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Influence of Phenobarbital on the Circadian Rhythm of Opiate Receptor in Rat Brain

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ABSTRACT

To investigate the influence of phenobarbital sodium on the action of morphine and on the diurnal rhythms of both opiate receptor binding and β-endorphin contents, the amount of specifically bound (^{3}H)-morphine and immunoreactive β-endorphin were measured in the midbrain of phenobarbital-treated rats at 4h intervals in a day. Rats were housed and adapted to a controlled cycle of either 12 h light-12 h dark or 24 h constant dark. After 3 weeks of adaptation, 0.5 ml of physiological saline or phenobarbital sodium (20mg/kg/day, i.p.) were administered twice a day for 2 weeks.

Highly significant diurnal rhythms of opiate receptor binding and β-endorphin were present in rat midbrain. In control group, the peak of maximum (^{3}H)-morphine binding was observed at 22:00 h, whereas the peak of β-endorphin content was found at 06:00 h. Even in the absence of time cues these diurnal rhythms persisted, but they were highly modified with respect to the wave form as well as differences in the timing of peak and nadir. In the phenobarbital-treated group, these diurnal rhythms were also modified in shape, phase and amplitude, as well as in timing of peak and nadir. In this group, 24 h mean of opiate receptor binding was significantly decreased, while the 24 h mean level of ß-endorphin content was highly increased.

However, Kd values in all experimental groups did not change. This indicates that differences in binding were not due to changes in the affinity, but in the number of binding sites. Statistical analysis of regression line indicates that changes of receptor binding were closely correlated with the changes of β-endorphin content.

These results suggest that phenobarbital may influence the action of morphine by changing the number of opiate receptors and that the modification of diurnal rhythm of opiate receptor by the agent is possibly due to changes of β-endorphin content.

Key Words: circadian rhythm, opiate receptor, β-endorphin, phenobarbital

INTRODUCTION

The biological responsiveness to a drug frequently exhibits a diurnal rhythm. Diurnal changes in brain functions are believed to modify many important behavioral phenomena, including responses to sedatives and many other drugs (Richter, 1977; Reinberg & Halberg, 1971). Several recent studies have indicated significant daily variations in the tissue levels of biologically active substances and the relationships of those rhythms to the physiological phenomena. Scheving and Pauly (1966), Hunter and Rigal (1966), and Dixit and Buckly (1967) observed daily fluctuations of a number of blood hormones

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in the rat. Friedman and Walker (1968), Hanin *et al.* (1978), and Scheving *et al.* (1978b) reported rhythmic changes in the concentrations of biogenic amines, including certain neurotransmitter substances, in the various parts of the central nervous system. In addition, striking diurnal changes have recently been demonstrated to occur with some receptors such as cholinergic, adrenergic, and opiate receptors (Kafka *et al.*, 1982; Naber *et al.*, 1980; Lee *et al.*, 1984; Chung *et al.*, 1984).

Diurnal rhythms of barbiturates effectiveness and anesthesia are also well documented. Davis (1962) and Vessel (1968) reported diurnal changes in barbiturate sleeping time in mice, while Scheving *et al.*, (1968a) studied the effect of different light-dark programs on the duration of pentobarbitone sodium anesthesia. Other workers have shown that barbiturates changed the diurnal rhythms of plasma catecholamines, histamine, dopamine and glucose concentrations of the rat (Friedman & Walker, 1969; Osowaya *et al.*, 1979). Moreover, a few recent studies indicate that barbiturates can modify the analgesic action of morphine, and opiate antagonist can influence the barbiturates anesthesia (Ossipov & Gebhart, 1984; Gilbert & Martin 1977; Horita *et al.*, 1978; Scheving, 1968c). But the interactions between barbiturates and narcotic analgesics was not been fully elucidated.

In this study, we measured the specific binding of ^{3}H-morphine and β-endorphin contents in the rat midbrain over 24 h period. Since significant diurnal rhythms throughout the day was found, we also examined whether chronic treatment with phenobarbital sodium modifies any of the characteristics of these rhythms.

MATERIALS AND METHODS

ANIMALS. Male Sprague-Dawley rats weighing about 180 g were used. Animals were maintained in a community cages under a controlled light-dark cycle (L:D, 12:12) or a constant darkness (D:D, 12:12). Food and water were given *ad libitum* After 3 weeks of adaptation, 0.5 ml of physiological saline or phenobarbital sodium (20 mg/kg/day)was administered intraperitoneally twice a day for 2 weeks.

TEST PROCEDURE. The amount of specifically bound (^{3}H)-morphine and immunoreactive ß-endorphin were measured at 4 h interval throughout a day.

