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GAMMA-VINYL-GABA POTENTIATES THE SEVERITY OF NALOXONE-PRECIPITATED ABSTINENCE SIGNS IN MORPHINE-DEPENDENT RATS

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Effects of gamma-vinyl-GABA (GVG), an antiepileptic drug that inhibits GABA transaminase and increases extracellular GABA concentrations in the brain, were investigated on the morphine abstinence syndrome (AS) in male Wistar rats. Two morphine pellets (75 mg morphine base in each) were implanted subcutaneously on the back of the rats. Seventy-two hours after the morphine implantation, naloxone (NL, 2 mg kg^{-1}) was injected intraperitoneally (i.p.) to induce precipitated morphine AS. GVG was administered at the doses of 250 mg kg^{-1} ($n = 11$) and 500 mg kg^{-1} ($n = 11$) i.p. 24 h prior to AS and at the dose of 500 mg kg^{-1} ($n = 13$) i.p. 6 h prior to AS. Immediately after NL injections, rats were observed for 5 min and AS signs (jumping, teeth chattering, wet dog shake, diarrhoea, ptosis and defecation) were assessed. The behavioural signs of GVG-treated rats were compared with the control groups ($n = 10$) during the AS. Jumping, wet dog shake, teeth chattering were found to be significantly increased in all of the GVG-treated groups. Ptosis was found to have increased in only 500 mg kg^{-1} GVG groups. GVG potentiated the severity of morphine AS signs. GVG does not seem to have any therapeutic potential for treatment of morphine abstinence unlike some other drugs that enhance GABAergic transmission.

KEY WORDS: morphine physical dependence, gamma-vinyl-GABA (GVG), Vigabatrin, GABA, inhibition, morphine abstinence syndrome.

INTRODUCTION

Chronic administration of opiates, such as morphine, produces tolerance and dependence, limiting their clinical use [1, 2]. Although the mechanisms underlying the development of physical dependence and the expression of abstinence syndrome (AS) are not clear, previous reports suggest that multiple receptors and neurotransmitter systems may be involved in the development of opiate dependence [1–3]. It has recently been shown that excitatory amino acids (EAA) and activation of *N*-methyl-D-aspartate (NMDA) receptors play an important role in the development of morphine physical dependence (MPD) and in the expression of naloxone (NL) precipitated morphine withdrawal syndrome [4–9]. NMDA receptor antagonists blocked NL precipitated morphine AS [4–9]. It has also been reported that acute morphine administration is proconvulsant, as well as anticonvulsant [10]. It has been hypothesized that morphine abstinence syndrome is an excessive excitation of various regions of the central nervous system [7, 8]. Although the role of EAA and NMDA receptors has been defined, the subtle mechanisms of inhibitory systems in the development and expression of morphine AS has not yet been clearly elucidated.

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous

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system released from GABAergic interneurons and many studies have demonstrated the importance of GABAergic transmission in epilepsy or other neurological disorders [11]. It is known that GABAergic transmission inhibits the excitation of the neurons in various parts of the central nervous system. It has been reported that benzodiazepine pretreatment, that enhances the GABAergic transmission, suppresses morphine withdrawal signs [12]. It is also reported that the GABAergic system plays an important role in the development of MPD and/or morphine tolerance, however, a clear picture of the role of GABAergic system has not yet been explained [13, 14]. Hence it is important to elucidate the role of excitatory and inhibitory systems in the mechanism of the development of MPD.

Vigabatrin (gamma-vinyl-GABA, GVG) is a potent antiepileptic drug that inhibits GABA transaminase activity and increases extracellular GABA levels in many regions of the mammalian brain up to tenfolds [15–17]. GVG antagonizes seizures in various models of epilepsy [18–21] and has an anxiolytic effect [22]. GVG is expected to suppress the excitation of the central nervous system and the signs of NL precipitated AS in the rodent model of MPD. If this were the case, GVG could be a drug of choice in the treatment of morphine withdrawal syndrome, like diazepam [23]. The present study was designed to elucidate whether GVG had an inhibitory effect on the signs of MPD.

