ELSEVIER

European Journal of Pharmacology 381 (1999) 129–133

ejp

www.elsevier.nl/locate/ejphar

Effects of GABAergic system on naloxone-induced jumping in morphine-dependent mice

Mohammad-Reza Zarrindast [a,*], Elaheh Mousa-Ahmadi [a,b]

[a] *Department of Pharmacology, School of Medicine, Tehran University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*
[b] *Neuroscience Research Center, Kerman University of Medical Sciences, P.O. Box 13145-784, Tehran, Iran*

Received 1 March 1999; received in revised form 21 July 1999; accepted 27 July 1999

Abstract

The effect of GABA (γ-aminobutyric acid system) receptor agonists and antagonists on naloxone-induced jumping in morphine-dependent mice was examined. Intraperitoneal (i.p.) or intracerebroventricular (i.c.v.) injection of different doses of the $GABA_B$ receptor agonist, baclofen (2.5, 5 and 10 mg/kg), reduced naloxone-induced jumping in morphine-dependent mice. The i.p. administration of the $GABA_B$ receptor antagonist, CGP35348 (*P*-[3-aminopropyl]-*p*-diethoxymethyl-phosphinic acid), but not the i.c.v. injection of the drug, increased naloxone-induced jumping. The antagonist also decreased the baclofen response. Administration of the $GABA_A$ receptor agonist, muscimol, but not the $GABA_A$ receptor antagonists bicuculline and picrotoxin, decreased the naloxone response in morphine-dependent animals. It is concluded that both $GABA_A$ and $GABA_B$ receptor subtypes may have an inhibitory influence on naloxone-induced withdrawal jumping in mice.

Keywords: Jumping; Morphine; Naloxone; GABA (γ-aminobutyric acid) agent; (Mouse)

1. Introduction

Effects of opioid drugs on GABA (γ-aminobutyric acid system) functions in the brain have been studied by several investigators. It has been reported that morphine causes an increase in whole brain GABA concentration in mice (Takanaka et al., 1976) and also an increase of GABA in discrete parts of the thalamus and in the spinal cord of the rat (Kuriyama and Yoneda, 1976).

Previously we have shown that drugs acting on GABA are able to alter the analgesia induced by morphine (Zarrindast and Djavdan, 1988) or stress (Zarrindast and Sabetkasai, 1992).

A major restricting factor in the clinical use of opioids is the fear of drug dependence (Weis et al., 1983) and their ability known to induce behavioural reinforcing effects (Bilsky et al., 1992). Chronic administration of morphine may modify central GABA receptors (Ticku and Huffman, 1980). It is well documented that GABA mediates a variety of pharmacological events including sedative, analgesic and anticonvulsant responses (De Feudis, 1982; Sawynok and Labella, 1982). GABA administration has been shown to facilitate the development of tolerance to and physical dependence on morphine in mice (Ho et al., 1976). Elevation of brain levels of GABA by aminooxyacetic acid enhanced the development of tolerance and physical dependence. On the other hand bicuculline, a GABA receptor antagonist, reduces tolerance and dependence (Ho et al., 1976). GABA functions through different receptor sites (Bowery et al., 1980, 1981). In the present study, effects of two different GABA receptor agonists and antagonists on naloxone-induced jumping behaviour in morphine-dependent mice was investigated.

2. Materials and methods

2.1. Animals

Male NMRI mice weighing 20–25 g were used in all experiments. The animals were housed in groups of seven under conditions of constant temperature (22°C–24°C) and in a light controlled room (light period, 0700–1900 h). The

* Corresponding author. Fax: +98-21-640-4377; e-mail: zarinmr(@)nrcgeb.ac.ir

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00546-4

animals had free access to food and water except during the experiments.

2.2. Induction of dependence

The animals were rendered dependent on morphine, using the method employed previously (Zarrindast and Farzin, 1996). Morphine sulfate was injected subcutaneously (s.c.) three times daily at 9, 13 and 17 h at doses of 50, 50 and 75 mg/kg, respectively. The higher dose at the third daily injection was aimed to minimize any overnight withdrawal. Morphine administration was carried out over a maximum of 3 days for all groups of mice. A dose of 50 mg/kg of morphine sulfate also was injected on the fourth day (1 h before naloxone injection).

