ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7): 639-644
http://www.chinaphar.com

Down-regulation of MAO-B activity and imidazoline receptors in rat brain following chronic treatment of morphine[1]

SU Rui-Bin[2], LI Jin, LI Xin, QIN Bo-Yi
(*Beijing Institute of Pharmacology and Toxicology, Beijing 100850, China*)

KEY WORDS morphine; monoamine oxidase; idazoxan; agmatine

ABSTRACT

AIM: To study the regulation of monoamine oxidase-B (MAO-B) activity and imidazoline receptors (I-R) during long term treatment of morphine. **METHODS**: MAO-B activity was detected by high performance liquid chromatography; I-R was detected by [^{3}H] idazoxan binding test. **RESULTS**: Idazoxan and morphine inhibited whole brain homogenate MAO-B activity in a dose-dependent manner, while agmatine, an endogenous imidazoline ligand, didn't affect the activity of MAO-B, and it had no effect on the inhibition of MAO-B activity by idazoxan or morphine. MAO-B activity of rats decreased markedly in all five brain regions detected (cerebral cortex, hippocampus, thalamus, cerebellum, and striatum) after chronic administration of morphine for 16 d ($P < 0.01$). Acute challenge with naloxone or idazoxan didn't influence MAO-B activity in morphine chronically treated rats. Although agmatine itself didn't affect MAO-B activity, co-administration of agmatine with morphine could reverse the effect of morphine on MAO-B activity. Chronic administration of morphine significantly decreased the density of [^{3}H] idazoxan binding sites and increased the binding affinity in cerebral cortex and cerebellum ($P < 0.05$ or $P < 0.01$). **CONCLUSION**: MAO-B activity was relevant to the abstinent syndrome of morphine dependent rats, but not related to the effect of agmatine on morphine analgesia; influence of agmatine on the pharmacological effects of morphine was based on its activation of imidazoline receptors.

[1] Supported by the National Natural Science Foundation of China (No 39970845) and Beijing Natural Science Foundation (No 7992028).
[2] Correspondence to SU Rui-bin, Ph D. Phn 86-10-6693-1621. Fax 86-10-6821-1656. E-mail QinBY@nic.bmi.ac.cn
Received 2000-12-05 Accepted 2001-03-18

INTRODUCTION

Many studies supported the hypothesis that MAO activity was an important factor influencing the effects of morphine (Mor). For example, Mor analgesia was potentiated by selective inhibition of brain MAO-B with deprenyl and antagonized by the MAO-A inhibitor clorgyline. Repeated administrations of MAO inhibitor pargyline could antagonize Mor analgesia, while acute administration of pargyline could enhance Mor analgesia both in tolerant and non-tolerant animals. Pargyline in a single dose could exacerbate jumping in mice undergoing abrupt withdrawal and enhance the withdrawal jumping response precipitated by naloxone (Nal) in Mor dependent mice, while pargyline didn't altering the process involved in the development of tolerance and physical dependence materially[1]. In recent years, imidazoline receptors (I-R) was found to be structurally related to MAO-B; 1) Chronic treatment with MAO-B inhibitors down-regulated I_2-imidazoline receptors in rat brain; 2) The regional distribution of I_2-imidazoline receptors correlated well with MAO-B; 3) Photolabeled I_2-imidazoline binding proteins could be immunoprecipitated with monoclonal antibodies to MAO-A and MAO-B; 4) In MAO-B knockout mice, [^{3}H]idazoxan binding was completely blocked; 5) Many I_2-imidazolines could inhibit MAO activity[2]. Furthermore, I-R and its endogenous ligand agmatine (Agm) were also thought to be an important system that influenced opioid effects. Agm could inhibit tolerance to and dependence on Mor and enhance Mor analgesia through activation of I-R and inhibition of nitric oxide synthase (NOS)[3-6]. I_2-imidazoline receptors were down-regulated in the brains of heroin addicts[7]. All these evidence inferred the important role of I-R and MAO-B on Mor tolerance and dependence, and imidazoline ligands might influence opioid effects through activation of I-R and inhibition of MAO activity.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6174-2629

In the present study, we investigated whether I-R density and MAO-B activity in different brain regions were changed after chronic treatment with Mor, whether Agm and idazoxan (Ida) could influence MAO-B activity, and whether this effect was correlated with its mechanism to influence Mor's effects, so that we could make clear the relationship between I-R and MAO-B during the development of Mor dependence.

