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Immunosuppressive effects of dihydroetorphine, a potent narcotic analgesic, in dihydroetorphine-dependent mice

Wei-Ran Wu [a,*], Ji-Wang Zheng [b], Na Li [a], Hui-Qing Bai [c], Ke-Ren Zhang [c], Ying Li [c]

[a] *Department of Pharmacology, Xi'an Medical University, Xi'an 710061, China*
[b] *Department of Neuropharmacology, National Institute on Drug Dependence, Beijing 100083, China*
[c] *Department of Immunology, Beijing Medical University, Beijing 100083, China*

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Abstract

The immunomodulatory effects of dihydroetorphine were systematically investigated in subchronically treated mice. In a dose-dependent fashion, dihydroetorphine (total doses at 444.5, 889 and 1778 μg/kg) lowered the increase of body weight, decreased the weight of the spleen and thymus, weakened the delayed-type hypersensitivity, reduced the generation of antibody-forming cells, inhibited splenic lymphocyte proliferation induced by concanavalin A and lipopolysaccharide, suppressed the production of interleukin-2 in the supernatant of splenocytes induced by concanavalin A, and depleted the ratio of $CD4^+$ and $CD8^+$ subpopulations. Moreover, the physical dependence on dihydroetorphine was also evaluated to confirm that the immunosuppression was concomitant with the addiction to the drug. These results demonstrate that subchronic treatment with dihydroetorphine dose dependently suppresses both humoral and cell-mediated immune function, and that the immunosuppressive effects of dihydroetorphine are much more potent than those of morphine.

Keywords: Dihydroetorphine; Immunosuppression; Dependence

1. Introduction

Dihydroetorphine, 7α-[1-(*R*)-hydroxy-1-methylbutyl] 6,14-endo-ethanotetrahydrooripavine, is a morphine-like thebaine derivative. It is a potent selective μ-opioid receptor agonist, whose affinity ratio for μ, δ and κ-opioid receptors is 1951:2:1 (Wang et al., 1991; Yuan et al., 1995). As the most powerful narcotic analgesic synthesized up to now, the ED_{50} of dihydroetorphine in mice is only 1/12 000 of that of morphine (Bentley and Hardy, 1967; Huang and Qin, 1982a). However, because of its possible high abuse potential and toxicity (Blane et al., 1967; Cowan et al., 1971; Jasinski et al., 1975), it had not been used in clinical therapy until it was exploited in China (Huang and Qin, 1982a,b).

Dihydroetorphine has been used not only as an analgesic, but also as a detoxification agent since the 1980s (Qin, 1996). As a potent analgesic, it has been used during and after operations and for the treatment of cancer, acute abdomen and other diseases (Qian and Zhang, 1983; Wu and Sun, 1991; Ren et al., 1993). As a detoxification agent, dihydroetorphine has been independently or jointly used in heroin and other opioid addictions (Sha et al., 1993; Su et al., 1994a,b). However, concomitant with its application, there was a sharp increase in the number of dihydroetorphine abusers with infections (Li et al., 1995; Liu et al., 1995) because its clinical use was actually based on insufficient assessment of its dependence and abuse liability (Huang and Qin, 1982b) and on unilateral evaluation of its therapeutic effects in morphine-dependent animals (Wang et al., 1992). Under the circumstances, recent research has paid more attention to its abuse potential (Zheng and Zhang, 1995; Wu et al., 1998a,b) and immunosuppressive effects (Wu et al., 1998a,b,c).

There were few data on the immunomodulatory effects and mechanisms of dihydroetorphine before our recent reports. We demonstrated that dihydroetorphine had potent

* Corresponding author. Group of Neuropharmacology, 1st Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 294 Tai-Yuan Rd., Shanghai 200031, China. Tel.: +86-21-6431-1833 ext. 309; Fax: +86-21-6437-0269; E-mail: wrwu@yahoo.com

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immunosuppressive effects in rats receiving automatic injections of the drug (Wu et al., 1998a), which were consistent with the physical dependence seen in i.v. abusers, in intravenous self-administration rats (Wu et al., 1998b), which were consistent with the psychic dependence seen in i.v. abusers, and in acutely administered mice (Wu et al., 1998c), which could be effectively blocked by the μ-opioid receptor antagonist naloxone and the α-adrenoceptor antagonist phentolamine, but not by the β-adrenoceptor antagonist propranolol. In the present study, for the first time, the immunosuppressive effects of dihydroetorphine were systematically investigated on both humoral and cell-mediated immune functions in subchronically treated mice.

