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Involvement of the Serotonergic System in the Prolongation of Pentobarbital Sleeping Time Produced by Prostaglandin D_2[1]

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HOLLINGSWORTH, E. B. AND G. A. PATRICK. *Involvement of the serotonergic system in the prolongation of pentobarbital sleeping time produced by prostaglandin D_2.* PHARMACOL BIOCHEM BEHAV 22(3) 365–370, 1985.—In the present study, the depressant and sedative actions of prostaglandin D_2 (PGD_2) were investigated. Intravenous (IV) administration of PGD_2 produced a significant decrease in the spontaneous locomotor activity of mice from 1 to 15 minutes following injection. Prostaglandin D_2 was also able to potentiate pentobarbital sleeping time at doses of 0.4 and 4.0 mg/kg when administered intravenously. Distribution studies with ^{3}H-PGD_2 (6 μCi, 4 mg/kg) showed that only 0.04% of the tritium administered could be found in brain at 5 min after the injection, and that only 50% of this was parent ^{3}H-PGD_2. The role of the serotonergic neurotransmitter system in the depressant action of PGD_2 was investigated with drugs which modulate this system. The ability of PGD_2 to potentiate pentobarbital sleeping time was diminished by pretreatment with agents that reduce brain level or synthesis rate of serotonin. Such agents include para-chlorophenylalanine (PCPA), a tryptophan hydroxylase inhibitor, 5,7-dihydroxytryptamine (5,7-DHT), a neurotoxin with selectivity for serotonergic neurons, and quipazine, a serotonergic autoreceptor stimulant. On the other hand, pretreatment with 5-hydroxytryptophan (5-HTP), the precursor of serotonin, further enhanced the potentiation of pentobarbital sleeping time by PGD_2. These data suggest that the depressant actions of PGD_2 are linked to the serotonergic neurotransmitter system.

Prostaglandin D_2 Serotonin Depressants Sedation Pentobarbital Sleeping time Locomotor activity

PROSTAGLANDIN E_1, E_2, and E_3 injected into the cerebral ventricles of unanesthetized cats produced sedation, stupor and signs of catatonia [13]. It was later found that PGD_2 was the predominant prostaglandin in the brains of rats and mice and that PGD_2 and $PGF_{2\alpha}$ were the principle prostaglandins in the guinea pig brain [1,2]. These findings prompted the investigation of the depressant effects of PGD_2 in cats, monkeys and rats [20]. A decrease in spontaneous locomotor activity in rats, at doses which produced no impairment of neuromuscular coordination in the rotorod test in mice, was reported after treatment with this prostaglandin. Administration of PGD_2 did not prevent electroconvulsive shock or pentylenetetrazol-induced seizures in mice. Bradycardia, diarrhea, hyperthermia, sedation and catatonia were seen in cats given PGD_2. Electroencephalographic recordings during intravenous infusion of PGD_2 resulted in a high voltage, slow wave pattern which is a characteristic of slow wave sleep. There was a decrease in spontaneous locomotor activity and an increase in the number of sleep episodes in monkeys infused with PGD_2 [20]. The relative abundance of this prostaglandin in brain and its possible role in sedation, depression, or unconsciousness make investigation of its sedative effect worthy of study.

The main focus in the present investigation is the ability of PGD_2 to potentiate pentobarbital sleeping time and the interaction of this prostaglandin with the serotonergic system. An earlier observation that inhibition of serotonin synthesis by para-chlorophenylalanine (PCPA) or by destruction of the serotonin-containing neurons in the raphe nucleus resulted in insomnia in cats [17] prompted us to investigate the role that serotonin might play in the depressant action of PGD_2.

METHOD

Animals

Male CD/3 mice (Flow Laboratories, Dublin, VA, 20–40 g) were kept on a 12-hour light/dark cycle and allowed food and water ad lib. Each animal was used only once.

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TABLE 1

EFFECT OF PGD_2 ON LOCOMOTOR ACTIVITY IN MICE*

	Number of Photocell Interruptions During Certain Time Intervals†				
	0–1 min	1–5 min	5–10 min	10–15 min	15–30 min
PGD_2	23.0 ± 2.7‡	37.6 ± 6.3‡	26.2 ± 5.6†	22.7 ± 6.6‡	88.3 ± 12.7
Vehicle§	56.1 ± 12.2	88.2 ± 10.9	66.5 ± 13.2	65.2 ± 16.6	125.0 ± 18.3

*PDG_2 was given IV in a dose of 4 mg/kg; n=6 per experimental group.
†Results are means ± S.E.M.
‡Significantly different from vehicle control at $p<0.05$.
§Vehicle was 4% ethanol in saline.

