Turk J Gastroenterol 1995; 6:327-331

How important is the intrinsic activity of benzodiazepine receptor ligands in the behavioral amelioration of hepatic encephalopathy?

Hepatik ensefalopatinin düzelmesinde benzodiazepin reseptör ligandlarının aktivitesinin önemi nedir?

H. ÇETİNKAYA, C. YURDAYDIN, Ö. PALAOĞLU, S. USANMAZ, Ö. UZUNALİMOĞLU

Departments of Gastroenterology and Pharmacology, Ankara University, Ankara, Turkey

ÖZET: *Hepatik ensefalopatide varlığı ileri sürülen artmış gamma aminobütirik asit(GABA)erjik tonüs, çeşitli dokulardaki yüksek düzeydeki 'endojen' benzodiazepin reseptör (BZR) ligandlarına bağlı olabilir. BZR antagonistleri ile deneysel hepatik ensefalopatide elde edilen olumlu etkiler bu hipotezi kuvvetle desteklemektedir. Bununla birlikte son zamanlardaki yayınlarda sadece invers agonist özellik taşıyan BZR antagonistlerinin etkili olduğu, buna karşılık 'pür' BZR antagonistlerinin etkisiz olduğu belirtildi. Teorik olarak invers agonist bir ligand nötral GABAerjik tonüsü de düşürür, bu da hepatik ensefalopatide gözlenen etkinin hastalığa özgü olmayabileceğini akla getirir. Eğer BZR antagonisteleri ile hepatik ensefalopatide gözlenen düzelme sadece bu ligandların invers agonist özelliklerine bağlı ise, bu intrensek aktivite nötral GABAerjik tonüs şartlarında sıçanlarda azalmış motor aktiviteyi arttırmalıydı. Bu düşünceden yola çıkılarak intrensek aktivite profilleri farklı üç BZR antagonistinin, nötral GABAerjik tonüste motor inaktivitenin mevcut olduğu, morfinle oluşturulmuş katalepsiye etkisi araştırıldı. Opiyat antagonisti nalokson'un katalepsiye ve üç BZR antagonistinin flunitrazepam ile oluşturulan sedasyona etkisi karşılaştırma için kullanıldı. Her üç BZR antagonisti kataleptik sıçanların motor inaktivitesine karşı etkisizdi. BZR antagonistleri ne ambulatuvar, ne stasyoner hareketlerde bir artışa yol açmadı. Nalokson katalepsiyi tamamen düzeltti ve her üç BZR antagonisti de flunitrazepam'ın yol açtığı nörolojik tabloyu normale çevirdi. Sonuçlar, tek başına parsiyel invers agonist intrensek aktivitenin sıçanlarda motor aktiviteyi arttırmadığını ve BZR antagonistleri ile hepatik ensefalopatide elde edilen düzelmenin hastalığa özgü olduğunu göstermektedir. Ayrıca hepatik ensefalopatide BZR antagonistleri ile elde edilen düzelmenin ligandların intrensek aktivitelerine değil fakat BZR antagonizmine bağlı olduğunu düşündürmektedir.*