MATERIAL AND METHODS

Animals and laboratory

Male Wistar inbred rats (250–300 g), that were kept in a temperature (22 ± 2°C) and humidity (62 ± 4%) controlled room in which a 12-h light/dark cycle was maintained. They were fed with a standard regimen (lab chow) *ad libitum*.

Drugs

Vigabatrin was a generous gift from the Merrill-Dow Institute (Strasbourg, France). Naloxone and morphine were purchased from Sigma (St. Louis, MO), Verenigde Pharmaceutische Fabrieken B.V. (Holland), respectively. Vigabatrin and naloxone were dissolved in saline and injected into rats i.p., an equal amount of saline was injected into the control groups.

Methods

Two pellets containing 75 mg morphine base (M) (total 150 mg) were subcutaneously (SC) implanted on the back of the rats lightly anaesthetised with ether to induce MPD, as previously described [8, 24–26]. Abstinence syndrome was precipitated by the injection of NL (2 mg kg^{-1}) i.p. 72 h after pellet implantation. The rats were assigned into seven groups at random.

The experimental groups were as follows (summarised in Table I):

1. No treatment group ($n = 8$). The rats in this group were implanted with placebo pellets which did not contain morphine, they only received NL (2 mg kg^{-1}) i.p. 72 h after the pellet implantation and observed.
2. The group that received only GVG ($n = 8$). This was a control group for GVG. The rats in this group were injected with GVG (500 mg kg^{-1}) i.p. and observed 24 h later.
3. The group that received GVG and NL ($n = 8$). The rats in this group were injected with GVG (500 mg kg^{-1}) i.p. and 24 h later, they were injected with NL (2 mg kg^{-1}) i.p. and observed.
4. Control group ($n = 10$). These rats were implanted with two morphine pellets (MP, each containing 75 mg morphine base). Seventy-two hours after the implantation of MP, AS was precipitated by the injection of NL (2 mg kg^{-1}) i.p.
5. GVG-250 mg kg^{-1}-24 h group ($n = 11$). These rats were implanted with two MP. Twenty-four hours before the precipitated morphine AS, they were injected with GVG (250 mg kg^{-1}) i.p. Twenty-four hours was chosen because after the injection of GVG, GABA levels peaked at the 24th hour [15]. Twenty-four hours after GVG administration, the AS was

Table I
The experimental groups and injection schedule

Group	n	*Morphine pellet*	*GVG*		*Naloxone (NL)*
			Dose (mg kg^{-1})	*Time before NL (h)*	
1	8	(—)	(—)	(—)	✓
2	8	(—)	500	−24	(—)
3	8	(—)	500	−24	✓
4	10	✓	(—)	(—)	✓
5	11	✓	250	−24	✓
6	11	✓	500	−6	✓
7	13	✓	500	−24	✓

precipitated by the injection of NL (2 mg kg^{-1}) i.p.

6. GVG-500 mg kg^{-1}-6 h group ($n = 13$). These rats were implanted with two MP. Six hours before the NL (2 mg kg^{-1}) induced precipitated AS, they were injected with GVG (500 mg kg^{-1}) i.p.
7. GVG-500 mg kg^{-1}-24 h ($n = 11$). These rats were implanted with two MP. Twenty-four hours before the NL injection, they were injected with GVG (500 mg kg^{-1}) i.p. Seventy-two hours after MP implantation, they were injected with NL (2 mg kg^{-1}) i.p. to induce the AS and were observed.

In the MP implanted groups, after the injection of NL, the rats were immediately placed in a metal cage (base area, 20×22 cm; height, 20 cm) and observed for 10 min by an experimenter blind to group identification. The withdrawal signs of jumping, wet dog shake, teeth chattering, diarrhoea, defecation and ptosis were counted or rated [8, 24–26]. During this observation period the number of jumps, wet dog shakes and defecations were counted. Teeth chattering and, diarrhoea or ptosis were rated 1–10 and 1, 2, 3, respectively according to their severity as reported before [8, 24–26]. General ethical considerations on the maintenance of and experimentation on the animals were obeyed throughout this study.