2. Materials and methods

2.1. Drugs and chemicals

Dihydroetorphine hydrochloride, morphine sulphate and naloxone sulphate were supplied by the National Institute on Drug Dependence (Beijing, China). [^{3}H]thymidine (20 Ci/mM) was purchased from the Shanghai Institute of Nuclear Research of the Chinese Academy of Sciences (Shanghai, China). Agarose, concanavalin A, lipopolysaccharide, and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide were purchased from Sigma Chemical (St. Louis, MO). Cytotoxic T lymphocyte line, fluorescein isothiocyanate conjugated anti-L3T4 and anti-LYT2 antibodies were supplied by the Department of Immunology of Beijing Medical University (Beijing, China).

2.2. Animals and drug administration

Female C57BL/6J mice (20–24 g) from the Experimental Animal Center of Beijing Medical University (Beijing, China) were used for all studies. Food pellets and water were provided ad libitum, and a 12-h light–dark cycle was maintained. The mice were housed in a constantly controlled temperature (22°–26°C) and relative humidity (40–60%). Mice were randomly divided into five groups ($n = 9$). The saline group was injected s.c. with 0.1 ml/10 g of 0.9% saline. The morphine group and the dihydroetorphine groups receiving a low total dose (dihydroetorphine$_l$), a median total dose (dihydroetorphine$_m$) and a high total dose (dihydroetorphine$_h$) were given progressively increasing daily doses as shown in Table 1. All mice were injected s.c. four times a day (q.i.d.) at 0800, 1200, 1600, 2000 h respectively for 14 days. On day 15, all mice were killed for the assessment of immune function. An additional three groups ($n = 8$) treated with saline, morphine, and the high total dose of dihydroetorphine (dihydroetorphine$_h$), administered as described above, were used to evaluate physical dependence on dihydroetorphine.

Table 1
Doses of dihydroetorphine injected in subchronically treated mice

Treatment group	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Total dose
Morphine (mg kg^{-1})	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	1778.0
Dihydroetorphine$_l$ (μg kg^{-1})	0.375	0.75	1.5	2	3	4	6	8	12	16	24	32	48	64	444.5
Dihydroetorphine$_m$ (μg kg^{-1})	0.75	1.5	2	3	4	6	8	12	16	24	32	48	64	96	889.0
Dihydroetorphine$_h$ (μg kg^{-1})	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	1778.0

Drugs were all dissolved with saline. Each animal was injected 0.1 ml/10 g, s.c., q.i.d.

Table 2
Body-weight changes of mice treated subchronically with dihydroetorphine

Treatment group	Total dose	Weight change (g)	Suppression (%)
Saline	–	6.43 ± 2.36	–
Morphine	1778 (mg kg^{-1})	2.80 ± 1.16^{c}	56.5
Dihydroetorphine$_{l}$	444.5 (μg kg^{-1})	1.53 ± 1.24^{c}	76.2
Dihydroetorphine$_{m}$	889 (μg kg^{-1})	0.67 ± 0.92^{cd}	89.6
Dihydroetorphine$_{h}$	1778 (μg kg^{-1})	0.06 ± 1.31^{ce}	99.1

The data represent the means ± S.D. ($n = 7$).
[c] $P < 0.001$ vs. saline group.
[d] $P < 0.05$, [e] $P < 0.01$ vs. morphine group.

2.3. Measurement of body weight

Each mouse was weighed on day 0, 3, 6, 9, 12 and 15 (before death) at 0800 to study the changes in body weight.

2.4. Measurement of spleen and thymus weights

After the mice were killed on day 15, the spleen and thymus of each mouse were collected and weighed immediately wet weight.

2.5. Measurement of delayed-type hypersensitivity

This assay was performed as described by Langrange et al. (1974). Each mouse was immunized with 200 μl sheep red blood cells (1×10^8 cells/animal, i.p.) on day 1. On day 14, the thickness of the left rear foot of each mouse was measured, and then the sole of the foot was injected with 20 μl sheep red blood cells (1×10^8 cells/animal, s.c.). The thickness of the foot was measured again 24 h later, and the change was calculated.