MATERIALS AND METHODS

Animals Male Wistar rats (200 g ± 20 g, Grade II, Certificate № 01-3039) were provided by the Experimental Animal Center of Academy of Military Medical Sciences.

Drugs and reagents Idazoxan was purchased from Research Biochemicals Inc (RBI, Wayland MA, USA). Naloxone hydrochloride and agmatine sulphate were got from Sigma Chemical Co (St Louis, MO, USA). [^{3}H]Idazoxan (1739 GBq/mmol) was obtained from Amersham International (Buckinghamshire, UK). Morphine hydrochloride was produced by Qinghai Pharmaceutical Factory, China. Methanol (HPLC grade) was from Fisher Chemicals (Fair Lawn, NJ, USA). Benzalamine hydrochloride and benzaldehyde were obtained from Beijing Chemical Plant, China. All other reagents were of analytical grade and purchased from commercial sources.

Determination of MAO-B activity by HPLC[8] Rat brain was removed rapidly and dissected into different brain regions (cerebral cortex, hippocampus, thalamus, cerebellum, and striatum), then frozen at −70 ℃. Before the experiments, the brain regions were homogenized in ten volumes of cold PBS (pH 7.4). Protein was determined by the method of Lowry, with bovine serum albumin as the standard.

The enzyme incubation mixture contained the following components in a total volume of 0.5 mL, 0.35 mL of sodium phosphate buffer (pH 7.4, 0.1 mol/L), 0.1 mL of brain homogenate, and 0.05 mL of benzylamine (20 mmol/L). The mixture was incubated at 37 ℃ water bathe for 30 min. The reaction was stopped by the addition of perchloric acid (1 mol/L) 50 μL. Protein was removed by centrifugation at 8000 × *g*. Aliquot volume of 20 μL was injected into the liquid chromatography.

The liquid chromatographic system consisted of a HP1100 series binary pump, a Zorbax ODS column (250 mm × 4.6 mm, 5 μm) fitted with a precolumn (45 mm × 4.6 mm). The eluted components were detected by ultraviolet absorption (UV) at 254 nm with a TSP UV2000 detector. The elution was carried out isocratically using 40 % methanol containing sodium phosphate 50 mmol/L and heptane-sulphonic acid 1 mmol/L. The pH was adjusted to 3.2 with hydrochloride. The flow rate was 1.5 mL/min. The enzyme activity was calculated as nmol benzaldehyde formed per min per mg protein.

Effects of Mor, Nal, Agm, and Ida on MAO-B activity of normal rats were observed on the whole brain homogenate with different drugs in the enzyme incubation mixture.

Male Wistar rats were treated with increasing doses of Mor (10 − 80 mg/kg, tid, sc) for 16 d. Then MAO-B activity of different brain regions was detected to evaluate the chronic effect of Mor on MAO-B activity.

Effect of Nal or Ida on MAO-B activity of Mor dependent rats was observed in homogenate of different brain regions. Male rats were pretreated with normal saline or increasing doses of Mor (10 − 50 mg/kg, tid, sc) for 5 d. Normal saline 1 mL/kg, Nal 5 mg/kg, or Ida 9 mg/kg were injected by ip 6 h after the last injection of Mor, the abstinent syndrome in the first 15 min were recorded and then the rats were decapitated.

Effect of chronic coadminstration of Ida or Agm with Mor on rats' MAO-B activity was observed on homogenate of different brain regions. Male rats were pretreated concomitantly with Agm 10 mg/kg or Ida 9 mg/kg by ip and increasing doses of Mor (10 − 50 mg/kg, tid, sc) for 5 d. After 6 h of the last injection, rats were decapitated for the experiment.

