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Antagonism of Alcohol Hypnosis by Blockade of Prostaglandin Synthesis and Activity: Genotype and Time Course Effects

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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**(1) 131–136, 1983.—Pretreatment of mice with prostaglandin synthetase inhibitors (PGSI's) antagonizes alcohol-induced behaviors. This study examined genetic and time course factors of this effect and studied the effects of a putative prostaglandin antagonist (SC-19220) on ethanol sleep time. Long Sleep (LS) and Short Sleep (SS) mice, lines bred for differential response to an hypnotic dose of ethanol, showed a four-fold difference in their dose-response curves for indomethacin antagonism of ethanol-induced hypnosis. Females of both lines required higher amounts of indomethacin relative to males. Indomethacin pretreated animals regained the righting response at a higher blood ethanol concentration than did saline pretreated animals. In addition, indomethacin pretreatment failed to alter the rate of ethanol disappearance from blood. In general, both lines showed effects with low doses of indomethacin at early time points and with high doses of indomethacin at later time points. Indomethacin did not antagonize ethanol-induced hyponosis if given after ethanol. In C3H mice, pretreatment with low doses of SC-19220, a dibenzoxazepine derivative, produced a significant decrease in ethanol sleep time, moderate doses produced no effect, and high levels increased sleep time. These results further substantiate and expand our previous reports. Possible mechanisms for the biphasic effects of indomethacin treatment are presented and discussed.

Prostaglandins Prostaglandin synthetase Ethanol CNS depression Behavior genetics

PROSTAGLANDINS (PGs) are normal constituents of mammalian CNS tissue [7]. However, research on PGs has focussed on the peripheral rather than the central nervous system due to methodological problems which has thus far limited the utility of currently available assays in analyzing brain tissue. In addition, PGs have been implicated as important factors in cardiovascular, reproductive and other peripheral functions and this has directed most PG research accordingly.

We have shown that pretreatment with a number of prostaglandin synthetase inhibitors (PGSIs), such as indomethacin or aspirin, significantly antagonizes the behavioral response to an hypnotic dose of ethanol and other alcohols, but not other sedative hypnotics, in adult males of a heterogeneous strain of mouse (HS/Ibg) [4]. The efficacy of each PGSI in producing this antagonism formed a perfect rank order correlation with its relative potency as an inhibitor of prostaglandin synthetase. Increases in waking blood alcohol levels indicated that the PGSI antagonism of ethanol depression was due to a PGSI-induced decrease in CNS sensitivity to the depressant effects of ethanol, and was not the result of an alteration of ethanol metabolism. An interesting finding of this study was that, for all PGSIs tested, an optimal dose for each PGSI was found, and further increases in dosage resulted in loss of the antagonism of ethanol's behaviors.

Hypnosis is only one of several interesting ethanol-related behaviors. This drug also produces a marked hypothermic effect [14]. We have found [5] that PGSI pretreatment significantly reduces ethanol- and pentobarbital-induced hypothermia in the HS, LS and SS lines of mice. Whether this is a centrally and/or peripherally mediated effect is not clear.

Another interesting aspect of sedative-hypnotic challenge is the biphasic behavioral dose-response curve found with these drugs. Relatively high doses of ethanol and pentobarbital, for example, act as behavioral depressants, while relatively low doses of these drugs produce a behavioral activation. Another recent study carried out in our laboratory demonstrated that pretreatment with a PGSI significantly decreases the behavioral activating effects of ethanol in C3H, LS and SS mice [13]. PGSI pretreatment had no effect on the activation caused by low doses of pentobarbital.

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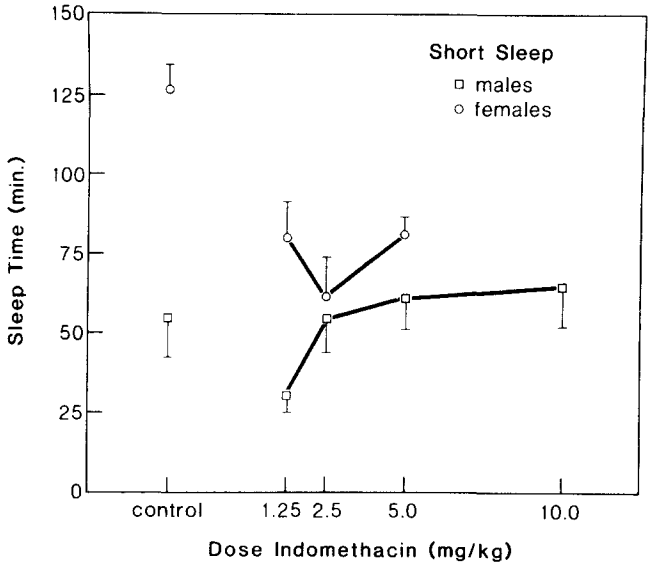