2.6. Assessment of humoral immune response

The humoral immune response was assessed by measuring the production of immunoglobulin M antibody-forming cells in response to sheep red blood cells with a modified method (Lepkowitz and Chiang, 1975; Pruett et al., 1992). Briefly, splenocyte suspensions were prepared in Hank's balanced salt solution by pressing the spleens through a stainless steel mesh (40 μm pores). Following two washes and centrifugations, the splenocytes from each mouse were resuspended in 5 ml Hank's balanced salt solution. Agarose (1%) was dissolved and mixed with an equal volume of 50% Hank's balanced salt solution in a water bath at 45°C, and distributed in small test tubes (5 ml/tube). Sheep red blood cells (50 μl; 1×10^8/tube) and 50 μl splenocyte suspension were added to each tube and mixed with the dissolved agarose, then immediately poured on a glass slide previously coated with 0.5% agarose. The slides were incubated for 1 h at 37°C, and then guinea pig complement (1/10, v/v) was added to the mixtures and incubated for another 1 h at 37°C. At the end of the culture period, the plaques of antibody-forming cells were quantified and expressed as antibody-forming cells/10^6 splenocytes.

2.7. Lymphocyte proliferation assay

A modification of the method of Bayer et al. (1990) was used. Spleens of mice were removed and placed in cold culture media (RPMI-1640) containing 1% fetal bovine serum and gentamicin (0.5 mg/ml). Cells were gently teased loose and passed through a stainless steel mesh (40 μm pores) to remove cell aggregates and connective tissue. Following two washes with phosphate-buffered saline, the cell suspensions were adjusted to a final concentration of 3×10^6 cells/ml in RPMI-1640 with 1% fetal bovine serum. To determine splenic lymphocyte proliferation, 200 μl of the cell suspensions was added to each well of a 96-well microtiter plates containing concanavalin A 5 μg/ml or lipopolysaccharide 1 μg/ml (final concentration). Cultures were carried out in triplicate and were incubated for 48 h at 37°C with 5% CO_2. Then [^{3}H]thymidine (0.2 μCi/well) was added and the cultures were incubated for an additional 24 h at 37°C. Radioactive deoxyribonucleic acids were collected on Whatman GF/C filters, using a cell harvester, and radioactivity was measured in a liquid scintillation counter (Model 1215, Beckman).

2.8. Lymphokine bioassay

To measure interleukin-2 production in cultures, a modification of the method of Bunjes et al. (1981) was used. Aliquots (1 ml/well) of the splenic cell suspensions (4 ×

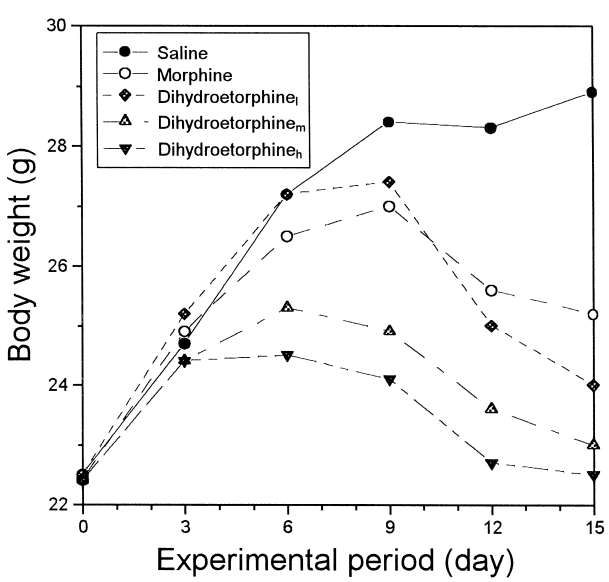

Fig. 1. The changes in body weight during 14 days of treatment with dihydroetorphine in mice. Data represent the means, $n = 9$.