Drugs and Drug Solutions

Prostaglandin D_2 was obtained from Upjohn Co. (Kalamazoo, MI). Parachlorophenylalanine, 5,7-dihydroxytryptamine, and 5-hydroxytryptophan were purchased from Sigma Chemical Co. (St. Louis, MO). Desipramine was donated by U.S.V. Pharmaceutical Corp. (Tuchaline, NY). Quipazine maleate was purchased from Packard Inst. Co., Inc. (Downers Grove, IL).

Sodium pentobarbital (J. T. Baker, Phillipsburg, NJ) was dissolved in 0.9% saline to yield a final concentration of 4 mg/ml. Prostaglandin D_2 (1 mg was dissolved in 100 μl of absolute ethanol. Aliquots of this PGD_2 stock solution were added to pentobarbital or saline to yield a final PGD_2 concentration of 0.4 mg/ml. Control animals received a 4% ethanol solution in saline (except during the pentobarbital dose-response experiments) with or without pentobarbital. The injection volume in all cases was 10 ml/kg. The dose of ethanol being injected IV into each animal was approximately 8 μl. The other drug preparations are described under their appropriate headings. They were dissolved in 0.9% saline unless otherwise noted.

Locomotor Activity Studies

The spontaneous locomotor activity of mice was determined by quantitating the number of interruptions of a photocell light beam (Autotron Inc., Danville, IL) in 13×7×3″ plastic cages. Mice interrupted the light beam which traversed the cage by ambulatory movement across it. Gross movement was measured cumulatively at various time intervals for 60 minutes after injection. Twelve mice were used for each dose with 2 per chamber. The mice were injected intravenously with saline-ethanol (control) or PGD_2 in saline-ethanol as described above.

^{3}H-Prostaglandin D_2 Distribution Studies

^{3}H-PGD_2 (6 μCi/mMole) plus unlabelled PGD_2 (4 mg/kg) were administered in saline in a total volume of 10 ml/kg. The animals were sacrificed 1 or 5 minutes after injection. The brain was divided down the midline fissure. One-half of the brain was oxidized using a Packard Oxidizer B306 (Downers Grove, IL). The radioactivity was collected in Monophase and measured using a Beckman L.S. 7000 scintillation counter (Fullerton, CA). The prostaglandins in the other half of the brain were extracted according to a conventional method [6]. This extract was chromatographed on a silica gel 60 plate and prostaglandins were visualized with iodine vapor. An aliquot of the ^{3}H-PGD_2 solution was co-chromatographed with the extracted samples. These methods were also performed on aliquots of serum from the animals. Portions of the hearts, livers and lungs, and aliquots of the serum were also carried through the oxidation procedures. The oxidation studies are reported as percent in each given tissue of the initial amount of radioactivity administered to the animal. The chromatography studies revealed the percentage of this tritium that had the same R_f value as the parent ^{3}H-PGD_2. This procedure permitted estimation of the quantity of parent PGD_2 present in each tissue.

Sleeping Times

A pentobarbital-PGD_2 solution or pentobarbital-ethanol solution (control) was administered IV. The dose of PGD_2 was 4 mg/kg except when determining the dose response relationship. The mice were placed on their backs after the injection and were watched continuously until they regained their righting reflex. The time point taken as the regaining of the righting reflex was when the mice turned over onto their stomachs and resisted being placed on their backs again. Sleeping time was measured as the time between injection and the mouse's regaining of the righting reflex, since animals lost their righting reflex almost immediately after the injection.

Neurotransmitter Modulation Studies

1. Fifty mg of parachlorophenylalanine was suspended in 10 ml saline with Tween-80 using a method reported previously [18]. This compound was administered at 150 mg/kg intraperitoneally (IP) two times per day for 3 days [26]. On the 4th day, the sleeping time studies were performed.
2. Quipazine maleate was administered IP, one-half hour prior to performing the sleeping time studies. The concentration of the solution was 5.0 mg of the quipazine salt per 10 ml saline [11].
3. 5-Hydroxytryptophan was dissolved in saline (25 mg/10 ml) and given IP (25 mg/kg) two hours prior to performing the sleeping time studies [22]. Carbidopa (50 mg/kg, IP) was given one-half hour before 5-HTP pretreatment.

