葛国庆、黄绮文 (上海延安制药厂，上海 200050，中国)

提要 6名志愿者交叉服用吲哚美辛缓释胶囊(25 mg bid)与普通胶囊(25 mg q 8 h)稳态血药浓度的波动指数(FI%)和生物利用度比较。结果表明，两种制剂的血药谷浓度和吸收程度相近($P>0.05$)，但缓释胶囊的血药峰浓度和FI(%)均明显小于普通胶囊($P<0.01$)。达峰时间也明显较迟($P<0.01$)，提示缓释胶囊有较好的缓释特性。

关键词 吲哚美辛；生物利用度；迟效制剂

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Effects of ohmefentanyl on CA1 field potentials in rat hippocampus slices

ZHAO Guo-Min, GU Jia-Jun, CHI Zhi-Qiang
(Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 200031, China)

ABSTRACT The effects of ohmefentanyl (OMF), a new opiate agonist with high affinity and high specificity for μ receptors, was examined on CA1 field potentials in the transverse hippocampal slices. OMF showed two effects upon the evoked population spikes (PS) recorded in stratum pyramidale: 1) a concentration-dependent increase in the amplitude of PS, which was largely reversed by naloxone, and 2) production of a naloxone-reversible additional PS at high stimulus intensities. No significant change was seen in field excitatory postsynaptic potential (EPSP) recorded simultaneously in stratum radiatum. The EC_{50} for OMF and morphine were 6.6 and 3700 nmol · L^{-1}, respectively. Thus OMF was 560 times more potent than morphine. The mechanism of augmentation by OMF of PS could be attributed to disinhibition as judged from the paired-pulse paradigm.

KEY WORDS ohmefentanyl; morphine; naloxone; hippocampus; evoked potentials

The rat hippocampus contained the major forms of endogenous opioid peptides as well as μ, δ, and κ opioid receptor types[1]. Opioid receptors were located in CA1, CA3, and the dentate gyrus[1,2]. Within each area, the density of receptors was found to be highest in and near the stratum pyramidale and granulosum, and all 3 major types of opioid receptors were increased in these layers. In the hippocampus, excitatory effects induced by opiates were found in CA1, CA3, and dentate gyrus. Opiate alkaloids or peptides applied to the hippocampus *in vivo* or *in vitro* increased the size of evoked field potentials[3-6]. It has been suggested that the δ receptor was the predominant receptor involved, inasmuch as [*D*-Ala^2, *D*-Leu^5]enkephalin (a relatively δ selective agonist) was more potent than morphine in producing the effect and morphine was considered to be the prototypic agonist for μ receptor[3].

OMF is a potent analgesic derived from fentanyl and was synthesized first in our laboratory[7]. The receptor binding assay and the assay carried out in isolated preparations indicated that OMF showed a high affinity and specificity for μ opioid receptor, and it was better than [*D*-Ala^2, MePhe^4, Gly-ol^5]enkephalin as a μ ligand[7-10]. Our aim was to characterize the pharmacological effects of OMF in the hippocampus slices and to assess the relationship between μ receptor and increased excitability of pyramidal cells using OMF, a novel and highly specific μ agonist.

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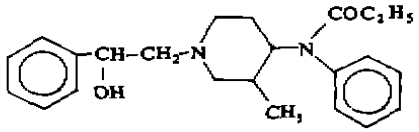

Ohmefentanyl

MATERIALS AND METHODS

Wistar rats, ♂, weighing 160± *s* 25 g, were anesthetized with ether and decapitated. Brains were cooled in chilled and oxygenated artificial cerebrospinal fluid (ACSF) for 1 min. Then the hippocampi were dissected free from surrounding tissues and transverse slices, 420 μm thick, were cut from the middle one-third of it with a McIlwain tissue chopper and transferred immediately to a recording chamber[11]. Slices were maintained at the interface between an atmosphere of warmed, humidified 95% O_2 + 5% CO_2 and the superfusing ACSF at 34.0± 0.5℃. The composition of ACSF was (mmol · L^{-1}): NaCl 124, KCl 3, KH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4 \cdot 7H_2O$ 2, $NaHCO_3$ 25, dextrose 10. ACSF was equilibrated with the gas mixture to maintain a pH at 7.4 and was delivered at a continuous rate of 1.2 ml · min^{-1}.

