PROSTAGLANDINS AND SLEEP. AWAKING EFFECT OF PROSTAGLANDINS AND SLEEP PATTERN OF ESSENTIAL FATTY ACIDS DEFICIENT (EFAD) RATS.

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ABSTRACT

The experiments were carried out to investigate the effects of prostaglandins (PGs) on the sleep pattern in the cat, and in normal and EFAD rats.

The data indicate that the duration of slow wave sleep (SWS) was significantly longer in EFAD rats compared with the normal rats. However, no difference in the REM sleep was observed between the two groups. Intraventricular (i.vc.) administration of PGE_1, PGE_2 and $PGF_{2\alpha}$ increased wakefulness without a significant alteration of REM sleep.

PGE_1 administered i.vc. did not alter the duration of SWS or REM sleep in the chronic cat, but induced ponto-geniculo-occipital (PGO) waves (spikes) which are the phasic phenomenon of REM sleep.

The fact that previous administration of 5-hydroxytryptophane abolished the PGE_1-induced PGO spiking, might indicate that this drug triggered the spikes mainly via the functional inhibition of the serotonergic system.

INTRODUCTION

Prostaglandins (PGs) are found diffusely throughout the central nervous system[1] and as long ago as 1964 it was shown that intraventricular (i.vc.) administration of PGs of the E type in the chicken and cats induces sedation, stupor and catatonia[2]. Similarly, a single subcutaneous injection of PGE_2 in rat rapidly exerted a sedative effect which was postulated to result from a reduction in cerebral blood flow[3]. The later experiments with PGE_1 administered intraventricularly caused drowsiness and facilitated sleep behaviour in monkeys and fowls[4,5], while in the rat REM sleep decreased, wakefulness increased but slow wave sleep (SWS) was not significantly altered[6]. These conflicting results are further complicated by the suggestion that PGE_1 given intraperitoneally induces a state of sedation, accompanied by the low voltage-high frequency EEG pattern resembling paradoxical sleep[7].

The most probable reason for these discrepancies of the effects of PGs on the sleep pattern are differences in the experimental animals, doses used and the routes of administrations of drugs.

Present experiments were undertaken to investigate the effect of PGs in the sleep pattern in the cat, normal rat and essential fatty acid deficient (EFAD) rats. The rat was selected as an experimental animal because it has been suggested that the rat sleep studies closely reflect results from human sleep studies[8]. However, in addition to the rats we also used the cats for the study of some phasic components of REM sleep such as PGO spikes (waves) which could not normally be

observed in the rat[9].

METHODS AND MATERIALS

Rats. Sleep cycles in rats were recorded by means of 5 chronically implanted epidural silver screw electrodes. Four of them were used for bilateral fronto-parietal derivation of the electrocorticogram (ECoG), and one as a reference. Two platinum ring electrodes in the neck muscle were used to derive the electromyogram (EMG).

The experiments were performed in a sound proof cabin, into which 4 animals were placed in separate cages. The rats remained in this cage for at least one night prior to the experiment, so they could adapt to the environment. On the day of the experiment, water and food were removed from the cages and the contacts in the headplugs of the rat were connected to a Grass EEG amplifier/recorder.

The states of vigilance were scored every 30 sec. for the first two hours before and four hours after i.vc. injection of drugs. Scoring was based on the registration of ECoG and EMG according to the criteria of Timo-laria et al[10].

The male EFAD rats of albino Wistar strain were taken from the mothers which were put on EFAD diet five days before the expected day of delivery. The fatty acid composition of the EFAD diet was described previously[11]. The control animals were of the same strain with a diet containing 3.5% of its calories as linoleic acid. In the EFAD group only those rats were selected showing deficiency symptoms such as reduction in growth, hair loss etc. The animals were 5-6 months old.

Implantation of an indwelling cannula for making injections into the lateral ventricle of the rat brain was similar to the method previously described[12]. To determine the accuracy of cannular placement, methylene blue was injected. The animals were sacrificed immediately after injection and the brain carefully dissected.

Cats. The acute experiments were performed in adult cats of either sex, weighing 3 to 3.5 kg. Under ether anaesthesia the trachea and a femoral vein were cannulated to enable artificial respiration and intravenous (i.v.) drug injections. The animals were immobilized by i.v. administration of 0,5 mg/kg pancuronium. Ether anesthesia was discontinued after fixation of the head of cat to the ear bars of a Kopf-stereotaxic instrument. Throughout the experiments the wound margins were infiltrated with xylocaine. The cannula used for i.vc. perfusion or administration of drug was inserted at point 2 mm posterior to the coronal suture and 0,5 mm lateral to the midline and lowered vertically 14 mm from the brain surface[13]. Tripolar electrodes were placed using the stereotaxic atlas of Snider and Niemer[14] in the lateral geniculate nucleus (A 6,0; L 10,5; H 2,5) and dorsal hypocampus (A 4,0; L 5,0; H 10,0). All electrodes had an interelectrode distance of 1 mm. Other monopolar electrodes were placed in the sensory motor cortex and visual cortex (P 5,0; L 2,0). In some experiments a perfusion of ventricles was performed to the can-

nulated aqueduct. The rate of infusion was 0,1 ml/min. In the acute cat experiments body temperature was recorded using a thermistor probe inserted rectally. Body temperature was recorded before and after injection of drug(s) at 15 min intervals.