The results were analysed by Kruskall–Wallis multiple comparison test, followed by Mann–Whitney *U*-test. The groups were compared with the control group and $P < 0.05$ was considered to be statistically significant.

RESULTS

The mean values ($\pm$ SE) and their statistical evaluation of the AS signs of groups 4–7 during the first 10 min immediately after 2 mg kg^{-1} of NL administration are shown in Figs. 1 and 2. The results of the observations of the behavioural changes in each group were as follows:

1. No treatment group. NL (2 mg kg^{-1}) did not induce any behavioural changes in this group. No major signs of AS (wet dog shake, teeth chattering, jumping, etc.,) were observed.
2. The group that received only GVG. GVG (500 mg kg^{-1}, i.p.) induced lethargy and immobility in rats 24 h after the injection. No other signs were observed.
3. The group that received GVG and NL. Twenty-four hours after the injection of GVG (500 mg kg^{-1}), NL (2 mg kg^{-1}) did not induce any significant behavioural changes in this group.
4. Control group. Seventy-two hours after the implantation of MP, they had the AS (jumping, teeth chattering, wet dog shake, etc.,) immediately after the injection of NL (2 mg kg^{-1}, i.p.) (Figs 1 and 2).
5. GVG-treated groups. GVG treatment, at all doses and time intervals, increased jumping [$H(3,41) = 21.46$, $P < 0.001$], wet dog shake [$H(3.41) = 8.28$, $P < 0.01$], teeth chattering [$H(3,41) = 22.75$, $P < 0.001$] compared to the control group [Fig. 1(A–C)]. It did not produce any significant changes on diarrhoea [$H(3,41) = 0.708$, $P > 0.05$] and defecation [$H(3,41 = 1.4$, $P > 0.05$] [Fig. 2(A,B)]. Ptosis was significantly different than the control group in 500 mg kg^{-1} GVG groups [$H = 6.36$, $P < 0.01$] (Fig. 2). GVG increased the severity of NL precipitated AS in morphine-dependent rats.

DISCUSSION

It was expected that GVG, an antiepileptic drug that increases extracellular levels of GABA in many parts of the brain [15, 17], would suppress the signs of morphine AS, however, we observed an opposite result.

Excitatory and inhibitory amino acids play an important role in the development and expression of MPD [5–9]. There is an altered release of excitatory and inhibitory neurotransmitters, e.g. glutamate [27] and/or GABA [14, 28] during the development of MPD. GABA levels are decreased in the ventral tegmental area [29], midbrain [30] and cortical slices [31] following morphine treatment. While GABA levels are found to be decreased, acetylcholine was reported to be increased in the cortex during morphine withdrawal [32]. Chronic morphine administration enhances $GABA_A$ receptor function and increases the benzodiazepine binding in the cortex [28]. Administration of diazepam (1, 2 and 4 mg kg^{-1}) injected 1 h before the NL challenge increased the number of jumping and wet dog shakes and the severity of abstinence syndrome [33]. Benzodiazepine pretreatment has also been reported to suppress the withdrawal [12] suggesting that a possible decrease of GABAergic inhibition may underlie MPD. Pentobarbital, that enhances GABAergic transmission, antagonize the antinociceptive effects of morphine [34].

During withdrawal, if GABA levels are decreased, the most important question is whether a reduction in GABA contributes to AS or results from it. When we administered GVG to elevate GABA levels in the brain prior to precipitation of AS by NL, we found unexpected results, similar to the findings of Baldino *et al.* [33].

Our findings seem to be in direct contradiction to that which Contreras *et al.* have reported [35]. They