FIG. 1. Effects of indomethacin pretreatment on ethanol-induced sleep time in SS males and females. F(males) (4,23)=2.600, $p=0.12$; paired comparisons: control vs. 1.25 mg/kg $p=0.004$. F(females) (3,16)=9.231, $p<0.001$; F(Quadratic)=12.099, $p<0.005$.

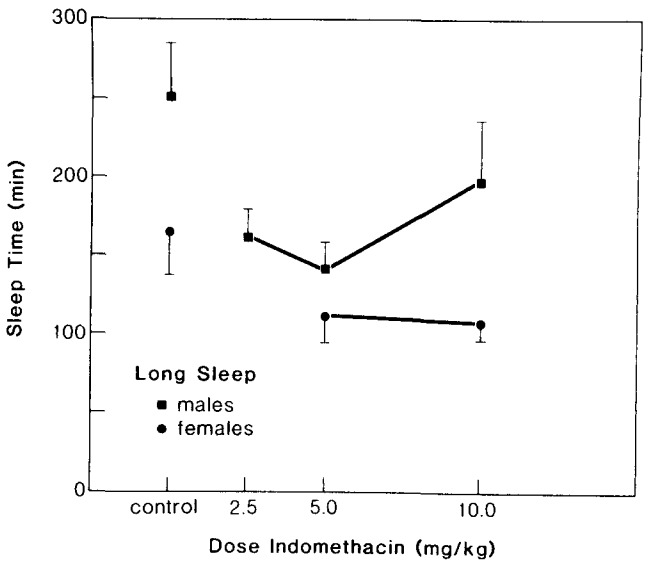

FIG. 2. Effects of indomethacin pretreatment on ethanol-induced sleep time in LS males and females. F(males) (3,17)=3.100, $p<0.05$; F(Quadratic)=7.075, $p<0.02$; F(females) (2,12)=3.684, $p=0.07$; paired comparison: control vs. indomethacin <0.05.

These results further support our hypothesis that the central effects of alcohol, but probably not other sedative hypnotics, are mediated by prostaglandins.

Collier *et al.* [3] have found that a 2.5% v/v ethanol solution stimulates prostaglandin production by prostaglandin synthetase extracted from bull seminal vesicles. If this process is operative in the brain, then inhibiting prostaglandin synthesis should inhibit the actions of alcohol. Our studies have shown, on a behavioral level, that at least the latter part of this hypothesis is true.

All of our previous work involving indomethacin antagonism of ethanol's effects utilized a 15 min pretreatment period. These studies also dealt with just one aspect of the PG system, that is, PG synthesis, and used only male animals. The purpose of this report is to describe experiments which [1] define the dose-response curve for indomethacin antagonism of ethanol-induced hypnosis in Long Sleep (LS) and Short Sleep (SS) male and female mice, animals bred selectively for differential sensitivity to a hypnotic dose of ethanol; (2) determine the time course of indomethacin antagonism of ethanol-induced hypnosis; and [3] describe the effects of a putative receptor blocker (SC-19220) on ethanol-induced hypnosis.

EXPERIMENT 1

METHOD

Animals

Adult HS/Ibg, SS/Ibg, and LS/Ibg male mice, and SS/Ibg and LS/Ibg female mice, all 60–100 days old were equally distributed within litters into the various control and indomethacin-treated groups. Animals were housed in sexually segregated litter groups on a 12:12 light-dark cycle (700 hr to 1900 hr light) with free access to Wayne Lab Blox and tap water.

Injection Procedure

Drug solutions were prepared as follows. Indomethacin was dissolved in a 1% ethanol, 1% polysorbate 80 solution at a concentration of 2.0 mg/ml, which allowed for the injection of 20 mg/kg dose in a volume of 0.01 ml/g body weight. Additional doses were made by dilution of the original stock solution. Control animals were injected with a 1% ethanol, 1% polysorbate 80 solution.