The arrangement of recording and stimulating electrodes in the experimental preparation is illustrated in Fig 1. Extracellular recordings were made using 2 glass microelectrodes (resistance 4-10 MΩ), filled with KAc 4 mol · L^{-1}, positioned in the CA1 cell body layer (stratum pyramidale) and dendritic field (stratum radiatum), respectively. A monopolar stainless steel stimulating electrode was placed in the stratum radiatum to activate pyramidal cell dendrites orthodromically via Schaffer collateral and / or commissural fibers. Constant current pulses (duration 0.1 ms, intensity 10-150 μA) were delivered to the stimulating electrode through a stimulus isolation unit at 0.25 Hz. Signals were amplified by MEZ-8201 or FW-2 microelectrode amplifier, displayed on a VC-9 oscilloscope, and stored on floppy disks of a AST / 286 computer for subsequent analysis. By varying the stimulus current, input / output (I / O) curves could be constructed that related the size of afferent volley to that of field EPSP slope, and the size of field EPSP slope to the amplitude of PS. The methods for measuring the size of PS, EPSP slope and afferent volley were showed in Fig 1. Opiate effects were expressed with the magnitude of leftward shift of the curves (Δ EPSP) that were calculated by the following equation:

$$\Delta EPSP = (EPSP_{40c} - EPSP_{40}) / EPSP_{50c}$$

where $EPSP_{40c}$ and $EPSP_{50c}$ are the measured EPSP slopes in mV · ms^{-1} that in the control condition produces 40 and 50% maximal PS, respectively, and $EPSP_{40}$ is the EPSP slope to elicit a 40% maximal PS in the presence of agonist[3,6]. Recording was begun at 60-90 min after placing slices in the chamber. Usually, control I / O curves were taken at time 0 and 20 min. At 30 min, superfusion with a known

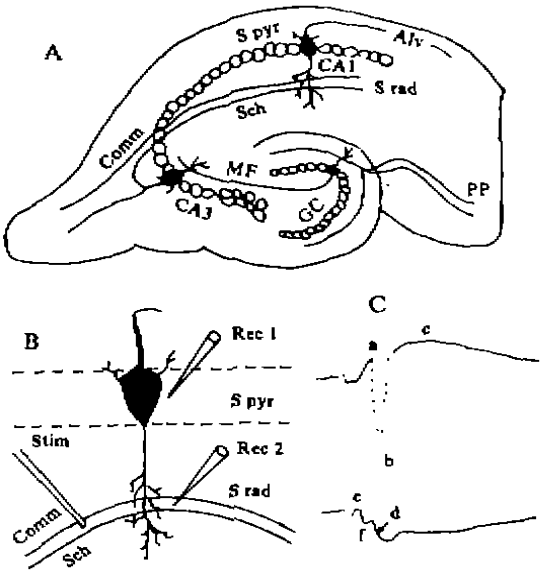

Fig 1. A) Transverse hippocampal slice. Alv = alveus; GC = granular cell; MF = mossy fibers; PP = perforant path. B) A pyramidal cell in CA1 with a stimulating electrode (Stim) and 2 recording electrodes. Constant current stimulus pulses activated Schaffer collaterals (Sch) and / or commissural afferent fibers (Comm). Evoked population spikes (PS) were recorded (Rec 1) in stratum pyramidale (S Pyr), dendritic field EPSP (Rec 2) were recorded in stratum radiatum (S Rad). C) Measurement of I / O relationship. PS amplitude was determined as \|(a + c) / 2 − b\| mV; simultaneously recorded dendritic field EPSP magnitude was measured as initial slope d (mV · ms^{-1}); the amplitude of triphasic afferent fiber volley was determined as (e-f) mV.

concentration of OMF or morphine was started and at time 60 min a third I / O curve was made. At 70 min, superfusion with agonist plus naloxone was started and at 100 min the final I / O curve was taken. A single agonist concentration was used for each slice.