After adaptation, the brain was removed rapidly and a half of the midbrain was homogenized using a motor-driven Teflon-pestle homogenizer in 19 volumes of ice-cold 10 mM Tris-HCl buffer, pH 7.4. All preparative procedures were performed at 4°C. Tissue preparations were incubated with or without varying concentrations of morphine or 10 μm of morphine for 5 min. Subsequently, (^{3}H)-morphine (specific activity 60 Ci/mM) was added to the reaction mixture and incubated for an additional 15 min period at 37°C. Bound drug was collected on membrane filter (pore size: 0.8 μm, nitrocellulose, Whatman) and washed immediately with 15 ml of the ice-cold Tris-HCl buffer. The filters were dissolved in 1.0 ml of ethyleneglycolmonoethylether and assayed for radioactivity by using liquid scintillation counting. Counting efficiency was monitored with the external standard channel-ratio which was calibrated with internal standards. The value obtained in the presence of 10μM morphine hydrochloride (non-saturable binding) was subtracted from that obtained in the absence of morphine (total binding) to calculate the saturable binding. Each experiment was performed in duplicate. Bmax and Kd values were calculated as described by Akera and Cheng (1980).

β-endorphin immunoreactivity was quantitated by radioimmunoassay. A half of midbrain was placed in an inverted petri dish on salted ice at -5°C to -10°C. The preparation was homogenized in 9 ml of 1 N acetic acid for 1 g of wet tissue. The homogenate was centrifuged at 10,000 g for 30 min. The supernatant was resuspended with equal volumes of 1 N acetone. The final suspension was evaporated at 20°C and submitted to radioimmunoassay procedure by using a NEW kit (NEK-003).

Protein was assayed by the method of Lowry *et al.* (1951).

Data were analyzed by student's t-test, q-ratio, one-way and two way analysis of variance.

The experiment was carried out from June to July.

Drugs used were: β-endorphin (I-125) and (^{3}H)-morphine sulfate from New Eng. Nuclear Co., morphine hydrochloride from Samsung Pharm., Korea and phenobarbital sodium from Cheil Pharm., Korea.

RESULTS

Saline injected rats showed opiate receptor diurnal rhythms.

Fig. 1 shows the unique diurnal patterns for specific (^{3}H)-morphine binding in control rats. The highest opiate binding occured during the early stage of dark phase (at 22:0 h), whilst a nadir binding was observed during the late phase of illumination period (at 18:00 h). Amplitudes defined as the ratio of peak and nadir to 24 h mean were +51.1% at peak and −26.6% at nadir. 24 mean value of binding was 0.45 ±0.03 pmol/mg protein (Table 1 and 2). One-way analysis of variance for each group indicated that there were significant changes over time($F = 3.9$, $P<0.05$). Furthermore, q ratio revealed that the value at 22:00 h was significantly different from the values at all other times (Table 3).

Fig. 2 shows the diurnal rhythm in binding to opiate receptor in the constant dark-adapted animals (D:D, 12:12). In this group, the rhythm also monophasic and changes occured in all characteristics of diurnal rhythm. There were shifting of phases, changes of wave forms (shape) and amplitudes (Table 1). 24 h mean value was significasntly decreased (Table 2). Two-way analysis of variance indicated that there were very significant changes over time ($F = 16.5$, $P<0.01$), as well as with dark-adaptation ($F = 6.8$,

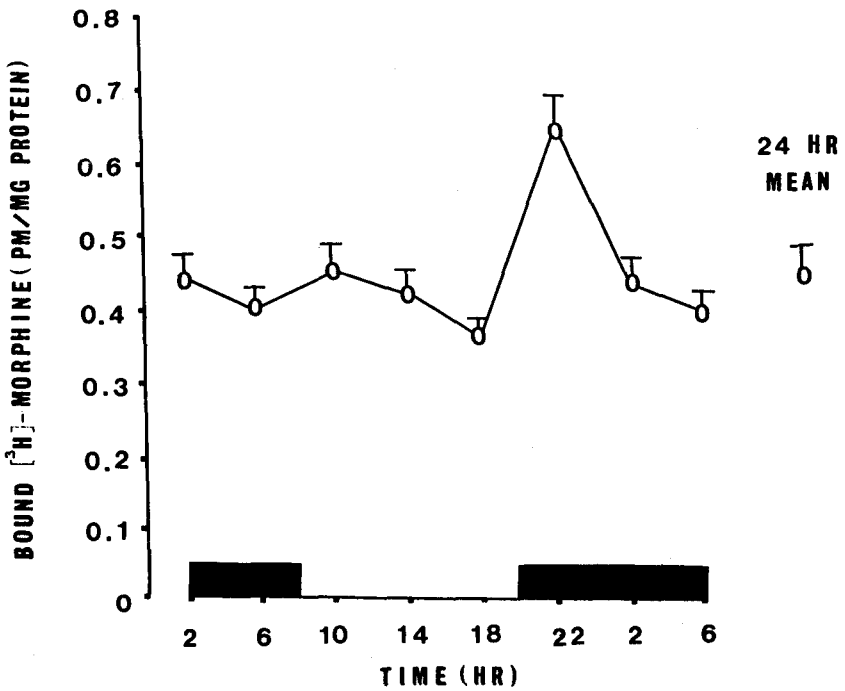

Fig. 1. Diurnal variation of opiate receptor binding (specifically bound ^{3}H-morphine) in the midbrain homogenates of control rat. Animals were adapted to the light-dark cycle (L:D, 12:12) for 3 weeks and 0.5ml of physiological saline solution was injected intraperitoneally twice a day for 2 weeks before experiments. Each point denotes the mean ± SEM from 6 experiments. The shaded areas indicate the dark phase of light-dark cycle.