To confine sleep time to approximately the same range, SS and LS mice received ethanol doses of 4.5 g/kg and 3.4 g/kg, respectively. The 4.5 g/kg dose was made by diluting 5.62 ml of absolute ethanol to 10 ml and injecting 0.01 ml/g body weight. The 3.4 g/kg dose was made by diluting 4.25 ml of absolute ethanol to 10 ml and injecting at the same volume.

To assess the time course for indomethacin antagonism of the depressant effects of ethanol, four indomethacin pretreatment time intervals were chosen: 24 hr, 18 hr, 12 hr, and 15 min. At 15 min, several indomethacin doses were used to determine the dose-response curve for antagonism of ethanol-induced hypnosis in the LS and SS mice. Finally, a group of HS mice received saline or indomethacin (5.0 mg/kg) 15 min after receiving ethanol (3.6 g/kg).

Indomethacin injections were given intraperitoneally (IP) at one of these time points prior to or after contralateral ethanol injection. SS mice were separated into control, 1.25 mg/kg, and 5.0 mg/kg groups for each time period and LS mice were separated into control, 5.0 mg/kg and 20.0 mg/kg groups. After pretreatment, mice were returned to their home cages and housed in the usual manner until the second injection.

Testing Procedure

Following administration of ethanol between 1100–1200 hr animals were placed on their backs in a V-shaped trough. The duration of loss of the righting reflex (sleep time) was used as

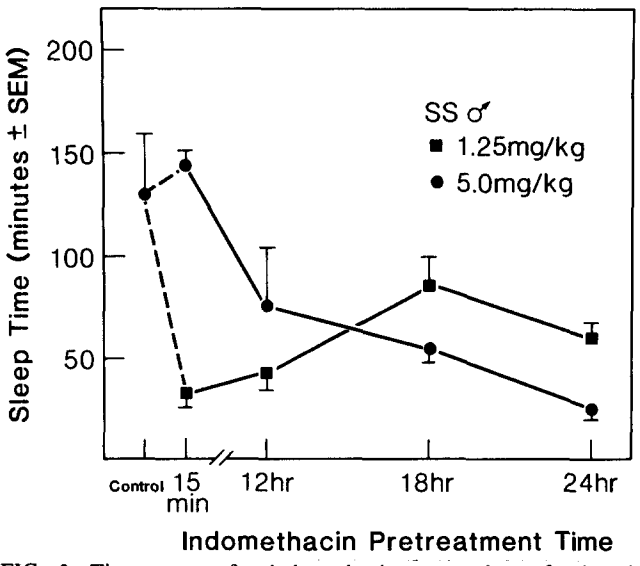

FIG. 3. Time course for indomethacin antagonism of ethanol-induced sleep time in SS/Ibg mice, F(1.25 mg/kg) (3,20)=2.010, $p<0.05$; F(5.0 mg/kg) (3,20)=4.368, $p<0.02$.

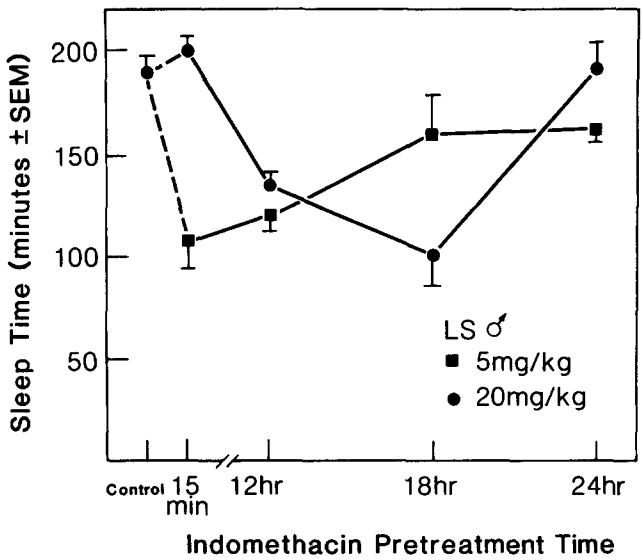

FIG. 4. Time course for indomethacin antagonism of ethanol-induced sleep time in LS/Ibg mice, F(5.0 mg/kg) (3,35)=5.649, $p<0.005$; F(20.0 mg/kg) (3,28)=10.298, $p<0.001$.

the measurement for ethanol effect. Animals were judged to be awake when they could right themselves three times in 30 sec. A 10 μl-blood sample was obtained at time of regaining the righting reflex by piercing the retro-orbital sinus with a capillary pipette. The sample was placed in a tube containing 0.990 ml of a 0.015% isopropanol solution which served as an internal standard. The tubes were stoppered immediately and stored on ice until analyzed for their ethanol content via gas chromatography as described elsewhere [4].