Table 3
Effects of dihydroetorphine on spleen and thymus weights in subchronically treated mice

Treatment group	Spleen weight (g)	Spleen weight/body weight	Thymus weight (g)	Thymus weight/body weight
Saline	0.15 ± 0.04	0.0049 ± 0.0012	0.12 ± 0.02	0.0042 ± 0.0006
Morphine	0.10 ± 0.03^{a}	0.0045 ± 0.0008	0.06 ± 0.02^{c}	0.0025 ± 0.0006^{c}
$\text{Dihydroetorphine}_{l}$	0.10 ± 0.03^{a}	0.0044 ± 0.0009	0.05 ± 0.02^{c}	0.0022 ± 0.0007^{c}
$\text{Dihydroetorphine}_{m}$	0.10 ± 0.02^{b}	0.0043 ± 0.0010	0.05 ± 0.01^{c}	0.0019 ± 0.0006^{c}
$\text{Dihydroetorphine}_{h}$	0.08 ± 0.04^{cd}	0.0035 ± 0.0006^{ad}	0.04 ± 0.01^{c}	0.0018 ± 0.0005^{cd}

The data represent the means ± S.D. ($n = 7$).
[a] $P < 0.05$, [b] $P < 0.01$, [c] $P < 0.001$ vs. saline group.
[d] $P < 0.05$ vs. morphine group.

10^6 cells/ml) were added to 24-well microtiter plates containing 1 ml concanavalin A (5 μg/ml). After the cultures in triplicate were incubated for 24 h at 37°C with 5% CO_2, the supernatants were harvested. Then 100 μl of diluted supernatant was added to 96-well microtiter plates containing 100 μl of cells of the active cytotoxic T lymphocyte line (7×10^4 cells/ml); in control wells 100 μl RPMI-1640 was substituted for the supernatant samples. All samples were incubated in triplicate for an additional 48 h and then 10 μl 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well. The optical density of the samples was measured with a microplate reader (Model 3550, BIO-RAD) at $A_{570\ nm}$.

2.9. Immunofluorescent staining for flow cytometric analysis

A modified method as described previously (Freier and Fuchs, 1993; Carr and France, 1993) was used. Splenocytes were adjusted to a concentration of 1×10^7 cells/ml and 100 μl was mixed with 100 μl of fluorescein isothiocyanate conjugated anti-L3T4 and 100 μl of fluorescein isothiocyanate conjugated anti-LYT2 antibodies to stain for $CD4^+$ and $CD8^+$ T cell subsets. The cells were incubated on ice for 30 min and were then washed and resuspended twice with phosphate-buffered saline containing 0.1% bovine serum albumin and 0.05 M azide. Then 100 μl of propidium iodide (0.1 mg/ml; diluted 1:15 in phosphate-buffered saline) was added to the resuspended cells, which were incubated on ice for another 30 min. Then the cells were washed and resuspended twice again. The final resuspensions were analyzed with a FACSCAN flow cytometer (Becton/Dickinson, San Jose, CA). A gate was set up to exclude nonviable cells from the analysis based on propidium iodide staining; 10 000 viable cells were analyzed.

2.10. Naloxone-precipitated jumping activity

To evaluate the physical dependence on dihydroetorphine of subchronically treated mice and to compare the dependence with that of morphine, additional groups of mice ($n = 8$) treated with saline, morphine, and dihydroetorphine (high total dose, $\text{dihydroetorphine}_{h}$) were precipitated with the μ-opioid receptor antagonist naloxone (4 mg/kg, s.c.) on day 15. The jumping times of mice between the 5th and 15th min after naloxone precipitation were calculated as described by Huang and Qin (1982b).

2.11. Statistical analysis

Data are expressed as means ± S.D. Statistical analyses were performed using an one-way analysis of variance followed by Dunnett's test to identify significant differ-

Table 4
Effects of dihydroetorphine on delayed-type hypersensitivity induced by injection of sheep red blood cells in subchronically treated mice

Treatment group	Total dose	Thickness changed (mm)	Suppression (%)
Saline	–	0.54 ± 0.16	–
Morphine	1778 (mg kg^{-1})	0.35 ± 0.10^{a}	35.2
$\text{Dihydroetorphine}_{l}$	444.5 ($\mu\text{g kg}^{-1}$)	0.34 ± 0.07^{a}	37.0
$\text{Dihydroetorphine}_{m}$	889 ($\mu\text{g kg}^{-1}$)	0.29 ± 0.11^{b}	46.3
$\text{Dihydroetorphine}_{h}$	1778 ($\mu\text{g/kg}^{-1}$)	0.27 ± 0.08^{bd}	50.0

The data represent the means ± S.D. ($n = 7$).
[a] $P < 0.05$, [b] $P < 0.01$ vs. saline group.
[d] $P < 0.05$ vs. morphine group.

