Phasic hippocampal activity during paradoxical sleep in the rat: selective suppression after diazepam administration

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Summary. The effects of diazepam on tonic (Tθ) and phasic (Pθ) components of the paradoxical-sleep hippocampal theta rhythm were studied in the rat. Results show that diazepam, a benzodiazepine analogue known to interfere with the putative neurotransmitter γ-aminobutyric acid (GABA) in the mammalian central nervous system, selectively abolishes Pθ. They confirm previous data according to which sedative/anaesthetic drugs cause an apparent dissociation of Tθ and Pθ of the paradoxical-sleep hippocampal theta rhythm. Moreover, they suggest a possible involvement of GABA in the neurochemical mechanisms underlying Pθ in the rat.

During paradoxical sleep (PS), electrical activity of the hippocampus of the rat is characterized by a well-known, rhythmical, slow activity (4–11 Hz) called the theta rhythm (θ). According to pharmacological data, this rhythm is subdivided into tonic (Tθ) and phasic (Pθ) components[1-3]. Tθ are characterized by low frequencies (4–7 Hz) and a low amplitude, and are associated with behavioral immobility. Since Tθ are abolished by atropine sulfate[2,4], their neurochemical basis could be cholinergic in nature. Pθ are characterized by a large amplitude and high frequencies (8–11 Hz) which generally occur in concomitance with bursts of rapid eye movements and twitches of somatic muscles. In contrast to Tθ, Pθ are not abolished by atropine sulfate[2,4,5]. But they are suppressed by tetrahydrocannabinols[1], (D-phe[7]) ACTH 4–10[6], and especially by barbiturates[2,7,8]. Because the central action of these pharmacological agents is not understood, the neurochemical basis of Pθ is still unknown and no hypothesis has been proposed.

The data that are reported in this paper show that diazepam, a benzodiazepine analogue which is commonly used as a sedative and anxiolytic agent, can selectively abolish Pθ in the rat in the same manner as barbiturates.

Since diazepam probably interferes with the putative neurotransmitter γ-aminobutyrate (GABA) in the mammalian central nervous system[9-12], the hypothesis that Pθ could be related to GABA mediation in this animal should be considered.

Methods. 9 male Sprague-Dawley rats (300 g) were used. Silver electrodes for chronic EEG and EMG recordings were implanted bilaterally over the frontal neocortex (2 mm anterior and 2 mm lateral to bregma) and in nuchal musculature. Under electrophysiological control, 2 stainless steel wires were implanted into the right dorsal hippocampus of the rat anaesthetized with ether. One wire aimed at the CA_1 area, the other at the dentate gyrus. Consequently, a clear theta rhythm (> 500 µV) could be recorded in bipolar derivation in all of the 9 rats during experimentation.

1 week after electrode implantation, a control recording was made after administration of isotonic saline. The next day another recording was made following i.p. administration of diazepam (4.5–7.5 mg/kg). Recordings lasted 3 h and were analyzed on the basis of these experimental conditions. Qualitative analysis concerned the first PS episodes. Quantitative analysis concerned the first 100 sec of PS. The number of theta waves was counted sec by sec. Thus, it was possible to calculate a) the average frequency of θ on 1-sec samples and b) the average percent of Tθ (4–7 Hz) and Pθ (8–11 Hz) of hippocampal θ. Data collected

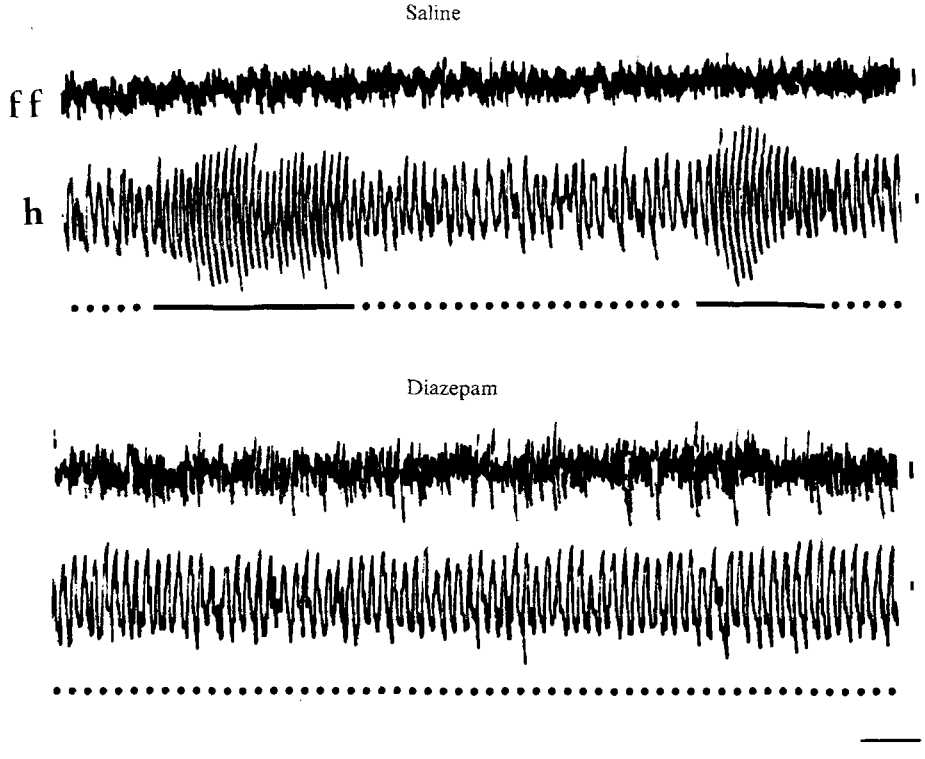

Fig. 1. Hippocampal (h) and frontal neocortical (ff) electrical activity during paradoxical sleep in rat Dr4 under control (Saline) and drugged (Diazepam) conditions. Tonic (Tθ) and phasic (Pθ) components of hippocampal theta rhythm are indicated under the hippocampal record by dotted and solid lines respectively. Diazepam selectively abolishes Pθ and causes a clear alteration of frontal neocortical activation characteristic of PS in undrugged rats. Calibration: 1 sec and 100 µV.

for each of these parameters were submitted to the Student t-test.