OMF hydrochloride was synthesized in our laboratory. Morphine hydrochloride was purchased from Qinghai Pharmaceutical Factory and naloxone hydrochloride was bought from Shanghai Medical University. All drugs were dissolved in ACSF just before superfusion.

RESULTS

Evoked potentials were recorded simultaneously in the pyramidal cell layer and apical dendritic field. In all slices, PS of 3 mV or more were evoked with an orthodromic stimulus current of <50 μA (50 μs, 0.25 Hz), and no additional PS was evoked over the entire range of the stimulus currents.

Superfusion of the slices with OMF (1–500 nmol · L^{-1}) increased the size of PS but left the field EPSP slope unaffected. An additional PS following the primary population spike was produced at high stimulus intensities when the concentration of OMF was higher than 10 nmol · L^{-1}. The effect of OMF was evident within 10 min after starting superfusion and reached a plateau during 15–25 min. I / O curves were constructed by varying the stimulus intensity. PS, EPSP wave shapes and I / O curves of a typical experiment at OMF 10 nmol · L^{-1} were shown in Fig 2 and 3. As demonstrated in Fig 3 A, B, the amplitude of PS was increased, and the EPSP threshold for evoking PS was reduced, but the relationship of the fiber volley to EPSP slope showed no discernible change after the application of OMF. Meanwhile, the maximum of PS increased only slightly after OMF. Usually the leftward shift of I / O relation curves was fully reversible by naloxone at the lower part, partially reversible at a slightly higher part, but not at all reversible or even enhanced it slightly in the upper portion of the curve (Fig 3 B). In only two slices the effect was completely reversible. The additional PS evoked by high stimulus intensities (Fig 3 C) was easily abolished in 5–10 min by superfusing with OMF plus naloxone at a concentration of 100 times as that of OMF. The effect of naloxone itself (1 μmol · L^{-1}, $n = 3$) was not significantly different from control. The observed effects at a wide concentration range of OMF could be reversed by naloxone, a specific narcotic antagonist, so they are mediated through opiate receptors. The leftward shift of I / O relationship curve between field EPSP and PS indicated that a given EPSP resulted in a larger PS than before exposure to OMF, namely, led to an increase in the excitability of CA1 pyramidal neurons.

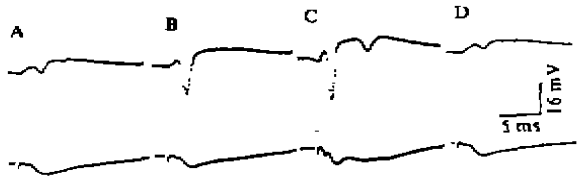

Fig 2. Effects of OMF on dendritic and somatic field potentials in CA1 region evoked by stimulating (30 μA, 0.1 ms, 0.25 Hz except 90 μA in panel C) Sch and / or Comm. Upper tracing showed PS recorded in S Pyr and lower tracing showed dendritic field EPSP recorded simultaneously in S Rad. Each panel was the digital average of 5 consecutive responses. A: Control. B: 30 min after superfusing the slice with OMF 10 nmol · L^{-1}. C: 30 min after superfusing with OMF. D: 30 min after superfusing with ACSF containing OMF + naloxone 1.0 μmol · L^{-1}.

A similar result was obtained by bath perfusion with morphine (1–20 μmol · L^{-1}) as a control.

The I / O relationship curve of EPSP to PS was plotted for each slice and the magnitude of leftward shift of curve was calculated in ΔEPSP for each drug concentration.

