EFFECTS OF 7-ETHOXYCARBONYL-6,8-DIMETHYL-4-HYDROXYMETHYL-1(2H)-PHTHALAZINONE (EG626) ON THE SPINAL TRIGEMINAL NUCLEUS, VENTRAL POSTEROMEDIAL NUCLEUS, AND SENSORY CORTEX

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Abstract—Effects of 7-ethoxycarbonyl-6,8-dimethyl-4-hydroxymethyl-1(2H)-phthalazinone (EG626) on the spinal trigeminal nucleus (STN), ventral posteromedial nucleus (VPM), and sensory cortex were examined in cats anesthetized with alpha-chloralose in comparison with the effects of morphine. EG626 produced a dose-dependent inhibition of the polysynaptic components of the cortical field potentials upon VPM stimulation and either facilitatory or inhibitory effects on the polysynaptic components of the VPM field potential upon stimulation of the medial lemniscus, while the drug failed to affect the STN field potential with trigeminal nerve stimulation. Morphine inhibited the postsynaptic components of the STN field potentials and to a lesser extent, the polysynaptic components of the cortical field potential; and the effects of morphine on the VPM field potential were similar to those seen with EG626. Pretreatment of the animal with naloxone antagonized the facilitatory effect on the VPM field potentials produced by morphine, but not those by EG626. Morphine and EG626 induced either a prolonged increase in the blood flow or transient increase followed by a decrease in the blood flow in the VPM. These results suggest that EG626 may impair the polysynaptic transmission and/or neuron excitability in the sensory cortex and the VPM at least partly due to the change in blood flow there as does morphine. Unlike morphine, however, EG626 did not produce any obvious effect on the STN.

7-Ethoxycarbonyl-6,8-dimethyl-4-hydroxymethyl-1(2H)-phthalazinone (EG626), synthesized by Ishikawa et al. (1), was shown to inhibit cyclic AMP phosphodiesterase (2) and to prevent edematous changes in rabbit aorta induced by angiotensin II and cholesterol (3, 4). It has been reported that EG626 is useful for the treatments of cerebellar ataxia (5), atherosclerotic disorders (5), and amyotrophic lateral sclerosis (6). In the course of clinical investigation, Mizukami (personal communication) found that EG626 is effective in treating sequelae of cerebral apoplexy such as thalamic pain. However, the reason why EG626 was able to relieve thalamic pain is still unclear; and furthermore, there is a lack of information regarding the detailed pathogenesis of thalamic pain.

We attempt to determine whether or not
EG626 would affect impulse transmission in the trigeminal afferent pathway in cats.

MATERIALS AND METHODS

1) Evoked field potentials: Thirty-two healthy adult cats of both sexes weighing 2.0 to 3.6 kg were used. All surgical procedures such as cannulation of the trachea and femoral vein and removal of the parieto-occipital skull and the tentorium were performed after the cats had been anesthetized with ether. After completion of these procedures, the ether anesthesia was replaced by that with alpha-chloralose (30 mg/kg, i.v.), and gallamine triethiodide (5 mg/kg/hr, i.v.) was given to immobilize the animals. All wound edges and pressure points were locally anesthetized with 8% lidocaine repeatedly throughout the experiment. Respiration was sustained artificially, and body temperature was maintained at 36.5 to 37.5 °C with a heating pad.

A glass-insulated silver wire microelectrode with an electrical resistance of approximately 1 megohm was used to record evoked field potentials in the spinal trigeminal nucleus (STN: P: 9.0, L: 5.5, H: −0.5) and ventral posteromedial nucleus (VPM: A: 8.0, L: 6.5, H: +1.3), according to the brain atlas of Snider and Niemer (7). Silver ball electrodes were used to record the field potentials on the sensory cortex (posterior sigmoid gyrus, S1). Concentric bipolar electrodes were stereotaxically inserted into the intracranial trigeminal nerve (TN: A: 6.0, L: 6.0, H: −8.0), medial lemniscus (ML: A: 4.0, L: 4.0, H: −0.5), and VPM.

Stimuli composed of square pulses with 0.05 msec duration and 5 to 30 V intensity were applied to the TN, ML, or VPM at intervals of 1.6 sec. At least 10 successive responses amplified and displayed on an oscilloscope (VC-9, Nihon Kohden) were photographed. Amplitude of the field potentials was measured from peak to peak and statistical significance was determined by the Student’s t-test and the chi-square test. The position of the electrodes was marked by passing a direct current of 20 μA for 20 sec and a histological verification made after termination of each experiment. Other details of the experiments were similar to those reported by Sasa et al. (8, 9).

