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European Journal of Pharmacology 353 (1998) 79–85

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Involvement of μ-opioid receptors and α-adrenoceptors in the immunomodulatory effects of dihydroetorphine

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Received 8 May 1998; accepted 12 May 1998

Abstract

The present study investigated the effects of acutely administered dihydroetorphine on mitogen-stimulated lymphocyte proliferation and lymphokine production in mice. These immune functions were significantly suppressed by dihydroetorphine at 24 μg/kg and 128 μg/kg in a dose-dependent fashion. This study further examined the involvement of μ-opioid receptors and α-adrenoceptors in the immunomodulatory effects of dihydroetorphine. The μ-opioid receptor antagonist, naloxone (4 mg/kg), and α-adrenoceptor antagonist, phentolamine (10 mg/kg), but not the β-adrenoceptor antagonist, propranolol (10 mg/kg), effectively blocked dihydroetorphine-induced suppression of splenic lymphocyte proliferation and lymphokine production. These results demonstrate that dihydroetorphine has significant immunosuppressive effects in mice and the mechanisms of these effects may lie in its interactions with opioid receptors and adrenergic pathways. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Dihydroetorphine; Lymphocyte proliferation; Lymphokine production; Naloxone; Phentolamine; Propranolol

1. Introduction

Dihydroetorphine, 7α-[1-(*R*)-hydroxy-1-methylbutyl]6,14-endo-ethanotetrahydrooripavine, is a potent morphine-like thebaine–oripavine derivative. Being an active μ-opioid receptor agonist, dihydroetorphine is one of the strongest narcotic analgesics synthesized up to now (Bentley and Hardy, 1967). However, because of its high abuse potential (Blane et al., 1967; Cowan et al., 1971; Jasinski et al., 1975), dihydroetorphine had not been clinically used until it was exploited in China (Huang and Qin, 1982a). Furthermore, dihydroetorphine has been used not only as an analgesic, but also as a detoxification agent (Qin, 1996), but this was based on insufficient assessments of its dependent liability (Huang and Qin, 1982b) and unilateral conclusions of its therapeutic effects in morphine-dependent animals (Wang et al., 1992). Consequently, concomitant with its use, the number of dihydroetorphine's abusers and infectious cases thereof rockets in clinical therapy (Liu et al., 1995) and among drug addicts (Li et al., 1995), which leads to further research on its abuse liability (Zheng and Zhang, 1995) and immunosuppressive effects (Wu et al., 1998a,b).

At present, the immunomodulatory effects of acute administration of dihydroetorphine on immune functions and its mechanisms are paid little attention to. Therefore, our present study was undertaken to examine the effects of acutely administered dihydroetorphine on immune functions in mice. Moreover, we also tried to clarify the receptors and related pathways possibly involved in its immunomodulatory effects.

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2. Materials and methods

2.1. Animals

Female BALB/c mice (18–22 g) from the Experimental Animal Center of Beijing Medical University (Beijing, China) were used throughout the study. The animals were housed six per cage to habituate to a constant temperature (24 ± 2°C) and humidity (50 ± 10%) on a 12-h light–dark cycle (light period 07:00 to 19:00 h). Food and water were available ad libitum.

2.2. Drugs and chemicals

Dihydroetorphine, morphine and naloxone were supplied by the National Institute on Drug Dependence (Beijing, China). Phentolamine was purchased from Ciba-Geigy (Basle, Switzerland). Propranolol was from Sigma (St. Louis, MO, USA). All drugs were dissolved in saline (0.9%).

[^{3}H]thymidine (20 Ci/mM) was purchased from the Shanghai Institute of Nuclear Research (Shanghai, China). Concanavalin A, lipopolysaccharide and 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide were from Sigma. The cloned mouse cytotoxic T lymphocytic line was supported by the Department of Immunology of Beijing Medical University (Beijing, China).

2.3. Treatments

Mice were randomly assigned to 12 treatment groups ($n = 6$). Drugs of each group were administered as shown in Table 1. Mice of the upper six groups were killed 30 min after receiving one injection of the respective drug. Each mouse of the other groups were administered one injection of naloxone, phentolamine or propranolol 30 min before receiving one injection of dihydroetorphine (24 or 128 μg/kg). Then another 30 min later, these mice were killed and their spleens were rapidly removed.

