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Reevaluation of the Effect of Phenobarbital on the Response to Pain in Rat

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ABSTRACT

Clinically, subhypnotic doses of barbiturates have been known to elicit hyperalgesia. In this experiment, effect of acute or chronic phenobarital treatment on the response to pain in rat was reevaluated by hot-plate method. To elucidate its mechanism, changes of β-endorphin contents and [3H]-morphine binding of the rat midbrain as well as functional opiate receptor in vas deferens were also measured.

Intraperitoneal injection of subanesthetic dose phenobarbital induced initial hyperalgesia followed by successive analgesia, while chronic phenobarbital-treatment decreased reactivity to pain.

Naloxone (10mg/kg, i.p.) markedly shortened hot plate latency period, and significantly inhibited the analgesic action of phenobarbital.

Single dose of phenobarbital did not affect β-endorphin contents and [3H]-morphine binding in rat midbrain, but in the chronic phenobarbital-treated groups, β-endorphin contents was increased, while B_{max} of opiate receptor binding was decreased. Moreover, very significant correlations among responses to pain, changes of β-endorphin contents and opiate receptor binding were observed. However, Kd values of opiate receptor bindings were not changed in all preparations.

In the chronic phenobarbital-treated vas deferens preparations, ID50 of morphine was increased with concomittant decrease of maximum effect. But pA_2 value for naloxone was not changed.

From these results, it is suggested that phenobarbital can produce analgesia due to changes of β-endorphin contents as well as functional opiate receptors by receptor regulation.

Key Words: Phenobarbital, response to pain, β-endorphin, opiate receptor.

INTRODUCTION

The barbiturates, unlike the gaseous and volatile anesthetics, lack significant ability to obtund the sense of pain without definite impairment of consciousness. By most experimental criteria, they are not classified as analgesics.

Clinically, it has been known that small doses of barbiturates antagonize the analgesic action of nitrous oxide or meperidine. Dundee (1960) and Clotton-Brock (1961) indicated that barbiturates in subhypnotic doses increased the reaction to painful stimuli.

The mechanism of this hyperalgesic phenomenon appears to be the removal of inhibitory systems on painful sensory input. Nicoll (1979) and other investigators (Gilman and Goodman, 1980) suggested that an inhibitory system in the diffuse ascending reticular system was blocked, permitting the secondary slow pain impulses to be facilitated.

In the last several years, specific opiate receptors and endogenous morphine-like substances have been found in the mammalian central nervous system. Several studies indicated that these endogenous opiate-like substances functioned in some manner to mediate or suppress pain sensation (Kaplan and Glick, 1979; Belluzi *et al.* 1976).

Recently, Akil *et al.* (1976) observed that naloxone partially anatagonized the analgesia produced by focal electrical stimulation of the periaqueductal grey area of the brain, using the tail-frick test to measure analgesia. Moreover, Finck *et al.*, (1977)

found that naloxone alters the depth of inhalational anesthesia, suggesting general anesthetics may release an endogenous morphine-like substances.

In our previous studies (Park *et al.*, 1985; Park and Eun, 1985) that phenobarbital can change the circadian rhythm of opiate receptor binding and β-endorphin contents in rat midbrain. In this paper, we report the effect of acute and chronic phenobarbital administration on the response to pain in rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dewley rats weighing bout 180g were used. Food and water were given ad libitum. Each animal group consisted of 6 rats. The drugs were dissolved in saline and injected intraperitoneally with control animals receiving saline. Solutions of drug or saline were injected in a volume of 0.1ml/100g of body weight.

Determination of Antinociception

Antinociception was determined by hot-plate method (Bowman and Rand, 1980) following a prescreening procedure for the threshold of pain of each animal. All screened rats were grouped according to their thermal response latencies.

Opiate Receptor Binding Assay

The amount of specifically bound [3H]-morphine was determined by the method of Goldstein *et al.* (1979).