Table 1. Diurnal variation of specific opiate receptor binding in control group and its modification by constant dark adaptation and phenobarbital treatment

Time (h)	Control		Constant dark adaptation		Phenobarbital sodium	
	Bmax	kd	Bmax	Kd	Bmax	Kd
2	0.44±0.03	0.85±0.07	0.58±0.05[a]	0.85±0.08	0.61±0.05[a]	0.87±0.09
6	0.38±0.03	0.82±0.07	0.52±0.04	0.84±0.08	0.32±0.03	0.86±0.07
10	0.46±0.04	0.85±0.08	0.45±0.04	0.84±0.09	0.26±0.01	0.84±0.08
14	0.42±0.04	0.82±0.07	0.14±0.01[b]	0.84±0.08	0.18±0.01[b]	0.85±0.07
18	0.33±0.03[b]	0.83±0.07	0.22±0.02	0.84±0.08	0.28±0.02	0.86±0.08
22	0.68±0.06[a]	0.85±0.08	0.27±0.02	0.85±0.08	0.34±0.03	0.85±0.08

Maximum ^{3}H-Morphine binding (pmol/mg protein) and Kd (nM) values were measured at 6 points of 4 hr interval throughout a day. Each point represents the mean ± SEM from 6 experiments. a: Peak. b: Nadir.

Table 2. 24 h mean of specific opiate receptor binding amplitude of peak and nadir in control and constant darkness and phenobarbital treatment in the midbrain homogenates of the rat

Treatment	Maximum binding (pmol/mg protein)	Amplitude	
		Peak	Nadir
control	0.45±0.03	+51.1	−26.6
constant darkness	0.36±0.03[a]	+61.1	−60.4
phenobarbital	0.33±0.03[a]	+84.3	−21.1

a: Significantly different from the control value ($P<0.05$).
Amplitude denotes % change from 24 h mean.

Table 3. Statistical analysis of circadian rhythm of specific opiate receptor binding in the midbrain homogenates of the rat

Treatment	One-way analysis of variance		q ratio test
	F value	P value	($P<0.01$)
control	3.99	0.05	22[a]>2, 6, 10, 14, 18
constant darkness	5.34	0.01	2[a], 6, 10>14, 18, 22
phenobarbital	6.06	0.01	2[a]>6, 10, 14, 18, 22

a: Peak.

$P<0.01$). The least significant difference test indicated that binding values at 02:00 h, 06:00 h and 10:00 h were significantly different from those at the other times (Table 3).

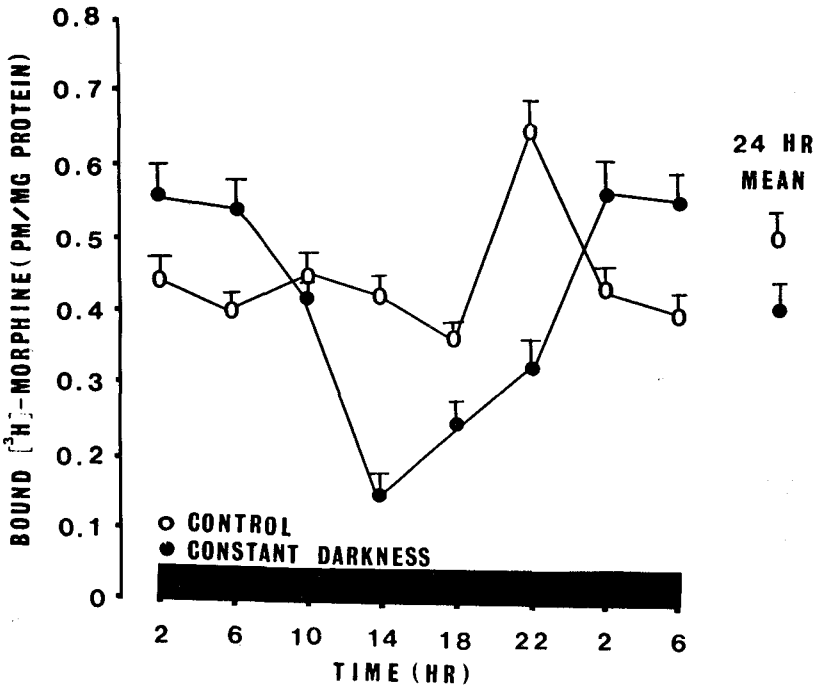

Fig. 2. Diurnal variation of specific opiate receptor binding and its modification by constant dark condition in the midbrain homogenates of the rat. Animals were adapted in constant dark state (D:D, 12:12) for 3 weeks and 0.5ml of physiological saline was injected intraperitoneally twice a day for 2 weeks before the experiments. Each point denotes the mean ± SEM from 6 experiments. Statistical analysis; One-way analysis of variance, $F = 5.34$ ($P<0.01$), q ratio test, 2, 6, 10>14, 18, 22 h ($P<0.01$). Two-way analysis of variance comparing control and constant dark group shows significant change over time ($F = 16.5$, $P<0.01$) and over treatment ($F = 6.8$, $P<0.01$).

