

Immunosuppressive effects of intravenous self administration of dihydroetorphine on lymphocyte functions in rats¹

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KEY WORDS dihydroetorphine; self administration; dependency (psychology); lymphocytes; interleukin-2; immunosuppression

AIM: To study the effects of dihydroetorphine (DHE) on lymphocyte functions in rats and to further assess the abuse potential of DHE. **METHODS:** An intravenous self administration (SA) procedure in rats was used to determine the SA liability of DHE. Concanavalin A (Con A)-stimulated lymphocyte proliferation and lymphokine production of rat splenocytes were measured. **RESULTS:** DHE $178 \pm 13 \mu\text{g}$ established a stable and typical rat model of psychological dependence, suppressed lymphocyte proliferation ($129 \pm 11 \text{ Bq}$) compared with control group ($620 \pm 36 \text{ Bq}$), and inhibited the activity of interleukin-2 (IL-2) ($A_{570} = 0.28 \pm 0.06$) compared with control group ($A_{570} = 0.51 \pm 0.03$). **CONCLUSION:** DHE had a high abuse potential and inhibited the Con A-induced lymphocyte proliferation and interleukin-2 production in rats.

Dihydroetorphine (DHE), a morphine-like thebaine-orphavine derivative, is a potent μ -receptor agonist^[1,2]. Although DHE is one of the strongest narcotic analgesics, its liability of addiction has not been evaluated discreetly^[3,4] until a large increase of its abuse in clinical use as analgesic and detoxification agent^[5] and in drug addicts^[6]. Furthermore, many infectious cases of DHE addicts have been reported^[5,6], but there were few reports on its immunomodulatory effects and mechanisms, and those pertinent to the dependence. Therefore, the aim of this work was to study the effects of DHE

intravenous self administration (SA) on lymphocyte functions in rats.

MATERIALS AND METHODS

Materials Wistar rats (δ , weighing $300 \pm s 20 \text{ g}$, clean, Certificate No 01-3056) were supplied by the Experimental Animal Center of Beijing Medical University. DHE, supplied by National Institute on Drug Dependence (NIDD), was dissolved in saline. [³H]Thymidine ($740 \text{ TBq} \cdot \text{mol}^{-1}$) was purchased from Shanghai Institute of Nuclear Research, Chinese Academy of Sciences. Concanavalin A (Con A) and 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide (MTT) were from Sigma Co. Cytotoxic T-lymphocyte line (CTLL) was supplied by Department of Immunology of Beijing Medical University. Self administration control system (SACS) including apparatus was designed and supplied by NIDD.

Rat model of SA SA methods^[7, 8] were modified. Each rat was surgically prepared with a chronic jugular cannula of silicone rubber tubing (0.6 mm inside diameter and 1.2 mm outside diameter). Through a saddle in which the rat was placed, its free end was connected to a swivel allowing the rat almost unrestrained in the chamber, then to a computer-controlled infusion pump. After surgery, the rats were housed individually during of the experiment in conditioning chambers at $22 - 26 \text{ }^\circ\text{C}$ under a 12-h automatic light cycle (7 am to 7 pm). The opposite wall of each chamber was equipped with one response lever, above which a small red stimulus light was mounted and one water tube which the rat could have free access to water was placed. Food pellets were removed before and delivered after the daily experimental period. The rats were allowed 7 d to recover and adapt to the experimental situation.

Then each rat was trained to press the lever at hourly intervals from 9 am to 3 pm daily for 6 d and then DHE $1 \mu\text{g} \cdot \text{kg}^{-1}$ was forced to be

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injected into each rat of the DHE group and 0.2 mL saline into each rat of the control group by the infusion pump. During other times, the lever was covered to prevent the rat from responding. From d 14 on, rats were given free access to the levers and the forced injections of the DHE group were ceased. The red stimulus light was illuminated during the daily experiment period from 9 am to 3 pm ($6 \text{ h} \cdot \text{d}^{-1}$). When the lever was pressed, the light was switched off and other presses would have no programmed responses until 0.2 mL solution was infused. During this experiment, rats were tested with different doses of DHE (2, 4, and $1 \mu\text{g} \cdot \text{kg}^{-1}$), and the fixed ratio was one to one (FR1), which meant the number of lever-pressing was equal to that of injections.