Analysis of Serotonin in Whole Brain

Following decapitation, the whole brain of the mouse was rapidly removed, weighed and homogenized in ice cold 0.4 N perchloric acid. The homogenate was centrifuged at 10,000 g for 10 min, and the homogenization and centrifugation processes were repeated with the pellet. The two supernatant portions were combined, and the pH was adjusted to 6.7 to

TABLE 2

DISTRIBUTION OF ^{3}H-PGD_2 IN THE TISSUES FOLLOWING INJECTION

Tissue	Time after ^{3}H-PGD_2 (6 μCi, 4 mg/kg, IV) Administration: 1 min	5 min
	Amount of tritium in tissue × 100* / Amount of tritium injected	
Brain	0.069 ± 0.007	0.035 ± 0.004
Serum	13.4 ± 2.3	3.0 ± 1.1
Heart	0.47 ± 0.09	0.12 ± 0.04
Liver	28.2 ± 4.0	29.4 ± 1.5
Lung	1.71 ± 0.26	0.93 ± 0.05
	Amount of Parent ^{3}H-PGD_2 × 100 / Amount of tritium in tissue	
Brain	63.3 ± 3	50 ± 8
Serum	78 ± 4	44 ± 7

*n=2 for heart, liver and lung values at the 1 min time point, n=3 for all other values.

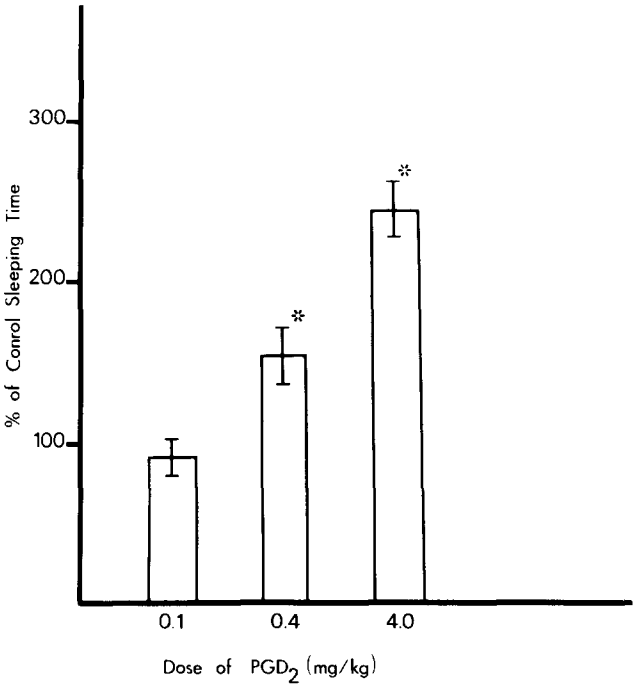

FIG. 1. The effect of PGD_2 on pentobarbital (40 mg/kg) sleeping time. Each bar represents the mean±S.E.M. percentage of a concomitantly tested control group (pentobarbital plus vehicle). (N=5 to 19). *Indicates significant deviation from appropriate control group. $p<0.05$.

6.9. The sample was then applied to a column of Amberlite CG-50 resin, from which the serotonin was eluted with 3N hydrochloric acid [5,8]. The quantity of serotonin in the sample was determined by fluorimetric analysis (Aminco-Bowman Spectrophotofluorometer; Silver Spring, MD) using an excitation wavelength of 305 nm and an emission wavelength of 540 nm [3]. Appropriate standards and tissue blanks were assayed concomitantly with the test samples.

Statistical Analysis

The locomotor activity study and serotonin brain levels were analyzed using a two-sided Student's *t*-test (unpaired groups). Analysis of Variance paired with a Bartlett's test followed by a multiple range *t*-test was used in analyzing the sleeping time studies. The null hypothesis was rejected for *p*-values less than 0.05 [30].

RESULTS

Locomotor Activity Studies

PGD_2 given alone decreased the locomotor activity of mice for approximately 15 minutes after the injection as shown in Table 1, which displays the number of photocell interruptions during certain time intervals. The depressant action of PGD_2 is present within the first 1 minute interval and seems to persist for at least 15 minutes after PGD_2 administration.