Table 4. Statistical analysis of the influence of time, constant dark adaptation and phenobarbital on the specific opiate receptor binding in the midbrain homogenates of the rat: two-way analysis of variance

Treatment	Two-way interaction			
	Treatment-dependent process		Time-dependent process	
	F value	P value	F value	P value
constant darkness	6.8	0.01	16.5	0.01
phenobarbital	6.3	0.01	13.8	0.01

Fig. 3 shows the diurnal rhythm of (^{3}H)-morphine binding in chronic phenobarbital-treated rats. Chronic phenobarbital-treatment changed the wave form, amplitudes, phase and 24 h mean value of binding of the diurnal rhythm. After chronic phenobarbital, the highest binding occured at the late stage of dark phase (at 02:00 h) as compared to the early stage (at 22:00 h) in controls. The amplitude of the rhythm was also increased. 24 h mean value of binding was significantly decreased (Tables 1 and 2). One-way analysis of variance for each group indicated the existnece of significant changes with

time ($F = 6.06$, $P<0.01$) and two-way analysis of variance indicated that there were significant changes over time ($F = 13.8$, $P<0.01$) and over treatment ($F = 6.30$, $P<0.01$). q ratio test showed that in phenobarbital-treated animals, binding values at 02:00 h. were significantly different from the values at all other times.

However, Kd values did not change in all experimental groups.

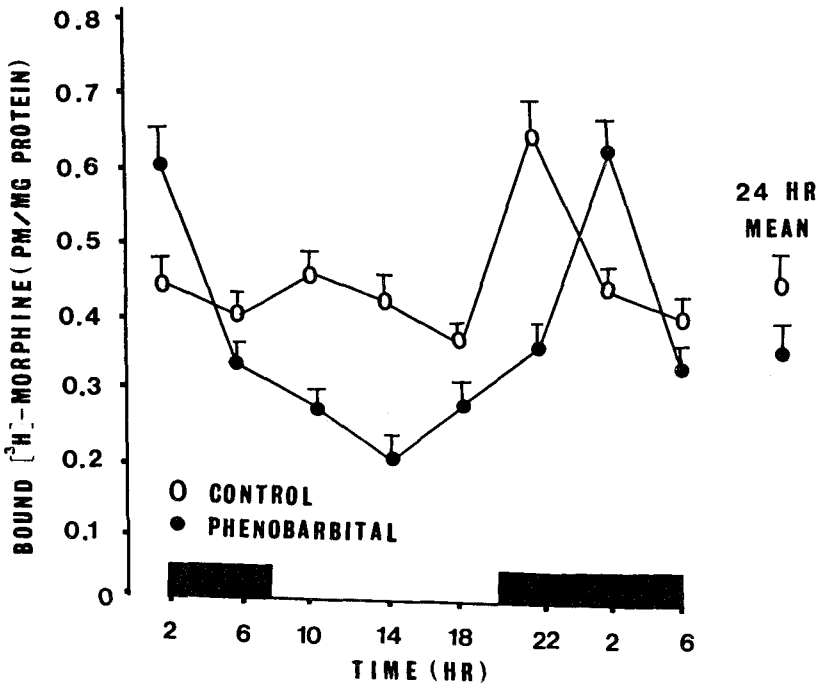

Fig. 3. Diurnal rhythm of specific opiate receptor binding and its modification by phenobarbital sodium in the midbrain homogenates of the rat. Animals were adapted to L:D, 12:12 cycle for 3 weeks and 20mg/Kg of phenobarbital sodium was injected intraperitoneally twice a day for 2 weeks before the experiments. Each point denotes the mean ± SEM from 6 experiments. Statistical analysis: One-way analysis of variance, $F = 6.06$ ($P<0.01$), q ratio test, 2>6, 10, 14, 18, 22 ($P<0.01$). Two-way analysis of variance comparing control and phenobarbital sodium-treated group shows significant change over time ($F = 13.8$, $P<0.01$) and over treatment ($F = 6.3$, $P<0.01$).

Table 5. Diurnal variation of immunoreactive β-endorphin in control and its modification by constant darkness and phenobarbital treatment in the midbrain homogenates of the rat

Time	Control	Constant darkness	Phenobarbital
	(fmol/mg protein)	(fmol/mg protein)	(fmol/mg protein)
2 h	38.3 ± 2.5	20.8 ± 1.9[b]	43.2 ± 4.0[b]
6 h	94.8 ± 7.7[a]	24.7 ± 2.1	47.7 ± 4.3
10 h	51.8 ± 3.8	39.5 ± 3.8	68.5 ± 6.6
14 h	39.3 ± 3.1	64.2 ± 5.7[a]	110.5 ± 9.8[a]
18 h	27.6 ± 2.2[b]	22.6 ± 2.1	48.7 ± 4.6

Total immunoreactive β-endorphin was measured at 6 points of 4 h interval throughout a day. Each value represents the mean ± SEM from 6 experiments. a: Peak. b: Nadir.

