*Pharmacology Biochemistry & Behavior*, Vol. 39, pp. 325–327. © Pergamon Press plc, 1991. Printed in the U.S.A. 0091-3057/91 $3.00 + .00

# Bay K 8644 Potentiates the Anxiolytic Effect of Ethanol

RABIA KIRAÇ AND LÜTFIYE EROĞLU*[1]

**Department of Pharmacology, Istanbul Medical Faculty, Çapa, İstanbul, Türkiye*

Received 5 July 1990

KIRAÇ, R. AND L. EROĞLU. *Bay K 8644 potentiates the anxiolytic effect of ethanol.* PHARMACOL BIOCHEM BEHAV **39**(2) 325–327, 1991.—The possible role of voltage-sensitive calcium channels (VSCCs) in the anxiolytic effect of ethanol was investigated using three different doses of ethanol (0.5, 1.0 and 2.0 g/kg) with calcium agonist Bay K 8644 (0.5 mg/kg) and calcium antagonist nifedipine (5 mg/kg) in rats. Ethanol produced an anxiolytic effect in a dose-dependent manner. The Bay K 8644-potentiated anxiolytic effect of ethanol, however, Bay K 8644 did not alter anxiety when used alone. Nifedipine itself showed an anxiolytic effect but did not change the ethanol-induced anxiolytic effect. This finding may lead to the consideration of the neurochemical mechanisms of the anxiolytic effects of ethanol and nifedipine as they vary from each other.

Ethanol Anxiety Calcium channels Bay K 8644 Nifedipine

THE anxiety-reducing property of ethanol is considered to be a major factor in its wide abuse throughout human history (3,4). Even though the anxiolytic action of ethanol has been shown both in laboratory animals (2) and humans (4), its nature is poorly defined.

However, Dalterio et al. (5) have demonstrated that diazepam, a benzodiazepine drug, potentiates the anxiolytic effect of ethanol. Besides, ethanol shares many neuropharmacological properties, including augmentation of GABAergic neurotransmission, with benzodiazepines and barbiturates (1, 21–23).

Recent studies have shown that ethanol and other sedative hypnotic drugs, such as barbiturates, chlordiazepoxide and chlorpromazine, inhibit voltage-dependent calcium influx (6,29). Furthermore, voltage-sensitive calcium channel (VSCC) antagonists have been reported to elicit a multitude of behavioral responses including anticonvulsant, antidepressant, neuroleptic-like, and even anxiolytic effects (27).

The present study was designed to test the possibility of VSCCs being involved in the anxiolytic action of ethanol, by using a channel agonist, Bay K 8644, and an antagonist, nifedipine, from dihydropyridine (DHP) group drugs. For this purpose, we used rats in our experiment and the elevated plus maze for the measurement of anxiety-related behavior.

## METHOD

### *Subjects*

Adult male Wistar rats, weighing 200–250 g, were housed 9–10 per cage in an animal colony facility for one week prior to being used in the experiment. The animals were maintained in a constant room temperature (21 ± 1°C) under a 12-hour light/dark cycle (light onset at 0600) with free access to commercial chow and tap water.

### *Apparatus*

Anxiety-related behavior was measured by the elevated plus maze. The plus maze consisted of two open arms, 50 × 10 cm, and two excluded arms, 50 × 10 × 40 cm, with an open roof, arranged such that two open arms were opposite to each other. The maze was elevated to a height of 50 cm. Each rat was placed in the central square of the maze facing one of the open arms. During a 5-min trial the number of entries into open and closed arms was measured, and the time spent in each type of arm was scored by two unaware observers.

### *Procedure*

The animals were randomly assigned to the following drug condition groups. Rats were pretreated intraperitoneally (IP) with either saline or Bay K 8644 (0.5 mg/kg) or nifedipine (5 mg/kg), followed 20 min later with an IP injection of either ethanol (0.5, 1.0 and 2.0 g/kg) or saline. Rats were then individually housed and were subjected to the elevated plus maze test 25 min after the second injections. The protocol was designed for 5 consecutive days, so that two rats from each group were run concurrently on each test day. All experiments were performed between 8:30–12:30 a.m.

### *Drugs*

Bay K 8644 (Bayer AG) was dissolved in Bay A 1040 placebo solvent (composition: 969 g polyethylene glycol 400, 60 g glycerine, 100 g water) for injection. Nifedipine (Doğu İlaç) was suspended in 1% carboxymethylcellulose. Both solutions were protected from light. Ethanol solutions were prepared by mixing 95% ethanol in isotonic saline to yield doses of 0.5 g/kg (4.3%

[1]Requests for reprints should be addressed to Prof. Dr. Lütfiye Eroğlu.