^{3}H-PGD_2 Distribution Studies

As shown in Table 2, one minute after animals were injected with ^{3}H-PGD_2 (4 mg/kg, 6 μCi, IV) 0.07% of the initial radioactivity injected into the animals was found in the brain. It was determined that 63% of this tritium (or 0.04% of the tritium injected) was parent compound, i.e., it had the same Rf value as ^{3}H-PGD_2 standard. One minute after the injection, there is approximately 116 ng of ^{3}H-PGD_2/g wet brain weight. Five minutes after the injection, only 0.04% of the initial radioactivity given to the animal is found in the brain. Fifty percent of this is the parent compound; therefore, at this time point the concentration of ^{3}H-PGD_2 is 44 ng/g wet brain weight. As shown in Table 2, at one and five minutes after sacrifice, 28% of the tritium label is found in the liver, 0.9–1.7% is found in the lung and 0.1–0.5% is found in the heart.

Sleeping Time Studies

Prostaglandin D_2 produces a dose-related potentiation of pentobarbital sleeping time (0.1, 0.4, 4.0 mg/kg) (Fig. 1). There is a significant potentiation ($p<0.05$) of sleeping times over control values at both 0.4 and 4.0 mg/kg. The remainder of the studies were performed at 4.0 mg/kg because this dose consistently produced a significant potentiation of sleeping time.

Neurotransmitter Modulation Studies

PCPA. After pretreating mice with this tryptophan hydroxylase inhibitor, there was a statistically significant decrease in the ability of PGD_2 to potentiate pentobarbital sleeping time (Fig. 2). There was no change in sleeping time between the pentobarbital-treated animals and those animals treated with PCPA and pentobarbital.

5-HTP. As can be seen in Fig. 3, pretreatment with this serotonin precursor results in a significant 19% increase in potentiation of pentobarbital sleeping time over the potentiation produced by PGD_2. 5-HTP pretreatment did not change the pentobarbital-induced sleeping times.

Quipazine. Quipazine caused a decrease in the potentiation of pentobarbital sleeping time by PGD_2, with a reduction

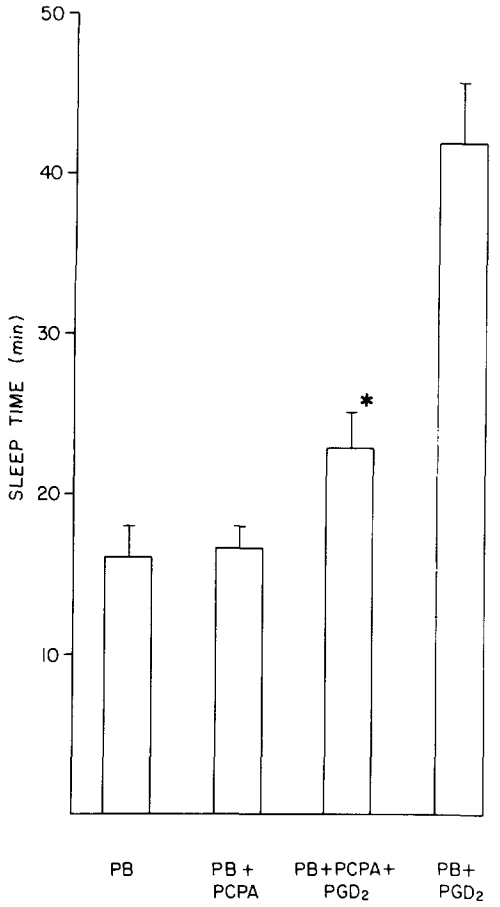

FIG. 2. The effect of PCPA pretreatment on pentobarbital (40 mg/kg) or pentobarbital plus PGD_2 (4 mg/kg) sleeping time. PCPA was administered (150 mg/kg, IP) two times per day for 3 days prior to the sleeping time studies. Results are means±S.E.M. (N=10 to 14). *Indicates significant deviation from other three treatment groups.