Table 6. 24 h mean of total immunoreactive β-endorphin and amplitude of peak and nadir in control, constant darkness and phenobarbital treatment in the midbrain homogenates of the rat

Treatment	24 h mean	Amplitude	
	(fmole/mg protein)	peak (%)	nadir (%)
control	46.7±3.6	+81.9	−40.9
constant darkness	35.9±3.1	+78.8	−42.1
phenobarbital	60.9±5.8	+81.4	−41.0

a: Significantly different from the control value ($P<0.05$).
Amplitude denotes % change from 24 h mean.

Table 7. Statistical analysis of circadian rhythm of total immunoreactive β-endorphin in the midbrain homogenates of the rat

Treatment	One-way analysis of variance		q ratio test
	F value	P value	($P_4<0.01$)
control	3.99	0.05	2[a]>6, 10, 14, 18, 22
constant darkness	5.34	0.01	14[a], 18>2, 6, 10, 2
phenobarbital	6.47	0.01	10, 14[a]>2, 6, 18, 22

a: Peak.

Fig. 4 shows the diurnal rhythm of beta-endorphin contents in the control rat midbrain: the highest β-endorphin content was observed during the end of dark phase, whereas the minimum content was observed during the end of light phase. Amplitudes were +81.9% at peak and −40.9% at nadir. 24 h mean value of ß-endorphin content were significantly changed over time ($F=3.99$, $P<0.05$). q ratio showed that the value at 06:00 h was significantly different from the others (Table 7).

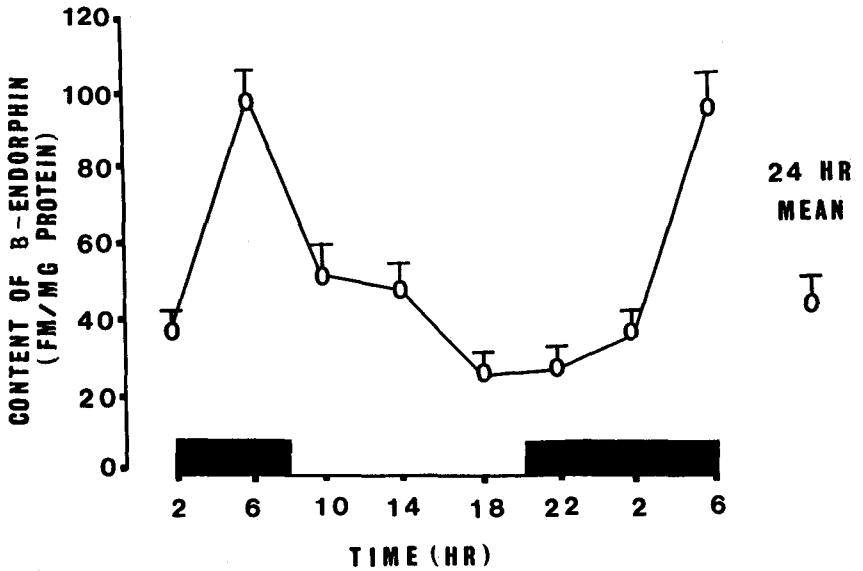

Fig. 4. Diurnal variation of β-endorphin in the midbrain homogenates of the control rat. Animals were adapted to L:D, 12:12 cycle for 3 weeks and 0.5ml of physiological saline was injected intraperitoneally twice a day 2 weeks before experiments. Each point denotes the mean ± SEM from 6 experiments. The shaded area indicates the dark phase of light-dark cycle.

Fig. 5 shows that constant dark adaptation modified the diurnal rhythm of β-endorphin content in phase, shape and 24 h mean value. The peak of β-endorpin content was noted at 14:00 h, whereas the minimum value was observed at 02:00 h (Tables 5 and 6). 24 h mean value of the content was markedly decreased. One-way analysis of variance indicated that there were highly significant changes over time ($F=5.34$, $P<0.01$) and the values at 02:00, 06:00 and 10:00 h were different from the others (Table 7). The interactons of adaptation and time were also significant (treatment: $F=6.41$, $P<0.01$, time: $F=5.68$, <0.01) (Table 8).

