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BRIEF COMMUNICATION

Increase of Paradoxical Sleep (PS) by Intraperitoneal Injection of Brain Extract From PS-Deprived Rats

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WETZEL, W , H STARK AND H MATTHIES *Increase of paradoxical sleep (PS) by intraperitoneal injection of brain extract from PS-deprived rats* PHARMACOL BIOCHEM BEHAV **27**(3) 537–539, 1987 —Intraperitoneal injection of a brain extract obtained from paradoxical sleep-(PS) deprived donor rats resulted in a small but significant increase of PS in normal recipient rats Brain extract of non-deprived control rats was without effect The results provide further evidence for the existence of a PS-inducing factor accumulating in the brain during PS deprivation

Paradoxical sleep Paradoxical sleep deprivation Paradoxical sleep inducing factor Brain extract Rats Intraperitoneal injection

AN increasing number of studies based on the classical concept of humoral regulation of sleep [8] have suggested that endogenous sleep-promoting substances may exist in blood [10], urine [7], cerebrospinal fluid (CSF) [13], and brain tissue [2, 11, 14] The main effect of these sleep factors, injected intracerebrally or systemically, is their influence on slow-wave sleep (SWS), with little or no effects on paradoxical sleep (PS) (for review, see [3, 5, 18]) Recently, however, it was shown that intraventricular infusion of CSF from PS-deprived donors can induce PS in PCPA pretreated cats [16,17] and in propranolol or α-methyldopa pretreated rats [1,4] and it was concluded that some PS-inducing factor accumulates in the CSF during PS deprivation

The purpose of the present study was to test whether it is possible to find effects on PS of a brain extract from PS deprived rats injected intraperitoneally in normal recipient rats In our first experiments we used the whole brain of donors and a simple extraction procedure without further purification steps Nevertheless, significant effects of the crude brain extract on PS in recipients were found

METHOD

Adult male Wistar rats weighing 220–320 g (donors) and 260–360 g (recipients), respectively, were used Recipient rats were implanted with cortical EEG electrodes and neck muscle EMG electrodes for sleep recording After recovery from surgery, the rats were habituated to the recording conditions for at least one week A 12 hr light-dark cycle was maintained, with lights on from 6 a m to 6 p m The room temperature was 23±1°C Food and water were available ad lib

Donor rats were PS deprived by the water tank method [9] The rats were placed on circular platforms with 7 cm diameter surrounded by water reaching to a level of 0 5–1 0 cm below the platform The water was changed daily in the morning Food was available ad lib The PS deprivation (PSD) was started at 7 30 a m and continued for 100 hours In previous experiments, using the same PS deprivation procedure, we found that PS was reduced by 95%, whereas SWS was reduced by 17% A second group of donor rats, kept in their home cages, was used (control rats, not PS-deprived) Donor rats were decapitated at 10 30 a m , the whole brains were removed and homogenized with two volumes of distilled water The homogenate was centrifuged at 40,000× g for 3 hours The supernatant was stored at −20°C and was used later for intraperitoneal administration in recipient rats

After two or more days of baseline recording following intraperitoneal injection of NaCl (1 0 ml/100 g body weight), recipients received 1 ml of the brain extract per 100 g body weight intraperitoneally immediately before the onset of the

TABLE 1

EFFECT OF BRAIN EXTRACT (BE) OF PS-DEPRIVED DONOR RATS (PSD) AND NON-DEPRIVED CONTROL RATS (CON) ON SLEEP IN RECIPIENT RATS DURING THE FIRST 4 HOURS OF RECORDING (08 00–12 00)

	Con (n=10)			PSD (n=10)		
	NaCl	BE	% Change	NaCl	BE	% Change
PS	23 5 ± 2 4	23 0 ± 3 1	−2 1%	25 2 ± 2 4	29 3 ± 2 6†	+16 3%*
SWS	175 2 ± 4 6	181 0 ± 5 3	+3 3%	171 1 ± 4 1	180 0 ± 3 8	+5 2%
W	41 3 ± 5 5	36 0 ± 5 3	−12 8%	43 7 ± 4 3	30 7 ± 3 4	−29 7%*

Results are means ± SEM (min) *$p<0$ 05 (BE-PSD vs NaCl), †$p<0$ 05 (BE-PSD vs BE-Con)

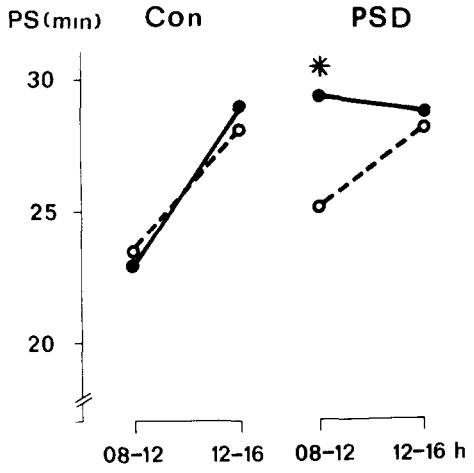

FIG 1 Effect of brain extract of PS-deprived donor rats (PSD) and non-deprived control rats (Con) on the amount of PS in recipient rats (PSD, n=10, Con, n=10) during the first and second 4 hr period following injection (○ NaCl baseline, ● brain extract, *$p<0$ 05)