After adaptation, the brain was removed rapidly and a half of the midbrain was homogenized using a motor-driven Tefron-pestle homogenizer in 19 volumes of ice-cold 50mM 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, [3H]-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: 0.8μm, nitrocellulose, Whatman) and washed immediately with 15ml of ice-cold Tris-HCl buffer. The filters were dissolved in 1.0 ml of ethyleneglycolmonomethylether and assayed for radioactivity by using liquid scintillation counting. Counting efficiency was monitered with the external standard channel-ratio value obtained in the presence of 10μM morphine hydrochloride (nonspecific binding) was substracted from that obtained in the absence of morphine (total binding) to calculate the specific binding. Each experiment was performed in duplicate. B_{max} and Kd values were calculated as described by Akera and Cheng (1980).

Beta-endorphin Radioimmunoassay

BETA-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 9ml of 1N acetic acid for 1g of wet tissue. The homogenate was centrifuged at 10,000 × g for 30 minutes. The supernate 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 NEN kit (NEK-003).

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

Determination of Affinity of the Opiate Receptor for Naloxone in Isolated Rat Vas Deferens.

Rats were stunned by a blow to the head and the vas deferens was immediately removed. The vas deferens was placed in the modified Krebs-Henseleit solution of the following composition: 118mM NaCl, 27.2mM $NaHCO_3$, 4.8mM KCl, 1.0mM KH_2PO_4, 1.2mM $MgSO_4$, 1.2mM $MgSO_4$, 1.8mM $CaCl_2$ and 11.1mM glucose. Subsequently, a strip of vas deferens was suspended between two parallel platinum electrodes placed 1 cm apart in the above solution saturated with 95% O_2-5% CO_2 gas mixture at 37°C (pH 7.4). Field stimulation (0.2 Hz, 10ms duration and supramaximal voltage) was applied and the force of isometric contraction was recorded by using a isometric force transducer (Narco, F-60) and physiograph (Narco, MK-IV). Resting tension was adjusted 0.5g. After a 60min equilibration, morphine was added to the incubation medium, and its effect on contraction was observed until a steady state was reached. Subsequently, morphine was washed five times with drug free solution, and another concentration of morphine was added to the incubation medium.

To study the affinity of the functional binding sites for naloxone, the effect of morphine was examined in the prsence of various concentration of naloxone, and pA_2 values were obtained using the method described by Arunlakshana and Schild (1959).

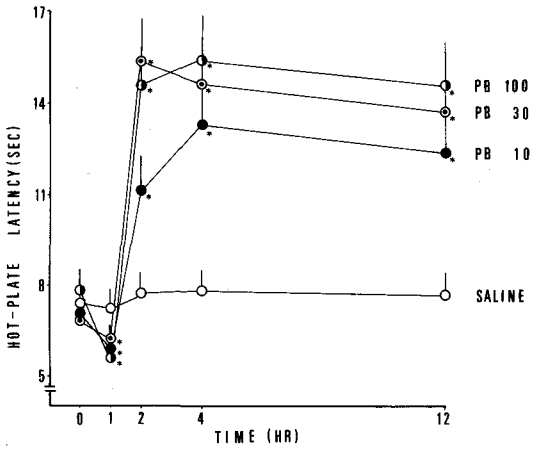

Fig. 1. Effects of acute administration of phenobarbital on the response to pain rats. Each point and vertical bar denotes the mean with SEM from 8 experiments. *: significantly different from the saline-treated group (p 0.05).

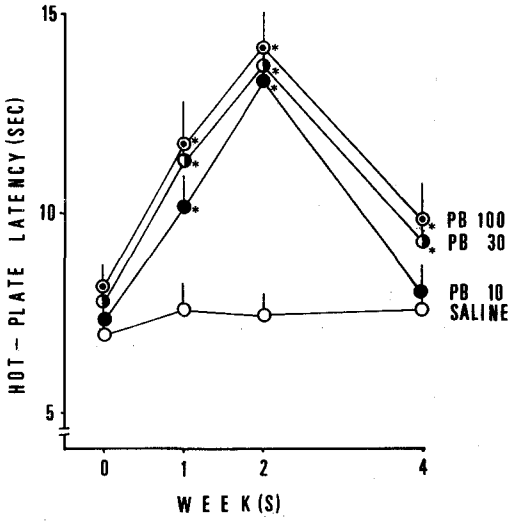

Fig. 2. Effects of chronic administration of phenobarbital on the response to pain in rats. other legends are same as Fig. 1.