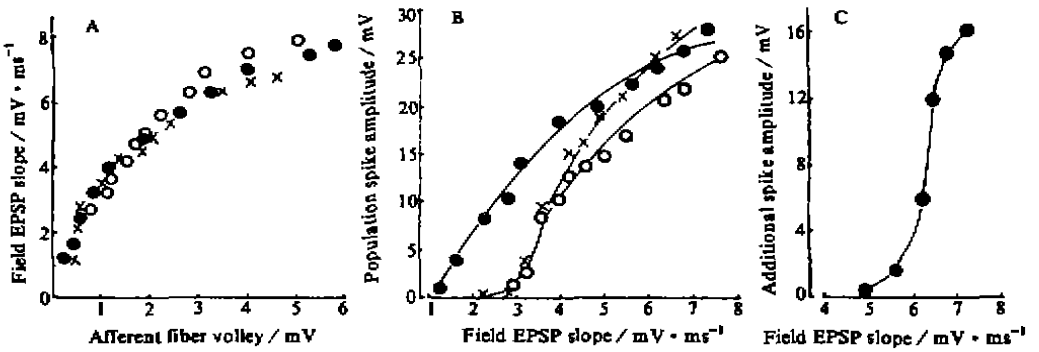

Fig 3. Input / output (I / O) curve of evoked pyramidal cell activity from one slice in CA1. (○) Control. (●) 30 min after OMF 10 nmol · L^{-1}. (✓) 30 min after OMF 10 nmol · L^{-1} + naloxone 1.0 μmol · L^{-1}. n=5 sweeps. A: related the size of field EPSP slope to afferent volley. B: showed amplitude of PS as a function of field EPSP slope. C: related size of additional PS to field EPSP slope.

Concentration–response curves for both opioids were shown in Fig 4. The leftward shift of I / O curves by OMF or morphine was dose–dependent. The concentration–response cruve for OMF rose over 2 to 3 magnitude orders of concentration and approached a maximum at a leftward shift of 0.4 to 0.5. By fitting these data with exponential curve we obtained the maximum of 0.424. The EC_{50} value (the concentration of half–maximum effect) and confidence interval for OMF or morphine were estimated by linear ragression of logarithm concentration–ΔEPSP of these data points[13]. The EC_{50} for OMF and morphine were 6.6 (2.0 – 23.4) nmol · L^{-1} and 3700 (2100 – 6300) nmol · L^{-1}, respectively. The slope of linear regression for OMF and morphine were 0.13 (0.08 – 0.18) and 0.15 (0.09 – 0.21), respectively. Hence OMF was found to be 560 times more potent than morphine as an opioid receptor agonist in CA1 of hippocampus.

In addition OMF 5 nmol · L^{-1} (n=3) also enhanced the PS evoked by antidromic stimulation of alveus, the efferent fibers from CA1 pyramidal cells.

Paired–pulse stimulation was used by several authors[12,13] to explore the mechanism involved in the excitatory action of opioid on the pyramidal cells. The alteration of excitability of the pyramidal cells after a conditioning pulse at various intervals was considered to reflect the activity of local inhibitory interneurons. In the present study, a double–pulse stimulation with identical intensity was given to Schaffer collaterals, and the effects of OMF (5 nmol · L^{-1}) on both the condition PS and the test PS were observed. As shown in Fig 5, perfusion of the slice with OMF caused: 1) and augmentation of condition population spike (A, C, from 2.2 to 2.7 mV); 2) a suppression of sequential inhibition or converting sequential inhibition to

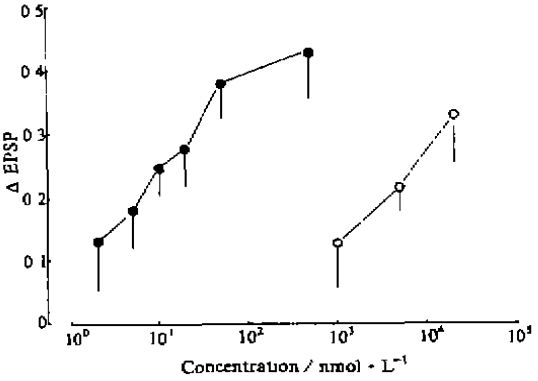

Fig 4. Concentration–response curves for slices superfused for 30 min with morphine (○) or OMF (●). n=5–7 slices. $\bar{x} \pm s$.

facilitation (A, C, the ratio of second PS versus the first increased from 0.21 to 1.3); 3) an slightly increase of sequential facilitation (B, D, the ratio from 1.3 to 1.6). Naloxone could reverse this effect completely (E, F). Similar effects were observed in 2 other slices.