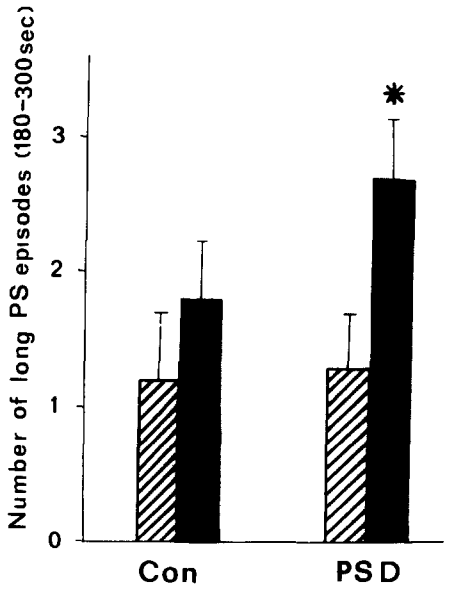

FIG 2 Number of long PS episodes (180–300 sec duration) during the first 4 hours following injection of brain extract (black columns) obtained from PS-deprived donor rats (PSD) and non-deprived control rats (Con) compared to NaCl injection (hatched columns) Values are means+SEM, n=10, *$p<0$ 01

8 hr recording period (8 a m –4 p m) Ten rats (PSD group) were treated with brain extract of PS-deprived donors, whereas another group of 10 rats (control group) received the brain extract of normal, non-deprived rats

Sleep records were evaluated visually according to standard criteria Each minute of the record was determined to be either wakefulness (W), slow-wave sleep (SWS), or paradoxical sleep (PS) Latencies, %-values, number and duration of sleep episodes were calculated and used for statistical evaluation by means of the Wilcoxon matched pairs signed rank test (brain extract vs NaCl), and Chi-square test (PSD brain extract vs control brain extract), respectively

RESULTS

Comparing the sleep parameters following administration of brain extract from PS-deprived rats (BE-PSD) and brain extract from non-deprived rats (BE-Con) with those following NaCl injection on the baseline day before revealed the following results The PS latency (time between the onset of recording and the first PS episode) was decreased from 60 8±10 3 min (NaCl) to 38 0±4 4 min (BE-PSD) in the PSD group (mean±SEM, $p<0$ 05) The corresponding values in the control group were 46 9±6 2 min (NaCl) and 62 1±21 4 min (BE-Con) (n s) Neither BE-PSD nor BE-Con did influence the SWS latency During the total recording time of 8 hours, the percentage of PS was changing from 11 4±1 0 to 12 4±1 1 in the PSD group ($p<0$ 05) and from 11 0±0 8 to 11 2±1 1 in the control group (n s) There were no significant effects on the 8 hr amounts of SWS and W

As shown by Fig 1 and Table 1, the main effect of BE-PSD was observed during the first 4 hours of recording PS was increased, W was decreased, and SWS was not significantly influenced During the first 2 hours of recording, the percentage of PS was changed from 7 4±1 3 (baseline) to 10 1±1 4 (BE-PSD) (+36 5% increase, $p<0$ 01) Although there were tendencies for the number and mean duration of PS episodes to increase after BE-PSD, these changes were not significant More PS episodes of 180–300 sec duration, however, were observed after BE-PSD treatment during the first 4 hours of recording (Fig 2) Similarly, during the second 4 hours of recording, the number of such long PS episodes increased from 1 9±0 5 (NaCl) to 3 0±0 5 (BE-PSD) ($p<0$ 05)

DISCUSSION

Various body fluids (CSF, blood, urine) but also brain tissues were used for investigation of endogenous sleep factors Thus, sleep-promoting material was extracted from the brain stem of rats [11] and from the brain stem and cortex of goats and sheep [14] Furthermore, it was shown that sleep factors or putative sleep factor containing extracts, usually injected intracerebrally, did exert their effects after intravenous or intraperitoneal administration [10–12, 15] In the present experiments we found that a crude brain extract, obtained from selectively PS-deprived rats and injected intraperitoneally, increases PS in recipient rats The effect was rather small than pronounced It should be noted, however, that normal (not insomniac) recipients, showing a high baseline level of PS during the time of recording (8 a m to 4 p m), were used Nevertheless, significant effects on different parameters of PS were found Although these effects (PS latency, PS percentage) were observed during the first hours after injection, certain effects seem to persist longer (occurrence of long PS episodes) On the other hand, the BE-PSD did not significantly affect SWS, although there was a small tendency towards an increase, perhaps as a consequence of a small SWS deficit resulting from the PS deprivation procedure Neither PS nor SWS were influenced by the brain extract obtained from non-deprived control rats (BE-Con)

Thus, our results support the idea that a paradoxical sleep inducing factor accumulates in the brain during PS deprivation [6] According to this hypothesis it was shown that transfer of small quantities of CSF from PS-deprived animals can influence PS in recipients pretreated with PCPA, propranolol, or α-methyldopa [1, 4, 16, 17] In our experiments, a brain extract volume obtained from about one donor was transferred to one recipient Thus, possibly by the larger quantity of transferred material, a PS influencing effect in normal, i e , not pretreated animals was found If the effect of a brain extract from PS-deprived animals can be confirmed by further behavioral investigations testing different doses of BE, different times of injection and recording, and more adequate donor controls, larger quantities of material will be available and this would stimulate further investigations on biochemical characterization of the postulated PS-inducing factor

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