Data were analyzed by student's t-test.

Drugs used were β-endorphin (I-125, New Eng. Nuc. Col), [3H]-morphine sulfate (New Eng. Nuc. Co.), morphine hydrochloride (Samsung Pharm), phenobarbital sodium (Cheil Pharm.), and naloxone hydrochloride (Sigma).

RESULTS

Effect of Phenobarbital on the response to pain

The selected rats showed similar duration of response to hot plate stimuli. Fig. 1 represents the effect of acute phenobarbital administration on the response to pain in rats. The administration of phenobarbital in subanesthetic doses produced initial hyperalgesia in as little as 5 min. after injection. This hyperalgesia was followed by analgesia, which lasted over 12 hours.

In the chronic phenobarbital treated groups, 10, 30 or 100mg/kg of phenobarbital was intraperitoneally administrated once a day for 1, 2 or 4 weeks. Hot plate latency was measured at 24 hrs after the last injection. Hot plate latency was significantly increased at 1 or 2 weeks after phenobarbital treatment, but the analgesic effect of phenobarbital was markedly diminished in the rat treated with phenobarbital for 4 weeks (Fig. 2).

Fig. 3 shows the influence of naloxone on the phenobarbital-induced analgesia. Naloxone (10mg/kg) induced hyperalgeaia, which lasted over 12 hrs.

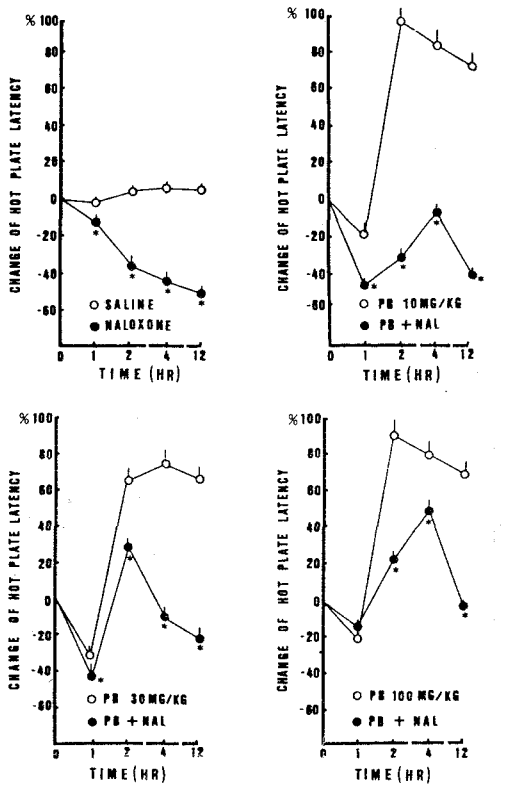

Fig. 3. Influence of naloxone (10 mg/kg) on phenobarbital-induced analgesia in rat.
Each point denotes mean ± SE from 6 experiments.
*: Statistically significant from phenobarbital-treated group ($P<0.05$).

