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Dihydroetorphine is a μ-Receptor-selective Ligand

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Abstract

The selectivity to opioid receptors of dihydroetorphine, a potent analgesic with only mild physical dependence, was investigated using radioligand binding assay and its analgesic activity in mice determined.

The relative affinity ratio of dihydroetorphine to μ-, δ- and κ- opioid receptors was 333 : 1 : 1. The analgesic effect of intracerebro-ventricular injection in mice could be antagonized by the μ-antagonist β-funaltrexamine but could not be antagonized by δ- and κ-selective antagonists naltrindole and nor-binaltorphimine.

We conclude that dihydroetorphine is a selective ligand for the μ-opioid receptor.

Dihydroetorphine is the first narcotic analgesic successfully developed and approved for production in China, and it is several thousand times more potent than morphine (Huang & Qin 1982a) as an analgesic, with a milder physical-dependence effect (Huang & Qin 1982b). Dihydroetorphine can inhibit the withdrawal symptoms of morphine-dependent animals without causing itself dependence during the treatment (Wang et al 1992). For this reason, dihydroetorphine has been successfully applied in the treatment of heroin addicts (Qin 1993; Qin et al 1994). Compared with the established morphine substitute methadone, dihydroetorphine has the advantage of quick onset, powerful effect in controlling abstinence symptoms and smooth withdrawal after several days of treatment. So far, there have been few reports on the characteristics of its binding to opioid receptors. We previously reported that dihydroetorphine was selective for the μ-opioid receptor (Wang et al 1991), but because the radioligands used at that time were not very selective for μ-, δ- and κ-receptors, the work needed further confirmation.

Materials and Methods

Radioligand binding study

Brains of Wistar rats (without cerebellum) were homogenized in 50 mM Tris-HCl buffer (25°C, pH 7·4) at 0°C for 30 s. The suspension was incubated at 35°C for 30 min and then centrifuged at 27 000 *g* for 20 min. The pellets were washed twice with Tris-HCl buffer and resuspended in this buffer. Protein concentration was determined by the Coman's method using bovine serum albumin standards (Bradford 1976).

The binding studies were carried out at 35°C in 50 mM Tris-HCl. Each assay (in triplicate) contained 0·5–0·8 mg protein, 50 μg bacitracin, the radioligand at the desired concentration with or without 10 μM etorphine, and other agents as indicated in the text, in a final volume of 1 mL. After incubation (30 min at 35°C), the reactions were terminated by rapid cooling and filtration.

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Mice in-vivo study

Kun-Ming mice (18–22 g, provided by the breeding centre in our Academy) of both sexes were used in the experiments.

Mouse tail-flick test

The thermal nociceptive stimulus was 55°C water with the latency to tail-flick or withdrawal taken as the endpoint. Mice with control latency of 1–3 s were selected for the experiment. After determination of control latency, the mice received graded intracerebroventricular doses of agonists or antagonists at various times. A cut-off time of 10 s was used; if the mouse failed to demonstrate a tail-flick in 10 s, the tail was removed from the water and that animal was assigned a maximum score. The percent analgesia at each time point was calculated as:

$$(\text{test latency} - \text{control latency})/(10 - \text{control latency}) \times 100$$

Mouse acetic acid abdominal constriction test

The method used was as previously described (Porreca et al 1987). Each mouse received intracerebroventricular administration of agonist, antagonist or saline at various times before intraperitoneal administration of acetic acid (0.6%, 10 mL kg^{-1}), and was then placed into an individual observation chamber. After 5 min, the mouse was observed for a further 5 min, and the number of abdominal constrictions displayed by each mouse was counted. Percent analgesia was determined according to the following formula:

$$(\text{mean number of constrictions in the control group} - \text{number of constrictions for each test mouse})/(\text{mean number of constrictions in the control group}) \times 100$$

where the control group was defined as all animals treated with saline.

Intracerebroventricular injections

Compounds were delivered (in a volume of 5 μL) to lightly anaesthetized mice using the modified methods as described by Pedigo et al (1975). The procedure involved cutting the

Table 1. K_D and B_{max} values of [^{3}H]DAGO, [^{3}H]DPDPE and [^{3}H]U69593 binding to rat brain membranes.