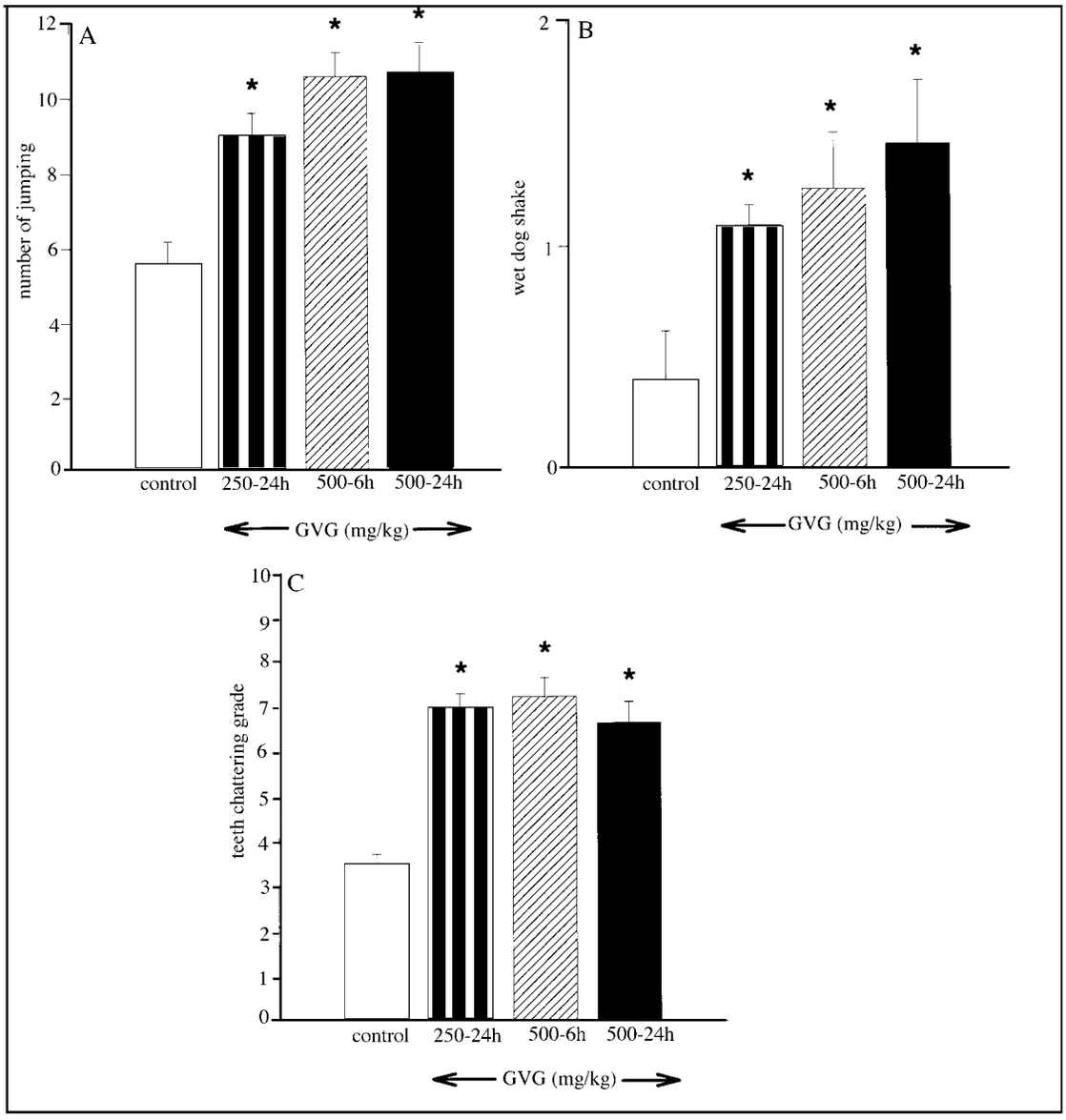

Fig. 1. Effect of GVG on major morphine abstinence signs. (A) Effect on number of jumping ($^*P < 0.05$). (B) Effect on wet dog shake ($^*P < 0.05$). (C) Effect on the severity of teeth chattering (1–10 scale) ($P < 0.05$). Observation was made for 10 min after the naloxone injection; 250-24 h stands for 250 mg kg^{-1} GVG injection 24 h before naloxone (NL, 2 mg kg^{-1}) precipitated abstinence syndrome; 500-6 h stands for 500 mg kg^{-1} GVG injection 6 h before NL injection; 500-24 h stands for 500 mg kg^{-1} GVG injection 24 h before NL injection.

found that GVG induced a decrease in the severity of the AS in mice, while another GABA-transaminase inhibitor γ-acetylenic GABA, in contrast, induced convulsions in the morphine-dependent mice. The rating and scoring of the signs of AS in this study was very different from our conventional way of scoring [7, 8, 24–26]; it was a protected/unprotected scale which is more difficult to assess the withdrawal. The findings of Contreras *et al.* is very hard to compare with our or other researchers' studies; discrepancies between these results may be related to different species of animals, observation intervals, doses, routes of injection or methods of scoring AS.