2.3. Naloxone-induced jumping

Groups of animals were observed for the occurrence of jumping after their 10th injection of morphine on day 4. One hour after the last dose of morphine (50 mg/kg), signs of abstinence were precipitated by a subcutaneous injection of naloxone; then animals were placed individually in a glass cylinder (25 cm, diameter; 40 cm, height) and the number of jumps was recorded for a 30-min period.

2.4. Chronic guide cannula implantation

Stainless-steel guide cannulas (23 gauge) were stereotaxically (David Koft Instruments, USA) implanted under anaesthesia with pentobarbital (60 mg/kg; intraperitoneal, i.p.), 5–7 days before the experiments. The guide cannulas were implanted in the left lateral ventricle at the following coordinates, based on the method of Jiang et al. (1990) with a minor modification: 2 mm lateral and 0.9 mm caudal to bregma at the depth of 3 mm. The drugs were injected in a volume of 2 μl over a period of 2 min, by means of an internal cannula (30 gauge) connected with polyethylene tubing to a 10-μl Hamilton syringe. The injection cannula was left in place for a further 1 min before being slowly withdrawn.

2.5. Drug treatment

The animals were treated as follows: group 1 received 10 injections of morphine as described in Section 2.2., in order to develop dependence to morphine. The number of jumps induced by naloxone was compared with those induced in group 2 that received 10 injections of saline instead of morphine. Groups 3, 4, 5 and 6 were dependent mice, which were used to test effects of GABA receptor agonists and antagonists on naloxone-induced jumping. Group 3 received different doses of muscimol, bicuculline or picrotoxin i.p. 15 min before naloxone. Group 4 received different doses of muscimol or bicuculline intracerebroventricularly (i.c.v.), 5 min before naloxone. Group 5 received different doses of baclofen or CGP35348 i.p., 15 min before naloxone. Gruop 6 received different doses of baclofen or CGP35348 i.c.v., 5 min before naloxone.

2.6. Drugs

The chemicals used were: Morphine sulfate (Mac-Farlan, Smith, England) naloxone hydrochloride (Dupont, Germany), baclofen, CGP35348 ([*P*-(3-aminopropyl)-*p*-diethoxymethyl-phosphonic acid], Ciba-Geigy, Switzerland), muscimol, (+)-bicuculline and picrotoxin (Sigma, Poole, England). (+)-Bicuculline was dissolved in saline with a drop of glacial acetic acid and the other drugs were dissolved in saline only. The drugs were injected i.p. in a volume of 10 ml/kg or i.c.v. in a volume of 2 μl/mouse, respectively. The i.p. injections of muscimol (0.5–2 mg/kg), baclofen (0.1–3 mg/kg), bicuculline (1–3 mg/kg), picrotoxin (1–3 mg/kg) and CGP35348 (75–350 mg/kg) were made 15 min before naloxone administration. The i.c.v. injections of muscimol (2–6 μg/mouse), baclofen (0.1–2 μg/mouse), bicuculline (1–4 μg/mouse) and CGP35348 (5–20 μg/mouse) were made 5 min before naloxone injection. The doses of drugs used had been shown to be active in previous studies (Zarrindast and Oveisi, 1987; Zarrindast and Farahvash, 1994; Sabetkasai et al., 1999).

2.7. Statistical analysis

Analyses of variance (ANOVAs) followed by Newman–Keuls test were used for analysis of the data. Differences between means were considered statistically significant if $P < 0.05$. Each point is the mean ± S.E.M. for seven mice.

3. Results

3.1. Naloxone-induced withdrawal jumping in morphine-dependent mice

The mice were divided randomly into two groups. One group received morphine (as described in the Method section) to induced dependence. The next group received saline (10 ml/kg) instead of morphine. Naloxone (2 mg/kg, s.c.) increased the number of jumps in morphine-dependent mice (41.0 ± 10; $N = 7$) as compared with non-dependent mice (0.2 ± 0.2; $N = 7$, $P < 0.0001$). The results showed that naloxone can induce jumping in morphine-dependent mice. We considered jumping behaviour as the sign of abstinence for further experiments in our study. Hyperactivity and straub-tail reaction were seen after morphine injections. Loss of weight (7%–16%) and death (1%–2%) occurred with chronic administration of morphine sulfate.

