EFFECT OF *l*-TETRAHYDROPALMATINE ON SLEEP-WAKING CYCLE OF CATS

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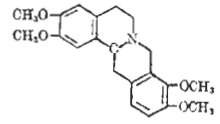
ABSTRACT After ip *l*-THP 30 to 80 mg/kg in cats, SWS-2, PS and PGO discharges were eliminated. At 80 mg/kg, *l*-THP interrupted the sleep waking cycle by inducing a notable state of excitation, which lasted 12-30 h. At 30 or 40 mg/kg, the effect of *l*-THP lasted 10 h; waking was decreased and SWS-1 increased with sedation, but the arousal response was maintained. This effect at 30 or 40 mg/kg is similar to that shown by other tranquillizing drugs or DA-receptor antagonists.

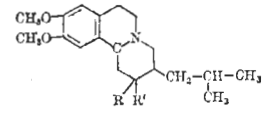
KEY WORDS *l*-tetrahydropalmatine; sleep-waking cycle; light slow wave sleep; deep slow wave sleep; paradoxical sleep; pontogeniculo-occipital discharges

Sleep-waking cycles in animals and man are composed of a waking phase, light slow wave sleep (SWS-1), deep slow wave sleep (SWS-2) and a paradoxical sleep phase (PS) with pontogeniculo-occipital discharges (PGO-waves)[1,2]. Brain catecholaminergic and serotoninergic systems are apparently involved in the regulation of the sleep-waking cycle[2-4], and the insomnia can be induced by a deficiency in brain 5-HT after 5,6-DHT, PCPA or electrical lesions. SWS-2 and PS are decreased or eliminated and permanent discharges of PGO waves and waking occur, but SWS-1 is often increased[4,5]. PS and PGO discharges are generated by reserpine and benzoquinolizine derivatives (eg, tetrabenazine and Ro-4-1284)[6,7], which deplete the brain stores of 5-HT, DA and NA. The depletion of the 5-HT stores is possibly of greater importance for PS and PGO activity induced by reserpine and Ro-4-1284.

l-Tetrahydropalmatine (*l*-THP), isolated from *Corydalis ambigua or Stephania viridiflaveus*, contains a benzoquinolizine structure, which is similar to that of tetrabenazine or Ro-4-1284. *l*-THP has a marked sedative-tranquillizing effect[8], and is used in clinical alleviation of pain and anxious insomnia[9]. *l*-THP possesses the characteristics of a DA-receptor antagonist[10], and slightly lowers the brain level of DA, while leaving 5-HT essentially unchanged. *l*-THP has therefore a different action from tetrabenazine or Ro-4-1284. In this study, we investigated the effect of *l*-THP on the sleep-waking cycle in cats.



l-THP



R and R′ = O, tetrabenazine
R = Et, R′ = OH, Ro-4-1284

MATERIAL AND METHODS

Three adult ♂ cats, weighing 3-3.2 kg, were implanted for polygraphic recordings of PGO discharges from lateral geniculate nucleus, EEG from frontal and occipital cortices, EEG from the nuchal musculature, and eye movements from the orbits. The scoring of the polygraphic records was carried out according to previously published criteria[11]. Awake: low voltage, fast cortical EEG activity, no spindles, presence of EMG activity; SWS-1: one or more spindles in 30 s with a primarily fast frequency low voltage background, EMG generally reduced, eye movements absent or slow; SWS-2: spindles present (rarely absent) with a primarily

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slow frequency high voltage background, EMG reduced or silent, eye movements absent or slow; and PS (or REM sleep): fast activity low voltage cortical EEG, EMG silent except for occasional myoclonic twitchings, rapid eye movements and PGO discharges. Each cat received either drug or vehicle in separate experimental sequences for twice trials after an interval of 1–2 months. Control polygraphic recordings of the sleep-waking cycle were made from 9:00 AM to 8:00 AM the next day for several consecutive days in box.

l-THP, as the free base, was dissolved in 0.1 *M* H_3PO_4 and then adjusted with 0.1 N NaOH to pH 5–5.5, containing 30 mg/ml for ip. The vehicle was made with H_3PO_4 and NaOH. Alpha-methyl-*p*-tyrosine (α-MPT, Sigma) was suspended in arabic gum and 0.9% NaCl solution prior to ip.