Table 5
Effects of dihydroetorphine on the antibody-forming cell response induced by sheep red blood cells in subchronically treated mice

Treatment group	Total dose	Antibody-forming cells/10^6 splenocytes	Suppression (%)
Saline	–	1862.1 ± 88.3	–
Morphine	1778 (mg kg^{-1})	1520.6 ± 74.8^{b}	18.3
Dihydroetorphine$_l$	444.5 (μg kg^{-1})	1548.8 ± 80.6^{b}	16.8
Dihydroetorphine$_m$	889 (μg kg^{-1})	631.0 ± 23.7^{cf}	66.1
Dihydroetorphine$_h$	1778 (μg kg^{-1})	416.9 ± 24.4^{cf}	77.6

The data represent the means ± S.D. ($n = 7$).
[b]$P < 0.01$, [c]$P < 0.001$ vs. saline group.
[f]$P < 0.001$ vs. morphine group.

ences between experimental groups. $P < 0.05$ was considered significant.

3. Results

3.1. Effects of dihydroetorphine on body weight

Dihydroetorphine given subchronically at total doses of 444.5, 889 and 1778 μg/kg s.c. significantly ($P < 0.001$) suppressed the increase in the body weight of mice in a dose-dependent fashion (Table 2). During the experimental period (14 days), the more the daily dose of dihydroetorphine was increased, the more the body weight was decreased (Fig. 1).

3.2. Effects of dihydroetorphine on the spleen and thymus weights

The spleen and thymus weights and the ratios of spleen weight/body weight and thymus weight/body weight were all decreased by subchronic treatment with dihydroetorphine at total doses of 444.5, 889 and 1778 μg/kg s.c. (Table 3).

3.3. Effects of dihydroetorphine on delayed-type hypersensitivity

Dihydroetorphine given subchronically at total doses of 444.5, 889 and 1778 μg/kg s.c. significantly weakened the delayed-type hypersensitivity induced by sheep red blood cells in a dose-dependent fashion (Table 4).

3.4. Effects of dihydroetorphine on antibody-forming cells

The antibody-forming cell response induced by sheep red blood cells was inhibited by dihydroetorphine given at total doses of 444.5, 889, and 1778 μg/kg s.c. in a dose-dependent fashion (Table 5).

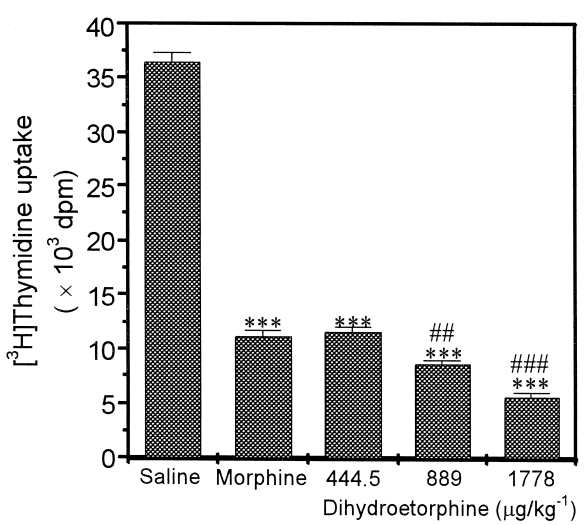

Fig. 2. Effects of dihydroetorphine on splenic lymphocyte proliferation induced by concanavalin A in subchronically treated mice. Data represent the means ± S.D., $n = 9$. $^{***}P < 0.001$ vs. saline group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. morphine group.

