	PS	IS	PS
Diazepam	0.0	6	94	12	88
	1.0	7	97	16	58
	3.0	64	44	56	46
Midazolam	0.0	5	95	12	88
	1.0	11	183	22	131
	3.0	47	110	67	49
Triazolam	0.0	4	96	13	87
	0.3	14	76	25	69
	1.0	38	56	40	14

The summation IS duration + PS duration after vehicle injection represents the value of 100%. By comparing the percentages of duration for each stage, it is obvious that diazepam and triazolam extended IS at the expense of PS in Wistar rats, this substitution being not always complete (i.e., the total duration IS + PS decreased from vehicle) in WAG/Rij rats. In contrast, the extension of IS promoted by midazolam was only partially at the expense of PS because this last stage also increased in duration.

IS: Intermediate stage of sleep; PS: paradoxical sleep.

induced at the low dose (0.3 mg/kg) a late secondary effect (during the fifth or sixth hour) on the IS amount (+78%) and episode duration (+103%) in WAG/Rij rats.

When PS recovered, it showed a late increase (during the fifth and/or sixth hour), which was dose dependent as follows: triazolam < midazolam < diazepam (Table 2). Only diazepam at the low dose (1 mg/kg) induced a late increase (during the sixth hour) of the theta rhythm frequency (12% and 10% in Wistar and WAG/Rij rats, respectively). The main difference concerns midazolam: alone among the three BDZ, it promoted extended IS (Fig. 1) but only partially at the expense of PS since this last stage was also increased in duration (Table 4). This fact explains the decrease of PS occurrence latency (Table 1) and the increase of PS amount (Table 2) after midazolam injection (at least at the dose of 1 mg/kg) as well as the absence of effect (at both doses) on the eye movements of PS (Fig. 1, Table 3).

DISCUSSION

BDZ induce IS patterns at the expense of PS in both rat strains. Regarding the physiological significance of IS (13) already mentioned in the introduction, it can be proposed that they promote a pharmacological cerveau isolé-like stage at the entrance of PS. To be strictly accurate, BDZ act on the mechanism responsible for the maintenance rather than for the onset of IS, because the increase in IS amount was mainly due to an increase in the mean duration of IS episodes and not of the number of episodes. In contrast, BDZ could act both on the onset and maintenance of PS, because they decreased both the facility to enter PS and the mean duration of PS episodes. Moreover, because BDZ decreased in frequency the hippocampal theta rhythm during IS and PS, they could interfere with the septal theta pacemaker (25,31). BDZ are able to increase an already relatively long-lasting IS in the WAG/Rij strain; however, less than in the Wistar strain but with a higher modulation of qualitative characteristics such as of spindles because their amplitude was longer-lasting decreased, whereas IS amount and duration were shorter-lasting increased.

Other differences have been shown between the two rat strains. They can be explained by a previous interstrain study (8): a) if BDZ decreased in frequency the hippocampal theta rhythm during IS only in WAG/Rij rats, it was probably because the control frequency was higher than in Wistar rats; b) if the occurrence level of IS prior to PS was increased only in Wistar rats, it was probably because the WAG/Rij strain showed PS quasi systematically preceeded by IS; c) if BDZ-extended IS was less important in WAG/Rij rats and less completely substituted for PS than in Wistar rats, whereas the first strain showed a longer duration for PS recovery with less stable episodes, it was probably because the WAG/Rij strain showed in control recordings a higher amount of IS and a lower amount of PS than Wistar rats. This last interstrain difference should be related to the GABA system because the BDZ binding site is a part of the GABAergic receptor (29) and the WAG/Rij strain is a genetically epileptic strain (37) with GABA disturbances (33). Lastly, the main difference between the three BDZ concerns midazolam, which is different from the others by its quantitative and qualitative modulations of PS: increase of PS amount and duration, decrease of its occurrence latency, absence of effects on the eye movements of PS. Thus, the midazolam-extended IS is not substituted for PS. This difference could be related to the BDZ receptor multiplicity (35) in regard to the heterogeneity of the structure of the $GABA_A$ receptor complex (9).

Thus, BDZ promote IS at the expense of PS as it was already the case with barbiturates (11). Nevertheless, if medium doses of pentobarbital (15–25 mg/kg) decreased also the frequency of hippocampal theta rhythm of extended IS, they were in contrast without any significant effects on the amplitude, frequency, and duration of frontal cortical spindles of extended IS (13), whereas BDZ changed the characteristics of these spindles.

Finally, it is worthwhile to mention that a stage in some way similar to IS has been found in humans. It also preceeds and follows PS and is characterized by unusual polygraphic pattern associations (for example K complexes and spindles alternating with PS patterns but without eye movement). This stage was called intermediate phase (23) and increases at the expense of PS during given mental illness syndromes: oligophrenia (17), schizophrenia (21), and acute paranoid disorder (24). Arousal during this stage is difficult and reveals a "feeling of indefinable discomfort, anxious perplexity and harrowing worry" (24) that could be related to a transient lowering of brain stem ascending influences inducing unusual and uncontrolled higher nervous processes (12). It would be interesting to study the effects of BDZ on this transitional phase in humans. Moreover, the accurate effects of BDZ on memory is still discussed [for review, see (20)]. Although the sometimes observed improvement in mnemonic performances could be mediated by an endogenous ligand of BDZ receptor (18), the BDZ-induced amnesia could result from the BDZ-induced PS pharmacological deprivation (39). A last argument on behalf of this hypothesis: zolpidem, an hypnotic agent of the new class of imidazopyridines thought to act through a novel BDZ receptor (39), does not extend IS at the expense of PS (15) and does not impair memory (19).

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