Pharmacology Biochemistry & Behavior, Vol 10, pp. 865–869 Printed in the U S.A.

Prostaglandin Synthetase Inhibitors Antagonize the Depressant Effects of Ethanol[1]

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(Received 23 August 1978)

GEORGE, F. R. AND A C. COLLINS. *Prostaglandin synthetase inhibitors antagonize the depressant effects of ethanol.* PHARMAC. BIOCHEM BEHAV 10(6) 865–869, 1979.—Several studies indicate that ethanol may depress the central nervous system by altering neurotransmitter release. Evidence obtained from the peripheral nervous system suggests that prostaglandins act as negative feedback inhibitors of transmitter release. If a similar process occurs in the brain, then perhaps ethanol affects transmitter release via a mechanism involving prostaglandins. Prostaglandin synthetase inhibitors were administered to adult HS/Ibg male mice prior to intraperitoneal injection of a hypnotic dose of either ethanol, propanol, or t-butanol. A significant decrease in the length of alcohol sleep time was found in the ethanol study, this was coupled with a significant increase in waking blood alcohol levels These results indicate that inhibition of prostaglandin synthesis alters CNS sensitivity to the depressant effects of alcohol When the same inhibitors were administered prior to other sedative hypnotics, i.e., pentobarbital and chloral hydrate, no effect was found. This suggests that prostaglandins may be specifically involved in the biochemical mechanism of alcohol depression.

Prostaglandins Prostaglandin synthetase Ethanol CNS depression Sedative hypnotics

A NUMBER of investigations have suggested that ethyl alcohol elicits at least a portion of its depressant effects on the central nervous system (CNS) by altering neurotransmitter function. One such alteration is an inhibition of transmitter release [10]. Ethanol has been shown to inhibit the release of several transmitters *in vitro*, with the order of sensitivity being acetylcholine>serotonin>dopamine>norepinephrine>glutamate>GABA [2]. The precise mechanism underlying this effect has not been elucidated. Several studies have also examined the influence of alcohol on *in vivo* neurotransmitter turnover rates. Ethanol-induced decreases in turnover have been reported for serotonin [9,16], norepinephrine [13,14], and dopamine [5]. Decreased transmitter turnover should occur as a consequence of inhibition of transmitter release.

Transmitter release under normal conditions appears to be controlled by several mechanisms. Recent interest has focused on presynaptic regulation of release by various neurotransmitters and neuromodulators. Evidence indicates that release may be regulated by either the released transmitter, other neurotransmitters, or—in some cases—prostaglandins. Numerous studies [1, 8, 15] indicate that prostaglandins, presumably formed postsynaptically, exert a negative feedback inhibition on transmitter release in the peripheral autonomic nervous system. Further evidence suggests that a similar process may occur in the brain [3,11]. The present study provides supportive behavioral evidence that prostaglandins serve as neuromodulators in the CNS and that altering the concentration of these agents influences a behavioral response to alcohols but not to other sedative hypnotics.

METHOD

Animals

Adult male HS/Ibg mice (60–100 days old) were randomly divided into the various control and drug-treated groups and were tested between 1300 and 1600 hr. Animals were housed in litter groups on wood shavings on a 12:12 light-dark cycle with free access to food and water.

[1]This work was supported by NIAAA research grant AA-03527, by NIGMS training grant GM-07305, and by a Research Scientist Development Award (AA-00029) to A. C. Collins. Indomethacin was obtained as a gift from Merck and Company, Inc. Flufenamic acid and mefenamic acid were a gift from Parke-Davis. We thank Debra S. Hood for technical assistance.

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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 the injection of a 20 mg/kg dose in a volume of 0.01 ml/g body weight. Aspirin, 3.0 mg/ml, was dissolved in warm water and cooled to room temperature before injection of 0.015 ml/g body weight. This resulted in a 45 mg/kg dose. Flufenamic acid, 5.0 mg/ml, was dissolved in a 1% polysorbate 80 solution prior to injection of 0.01 ml/g body weight (a dose of 50 mg/kg). Acetaminophen, 4.5 mg/ml, was dissolved in warm water and cooled to room temperature before injection of 0.01 ml/g body weight. This produced a 45 mg/kg dose. Mefenamic acid, 1.0 mg/ml, was dissolved in 1% polysorbate 80 and 1% ethanol at basic pH and buffered to pH 7.3 before injection of 0.01 ml/g body weight (a dose of 10 mg/kg). Additional doses of all drugs were made by dilution of the respective stock solution.