These unexpected effects of GVG on NL precipitated AS may be explained by three mechanisms. First, by the untoward effects of extracellular GABA increase; second, by a possible enhanced glutamatergic activity induced by GVG and/or GABA increase; third, by a possible direct proconvulsant effect of GABA to specific areas like, the *superior colliculus* [20]. Normally, if there is an excessive excitation of neurons in some specific regions of the brain during AS, then the enhancement of GABAergic transmission would be expected to suppress it. However, an increase in the extracellular GABA levels may not always induce an overall increase in the $GABA_A$ mediated inhibition, because an extra-

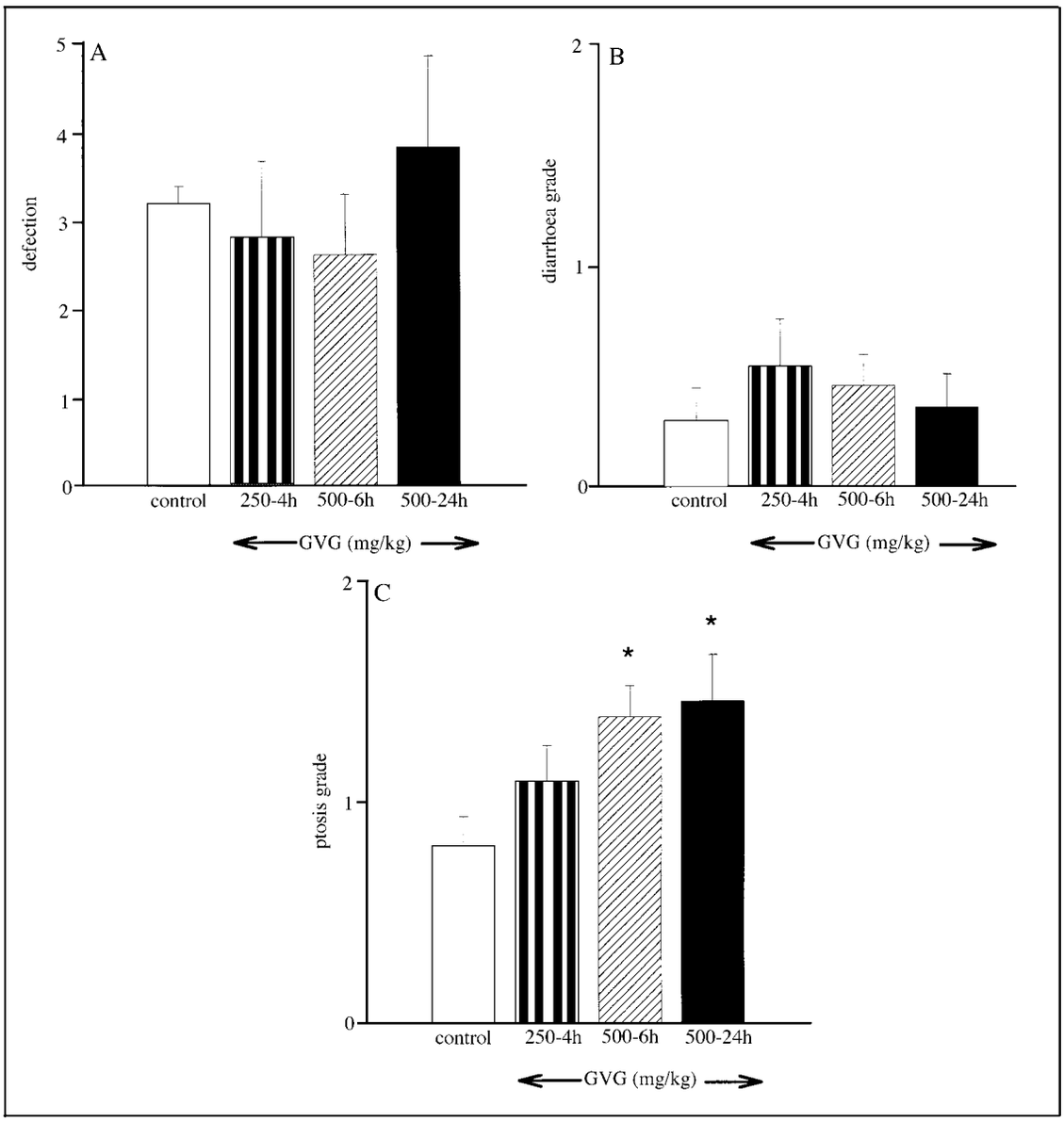

Fig. 2. Effect of GVG on minor morphine abstinence signs. A) Effect on number of defecation B) Effect on diarrhoea grade (1–3 scale) C) Effect on ptosis (1–3 scale). $^{*}P < 0.05$.

cellularly increased GABA level may not always enhance the GABA function in some GABAergic synapses. In a microdialysis study we found that increased extracellular GABA levels, even in substantia nigra [18], did not antagonize pentylenetetrazol induced tonic–clonic seizures [17]. Jackson *et al.* [36] also found that GVG (in 250–500 μM concentrations) induced a loss of paired pulse inhibition in the CA1 region of rat hippocampal slices, without having any specific binding or antagonistic effects to $GABA_A$ receptor. In another study, GVG caused a loss of paired pulse inhibition (at 15-ms interpulse interval) in the dentate gyrus of the rat hippocampus *in vivo* and *in vitro*; this effect was blocked by $GABA_B$ antagonists 2-OH-saclofen and CGP 35348 [37]. These paradoxical effects of GVG may be attributed to the presynaptic inhibition of GABA release at the GABAergic synapses by the excessive stimulation of presynaptically located $GABA_B$ autoreceptors. Thus, GVG, indirectly, may be reducing the synaptically