Pharmacology Biochemistry & Behavior, Vol. 25, pp. 739–742, 1986. © Ankho International Inc. Printed in the U.S.A. 0091-3057/86 $3.00 + .00

Hypoglycemia and Hypothermia Induced by Ethanol: Antagonism by Indomethacin[1]

GINA STRUFFALDI MORATO,[2] MARIA LUCIA OLIVEIRA SOUZA, MARIA LAURA NOGUEIRA PIRES AND JANDIRA MASUR[3]

Departamento de Psicobiologia, Escola Paulista de Medicina
Rua Botucatu, 862, 04023, Sao Paulo, SP, Brasil

Received 25 June 1986

MORATO, G. S., M. L. O. SOUZA, M. L. N. PIRES AND J. MASUR. *Hypoglycemia and hypothermia induced by ethanol: Antagonism by indomethacin.* PHARMACOL BIOCHEM BEHAV **25**(4) 739–742, 1986.—The influence of pretreatment with 5.0 or 10.0 mg/kg of indomethacin, a prostaglandin synthetase inhibitor, on the alterations in body temperature produced by 3.0 and 4.0 g/kg of ethanol, was studied in food-deprived and free-feeding rats. A partial antagonism of ethanol's hypothermic effect resulted from indomethacin pretreatments and this effect was found to be ethanol dose-dependent. This result could account for the conflicting reports in the literature on the effectiveness of indomethacin in antagonizing ethanol-induced hypothermia. Indomethacin (5.0 mg/kg) also antagonized ethanol-induced hypoglycemia in 48 hr starved rats. The relationship between two effects of ethanol, hypothermia and hypoglycemia, is discussed.

Ethanol-induced hypothermia Ethanol-induced hypoglycemia Indomethacin and ethanol

AN increasing body of evidence suggests that prostaglandins are involved in the molecular mechanisms through which ethanol influences some peripheral and central nervous system functions [1, 3, 16]. It is known that aspirin and other prostaglandin synthetase inhibitors (PGSI) [22] can antagonize some effects of alcohol such as sleep time [2, 4, 6], hyperactivity [15] and prenatal malformations [14] in animals.

Some authors have suggested that hypothermia occurs as a consequence of the ethanol-induced hypoglycemia [7]. However, Kalant and Le [9] recently suggested that "there is rather more persuasive evidence that severe and prolonged hypothermia may cause hypoglycemia." Therefore, it is likely that hypothermic and hypoglycemic effects produced by ethanol are casually linked. Although hypoglycemia induced by ethanol in fasted animals has been reported [18–21] to our knowledge there is no data concerning the effects of PGSI on this response.

Reports of the effect of PGSI on ethanol-induced hypothermia have been inconsistent. For instance, George *et al.* [5] found that PGSI significantly reduced ethanol-induced hypothermia in mice, while Pohorecky *et al.* [12] and Greizerstein [6] observed no effect of PGSI on ethanol-induced hypothermia in rats and mice, respectively.

In view of the conflicting reports on the effects o aspirin-like drugs on ethanol-induced hypothermia and considering the possibility that this hypothermic effect can lead to hypoglycemia, the purpose of this study was to re-examine ethanol-induced hypothermia and hypoglycemia in rats and to determine whether such actions are reliably influenced by the PGSI indomethacin.

METHOD

Animals

One hundred and thirty-six male Wistar rats, from our own colony, 90 days old and weighing 250–300 g were used. After weaning at 25 days of age, they were housed 3 to a cage (wire, 30×20×15 cm) in a room maintained at a constant temperature (23±1°C) and on a 12 hr dark-light cycle (lights on from 7:00 to 19:00 hr). Free access to tap water and standard laboratory chow, except in fasting experiments, was made available.

Drugs

Ethanol for analysis (Carlo Erba Lab) and indomethacin (Indocid® Merck Lab) were used. Ethanol was diluted in

[1]This work was partially supported by Conselho Nacional de Desenvolvimento Cientifico e Technologico (CNPq) and Associação Fundo de Incentivo a Psicofarmacologia (AFIP).

[2]Permanent address: Departamento de Farmacologia, CCB, Universidade Federal de Santa Catarina, Rua Ferreira Lima, 26, 88000 Florianopolis, SC, Brasil.

[3]Career Investigator of CNPq.