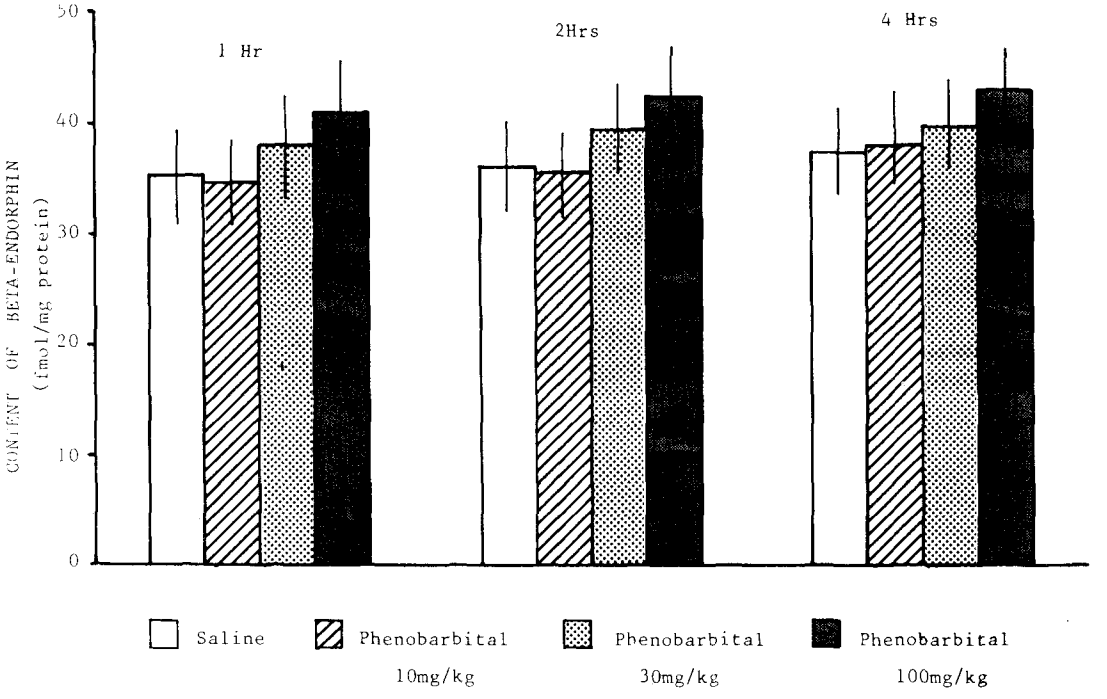

Fig. 4. Effects of acute administration of phenobarbital on the β-endorphin content in rat midbrain. Each value and vertical bar denotes the mean and SEM from 8 experiments.

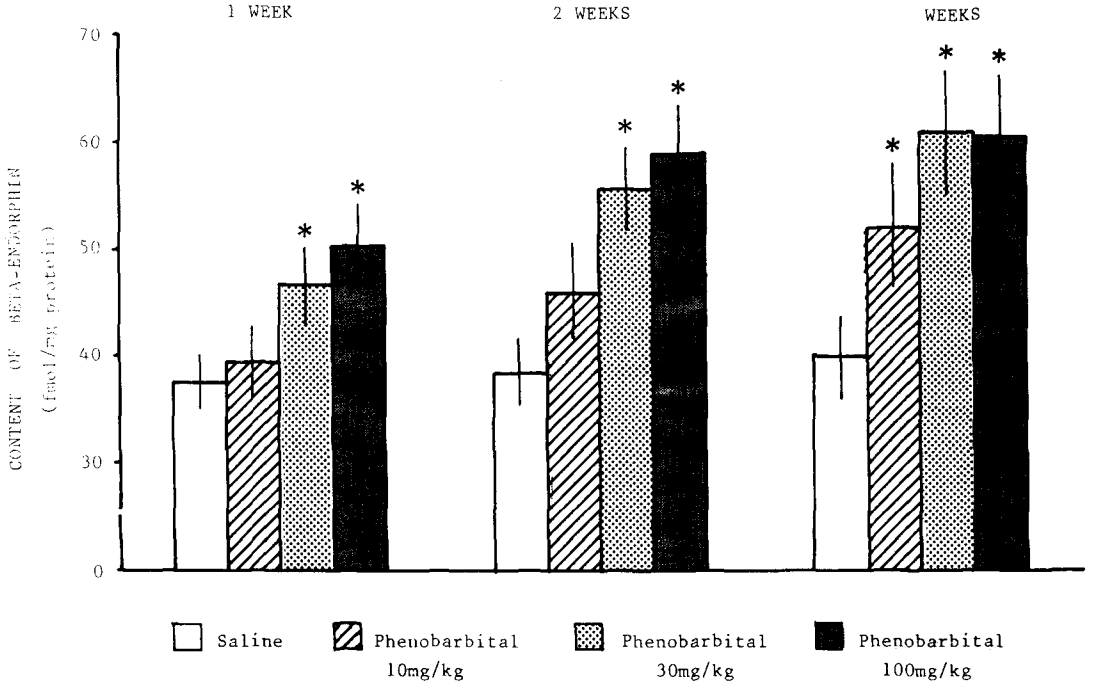

Fig. 5. Effects of chronic administration of phenobarbital on the β-endorphin content in rat midbrain. *: Significantly different from the saline-treated group (p 0.05). Other legends are same as Fig. 4.