released, active GABA at some parts of the brain, instead of increasing the inhibition. Although GVG is a potent antiepileptic drug [16], and has reduced seizure frequency by approximately 50–60% [38, 39], it has also been reported to exacerbate primary generalized seizures [40] and precipitate status epilepticus [41], possibly by means of similar mechanism mentioned above.

A second explanation of these effects may be through an interaction with glutamatergic system. GVG inhibits glutamic acid decarboxylase, an enzyme that converts glutamate, an excitatory amino

acid, to GABA [16]. Increased extracellular GABA levels may also be limiting the rate of this enzyme, resulting in increased glutamate, which is the precursor of GABA, in the presynaptic pools. As reported before [7, 8] combined with the possible altered release of glutamate modulated by the chronic administration of morphine [3, 7, 8, 27], an acute excessive release of this glutamate pool may induce a severe AS.

A third explanation may be the possible proconvulsant and/or excitatory effect of increased GABA levels at some specific areas of the brain, like *superior colliculus*, where antagonism of the GABAergic system is inhibitory and anticonvulsant [42], may prevail over the inhibitory GABAergic system [11, 20, 42]. Another important point may be a possible interaction between the increased extracellular GABA levels (or GVG itself) with morphine during the development of MPD, however, there are no reports explaining such an interaction. Further research to elucidate the mechanism of how morphine AS becomes more severe when extracellular GABA levels in the brain is increased may give important clues about the development of morphine AS and the interaction between the opioid and GABAergic system. GVG does not seem to have any therapeutic potential for treatment of morphine abstinence in contrast to other drugs that enhance GABAergic transmission, like benzodiazepines.