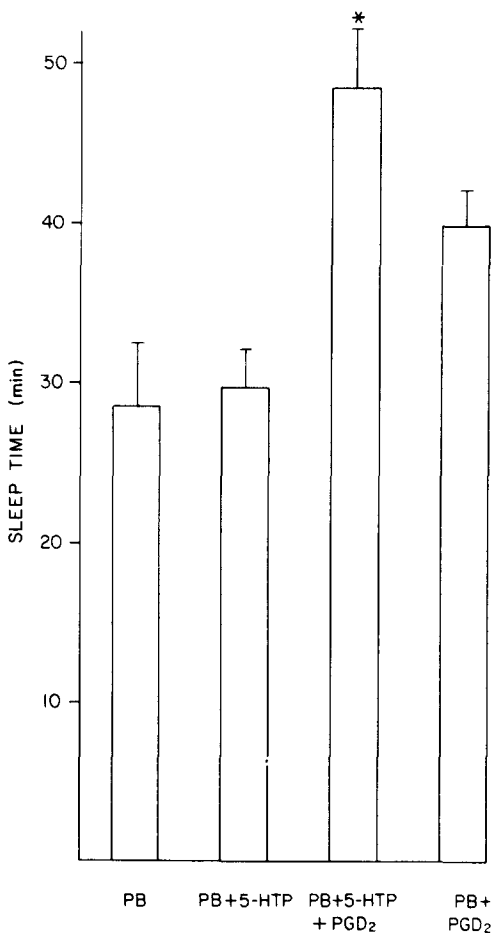

FIG. 3. The effect of 5-HTP pretreatment on pentobarbital (40 mg/kg) or pentobarbital plus PGD_2 (4 mg/kg) sleeping time. 5-HTP was administered (25 mg/kg, IP) two hours prior to performing the sleeping time studies. Results are means±S.E.M. (N=11 to 13). *Indicates significant deviation from other three treatment groups.

of mean sleeping times by more than 10 minutes ($p<0.05$; Fig. 4). Quipazine pretreatment alone produced a slight but not significant increase in pentobarbital sleeping time over control values.

Whole Brain Serotonin Levels

After pretreatment with PCPA there was a significant decrease in whole brain serotonin level while pretreatment with 5-HTP produced a significant increase in the level of serotonin (see Table 3).

DISCUSSION

In the present study, prostaglandin D_2 decreased the spontaneous locomotor activity of mice. On gross observation, the mice were not asleep, but rather their normal activity was depressed. The locomotor activity studies showed that there was a significant decrease in the activity of prostaglandin-treated animals. The difference between the activity values from 15 to 30 minutes after treatment was not significantly different for vehicle and PGD_2-treated groups; therefore, it seems that the depressant action of PGD_2 begins to subside by this time. Other investigators have also noted the ability of PGD_2 to decrease locomotor activity in the rat [20]. They reported a decrease to 42–60% of control values during the 15–30 minute time period [20]. A decrease in spontaneous locomotor activity has also been measured after the administration of PGE_1, E_2, or F_{2a} to rats and mice. PGE_2 appeared to be the most potent of the three in decreasing the locomotor activity [23,29].

Distribution studies with tritiated prostaglandin D_2 were performed to determine the amount of ^3H-PGD_2 which can be found in the brain at one and five minutes after IV administration of ^3H-PGD_2. The amount of parent ^3H-PGD_2 remaining in the serum during this time was also determined. Based on the distribution data using the tritium label, only around 44 ng/g (^3H-PGD_2/g wet brain weight) was present in the brain at 5 minutes after injection. In comparison, it has been reported that the maximum prostaglandin synthetic capacity of mouse brain homogenates is approximately 500 ng/g [1]. At one minute after the injection of ^3H-PGD_2, 78% of the tritium in the blood corresponded to the parent compound, while at 5 minutes after injection only 44% of the

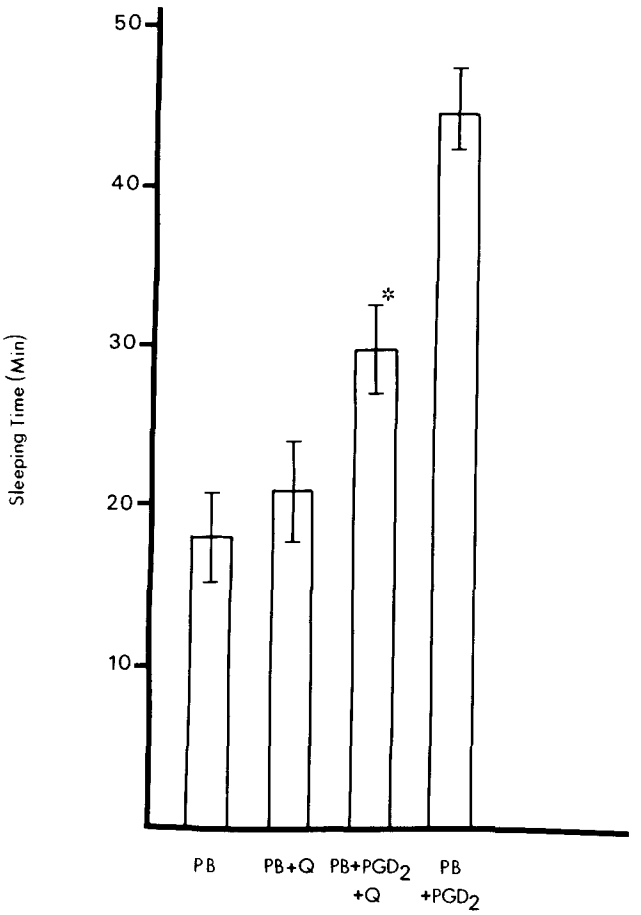

FIG. 4. The effect of quipazine pretreatment on pentobarbital (40 mg/kg) or pentobarbital plus PGD_2 (4 mg/kg) sleeping time. Quipazine was administered (5 mg/kg, IP) one-half hour prior to performing the sleeping time studies. Results are means±S.E.M. (N=12 to 16). *Indicates significant deviation from other three treatment groups.