Anahtar kelimeler: **Hepatik ensefalopati, benzodiazepin reseptör ligandları**

SUMMARY: *The postulated increased gamma aminobutyric acid(GABA)ergic tone in hepatic encephalopathy may arise as a result of elevated levels of 'endogenous' agonist benzodiazepine receptor (BZR) ligands. Important support for this hypothesis comes from BZR antagonist-induced ameliorations in experimental hepatic encephalopathy. However, recent studies in animal models of hepatic encepaphalopathy suggest that only BZR antagonists with inverse agonist properties improve hepatic encephalopathy whereas 'pure' BZR antagonists have no effect. Theoretically, an inverse agonist ligand could decrease also a neutral GABAergic tone, raising the question of the specificity of the effect. If the behavioral improvement of hepatic encephalopathy by BZR antagonists depend solely on their inverse agonist properties this intrinsic activity should increase motor activity in rats with diminished motor activity at neutral GABAergic tone. Hence, the effects of three BZR antagonists with different intrinsic activity profiles on morphine-induced catalepsy, a state of motor inactivity at neutral GABAergic tone, was investigated. The effects of the opioid antagonist naloxone on morphine-induced catalepsy and the effects of the three BZR antagonists on flunitrazepam-induced sedation were evaluated for comparison. All three BZR antagonists did not affect the motor inactivity of cataleptic rats. Neither ambulatory motor activity nor stationary movements increased after administration of BZR antagonists. Naloxone completely reversed the catalepsy, and all three BZR antagonists restored the flunitrazepam-induced decrased neurological score to normal. The results indicate that partial inverse agonist intrinsic activity per se do not increase motor activity in rats, and that ameliorations achieved with BZR antagonists in experimental HE are disease specific. The results further suggest that BZR antagonist-induced improvements in HE may depend not on intrinsic activity of the ligands but on BZR antagonism.*

Key words: **Hepatic encephalopathy, benzodiazepine receptor ligands**

Geliş tarihi: 5.8.1995, Kabul tarihi: 4.9.1995

THE gamma-aminobutyric acid (GABA) hypothesis of the pathogenesis of hepatic encephalopathy (HE) suggests that an increased GABAergic tone is at least partially responsible for the neural inhibition of HE (1). Elevated levels of endogenous benzodiazepine receptor (BZR) ligands may contribute to an increased GABAergic tone (2-4). Important support for this hypothesis comes from BZR antagonist-induced ameliorations in human (5-7) and experimental HE (8-10). However, recent studies in animal models of HE suggest that only BZR antagonists with inverse agonist properties improve HE whereas 'pure' BZR antagonists have no effect (11, 12). Theoretically, an inverse agonist ligand could decrease not only an increased but also a neutral GABAergic tone, raising the question of the specificity of the effect, especially if one considers the paucity of data linking GABA itself (13-16) or its receptor (14, 17) to an increased GABAergic tone in HE.

Studies demonstrating ameliorations of HE in animal models of HE largely depend on quantification of motor activity of rats in animal activity meters (6-8). If the behavioral improvement of HE by BZR antagonists depend solely on their inverse agonist properties it should be possible to increase the motor activity of rats with a neutral GABAergic tone at a state of diminished motor activity, such as morphine-induced catalepsia, with BZR ligands possessing inverse agonist intrinsic activity. Therefore, the effects of a pure BZR antagonist, flumazenil, two BZR antagonists with inverse agonist properties, Ro 15-3505 and Ro 15-4513, on morphine-induced catalepsia was explored in this study. Furthermore, the effect of the opioid antagonist naloxone on morphine-induced catalepsia and the effects of the three BZR antagonists on flunitrazepam-induced sedation were investigated for comparison.

MATERIAL and METHODS

Adult male Sprague-Dawley rats weighing 200-300g were used in the study. Rats were fed with standard rat chow diet ad libitum, allowed free access to water, and housed in standard facilities with a 12 h day/night cycle at a room temperature of 20°C.

Catalepsia was induced by the administartion of 80mg/kg morphine sulphate intraperitoneally (i.p.). A sedation state was induced in rats with i.p. application of 5mg/kg flunitrazepam. After administration of flunitrazepam rats were tested neurologically according to Bures et al (18). Neurological testing is based on examination of 14 different reflexes including pain, grasping, position, and corneal reflexes where every positive reflex is represented by one point; thus a normal rat has a score of 14 points.