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REFERENCES

1. Stolerman I. Drugs of abuse: behavioral principals, methods and terms. *TIPS* 1992; **13:** 170–6.
2. Koob GF. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *TIPS* 1992; **13:** 177–84.
3. Olson GA, Olson RD, Kastin AJ. Endogenous opiates: 1993. *Peptides* 1994; **15:** 1513–56.
4. Trujillo KA, Akil H. Inhibition of opiate tolerance by non-competitive antagonists. *Brain Res* 1994; **633:** 178–88.
5. Trujillo KA, Akil H. Inhibition of morphine tolerance and dependence by the NMDA receptor antagonist MK-801. *Science* 1991; **261:** 85–6.
6. Tanganelli S, Antonelli T, Morari M, Bianchi C, and Beani L. L-Glutamate antagonists prevent morphine withdrawal in mice and guinea pigs. *Neurosci Lett* **122:** 270–2.
7. Koyuncuoğlu H, Sayin Ü, Dizdar Y, Aricioglu F. Effects of competitive NMDA receptor blocker CGP 39551 on morphine physical dependence. *Tr J Med Sci* 1994; **21:** 163–8.
8. Koyuncuoğlu H, Dizdar Y, Aricioglu F, Sayin Ü. Effects of MK-801 on morphine physical dependence: attenuation and intensification. *Pharm Biochem Behav* 1992; **43:** 487–90.
9. Popik P, Skolnick P. The NMDA antagonist memantine blocks the expression and maintenance of morphine dependence. *Pharmacol Biochem Behav* 1996; **53:** 791–7.
10. Foote F, Gale K. Proconvulsant effect of morphine on seizures induced by pentylenetetrazol in the rat. *Eur J Pharmacol* 1984; **105:** 179–84.
11. Gale K. GABA in epilepsy: pharmacological basis. *Epilepsia* 1989; **30 (Suppl 3):** S1–S11.
12. Suzuki T, Tsuda M, Narita M, Funada M, Mizoguchi H, Misawa M. Diazepam pretreatment suppresses morphine withdrawal signs. *Life Sci* 1995; **58:** 349–57.
13. Bartlett SE, Smith MT. Effects of morphine-3 glucuronide and morphine on the K^+ evoked release of [^{3}H]-glutamic acid and [^{14}C]-GABA from rat brain synaptosomes. *Life Sci* 1996; **58:** 447–54.
14. Raman AF, Takahashi M, Kaneto H. Role of GABAergic systems in the development of morphine tolerance in formalin-treated mice. *Japan J Pharmacol* 1995; **68:** 207–11.
15. Halonen T, Pitkanen A, Riekkinen PJ. Administration of vigabatrin affects the levels of inhibitory and excitatory amino acids in rat cerebrospinal fluid. *J Neurochem* 1990; **55:** 1870–3.
16. Hammond EJ, Wilder BJ. Gamma Vinyl GABA. *Gen Pharmacol* 1985; **16:** 441–7.
17. Sayin Ü, Timmerman W, Westerink BHC. The significance of extracellular GABA in the substantia nigra of the rat during seizures and anticonvulsant treatment. *Brain Res* 1995; **669:** 67–72.
18. Iodorala MJ, Gale K. Substantia nigra: site of anticonvulsant activity mediated by GABA antagonists. *Science* 1982; **218:** 1237–40.
19. Kendal DA, Fox DA, Enna SJ. Effect of GVG on bicuculline induced seizures. *Neuropharmacology* 1981; **20:** 351–5.
20. Sayin Ü, Cengiz S, Altug T. Vigabatrin as an anticonvulsant against pentylenetetrazol seizures. *Pharmacol Res* 1993; **28:** 325–31.
21. Stevens J, Philips I, Beuropaire R. Gamma vinyl GABA in endopiriform area suppresses kindled amygdala seizures. *Epilepsia* 1988; **29:** 404–11.
22. Sayin Ü, Purali N, Özkan T, Altug T, Buyukdevrim S. Vigabatrin has an anxiolytic effect in the elevated plus maze test of anxiety. *Pharmacol Biochem Behav* 1992; **43:** 529–35.
23. Koyuncuoğlu H, Saydam B. The treatment of heroin addicts with dextromethorphan: a double-blind comparison of dextromethorphan with chlorpromazine. *Int J Clin Pharmacol Ther and Toxicol* 1990; **28:** 147–52.
24. Blaesig J, Herz A, Reinhold K, Zielgaensberger S. Development of physical dependence on morphine in respect to time and dosage and quantification of the precipitated abstinence syndrome in rats. *Psychopharmacologia* 1973; **33:** 19–38.
25. Way EL, Loh HH, Shen F. Simultaneous quantitative assessment of morphine tolerance and physical dependence. *J Pharmacol Exp Ther* 1969; **167:** 1–8.
26. Aricioglu-Kartal F, Uzbay IT. Inhibitory effect of agmantine on naloxone-precipitated abstinence syndrome in morphine dependent rats. *Life Sci* 1997; **61:** 1775–81.
27. Aghajanian GK, Kogan JH, Moghaddam B. Opiate withdrawal increases glutamate and aspartate efflux

