Pharmacology Biochemistry & Behavior, Vol. 8, pp. 197–201. Printed in the U.S.A.

Intoxicating Effects of Three Aliphatic Alcohols and Barbital on Two Rat Strains Genetically Selected for Their Ethanol Intake

AINO MALILA

Research Laboratories of the State Alcohol Monopoly (Alko), Box 350, SF-00101 Helsinki 10, Finland

(Received 11 June 1977)

MALILA, A. *Intoxicating effects of three aliphatic alcohols and barbital on two rat strains genetically selected for their ethanol intake.* PHARMAC. BIOCHEM. BEHAV. 8(2) 197–201, 1978. – Intoxicating effects of ethanol, isopropanol, tert. butanol and barbital were studied by comparing performances on the tilted plane of ethanol preferring AA (Alko, Alcohol) and ethanol avoiding ANA (Alko, Non-Alcohol) rat strains raised by genetic selection for their voluntary ethanol intake. The motor coordination of AA rats was found to be less affected than that of ANA rats by all three alcohols and barbital. The results indicate a marked genetic difference in neural tolerance to the alcohols and barbital, and suggest that neural tolerance to alcohols plays a role in determining the ethanol preference of AA rats and ethanol aversion of ANA rats.

Ethanol Isopropanol Tert. butanol Barbital Genetically selected rat strains Neural tolerance

IT is well documented that inbred mouse strains differ significantly in their ethanol preference. The C57BL strain has a high preference for ethanol, DBA and BALB strains display a high ethanol aversion, while other strains show intermediate degrees of ethanol preference [24]. It has also been noted that the sleeping time of the ethanol-preferring C57BL strain is one third that of the ethanol-avoiding BALB strain, after administration of an anesthetic ethanol dose [20]. The fact that no difference was found in ethanol concentrations in the brain suggests that the strains differ in their neural tolerance to ethanol. Elsewhere [10], blood ethanol clearance affected by different enzyme activities of liver has been cited as the major factor influencing sleeping time after ethanol. And it has also been suggested that acetaldehyde, the metabolic product of ethanol, is responsible for the differences in sleeping times of the inbred mouse strains [6]. However, two mouse strains selectively bred for differences in sleeping time have been shown to have identical rates of ethanol and acetaldehyde metabolism [17].

Several authors [27, 28, 29] have now verified that mice with high preference for ethanol display greater behavioral and neural tolerance to ethanol than mice with low preference. From these findings it has been concluded that neural tolerance may play a role in determining ethanol intake among inbred mouse strains. However, the inbred mice tested have been genetically selected for characteristics other than ethanol intake. The correlations found with ethanol intake have therefore been produced quite coincidentally and can be considered questionable.

In the Research Laboratories of Alko two rat strains, an ethanol-preferring AA (Alko, Alcohol) and an ethanol-avoiding ANA (Alko, Non-Alcohol) strain, have been raised by selective outbreeding for their different ethanol preferences [13,14]. The process of this selection has been described in detail elsewhere [15]. Recent findings suggest a difference in ethanol tolerance between these rat strains, the strain preferring ethanol possessing a greater neural tolerance than the strain avoiding ethanol [26]. The rat strains also display differences in ethanol and acetaldehyde metabolism, which may explain their drinking behavior [12]. Female AA rats eliminate ethanol faster than female ANA rats, but no differences were found in males; at the same time ANA rats of both sexes display a higher acetaldehyde concentration after ethanol than AA rats. Differences have also been noted in 5-hydroxytryptamine (5–HT), 5-hydroxyindolyacetic acid (5–HIAA) and dopamine (DA) contents in the brain [1, 2, 3]. The present study was undertaken to investigate whether the difference in ethanol tolerance of AA and ANA rats could be generalized to include other alcohols and general depressants. Of the alcohols used, ethanol is a primary alcohol which is metabolized to acetaldehyde, isopropanol a secondary alcohol which is converted to acetone, and tert. butanol a tertiary alcohol, which is a nonmetabolized alcohol eliminated mainly by excretion [11]. The barbiturate used, barbital (the sodium salt of 5,5-diethylbarbituric acid) is a long-acting, nonmetabolized drug [30]. An important advantage of using tert. butanol and barbital is that the effects of the drugs per se can be separated from the effects of their metabolites.