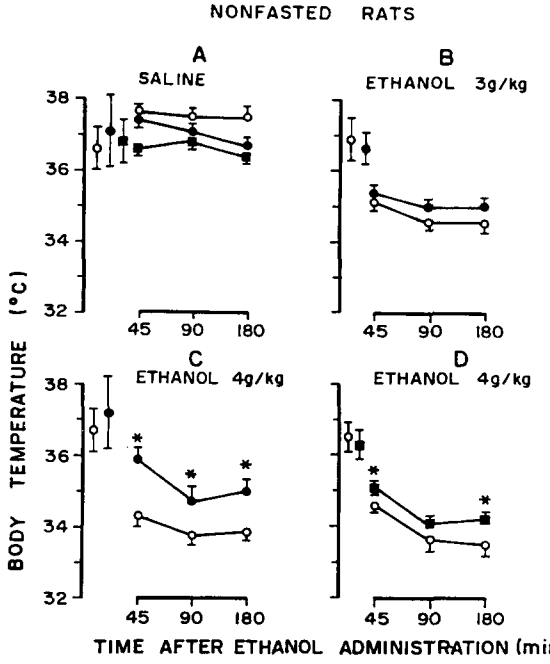

FIG. 1. Mean body temperature of free-feeding rats injected with saline or ethanol (3.0 or 4.0 g/kg) 20 min after pretreatment with saline (○), 5.0 (●) or 10.0 (■) mg/kg of indomethacin. The isolated points indicate the mean basal values of each group, immediately before injections. Vertical bars over isolated points indicate 0.99 confidence limits. Vertical bars in the curves represent standard errors. *$p<0.05$.

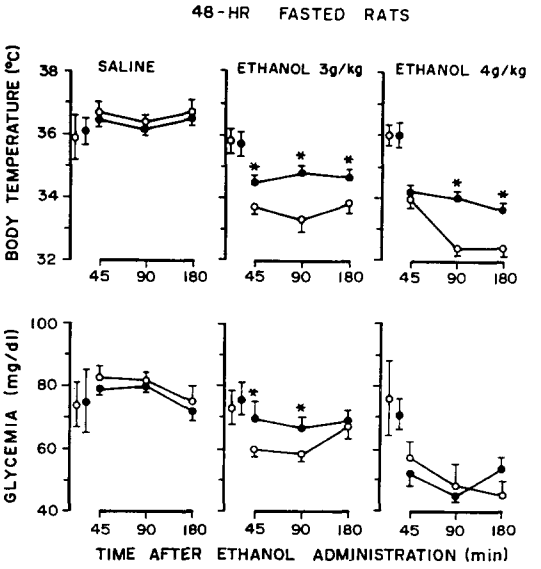

FIG. 2. Mean body temperature and blood glucose levels of 48 hr food-deprived rats injected with saline or ethanol (3.0 or 4.0 g/kg) 20 min after pretreatment with saline (○) or 5.0 mg/kg of indomethacin (●). The isolated points indicate mean basal values of each group immediately before injections. Vertical bars over isolated points indicate 0.99 confidence limits. Vertical bars in the curves represent standard errors. *$p<0.05$.

saline (0.9% NaCl) to the concentration of 20% w/v, and indomethacin was dissolved in distilled water immediately before the experiments.

Blood Glucose Determinations

Blood glucose was determined through the reaction of a blood drop, collected from the tail, with a Dextrostix strip using a dextrometer reflectance colorimeter [13].

Temperature Measurements

Temperature was monitored through a digital thermometer. The animals were placed in plastic restraining cages and rectal temperature was taken by inserting a Vaseline-lubricated thermistor probe 2.5 cm into the rectum until the measure was stable (30 sec).

Statistical Analysis

The values of body temperature and blood glucose after drug administration were compared to basal values (before drug) using the 0.99 confidence interval for small samples according to Hardyck and Petrinovich [8]. Comparisons between groups were performed using the unpaired Student's *t*-test. $p<0.05$ was used as the significance level.

EXPERIMENT I

Effect of indomethacin pretreatment on hypothermia induced by ethanol in free-feeding rats.

Procedure

Rats were injected with 5.0 or 10.0 mg/kg of indomethacin or saline (volume 0.1 ml/100 g body weight) intraperitoneally (IP). Twenty minutes later they received ethanol 3.0 or 4.0 g/kg (volume 1.5 or 2.0 ml/100 g body weight, respectively) or saline, IP. Body temperature was determined immediately before indomethacin, and 45, 90 or 180 min after ethanol administration. All experiments were carried out between 13:00 and 18:00 hr to avoid circadian effects.