Ethanol Metabolism

Separate groups of LS and SS animals were pretreated with indomethacin to assess the effects of this compound on ethanol elimination. Fifteen minutes prior to IP injection of 2.5 g/kg ethanol given a volume of 0.01 ml/g, SS animals (4 males, 4 females) were injected with 1.25 mg/kg indomethacin and LS animals (4 males, 4 females) were injected with 5 mg/kg indomethacin. Blood samples (10 μl) were taken, as described previously, 60, 100, 140 and 180 minutes after ethanol administration. Blood ethanol content and rates of ethanol elimination were determined. In addition, the apparent volume of distribution (V_d) was estimated from the elimination curve.

RESULTS

The dose-response portion of this experiment revealed several interesting results. Within each mouse line, the dose of indomethacin most effective in reducing sleep time in females was approximately twice the most effective dose for males, as shown in Figs. 1 and 2. In SS mice, the most effective indomethacin doses were 1.25 mg/kg and 2.5 mg/kg in males and females, respectively. The most effective dose of this PGSI in LS males was 5.0 mg/kg, while 5.0 mg/kg and 10.0 mg/kg doses were nearly equally effective in females. Within sex groups, LS mice required an indomethacin dose approximately four times that of the SS mice to optimally antagonize the effects of ethanol.

The time course study indicates that in SS males a 1.25 mg/kg dose of indomethacin was effective in reducing alcohol sleep time up to 12 hr pretreatment time, but not at 18 hr or 24 hr (Fig. 3). Indomethacin at 5.0 mg/kg was effective at 18 hr and 24 hr, but not at 12 hr or less. Similarly, the 5.0 mg/kg indomethacin dose was effective in LS males for up to 12 hr pretreatment time, but not at 18 hr or 24 hr (Fig. 4). The 20.0 mg/kg dose was effective at the 12-hr and 18-hr time points only. These results are summarized in Table 1. Waking blood alcohol (WBA) levels in all cases suggest an indomethacin-induced decrease in CNS sensitivity to ethanol. WBA levels for LS male controls were 225 mg%, 214 mg% for LS female controls, 423 mg% for SS male controls and 451 mg% for SS female controls. Values for percentage change from control for optimum indomethacin groups in males are presented in Table 1. Similar effects were found in females. These results agree with our previous data indicating that PGSIs decrease CNS sensitivity to alcohols [4].

Table 2 presents the results of the ethanol elimination studies for the LS and SS mice. Indomethacin had no effect on elimination rate in either line of mouse. Table 2 also presents the V_d calculated from the elimination curves for each line. V_d was calculated using the equation $V_d=\text{Dose}/C_o$ where C_o is the extrapolated blood concentration at zero time. Indomethacin pretreatment failed to alter the V_d for ethanol in LS mice. However, a modest and statistically significant ($p<0.05$, student's t) increase in V_d was seen in indomethacin-pretreated SS mice.

Post-treatment with indomethacin produced no change in ethanol-induced sleeptime in HS mice. Control mice slept 92±18 minutes (mean±SEM) while the indomethacin group slept 105±7 minutes, F(1,6)=0.290, n.s. The same dose of indomethacin was used which had previously been reported to maximally antagonize sleep time in HS males when administered 15 min prior to ethanol injections [4].

EXPERIMENT 2

This experiment examined the effects of a putative PG antagonist on ethanol-induced sleep time.

TABLE 1

EFFECT OF PRETREATMENT TIME ON SLEEP TIME (MIN) AND WBA (mg%) LEVELS IN SS AND LS MALE MICE

Mouse Line	Dose Ethanol (g/kg)	Dose Indomethacin (mg/kg)	Optimum Time Point (hr)	Control (+SEM) Sleep Time	Indomethacin (+SEM) Sleep Time†	Indomethacin (+SEM) % Control WBA†
SS	4.5	5.0	24	133 ± 33	24 ± 7	125
SS	4.5	1.25	12	133 ± 33	45 ± 9	126
LS	3.4	20.0	18	191 ± 9	103 ± 18	131
LS	3.4	5.0	12	191 ± 9	125 ± 10	132

†Analysis of Variance = SS: Sleep F(Time)=7.067, df=2,31, p=0.003; WBA, F(Time)=3.928, df=2,31, p=0.03. LS: Sleep F(Time)=3.244, df=2,28, p=0.05; WBA F(Time)=11.264, df=2,28, p=0.001.