METHOD

Intact female (13 AA and 14 ANA) and male (13 AA and 12 ANA) rats of the F_{26}-generation were used. At the beginning of the experiments the rats were 3 months old, weighing 200–250 g. They were housed 6 to 7 per cage at 24–25°C on a day-night schedule of 12:12 hr. Standard

0091-3057/78/0802-0197$00.75

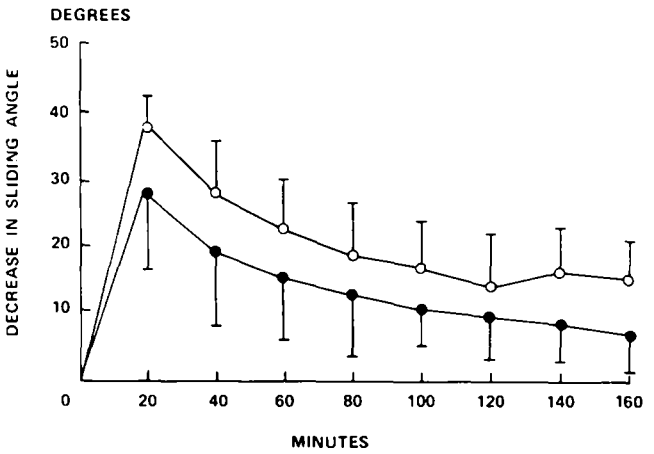

FIG. 1. Effect of 2.5 g/kg ethanol on motor performances of the two rat strains on the tilted plane expressed as a decrease in sliding angle (in degrees) relative to their own control values. Solid circles (●—●—●—●) represent the performance of the AA rats preferring ethanol and open circles (○—○—○—○) the performance of the ANA rats avoiding ethanol. Each point in the figure represents the mean of 26 rats. Vertical bars represent + or − SD.

food (Astra Ewos®, Ab Astra, Södertälje, Sweden) and water were available ad lib.

Since equimolar doses of aliphatic alcohols produce different degrees of intoxication [22,32], pilot studies were conducted to determine test doses which would be equally intoxicating. The test doses were as follows: ethanol, 2.5 g/kg b.w. as a 10% (w/v); isopropanol, 1.8 g/kg b.w. as a 10% (w/v); tert. butanol, 0.8 g/kg b.w. as a 5% (w/v); and barbital (the sodium salt of 5,5-diethylbarbituric acid), 120 mg/kg b.w. as a 2% (w/v) solution. The drugs were diluted with saline and administered intraperitoneally.

Motor coordination of the animals was measured by the standardized tilted plane test in a motorized form [5]. During 5 sec the plane was tilted at a constant speed from horizontal to vertical by a motor. The testing person stopped movement of the plane when the rat slid off and recorded the sliding angle. The extent of intoxication was expressed as the decrease in mean sliding angle (in degrees) relative to the control value. The alcohol tests were carried out eight times at 20-min intervals after injection of the alcohols. The first barbital test was performed 40 min after the administration of barbital and the next seven at 20-min intervals. Blood samples were taken from the tip of the rat's tail to measure the alcohol concentration after the last testing on the plane (160 min after the alcohol injections). All tests were performed in a silent, lighted room between 9:00 a.m. and 3:00 p.m. The time between the different series of tests was at least seven days in order to prevent overlapping of the effects.

A blood sample of 0.1 ml was taken from the tip of the rat's tail and pipetted into ice-cold perchloric acid (0.6 N) [12]. The precipitates were centrifuged at 4000 g for 15 min at 4°C and the supernatants were used for chromatographic analyses of alcohols. Ethanol, tert. butanol, isopropanol and its metabolite, acetone, were all measured with a Perkin-Elmer F 40 gas chromatograph with application of the head-space technique.

Statistical comparisons were made by means of a two-way analysis of variance and Student's *t*-test.

TABLE 1

ALCOHOL CONCENTRATIONS ± SD (mM) AFTER THE TILTED PLANE TEST

Alcohol or its metabolite	AA rats	ANA rats
ethanol	45.4 ± 2.9 (n = 26)	45.8 ± 2.4 (n = 25)
tert. butanol	13.9 ± 1.5 (n = 26)	14.2 ± 1.0 (n = 25)
isopropanol	19.7 ± 1.9 (n = 26)	18.0 ± 1.5* (n = 24)
acetone	9.7 ± 1.2 (n = 26)	11.6 ± 1.0† (n = 24)

*$p<0.005$, number of rats in parentheses.
†$p<0.001$.