3.2. Effects of $GABA_A$ receptor agonist and antagonist on naloxone-induced jumping behaviour in morphine-dependent mice

The i.p. administration of the $GABA_A$ receptor agonist, muscimol (0.5, 1 and 2 mg/kg) [$F(3,24) = 6.5$, $P < 0.01$], but not of the $GABA_A$ receptor antagonist, bicuculline (1, 2 and 3 mg/kg, i.p.) [$F(3,24) = 1.8$, $P > 0.05$], when administered 15 min before naloxone (2 mg/kg, s.c.) significantly decreased the jumping response induced by naloxone in morphine-dependent animals. Various doses of picrotoxin (1, 2 and 3 mg/kg) [$F(3,24) = 0.5$, $P > 0.5$] did not alter the naloxone response (Fig. 1).

The i.c.v. injection of muscimol (2, 4 and 6 μg/mouse) [$F(3,24) = 6.9$, $P < 0.0001$] or bicuculline (1, 2 and 4 μg/mouse)[$F(3,24) = 5.3$, $P < 0.01$], 5 min before naloxone, also reduced naloxone-induced jumping in the morphine-dependent mice (Fig. 2). The data may indicate that a $GABA_A$ receptor mechanism can alter naloxone-induced jumping behaviour.

ANOVA also indicated a significant difference between morphine-dependent animals that were received saline and those that received (i.p.) the $GABA_B$ receptor agonist, baclofen (0.1, 2 and 3 mg/kg) [$F(3,24) = 10.0$, $P < 0.001$] or the GABA receptor antagonist, CGP35348 (75, 100 and

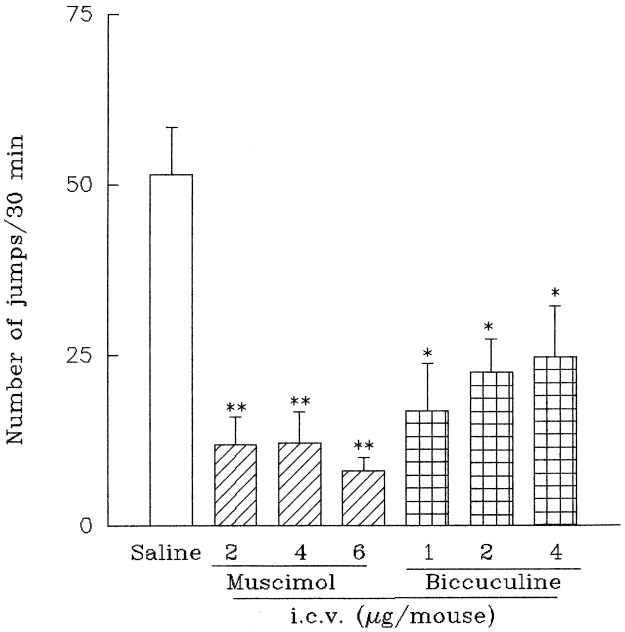

Fig. 2. Effect of i.c.v. administration of $GABA_A$ receptor agonist and antagonist on jumping induced by naloxone in morphine-dependent mice. Animals were made dependent as described in the Methods section. All the dependent-mice received naloxone (2 mg/kg) to induce jumping. The animals received saline (2 μl/mouse), different doses of muscimol (2, 4 and 6 μg/mouse) or bicuculline (1, 2 and 4 μg/mouse) 5 min before naloxone administration. Each group comprised seven mice. Data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ different from the saline control group.