Ligand binding test[9] Male Wistar rats were treated with increasing doses of Mor (10 − 80 mg/kg, tid, sc) for 16 d. At the 17th day, the rats were decapitated, their brains removed rapidly and dissected into different brain regions (cerebral cortex, thalamus, and cerebellum), then frozen at −70 ℃ until required. The tissue samples were homogenized in 5 mL of ice-cold Tris-sucrose buffer (Tris-HCl 5 mmol/L, sucrose 320 mmol/L, $MgCl_2$ 1 mmol/L, pH 7.1). The homogenate was centrifuged at 4 ℃, 1500 × *g* for 10 min and the supernatant was then centrifuged at 33 000 × *g* for 25 min. The resulting pellet was washed twice with fresh incubation buffer (Tris-HCl 50 mmol/L, ascorbic acid 0.1 %, pH 7.5). The final pellet was resuspended in an appropriate volume of this buffer to a final protein content of 1 g/L.

Binding of [^{3}H] idazoxan to I-R in rat brain was done in the presence of (−)-noradrenaline bitartrate 1 μmol/L and 0.1 % of ascorbic acid to prevent the binding of the radioligand to α_2-adrenoceptors. Nonspecific binding was determined in the presence of 100 μmol/L idazoxan. Brain samples (0.25 g/L) were incubated with 6 increasing concentrations of [^{3}H] idazoxan (2 − 64 nmol/L) as above. The total binding was determined as above and plotted as a function of the drug concentration. The specific binding was defined as the difference between total binding and nonspecific binding and was plotted as a function of increasing concentrations of the radioligand.

Total [^{3}H] idazoxan binding to I-R was measured in 0.2 mL aliquots (Tris-HCl 50 mmol/L, pH 7.5), which was incubated in shaking for 60 min at 25 ℃. Incubation was terminated by diluting the samples with 5 mL of ice-cold incubation buffer. Bound and free radioligands were separated by vacuum filtration through Whatman GF/C glass fiber filters. Then the filters were rinsed twice with 5 mL of incubation buffer, air-dried, transferred to minivials containing 5 mL of scintillation liquid and counted for radioactivity by liquid scintillation spectrometry at 42 % efficiency.

Statistics The concentrations of benzaldehyde in the incubation mixture were calculated with reference to the peak height of external standard. Analysis of saturation isotherms (K_d: dissociation constant; B_{max}: maximum density of binding sites) was performed by computer-assisted nonlinear regression. Results are expressed as $\bar{x} \pm s$. Statistical significance between control and experimental groups were computed by t-test. Difference was judged significant at $P < 0.05$.

RESULTS

Detection of MAO-B activity Ida inhibited MAO-B activity dose-dependently and at the concentration of 1 mmol/L the MAO-B activity decreased to 60 % (Tab 1). This effect of Ida seemed not to be mediated through I-R but by the direct action on MAO-B, since Agm alone didn't affect the activity of MAO-B even at the highest concentration (1 mmol/L), and it had no effect on the inhibition of MAO-B activity by Ida. Nal didn't influence the MAO-B of normal rats (data not show).

Mor alone inhibited the activity of MAO-B to about 72 % at the highest dose. A synergism effect between

Tab 1. Effect of drugs on the whole brain MAO-B activity in naive rats. $n = 6$. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* NS.