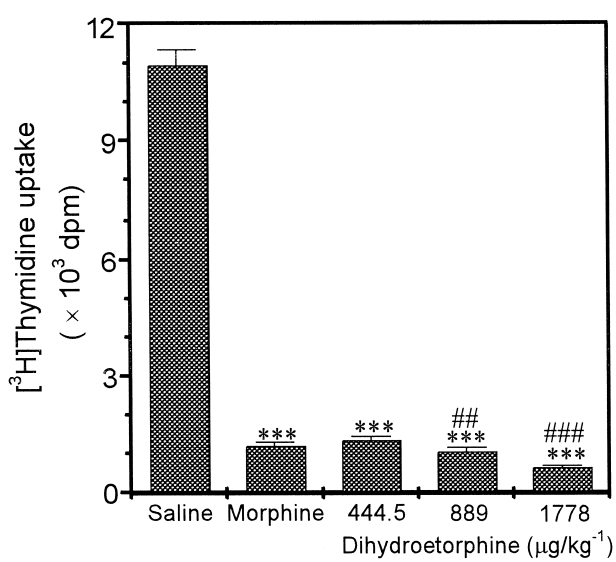

Fig. 3. Effects of dihydroetorphine on splenic lymphocyte proliferation induced by lipopolysaccharide in subchronically treated mice. Data represent the means ± S.D., $n = 9$. $^{***}P < 0.001$ vs. saline group. $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. morphine group.

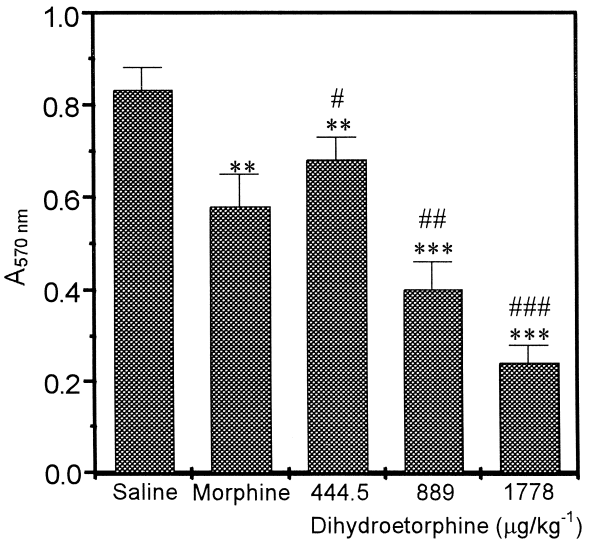

Fig. 4. Effects of dihydroetorphine on the concanavalin A-stimulated production of interleukin-2 in the supernatants of splenocytes from subchronically treated mice. Data represent the means ± S.D., $n = 9$. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. saline group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. morphine group.

3.5. Effects of dihydroetorphine on lymphocyte proliferations

Dihydroetorphine given subchronically at total doses of 444.5, 889 and 1778 μg/kg s.c. dose dependently suppressed the splenic lymphocyte proliferation induced by concanavalin A (5 μg/ml). [^{3}H]thymidine uptake was decreased by 68.5%, 76.5% and 84.5% respectively ($P < 0.001$) compared with that of the saline group, and was also significantly different from that of the morphine group, which was reduced by 69.5% (Fig. 2). The splenic lymphocyte proliferation induced by lipopolysaccharide (1 μg/ml) was also suppressed. [^{3}H]thymidine uptake was reduced by 87.8%, 90.4% and 94.4% respectively ($P < 0.001$) compared with that of the saline group, and the [^{3}H]thymidine uptake of the dihydroetorphine$_m$ and dihydroetorphine$_h$ groups were also significantly different from that of the morphine group, which was reduced by 89.4% (Fig. 3).

Table 7
Naloxone-precipitated jumping activity in mice pretreated subchronically with dihydroetorphine

Treatment group	Total dose	Jumping times
Saline	–	0.33 ± 0.52
Morphine	1778 mg kg^{-1}	74.00 ± 5.69^{c}
Dihydroetorphine$_h$	1778 μg kg^{-1}	50.67 ± 5.28^{cf}

The data represent the means ± S.D. ($n = 8$).
$^{c}P < 0.001$ vs. saline group.
$^{f}P < 0.001$ vs. morphine group.

3.6. Effects of dihydroetorphine on lymphokine production

Dihydroetorphine given subchronically at total doses of 444.5, 889 and 1778 μg/kg s.c. dose dependently suppressed the concanavalin A (5 μg/ml)-induced production of interleukin-2 in the supernatant of splenocytes in mice. The A_{570nm} values were reduced by 18.1%, 51.8% and 71.1% respectively compared with those of the saline group and were also significantly different from that of the morphine group, which was reduced by 30.1% (Fig. 4).