Lymphocyte proliferation assay The method⁽⁹⁾ was modified. After the experimental duration, the spleens of rats were excised removed rapidly. The splenocyte suspensions were washed and adjusted to a final concentration of $3 \times 10^9 \text{ cells} \cdot \text{L}^{-1}$ in RPMI-1640 with 1 % fetal bovine serum. Then the cell suspensions ($200 \mu\text{L}$ per well) were added to 96-well microtiter plates containing Con A $5 \text{ mg} \cdot \text{L}^{-1}$ (final concentration). Cultures were triplicated and incubated with 5 % CO_2 at $37 \text{ }^\circ\text{C}$ for 48 h. Then [^3H]thymidine (7.4 kBq per well) was added and the cultures were incubated for additional 24 h. Radioactive deoxyribonucleic acids were collected on Whatman GF/C filters, using a cell harvester and measured by a Beckman 1215 liquid scintillation counter.

Interleukin-2 bioassay The method⁽¹⁰⁾ was modified and used. The splenic cell suspension ($4 \times 10^9 \text{ cells} \cdot \text{L}^{-1}$, 1 mL per well) was added to 24-well microtiter plates containing Con A $5 \text{ mg} \cdot \text{L}^{-1}$ (final concentration). After the triplicated cultures were incubated with 5 % CO_2 at $37 \text{ }^\circ\text{C}$ for 48 h, supernatants were harvested. Diluted supernatants ($100 \mu\text{L}$) were added to 96-well microtiter plates containing $100 \mu\text{L}$ active CTLL cells ($7 \times 10^7 \text{ cells} \cdot \text{L}^{-1}$), and in control wells $100 \mu\text{L}$ RPMI-1640 were substituted for supernatants. All samples were triplicated and incubated for an additional 40 – 48 h until all cells in control wells were dead, then MTT $10 \mu\text{L}$ was added into each well.

Samples were detected with a Bio-Rad 3550 microplate reader at 570 nm.

Statistical analysis Data were expressed as $\bar{x} \pm s$ and analyzed using *t* test.

RESULTS

DHE self administration by rats

Controlled by the schedule of reinforcement, DHE SA by rats was reliably established. During the experiment, the total doses of DHE were maintained at $178 \pm 13 \mu\text{g}$, but the values of the daily response rates, which were equal to the times of lever-pressing, were inversely proportional to those of graded doses (Tab 1, Fig 1).

Tab 1. Response rates (RR) and total daily doses (TDD) of DHE self administration by rats. $n = 6, \bar{x} \pm s$. ^c $P < 0.01$ vs $1 \mu\text{g} \cdot \text{kg}^{-1}$. ^d $P < 0.01$ vs $2 \mu\text{g} \cdot \text{kg}^{-1}$.

DHE/ $\mu\text{g} \cdot \text{L}^{-1}$	RR/rate $\cdot \text{d}^{-1}$	TDD/ μg
1	31.1 ± 2.4	10.6 ± 2.6
2	16.8 ± 2.8^c	11.6 ± 2.2
4	9.1 ± 1.1^d	11.6 ± 1.7

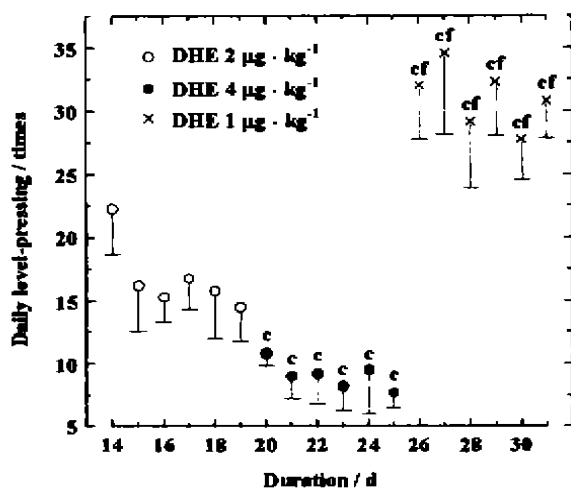


Fig 1. Times of daily lever-pressing of DHE self administration by rats. $n = 6, \bar{x} \pm s$. ^c $P < 0.01$ vs $2 \mu\text{g} \cdot \text{kg}^{-1}$. ^{cf} $P < 0.01$ vs $4 \mu\text{g} \cdot \text{kg}^{-1}$.