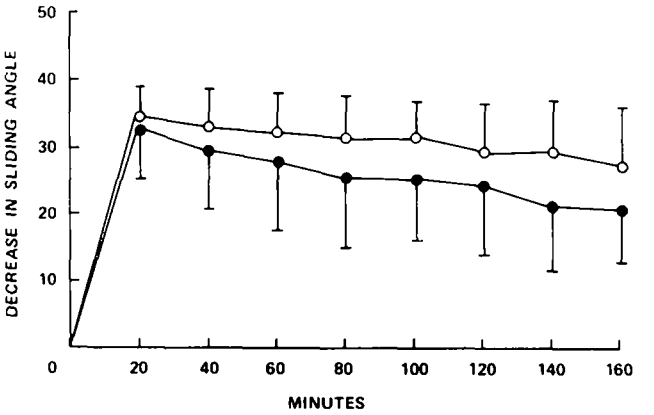

FIG. 2. Effect of 1.8 g/kg isopropanol on motor performance of the two rat strains on the tilted plane expressed as a decrease in sliding angle (in degrees) relative to their own control values. Solid circles (●—●—●—●) represent the performance of the AA rats preferring ethanol and open circles (○—○—○—○) the performance of the ANA rats avoiding ethanol. Each point in the figure represents the mean of 26 rats. Vertical bars represent + or − SD.

RESULTS

The effects of the three aliphatic alcohols and barbital on the performance of the rats on the tilted plane are depicted in Figs. 1–4. The impairment of performance after drugs is expressed as the decrease in the mean sliding angle relative to the rats' own control values, so that higher points in the figures indicate greater intoxication. The results are presented in this form because the rat strains tested differed in their control values on the tilted plane. The mean sliding angle for the AA rats was 81° ± 2° and for the ANA rats 75° ± 4° ($t = 5.79$, $df = 50$, $p<0.001$). As shown in Fig. 1, the ANA rats were more affected by ethanol than the AA rats. Comparisons by means of the two-way analysis of variance showed the strain difference in ethanol intoxication to be highly significant, $F(1,50) = 19.73$, $p<0.001$. Blood ethanol concentrations, shown in Table 1, were equal in the two strains, thus excluding the effects of different elimination rates of ethanol. The AA rats also performed significantly better than the ANA rats in the isopropanol experiment, as seen in Fig. 2, $F(1,50) = 7.18$, $p<0.01$. Whereas some differences are seen

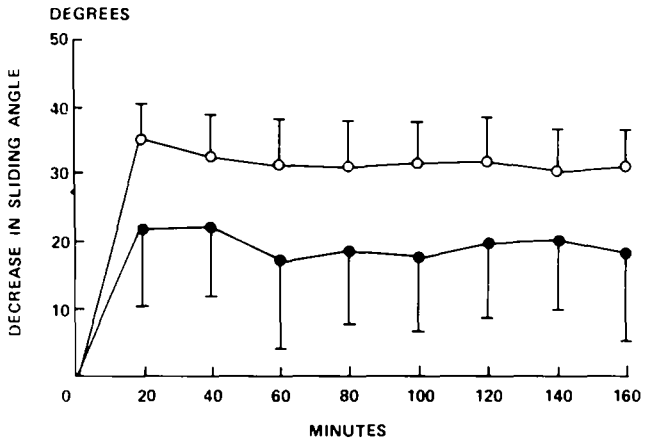

FIG. 3. Effect of 0.8 g/kg tert. butanol on motor performances of the two rat strains on the tilted plane expressed as a decrease in sliding angle (in degrees) relative to their own control values. Solid circles (●—●—●—●) represent the performance of the AA rats preferring ethanol and open circles (○—○—○—○) the performance of the ANA rats avoiding ethanol. Each point in the figure represents the mean of 26 rats. Vertical bars represent + or − SD.