TABLE 2

EFFECT OF INDOMETHACIN DISTRIBUTION ON ELIMINATION OF ETHANOL IN SS AND LS MICE

Mouse Line	Group	V_d	EtOH Elimination Rate (mg%/hr)
SS	Control	24.8 ± 0.6	84 ± 6
	1.25 mg/kg indomethacin	27.0 ± 0.6*	79 ± 6
LS	Control	25.2 ± 1.6	78 ± 12
	5.0 mg/kg indomethacin	24.2 ± 2.1	96 ± 24

Each point represents the mean value obtained from 8 animals.
Data are reported as mean ± S.E.M.
*Significantly different from untreated control.
Animals were injected with a 2.5 g/kg ethanol dose.

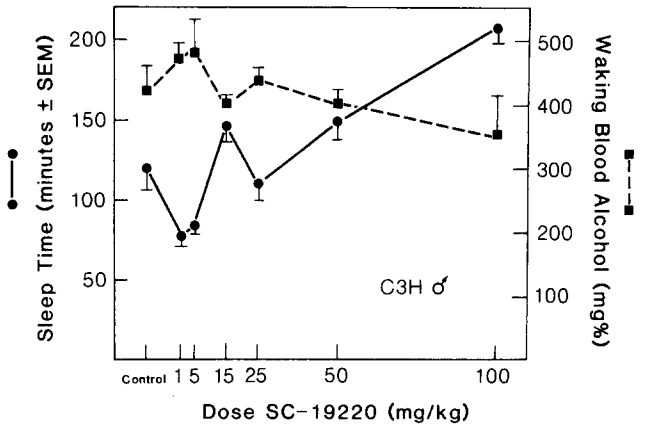

FIG. 5. Effects of SC-19220 pretreatment on ethanol-induced sleep time, F(6,32)=16.017, p<0.00001 and waking blood ethanol levels, F(6,32)=5.596, p<0.0005, in C3H/2Ibg males.

Animals

Thirty-nine adult C3H/2Ibg male mice (60–100 days old) were randomly divided into the various control and drug-treated groups. Animals were maintained and treated as described in Experiment 1.

Injection Procedure

Drug solutions were prepared as follows: 1-acetyl-2-(8-chloro-10, 11-dihydrodibenz [b,f] [1,4] oxazepine-10-carbonyl) hydrazine (SC-19220 Searle) was prepared by dissolving 10 mg/ml of drug in a warm, 1% polysorbate 80, 0.9% saline solution. Ethanol, 4.0 g/kg was prepared by diluting 5.0 ml absolute ethanol to 10.0 ml with 0.9% saline. Drugs were injected IP at a volume of 0.01 ml/g body weight. SC-19220 was cooled to room temperature and administered 15 min prior to ethanol injection. Mice were then tested for sleep time and WBA levels as described above.

RESULTS

The results from Experiment 2 are shown in Fig. 5. A significant overall effect was found. Subsequent post-hoc analysis (Tukey-B) showed that SC-19220 doses of 1.0 mg/kg and 5.0 mg/kg significantly reduced ethanol-induced sleep time whereas a 100 mg/kg produced a significant increase in sleep time. WBA levels were significantly higher in the 1.0 mg/kg and 5.0 mg/kg groups and significantly lower in the 100 mg/kg groups. These results are consistent with an effect of SC-19220 on CNS sensitivity to ethanol rather than a change in ethanol metabolism.

GENERAL DISCUSSION

The results obtained provide further evidence that ethanol-induced hypnosis is mediated to a significant extent by the PG system. WBA levels were again consistent with a CNS effect rather than a metabolic effect. When the possible influence of indomethacin on ethanol elimination was measured, no effect was seen. However, indomethacin may alter the estimated V_d. Estimating V_d from an elimination curve obtained following IP injection is likely to provide only a crude estimate of this pharmacokinetic parameter. However, a modest increase in V_d was seen in SS mice. No change was seen in LS mice. This difference may account for some of the difference between the lines in sensitivity to indomethacin antagonism of alcohol's actions. Additional studies of in-

domethacin's potential influence on ethanol's V_d will require intravenous infusion. It is clear that if indomethacin does, indeed, change the V_d of ethanol that the effect is a modest one and is not likely to be the predominant factor in altering alcohol's actions. These results also suggest that indomethacin does not produce its antagonistic effect by decreasing brain ethanol levels, since the change in V_d, if any, is in a direction opposite to that expected if this were the case. However, since our estimate of V_d was obtained in a non-ideal manner, and since indomethacin has significant effects on cerebral blood flow [15], a change in brain ethanol concentration large enough to account for the large indomethacin effects on sleep time, while unlikely, cannot be completely ruled out from the results of this study. Post-treatment with indomethacin produced no effect, suggesting that PG synthesis or activity must be inhibited before administration of alcohol to produce the antagonistic effect.