Effect on lymphocyte proliferation DHE $178 \pm 13 \mu\text{g}$ suppressed Con A-activated splenic lymphocyte proliferation by 79.2 % (Tab 2).

Effect on IL-2 production DHE $178 \pm 13 \mu\text{g}$ inhibited the activity of IL-2 in the supernatant of Con A-activated splenocytes in

rats. The value of A_{570} was decreased by 45.1 % (Tab 2).

Tab 2. Effects of DHE self administration on splenic lymphocyte proliferation and IL-2 production in the supernatant of splenocytes induced by Con A in rats. $n = 6$, $\bar{x} \pm s$. $^c P < 0.01$ vs saline.

Groups	Total dose of DHE/ μg	[^3H]thymidine uptake, Bq/well	A_{570}
Saline	0	620 \pm 36	0.51 \pm 0.03
DHE	178 \pm 13	129 \pm 11 ^c	0.28 \pm 0.06 ^c

DISCUSSION

The SA model established here was designed to obtain a quantitative index of drug-seeking behavior and to evaluate the abuse potential of DHE in rats. The results indicated that DHE induced powerful psychological dependence and high abuse liability, which accorded with its physical dependence in rats^[11] and the actual abuse in human^[6]. On this SA model. By this model, the specific conditions of DHE's addicts were mimicked, thus the conclusions of this study were more reasonable and convincing. The results that DHE inhibited the Con A-activated splenic lymphocyte proliferation by 79.2 % and the activity of IL-2 in the supernatant of splenocytes by 45.1 % in rats suggested that DHE had potent immunosuppressive effects. The present data demonstrated the immunosuppressive effects of DHE on T-lymphocytes. It suppressed the proliferative response of splenocytes to the T-cell mitogen Con A, and further inhibited T-cells to activate and secrete IL-2. IL-2, which is mainly secreted by T_h cells, plays a versatile role in immune responses. IL-2 can accelerate the proliferation of T-cells, maintain the survival of T-cells *in vitro*, enhance the activities of T_c , T_s , NK, and K cells, induce the productions of γ -IFN and BCGF, activate B cells to produce antibodies and antineoplastic LAK cells^[9]. Because these key steps were suppressed by DHE, many other steps and pathways of immune responses could not be triggered.

The present results are consistent with our other data^[11,12] and unpublished research, which demonstrated that DHE led to physical dependence in mice and rats. DHE also had extensive and powerful immunosuppressive effects

in acutely and chronically administrated mice and rats. Furthermore, its immunosuppressive effects and abuse liability are parallel and stronger than those of morphine^[11]. Based on our research^[12], it can be supposed that the mechanisms of DHE immunosuppression may lie in the direct binding on opiate receptors on cells of immune system, activating opiate receptors in central nervous system, modulating releases of neurotransmitters in sympathetic nervous system and hormones in hypothalamo-pituitary-adrenal axis, and interacting with mediators in other indirect pathways.

In conclusion, DHE has a high abuse potential and potent immunosuppressive effects in SA rats, which are consistent with circumstances of DHE addicts. DHE must be administered strictly and used discreetly.

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REFERENCES

- Bentley KW, Hardy DG. Novel analgesics and molecular rearrangements in the morphine-thebaine group. III. Alcohols of the 6, 14-endo-ethenotetrahydro-orphavine series and derived analogues of *N*-allylnormorphine and norcodeine. *J Am Chem Soc* 1967; 89: 3281-92.
- Blane GF, Boura ALA, Fitzgerald AE, Lister RE. Actions of etorphine hydrochloride (M99): a potent morphine-like agent. *Br J Pharmacol Chemother* 1967; 30: 11-22.
- Huang M, Qin BY. Physical dependence of dihydroetorphine in mice and monkeys. *Acta Pharmacol Sin* 1982; 3: 81-4.
- Wang DX, Liu YS, Qin BY. Experimental therapeutic effects of dihydroetorphine in morphine-dependent rats and monkeys. *Chin J Pharmacol Toxicol* 1992; 6: 36-40.
- Qin BY. Advances of dihydroetorphine: from analgesia to detoxification. *Drug Dev Res* 1996; 39: 131-4.
- Liu ZM, Cao JQ, Shi F, Cai ZJ. An epidemiological study on dihydroetorphine abuse. *Chin Bull Drug Depend* 1995; 4: 223-31.
- Spealman RD, Goldberg SR. Drug self administration by laboratory animals: control by schedules of reinforcement. *Annu Rev Pharmacol Toxicol* 1978; 18: 313-39.
- Wang WP, Xie L, Zheng JW, Cai ZJ. A continuous self administration model under fixed ratio in rats. *Chin Bull Drug Depend* 1994; 3: 214-7.
- Bayer BM, Dauassin S, Hernandez M, Irvin L. Morphine inhibition of lymphocyte activity is mediated by an opioid dependent mechanism. *Neuropharmacology* 1990; 29: 369-74.
- Bunjes D, Hardt C, Rollingshoff M, Wagner H. Cyclosporin A mediates immunosuppression of primary cytotoxic T cell