Study Design

Effect of three BZR antagonists and the opioid antagonist naloxone on catalepsia:

The effect of three BZR antagonists, flumazenil, Ro 15-3505, Ro 15-4513 and the opioid antagonist naloxone on catalepsia was evaluated by quantitating changes in motor activity of rats. Motor activity of single rats was determined using an open field activity monitor (Opto-Varimex Minor, Columbus Instruments, Columbus, OH). This device is based on the the number of interruptions of a grid of infrared beams placed 1cm apart by freely moving rats (ambulatory movements) or by head shakes, whisker twitches etc. (stationary movements) . Results are expressed as number of beam interruptions (counts) per 10 minutes. Each rat was tested twice in the open field. Animal activity was monitored for 10 minutes before and for another 10 minutes after administration of the particular drug or its vehicle. Flumazenil, Ro 15-3505 and Ro 15-4513 were administered at a dose of 10mg/kg and naloxone at a dose of 1mg/kg.

Effect of BZR antagonists on flunitrazepam-induced sedation:

After administration of 5mg/kg flunitrazepam rats were tested neurologically. Neurological testing was repeated 5 minutes after administration of 10 mg/kg of flumazenil or Ro 15-3505 or Ro 15-4513.

Materials

Ro 15-3505 was a gift from Dr. Peter Sorter (Hoffmann-LaRoche, Nutley, NJ) and naloxone was a gift from Dr. Kenner Rice (National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, National Institutes of Health). Ro 15-4513 and flumazenil were purchased from Research Biochemicals Inc. (Natick, MA). Flunitrazepam and morphine sulfate was purchased from Sigma Chemical Co. (St. Louis, MO). Flunitrazepam, flumazenil, Ro 15-3505 and Ro 15-4513 were dissolved in a 3% Tween solution with saline. Morphine sulfate and naloxone were dissolved in saline.

Statistics

Comparison between predrug and postdrug examination periods were performed with the Student's t test.

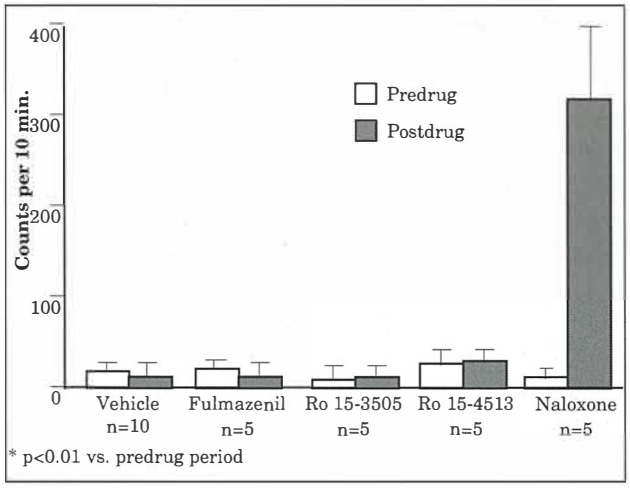

Figure 1. Effect of three BZR antagonists and the opioid receptor antagonist naloxone on ambulatory movements in morphine sulfate-induced catalepsy.

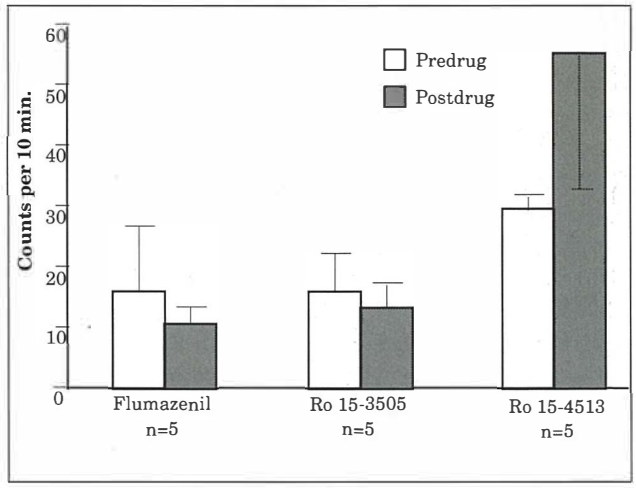

Figure 2. Effect of three BZR antagonists on stationary movements in morphine sulfate-induced catalepsy.