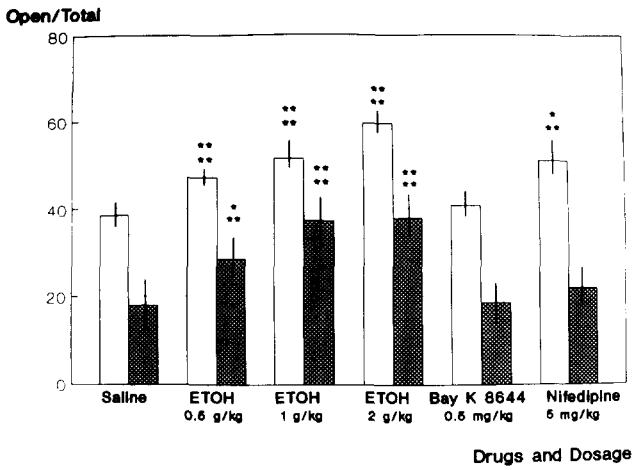


FIG. 1. Effects of three doses of ethanol, Bay K 8644 and nifedipine on mean (±SEM) percentage of open arm entries (white bars) or time (s) spent in the open arms (hatched bars) in rats placed at the centre of a plus maze and given a 5-min test 45 min after drug treatments. ***$p<0.001$, Students $t$-test, compared with control. ****$p<0.001$, Students $t$-test, compared with control.

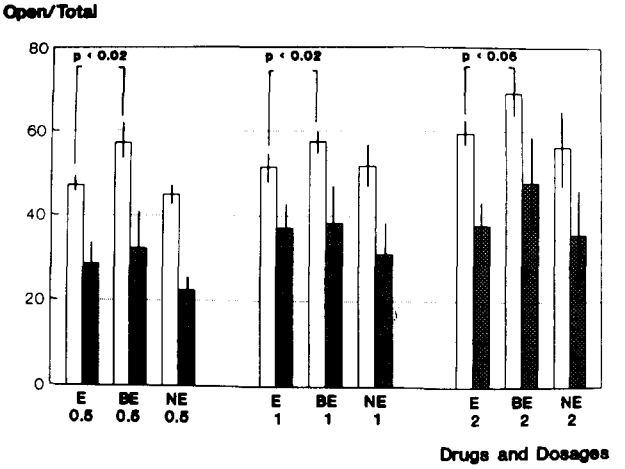


FIG. 2. Mean (±SEM) percentage of open arm entries (white bars) or time (s) spent in the open arms (hatched bars) in rats at the centre of a plus maze and given a 5-min test. The animals were pretreated with either Bay K 8644 (B; 0.5 mg/kg), or nifedipine (N; 5 mg/kg), or saline and were treated with 0.5, 1 and 2 g/kg doses of ethanol (E) 20 min later. The test was performed 45 min after first injections.

v/v), 1 g/kg (8.6% v/v), and 2 g/kg (17.1% v/v). All solutions were prepared immediately before use. Data were analysed according to Student's $t$-test.

## RESULTS

Figures 1–2 show the open arm entries and the time spent on the open arms, expressed as a percentage of the total entries or time. The percentage of open/total arm entries and time spent in the arms was taken as the measure of anxiety in the elevated plus maze test; the higher the score was the lower the anxiety (13,24). As seen in Fig. 1, ethanol (0.5, 1.0 and 2.0 g/kg) dose-dependently increased both percentages of open/total arm entries, and Bay K 8644 did not affect any parameter (Fig. 1).

Pretreatment with Bay K significantly increased anxiolytic effects of ethanol at all doses studied, while nifedipine pretreatment caused no effect (Fig. 2).

## DISCUSSION

In the present study, ethanol produced an anxiolytic effect in a dose-dependent manner. Bay K 8644, a calcium channel agonist of the dihydropiridine (DHP) class, potentiated the anxiolytic effect of ethanol but did not alter anxiety when used alone. On the other hand, nifedipine, a DHP calcium channel antagonist, showed an anxiolytic property but failed to modify ethanol-induced anxiolytic effects.

In the recent years, studies on the interaction between ethanol and DHP calcium channel antagonists have been accumulating. It has been shown that nimodipine potentiates both ethanol-induced hypothermia and motor incoordination in mice (16). Nifedipine prolongs ethanol-induced sleep time and reduces the audiogenic seizure response during withdrawal from chronic ethanol treatment in rats (25). Both studies suggested that ethanol may suppress the calcium influx, and the inhibition of VSCCs can exaggerate ethanol-induced effects. Engel et al. (11) reported that nifedipine completely antagonizes the behavioral stimulation produced by ethanol in mice and that the stimulatory effect of ethanol may involve an enhancement of calcium-mediated mechanisms. In addition to these findings, in the previous studies calcium significantly increased ethanol-induced sleeping time and behavioral intoxications in mice and rats (12,18).