Results. Following diazepam administration, drowsiness and slow-wave sleep appeared. After a delay comparable to that observed in undrugged rats ($t=0.31$, $p>0.1$, $df=8$), drugged rats appeared to enter PS as was evidenced by the appearance of θ in the hippocampus and by the coincident abolition of EMG activity. Clear changes were observed in hippocampal and frontal neocortical activity (figure 1).

1. Hippocampal activity. Pθ, which normally occur in control rats, were absent in diazepam-treated rats. Hippocampal EEG were characterized by slow and regular theta waves (Tθ), present during the entire PS phase. Quantitative analysis confirmed these data (figure 2) and revealed a drastic reduction of Pθ from 25.6% before treatment to 0% after treatment. Conversely, there was a significant increase in the average percent of Tθ ($t=5.9$, $p<0.001$, $df=8$). As a result, the average θ frequency was significantly lower ($\bar{X}=5.6$ Hz, range 5.4–5.8 Hz, $df=8$) than that observed in control rats during PS ($\bar{X}=6.9$ Hz, range 6.4–7.4 Hz, $df=8$) ($t=6.48$, $p<0.001$, $df=8$).

2. Frontal neocortical activity. Frontal neocortical activation characteristic of PS in undrugged rats was affected by administration of diazepam. Following drug administration, the frontal neocortex showed continuous large-amplitude wave activity associated with fast activity during the entire PS phase. No systematic quantitative analysis of frontal neocortical activity obtained before and after drug administration was performed. Nevertheless, preliminary data indicated that after diazepam administration the frequency was lowered by 42.5% and the amplitude was increased by 300% when compared to the frequency and amplitude mesured in rats before drug administration.

Discussion. These data show that diazepam selectively abolishes Pθ as barbiturates do in the rat[2,8]. They confirm results reported by other authors according to which sedative/anaesthetic drugs cause an apparent dissociation of tonic and phasic components of PS hippocampal θ[1,2,8]. Moreover, they suggest a possible neurochemical basis for Pθ. Indeed, in the recent past, attention has been focused on the effects of the benzodiazepines on pre-and postsynaptic responses to putative neurotransmitter aminoacids, especially to GABA[9,10]. A number of investigations have suggested that the benzodiazepines exert a primary action on GABA-containing neurons, some of which in turn may regulate transmission at monoaminergic synapses[10]. In particular, diazepam is able to potentiate the hippocampal recurrent inhibition which is mediated by the GABA-ergic basket cells[13]. On the basis of these later data and after having taken into account the Pθ-suppressing effect of diazepam, it seems reasonable to postulate that Pθ could be dependent on GABA-ergic mechanisms. The demonstration that barbiturates having EEG effects similar to those of diazepam were also able to potentiate GABA-ergic recurrent inhibition in the rat[13] and the cat[14,15] hippocampus supports this hypothesis. As a preliminary test, GABA antagonists, such as picrotoxin or bicuculline could be injected in diazepam-treated rats in an attempt to reverse the Pθ-suppressing effect of diazepam. The ability of bicuculline to antagonize the diazepam-prolonged GABA-ergic recurrent inhibition in hippocampal neurons of the cat[15] is stimulating in this respect. A similar study is now in progress in our laboratory.

Preliminary data relative to cortical EEG seem to indicate that diazepam is able to affect frontal neocortical activation during PS in the rat in the same way as some barbiturates[7,8,16]. It is difficult to give a neurochemical interpretation of this finding on the basis of these fragmentary data alone. But if they could be confirmed and enlarged, then these data could suggest a new perspective for the possible neurochemical mechanisms involved in frontal neocortical activation, such as, for example, the GABA-system. The indication that the density and the distribution of the benzodiazepine receptors in the neocortex are very similar to those of the GABA-receptors[17,18] supports this hypothesis. Another positive argument is the fact that diazepam has been shown to enhance GABA-mediated inhibition of facilitation of intracortical response in the cat[19].

In conclusion, present neuropharmacological data support the hypothesis that GABA could be an intermediate involved in the control of Pθ and possibly of frontal neocortical activation mechanisms during PS in the rat.

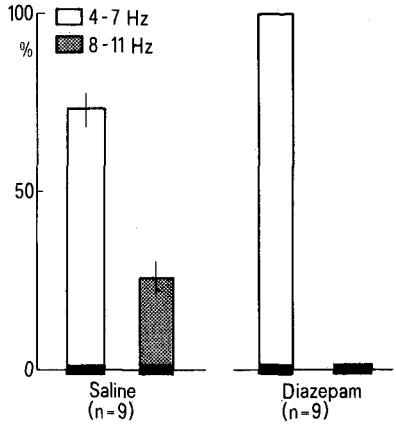

Fig. 2. Percent of tonic (4–7 Hz) and phasic (8–11 Hz) components of hippocampal theta rhythm (θ) during paradoxical sleep in control (Saline) and drugged (Diazepam) rats. There is a drastic reduction in the occurrence of phasic components of θ in drugged rats.

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