Table 1
Design of drug administration

Treatment group	Dose	Administration
Saline (control)	–	s.c.
Naloxone (Nal)	4 mg/kg	s.c.
Phentolamine (Phe)	10 mg/kg	i.p.
Propranolol (Pro)	10 mg/kg	i.p.
Dihydroetorphine$_1$ (DHE_1)	24 μg/kg	s.c.
Dihydroetorphine$_2$ (DHE_2)	128 μg/kg	s.c.
Nal + DHE_1	4 mg/kg + 24 μg/kg	s.c. + s.c.
Phe + DHE_1	10 mg/kg + 24 μg/kg	i.p. + s.c.
Pro + DHE_1	10 mg/kg + 24 μg/kg	i.p. + s.c.
Nal + DHE_2	4 mg/kg + 128 μg/kg	s.c. + s.c.
Phe + DHE_2	10 mg/kg + 128 μg/kg	i.p. + s.c.
Pro + DHE_2	10 mg/kg + 128 μg/kg	i.p. + s.c.

Mice were randomly assigned to 12 treatment groups ($n = 6$). Each mouse of the upper six groups was given one injection of the respective drug. Each mouse of the remaining groups was respectively given one injection of naloxone, phentolamine or propranolol 30 min before receiving one injection of dihydroetorphine (24 or 128 μg/kg).

2.4. Lymphocyte proliferation assay

A modification of the method of Bayer et al. (1990) was used. Spleens were removed and placed in cold culture media (RPMI-1640) containing 1% fetal bovine serum and gentamicin (0.5 mg/ml). Spleens were gently teased apart 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/well of the cell suspensions was added to 96-well microtiter plates containing 5 μg/ml concanavalin A (final concentration) or 1 μg/ml lipopolysaccharide (final concentration). Cultures were incubated in triplicate 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. Radioactive deoxyribonucleic acids were collected on Whatman GF/C filters, using a cell harvester and measured in a liquid scintillation counter (Model 1215, Beckman) at an efficiency of 36%.

2.5. Lymphokine bioassay

To measure interleukin-2 in culture, a modification of the method of Bunjes et al. (1981) was used. The splenic cell suspensions (4×10^6 cells/ml) were added to 24-well microtiter plates (1 ml/well) containing 1 ml concanavalin A (5 μg/ml). After the triplicate cultures were incubated for 24 h at 37°C with 5% CO_2, the supernatants were harvested. Diluted supernatants, 100 μl, were added to 96-well microtiter plates containing 100 μl active cloned mouse cytotoxic T lymphocytic line cells (7×10^4 cells/ml), and 100 μl RPMI-1640 replaced supernatants in the control wells. All samples were incubated in triplicate for an additional 48 h until all cells in the control wells were dead, then 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (10 μl) was added into each well. Samples were analyzed in a microplate reader (Model 3550, Bio-Rad) at $A_{570\ nm}$.

2.6. Statistical analysis

The data are means ± S.E.M. of n independent observations. Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Student's–Newman–Keuls test.

3. Results

3.1. Immunosuppressive effects of acute administration of dihydroetorphine in mice

3.1.1. Effects of dihydroetorphine on lymphocyte proliferation

Dihydroetorphine (24 or 128 μg/kg) dose-dependently suppressed the mouse splenic lymphocyte proliferation induced by concanavalin A (5 μg/ml). Dihydroetorphine significantly reduced [^{3}H]thymidine uptakes by 51.4% ($P < 0.001$) and 76.9% ($P < 0.001$) (Fig. 1A). Dihydroetorphine also suppressed the lymphocyte proliferation induced by lipopolysaccharide (1 μg/ml). The [^{3}H]thymidine uptakes were reduced by 39.2% ($P < 0.001$) and 71.5% ($P < 0.001$) (Fig. 1B).

3.1.2. Effects of dihydroetorphine on lymphokine production

Dihydroetorphine (24 or 128 μg/kg) dose-dependently suppressed the concanavalin A (5 μg/ml)-induced production of interleukin-2 in the supernatant of splenocytes in mice. The values of $A_{570\ nm}$ were reduced by 65.2% ($P < 0.001$) and 82.5% ($P < 0.001$) (Fig. 1C).