All chronic experiments were carried out in cats of either sex weighing 2-3,5 kg. The cat under nembutal anaesthesia (40 mg/kg) was placed in the stereotaxic instrument and its skull was exposed. A small hole was drilled in the skull and the tip of the needle guide (22 gauge) was directed stereotaxically to the right lateral geniculate, while a tripolar electrode was placed in the left lateral geniculate. Other monopolar electrodes were placed in the frontal and occipital cortex. All cats had the electrodes implanted in the neck muscle for EMG recording, and the usual aseptic surgical techniques were followed.

Drugs. Prostaglandins E_1, E_2 and $F_{2\alpha}$ were dissolved in 0,2 mg/ml sodium bicarbonate to avoid using ethanol, which might influence neuronal activity[4]. Further dissolving was done with the arteficial cerebrospinal fluid (ACF). Neutral solution warmed to 37°C was injected slowly under aseptic conditions in a volume of 0,1-0,2 ml and washed in with 0,1 ml ACF. The PGs were administered into the right lateral geniculate region in a volume of 20 µl by use of a Hamilton microsyringe. The control animals received the same volume of solvent by the same route. The following drugs were also given: atropine sulphate (Merck), 5-hydroxytryptophane ethylester hydrochloride (Sigma), pancuronium bromide (Organon), and pentobarbital sodium (Abbott). The doses quoted in the text refer to the salts.

RESULTS

Sleep pattern of the EFAD rats. The sleep/waking cycle was followed in 8 normal and 6 EFAD rats in the period between 10 a.m. to 5 p.m. The data indicate that EFAD rats had a significantly longer duration of SWS than the normal rats. However, no significant differences in the REM sleep were observed (Table 1).

Table 1. Duration of sleep/waking activity in 6 EFAD rats and 8 normal rats. Results are tabulated as percentage of the recorded time (7 hrs).

Sleep/waking activity	Normal rats	EFAD rats
Alerted	43,1 ± 7,3	19,6 ± 9,2*
SWS	47,8 ± 5,8	70,2 ± 8,4*
REM	9,1 ± 1,6	10,2 ± 2,4

* $P<0.01$

Intraventricular administration of PGs in the EFAD rats. Administration of PGE_1 (50-100 µg/kg) in the same groups of 8 normal and 6 EFAD rats induced a significant decrease of SWS (Table 2). Administration of $PGF_{2\alpha}$ (0.1-1 mg/kg) also increased the wakefulness in both groups of animals but this was statistical significance only in the EFAD group of rats. Compared with the REM sleep no significant alterations were observed.
Behaviourally, after administration of PGs the rats of both groups were sedated, with closed eyes and decreased exploratory and locomotor activity.

Table 2. Duration of sleep/waking activity after intraventricular administration of PGE_1 (100 µg/kg) in 6 EFAD rats and 8 normal rats. Results are tabulated in percentage of the recorded time (7 hrs).

Sleep/waking activity	Normal rats	EFAD rats
Alerted	61,3 ± 5,4*	49,6 ± 6,1*
SWS	31,1 ± 6,2*	42,0 ± 5,4*
REM	7,6 ± 4,2	9,4 ± 2,3

* $P<0.05$

Effect of PGE_1 in the cat. Intraventricular administration of PGE_1 (16-64 µg/kg) in the 8 chronic cat did not alter duration of SWS or REM sleep in the time interval of 6 hours. However, i.vc. administration of PGE_1 induced a PGO spiking from the lateral geniculate region and occipital cortex. The episodes of the spiking appeared 10-20 min after an administration of drug and lasted 30 min to several hours. During this period of prostaglandin-induced PGO waves the animal was behaviourally and electro-encephalographically either awake or in SWS. However, the frequency of PGO spikes in these two stages of vigilance was significantly less prominent than in the REM sleep (Fig. 1). Atropine (0,1-1 mg/kg i.v.) pretreatment 15 min before PGE_1 did not abolish a drug induced PGO spiking. However, the same pretreatment with 5-hydroxytryptophane (0,5-5 mg/kg i.v.) abolished the PGO spiking induced by PGE_1.
PGE_1 (5-20 µg/kg) induced an unilateral PGO spiking within 15 min after administration into the geniculate region.