RESULTS

Flumazenil, Ro 15-3505, Ro 15-4513, naloxone and their vehicles had no effect on ambulatory or stationary movements of control rats. Catalepsia developed in rats after 1 to 30 minutes of administration of 80mg/kg morphine sulfate. All three BZR antagonists used in this study did not affect the motor inactivity of cataleptic rats . Neither ambulatory motor activity (fig. 1) nor stationary movements (fig. 2)increased after administration of the three BZR antagonists. Vehicle 1 (saline) and vehicle 2 (3% Tween solution) had also no effect. In contrast, 1mg/kg naloxone, as expected, completely reversed the catalepsia (figure 1).

After administration of 5mg/kg flunitrazepam rats developed marked ataxia, and at examination according to Bures et al (13) a decreased neurological score was noted (10.2±0.3, x±SEM, n:15). Rats immediately returned to normal after treatment with flumazenil, Ro 15-3505 and R0 15-4513 (figure 3).

DISCUSSION

In this study, neither a 'pure' BZR antagonist, flumazenil, nor two BZR antagonists with inverse agonist properties, Ro 15-3505 and Ro 15-4513, were able to improve the cataleptic state of rats induced by morphine sulfate. This underlines once again the specificity of BZR antagonist-induced ameliorations of HE in animal models of HE as has been demonstrated by the ineffectiveness of BZR antagonists in uremic encephalopathy (8).

With almost no intrinsic activity, the 'pure' BZR antagonist flumazenil is not expected to improve motor inactivity at neutral GABAergic tone. Ro 15-3505 has only slight partial inverse agonistic intrinsic activity (19, 20) which may not be enough to decrease GABAergic tone sufficiently to culminate in neural excitation. Ro 15-4513, with its more pronounced inverse agonistic activity compared to Ro 15-3505 (19-21) would have been expected to induce a decreased GABAergic tone reflected clinically by neural excitation. This was not seen since Ro 15-4513 too, had no effect on the motor inactivity of cataleptic rats. In contrast, 1mg/kg naloxone totally abolished the cataleptic state excluding the possibility that the rats were too sick to responde. In addition, all three BZR antagonists restored the flunitrazepam-induced decreased neurological score to normal.

These observations suggest that the intrinsic activity of BZR ligands do not lead to non-specific

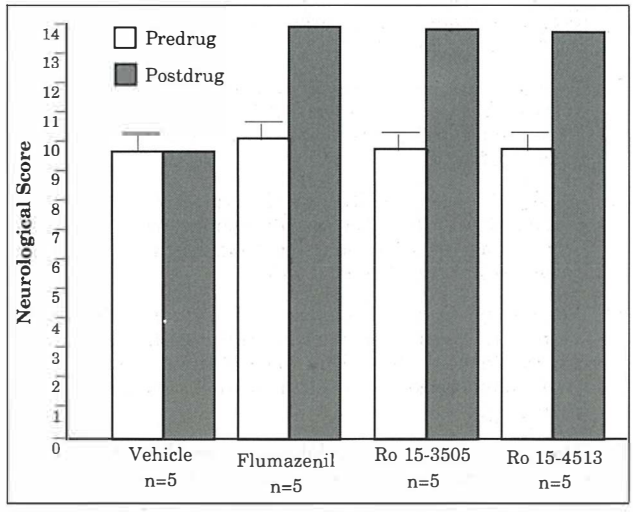

Figure 3. Effect of three BZR antagonists on flunitrazepam-induced sedation.