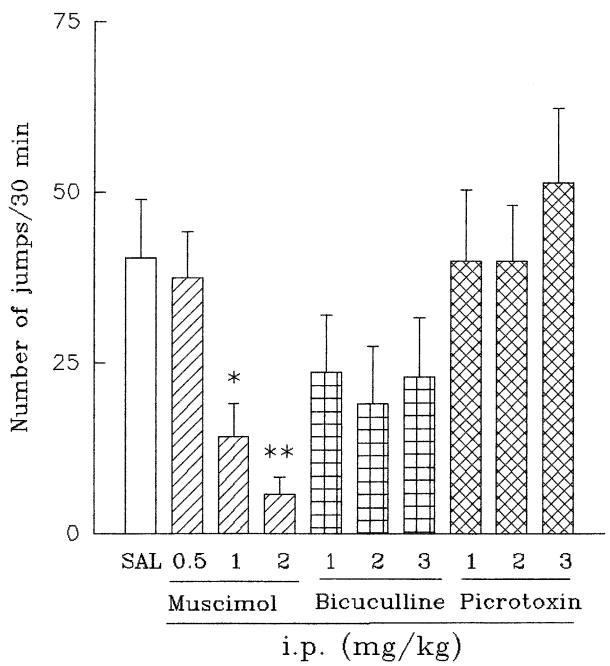

Fig. 1. Effects of $GABA_A$ receptor agonist and antagonist on naloxone-induced jumping in morphine-dependent mice. Mice were made dependent as described in the Methods section. All the dependent-mice received naloxone (2 mg/kg) to induce jumping. The animals received (i.p.) saline, muscimol (0.5, 1 and 2 mg/kg), bicuculline (1, 2 and 3 mg/kg) and picrotoxin (1, 2 and 3 mg/kg) 15 min before naloxone administration. Each group comprised of seven mice. Data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ different from the saline control group.

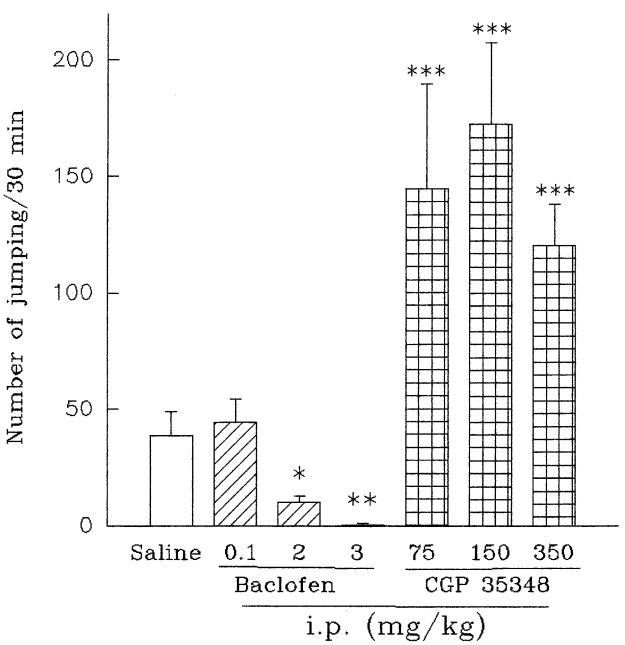

Fig. 3. Effects of $GABA_B$ receptor agonists and antagonists on jumping induced by naloxone in morphine-dependent mice. The animals received (i.p.) saline, baclofen (0.1, 2 and 3 mg/kg) or CGP35348 (75, 150 and 350 mg/kg) 15 min before naloxone administration. Each group comprised of seven mice. Data are means ± S.E.M. $^{*}P$,0.05, $^{**}P < 0.01$ different from saline control group.