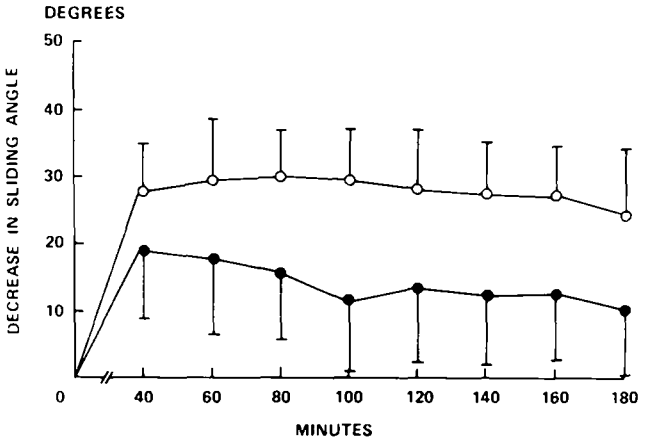

FIG. 4. Effect of 120 mg/kg barbital (the sodium salt of 5,5-diethylbarbituric acid) on motor performances of the two rat strains on the tilted plane expressed as a decrease in sliding angle (in degrees) relative to their own control values. Solid circles (●—●—●—●) represent the performance of the AA rats preferring ethanol and open circles (○—○—○—○) the performance of the ANA rats avoiding ethanol. Each point in the figure represents the mean of 26 rats. Vertical bars represent + or − SD.

(Table 1) in isopropanol metabolism of the rat strains, for blood isopropanol concentration of the AA rats was higher than that of the ANA rats ($t = 3.44$, $df = 48$, $p<0.005$) and the reverse was true for blood concentrations of acetone, the metabolic product of isopropanol ($t = 6.01$, $df = 48$, $p<0.001$). Figure 3 shows that tert. butanol inebriated the ANA rats more than the AA rats, the strain difference being highly significant, $F(1,50) = 35.42$, $p<0.001$. Blood concentrations of tert. butanol were identical in the rats after the tilted plane test. Barbital affected the rats in the same way as the three alcohols, having a greater intoxicating effect on the ANA rats than the AA's, as shown in Fig. 4. The strain difference was highly significant, $F(1,50) = 33.93$, $p<0.001$. Because barbital is a nonmetabolized, long-acting barbiturate, metabolism can be assumed not to affect the test results and it was not measured in the present study.

DISCUSSION

The standardized tilted plane test was used in the present study. Reliability of the test is indicated by the fact that the effect of ethanol on performance has been found proportional to the ethanol dosage [5]. In spite of the simplicity of the test the performance on the tilted plane probably is a complicated function composed of vestibular and grasping reflexes including a strong cortical component [5]. The AA rats displayed a better motor coordination than the ANA rats in the control test, indicating perhaps the greater motor activity of the former. The present experiments also revealed a marked strain difference in alcohol intoxication induced by three aliphatic alcohols: ethanol, isopropanol and tert. butanol. In all cases the ethanol preferring rats were less intoxicated than the ethanol avoiding rats by the same alcohol dose. Different rates of metabolism cannot account for the differences in intoxication induced by ethanol and tert. butanol, but rather different neural tolerance probably is involved. Because no aldehyde develops from tert. butanol, the results from the tert. butanol test suggest that aldehyde does not play a major role in alcohol intoxication of these rat strains. Therefore the difference reported in blood acetaldehyde amounts of the AA and ANA rats [12] cannot explain the differences in ethanol intoxication of the rats either. The results of the isopropanol test, on the other hand, reflect isopropanol-acetone intoxication, because isopropanol causes acetonemia [32]. The difference in intoxication may thus be due in part to different metabolic rates of isopropanol and acetone in the AA and ANA rats.

Recently two rat strains comparable to the AA and ANA rats have been developed by selective inbreeding for their different ethanol preferences [21,23]. No data yet exist regarding their tolerance to ethanol, but no differences in the ethanol and acetaldehyde metabolisms of the strains were observed.

The different neural tolerance to alcohols observed in the present study is in agreement with the results obtained with some inbred mouse strains. The mouse strain preferring ethanol was noted to have a much higher neural and behavioral tolerance to ethanol than the strains avoiding ethanol. Neural tolerance was determined in those experiments by measuring ethanol-induced sleeping time [20,27], ethanol-induced depression of the jaw-jerk reflex [28,29] and nest-building behavior [28]. Besides this, the selection and tolerance of a three-carbon alcohol, 1,2-propanediol (propylene glycol), by these mouse strains was reported to be in the same direction and order of magnitude as their selection and tolerance of ethanol [18]. Moreover, genetically raised mouse strains, short- and long-sleeping, differed in their sleeping times induced by methanol, butanol and tert. butanol [16].