3.2. Involvement of μ-opioid receptors in dihydroetorphine-induced immunosuppression

3.2.1. Effects of naloxone on the lymphocyte proliferation suppressed by dihydroetorphine

A single administration of the μ-opioid receptor antagonist, naloxone (4 mg/kg), effectively antagonized the suppressive effects of dihydroetorphine (24 or 128 μg/kg) on mouse splenic lymphocyte proliferation induced by concanavalin A (5 μg/ml). The reduced [^{3}H]thymidine uptakes were increased by 87.4% ($P < 0.01$) and 243.7% ($P < 0.001$) (Fig. 2A). The lipopolysaccharide (1 μg/ml)-induced lymphocyte proliferation suppressed by dihydroetorphine was also blocked. The reduced [^{3}H]hymidine uptakes were increased by 46.7% ($P < 0.01$) and 204.4% ($P < 0.001$) (Fig. 2B).

3.2.2. Effects of naloxone on the production of interleukin-2 suppressed by dihydroetorphine

A single administration of naloxone (4 mg/kg) effectively antagonized the suppression by dihydroetorphine (24 or 128 μg/kg) of the production of interleukin-2 in the supernatant of splenocytes with concanavalin A (5 μg/ml) in mice. The reduced values of $A_{570\ nm}$ were increased by 121.4% ($P < 0.01$) and 142.9% ($P < 0.01$) (Fig. 2C).

3.3. Involvement of α-adrenoceptors in dihydroetorphine-induced immunosuppression

3.3.1. Effects of phentolamine on the lymphocyte proliferation suppressed by dihydroetorphine

A single administration of the α-adrenoceptor antagonist, phentolamine (10 mg/kg), effectively antagonized

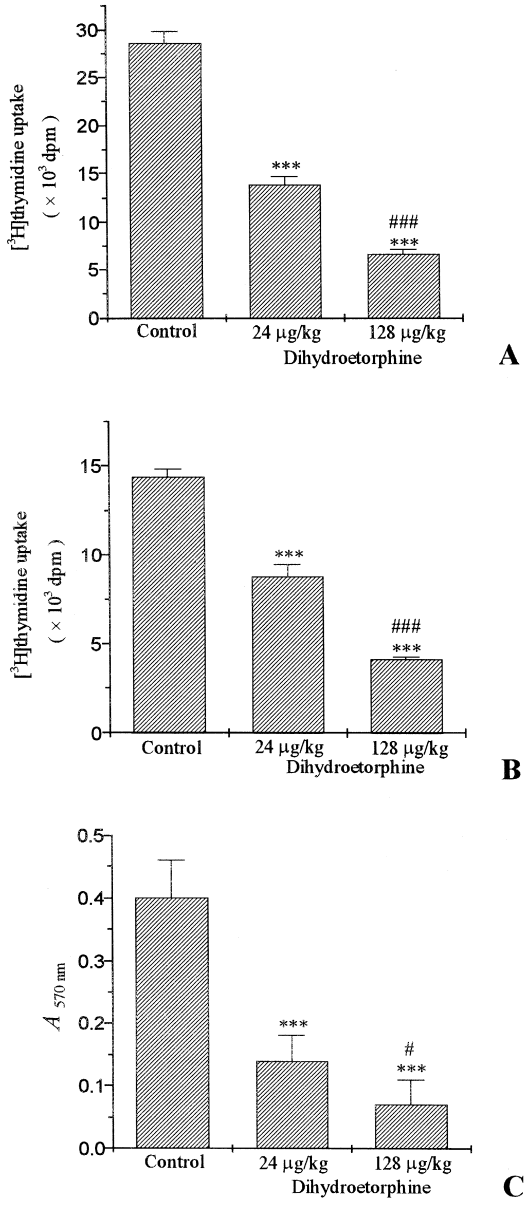

Fig. 1. (A) Effects of dihydroetorphine on splenic lymphocyte proliferation induced by concanavalin A in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{###}P < 0.001$ vs. 24 μg/kg dihydroetorphine. (B) Effects of dihydroetorphine on splenic lymphocyte proliferation induced by lipopolysaccharide in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{###}P < 0.001$ vs. 24 μg/kg dihydroetorphine. (C) Effects of dihydroetorphine on the production of interleukin-2 in the supernatant of splenocytes with concanavalin A in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{#}P < 0.05$ vs. 24 μg/kg dihydroetorphine.