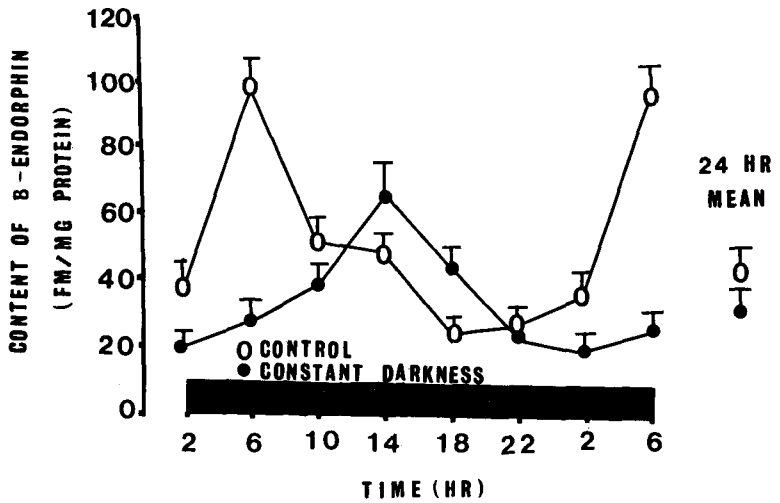

Fig. 5. Diurnal variation of β-endorphin and its modification by constant dark condition in the midbrain homogenates of the rat. Animlas were adapted to D:D, 12:12 cycle for 3 weeks and 0.5ml of physiological saline was injected intraperitoneally twice a day for 2 weeks before experiments. Each point denotes the mean ± SEM from 6 experiments. Statistical analysis: One-way analysis of variance, $F=4.28$ ($P<0.05$), q ratio test, 14, 18>2, 6, 10, 22 ($P<0.01$). Two-way analysis of variance comparing control and constant dark-group shows significant change over time ($F=5.68$, $P<0.01$) and adapted ($F=6.41$, $P<0.01$).

Table 8. Statistical analysis of the influence of time, constant dark adaptation and phenobarbital on the total immunoreactive β-endorphin in the midbrain homogenates of the rat: two-way analysis of variance

Treatment	Two-way interaction			
	Treatment-dependent process		Time-dependent process	
	F value	P value	F value	P value
constant darkness	6.41	0.01	5.68	0.01
phenobarbital	6.5	0.01	5.27	0.01

Fig. 6 shows the diurnal rhythm of β-endorphin contents in chronic phenobarbital-treated group. Chronic phenobarbital treatment changed the diurnal rhythms of β-endorphin content in wave form, amplitudes, phase and 24 h mean. In this group the highest content of β-endorphin was observed at 14:00 h, while the nadirs were noted at 18:00 h. 24 h mean value was significantly increased (Table 5 and 6). One-way analysis of variance indicated that there were highly significant changes over time

($F = 6.47$, $P<0.01$). The least significant difference test showed that 10:00 h and 14:00 h were different from all other time points (Table 7). Two-way analysis of variance indicated that the interactions of treatment with time were also significant (treatment: $F = 6.50$, $P<0.01$, time: $F = 5.27$, $P<0.01$) (Table 8).

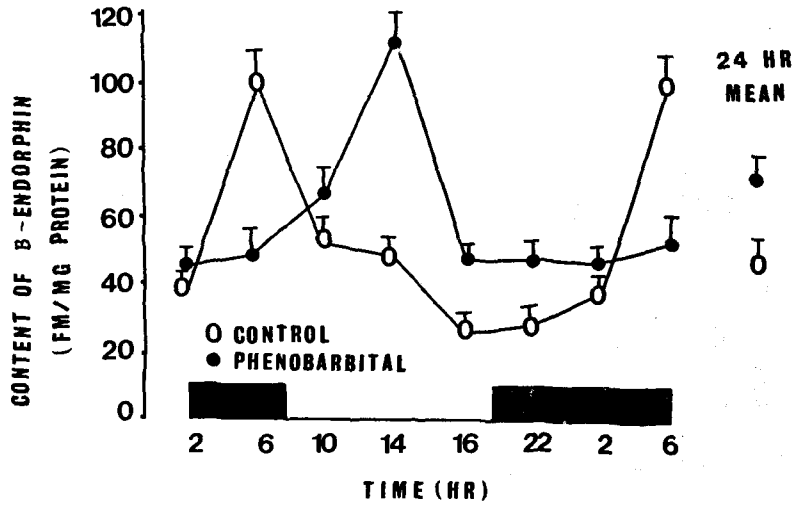

Fig. 6. Diurnal variation of β-endorphin and its modification by phenobarbital in the midbrain homoge the rat. Animals were adapted to L:D, 12:12 cycle for 5 weeks and 20mg/kg of phenobarbital sodium was injected intraperitoneally twice a day for 2 weeks before experiments. Statistical analysis: One-way analysis of variance, $F = 6.47$, $P<0.01$, q ratio test, 10, 14>2, 6, 18, 22 ($P<0.01$). Two-way analysis of variance comparing control and phenobarbital-treated group shows significant change over time ($F = 5.27$, $P<0.01$) and treatment ($F = 6.50$, $P<0.01$).

Fig. 7, 8 and 9 show correlation between β-endorphin content and specific opiate receptor binding in control, constant dark-adapted and chronic phenobarbital-treated rats. There were significant negative correlations between the opiate bindings and β-endorphin content.