In the presence of naloxone, analgesic action of phenobarbital was significantly inhibited. Effect of phenobarbital on the immunoreactive β-endorphin content and specific opiate binding in the rat midbrain.

The above data suggest the involvement of opiate system in the penobarbital-induced analgesia. In these experiments, the immunoreactive β-endorphin contents as well as specific opiate receptor binding were measured after acute or chronic administration of phenobarbital.

Fig. 4 represents the effect of acute administration of phenobarbital on the immunoreactive β-endorphin contents in the rat midbrain. β-Endorphin contents in saline-administered control animals were 35.4±3.5 fmol/mg protein. Single injection of phenobarbital tended to increase the immunoreactive β-endorphin contents. This value was not significantly different from that of control group. However, Chronic pretreatment with phenobarbital significantly increased the immunoreactive β-endorphin contents in rat midbrain (see Fig. 5).

The specific [3H]-morphine bindings were carried out in the same preparations used in determination of β-endorphin contents. Single injection of phenobarbital did not affect the B_{max} and Kd values of opiate receptor. But in the chronic phenobarbital-treated preparations, B_{max} of opiate receptor was significantly decreased (see Table 1 and 2). There are significant correlation among hot plate latency, β-endorphin contents and opiate receptor binding (Fig. 6).

Table 1. Influence of single treatment of phenobarbital on the specific [^{3}H]-morphine binding in rat midbrain

Dose (mg/kg)		After Treatment of Phenobarbital 1 hr	2 hrs	4 hrs
Saline	B_{max}	0.51±0.05	0.47±0.04	0.52±0.05
	Kd	0.81±0.08	0.79±0.08	0.80±0.07
PB 10	B_{max}	0.49±0.04	0.52±0.05	0.49±0.05
	Kd	0.77±0.08	0.85±0.08	0.79±0.07
PB 30	B_{max}	0.51±0.04	0.49±0.04	0.48±0.06
	Kd	0.79±0.09	0.82±0.08	0.79±0.08
PB 100	B_{max}	0.49±0.05	0.48±0.05	0.50±0.06
	Kd	0.83±0.09	0.79±0.08	0.82±0.09

Each value represents the mean of maximum binding (Bmax, pmole/mg protein) or dissociation constant (kd, nM) with SE from 6 experiments.

Table 2. Influence of phenobarbital treatment on the specific [^{3}H]-morphine binding in rat midbrain

Dose (mg/kg)	Periods of Phenobarbital Treatment 1 wk		2 wks		4 wks	
	B_{max}	Kd	B_{max}	Kd	B_{max}	Kd
Saline	0.51±0.03	0.85±0.08	0.49±0.02	0.88±0.07	0.48±0.04	0.79±0.08
FB 10	0.53±0.03	0.88±0.10	0.45±0.03	0.79±0.09	0.48±0.05	0.89±0.11
PB 30	0.50±0.03	0.77±0.09	0.36±0.04[a]	0.89±0.09	0.34±0.03[a]	0.79±0.08
FB 100	0.44±0.03[a]	0.88±0.08	0.31±0.03[a]	0.86±0.07	0.27±0.02[a]	0.90±0.09

Each value represents the mean of maximum binding (Bmax, pmol/mg protein) and dissociation constant (Kd, nM) with SE from 6 experiments.
a: Statistically different from the saline-treated group ($P<0.05$).

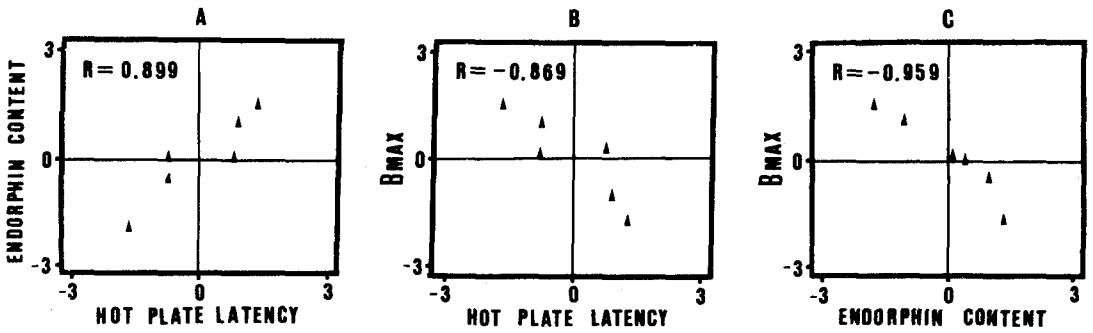

Fig. 6. Correlations among hot plate latency, β-endorphin content and [^{3}H]-morphine binding in chronic phenobarbital-treated rats.