the suppressive effects of dihydroetorphine (24 or 128 μg/kg) on the mouse splenic lymphocyte proliferation induced by concanavalin A (5 μg/ml). The reduced [^{3}H]thymidine uptakes were increased by 61.8% ($P < 0.01$) and 189.1% ($P < 0.001$) (Fig. 3A). The lipopolysaccharide (1 μg/ml)-induced lymphocyte proliferation suppressed by dihydroetorphine was also blocked. The reduced

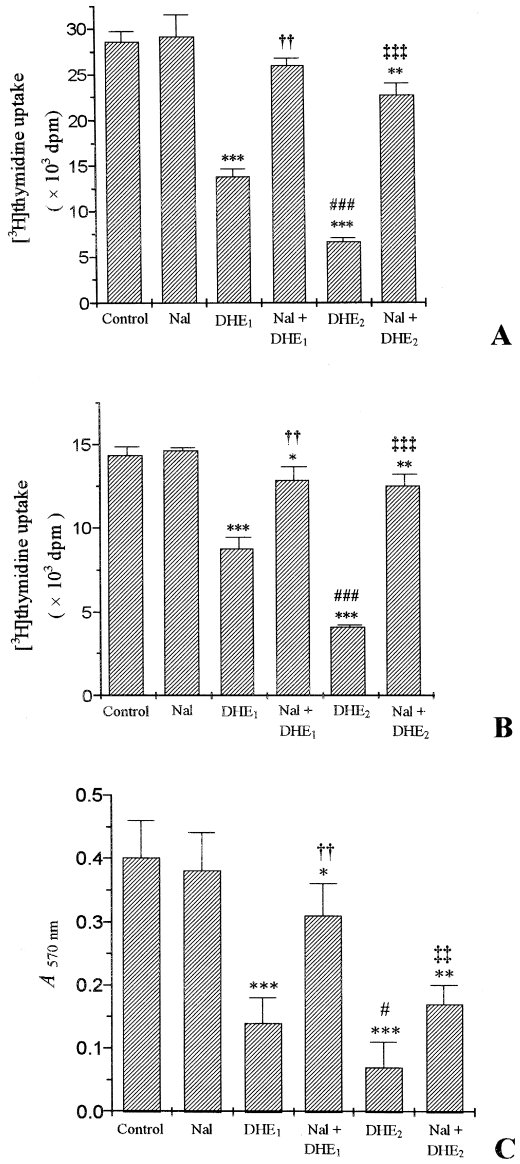

Fig. 2. (A) Effects of naloxone on the concanavalin A-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger\dagger}P < 0.01$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger\ddagger}P < 0.001$ vs. DHE_2 (128 μg/kg dihydroetorphine). (B) Effects of naloxone on the lipopolysaccharide-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger\dagger}P < 0.01$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger\ddagger}P < 0.001$ vs. DHE_2 (128 μg/kg dihydroetorphine). (C) Effects of naloxone on the production of interleukin-2 in the supernatant of splenocytes with concanavalin A suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#}P < 0.05$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger\dagger}P < 0.01$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger}P < 0.01$ vs. DHE_2 (128 μg/kg dihydroetorphine).