responses by impairing the release of interleukin 1 and interleukin 2. Eur J Immunol 1981; 11: 657-61.

- 11 Wu WR, Li Y, Zhang KR, Wang GL, Xie L, Bai HQ. Effects of dihydroetorphine on immune functions in rats treated by automatical intravenous injection. Chin Bull Drug Depend 1998; 7: in press.
- 12 Wu WR, Zheng JW, Li FY, Li Y, Zhang KR, Bai HQ. Involvement of μ -opioid receptors and α -adrenoceptors in the immunomodulatory effects of dihydroetorphine. Eur J Pharmacol 1998; in press.

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二氢埃托啡自身给药对大鼠淋巴细胞功能的免疫抑制作用¹

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关键词 二氢埃托啡; 自身给药; 依赖(心理学); 淋巴细胞; 白细胞介素-2; 免疫抑制

目的: 研究二氢埃托啡(DHE)对大鼠淋巴细胞功能的影响及其精神依赖性潜力。 **方法:** 用大鼠静脉自身给药模型评价精神依赖性; 用淋巴细胞增殖反应和白细胞介素-2活性检测免疫功能。 **结果:** DHE(总剂量 $178 \pm 13 \mu\text{g}$)自身给药使大鼠形成稳定的精神依赖性, 且显著抑制由刀豆球蛋白刺激的淋巴细胞增殖反应(DHE组 $129 \pm 11 \text{ Bq}$; 对照组 $620 \pm 36 \text{ Bq}$)和白细胞介素-2活性(DHE组: $A_{570} = 0.28 \pm 0.06$; 对照组: $A_{570} = 0.51 \pm 0.03$)。 **结论:** DHE具有高度精神依赖性潜力, 且能明显抑制大鼠淋巴细胞增殖和白细胞介素-2活性。

Effects of nitroquine on ultrastructures and cytochrome oxidase of exoerythrocytic *Plasmodium yoelii* in rat liver¹

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KEY WORDS antimalarials; nitroquinolines; *Plasmodium yoelii*; electron microscopy; cytochrome-c oxidase; liver

AIM: To study the effects of nitroquine acetate (NA) on the ultrastructures and cytochrome-c oxidase(CCO) of exoerythrocytic forms (EEF) of *Plasmodium yoelii*. **METHODS:** Rats were inoculated with sporozoites directly into the liver. After 48 h rats were killed. Rat liver thin sections were incubated in histochemical reaction medium, then examined by transmission electron microscopy. NA ($2 \text{ mg} \cdot \text{kg}^{-1}$) was fed to rats 3.5 h and 14 h before killing the rats. **RESULTS:** At 3.5 h, in the parasites there appeared swelling and proliferation of mitochondria, dilation of endoplasmic reticulum,

and reduction of the electron density of parasites' nuclei. The structures of the parasites disintegrated to form many autophagocytes 14 h after exposure to NA. The reaction products of CCO still existed until 14 h after using NA. **CONCLUSION:** CCO was not the starting point of NA action. NA interferes with the structure and function of the cytoplasm and nucleus of malaria parasites and exerts its antimalarial effects in many aspects.

Exoerythrocytic forms (EEF) of malarial parasites result in relapses of malaria. Nitroquine { 2, 4-diamino-6-[(3, 4-dichlorobenzyl) nitros-amino] quinazoline } acetate (NA, CI-679) is an antimalarial drug, which is effective on the erythrocytic, exoerythrocytic, and sporogonic stages of many malaria parasites including plasmodia in human. Its mechanism of action is related to inhibiting DNA and protein synthesis of *Plasmodium*^[1,2]. Ultrastructural

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