increases in motor activity of rats. Hence, the most likely explanation of a behavioral amelioration in rats with HE by BZR antagonists may not be their intrinsic activity but their ability to displace agonist BZR ligands from their receptors. Recently, two studies questioned the roles of endogenous BZR ligands contributing to the pathophysiology of HE in the thioacetamide rat (22, 23). In the neurochemical study by Widler et al (22) no difference of BZR ligand levels was found between control and HE rats with a radioreceptor assay. A major critique to the study was the different extraction technique they used which may have interfered with their results (24, 25). In the behavioral study by Püspök et al (23) a pure BZR antagonist, Ro 14-7437, in contrast to Ro 15-4513, had no effect on HE. In addition, Ro 14-7437 blocked the beneficial effect of Ro 15-4513 on HE. They concluded that agonist BZR ligands do not contribute to the increased GABAergic tone of HE in the thioacetamide rat model but interestingly, DMCM (methyl-6,7-dimethoxy-4-ethyl-ß-carboline 3-carboxylate), a full inverse agonist, was also ineffective in HE. It is conceivable that in HE partial inverse agonist activity may restore the increased GABAergic tone towards normal (i.e. neutral GABAergic or less increased GABAergic tone) which may behaviorally be observed as an increase in ambulatory movements of rats. The lack of an increase in ambulatory movements after administration of a partial inverse agonist at neutral GABAergic tone may then be due to the irritable, preconvulsive state of a decreased GABAergic tone (21). Although this logic makes sense, it is unlikely to be the case, since we were unable to observe an increase in stationary movements characteristic of a decreased GABAergic tone (10).

In summary, the lack of effect of flumazenil in contrast to Ro 15-4513 in the behavioral amelioration of HE in rats was interpreted in previous studies ((11, 12) as such that an inverse agonist activity is necessary for an amelioration of HE in rats, and hence that agonist BZR ligands do not contribute to HE in these animal models. Our results question these interpretations, and suggest that an inverse agonist intrinsic activity per se is not the prerequisite of a beneficial effect of BZR antagonists in rat models of HE.

REFERENCES

1. Yurdaydin C, Jones EA: Hepatic encephalopathy. In: Principles and Practice of Gastroenterology and Hepatology, 2nd edition, G. Gitnick, ed., Elsevier Science Publishing Co., Inc. 1994; pp.985-95
2. Mullen KD, Szauter KM, Kavinsky-Russ K. "Endogenous" benzodiazepine activity in body fluids of patients with hepatic encephalopathy. Lancet 1990; 336:81-83
3. Olasmaa M, Rothstein JD, Guidotti A, Weber RJ, Paul SM, Spector S, Zeneroli ML, et al. Endogenous benzodiazepine receptor ligands in human and animal hepatic encephalopathy. J Neurochem 1990; 55:2015-23
4. Basile AS. The contribution of endogenous benzodiazepine receptor ligands to the pathogenesis of hepatic encephalopathy. Synapse 1991; 7:141-50
5. Grimm G, Ferenci P, Katzenschlager R, Madl C, Schneeweiss B, Laggner AN, Lenz K, Gangl A. Improvement of hepatic encephalopathy treated with flumazenil. Lancet 1988; 2:1392-4
6. Bansky G, Meier PJ, Riederer E, Walser H, Ziegler WH, Schmid M. Effects of the benzodiazepine receptor antagonist flumazenil in hepatic encephalopathy in humans. Gastroenterology 1989; 97:744-50
7. Pomier-Layrargues G, Giguère JF, Lavoie J, Perney P, Gagnon S, D'Amour M, Wells J, Butterworth RF. Flumazenil in cirrhotic patients in hepatic coma: a randomized double-blind placebo-controlled crossover trial. Hepatology 1994; 19:32-7
8. Basset ML, Mullen K, Skolnick P, Jones EA. Amelioration of hepatic encephalopathy by pharmacological antagonism of the GABAA-benzodiazepine receptor complex in a rabbit model of fulminant hepatic failure. Gastroenterology 1987; 93:1069-77
9. Gammal SH, Basile AS, Geller D, Skolnick P, Jones EA. Reversal of the behavioral and electrophysiological abnormalities of an animal model of hepatic encephalopathy by benzodiazepine receptor ligands. Hepatology 1990; 11:371-8
10. Yurdaydın C, Gu Z-Q, Nowak G, Fromm C, Holt AG, Basile AS. Benzodiazepine receptor ligands are elevated in an animal model of hepatic encephalopathy: relationship between brain concentration and severity of encephalopathy. J Pharmacol Exp Ther 1993; 265:565-71
11. Steindl P, Püspök A, Druml W, Ferenci P. Beneficial effect of pharmacological modulation of the GABAA-benzodiazepine receptor on hepatic encephalopathy in the rat) comparison with uremic encephalopathy. Hepatology 1991; 14:963-8
12. Bosman DK, Van Den Buijs CACG, DeHaan JG, MAAS MAW, Chamuleau RAFM. The effects of benzodiazepine-receptor antagonists and partial inverse agonists on hepatic encephalopathy in the rat. Gastroenterology 1991; 101:772-81
13. Ferenci P, Ebner J, Zimmermann C, Kikuta C, Roth E, Häussinger D. Overestimation of serum concentrations of g-aminobutyric acid in patients with hepatic encephalopathy by the g-aminobutyric acid-radioreceptor assay. Hepatology 1988; 8:69-72
14. Maddison JE, Dodd PR, Morrison M, Johnston GAR, Farrell GC. Plasma GABA, GABA-like activity and the brain GABA-benzodiazepine receptor complex in rats with chronic hepatic encephalopathy. Hepatology 1987; 7:621-8
15. Zimmermann C, Ferenci P, Pifl C, Yurdaydin C, Ebner J, Lassmann H, Roth E, Hörtnagl H. Hepatic encephalopathy in thioacetamide-induced acute liver failure in rats: Characterisation of an improved model and study of aminoacid-ergic neurotransmission. Hepatology 1989; 9:594-601