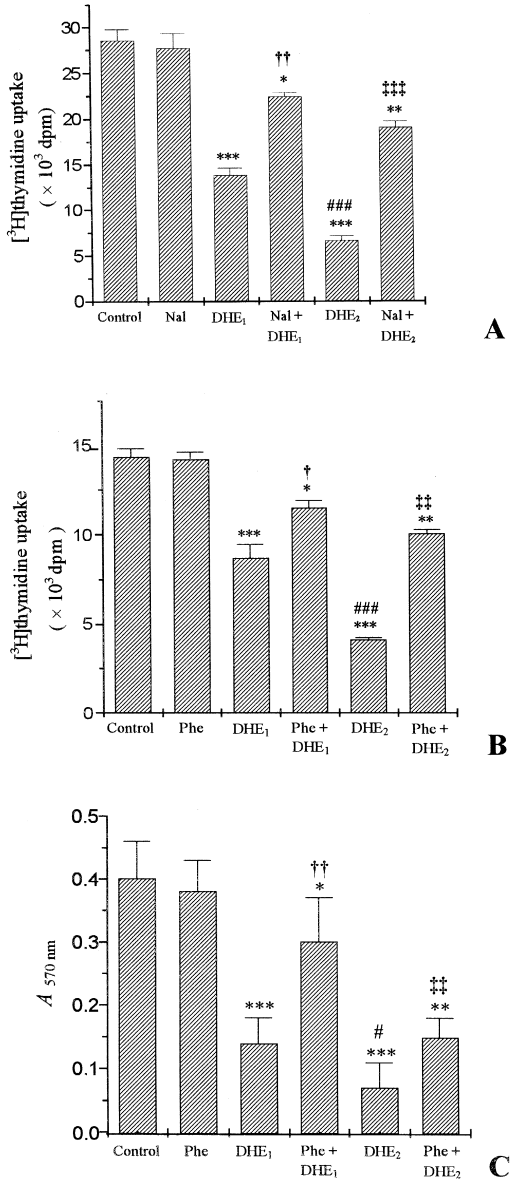

Fig. 3. (A) Effects of phentolamine on the concanavalin A-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger\dagger}P < 0.01$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger\ddagger}P < 0.001$ vs. DHE_2 (128 μg/kg dihydroetorphine). (B) Effects of phentolamine on the lipopolysaccharide-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#\#\#}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger}P < 0.05$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger}P < 0.01$ vs. DHE_2 (128 μg/kg dihydroetorphine). (C) Effects of phentolamine on the production of interleukin-2 in the supernatant of splenocytes with concanavalin A suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control, $^{\#}P < 0.05$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\dagger\dagger}P < 0.01$ vs. DHE_1 (24 μg/kg dihydroetorphine), $^{\ddagger\ddagger}P < 0.01$ vs. DHE_2 (128 μg/kg dihydroetorphine).

[^{3}H]thymidine uptakes were increased by 31.8% ($P < 0.05$) and 145.8% ($P < 0.01$) (Fig. 3B).

3.3.2. Effects of phentolamine on the production of interleukin-2 suppressed by dihydroetorphine

A single administration of phentolamine (10 mg/kg) effectively antagonized the suppression by dihydroetorphine (24 or 128 μg/kg) of the production of interleukin-2 in the supernatant of splenocytes with concanavalin A (5 μg/ml) in mice. The reduced values of $A_{570\ nm}$ were increased by 114.3% ($P < 0.01$) and 114.3% ($P < 0.01$) (Fig. 3C).