All animals treated with ethanol received a 3.6 g/kg dose, which was made by diluting 4.5 ml of absolute ethanol to 10 ml and injecting .01 ml/g body weight. Those animals which were treated with propanol received a 2.0 g/kg dose, while those treated with t-butyl alcohol (t-butanol) received a 1.8 g/kg dose. Both of these alcohols were prepared in a fashion similar to that described for ethanol. In those studies using pentobarbital, a 6.0 mg/ml solution of sodium pentobarbital in distilled water was prepared. Animals were injected with 0.01 ml/g body weight, which resulted in a 55.6 mg/kg dose. Chloral hydrate was dissolved in distilled water at a concentration of 35 mg/ml, and 0.01 ml/g was injected resulting in a 350 mg/kg dose.

In order to assess the effect of prostaglandin synthetase inhibition on sedative hypnotic-induced sleep time, animals were injected intraperitoneally with either a prostaglandin synthetase inhibitor or a control solution before contralateral injection of the sedative hypnotic drug. Indomethacin was injected 15 min, and aspirin 30 min before the sedative hypnotic. All other synthetase inhibitors were administered 45 min before the sedative hypnotic. Control animals for the indomethacin and mefenamic acid groups were injected with a 1% ethanol, 1% polysorbate 80 solution 15 min or 45 min before the sedative hypnotic, while aspirin and acetaminophen controls received a distilled water injection 30 min before drug treatment. Flufenamate controls received a 1% polysorbate 80 solution 45 min before drug treatment.

Testing Procedure

Following administration of the sedative hypnotic drug, animals were placed on their backs in a V-shaped trough. The duration of loss of the righting reflex (sleep time), an efficient measure of sedative hypnotic effect, was recorded. Animals were judged to be awake when they could right themselves three times in 30 sec. For the ethanol studies, a 40-μl blood sample was obtained at time of regaining the righting reflex by piercing the retro-orbital sinus with a capillary pipet. The sample was placed in a tube containing 0.96 ml of a 0.015% solution of isopropanol which served as an internal standard. The tubes were stoppered immediately and stored on ice until analyzed for their ethanol content.

The stoppered tubes containing the blood samples were incubated at 65°C for 15 min, at which time a 1.0 ml aliquot of head space gas was injected into a Beckman GC-45 gas chromatograph equipped with a 4 ft × 1/8 in Porapak Q column. Helium served as a carrier gas and had a flow rate of 55 ml/min. Air and hydrogen flow rates were 300 and 44 ml/min, respectively. The inlet temperature was maintained at 150°C, while column and detector temperatures were 140° and 195°C, respectively. Peak areas were computed by triangulation and compared with ethanol standards which were prepared and run daily. Under these conditions, a linear relationship existed between peak area and the amount of ethanol in standard solutions.

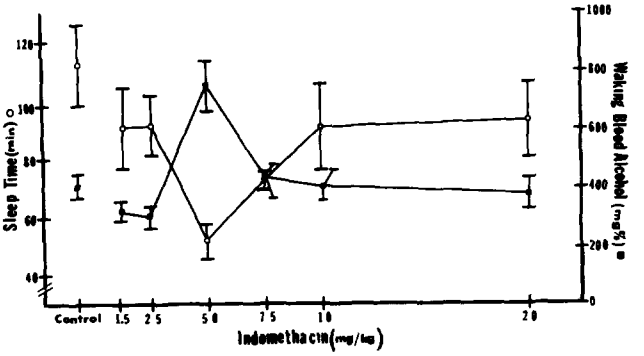

FIG. 1. Effects of indomethacin pretreatment on ethanol-induced sleep time, $F(6,28)=2.416$, $p<0.05$ and waking blood ethanol levels, $F(6,56)=6.671$, $p<0.0001$