in the locus coeruleus: an *in vivo* microdialysis study. *Brain Res* 1994; **636:** 126–30.
28. Lope F, Miller LG, Thompson ML, Schatzki A, Chesley S, Greenblatt DJ, Shader RI. Chronic morphine administration augments benzodiazepine binding and GABA-A receptor function. *Psychopharmacology* 1990; **101:** 545–9.
29. Klitenick MA, DeWitte P, Kalivas PW. Regulation of somatodentritic dopamine release in the ventral tegmental area by opiates and GABA: an in vivo microdialysis study. *J Neurosci* 1992; **12:** 2623–32.
30. Renno WM, Mullet MA, Beitz AJ. Systemic morphine reduces GABA release in the lateral but not in the medial portion of the midbrain periaquaductal gray of the rat. *Brain Res* 1992; **594:** 221–32.
31. Beani L, Bianchi C, Ferraro L, Morari M, Simonato M, Spatullo G, Tanganelli S. Alpha-2-adrenoceptor-mediated decreases in GABA outflow in cortical slices and synaptosomes during morphine tolerance. *J Pharmacol Exp Ther* 1991; **258:** 472–6.
32. Antonelli T, Beani L, Bianchi C, Rando S, Simonato M, Tanganelli S. Cortical acetylcholine release is increased and GABA outflow is reduced during morphine withdrawal. *Br J Pharmacol* 1986; **89:** 853–60.
33. Baldino F, Cowan A, Geller EB, Adler M. The effects of antipsychotic and antianxiety drugs on the morphine abstinence syndrome in rats. *J Pharmacol Exp Ther* 1979; **208:** 63–6.
34. Wang FS, Fujimoto JM. Pentobarbital administered intracerebroventricularly antagonizes morphine-induced antinociception in mice. *J Pharmacol Exp Ther* 1993; **265:** 1361–8.
35. Contreras E, Tamayo L, Quijada L. The effects of the irreversible inhibition of GABA transaminase upon some morphine effects. *Neuropharmacology* 1979; **18:** 309–13.
36. Jackson M, Trevor D, Esplin B, Capek R. Acute effects of gamma-vinyl-GABA on hippocampal inhibition *in vitro*. *Brain Res* 1994; **651:** 85–91.
37. Sayin Ü, Rutecki PA, Mellanby J, Sutula T. Gamma-Vinyl-GABA impairs paired pulse inhibition in the rat dentate gyrus *in vivo* and *in vitro*. *Epilepsia* 1997; **38 (S8):** 110–11.
38. Loiseau P, Hardenberg JP, Pester M, Guyot M, Schechter P, ell GP. Double blind placebo controlled study of vigabatrin in drug resistant epilepsy. *Epilepsia* 1986; **27:** 115–20.
39. Reynolds EG, Ring H, Heller A. A controlled trial of gamma vinyl GABA in drug resistant epilepsy. *Br J Clin Pharmacol* 1988; **33:** 937–46.
40. Luna D, Dulac O, Pajot N, Beaumont D. Vigabatrin in the treatment of childhood epilepsies: a single-blind placebo controlled study. *Epilepsia* 1989; **30:** 430–7.
41. de Krom MC, Verduin N, Visser E, Kleijer M, Scholtes F, De Groen JH. Status epilepticus during vigabatrin treatment: a report of three cases. *Seizure* 1995; **2:** 159–62.
42. Dean P, Gale K. Anticonvulsant action of GABA receptor blockade in the nigrotectal target region. *Brain Res* 1989; **477:** 391–5.