Table 3. Influence of phenobarbital treatment on the inhibitory effect of morphine in the isolated vas deferens of rat

Treatment	ID_{50} of Morphine (μm)	Maximum effect (mg)
Saline	0.31 ± 0.01	150.5 ± 9.7
Phenobarbital		
10mg/kg	0.51 ± 0.03*	64.7 ± 5.2*
1 wks	0.57 ± 0.06*	99.9 ± 7.2*
4 wks	0.55 ± 0.04*	90.5 ± 8.5*
30mg/kg		
1 wk	0.35 ± 0.01	115.5 ± 10.1*
2 wks	0.46 ± 0.05*	90.4 ± 7.6*
4 wks	0.5 ± 0.03*	86.5 ± 6.9*
100mg/kg		
1 wk	0.57 ± 0.04*	68.2 ± 5.3*
2 wks	0.48 ± 0.04*	99.4 ± 7.5*
4 wks	0.69 ± 0.06*	79.7 ± 7.0*

Each value represents the mean ± SE from 6 experiments.
*: Statistically different from the value of saline-treated group ($P<0.05$).

Table 4. Influence of phenobarbital on the pA_2 value for naloxone in the isolated rat vas deferens

Treatment	pA_2 value for Naloxone
Saline	10.09
Phenobarbital	
10 mg/kg	
1 wk	9.66
2 wks	9.95
4 wks	11.72
30 mg/kg	
1 wk	9.94
2 wks	9.96
4 wks	10.21
100 mg/kg	
1 wk	9.96
2 wks	9.65
4 wks	11.28

Each value represents the mean ± SE from 6 experiments.

Effect of phenobarbital on the action of morphine in isolated vas deferens

Rats were treated with phenobarbital from 1 to 4 weeks. Field stimulation was applied to the preparations. Dose-response curves were obtained from 5 successive doses of morphine in the absence or in the presence of various doses of naloxone. Chronic phenobarbital-pretreatment significantly increased ID50 of morphine and markedly decreased maximum effect. But the pA2 value for naloxone was not changed.

The results were summerized in Table 3 and 4.

DISCUSSION

The results of present experiment indicate that subanesthetic dose of phenobarbital can produce initial hyperalgesia followed by analgesia and chronic phenobarbital-treatment decreased reactivity to pain. Barbiturates can produce all degrees of depression of CNS, ranging from mild sedation to general anesthesia. Moreover, these drugs have various effects on CNS such as on anxiety, motor activity, EEG pattern and stages of sleep (Goodman *et al.*, 1980). In the response to pain, barbiturates have known to have little or no direct effects on relieving pain. Rather than an analgesic action, subhypnotic doses of barbiturates increase the sensitivity to pain (Dundee, 1960; Clotton-Brock, 1961). This anti-analgesic action applies principally to deep somatic pain, and especially to visceral pain. The mechanism of this phenomenon appears to be the removal of inhibitory systems on painful input. Brazier (Brazier, 1954) has suggested that an inhibitory system in the diffuse ascending reticular system is blocked, permitting the secondary slow pain impulses to be facilitated. Recently, it has been revealed that barbiturates act throughout the CNS, although not with equal potency in all regions. Pertinent to their sedative-hypnotic effects is the fact that the mesencephalic-reticular activating system is eaquisitively sensitive to these drugs. In whatever region

of the neuraxis, nonanesthetic doses preferentially suppress polysynaptic responses. Facilitation is diminished, and inhibition is usually enhanced. The crucial role of GABA and GABA-ergic involvement has been indicated to the action of barbiturates (MacDonald and MacLean, 1982; Olsen, 1982; Study and Barker, 1981).