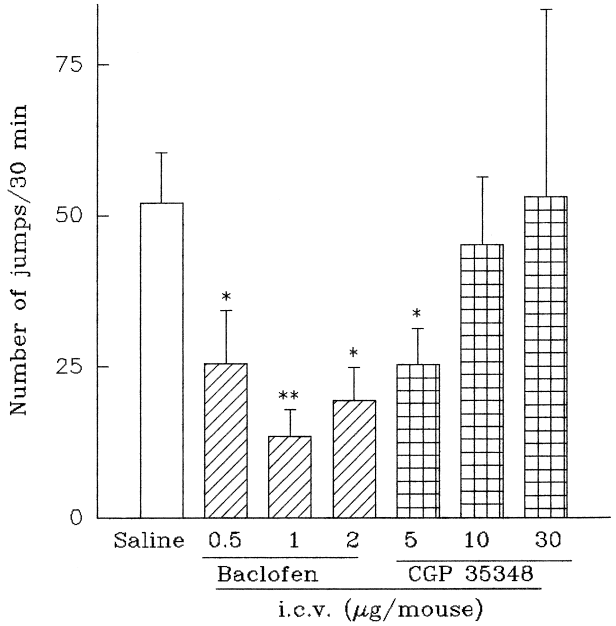

Fig. 4. Effect of i.c.v. administration of GABA receptor agonist and antagonist on jumping induced by naloxone in morphine-dependent mice. Animals were made dependent as described in the Methods section. All the dependent mice received naloxone (2 mg/kg) to induce jumping. The animals received saline (2 μl/mouse), different doses of baclofen (0.5, 1 and 2 μg/mouse) or bicuculline (5, 10 and 30 μg/mouse) 5 min before naloxone administration. Each group comprised seven mice. Data are means ± S.E.M. $^{*}P < 0.05$, $^{**}P < 0.01$ different from the saline control group.

350 mg/kg, i.p.)[$F(3,24) = 2.6$, $P < 0.05$], 15 min prior to naloxone administration (Fig. 3). Further analysis indicated that baclofen decreased, while a higher dose of CGP35348 increased naloxone-induced jumping behaviour.

The i.c.v. injection of baclofen (0.5, 1 and 2 μg/mouse) [$F(3,24) = 7.5$, $P < 0.001$] but not of CGP35348 (5, 10 and 20 μg/mouse)[$F(3,24) = 2.3$, $P > 0.05$] reduced naloxone-induced jumping in the morphine-dependent mice (Fig. 4). It thus seems likely that a $GABA_B$ receptor mechanism can alter naloxone-induced jumping behaviour.

4. Discussion

The role of several neurotransmitters including serotonin, dopamine, GABA, adenosine aspartate, and excitatory amino acid, in morphine tolerance and withdrawal has been investigated. They seem to be involved in morphine tolerance and dependence (Bhargava, 1994). A number of studies have also demonstrated that dopamine contributes to the expression of reward or morphine reinforcement (Bozarth and Wise, 1981). Interactions of GABA with dopaminergic pathways (Zarrindast and Moghadampour, 1991; Zarrindast and Farahvash, 1994), and adenosine (Sabetkasai and Zarrindast, 1993) also may indicate a greater complexity of the role of these neurotransmitters. It has been suggested that GABA-ergic and opiopeptidergic systems are interconnected through μ-opioid receptors (Desarmenien et al., 1984). GABA administration has also been shown to facilitate the development of tolerance and physical dependence (Ho et al., 1976). The present results indicate that both baclofen and muscimol, which are GABA receptor agonists (Bowery et al., 1983, 1984), inhibit naloxone-induced jumping behaviour in morphine-dependent mice in a dose-dependent manner. The data also may indicate that GABA receptor mechanism(s) are involved in morphine jumping behaviour.

The central inhibitory neurotransmitter, GABA (De Feudis, 1977; Johnston 1978; Hösli and Hösli, 1978), is found in all areas of the human brain (Perry et al., 1971). It is well established that the GABA system is a target for a variety of centrally pharmacological agents including sedatives, analgesics and anticonvulsants (De Feudis, 1982; Sawynok and Labella, 1982). GABA receptors in the brain have been classified as $GABA_A$ and $GABA_B$ (Bowery et al., 1980, 1981; Hill and Bowery, 1981). Whereas $GABA_A$ receptors are directly associated with a chloride channel, the $GABA_B$ receptors seem to be G-protein coupled and linked to Ca^{2+} or K^+ channels (Bormann, 1988). However, a third class of GABA binding site, the $GABA_C$ sites, resembles the $GABA_A$ receptor but is insensitive to bicuculline (Drew et al., 1984).