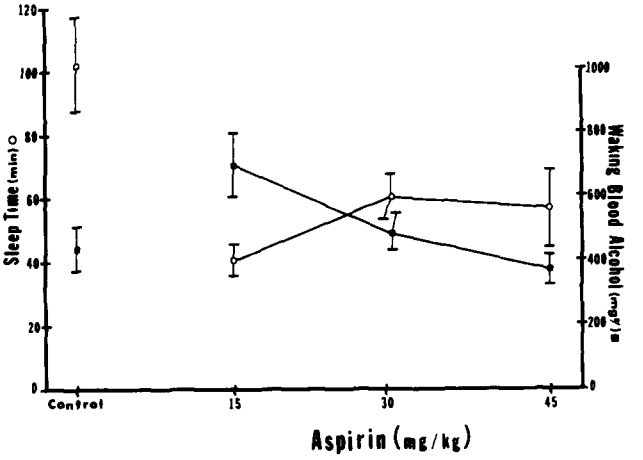

FIG 2. Effects of aspirin pretreatment on ethanol-induced sleep time, $F(3,30)=4.320$, $p<0.02$ and waking blood ethanol levels, $F(3,30)=3.196$, $p<0.05$

RESULTS

The effects of several doses of indomethacin on ethanol sleep time are shown in Fig. 1, along with mean waking blood ethanol concentrations. An overall decrease in sleep time was observed. The most significant effect was seen at the indomethacin dose of 5.0 mg/kg, where a 54% decrease in mean sleep time was observed. This alteration of sleep time is most likely attributable to a decrease in CNS sensitivity to the hypnotic actions of ethanol, since at the 5.0 mg/kg dose there was a significant increase in waking blood ethanol concentration. The animals treated with the 5.0 mg/kg dose of indomethacin regained the righting reflex at a blood ethanol concentration nearly double that seen in control animals. Thus, it appears that inhibition of prostaglandin production

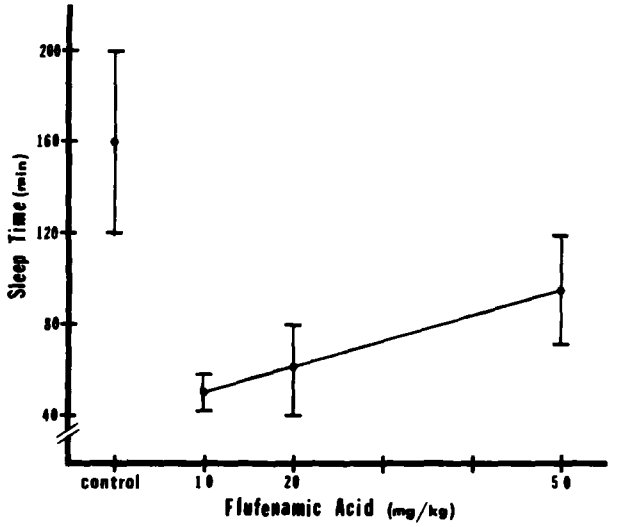

FIG. 3. Effects of flufenamic acid pretreatment on ethanol-induced sleep time, $F(3,16)=3.237$, $p<0.05$.

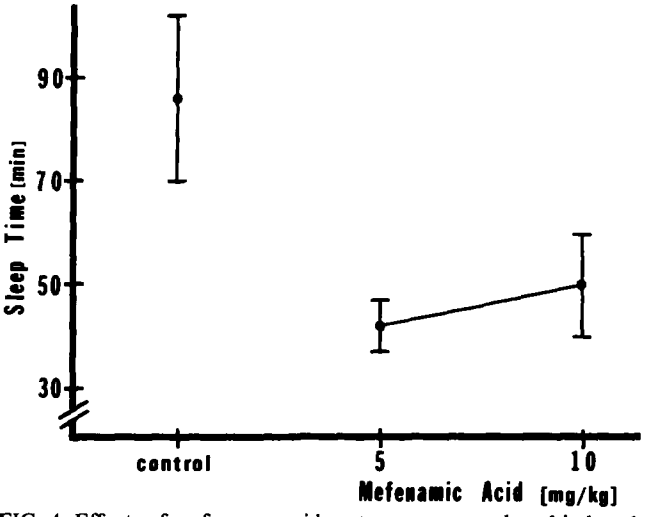

FIG. 4. Effects of mefenamic acid pretreatment on ethanol-induced sleep time, $F(2,22)=3.871$, $p<0.05$.