In current study, naloxone markedly inhibited analgesic action of phenobarbital, and chronic phenobarbital treatment increased β-endorphin content and decreased B_{max} of opiate receptor binding in rat midbrain. Moreover, Scatchard analysis indicated the variation in binding are due to changes in the number of binding site and statistical analysis of regression line revealed that analgesic effect of phenobarbital are closely correlated with changes of β-endorphin contents and opiate receptor bindings. The endorphin exhibits several potent actions of interest to the pharmacological characterization of their receptors and their function. The actions after intracerebroventricular injection consist of akinesia, analgesia, hypothermia and hyperglycemia (Bloom *et al.*, 1976; Feldberg and Smyth, 1976; Guillemin *et al.*, 1977; Jacquet and Marks, 1976). When β-endorphin is intravenously administered in the dose range which produces the maximal stress-induced elevation of plasma β-endorphin, little or no CNS actions can be seen: however at higher doses, particularly in mice and cats, β-endorphin produces analgesia (Feldberg and Smyth, 1977). At the cellular level in CNS, iontophoretically administered β-endorphin produces naloxone-reversible depression of test cell in most brain regions (Nicoll *et al.*, 1977). The antibarbiturate effect of naloxone was first described in man and more recently confirmed in the rat (Furst *et al.*, 1977; Horita and Carino, 1978). Gilbert and Marton (1977) demonstrated that naltrexone antagonized some of the actions of phenobarbital in the spinal dog preparation. During the last decade, many reports indicating interactions of naloxone and naltrexone with various nonopiate drugs have appeared (Finck *et al.*, 1977; Horita and Carino, 1975; Jacob *et al.*, 1972; Berkowitz *et al.*, 1976). In none of these findings was there convincing evidence that endorphins or opiate receptors were involved in the interactions. In the previous reports, we demonstrated that phenobarbital changed the circadian rhythms of β-endorphin contents and opiate receptor binding in rat brain. The current study supports this view and provides additional evidence that phenobarbital may produce analgesia due to changes of β-endorphin contents as well as functional opiate receptors.

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=국문초록=

백서에서 동통에 미치는 Phenobarbital 효과의 재평가

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소병겸, 김기원, 고명규, 양원모, 조규박

백서에서 열판법을 이용하여 과민동통을 일으키는 약물로 알려진 phenobarbital의 동통에 대한 효과를 재검토하고 그 기전을 알고저 phenobarbital 단기 또는 장기처리에 의한 뇌내 β-endorphin함량, opiate 수용체 및 시험관내 실험으로 functional opiate 수용체의 변동유무를 검토하여 다음과 같은 결과를 얻었다.

1) 마취에 미달하는 용량의 phenobarbital 1회 복강내 투여는 일시적인 HPL단축에 이어 이를 지연시켰고 phenobarbital 장기처리는 HPL을 현저히 지연시켰다.

2) Naloxone 자체는 HPL을 현저히 단축시켰고, naloxone처리는 phenobarbital의 HPL 지연 효과를 억제하였다.

3) Phenobarbital 1회 복강내 투여는 뇌내 β-endorphin 함량에 영향을 미치지 못하였으나 phenobarbital 장기처리는 이를 현저히 증가시켰다.

4) Phenobarbital 1회 복강내 투여는 [3H]-morphine binding에 영향을 미치지 못하였으나, phenobarbital 장기처리는 Kd치와는 달리 Bmax를 현저히 감소시켰다.

5) Phenobarbital 장기처리에 의한 HPL변동, 뇌내 β-endorphin함량변동 그리고 opiate receptor Bmax변동 삼자간에는 유의한 상관관계가 있었다.

6) 적출vas deferens 표본에서 phenobarbital 장기처리는 morphine의 ID50은 증가시키고 maximum effect는 감소시키나 naloxone에 대한 pA_2치에는 영향을 미치지 못하였다.

이상의 실험성적은 phenobarbital이 일시적인 과민동통 효과에 이어 진통효과를 갖고 있으며, phenobarbital의 진통효과는 뇌내 β-endorphin함량 증가와 이로인한 functional opiate 수용체의 숫적 변동에 기인함을 시사하였다.