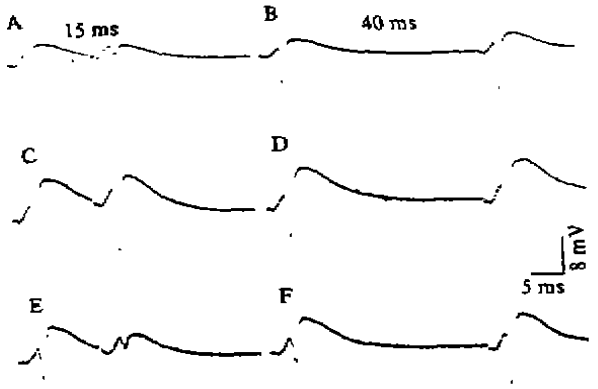

Fig 5. Paired-pulse inhibition (A) and facilitation (B) from paired-pulse stimulation to S Rad at interpulse intervals of 15 and 40 ms, respectively. The inhibition was reduced or converted to facilitation (C), and the facilitation enhanced slightly (D) 20 min after superfusion of OMF 5 nmol · L^{-1}. Naloxone 0.5 μmol · L^{-1} reversed completely the effects of OMF (E, F).

DISCUSSION

We have found that superfusion of transverse hippocampal slices *in vitro* with OMF, a novel μ selective opiate ligand, led to an increase in the excitability of CA1 pyramidal neurons in a concentration-dependent manner and the effect could be reversed by naloxone; While, it was not accompanied by any significant change in field EPSPs determined simultaneously in the dendritic layer. These results were consistent with and extended the previous studies[3-6] of opioid effects on the hippocampus *in vitro*. The fact that EC_{50} of OMF was 6.6 nmol · L^{-1} and OMF was 560 times more potent than morphine indicated that OMF had an extra high affinity with opioid receptors. This was in accordance with the results from receptor binding assays[9-10].

In view of OMF's high specificity for μ receptor and its low concentration applied in ACSF, it was suggested that the observed opioid receptor-mediated increase in pyramidal neuron excitability was attributable to μ receptor. In accordance with the results with δ agonists[13] it appeared that μ selective ligands were similar to δ specific ligands in inducing the leftward shift of I / O curves and producing additional PS. In other words, both receptor subtypes might subserve similar functions in CA1 area of hippocampus.

I / O relationship curve obtained between the presynaptic fiber volley and the field EPSP slope was not changed by OMF, this result suggested that the increased excitatory transmitter release was not involved in the mechanism by which opioids excited the pyramidal cells. The fact that OMF enhanced the antidromically activated PS was consistent with the above result.

According to the paired-pulse paradigm, the concomitant reduction of inhibition after conditioning stimulus was in accordance with a disinhibition mechanism of opioid action on pyramidal cells[14,15].

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羟甲芬太尼对大鼠海马切片 CA1 区场电位的影响

赵国民、顾家骏、池志强 (中国科学院上海药物研究所、上海 200031、中国)

提要 羟甲芬太尼(OMF)是 μ 阿片受体的专一性激动剂. OMF 对离体海马切片 CA1 区诱发场电位的影响表现为: 场兴奋性突触后电位不变而群峰电位增大; 在高刺激强度时产生第二群峰. OMF 和吗啡的 EC_{50} 分别是 6.6 和 3700 nmol · L^{-1}. 因此, OMF 的作用比吗啡强 560 倍. 成对刺激实验表明 OMF 提高锥体细胞兴奋性的机制可能与去抑制有关.

关键词 羟甲芬太尼; 吗啡; 纳络酮; 海马; 诱发电位

Corrigendum

1 1992 Jan; **13** (1) : 15. Tab 1 should be supplemented with

	Control	Cimetidine / mmol · L^{-1} 0.01	0.1	1
—	—	—	—	—
Vulnerable period / ms				
Perfusion	1.7 ± 1.4	$1.6 \pm 1.3^{*}$	$1.5 \pm 1.2^{*}$	$1.2 \pm 1.0^{*}$
Reperfusion	$5.7 \pm 2.4^{+++}$	$5.0 \pm 2.2^{*+++}$	$2.3 \pm 1.7^{***++}$	$2.0 \pm 1.7^{***+}$

2 1992 Jan; **13** (1) : 36, Fig 1, legend: (□) should be (×).

3 1992 Mar; **13** (2) : 181, Fig 2: Panel B should be C; Panel C should be B.