Since muscimol is a $GABA_A$ receptor agonist (Bowery et al., 1984) and baclofen acts on $GABA_B$ receptors (Bowery et al., 1983, 1984), it can be speculated that decreases in naloxone-induced jumping are mediated through both the $GABA_A$ and $GABA_B$ receptors. The inhibitory response induced by either i.p. or i.c.v. administration of the agonists, therefore, makes it likely that the responses to drugs are mediated through central GABA receptor mechanism(s). Our data are consistent with data which shows that chronic administration of morphine may modify central GABA receptors (Ticku and Huffman, 1980), and the blockade of postsynaptic GABA receptors by bicuculline inhibits the development of tolerance and dependence (Ho et al., 1976), indicating that the central GABA system is involved in the expression of jumping behaviour.

The i.c.v. but not i.p. administration of the competitive $GABA_A$ receptor antagonist, bicuculline, or the non-competitive $GABA_A$ receptor antagonist, picrotoxin (Ticku and Maksay, 1983), also decreased noloxone-induced jumping. Bicuculline has been shown to release GABA, which in turn may reduce jumping behaviour. The present data showed that i.p. injection of the higher dose of the $GABA_B$ receptor antagonist, CGP35348 (Olpe et al., 1990), increased naloxone-induced jumping. This may indicate that a $GABA_B$ receptor mechanism has a dominant inhibitory influence on this behaviour. However, the i.c.v. injection of the antagonist did not alter the naloxone-induced jumping. Therefore, a possibility may exist that the response induced by i.p. administration of a higher dose of

the drug is mediated through a peripheral mechanism. To clarify the exact mechanism(s) involved will require further work.

Acknowledgements

The authors wish to thank Dr. Y. Fathollahi and Dr. M. Sahebgharani for their assistance in preparing this manuscript.

References

Bhargava, M.N., 1994. Diversity of agents that modify opioid tolerance, physical dependence, abstinence syndrome, and self-administration behaviour. Pharmacol. Rev. 46, 293–324.

Bilsky, E.J., Montegut, M.J., Delong, C.L., Reid, L.D., 1992. Opioidergic modulation of cocaine conditioned place preference. Life Sci. 50, 85–90.

Bormann, J., 1988. Electrophysiology of GABAA and GABAB receptor subtypes. Trends Neurosci. 11, 112–116.

Bowery, N.G., Hill, D.R., Hudson, A.L., Doble, A., Middlemiss, D.N., Shaw, J., Turnbull, M.J., 1980. (−)-Baclofen decreases neurotransmitter release in the mammalian CNS by an action at a novel receptor. Nature 283, 92–94.

Bowery, N.G., Doble, A., Hill, D.R., Hudson, A.L., Shaw, J.S., Turnbull, M.J., Warrington, R., 1981. Bicuculline insensitive GABA receptors on peripheral autonomic nerve terminals. Eur. J. Pharmacol. 71, 53–70.

Bowery, N.G., Hill, D.R., Hudson, A.L., 1983. Characteristics of GABAB receptors binding sites on rat whole brain synaptic membranes. Br. J. Pharmacol. 78, 191–206.

Bowery, N.G., Price, G.W., Hudson, A.L., Hill, D.R., Wilkin, G.P., Turnbull, M.J., 1984. GABA receptor multiplicity: visualization of different receptor types in the mammalian CNS. Neuropharmacology 23, 219–231.

Bozarth, M.A., Wise, R.A., 1981. Heroin reward is dependent on a dopaminergic substrate. Life Sci. 29, 1881–1886.

De Feudis, F., 1977. GABA-ergic in the vertebrate nervous system. Prog. Neurobiol. 9, 123–145.

De Feudis, F.V., 1982. GABAergic analgesia — a naloxone insensitive system. Pharmacol. Res. Commun. 14, 383–390.

Desarmenien, M., Felz, P., Occhipiti, G., Santangeto, F., Schlicher, R., 1984. Coexistence of $GABA_A$ and $GABA_B$ receptors on AS and C primary afferents. Br. J. Pharmacol. 81, 327–333.

Drew, C.A., Johnston, G.A.R., Weatherby, R.P., 1984. Bicuculline insensitive GABA receptors: studies on the binding of (−)-baclofen to rat cerebellar membranes. Neurosci. Lett. 52, 317–321.

Hill, D.R., Bowery, N.G., 1981. 3*H*-Baclofen and 3*H*-GABA bind to bicuculline-insensitive GABAB sites in rat brain. Nature 290, 149–152.