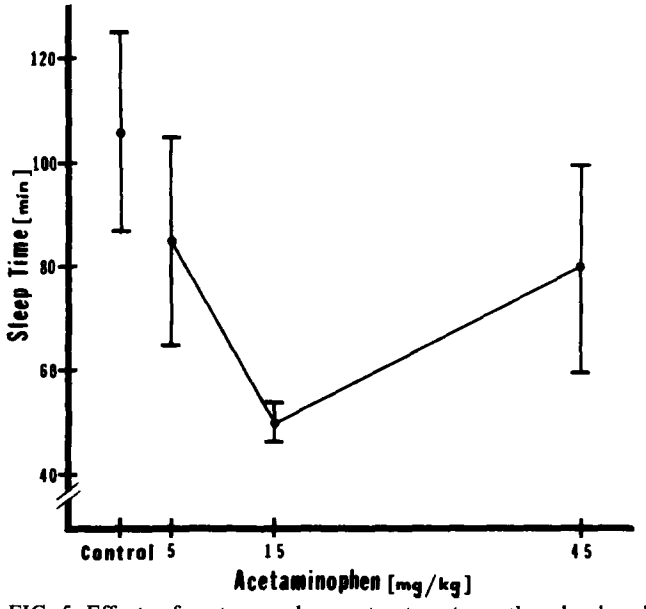

FIG. 5. Effects of acetaminophen pretreatment on ethanol-induced sleep time, $F(3,23)=1,376$, $p>0.1$. Although the overall test was not significant due to greater variability with this drug, an *a priori* hypothesis describing 15 mg/kg as the optimum dose was correct (*a priori* contrast, $t=3.050$, $p=0.006$).

has the potential for decreasing CNS sensitivity to the depressant actions of ethanol.

This conclusion is supported by the observation that another prostaglandin synthetase inhibitor, aspirin, also caused an overall decrease in duration of ethanol-induced sleep time (see Fig. 2). The greatest effect occurred at a dose of 15 mg/kg, where a 60% reduction in sleep time was observed. As with indomethacin, a biphasic response was seen in that higher doses had no significant effect. The decrease in sleep time was accompanied by a significant elevation in waking blood ethanol level.

We subsequently tested the effects of three additional prostaglandin synthetase inhibitors—flufenamic acid, mefenamic acid, and acetaminophen—on ethanol-induced sleep time. These compounds differ in their ability to inhibit prostaglandin synthetase; their order of potency is indomethacin>mefenamic acid>flufenamic acid>aspirin~acetaminophen [6]. Effects of these drugs on ethanol-induced sleep time in HS mice are depicted in Fig. 3 (flufenamic acid), Fig. 4 (mefenamic acid), and Fig. 5 (acetaminophen).

The results obtained with the additional drugs, as well as with indomethacin and aspirin, are summarized in Table 1. All of these agents counteracted the depressant action of ethanol in a biphasic manner. An optimum range or dose was found for each drug, above and below which no effect was observed. This biphasic aspect of the dose-response curve was significant for all of the drugs tested. In addition, the effective range of each drug correlated perfectly with its potency for inhibition of prostaglandin synthetase. Waking blood ethanol levels for animals treated with flufenamic acid, mefenamic acid, and acetaminophen were also consistent with the hypothesis that these agents decrease CNS sensitivity to the depressant effects of ethanol.

Since all of these prostaglandin synthetase inhibitors appeared able to decrease CNS sensitivity to the depressant effects of ethanol, it seemed possible that inhibition of prostaglandin production might decrease CNS sensitivity to depressant drugs in general. It therefore was desirable to determine whether administration of a prostaglandin synthetase inhibitor would alter the depressant actions of other sedative hypnotics. First, the effects of three doses of indomethacin in sleep time induced by pentobarbital were assessed. The procedure was exactly the same as for the ethanol experiments. No effect was observed at any of the doses used (see Table 2). Similarly, as shown in Table 3, a 5.0 mg/kg indomethacin dose, chosen because it was the most effective in altering ethanol-induced sleep time, had no effect on sleep time induced by chloral hydrate (350 mg/kg). These data suggest that specific interactions may exist between alcohol and prostaglandin function in the CNS.

This conclusion is strengthened by the observation that inhibition of prostaglandin synthetase antagonizes the de-

TABLE 1

EFFECTS OF SEVERAL PROSTAGLANDIN SYNTHETASE INHIBITORS ON ETHANOL SLEEP TIME

Drug	Relative Potency	Optimum Dose (mg/kg)	Total N	Sleep Time (% Decrease from Control)
Indomethacin	I	5 0	75	54*
Mefenamic Acid	II	5.0–10.0	25	51*
Flufenamic Acid	III	10 0	20	68*
Aspirin	IV	15.0	34	61†
Acetaminophen	IV	15 0	27	52†

*$p<0$ 05
†$p<0$ 01.

