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 system.
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...
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 [8, 24–26] 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.

The mean values (±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)]. Posisis 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 GABAₐ 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]. Benzo diazepine 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-
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_A 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_A 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

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**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.
acid, to GABA [16]. Increased extracellular GABA levels may also be limiting the rate of this enzyme, resulting in increased glutamate, which is the pre-cursor 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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