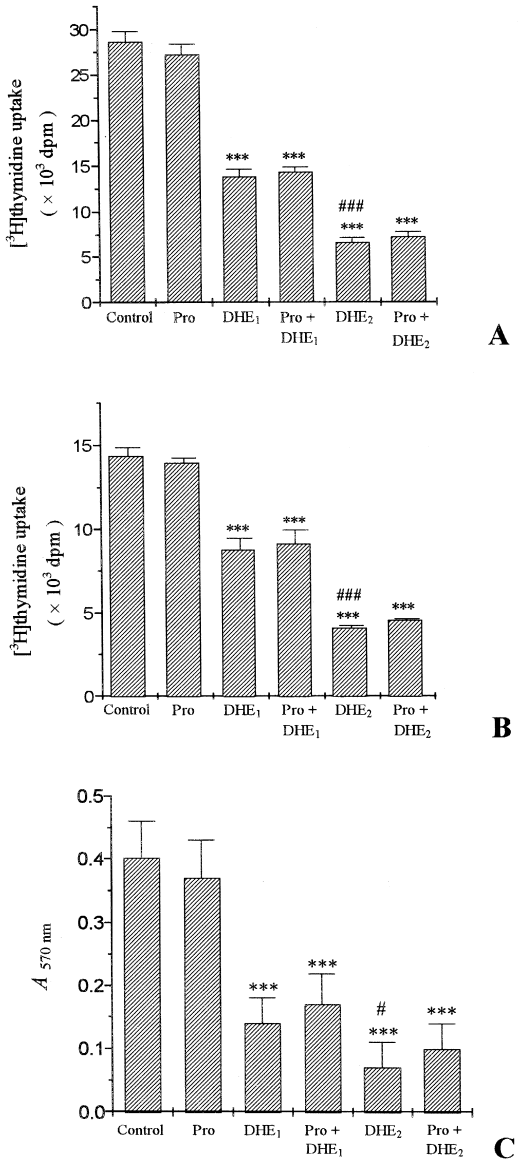

Fig. 4. (A) Effects of propranolol on the concanavalin A-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{###}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine). (B) Effects of propranolol on the lipopolysaccharide-induced splenic lymphocyte proliferation suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{###}P < 0.001$ vs. DHE_1 (24 μg/kg dihydroetorphine). (C) Effects of phentolamine on the production of interleukin-2 in the supernatant of splenocytes with concanavalin A suppressed by dihydroetorphine in mice. Data are presented as means ± S.E.M., $n = 6$, $^{***}P < 0.001$ vs. control, $^{#}P < 0.05$ vs. DHE_1 (24 μg/kg dihydroetorphine).

3.4. Involvement of β-adrenoceptors in dihydroetorphine-induced immunosuppression

3.4.1. Effects of propranolol on the lymphocyte proliferation suppressed by dihydroetorphine

A single administration of the β-adrenoceptor antagonist, propranolol (10 mg/kg), did not significantly block the suppressive effects of dihydroetorphine (24 or 128 μg/kg) on the mouse splenic lymphocyte proliferation induced by concanavalin A (5 μg/ml). The reduced [^{3}H]thymidine uptakes were only increased by 3.5% ($P > 0.05$) and 8.6% ($P > 0.05$) Fig. 4A). The lipopolysaccharide (1 μg/ml)-induced lymphocyte proliferation suppressed by dihydroetorphine was not blocked either. The reduced [^{3}H]thymidine uptakes were increased by 4.1% ($P > 0.05$) and 11.8% ($P > 0.05$) (Fig. 4B).

3.4.2. Effects of propranolol on the production of interleukin-2 suppressed by dihydroetorphine

A single administration of propranolol (10 mg/kg) did not significantly block the suppression by dihydroetorphine (24 or 128 μg/kg) of the production of interleukin-2 in the supernatant of splenocytes with concanavalin A (5 μg/ml) in mice. The reduced values of $A_{570\ nm}$ were increased by 21.4% ($P > 0.05$) and 42.9% ($P > 0.05$) (Fig. 3C).

4. Discussion

It is well-known that there are a great many reports about the immunomodulatory effects and mechanisms of acutely administered opiates in rodents. Acute administration of morphine can suppress phagocytic functions (Tubaro et al., 1983), inhibit the cytolytic production of natural killer cells (Shavit et al., 1986; Weber and Pert, 1989), depress T- and B-cell mitogen-stimulated lymphocyte proliferations (Bayer et al., 1990) and decrease cytokine productions such as interleukin-2 and interferon-γ (Lysle et al., 1993). Furthermore, several main possibilities in the series of complex steps have been suggested as mechanisms: (1) directly combining with opioid receptors (Carr et al., 1989; Ovadia et al., 1989) and specific non-opiate receptors (Hazum et al., 1979) on cells of the immune system; (2) directly combining with opioid receptors in the central nervous system (Shavit et al., 1986; Weber and

Pert, 1989); (3) indirectly interacting with the hypothalamic–pituitary–adrenal axis to alter central corticotrophin-releasing factor and production of adrenocorticotropic hormones (Fuchs and Pruett, 1993; Freier and Fuchs, 1994); (4) indirectly interacting with the sympathetic nervous system to alter sympathetic outflow (Appel et al., 1986; Fecho et al., 1993).

However, although dihydroetorphine is a potent selective μ-opioid receptor agonist, with μ, δ, κ-opioid receptor affinity ratios 1951:2:1 (Wang et al., 1991; Yuan et al., 1995), its immunomodulatory effects have seldom been reported upon. In our other recent studies, the immune functions of rats were significantly suppressed by 72-h computer-controlled automatic intravenous injection of dihydroetorphine (Wu et al., 1998a) and by intravenous self-administration of dihydroetorphine (Wu et al., 1998b) in rats. Our unpublished results also suggested that chronic administration of dihydroetorphine at different total doses (444.5, 889, 1778 g/kg, s.c.) significantly inhibited both humoral and cell-mediated immune functions in mice, which included decrease of body weight, and of spleen and thymus weights, suppression of delayed-type hypersensitivity, abatement of the production of plaque-forming cells, inhibition of lymphocyte proliferation and lymphokine production, and decrease of the ratio of CD4 and CD8. Furthermore, the immunosuppressive effects of dihydroetorphine were at least 1000 times as strong as those of morphine at the same doses (Wu et al., 1998a; Wu et al., unpublished data). Nevertheless, the immunomodulatory effects, and their mechanisms, of acutely administered dihydroetorphine have not been examined.