RESULTS

Expt 1. Within 10 min after ip *l*-THP 80 mg/kg, one conscious cat, standing calmly, showed sedation with salivation. Its nuchal muscular tonicity was relaxed and its head was lowered to the floor. The cat sometimes sat and sometimes laid with the abdominal attitude, SWS-1 and short awake periods were observed alternatively. The cat became aroused after 3 h. It walked around in the box with frequent and vigorous eye movements and nuchal muscular activities (Fig 1). No PGO discharges, SWS-2 and PS were seen (Fig 2) and only a predominately wake state (77%, Tab 1) occurred. The excitatory state lasted approximately 10 h. After that the excitation reduced: eye movements ceased suddenly (Fig 1), nuchal muscular activities decreased and a short episode of SWS-1 appeared. Subsequently, the cat showed a waking EEG for 4 h, and then a waking EEG and SWS-1 appeared alternatively until early next morning (from 2.00–8.00 AM).

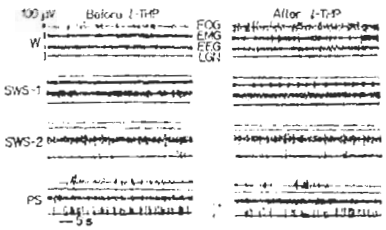

Fig 1. Effect of *l*-THP on sleep-waking of cat 81. Polygraphic characteristics of waking (W), light slow wave sleep (SWS-1), deep slow wave sleep (SWS-2) and paradoxical sleep (PS). EOG: electro-oculogram; EMG: electromyogram; EEG: Cortical electroencephalogram; LGN: lateral geniculate nucleus. After ip *l*-THP 80 mg/kg, W state lasted 10 h, and then SWS-1 occurred; 20 h later, SWS-2 occurred; 30 h later, typical PS appeared.

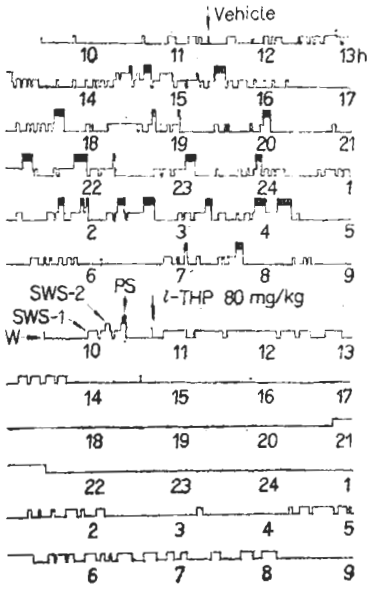

Fig 2. Effect of *l*-THP on sleep-waking cycle of cat. Vehicle was ip at 11:20 AM and ip *l*-THP 80 mg/kg at 10:40 AM next day. Recording started at 9:00 AM until 8:00 AM next day.

About 20 h later, SWS-2 occurred again and frequently; subsequently a few PGO discharges followed, and then increased gradually. Only after 31 h did the typical PS phase reappear (waking EEG, PGO dischages, relaxation of nuchal muscular tonicity and eye movements).

Similar results were observed in another conscious cat.

In summary, the effects of *l*-THP on the sleep-waking cycle presented two main characteristics: 1) a long continuous excitatory state with waking EEG and EMG, lasting for more than 20 h; 2) SWS-2, PS, and PGO discharges