Our previous studies have indicated that PGSI antagonism of ethanol's effects is biphasic in nature, that is, there exists an optimum PGSI dose or dose range above and below which there is little or no effect. The present results substantiate this finding. The time course experiment suggests that over a period of 12–24 hours a sufficient percentage of high indomethacin dose is metabolized to unmask its antagonistic effect on ethanol. There are a number of potential reasons why high doses of PGSIs may not decrease alcohol's actions. For example, recent studies have shown that indomethacin is a calcum ion antagonist [11], inhibits protein kinase [12] and affects cerebral blood flow [15], at doses similar to the higher doses used in our studies. Non-linear dose-response curves would arise if any of these effects, or other unknown effects, worked in an opposing manner to the effects of decreased PG production. Inhibition of the cyclooxygenase by PGSIs appears to be irreversible and is overcome by de novo synthesis of new enzyme. However, the higher dose indomethacin effects are reversible, which could explain the present time course results. As excess levels of indomethacin are metabolized, the high dose effects of this agent will decrease accordingly. Only the irreversible effects, that is, inhibition of PG synthesis, will remain.

Interestingly, in the LS mice the high indomethacin dose (20 mg/kg) became effective at 12 hr, whereas the high dose in the SS mice became effective at 18 hr. This may suggest a differential rate of indomethacin metabolism between these lines and may explain, in part, the difference between the lines in sensitivity to indomethacin antagonism of ethanol's actions. In addition, the high dose had lost its effect in the LS by 24 hr.

Pretreatment with a putative PG antagonist, SC-19220, a dibenzoxazepine derivative, also proved effective in decreasing sleep time, again in a dose-dependent manner. Interestingly, a very high dose (100 mg/kg) of this drug produced a significant increase in ethanol sleep time. WBA levels indicate that this response was due to a SC-19220 induced increase in CNS sensitivity to ethanol. SC-19220 has been shown to block PG activity without affecting PG synthesis, suggesting that this agent is a PG receptor blocker, or PG antagonist [16]. Previous work has also shown this compound to be effective peripherally *in vivo* at concentrations similar to those found to decrease sleep time in the present study [17]. However, our results should be taken with caution since the true mechanism of action of this agent and its *in vivo* antiprostaglandin activity are not completely established.

The high dose effects of PG system antagonists, that is, agents which disrupt and decrease normal functioning of some aspect of the PG system, on other systems is one possible explanation for the consistent non-linear responses we have obtained. However, the present results with SC-19220 present another possible explanation. We [5] have shown that indomethacin acts not only as an anti-pyretic, i.e., a blocker of hyperthermia, but also as an antagonist of agents which induce hypothermia. These effects were seen at an indomethacin dose that did not alter the temperature of normothermic mice. Wang [19] has reported that pyrogen decreases the activity of thermoregulatory hypothalamic neurons which respond to heating with increased activity but increases activity of similar neurons which respond to heating by decreasing activity. PGSI administration antagonizes both of these effects causing the discharge rate to return to normal. Horrobin [8] has observed that dose-response curves for PGs are frequently curvilinear, or bell-shaped. In other words, very high and/or very low levels of PGs may produce the same final result, which may be opposite to the effects of moderate PG levels. These facts suggest a role for central PGs as neuroregulators which maintain neural activity within a physiologically normal range. This effect may involve PG mediation of neurotransmitter release [6,18]. Dramatic changes in PG levels, whether increases or decreases, may produce common outcomes.

While the hypothesis that the CNS actions of PGs and PG inhibitors are characterized by a bell-shaped dose-response curve is speculative, it is based on findings from our laboratory and several others. Since it appears possible that PGs play an important role in the mediation of alcohol's effects, an increased understanding of the role of PGs in the CNS seems imperative. We hope that results and ideas presented here will aid in this process.

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