The present results that the concanavalin A and lipopolysaccharide-induced lymphocyte proliferation and the production of interleukin-2 were all significantly suppressed by a single administration of dihydroetorphine (24 or 128 g/kg) demonstrated that the functions of both T and B splenic lymphocytes were inhibited. Dihydroetorphine suppressed the proliferative response of splenocytes to the T-cell mitogen, concanavalin A, and B-cell mitogen, lipopolysaccharide; it further inhibited T-cell-activated and -secreted interleukin-2. Interleukin-2, which is mainly secreted by helper T-cells, plays a versatile role in immune responses. Interleukin-2 can accelerate the proliferation of T-cells, maintain the survival of T-cells in vitro, enhance the activities of cytotoxic T-cells, suppressor T-cells, natural killer cells and killer cells, induce the production of interferon-γ and B-cell growth factors, activate B-cells to produce antibodies, and produce antineoplastic lymphokine-activated killer cells in vivo (Bayer et al., 1990). Because these key steps are suppressed by dihydroetorphine, many other steps and pathways of immune responses cannot be triggered. The finding that the μ-opioid receptor antagonist, naloxone (4 mg/kg), and a α-adrenoceptor antagonist, phentolamine (10 mg/kg), effectively antagonize the immunosuppressive effects of dihydroetorphine suggested that μ-opioid receptors and α-adrenergic pathways play pivotal roles in the immunomodulatory effects of dihydroetorphine. Moreover, the fact that the β-adrenoceptor antagonist, propranolol (10 mg/kg), did not effectively antagonize the effects suggested that a β-adrenergic pathway might not be involved in these acute immunosuppressions.

Studies of acute morphine exposure in vivo, yielded convincing evidence to suggest both that its effects on immunocompetence were centrally mediated through receptors in the periaqueductal gray matter of the mesencephalon (Weber and Pert, 1989) and that the pathway involving the sympathetic nervous system (Fecho et al., 1993), but not the hypothalamic–pituitary–adrenal axis (Bayer et al., 1990; Bryant et al., 1991), was apparently involved in acute morphine effects. However, the actual and exact mechanisms of morphine immunosuppression are still obscure and open to argument. Therefore, although the present study indeed provided evidence for further research on the immunomodulatory effects and mechanisms of dihydroetorphine, to find whether central or peripheral opioid receptors and whether the sympathetic nervous system or the hypothalamic–pituitary–adrenal axis mainly mediates the immunomodulation by dihydroetorphine requires more investigation. Nevertheless, summarizing our present results and recent research (Wu et al., 1998a,b; Wu et al., unpublished data), we discover that the immunosuppressive effects of dihydroetorphine are much stronger than those of morphine at the same doses. Moreover, we suppose that the immunomodulatory mechanisms of potent narcotic analgesics like dihydroetorphine are similar to those of opiates like morphine, i.e., acutely given dihydroetorphine may directly combine with opioid receptors and adrenoceptors on cells of immune system to exert its immunomodulatory effects. It may also combine with opioid receptors in the central nervous system, then indirectly interact with the sympathetic nervous system to alter sympathetic outflow to exert its effects in vivo.

5. Conclusion

These results demonstrate that acute administration of dihydroetorphine has potent immunosuppressive effects in mice, which are consistent with those in other experimental animals and in dihydroetorphine addicts. The data further clarify that μ-opioid receptors and α-adrenoceptors are involved in the effects and that the pathway involving the sympathetic nervous system contributes to dihydroetorphine-induced immunosuppression following acute treatment, which may explain the occurrence of infectious cases in dihydroetorphine addicts. Further investigation on dihydroetorphine to characterizes the relations between opioid receptors, sympathetic nervous system and the hypothalamic–pituitary–adrenal axis should urgently be carried out, which may lead to effective therapeutic strategies in dihydroetorphine-induced immunosuppressed patients.

Acknowledgements

This work was supported by and carried out in the National Institute on Drug Dependence of China (NIDD). The authors wish to thank the staff of NIDD.

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