[^{3}H]Ligand (nM)	K_D (nM)	B_{max} (fmol (mg protein)$^{-1}$)
DAGO (0·5–10)	2·22 ± 0·29	174 ± 8
DPDPE (0·5–16)	7·69 ± 0·95	103 ± 4
U69593 (0·5–10)	4·55 ± 0·51	52 ± 6

Concentration range of tritiated ligands using eight points (mean ± s.e.).

scalp of the mouse, locating the bregma and injecting 2 mm caudal and 2 mm lateral to the bregma at a depth of 3 mm using a microlitre syringe.

Antagonism studies

Norbinaltorphimine and naltrindol were co-administered intracerebroventricularly with agonists, and analgesia was determined after 10 min. β-Funaltrexamine was administered by the same route 24 h before the agonists.

Drugs

Dihydroetorphine, etorphine and heroin, were synthesized by our institute; ohmefentanyl was a generous gift of Dr Chi Zhi-qiang (Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai); morphine was purchased from Qing-Hai Pharmaceutical Factory; methadone was purchased from Tian-Jing Central Pharmaceutical Factory; [D-Ala^2, *N*-Me-Phe^4, Gly-ol^5]-enkephalin (DAGO), [D-Pen^{2,5}]enkephalin (DPDPE) and bacitracin were from Sigma; U-50488H was from Upjohn, USA; β-funaltrexamine, naltrindole and norbinaltorphimine were from Research Biochemicals Inc, USA; [^{3}H]DAGO (2·03 TBq mmol^{-1}), [^{3}H]DPDPE (1·11 TBq mmol^{-1}) and [^{3}H]U69593 (1·84 TBq mmol^{-1}) were from New England Nuclear, USA. All compounds were dissolved in distilled water. Peptide solutions were frozen in small portions until immediately before use. β-Funaltrexamine, norbinaltorphimine and naltrindole were dissolved in distilled water just before use. The structures of the key drugs are shown in Fig. 1.

Data analysis

The K_D and B_{max} values in the saturation-binding study, IC50 values in the competitive-binding study and ED50 values in the mice in-vivo study were determined using the computer program described by Zhang et al (1988). K_i values were determined according to the following formula:

$$K_i = IC50/(1 + L/K_a),$$

where L was the radioligand concentration. In the in-vivo study, all data points shown are the means of 8–10 mice and the error bar represents the s.e. Respective groups were compared using analysis of variance and, where significant, differences were indicated, followed by Student's *t*-test for unpaired data.

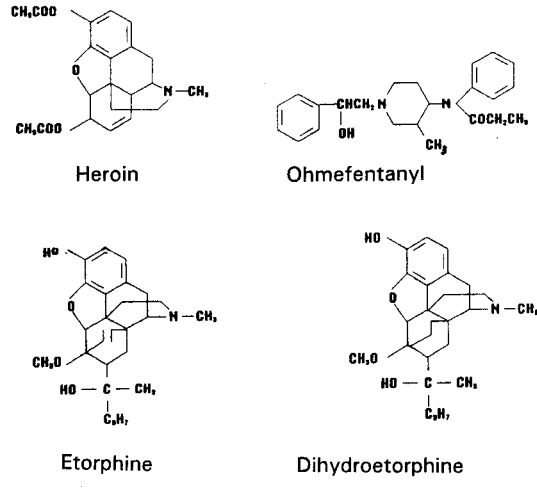

FIG. 1. Structure of drugs used in the study.

Results and Discussion

Radioligand-binding study

Equilibrium binding parameters of radiolabelled DAGO, DPDPE and U69593 to rat brain membrane fractions are reported in Table 1. Scatchard analysis of the binding isotherms showed the occurrence of only a single class of binding sites for each ligand.