3.7. Effects of dihydroetorphine on lymphocyte subsets

Dihydroetorphine given subchronically at total doses of 444.5, 889 and 1778 μg/kg s.c. dose dependently decreased the number of CD4$^+$ cells, increased the number of CD8$^+$ cells, and thus reduced the ratio of CD4/CD8 by 38.3%, 51.9% and 59.0% respectively compared with the ratio of the saline group. Morphine at a total dose 1778 mg/kg s.c. also decreased the number of CD4$^+$ cells, increased the number of CD8$^+$ cells, and decreased the ratio of CD4/CD8 by 32.2%, but its effects were weaker than those of dihydroetorphine (Table 6).

3.8. Effects of naloxone-precipitated jumping activity

Dihydroetorphine given subchronically at a total dose of 1778 μg/kg s.c. and morphine at a total dose of 1778 mg/kg s.c. both made mice physically dependent. The jumping times of the morphine group were more than

Table 6
Modulation of phenotypically defined splenic lymphocyte subpopulations in mice treated subchronically with dihydroetorphine

Treatment group	Phenotype marker		CD4/CD8	Suppression (%)
	CD4	CD8		
Saline	34.94 ± 2.83	19.12 ± 0.76	1.83 ± 0.08	–
Morphine	27.51 ± 1.23^{b}	22.13 ± 1.03	1.24 ± 0.06^{b}	32.2
Dihydroetorphine$_l$	29.22 ± 2.87^{b}	26.14 ± 2.03^{bd}	1.13 ± 0.15^{bd}	38.3
Dihydroetorphine$_m$	25.14 ± 1.21^{cd}	28.68 ± 1.23^{cf}	0.88 ± 0.03^{cf}	51.9
Dihydroetorphine$_h$	22.50 ± 1.94^{ce}	30.40 ± 2.08^{cf}	0.75 ± 0.10^{cf}	59.0

Values represent the means ± S.D. ($n = 6$) in percent positive cells per 10 000 gated events.
$^{b}P < 0.01$, $^{c}P < 0.001$ vs. saline group.
$^{d}P < 0.05$, $^{e}P < 0.01$, $^{f}P < 0.001$ vs. morphine group.

those of the dihydroetorphine$_h$ group ($P < 0.001$) (Table 7), but dihydroetorphine was much more potent than morphine, at the same doses, in inducing dependence.

4. Discussion

The present study systematically investigated the effects of dihydroetorphine on immune functions in subchronically treated mice. To mimic the actual situation in dihydroetorphine addicts, and taking into consideration the ED_{50}, half-life and tolerance to dihydroetorphine, we designed an animal model of dihydroetorphine-dependence in mice, i.e., dihydroetorphine was administered (s.c., q.i.d.) for 14 days at progressively increasing daily doses, to examine the immunomodulation by dihydroetorphine and compare it with that of morphine. As primary immune parameters, body weight, spleen/body and thymus/body weight ratios were used. Dihydroetorphine caused a dose-dependent inhibition of the increase in body and spleen and thymus weights, which indicated that it could cause immunosuppression. This inhibition of weight gain is consistent with the clinical symptoms of dihydroetorphine addicts, such as thinness and loss of body weight (Wang et al., 1994; Qin, 1996). Cell-mediated immune function was assessed by determining sheep red blood cell-induced delayed-type hypersensitivity, concanavalin A-induced splenic lymphocyte proliferation and lymphokine production. The results showed that dihydroetorphine significantly inhibited the delayed-type hypersensitivity response induced by sheep red blood cells and the proliferative response of splenocytes to the T-cell mitogen concanavalin A. It also inhibited the T-cell activation and the secretion of interleukin-2 in a dose-dependent fashion. Humoral-mediated immune function was assessed by measuring the sheep red blood cell-induced antibody-forming cell response and the splenic lymphocyte proliferation response to the B-cell mitogen lipopolysaccharide. The data indicated that dihydroetorphine significantly suppressed these responses in a dose-dependent fashion. The results suggested that dihydroetorphine indirectly reduced the production of antibody-forming cells, mediated by inhibition of helper T-cell function, and directly inhibited B-cell function and activated suppressor T-cell function. It was supposed that its suppression of B-cell proliferation was related to its inhibition of interleukin-2 secretion by helper T-cells. Furthermore, flow cytometric analysis of cells labelled with monoclonal antibody was used to define the two major subpopulations of splenocytes and their responses to dihydroetorphine. The data demonstrated that dihydroetorphine dose dependently decreased the total number of $CD4^+$ cells, increased the total number of $CD8^+$ cells, and hence significantly decreased the CD4/CD8 ratio. The data verified the supposition that dihydroetorphine could inhibit Th and activate Ts functions to exert further immunosuppressive effects.