Drug	Concentration/ μmol·L^{-1}	MAO-B activity/ nmol·min·mg^{-1} protein	Ratio of drug to NS
NS		1.2 ± 0.5	1.0
Agm	10	1.3 ± 0.3	1.1
Agm	100	1.5 ± 0.5	1.2
Agm	1000	1.21 ± 0.22	1.0
Ida	10	1.2 ± 0.4	1.0
Ida	100	1.1 ± 0.3	0.9
Ida	1000	0.71 ± 0.14^b	0.6
Ida + Agm	10 + 100	1.2 ± 0.3	1.0
Ida + Agm	100 + 100	1.0 ± 0.4	0.8
Ida + Agm	100 + 1000	0.8 ± 0.3^b	0.7
Mor	10	0.98 ± 0.23	0.8
Mor	100	0.94 ± 0.24	0.8
Mor	1000	0.86 ± 0.24^b	0.7
Mor + Agm	100 + 10	0.94 ± 0.26	0.8
Mor + Agm	100 + 100	0.89 ± 0.29	0.7
Mor + Agm	100 + 1000	0.91 ± 0.25	0.8
Mor + Ida	100 + 10	0.85 ± 0.22^b	0.7
Mor + Ida	100 + 100	0.77 ± 0.17^c	0.6
Mor + Ida	100 + 1000	0.56 ± 0.14^c	0.5
Mor + Agm + Ida	100 + 100 + 10	0.81 ± 0.23^b	0.7
Mor + Agm + Ida	100 + 100 + 100	0.80 ± 0.22^b	0.7
Mor + Agm + Ida	100 + 100 + 1000	0.61 ± 0.19^c	0.5

NS: normal saline; Agm: agmatine; Ida: idazoxan; Mor: morphine.

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

Ida and Mor was observed, MAO-B activity decreased to 47 %. Agm didn't influence the effect of Mor on MAO-B (Tab 1).

MAO-B activity of all the five brain regions (cerebral cortex, hippocampus, thalamus, cerebellum, and striatum) decreased after 16 d treatment of Mor (Tab 2).

Tab 2. Effect of chronic Mor treatment on MAO-B activity of different brain regions in rats. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **NS.**

Brain regions	MAO-B activity/nmol·min·mg^{-1} protein	
	NS	Mor
Cerebral cortex	0.85 ± 0.09	0.72 ± 0.06^c
Hippocampus	0.83 ± 0.17	0.73 ± 0.07^c
Thalamus	0.64 ± 0.07	0.59 ± 0.09^c
Striatum	0.77 ± 0.06	0.68 ± 0.09^c
Cerebellum	0.85 ± 0.05	0.73 ± 0.12^c

NS: normal saline; Mor: morphine.

The MAO-B activity of rats decreased significantly in cerebellum and cerebral cortex after chronic administration of Mor for 5 d ($P < 0.01$). Acute challenge with Nal led to typical abstinent syndrome such as jumping, writhing, wet dog shakes, teeth chatting, ptosis, diarrhea, and hostility on handling. Ida induced some of these symptoms, but not severe as Nal (data not show). Ida and Nal didn't further affect MAO-B activity of Mor dependent rats in all brain regions (Tab 3).

Agm co-administrated with Mor could prevent most of the abstinent syndrome precipitated by Nal in Mor dependent rats (data not show). In addition, although Agm itself didn't affect MAO-B activity *in vitro*, co-administration of Agm with Mor could reverse the effect of Mor on MAO-B activity. This action might be related to its preventing effect against substance dependence on Mor. Chronic treatment with Ida didn't influence MAO-B activity in Mor-dependent rats (Tab 4).

Receptor binding assay saturation binding studies were performed with brain regions including cerebral cortex, thalamus, and cerebellum of Mor-dependent rats to determine the density and affinity of [^{3}H] idazoxan binding sites labeled by Ida. The study revealed chronic administration of Mor significantly decreased the density of [^{3}H] idazoxan binding sites (B_{max} decreased by 44.2 % and 55.3 % for cerebral cortex and cerebellum respectively, $P < 0.05$, Tab 5) compared with controls. In addition to the decrease of B_{max} of the [^{3}H idazoxan binding sites, the affinity increasd significantly ($P < 0.05$ or $P < 0.01$, Tab 5). The density (B_{max}) and the affinity (K_d) of I-R in the thalamus changed either, but not significantly.