2) Blood flow in the VPM: Five healthy adult cats of both sexes weighing 3.0 to 3.5 kg were anesthetized with sodium pentobarbital (35 mg/kg, i.p.). Whenever necessary, an additional dose of sodium pentobarbital was given through the cannula inserted into the femoral vein. A needle type element (NT-100, Unique Medical) was inserted into the VPM of the cat fixed in the stereotaxic apparatus. Blood flow was measured using a UM-meter (UM-2000, Unique Medical) according to the method described by Yamaura et al. (10). The value of blood flow was expressed as a percent of the level obtained in the untreated condition (100%) and that of death was 0%.

3) Drugs used: EG626 was dissolved in 10% ethanol at a concentration of 0.1% (1 mg/ml). It was confirmed in a preliminary study that the vehicle, in the volume used in the present experiment, did not modify the neuronal activities. Morphine hydrochloride and naloxone hydrochloride were dissolved in distilled water. EG626 in doses of 0.5, 1, 2, and 3 mg/kg and morphine in doses of 1, 2, and 4 mg/kg were given intravenously in 60 min-intervals. Naloxone in a dose of 0.1 mg/kg was given intravenously 5 min prior to administration of 2 mg/kg of EG626 and 1 mg/kg of morphine.

RESULTS

1) Effects of EG626 and morphine on field potentials in the STN: The field potentials of the STN produced by stimulation of the trigeminal nerve were composed of two waves: pre- and postsynaptic components.
as illustrated in Fig. 1. Mean peak latencies of pre- and postsynaptic components in four preparations were 0.81±0.09 and 2.16±0.17 msec, respectively. EG626 and morphine in doses up to 3 and 4 mg/kg, respectively, had no effect on the amplitude and latency of the presynaptic component during a 30 min observation period. The amplitude of the postsynaptic component was dose-dependently reduced by morphine. The mean amplitude of the potentials in 4 cats was significantly decreased to 80.7±7.49% of the control 10 min after administration of 4 mg/kg of morphine. The reduction of the STN potential with 4 mg/kg of morphine persisted for 60 min. In contrast to morphine, EG626 in doses up to 3 mg/kg had no effect on the STN field potentials (Table 1).

2) Effects of EG626 and morphine on field potentials in the VPM: As shown in the inset of Fig. 2, the field potentials of the VPM produced by stimulation of the ML were usually composed of 3 waves: P-, N1-, and N2-waves. Mean peak latencies of the P-, N1-, and N2-waves were 0.73±0.03, 1.56±0.02, and 3.68±0.04 msec, respectively (N=15). There were no alterations in the amplitude and latency of the P-wave with either EG626 or morphine in doses up to 2 and 4 mg/kg, respectively. When EG626 in doses of 0.5, 1, and 2 mg/kg was given, a significant enhancement of the amplitudes of the N1- and N2-waves was observed in 5 out of 8 animals examined. However, the N1- and N2-waves in the remaining 3 animals were significantly reduced by EG626. These effects of EG626 on the N1- and N2-waves were dose-dependent and disappeared 60 min later. Morphine produced similar effects on these N1- and N2-waves. A significant enhancement of the N1- and N2-waves of the VPM field potentials was observed in 4

Table 1. Effects of EG626 and morphine on amplitudes of field potentials in the spinal trigeminal nucleus produced by trigeminal nerve stimulation.

| Drug    | Dose (mg/kg) | Presynaptic component | Postsynaptic component |
|---------|--------------|-----------------------|------------------------|
| EG626   | 1            | 100.0±0.0             | 98.6±1.46              |
|         | 2            | 100.3±0.25            | 98.4±0.96              |
|         | 3            | 99.8±0.80             | 92.9±1.29              |
| Morphine| 1            | 100.4±0.78            | 97.4±1.88              |
|         | 2            | 99.5±0.28             | 90.2±2.09              |
|         | 4            | 99.2±1.07             | 80.7±4.79*             |