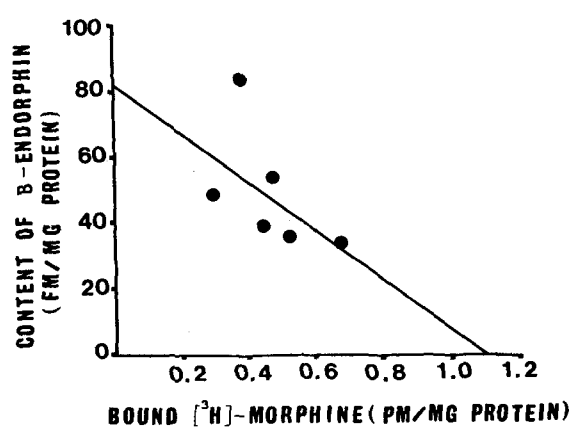

Fig. 7. Correlation between β-endorphin content and specific opiate receptor binding in the midbrain homogenates of the control rat.

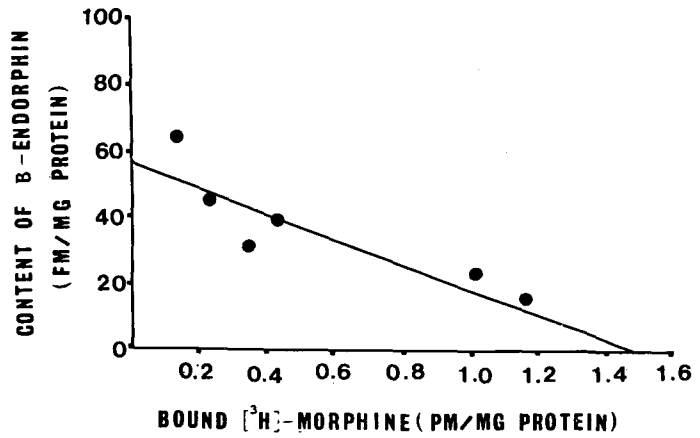

Fig. 8. Correlation between β-endorphin content and specific opiate receptor binding in the midbrain homogenates of the constant dark adapted rat.

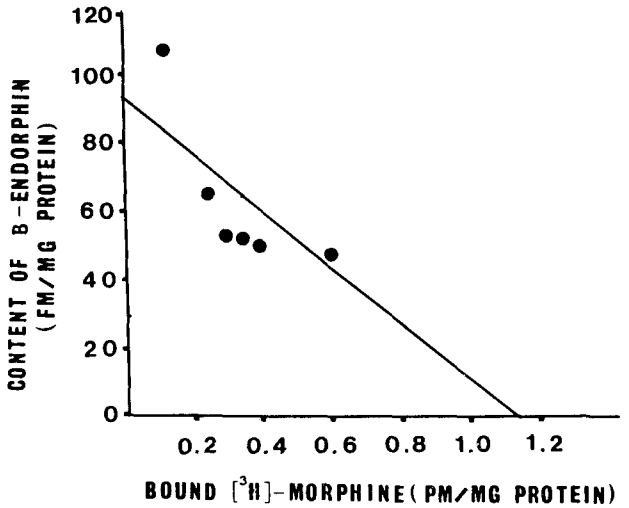

Fig. 9. Correlation betwen β-endorphin content and specific opiate receptor binding in the midbrain homogenates of the constant dark adapted rat.

DISCUSSION

This study demonstrates that opiate receptor binding and β-endorphin content of rat midbrain exhibited circadian rhythms with physiological changes over a few hours. The highly significant circadian rhythms of opiate receptor bindings were similar to the bimodal motor activity in nocturnal rodents (Aschoff, 1954; Pittendrigh & Daan, 1976), different sensitivity to pain and responsiveness to analgesics on the time of day in human (Dawes, 1974; Frederickson *et al.*, 1977). In our previos study (Lee *et al.*, 1984; Jung *et al.*, 1984), we also observed similar rhythms of (^{3}H)-morphine binding and β-endorphin content in rat midbrain, which were modified by some centrally acting drugs. However, Park *et al.* (1984) reported different rhythms in rat pineal gland.

Prolonging the dark phase resulted in changes of wave forms, amplitudes and 24 h mean values of opiate receptor binding and β-endorphin content. The designation of a function as circadian rhythm implies or means that a major crest of activity followed by a trough during a period of approximately 12 h of light followed by 12 h of darkness. Consquently, one can predict the local clock time at which the crest, trough or any other phase of the rhythm will occur under identical standardized conditions. If the light-dark cycle is discontinued and the animal is subject to continuous light or darkness, the rhythm of the physiological function measured now may have a frequency close to but different from 24 h; consequently, the peak and trough will gradually drift in relation to local clock time. This finding is consistent with an endogenous rhythm.

Scatchard analysis indicated that the variations in binding are due to changes in the number of binding site and not in affinity. Moreover, statistical analysis of regression line revealed that changes of receptor binding were closely correlated with changes in β-endorphin content: alternatively, these can be interpreted by receptor regulation due to change of β-endorphin content.