Tab 3. Effects of Nal (5 mg/kg, ip) or Ida (9 mg/kg, ip) on brain MAO-B activity in Mor-dependent rats. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **Mor.**

Brain regions	MAO-B activity/nmol·min·mg^{-1} protein			
	NS	Mor	Mor + Nal	Mor + Ida
Cerebral cortex	1.35 ± 0.09^c	1.16 ± 0.06	1.06 ± 0.16	1.25 ± 0.04
Hippocampus	0.84 ± 0.08^c	0.75 ± 0.03	0.69 ± 0.05	0.74 ± 0.06
Thalamus	0.59 ± 0.07	0.59 ± 0.04	0.56 ± 0.07	0.59 ± 0.04
Striatum	0.62 ± 0.08	0.62 ± 0.05	0.56 ± 0.07	0.59 ± 0.07
Cerebellum	1.21 ± 0.25	1.07 ± 0.17	0.96 ± 0.06	0.97 ± 0.08

NS: normal saline; Mor: morphine; Nal: naloxone; Ida: idazoxan.

Tab 4. Effects of coadministration of Mor with Agm (10 mg/kg, ip)- or Ida (9 mg/kg, ip)-pretreatment on brain MAO-B activity. $n = 6$. $\bar{x} \pm s$. $^cP < 0.01$ *vs* **Mor.**

Brain regions	MAO-B activity/nmol·min·mg^{-1} protein			
	NS	Mor	Mor + Agm	Mor + Ida
Cerebral cortex	0.91 ± 0.08^c	0.59 ± 0.08	0.72 ± 0.07^c	0.58 ± 0.05
Hippocampus	0.66 ± 0.09^c	0.53 ± 0.05	0.72 ± 0.03^c	0.59 ± 0.08
Thalamus	0.72 ± 0.07	0.77 ± 0.07	0.82 ± 0.03	0.79 ± 0.14
Striatum	0.69 ± 0.16	0.64 ± 0.21	0.64 ± 0.26	0.79 ± 0.14
Cerebellum	1.03 ± 0.08^c	0.83 ± 0.09	0.97 ± 0.04^c	0.81 ± 0.16

NS: normal saline; Mor: morphine; Agm: agmatine; Ida: idazoxan.

Tab 5. Regulation of the quantity and binding affinity of I_2-imidazoline receptors by chronic morphine treatment. $x \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* NS.

Brain regions	n	B_{max}/fmol·mg^{-1} protein		K_d/nmol·L^{-1}	
		NS	Mor	NS	Mor
Cerebral Cortex	4	172 ± 29	96 ± 40^b	72 ± 14	37 ± 11^b
Thalamus	3	81 ± 13	62 ± 9	28 ± 10	14.4 ± 1.9
Cerebellum	3	171 ± 7	81 ± 15^b	66 ± 14	28 ± 11^c

DISCUSSION

Recently, I_2-imidazoline receptors were supposed to locate exclusively on MAO-B[2]. However, other studies suggested that I_2-imidazoline receptors were not directly related to the site of action of these drugs on MAO activity, since most imidazoline/guanidine drugs were only weak inhibitors of MAO and the nature of the interactions was most probably through competitive/catalytic site related mechanisms[10]. Furthermore, imidazoline-binding domain on MAO was proved to distinct from the enzyme active site recognizing the mechanism-based inhibitors such as pargyline and deprenyl[11]. In this study, we found that Ida could inhibit MAO-B activity, while Agm, the endogenous ligand of I-R, didn't influence MAO-B activity and had no effect on the inhibition of MAO-B by Ida. The result indicated that the inhibition of MAO-B by Ida was not exerted through blockade of I-R but through it's direct action on MAO-B.

Mor could inhibit MAO-B activity and MAO-B inhibitors such as deprenyl and pargyline were found to enhance Mor analgesia[1,12], thus MAO-B was supposed to be an important factor influencing the effects of Mor, and it was possible that all MAO-B inhibitors might enhance Mor analgesia. However, in our study Agm didn't affect MAO-B activity but enhanced Mor analgesia[4,13], while Ida inhibited MAO-B activity and antagonized Mor analgesia[14]. Thus the effects of Agm and Ida on Mor analgesia were through activation or inhibition of I-R, but not related to their effects on MAO-B activity; and the effects of pargyline or deprenyl on enhancing Mor analgesia mingt depend on some other mechanisms instead of their direct inhibitory effect on MAO-B activity.