Peak-to-peak amplitude is given as the mean of 10 successive potentials before and 10 min after administration of the drug. The mean amplitude before giving the drug was taken as 100%. Each value represents the mean±S.E. (N=4 animals). *: Significantly different from the value before the drug at P<0.01 (Chi-square test).
out of 7 cats given 1, 2, and 4 mg/kg of morphine. In the remaining 3 animals, a significant inhibition of N\textsubscript{1} and N\textsubscript{2}-waves was apparent after administration of the drug. The potentiating effect of morphine on the field potentials of the VPM was more potent

![Graph showing effects of EG626 and morphine on field potentials](image)

**Fig. 2.** Dual effects of EG626 and morphine on amplitudes of field potentials in the ventral posteromedial nucleus produced by stimulation of the medial lemniscus. Peak-to-peak amplitude is given as the mean of 10 successive potentials. The mean amplitude before giving the drug was taken as 100%. P, N\textsubscript{1}, and N\textsubscript{2} indicate P-, N\textsubscript{1}-, and N\textsubscript{2}-waves, respectively (see text). ○: Morphine, ●: EG626 Calibration: 2 msec, 0.1 mV

**Table 2.** Effects of naloxone on potentiation by EG626 and morphine of field potentials in the ventral posteromedial nucleus produced by stimulation of the medial lemniscus

| Treatment                  | P     | N\textsubscript{1}     | N\textsubscript{2}     |
|----------------------------|-------|------------------------|------------------------|
| EG626 2 mg/kg              | 99.9±0.10 | 122.8±6.79         | 143.7± 9.47         |
| Naloxone 0.1 mg/kg + EG626 | 99.9±0.13 | 123.8±4.34          | 144.1±11.19        |
| Morphine 1 mg/kg           | 99.8±0.42 | 160.3±6.99          | 166.9± 6.30        |
| Naloxone 0.1 mg/kg + Morphine 1 mg/kg | 100.2±0.25 | 121.0±5.79*         | 116.5± 3.96**    |

Peak-to-peak amplitude is given as the mean of 10 successive potentials before and 10 min after administration of the drug. The mean amplitude before giving the drug was taken as 100%. Each value represents the mean±S.E. (N=4 animals). * and **: Significantly different from the values before naloxone at P<0.01 and 0.001, respectively (Student's t-test). P, N\textsubscript{1}, and N\textsubscript{2} indicate P-, N\textsubscript{1}-, and N\textsubscript{2}-waves, respectively (see text).
and longer lasting than those of EG626.

The enhancement of N1- and N2-waves with 1 mg/kg of morphine was significantly antagonized by 0.1 mg/kg of naloxone administered 5 min prior to the narcotic application (Table 2). The potentiating effect of EG626 at a dose of 1 mg/kg remained unaffected by pretreatment with the same dose of naloxone.

3) Effects of EG626 and morphine on field potentials in the sensory cortex: The field potentials in the sensory cortex produced by stimulation of the VPM were usually composed of 5 waves: P-, N1-, N2-, N3-, and N4-waves, as demonstrated in Fig. 3. Mean peak latencies of the P-, N1-, N2-, N3-, and N4-waves were 0.39±0.02, 1.11±0.04, 1.66±0.07, 2.75±0.26, and 5.20±0.07 msec, respectively (N=9). The amplitudes of the P-, N1-, and N2-waves were not significantly affected by either EG626 or morphine up to doses of 3 and 4 mg/kg, respectively. However, a dose-dependent and significant inhibition of the N3- and N4-waves was observed with 2 and 3 mg/kg of EG626 (Table 3). The inhibitory effect of EG626 on the field potentials lasted for 30 to 60 min. In contrast, only the highest dose of 4 mg/kg of morphine inhibited the N2- and N4-waves and significantly decreased the mean amplitudes of the N3- and N4-waves to 84.9 and 78.8%, respectively (Table 3).

4) Effects of EG626 and morphine on blood flow in the VPM: Figures 4 and 5 show representative recordings of the effects of EG626 and morphine on blood flow in the VPM. A significant increase in blood flow in the VPM was noted after giving 2 mg/kg of EG626 i.v. and with 1 mg/kg of morphine in 3 and 3 out of 5 cats, respectively. The mean of the maximum increase in blood flow was calculated to be 55.7±5.51% in the case of 2 mg/kg of EG626 (N=3) and 57.7±10.55% in the case of morphine (N=3). The duration of action at half maximum was 33.0±6.50 min.