DAGO, DPDPE and U69593 are commonly known as the μ-, δ- and κ-receptor-selective agonists (Handa et al 1981; Von Voigtlander et al 1983; Cotton et al 1985), so that the binding of [^{3}H]DAGO, [^{3}H]DPDPE and [^{3}H]U69593 to the brain membrane fractions represents the binding to μ-, δ- and κ-receptors respectively. The competitive inhibition effect of dihydroetorphine and several other opioid agonists against the binding [^{3}H]DAGO, [^{3}H]DPDPE and [^{3}H]U69593 were conducted at the tritiated ligand concentration equal to their K_D values. The results are shown in

Table 2. The inhibition effects of dihydroetorphine and other opioid agonists against the binding of [^{3}H]DAGO, [^{3}H]DPDPE and [^{3}H]U69593 to rat brain membranes.

Agonists	K_i value (M)		
	[^{3}H]DAGO	[^{3}H]DPDPE	[^{3}H]U69593
Dihydroetorphine	(9·69 ± 0·45) × 10^{-12}	(3·23 ± 1·15) × 10^{-9}	(2·24 ± 0·93) × 10^{-9}
Ohmefentanyl	(8·04 ± 1·53) × 10^{-11}	(4·64 ± 2·14) × 10^{-8}	(1·08 ± 0·11) × 10^{-6}
DAGO	(4·87 ± 1·89) × 10^{-10}	(4·30 ± 0·36) × 10^{-8}	(1·63 ± 0·75) × 10^{-6}
Etorphine	(3·62 ± 0·03) × 10^{-10}	(1·62 ± 0·01) × 10^{-9}	(3·75 ± 0·74) × 10^{-9}
Morphine	(3·58 ± 1·28) × 10^{-9}	(1·70 ± 0·36) × 10^{-7}	(8·53 ± 2·78) × 10^{-7}
Methadone	(4·76 ± 1·45) × 10^{-9}	(1·01 ± 0·14) × 10^{-7}	(7·31 ± 2·88) × 10^{-7}
Heroin	(7·36 ± 2·24) × 10^{-9}	(8·88 ± 0·40) × 10^{-8}	(1·19 ± 0·13) × 10^{-7}

Mean ± s.e., n = 3.