Results

The effects of ethanol alone or in combination with indomethacin on body temperature in nonfasted rats are shown in Fig. 1 (Panels A to D). Administration of indomethacin alone (5.0 and 10.0 mg/kg) had no effect on body temperature (Panel A). As expected both doses of ethanol led to a significant and sustained fall in body temperature. The effects of pretreatment with indomethacin varied according to the dose of ethanol used. Thus, 5.0 mg/kg of indomethacin did not affect the hypothermic action of 3.0 g/kg of alcohol (Panel B) but produced a partial though significant antagonism on the effect of ethanol 4.0 g/kg (Panel C). The dose of 5.0 mg/kg of indomethacin seemed to be more effective in antagonizing the ethanol effect since indomethacin 10.0 mg/kg did not promote a significant antagonism of ethanol hypothermia at 90 min after alcohol injection (Panel D).

EXPERIMENT II

Influence of indomethacin pretreatment on glycemic and

thermic alterations induced by ethanol in 48 hr food-deprived rats.

Procedure

Following food deprivation of 48 hr, rats were injected with 5.0 mg/kg indomethacin or saline, IP. Twenty minutes later they were administered ethanol 3.0 or 4.0 g/kg or saline through the same route. Blood glucose and body temperature were determined immediately before indomethacin and 45, 90 or 180 min after ethanol administration.

Results

As can be seen in Fig. 2 ethanol produced a dose-dependent hypothermia in 48 hr food-deprived rats. Indomethacin by itself had no effect on body temperature. The hypothermic effect of ethanol in this group appeared to be more pronounced than that produced in non food-deprived rats (Fig. 1). Thus, while body temperature fell to about 34°C after the injection of 4.0 g/kg in free-feeding rats, it dropped to near 32°C in fasted rats challenged with the same dose of ethanol. On the other hand it is important to note that pretreatment with 5.0 mg/kg indomethacin efficiently antagonized the hypothermic action of 3.0 and 4.0 g/kg of ethanol in food-deprived rats.

As expected, administration of hypnotic doses of ethanol resulted in hypoglycemia in 48 hr food-deprived rats (Fig. 2). Pretreatment with indomethacin significantly reversed the hypoglycemia induced by 3.0 g/kg of ethanol. This experiment was replicated with similar results. However, indomethacin failed to antagonize the hypoglycemic effect induced by ethanol 4.0 g/kg. As can also be observed in Fig. 2, indomethacin by itself did not affect blood glucose levels.

DISCUSSION

The present results confirm the study by George *et al.* [5], by showing that ethanol-induced hypothermia in rats can be partially antagonized by indomethacin, a potent PGSI. The data also indicate that the antagonizing effect of indomethacin is dependent on the dose of ethanol since pretreatment with the PGSI did not alter the hypothermia observed with the lower dose of ethanol (3.0 g/kg) in free-feeding rats. Interestingly, in the 48 hr food-deprived rats indomethacin was effective in antagonizing the hypothermia induced by both doses of ethanol (3.0 and 4.0 g/kg). In fact, acute food deprivation increases the effect of ethanol [10], probably due to the reduction of the rate of NADH reoxidation to NAD [17], an important limiting factor in the metabolism of ethanol [11]. While free-feeding rats treated with 3.0 g/kg of ethanol had body temperatures near 35°C, the body temperature of food-deprived animals dropped to nearly 33.5°C. Our findings suggest that the antagonizing effect of PGSI depends on the magnitude of the ethanol-induced hypothermia. This observation provides experimental support for George *et al.*'s [5] hypothesis that attributes the negative findings reported in the literature [12] to an insufficient decay in body temperature.

Another salient finding of this study was the observation that indomethacin significantly antagonized ethanol-induced hypoglycemia in 48 hr food-deprived rats. PGSI pretreatment effectively antagonized hypoglycemia induced by 3.0 g/kg of ethanol while no antagonism was seen when the ethanol dose was raised to 4.0 g/kg. Thus, differences between the thermic and the glycemic response to ethanol were noted since a clear antagonizing effect of indomethacin was observed for the body temperature response to 4.0 g/kg ethanol, while no antagonism of the glycemic response to 4.0 g/kg ethanol was detected. This dissociation could indicate different mechanisms mediating ethanol-induced body temperature and blood glucose alterations. In opposition to this interpretation are previous findings showing concomitant reversal of ethanol-induced hypothermia and hypoglycemia by increasing room temperature [19,20]. This relationship is so close that by raising the ambient temperature from 21 to 32°C, 4.0 g/kg of ethanol induced no hypothermia, and hypoglycemia was shifted to hyperglycemia in 48 hr food-deprived rats [20]. Moreover, it was observed that rats which were tolerant to the hypothermic effect of ethanol showed decreased glycemic response [19]. Considering the evidence in favor of a close relationship between the glycemic and thermic alterations induced by ethanol, the present data indicating that antagonism of ethanol-induced hypothermia is not necessarily accompanied by attenuation of the glycemic response to ethanol has to be cautiously interpreted.