Chronic Mor treatment could increase the rate of catecholamine synthesis, induce the release of catecholamine, and decrease the metabolism of the monoamines through inhibition of MAO activity[15]. Pargyline by a single dose could exacerbate jumping in mice undergoing abrupt withdrawal, and enhance the withdrawal jumping response precipitated by Nal in Mor-dependent mice[1]. All these result inferred the important role of MAO-B on the abstinent syndrome of Mor-dependent rats. We also observed the decrease of MAO-B activity in all brain regions of Mor dependent rats. Nal could precipitate the abstinent syndrome, this might depend on the effect of Nal on the release of catecholamines since Nal didn't affect the brain MAO-B activity. Ida could also induce the abstinent syndrome of Mor dependent rats[15], but this effect might be related to its inhibition on MAO-B activity although no further change of the brain MAO-B activity was observed after acute challenge with Ida. Chronic coadministration of Agm with Mor prevented the down-regulation of MAO-B activity, this effect might be exerted through I-R and relevant to its inhibitory effect on the abstinent syndrome of Mor-dependent rats. However, the exact mechanism of the influence of Agm on MAO-B activity after chronic administration still needs to be clarified.

I_2-imidazoline receptors were down-regulated in the brain of heroin addicts[7]. Consistent with this study, I_2-imidazoline receptors were also down-regulated in cerebral cortex and cerebellum of Mor-dependent rats in our study, this down-regulation demonstrated the presence of a functional interaction between I-R and the opioid receptors. Furthermore, both MAO-B and I-R were down-regulated after chronic Mor treatment, this result inferred the structurally relationship between MAO-B and I-R. The down-regulation of MAO-B activity and I-R were marked in cerebral cortex and cerebellum after Mor treatment, this indicated that these two brain regions were the earliest regions influenced by Mor.

In conclusion, our study indicated that inhibition of MAO-B activity was relevant at least partly to the precipitation of abstinent syndrome of morphine dependent rats. Influence of agmatine and idazoxan on morphine analgesia was through activation or blockade of imidazoline recep-

ISSN 0253-9756 Acta Pharmacol Sin 中国药理学报 2001 Jul; 22 (7)
E-mail aps@mail.shcnc.ac.cn Phn/Fax 86-21-6474-2629

tors, but not through their direct action on MAO-B. Down-regulation of imidazoline receptors and MAO-B activity after morphine treatment indicated MAO-B was structurally related to but functionally different from imidazoline binding proteins, and imidazoline receptors might be another receptors influencing the effect of opioids.