Ho, I.K., Loh, H.H., Way, E.L., 1976. Pharmacological manipulation of gamma-aminobutyric acid (GABA) in morphine analgesia, tolerance and physical dependence. Life Sci. 18, 1111–1124.

Hösli, L., Hösli, E., 1978. Action and uptake of neurotransmitters in CNS tissue culture. Rev. Physiol. Biochem. Pharmacol. 81, 135–188.

Kuriyama, K., Yoneda, Y., 1976. Alteration in distribution and metabolism of gamma aminobutyric acid (GABA) in the central nervous system following morphine administration. Jpn. J. Pharmacol. (Suppl. 26), 18 pp.

Jiang, G., Mosberg, H.I., Porreca, F., 1990. Selective modulation of morphine antinociception, but not development of tolerance, by δ receptor. Eur. J. Pharmacol. 186, 137–141.

Johnston, J.A.R., 1978. Neuropharmacology of amino acid inhibitory transmitters. Ann. Rev. Pharmacol. Toxicol. 18, 269–289.

Olpe, H.R., Karlsson, G., Pozza, M.F., Brugger, F., Steinmann, N., Van Riezen, H., Fagg, G., Hall, R.G., Froestel, W., Bittigr, H., 1990. CGP 35348: a centrally active blocker of GABAB receptors. Eur. J. Pharmacol. 187, 27–38.

Perry, T.L., Berry, K., Hansen, S., Diamond, S., Mok, C., 1971. Regional distribution of amino acids in human brain obtained at autopsy. J. Neurochem. 18, 513–519.

Sabetkasai, M., Zarrindast, M.R., 1993. Antinociception: interaction between adenosine and GABA systems. Arch. Int. Pharmacodyn. 322, 14–22.

Sabetkasai, M., Khansefid, N., Yahyavi, S.H., Zarrindast, M.R., 1999. Baclofen and antidepressant-induced antinociception in formalin test: possible GABAB mechanism involvement. Psychopharmacology 142, 426–431.

Sawynok, J., Labella, F.S., 1982. On the involvement of GABA in the analgesia produced by baclofen, muscimol and morphine. Neuropharmacology 21, 397–403.

Takanaka, A., Tzeng, S.F., Ho, I.K., 1976. Acute and chronic effects of morphine on glutamic decarboxylase discrete brain areas of the mouse. Neuosci. Abstr. 2, 617.

Ticku, M.K., Huffman, R.D., 1980. The effects of acute and chronic morphine administration on GABA receptor binding. Eur. J. Pharmacol. 68, 97–106.

Ticku, M.K., Maksay, G., 1983. Convulsant/depressant site of action at the allosteric benzodiazepine-GABA receptor-ionophore complex. Life Sci. 33, 2363–2375.

Weis, O.F., Sriwatanakul, K., Alloza, J.L., Weintraub, M., Lasagna, L., 1983. Attitudes of patients, housestaff, and nurses toward postoperative analgesic care. Anesth. Analg. 62, 70–74.

Zarrindast, M.R., Djavdan, M., 1988. $GABA_A$-antagonists and baclofen analgesia. Gen. Pharmacol. 19, 703–706.

Zarrindast, M.R., Farahvash, H., 1994. Effects of GABA-ergic drugs on penile erection induced by apomorphine in rats. Psychopharmacology 115, 249–253.

Zarrindast, M.R., Farzin, D., 1996. Nicotine attenuates naloxone-induced jumping behaviour in morphine-dependent mice. Eur. J. Pharmacol. 298, 1–6.

Zarrindast, M.R., Moghadampour, E., 1991. Influences of dopamine agonists and antagonists on baclofen antinociception in mice. Arch. Int. Pharmacodyn. 309, 42–50.

Zarrindast, M.R., Oveisi, Y., 1987. $GABA_A$ and $GABA_B$ receptor sites involvement in rat thermoregulation. Gen. Pharmacol. 19, 223–226.

Zarrindast, M.R., Sabetkasai, M., 1992. Stress-induced antinociception and GABAergic mechanism. Arch. Int. Pharmacodyn. 318, 5–12.