In fact, ethanol has been found to have a markedly inhibitory effect on VSCCs in several preparations (8, 18, 20). However, the molecular basis for the inhibitory effect of ethanol on calcium currents is unclear. It has been suggested either that structural changes in the lipid bilayer surrounding the channels might occur or that ethanol may directly affect an "activation gate" portion of the channel protein (20).

Recently, the measurement of intracellular calcium by fluorescent calcium chelators (i.e., Fura 2) showed that ethanol, in relatively high concentrations, does increase resting calcium in synaptosomes (7,26). The increase in intracellular calcium is due, at least in part, to the release of calcium from intracellular stores instead of the mechanism that involves $IP_3$ (19). Thus ethanol-induced inhibition of calcium entry may occur through an elevation of intracellular calcium which may in turn inactivate calcium channels (18).

Sensitivity to calcium-dependent inactivation has been shown to vary between the different channel types in rat sensory neurons (10), with L channels being more susceptible and T channels resistant. However, it is currently unknown which types of calcium channels are inhibited by ethanol. DHP calcium channel agonists and antagonists are selective for the L type, and ω-conotoxin prolongs ethanol-induced sleep in a dose-dependent manner (18). Thus the possible interaction between ethanol and ω-conotoxin-sensitive sites should not be excluded.

An interesting question is whether ethanol inhibits VSCCs by elevating intracellular calcium and or by directly acting on the channels or surrounding lipids.

In the case of the former, ethanol, at three different doses we used, might enhance intracellular calcium, which in turn blocks VSCCs in the permeation pore itself. The pharmacological importance of this action deserves comment. However, previous reports suggest that the sedative effects of ethanol and pentobarbital are related to decreased neuronal calcium influx (14, 15, 17). Thus the inhibition of calcium currents could also be responsible for the anxiolytic action of ethanol. Bay K 8644, while unable to enhance calcium in the appropriate microenvironment by itself, in this case could add to the average increase

in calcium concentrations produced by ethanol and thus potentiate the anxiolytic effect of ethanol. Possibly, the anxiolytic threshold of calcium entry blockage was reached to the ceiling level by nifedipine alone; then secondary treatment with ethanol might not enhance it further.

On the other hand, there is some evidence that, in a variety of brain regions, ethanol can suppress the excitatory effects of glutamate (8). The calcium channels activated by the NMDA receptor, a subtype of glutamate receptors, are highly sensitive to ethanol. Ethanol produces a concentration-dependent inhibition of NMDA-stimulated calcium entry (9). This mechanism may contribute to the anxiolytic action of ethanol, since NMDA agonists reduced sleep time, whereas antagonists increase it in mice (30).

In conclusion, although the present data may indicate at least a partial involvement of neuronal calcium channels in the anxiolytic action of ethanol, further research will be required to clarify the underlying mechanism.

ACKNOWLEDGEMENTS

For a generous supply of Bay K 8644, we are indebted to Prof. F. Hoffmeister, Dr. F. J. Morich and Dr. F. Seuter at Bayer AG, Wuppertal, FRG.