In addition, naloxone-precipitated jumping activity was used to assess the dependence on dihydroetorphine and to examine the relation between immunosuppression and dependence on opiates with different analgesic properties. Dihydroetorphine induced significant physical dependence in mice. Moreover, for the same dose, the physical dependence potential of dihydroetorphine was nearly 500 times stronger than that of morphine, and similarly dihydroetorphine induced much more potent immunosuppressive effects than morphine did. The immunosuppressive potency of dihydroetorphine was nearly 4000 times greater than that of morphine. These data are consistent with our previous results for the immunosuppressive effects of dihydroetorphine in rats (Wu et al., 1998a,b,c) and in its abusers (Cao et al., 1995; Chen et al., 1995; Liu et al., 1995). Deneau and Seevers (1964) pointed out that the analgesic potency of a narcotic agent was related to its abuse potential, to which we can now add that it is also related to the immunosuppressive effect of the agent. Although there were many arguments for the dissociation of opiate analgesia, tolerance and dependence (Ling et al., 1984; Kaneto et al., 1985; Shen and Crain, 1994), their relation with immunomodulation has not been paid attention. Our recent research (Wu et al., 1998a,b) and the present study concentrated on the immunomodulatory effects of dihydroetorphine, and compared its immunosuppressive and dependence potential with that of morphine. Our data demonstrated that the immunosuppression produced by dihydroetorphine was in proportion to its dependence, which were both much stronger than those of morphine. Moreover, other data (Zheng and Zhang, 1995; Wu et al., 1998b) also show that the psychic dependence potential of dihydroetorphine is nearly 500 times greater than that of morphine, which was parallel to its physical dependence potential reported in this study. Thus, we hereby advance a new viewpoint that the immunosuppressive potency of opiate analgesics is directly interrelated with their dependence potential in vivo.

Our present results on the immunosuppression caused by dihydroetorphine are also consistent with data for morphine. In rodents, exposure to morphine in vivo can significantly decrease the weights of the body, spleen and thymus (Bryant et al., 1987), suppress the antibody-forming cell production stimulated by sheep red blood cells (Pruett et al., 1992), inhibit mitogen responses to concanavalin A and lipopolysaccharide (Bryant et al., 1988; Bayer et al., 1990), decrease the cytokine production (Lysle et al., 1993), and reduce the ratio of CD4 to CD8 cells (Freier and Fuchs, 1993). Furthermore, the immunosuppressive mechanisms of dihydroetorphine identified in one of our previous reports (Wu et al., 1998c) are also consistent with those of morphine (Bayer et al., 1990; Bryant et al., 1991; Fuchs and Pruett, 1993; Freier and Fuchs, 1994), though there is still discussion about whether these opiates exert differential effects and have different mechanisms at central and peripheral opiate receptors, α- and β-pathways,

when administered acutely, subchronically or chronically in vivo or in vitro. In general, the immunosuppressive effects and mechanisms of dihydroetorphine in vivo are similar to those of morphine. Therefore, from this point of view, our novel viewpoint on interrelations between the immunosuppression by and the dependence on opiate analgesics is also underpinned.

In summary, the present study systematically demonstrated the powerful immunosuppressive effects of dihydroetorphine on both humoral and cell-mediated immune functions in dihydroetorphine-dependent mice. The results suggest that there is a relation between immunosuppression by and the dependence on opiates and provide evidence to support further preclinical research and the clinical use of dihydroetorphine. More studies on dihydroetorphine should be undertaken to examine the immunomodulatory effects and mechanisms both in vivo and in vitro, and to determine the relation between the analgesia, dependence and immunosuppression caused by opiates.

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