### Table 3. Effects of EG626 and morphine on the amplitudes of field potentials in the sensory cortex produced by stimulation of the ventral posteromedial nucleus

| Drug  | Dose (mg/kg) | P    | N1    | N2    | N3    | N4    |
|-------|--------------|------|-------|-------|-------|-------|
| EG626 | 1            | 100.0±0.04 | 99.6±0.45 | 96.4±0.92 | 98.0±1.36 | 91.6±0.62 |
|       | 2            | 100.1±0.14 | 98.0±0.59 | 94.3±2.44 | 81.2±1.49** | 88.0±3.57** |
|       | 3            | 100.0±0.04 | 95.3±0.91 | 89.1±2.07 | 75.1±2.97**** | 75.2±3.07**** |
| Morphine | 1        | 99.8±0.20 | 99.8±0.64 | 100.6±0.99 | 99.4±1.12 | 99.9±1.82 |
|       | 2            | 99.8±0.42 | 98.9±0.42 | 97.5±1.12 | 98.2±1.18 | 95.0±0.91 |
|       | 4            | 99.8±0.25 | 91.8±1.28 | 86.1±2.72 | 84.9±4.78* | 78.6±3.02*** |

Peak-to-peak amplitude is given as the mean of 10 successive potentials before and 10 min after administration of the drug. Each value represents the mean±S.E. (N=5 cats given EG626 and 4 given morphine). *, **, and ***: Significantly different from values before drug at P<0.05, 0.01, and 0.001, respectively (Chi-square test). P, N1, N2, N3, and N4 indicate P-, N1-, N2-, N3- and N4-waves, respectively (see text).
**DISCUSSION**

Morphine inhibited the postsynaptic component of the STN field potentials evoked by TN stimulation. These findings are in accord with the results obtained by Mizoguchi (11). The inhibitory effect of morphine on the STN field potentials is probably due to a blockade of the polysynaptic transmission since this narcotic produced an inhibition of the STN neuron with a long latency (12), but it had no effects on the STN relay neuron (13). Similarly, morphine inhibited the N$_3$- and N$_4$-waves of the cortical field potentials without effects on the P-, N$_1$- and N$_2$-waves, thereby suggesting that this opiate may also impair polysynaptic transmission in the sensory cortex. In contrast to morphine, EG626 was devoid of effects on the STN field potentials and had a more potent action on the N$_3$- and N$_4$-waves of the cortical field potentials. Therefore, EG626 may more selectively interfere with polysynaptic transmission in the sensory cortex.

Morphine produced both an inhibition of the VPM potentials in several cats and an enhancement of the potentials in other animals, while this drug inhibited the STN and cortical potentials. These variable effects on the VPM were also observed by Chin and Domino (14). The effects of EG626 on the VPM field potentials were similar to those obtained with morphine. However, since an enhancement of the VPM potentials with EG626 was not antagonized by naloxone, it is unlikely that the effect was mediated by opiate receptor(s). Possible mechanisms underlying the potentiating effect of EG626 on the VPM include: (i) a direct stimulating effect on VPM, (ii) a stimulating effect on a more distant facilitatory area, (iii) depression of an inhibitory system, and (iv) indirect action resulting from changes in blood flow.
Since the facilitatory effect of EG626 on the VPM field potentials was inconsistent and varied with the animal, it seems unlikely that the effect resulted from direct action such as stimulatory and/or disinhibitory effects on the VPM neurons. The time course of the enhancement of the VPM field potential with EG626 was parallel with that of an increase in the VPM blood flow. An enhancement of the field potential in the vestibular nucleus and an increase in responsiveness of vestibular neurons have been observed with intravenous administration of cinnarizine which is a vasodilator, suggesting that the facilitation may be due to an increase in blood flow of the vertebral artery (15). It is therefore suggested that the enhancement of the VPM field potential with EG626 may be at least partly due to an indirect action resulting from an increase in blood flow there. In some cats, EG626 as well as morphine reduced the amplitude of the VPM field potentials. However, it seems unlikely that the reduction of the VPM field potentials with EG626 and morphine may be due to a decrease in the VPM blood flow because the blood flow was transiently reduced, and reversion to control levels was seen in 10 to 20 min when the field potentials were still inhibited. In summary, our results suggest that the effects of EG626 on the sensory cortex and VPM may be related to the relief of thalamic pain.

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