16. Moroni F, Riggio O, Carla V, Festuccia V, Ghinelli F, Marino IR, Merli M, et al. Hepatic encephalopathy: lack of changes of g-aminobutyric acid content in plasma and cerebrospinal fluid. Hepatology 1987; 7:816-20

17. Rössle M, Deckert J, Jones EA. Autoradiographic analysis of GABA/benzodiazepine receptors in an animal model of acute hepatic encephalopathy. Hepatology 1989; 10:143-7

18. Bures J, Buresova O, Hunton JP. Innate and motivate behaviour. In: Techniques and basic experiments for the study of brain and behaviour. New York: Elsevier North Holland Inc., 1976:37-45

19. Haefely W, Kyburz E, Gerecke M, Möhler H. Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists. Adv Drug Res 1985; 14:165-322

20. Gardner CR. Pharmacological profiles in vivo of benzodiazepine receptor ligands. Drug Dev Res 1988; 12:1-28

21. Jones EA. Benzodiazepine receptor ligands and hepatic encephalopathy: Further unfolding of the GABA story. Hepatology 1991; 14:1286-90

22. Widler P, Fisch HV, Schoch P, Zimmermann AM, Schläepfer T, Reichen J. Increased benzodiazepine-like activity is neither necessary nor sufficient to explain acute hepatic encephalopathy in the thioacetamide rat. Hepatology 1993; 18:1459-64

23. Püspök A, Herneth A, Steindl P, Ferenci P. Hepatic encephalopathy in rats with thioacetamide-induced acute liver failure is not mediated by endogenous benzodiazepines. Gastroenterology 1993; 105:851-7

24. Basile AS, Jones EA. The involvement of benzodiazepine receptor ligands in hepatic encephalopathy (letter). Hepatology 1994; 20:541-2

25. Gacad R, Kaminsky-Russ K, Mullen KD. Detection of benzodiazepine-like activity in hepatic encephalopathy requires an initial lipophilic extraction procedure (letter). Hepatology 1994; 544-5