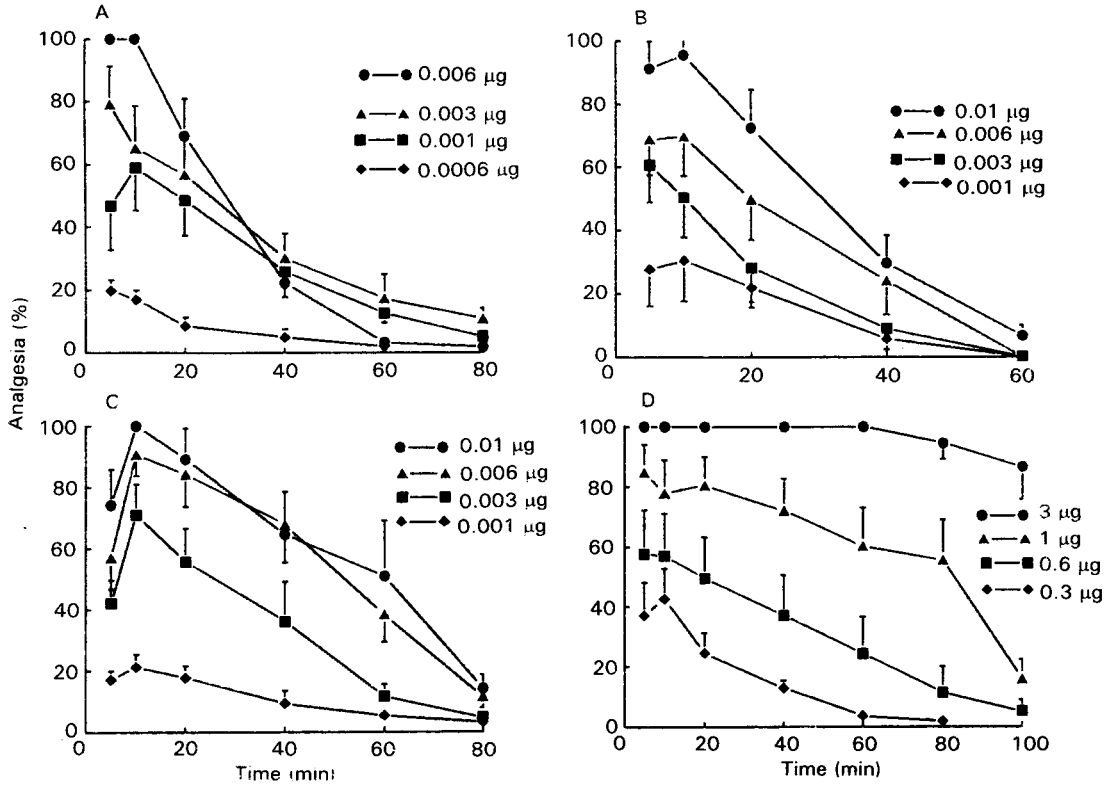

FIG. 2. Time course of analgesia (55°C warm water tail-flick test) of: A, dihydroetorphine; B, etorphine; C, ohmefentanyl; D, morphine after intracerebroventricular injection (μg per mouse) in mice. Values represent mean ± s.e.

Table 2. The relative affinity ratio of dihydroetorphine to μ-, δ- and κ-opioid receptors was 333 : 1 : 1. Compared with DAGO and ohmefentanyl, the most selective μ-opioid receptor agonists, the K_i value of dihydroetorphine against [^{3}H]DAGO was lowest, but because dihydroetorphine also had high affinity to δ- and κ-opioid receptors, the selectivity of dihydroetorphine to μ-opioid receptors was not outstanding. When comparing the selectivity for μ- and δ-receptors among these ligands, the selectivity of dihydroetorphine to μ-opioid receptors was between that for DAGO and ohmefentanyl.

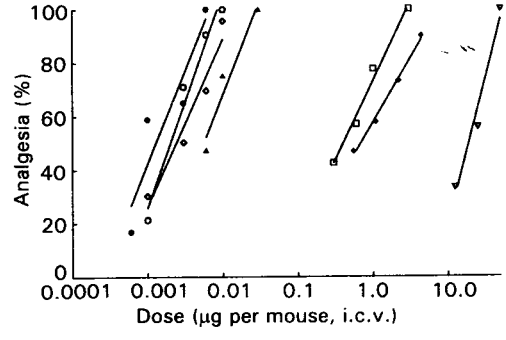

FIG. 3. Analgesic dose–response lines of dihydroetorphine and other opiate agonists. The regression lines represent responses seen 10 min after intracerebroventricular injection. The analgesic effects for all agonists were tested in mice on 55°C warm water tail-flick assay with the exception of that for U-50488H, which was tested in the acetic acid abdominal constriction assay. ●, Dihydroetorphine; ○, ohmefentanyl; ◇, etorphine; ▲, DAGO; □, morphine; ◆, U-50488H; △, DPDPE.

Etorphine is an analogue of dihydroetorphine, but etorphine is known as a universal ligand (Magnan et al 1982), binding equally to μ-, δ- and κ-receptors. Our results indicated that the minor change in structure can result in changes in the characteristic receptor binding. Other ligands such as morphine, heroin and methadone have affinities to μ-, δ- and κ-opioid receptors in the order $\mu > \delta > \kappa$, but the differences are small.

Mice in-vivo study

For further research on dihydroetorphine analgesia in-vivo, we conducted the mice in-vivo analgesic study. Light ether anaesthesia and the manipulation associated with the injection had no effect on mice tail-flick latency. Dihydroetorphine and most of the other agonists showed an analgesic effect at 5 min after administration, and reached a maximum at 10 min (Fig. 2). Dose–response lines for intracerebroventricular analgesia at 10 min were constructed from time–response curves and are shown in Fig. 3. The dose–response relationship for each of the agonists showed linear correlation, with the effect of dihydroetorphine found to be the most potent. The ED50 value (and 95% confidence limit) of each agonist was calculated from each line and is reported in Table 3. The effect of U-50488H on the tail-flick response was weak and did not reach 100% analgesia even at high doses. However, in the acetic acid abdominal constriction assay, a lower dose of U-50488H could produce evident analgesia (Table 3).