ACKNOWLEDGEMENTS

We thank Drs. Reinaldo Naoto Takahashi and Elisaldo Araujo Carlini for valuable suggestions on the preparation of this manuscript.

REFERENCES

1. Collier, H. O. J., W. J. McDonald-Gibson and S. A. Saeed. Stimulation of prostaglandin biosynthesis by capsaicin, ethanol and tyramine. *Lancet* **1**: 702, 1975.
2. George, F. R. and A. C. Collins. Prostaglandin synthetase inhibitors antagonize the depressant effects of ethanol. *Pharmacol Biochem Behav* **10**: 865–869, 1979.
3. George, F. R. and A. C. Collins. Ethanol's behavioral effects may be partly due to increases in brain prostaglandin production. *Alcohol: Clin Exp Res* **8**: 143–146, 1985.
4. George, F. R., T. C. Howerton, G. I. Elmer and A. C. Collins. Antagonism of alcohol hypnosis by blockade of prostaglandin synthesis and activity: Genotype and time course effects. *Pharmacol Biochem Behav* **19**: 131–136, 1983.
5. George, F. R., S. J. Jackson and A. C. Collins. Prostaglandin synthetase inhibitors antagonize hypothermia induced by sedative hypnotics. *Psychopharmacology (Berlin)* **74**: 241–244, 1981.
6. Greizerstein, H. B. Ethanol and indomethacin interactions in motor impairment, hypnosis, and body temperature. *Psychopharmacology (Berlin)* **84**: 101–104, 1984.
7. Haight, J. S. J. and W. R. Keatinge. Failure of thermoregulation in the cold during hypoglycaemia induced by exercise and ethanol. *J Physiol* **229**: 87–97, 1973.
8. Hardyck, C. D. and L. F. Petrinovich. *Introduction to Statistics for the Behavioral Sciences*. Philadelphia: W. B. Saunders Company, 1969, pp. 96–98.
9. Kalant, H. and A. D. Le. Effects of ethanol on thermoregulation. *Pharmacol Ther* **23**: 313–364, 1984.
10. Masur, J. and M. J. Ribeiro. Chronic starvation impairs in the effects of depressant drugs on CNS of rats. *Pharmacology* **23** 64–68, 1981.
11. Pirola, R. C. *Drug Metabolism and Alcohol*. Baltimore: University Park Press, 1978, pp. 55–79.

12. Pohorecky, L. A., J. Brick and J. Y. Sun. Serotoninergic involvement in the effect of ethanol on body temperature in rats. *J Pharm Pharmacol* **28:** 157–159, 1976.
13. Preece, M. A. and R. G. Newall. Dextrostix-Eyetone in the insulin hypoglycaemia test. *Br Med J* **2:** 152–154, 1977.
14. Randall, C. L. and R. F. Anton. Aspirin reduces alcohol-induced prenatal mortality and malformations in mice. *Alcohol: Clin Exp Res* **8:** 513–515, 1984.
15. Ritz, M. C., F. R. George and A. C. Collins. Indomethacin antagonizes ethanol but not pentobarbital-induced behavioral activation. *Subst Alcohol Actions Misuse* **2:** 289–299, 1981.
16. Rotrosen, J., D. Mandio, D. Segarnick, L. J. Traficante and S. Gershon. Ethanol and prostaglandin E_1: Biochemical and behavioral interactions. *Life Sci* **26:** 1867–1876, 1980.
17. Smith, M. E. and H. W. Newman. The rate of ethanol metabolism in fed and fasting animals. *J Biol Chem* **234:** 1544–1549, 1959.
18. Souza, M. L. O. and J. Masur. Blood glucose and body temperature alterations induced by ethanol in rats submitted to different levels of food deprivation. *Pharmacol Biochem Behav* **15** 551–554, 1981.
19. Souza, M. L. O. and J. Masur. Does hypothermia play a relevant role in the glycemic alterations induced by ethanol? *Pharmacol Biochem Behav* **16:** 903–908, 1982.
20. Souza, M. L. O. and J. Masur. Ethanol induces hyper and hypoglycemia in both fasted and nonfasted rats dependent on the ambient temperature. *Pharmacol Biochem Behav* **20:** 649–652 1984.
21. Tramill, J. L., P. E. Turner, G. Harwell and S. F. Davis. Alcoholic hypoglycemia as a result of acute challenges of ethanol *Physiol Psychol* **9:** 114–116, 1981.
22. Vane, J. R. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature* **231:** 232–235, 1971