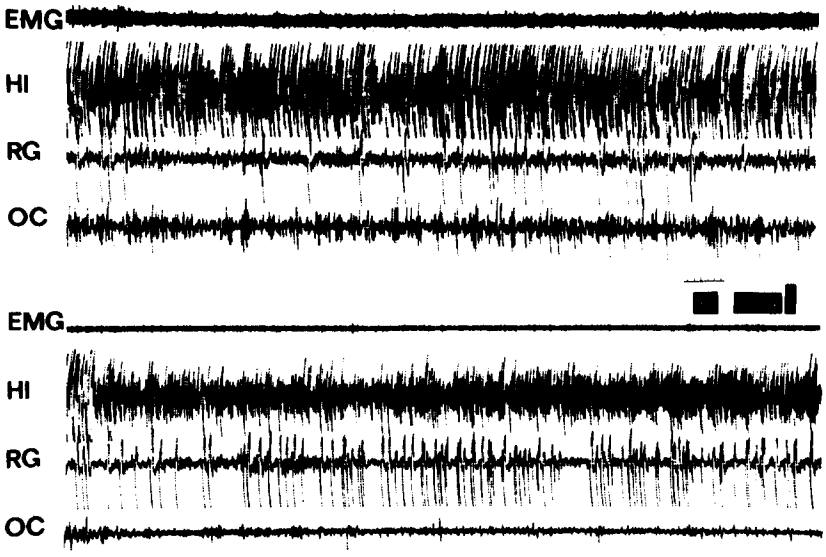

Fig. 1. The PGO waves in the right geniculate (RG) of an alerted cat 10 min after i.vc. administration of PGE_1 (32 µg/kg, Fig. above) and the PGO waves in the same cat in REM sleep (Fig. down). EMG = electromyogram; HI = Hippocampus; OC = occipital cortex.

Similarly an i.vc. administration of PGE_1 (16-60 µg/kg) in the acute cat also induced an increased PGO spiking lasting from 10 min to 1 hr. In this preparation an increased temperature (mean 1,8°C) was observed after administration of PGE_1.

DISCUSSION

It is relevant to point out that EFA deficiency in the rat is associated with the longer duration of the SWS. The exact cause of this is unknown but probably could be attributed to the considerable changes in the polyunsaturated fatty acid distribution, phospholipid content and other disturbances in the rat brain induced by an EFAD diet[15].

Another interesting point is that the duration of SWS in the EFAD rats can be decreased by i.vc. administration of PGs. The desynchronizing effect of PGE_1, PGE_2 and $PGF_{2\alpha}$ indicate that these drugs besides their behavioural sedative effect are primarily electroencephalographically alerting substances in the rat. This might eventually explain

a desynchronization of EEG observed by Haubrich et al.[7] after i.p. administration of PGE_1 to the rat. A similar observation was reported by Masek et al.[6] who found that PGE_1 (50 µg/kg) administered intracysternally induced a sedation associated with the increased wakefulness. However, these authors did not find a significant decrease of SWS after administration of PGE_1, probably due to the relatively low doses used, compared with the doses in our experiments. The observation that some patients receiving $PGF_{2\alpha}$ showed epileptiform spike activity[16] might be due to a common excitatory background responsible also for the increased wakefulness.

We observed an increase in temperature in the acute experiments on the cat, after i.vc. administration of PGE_1. It is known that PGE_1 injected into the cerebral ventricles of an unanesthetized cat produces a prompt elevation of body temperature [17] and that changes in the activity of thermoregulatory hypothalamic structures influence the sleep cycle[18]. However, in spite of this fact, PGE_1-induced hyperthermia and changes in sleep pattern are not necessarily causally related. Elucidation of this aspect of problem requires an additional and different experimental approach than that applied in this study.

It is also of interest to note that many authors observed that PGs prolonged the "sleep" induced by hypnotic drugs by measuring the righting reflex[19,20]. We consider that this observation is not in contradiction with the suggested waking properties of PGs, since the righting reflex in the rat is rather a measure of the general behaviour and/or motor abilities than of the sleep/waking cycles.

It is evident that PGs are not REM sleep inducers in the rat or in the cat. However, it is of interest to note that PGO spiking - a phasic element of REM sleep - is increased in the cat. Evidently, a specific mechanism responsible for the PGO spiking in the cat is triggered by PGE_1, but it was not sufficient to generate and maintain the complex mechanism responsible for the REM sleep.

It is known that a noradrenergic system originating in the locus coeruleus, together with the more powerful 5-hydroxytryptamine system, act as inhibitory modulators of the PGO spikes[21]. However, an involvement of the noradrenergic system in this case is unlikely, since PGE_1 decreased the NA concentrations in the brain, but had no effect on the NA turnover[19]. The role of the cholinergic system in the elaboration of the PGO waves is not clear, but it seems it is not essential. In fact, only drugs acting on the nicotinic receptors changed the amplitude, though not the density, of PGO waves[21]. This might explain the failure of atropine to modulate the prostaglandine induced PGP spiking in our experiments.

The fact that previous administration of 5-HTP completely abolished the effect of PGE_1 might indicate that this drug triggered PGO spiking mainly via a functional inhibition of the serotoninergic system.

However, an additional direct effect upon the neurons could not be excluded, since PGE_1 also induced spiking after local application of drug and excitatory action of PGE_1 on brain stem neurons is already established[22].

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