Tab 1. Effect of *l*-THP on % of sleep-waking cycle of cats in 23 h

ip	SWC	9:00-21:00	21:01-8:00 next
Vehicle	PS	5.3	11.4
	SWS-2	4.4	0.1
	SWS-1	31.2	28.5
	S	35.6	28.6
	W	59.1	60.0
l-THP	PS	0	0
	SWS-2	0	0
80 mg/kg	SWS-1	22.9	25.1
	S	22.9	25.1
	W	77.1	74.9
Next	PS	4.3	10.3
23 h	SWS-2	9.2	2.6
	SWS-1	35.2	23.8
	S	44.4	26.4
	W	51.3	63.3
Control	PS	12.1	17.4
	SWS-2	11.5	9.1
	SWS-1	26.0	33.9
	S	37.5	43.0
	W	50.4	39.6
l-THP	PS	0	2.5
	SWS-2	0	2.0
30 mg/kg	SWS-1	79.1	40.0
	S	79.1	42.0
	W	20.9	55.5
Vehicle	PS	11.3	16.7
	SWS-2	13.1	6.3
	SWS-1	28.2	29.2
	S	41.3	36.5
	W	47.4	46.8

S = SWS-1 + SWS-2; SWC = sleep-waking cycle

were suppressed during this time. It was hypothesized that the effects of *l*-THP may be related to brain catecholaminergic systems.

Expt 2. α-MPT was used to block CA biosynthesis and to verify the hypothesis. α-MPT 200 mg/kg was injected ip 1 h before *l*-THP 80 mg/kg. The results were very similar to those of Expt 1 (without α-MPT). The cats still displayed an excited behavior with a waking EEG and no PGO discharges, SWS-2 and PS were as long as in Expt 1. Hence the blockade of the biosynthesis of CA modified neither the state of excitation nor the change to the sleep-waking cycle induced by *l*-THP.

Expt 3. After ip *l*-THP 30-40 mg/kg, 2 cats appeared to be sedated, but the arousal response remained. SWS-1 was increased to 70-79% (Tab 1) against a waking EEG during the day, but SWS-2 and PS disappeared or markedly decreased over the 23 h period. Therefore the behavioral characteristics of cats were thus dependent on the dose of *l*-THP, ie, sedation at 30-40 mg/kg, and excitation at 80 mg/kg. The duration of suppression of SWS-2 and PS were also dependent on the dose of *l*-THP injected. These suppressions induced by *l*-THP 80 mg/kg were longer than that induced by *l*-THP 30-40 mg/kg.

DISCUSSION

Reserpine and the benzoquinolizine derivatives (eg, tetrabenazine, Ro-4-1284) are shown to be able to induce generalized PGO discharges[6,7], which closely resemble those occurring during PS[7]. The mechanisms of generation of these PGO discharges implicate some gating effect from brain 5-HT and CA stores which are depleted by these drugs. It seems likely that the induced deficiency in brain 5-HT, in particular, is a direct cause of the increased PGO discharges[7]. It suggest that there is a strong inhibition originating from 5-HT neurons on the generation of PGO discharges. On this basis, a tentative explaination is possible as to why *l*-THP and Ro-4-1284 have very different effects on the sleep-waking cycle. In contrast to Ro-4-1284 which could markedly generate PGO discharges[6], *l*-THP virtually completely eliminated PGO discharges, although the partial chemical structure of both compounds is very similar.

The main reason for this difference probably lies in the role of brain 5-HT, which is lowered by Ro-4-1284[6], but not by *l*-THP[10]. This possibility lends a support to the 5-HT deficiency theory in the genesis of PGO discharges.

The suppression of PS and SWS-2 seemed to be a stable feature of *l*-THP administration

in the cat, and was dependent neither on the excitory behavior which occurred at large dose nor on sedation with arousal reaction which was present after *l*-THP 30–40 mg/kg. This sedation with increase in SWS–1, and decrease in waking closely resembled the tranquillizing effect induced by reserpine[7]. Owing to the suppression of PS and SWS–2, *l*-THP is obviously not to induce a physiological sleep, although its sleep triggering properties have been seen in rats, mice, dogs, monkeys or men[9]. This sleep triggering effect may be a characteristic of a DA-receptor blocker[10], because some DA-receptor blockers also increase SWS and decrease wakefulness in rats[12-14].

Behavioral excitation resulting from the release of brain CA, can be totally suppressed with α-MPT[15] which effectively blocks the biosynthesis of CA. But we have shown that pretreatment with α-MPT had no effect on the excitatory phase evoked by *l*-THP. Whether the release of CA is likely involved in the mechanism of behavioral excitation and waking EEG induced by *l*-THP to be solved.

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