The results of the experiments with μ-, δ- and κ-selective

Table 3. Analgesic ED50 values (and 95% confidence limit) for opiate agonists after intracerebroventricular administration.

Agonists	ED50 (ng per mouse)	
	Tail-flick assay	Abdominal constriction assay
Dihydroetorphine	1·05 (0·15–7·27)	1·35 (0·86–2·14)
Ohmefentanyl	1·83 (0·29–1·17)	3·95 (1·17–13·37)
Etorphine	2·21 (0·56–8·76)	3·63 (0·24–54·01)
DAGO	6·92 (0·77–6·24)	
Morphine	462·55 (123·64–1730·49)	
U-50488H	64574·46 (1165·29–357839·30)	722·40 (344·41–1515·24)
DPDPE	16618·36 (259·73–1063301·00)	

Table 4. The percent analgesia of agonists (ED50) coadministered with different antagonists.

Treatment		Agonists				
		DAGO	Ohmefentanyl	Dihydroetorphine	Etorphine	Morphine
Saline		100	97·2 ± 2·8	100	100	100
Funaltrexamine[a,c]	50	77·9 ± 11·7*	73·9 ± 9·2*	64·5 ± 11·7*	50·4 ± 11·5*	64·1 ± 13·9*
(ng per mouse)	100	52·9 ± 12·1***	55·5 ± 10·1***	53·6 ± 8·2**	40·9 ± 8·6***	60·9 ± 10·5**
	250	24·5 ± 2·7***	30·1 ± 7·8***	23·1 ± 3·5***	33·7 ± 5·5***	34·2 ± 8·1***
		DPDPE				
Saline		99·2 ± 0·8	97·2 ± 2·8	100	100	100
Naltrindole[b,c]	5	79·6 ± 14·2				
(μg per mouse)	10	47·4 ± 10·5***				92·1 ± 5·7
	20	24·4 ± 11·2***	100	100	81·6 ± 9·5	75·2 ± 9·4
	40		96·4 ± 3·1	100	66·4 ± 6·8*	45·3 ± 12·9***
		U-50488H				
Saline		89·9 ± 3·5	97·2 ± 2·8	100	100	
nor-binaltorphimine[b,d]	1	82·0 ± 6·2				
(μg per mouse)	3	49·4 ± 10·6***				
	5				93·6 ± 4·1	
	10	31·3 ± 8·5**			77·7 ± 10·2*	
	20		96·8 ± 2·6	99·9 ± 0·1	80·3 ± 5·8**	
	40		98·7 ± 1·5	90·5 ± 5·9	55·3 ± 5·9***	

[a] Intracerebroventricularly 24 h before testing. [b] Intracerebroventricularly with agonists 10 min before testing. [c] In mouse tail-flick test. [d] In mouse acetic acid abdominal constriction test. Mean ± s.e. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs saline, n = 10.

antagonists are shown in Table 4. β-Funaltrexamine significantly antagonized the effect of intracerebroventricular DAGO, morphine, etorphine, ohmefentanyl and dihydroetorphine. Naltrindole antagonized the effect of DPDPE, morphine and etorphine without affecting the effect of ohmefentanyl or dihydroetorphine even at high doses. As for naltrindole in the mouse acetic acid abdominal constriction test, norbinaltorphimine significantly antagonized the effect of U-50488H and etorphine without altering the effects of ohmefentanyl or dihydroetorphine. None of the antagonists produced any observable effect when given alone.

The μ-opioid receptor is known to play a role in physical dependence; opioid agonists with evident physical dependence such as morphine and heroin have a high affinity for the μ-opioid receptor. Our results show dihydroetorphine is selective for the μ-opioid receptor. This rules out the possibility that dihydroetorphine's low physical dependence is due to any action at opioid receptors other than the μ-receptor. However, it may also be possible that dihydroetorphine acts at a different μ-receptor subtype as compared with morphine or heroin. Ling et al (1984) have reported that a μ_1-receptor may have little connection with the development of physical dependence; that is, physical dependence is mainly mediated by the μ_2-receptor.

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