Barbital like alcohols had a greater inebriating effect on the ANA rats than on the AA's. Although structurally dissimilar, both alcohols and barbiturates are classified as general depressants and their major action is believed to be exerted on the same nervous system structure, the reticular activating system [30,33]. The findings concerning the action of alcohols on the squid axon membrane [4,25] and of barbiturates on the lobster axon membrane [8] suggest a similar mechanism of action. However, the actual mech-

anism of action, both of alcohols and barbiturates, is still unexplained. Several contradictory findings with regard to the actions of barbiturates on inbred mouse strains have been reported. The C57BL mice were found to sleep less than the DBA and BALB mice after injection of hexobarbital [9,19]. A greater sensitivity to pentobarbital was reported for the C57BL mice than for the BALB and DBA mice [27,31]. On the other hand, the DBA mice were more susceptible to intoxication by phenobarbital than the C57BL mice in an experiment for inducing phenobarbital dependence [7]. No differences in sleeping times induced by pentobarbital were found between short- and long-sleeping mice [16,31]. Genetic differences in responses to barbiturates seem to be evident both among inbred mouse strains and outbred rat strains. However, further studies are needed before the effects of barbital on the two rat strains can be generalized to include all barbiturates.

The present results comparing the two rat strains have revealed a marked genetic strain difference in intoxication induced by the three aliphatic alcohols and one barbiturate. In all cases except partly in the isopropanol experiment the AA rats displayed a greater neural tolerance than the ANA rats. Further, from the tert. butanol test it can be concluded that aldehyde does not play a significant role in alcohol intoxication of these rats. The findings also suggest that the different drinking behaviors of the rat strains can be explained on the basis of a different neural tolerance to alcohol. Possibly these two rat strains, with their different ethanol preference and different neural tolerance to alcohols and barbital, will prove useful tools for elucidating the mechanisms of action of alcohols and other general depressants such as barbiturates.

ACKNOWLEDGEMENTS

The author wishes to thank Prof. H. Wallgren and Dr. K. Eriksson for valuable discussions. The study was supported by The Finnish Foundation for Alcohol Studies.