REFERENCES

1 Iwamoto ET, Ho IK, Way EL. Effect of pargyline on morphine tolerance and physical dependence development in mice. Eur J Pharmacol 1976; 38: 261 – 8.
2 Remaury A, Ordener C, Shih J, Parini A. Relationship between I_2 imidazoline binding sites and monoamine oxidase B in liver. Ann NY Acad Sci 1999; 881: 32 – 4.
3 Li J, Li X, Pei G, Qin BY. Agmatine inhibited tolerance to and dependence on morphine in guinea pig ileum *in vitro*. Acta Pharmacol Sin 1998; 19: 564 – 8.
4 Li J, Li X, Pei G, Qin BY. Analgesic effect of agmatine and its enhancement on morphine analgesia in mice and rats. Acta Pharmacol Sin 1999; 20: 81 – 5.
5 Li J, Li X, Pei G, Qin BY. Effects of agmatine on tolerance to and substance dependence on morphine in mice. Acta Pharmacol Sin 1999; 20: 232 – 8.
6 Li J, Li X, Pei G, Qin BY. Correlation between inhibitions of morphine withdrawal and nitric oxide synthase by agmatine. Acta Pharmacol Sin 1999; 20: 375 – 80.
7 Sastre M, Ventayol P, Garcia-Sevilla JA. Decreased density of I_2-imidazoline receptors in the postmortem brain of heroin addicts. Neuroreport 1996; 7: 509 – 12.
8 Nissinen E. Determination of monoamine oxidase B activity by high-performance liquid chromatography. J Chromatography 1984; 319: 156 – 9.
9 Alemany R, Olmos G, Garcia-Sevilla JA. Labeling of I_{2B}-imidazoline receptors by [^{3}H] 2-(2-benzofuranyl)-2-imidazoline (2-BFI) in rat brain and liver: characterization, regulation and relation to monoamine oxidase enzymes. Naunyn Schmiedebergs Arch Pharmacol 1997; 356: 39 – 47.
10 Ozaita A, Olmos G, Boronat MA, Lizcano JM, Unzeta M, Garcia-Sevilla JA. Inhibition of monoamine oxidase A and B activities by imidazol (ine)/guanidine drugs, nature of the interaction and distinction from I_2-imidazoline receptors in rat liver. Br J Pharmacol 1997; 121: 901 – 12.
11 Raddatz R, Savic SL, Lanier SM. Imidazoline binding domains on MAO-B localization and accessibility. Ann NY Acad Sci 1999; 881: 26 – 31.
12 Garzon J, Moratalla R, Del Rio J. Potentiation of the analgesia induced in rats by morphine or [*D*-Ala^2]-MET-Enkephalinamide after inhibition of brain type B monoamine oxidase: the role of phenylethylamine. Neuropharmacology 1980; 19: 723 – 9.
13 Kolesnikov Y, Jain S, Pasternak GW. Modulation of opioid analgesia by agmatine. Eur J Pharmacol 1996; 296: 17 – 21.
14 Su RB, Li J, Gao K, Pei G, Qin BY. Influence of idazoxan on analgesia, tolerance and physical dependence of morphine in mice and rats *in vivo*. Acta Pharmacol Sin 2000; 21: 1011 – 5.
15 Catravas GN, Takenaga J, McHale CG. Effect of chronic administration of morphine on monoamine oxidase activity in discrete regions of the brain of rats. Biochem Pharmacol 1977; 26: 211 – 4.

吗啡长期给药后大鼠脑内 MAO-B 活性及咪唑啉受体的下调[1]

苏瑞斌[2], 李 锦, 李 昕, 秦伯益
(北京毒物药物研究所, 北京 100850, 中国)

关键词 吗啡; 单胺氧化酶; 咪唑克生; 胍丁胺

目的: 探讨吗啡长期给药处理后大鼠不同脑区 MAO-B 活性及咪唑啉受体含量的变化. **方法**: 用[^{3}H]咪唑克生配体结合试验测定咪唑啉受体含量, 用高效液相色谱法测定 MAO-B 活性. **结果**: 咪唑克生和吗啡能剂量依赖性地抑制大鼠脑匀浆 MAO-B 活性. 咪唑啉受体的内源性配体胍丁胺既不影响 MAO-B 活性, 也不影响咪唑克生及吗啡对 MAO-B 活性的抑制作用. 吗啡连续给药 16 d 后大鼠大脑、海马、丘脑、纹状体及小脑内 MAO-B 活性均显著下调($P<0.01$). 纳洛酮及咪唑克生单次给药对吗啡依赖大鼠上述脑区 MAO-B 活性均没有进一步影响; 胍丁胺伴随吗啡给药后能显著抑制吗啡降低 MAO-B 活性的作用. 吗啡连续给药后大鼠皮层和小脑咪唑啉受体数量减少而亲和力上调($P<0.05$ 或 $P<0.01$). **结论**: MAO-B 活性与吗啡依赖大鼠发生戒断综合征相关, 但与胍丁胺对吗啡镇痛作用的影响无关; 胍丁胺对吗啡药理作用的影响与其激活咪唑啉受体有关.

(责任编辑 吴民淑)