Phenobarbital can produce all degrees of depression of CNS, ranging from mild sedation to general anesthesia. Moreover, this drug has been well known to have various effects on CNS such as on anxiety, motor activity, EEG pattern and stages of sleep (Gilman *et al.*, 1980). In the current study, phenobarbital increased 24 h mean of β-endorphin content and changed the charateristic parameters of circadian rhythms of opiate receptor binding and β-endorphin content. In addition to emulating various elements of the pharmacological profile of opiates, the endorphins exhibit several potent actions of interest to the pharmacological characterization of their receptors and their function. These actions after intracerebroventricular injection consist of akinesia, analgesia, hypothermia and hyperglycemia (Bloom *et al.*, 1976; Feldberg & Smyth, 1976b; Guillemin *et al.*, 1977; Jacquet & Marks, 1976). When β-endorphin is intravenously administered in the dose range which produces the maximal stress-induced elevations of plasma β-endorphin, little or no CNS actions can be seen; however at much higher values, β-endorphin i.v. can produce secretion of prolactin and growth hormone (Dupont *et al.*, 1977; River *et al.*, 1977) and at much higher doses, particulary in mice and cats, β-endorphin produces analgesia (Feldberg & Smyth, 1977). At the cellular level in CNS, β-endorphin induces in rat CNS a profound seizure state whose threshold is lower than that for analgesic action (Henriksen *et al.*, 1977), and iontophoretically administered β-endorphin produces naloxone-reversible depression of test cell in most brain regions (Nicoll *et al.*, 1977). Briefly summarized, β-endorphin can modulate the function of CNS, pain perception and behaviors.

In a single or small dose, the barbiturates are hyperalgesic and increase the reaction to painful stimuli. Hence, they can not be relied upon to produce sedation or sleep in the presence of even moderate pain. Barbiturates are believed to antagonize analgesics in experimental animals, but Geller and associates (1977) found no such interaction. With regard to these observations, other findings can be interpreted as a different patterns of action of barbiturates. Decrease of maximum (^{3}H)-morphine binding and increase of 24 h mean of β-endorphin content by chronic phenobarbital treatment will alter pain perception.

In the present study, chronic phenobarbital treatment changed the circadian rhythms of (^{3}H)-morphine binding and β-endorphin content in shape, phase and amplitudes. It has been well established that barbiturates show marked rhythms of effectiveness and toxicity. The largest doses of phenobarbital are needed at night to anesthetize rodents (Davis, 1962; Elmen & Kem, 1963), but they are most susceptable to potentially lethal doses at this time (Pauly & Scheving, 1963). Moreover, Friedman and Walker (1969) and Owasoyo *et al.* (1979) reported that barbiturates changed the circadian levels of brain biogenic amines. It is apparent from the results of this study that phenobarbital exerts its effect on β-endorphin content and changes the rhythm of opiate receptor. These effects may influence the function of CNS including pain perception, the actons of narcotics and other centrally acting drugs.

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=국문초록=

백서의 뇌내 Opiate 수용체의 일중 변동에 미치는 Phenobarbital의 영향

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실험적으로 명암에 적응시킨 백서의 뇌내 specific opiate binding과 β-endorphin 함량 일중변동에 미치는 지속적인 암적응, phenobarbital 장기처리의 영향을 관찰하고 opiate recptor binding과 B-endorphin 함량 양자간의 관계를 추구하여 다음과 같은 성적을 얻었다.

1. L : D, 12 : 12 주기에 적응시킨 대조군에서 maximum (^{3}H)-morphine binding과 뇌내 β-endorphin 함량은 각각 22시 및 06시에 최고에 달하는 매우 특이한 일중변동을 보였고 24시간 평균 (^{3}H)-morphine binding 및 뇌내 β-endorphin 함량은 각각 0.45+0.03 pmol/mg protein과 46.7+3.6 fmol/mg protein이었다.
2. D : D, 12 : 12 주기에 적응시킨 표본에서 maximum (^{3}H)-morphine binding과 뇌내 β-endorphin 함량은 대조군에서와는 달리 02시 및 14시에 최고에 달하는 유의한 일중변동을 보였고, 24시간 평균 maximum (^{3}H)-morphine binding치는 0.36+0.03 pmol/mg protein으로 대조군에 비하여 유의하게 감소를 하였으며, 24시간 평균 뇌내 β-endorphin치는 35.9+3.1 fmol/mg protein으로 대조군에 비하여 유의한 감소를 보였다.
3. Phenobarbital처리 표본에서 maximum (^{3}H)-morphine binding과 뇌내 β-endorphin 함량은 각각 02시 및 14시에 최고 그리고 14시 및 02시에 최저에 달하는 대조군과는 다른 일중변동을 보였고, 24시간 평균 maximum (^{3}H)-morphine binding치는 0.33+0.03 pmol/mg protein으로 대조군에 비하여 현저한 감소를 보였다.
4. 전 실험군에서 opiate receptor binding의 Kd치는 변동하지 않았다.
5. 전 실험군에서 maximum (^{3}H)-morphine binding은 β-endorphin 함량과 유의한 역상관관계가 있었다.

이상의 실험성적은 phenobarbital이 뇌내 β-endorphin 함량과 opiate 수용체의 숫적 변동을 일으켜 morphine의 약리적 작용을 변동시킬 수 있으며, 이와 더불어 opiate 수용체와 β-endorphin 함량 일중변동을 변화시킬 수 있음을 보여준다.