REFERENCES

1. Ahtee, L. and K. Eriksson. 5-Hydroxytryptamine and 5-hydroxyindolyacetic acid content in brain of rat strains selected for their alcohol intake. *Physiol. Behav.* 8: 123–126, 1972.
2. Ahtee, L. and K. Eriksson. Regional distribution of brain 5-hydroxytryptamine in rat strains selected for their alcohol intake. *Ann. N.Y. Acad. Sci. U.S.A.* 215: 126–134, 1973.
3. Ahtee, L. and K. Eriksson. Dopamine and noradrenaline content in the brain of rat strains selected for their alcohol intake. *Acta physiol. scand.* 43: 563–565, 1975.
4. Armstrong, C. M. and L. Binstock. The effects of several alcohols on the properties of the squid giant axon. *J. gen. Physiol.* 48: 265–277, 1964.
5. Arvola, A., L. Sammalisto and H. Wallgren. A test for level of alcohol intoxication in the rat. *Q. Jl. Stud. Alc.* 19: 563–572, 1958.
6. Belknap, J. K., J. W. MacInnes and G. E. McClearn. Ethanol sleep times and hepatic alcohol and aldehyde dehydrogenase activities in mice. *Physiol. Behav.* 9: 453–457, 1972.
7. Belknap, J. K., S. Waddingham and G. Ondrusek. Barbiturate dependence in mice induced by a simple short-term oral procedure. *Physiol. Psychol.* 1: 394–396, 1973.
8. Blaustein, M. P. Barbiturates block sodium and potassium conductance increases in voltage-clamped lobster axons. *J. gen. Physiol.* 51: 293–307, 1968.
9. Catz, C. and S. J. Yaffe. Strain and age variations in hexobarbital response. *J. Pharmac. exp. Ther.* 155: 152–156, 1967.
10. Damjanovich, R. P. and J. W. MacInnes. Factors involved in ethanol narcosis: Analysis in mice of three inbred strains. *Life Sci.* 13: 55–65, 1973.
11. Derache, R. Toxicology, pharmacology and metabolism of higher alcohols. In: *Alcohols and Derivatives*, Vol. 1, Int. Encycl. Pharmac. Ther. Sec. 20, edited by J. Trémoliéres. London: Pergamon Press, 1970.
12. Eriksson, C. J. P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their alcohol preference. *Biochem. Pharmac.* 22: 2283–2292, 1973.
13. Eriksson, K. Genetic selection for voluntary alcohol consumption in the albino rat. *Science* 159: 739–741, 1968.
14. Eriksson, K. Factors affecting voluntary alcohol consumption in the albino rat. *Ann. Zool. fenn.* 6: 227–265, 1969.
15. Eriksson, K. Behavioral and physiological differences among rat strains specially selected for their alcohol consumption. *Ann. N.Y. Acad. Sci. U.S.A.* 197: 32–41, 1972.
16. Erwin, V. G., W. D. W. Heston, G. E. McClearn and R. A. Deitrich. Effect of hypnotics on mice genetically selected for sensitivity to ethanol. *Pharmac. Biochem. Behav.* 4: 679–683, 1976.
17. Heston, W. D.W., V. G. Erwin, S. M. Anderson and H. Robbins. A comparison of the effects of alcohol on mice selectively bred for differences in ethanol sleep-time. *Life Sci.* 14: 365–370, 1974.
18. Hillman, M. G. and C. W. Schneider. Voluntary selection of and tolerance to 1,2-propanediol (propylene glycol) by high and low ethanol-selecting mouse strains. *J. comp. physiol. Psychol.* 88: 773–777, 1975.
19. Jay, G. E., Jr. Variation in response of various mouse strains to hexobarbital (Evipal). *Proc. Soc. exp. Biol. Med.* 90: 378–380, 1955.
20. Kakihana, R., D. R. Brown, G. E. McClearn and I. R. Tabershaw. Brain sensitivity to alcohol in inbred mouse strains. *Science* 154: 1574–1575, 1966.
21. Li, T.-K. and L. Lumeng. Alcohol metabolism of inbred strains of rats with alcohol preference and nonpreference. *Second International Symposium on Alcohol and Aldehyde Metabolizing Systems*, October 15–17, 1976, Natl. Inst. on Alcohol Abuse and Alcoholism, University of Pennsylvania, Johnson Res. Found., Abstr. 42, p. 19.
22. Lindbohm, R. and H. Wallgren. Changes in respiration of rat brain cortex slices induced by some aliphatic alcohols. *Acta pharmac. tox.* 19: 53–58, 1962.
23. Lumeng, L., T. D. Hawkins and T.-K. Li. New strains of rats with alcohol preference and nonpreference. *Second International Symposium on Alcohol and Aldehyde Metabolizing Systems.* October 15–17, 1976, Natl. Inst. on Alcohol Abuse and Alcoholism, University of Pennsylvania, Johnson Res. Found., Abstr. 38, p. 18.
24. McClearn, G. E. and D. A. Rodgers. Differences in alcohol preference among inbred strains of mice. *Q. Jl. Stud. Alc.* 20: 691–695, 1959.
25. Moore, J. W., W. Ulbricht and M. Takata. Effect of ethanol on the sodium and potassium conductances of the squid axon membrane. *J. gen. Physiol.* 48: 279–295, 1964.

26. Nikander, P. and L. Pekkanen. An inborn alcohol tolerance in alcohol preferring rats. The lack of relationship between tolerance to ethanol and the brain microsomal (Na^+K^+) ATPase activity. *Psychopharmacology* **51**: 219–223, 1977.
27. Randall, C. L. and D. Lester. Differential effects of ethanol and pentobarbital on sleep time in C57BL and BALB mice. *J. Pharmac. exp. Ther.* **188**: 27–33, 1974.
28. Schneider, C. W., S. K. Evans, M. B. Chenoweth and F. L. Beman. Ethanol preference and behavioral tolerance in mice: Biochemical and neurophysiological mechanisms. *J. comp. physiol. Psychol.* **82**: 466–474, 1973.
29. Schneider, C. W., P. Trzil and R. D'Antrea. Neural tolerance in high and low ethanol selecting mouse strains. *Pharmac. Biochem. Behav.* **2**: 549–551, 1974.
30. Sharpless, S. K. Hypnotics and sedatives. I. The barbiturates. In: *The Pharmacological Basis of Therapeutics*, 3rd edition, edited by L. S. Goodman and A. Gilman. New York: MacMillan Company, 1965.
31. Siemens, A. J. and A. W. K. Chan. Differential effects of pentobarbital and ethanol in mice. *Life Sci.* **19**: 581–590, 1976.
32. Wallgren, H. Relative intoxicating effects on rats of ethyl, propyl and butyl alcohols. *Acta pharmac. tox.* **16**: 217–222, 1960.
33. Wallgren, H. and R. Tirri. Studies on the mechanism of stress-induced reduction of alcohol intoxication in rats. *Acta pharmac. tox.* **20**: 27–38, 1963.