## REFERENCES

1. Barker, J. L.; MacDonald, R. BDZs specifically modulate GABA-mediated postsynaptic inhibition in cultured mammalian neurones. Nature 271:563–566; 1978.
2. Becker, H. C.; Flaherty, C. F. Influence of ethanol on contrast in consummatory behavior. Psychopharmacology (Berlin) 77:253–258; 1982.
3. Cappell, H. An evaluation of tension models of alcohol consumption. In: Gibbins, R. J.; Israel, Y.; Kalent, H.; Popham, R. E.; Schmidt, W.; Smart, R. G., eds. Research advances in alcohol and drug problems. New York: John Wiley and Sons; 1975:177–209.
4. Cappell, H.; Herman, P. Alcohol and tension reduction, a review. Q. J. Stud. Alcohol. 33:33–64; 1972.
5. Dalterio, S. L.; Wayner, M. J.; Geller, I.; Hartman, R. J. Ethanol and diazepam interactions on conflict behavior in rats. Alcohol 5:471–476; 1989.
6. Daniell, L. C.; Leslie, S. W. Inhibition of fast phase calcium uptake and endogenous norepinephrine release in rat brain region synaptosomes by ethanol. Brain Res. 377:18–28; 1986.
7. Davidson, M.; Wilce, P.; Shanley, B. Ethanol and synaptosomal calcium homeostasis. Biochem. Pharmacol. 39:1283–1288; 1990.
8. Deitrich, R. A.; Dunviddie, T. V.; Harris, R. A.; Erwin, V. G. Mechanism of action of ethanol: Initial central nervous system actions. Pharmacol. Rev. 41:489–537; 1989.
9. Dildy, J. E.; Leslie, S. W. Ethanol inhibits NMDA-induced increases in free intracellular calcium in dissociated brain cells. Brain Res. 499:383–387; 1989.
10. Dupont, J. L.; Boscu, J. L.; Feltz, A. Effect of internal calcium concentration on calcium currents in rat sensory neurons. Pflugers Arch. 406:433–435; 1986.
11. Engel, J. A.; Fahlke, C.; Hulthe, P.; Hard, E.; Johannessen, K.; Snape, B.; Svensson, L. Biochemical and behavioral evidence for an interaction between ethanol and calcium channel antagonists. J. Neural Transm. 74:181–193; 1988.
12. Erickson, C. K.; Tyler, T. D.; Harris, R. A. Ethanol: Modification of acute intoxication by divalent cations. Science 199:1219–1220; 1978.
13. File, S. E. Behavioral changes persisting into adulthood after neonatal benzodiazepine administration in the rat. Neurobehav. Toxicol. Teratol. 8:453–461; 1986.
14. Harris, R. A.; Bruno, P. Membrane disordering by anesthetic drugs: Relationship to synaptosomal sodium and calcium influxes. J. Neurochem. 44:1274–1281; 1985.
15. Heyer, E. J.; MacDonald, R. L. Barbiturate reduction of calcium-dependent action potentials: Correlation with anesthetic action. Brain Res. 236:157–171; 1982.
16. Isaacson, R. L.; Molina, J. C.; Draski, L. J.; Johnston, J. E. Nimodipine's interactions with other drugs: I. Ethanol. Life Sci. 36: 2195–2199; 1985.
17. Leslie, S. W.; Barr, E.; Chandler, J.; Farrar, R. P. Inhibition of fast and slow-phase depolarization-dependent synaptosomal calcium uptake by ethanol. J. Pharmacol. Exp. Ther. 225:571–575; 1983.
18. Leslie, S. W.; Brown, L. M.; Dildy, J. E.; Sims, J. S. Ethanol and neuronal calcium channels. Alcohol 7:233–236; 1990.
19. Machu, T.; Woodward, J. J.; Leslie, S. W. Ethanol and inositol 1,4,5-triphosphate mobilize calcium from rat brain microsomes. Alcohol 6:431–436; 1989.
20. Michaelis, M. L. The effects of ethanol on synaptic membrane calcium transporting systems. In: Sun, G. Y., et al., eds. Molecular mechanisms of alcohol. Clifton, NJ: The Humana Press; 1989:57–67.
21. Nestoros, J. N. Ethanol specifically potentiates GABA-mediated neurotransmission in feline cerebral cortex. Science 209:708–710; 1980.
22. Nestoros, J. N. Ethanol selectively potentiates GABA-mediated inhibition of single feline cortical neurons. Life Sci. 26:519–523; 1980.
23. Nishimura, Ch.; Ohkuma, S.; Tamura, J. T.; Kuriyama, K. A. Modulatory role of mercurials-sensitive sulfhydryl groups in synaptic GABA receptor binding. Neurochem. Int. 4:413–418; 1982.
24. Pellow, S.; Chopin, P.; File, S. E.; Briley, M. Validation of open: closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. Neurosci. Methods 14:149–167; 1985.
25. Pucilowski, O.; Krzascik, P.; Trzaskowske, E.; Kostowski, W. Different effect of diltiazem and nifedipine on some central actions of ethanol in the rat. Alcohol 6:165–168; 1989.
26. Rezazadeh, S. M.; Woodward, J. J.; Leslie, S. W. Fura-2 measurement of cytosolic free calcium in rat brain cortical synaptosomes and the influence of ethanol. Alcohol 6:341–345; 1989.
27. Soubrie, P. Neurophysiological profiles of calcium antagonists. Fundam. Clin. Pharmacol. 3:71s–78s; 1989.
28. Sutoo, D.; Akiyama, K.; Iimura, K. Effect of calmodulin antagonists on calcium and ethanol-induced sleeping time in mice. Pharmacol. Biochem. Behav. 23:627–631; 1985.
29. Stokes, J. A.; Harris, R. A. Alcohols and synaptosomal calcium transport. Mol. Pharmacol. 22:99–104; 1982.
30. Wiesner, J. B.; Ruth, J. A. NMDA receptors are involved in the acute response to ethanol in LS and SS mice. Soc. Neurosci. Abstr. 14:942; 1988.