TABLE 2

EFFECT OF INDOMETHACIN ON PENTOBARBITAL (55 6 mg/kg) SLEEP TIME*

Group	Dose (mg/kg)	N	Sleep time (min ± SE)
Control	Saline	8	90 ± 8.7
Indomethacin	2 5	8	106 ± 10 9
Indomethacin	5.0	8	111 ± 12 4
Indomethacin	7.5	8	100 ± 9.8

*F(3,28) = 0.804, n.s.

TABLE 3

EFFECT OF INDOMETHACIN ON CHLORAL HYDRATE (350 mg/kg) SLEEP TIME

Group	N	Sleep Time (min ± SE)
Saline control	6	68±7 0
Indomethacin (5 0 mg/kg)	6	62±6 4

TABLE 4

EFFECT OF INDOMETHACIN OR ASPIRIN ON PROPANOL (2 0 g/kg) SLEEP TIME*

Group	Dose (mg/kg)	N	Sleep Time (min ± SE)
Control	Saline	5	132±17 8
Indomethacin	5.0	5	67± 3 9
Aspirin	15.0	6	73±12 3

*F(2,13) = 11.45, p = 0.0014

TABLE 5

EFFECT OF ASPIRIN ON T-BUTANOL (1 8 g/kg) SLEEP TIME*

Group	N	Sleep Time (min ± SE)
Saline control	6	310± 3 1
Aspirin (15.0 mg/kg)	4	136±25 0

*F(1,8) = 74.12, $p<0.0001$

pressant effects of both propanol and t-butanol (Tables 4 and 5). The 5.0 mg/kg dose of indomethacin resulted in a 49% decrease in propanol sleep time. Similarly, aspirin at 15 mg/kg resulted in a 45% reduction in propanol-induced sleep time and a 56% reduction in sleep time induced by t-butanol. Thus, the effect of prostaglandin synthetase inhibitors on drug-induced hypnosis may be specific for alcohols.

DISCUSSION

The results obtained clearly demonstrate that prostaglandin synthetase inhibitors, at least at some doses, decrease sensitivity to the depressant actions of alcohols. This decreased sensitivity is clearly an altered CNS sensitivity since the animals regain the righting reflex at a higher blood alcohol concentration. The data obtained in these experiments also support the notion that prostaglandins are involved in regulating CNS function. If, as recently proposed [3], their role in the brain is similar to that observed in the peripheral nervous system (i.e., one of inhibiting transmitter release), the results of the present study suggest that ethanol and other alcohols may inhibit transmitter release via a mechanism involving prostaglandins. Collier *et al.* [4] have noted 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, it may be that ethanol decreases transmitter release by stimulating prostaglandin production. Inhibiting this effect could explain our observation that all prostaglandin synthetase inhibitors we studied antagonized the depressant effects of alcohols. The observation that depression caused by other sedative hypnotics was not affected by a synthetase inhibitor suggests that these agents induce their depressant effects in a manner different from that of alcohols. This agrees with results of previous research [7].

In view of the fact that relatively high doses of alcohols are required to elicit pharmacological effects, many investigators have suggested that alcohols exert their effects by a nonspecific interaction with biological membranes. Since prostaglandin synthetase is a membrane-bound enzyme complex, it may be that one of the results of interaction between alcohols and membranes is an activation of this

complex, which results in increased prostaglandin production and a subsequent decrease in presynaptic transmitter release.

The experiment carried out with t-butanol may have special significance. This compound may not be metabolized to an intermediate aldehyde as are ethanol and propanol. Since indomethacin antagonized the depressant actions of t-butanol, it seems likely that aldehyde metabolites are not involved in the interaction between prostaglandin synthetase inhibitors and alcohols.

A viable explanation for the observation that all of the prostaglandin synthetase inhibitors tested caused a reduction in sleep time at low doses but not at higher doses is not readily apparent. Since all of these drugs showed the same pattern with ethanol, it is tempting to speculate that this phenomenon, whatever its cause, might be characteristic of all prostaglandin synthetase inhibitors. Perhaps a modest reduction in prostaglandin production has an effect which serves to decrease ethanol's depressant actions, whereas a greater reduction results in other physiological changes which combat the initial effect. Alternatively, perhaps higher doses of the synthetase inhibitors affect another biochemical system in a way which opposes the prostaglandin effect. For example, high concentrations of indomethacin have been observed to inhibit prostaglandin stimulation of adenylate cyclase [12]. Studies are currently underway in our laboratory to investigate further the interactions between ethanol and prostaglandins.

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