labeled material was ^{3}H-PGD_2. The levels of ^{3}H-PGD_2 in both the brain and serum were decreasing with time. These studies support the contention that an IV injection of PGD_2 at a dose of 4 mg/kg does not produce an "exogenous prostaglandin" concentration in the brain exceeding that which brain homogenates are able to synthesize.

As a further measure of depressant activity, we examined the ability of PGD_2 to potentiate pentobarbital sleeping time. The dose response relationship in Fig. 1 exhibits a significant increase in pentobarbital sleeping time over control at doses of 0.4 and 4 mg/kg PGD_2 given IV. Many of the prostaglandins, such as PGE_1, F_{2a}, E_2, F_{1a}, A_1, A_2, and B_1, have been shown to potentiate pentobarbital sleeping time [13,25]. The remainder of the study was carried out using PGD_2 dose of 4 mg/kg, because this dose most consistently increased pentobarbital sleeping time.

PGD_2 alone has sedative properties, and it also exhibits this interesting potentiation of barbiturate sleeping time. In light of these facts, it was decided to examine the interaction of PGD_2 with the serotonergic neurotransmitter system, a system previously linked with sleep [17,19] and previously associated with the sedative action of other prostaglandins.

TABLE 3

EFFECT ON NEUROMODULATORS ON LEVEL OF SEROTONIN IN WHOLE BRAIN

Treatment	N	Serotonin Level (μg/g tissue)
Control*	9	0.47 ± 0.03
PCPA	6	0.27 ± 0.02†
5-HTP	6	0.57 ± 0.03†

*The appropriate vehicle was administered intraperitoneally.
†Significantly different from control at $p<0.05$.

For example, PGE_1 has been shown to cause sedation and increase 5-hydroxytryptamine turnover in rat brain [12]. The same investigators commented that their findings were consistent with the view that PGE_1 may elicit its sedative effect by increasing the rate of formation of a deaminated metabolite of 5-hydroxytryptamine [12]. Along with its sedative properties, it has been demonstrated that PGF_{2a} increased 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) levels in rat brain [24], but no causative relationship has been suggested. In order to examine the relationship between PGD_2 and serotonin, the mice were pretreated appropriately with drugs which affect the serotonergic system, and sleeping time studies were performed.

PCPA is a drug which inhibits tryptophan hydroxylase [16,21], the rate-limiting enzyme involved in the synthesis of serotonin. Inhibition of this enzyme decreases serotonin synthesis and lowers the endogenous level of serotonin [18,19]. It has been shown in the present study that PCPA-pretreatment dramatically decreased the ability of PGD_2 to potentiate barbiturate sleeping time, while having no effect on the control pentobarbital sleeping time. These data suggest that normal synthesis or levels of 5HT in brain may be necessary for the effect of PGD_2.

Administration of the serotonin precursor 5-hydroxytryptophan increases the rate of serotonin synthesis and the levels of serotonin in brain [4,28]. The serotonin initially formed from 5-HTP is not located in the neuron, but is believed to be extraneuronal [9]. The possibility still exists that this 5HT formed can act on the postsynaptic serotonergic receptors [9]. The animals pretreated with 5-HTP were also given carbidopa, a decarboxylase inhibitor that does not cross the blood-brain barrier. Cardidopa was given in order to minimize the use of 5-HTP in peripheral synthesis of serotonin and, therefore, to localize the action of 5-HTP mainly in the central nervous system. In the presence of 5-HTP and PGD_2 the pentobarbital sleeping time of the mice was significantly longer than the sleeping time of the pentobarbital-treated animals and also longer than that of the animals given pentobarbital and PGD_2. There was no difference between sleeping times of control mice and those pretreated with 5-HTP. Therefore, our data suggest that when the level of serotonin in the central nervous system (CNS) is increased, the sedative effect of PGD_2 is enhanced.

Quipazine has been reported to decrease release of 5HT [17] and the level of 5-HIAA, a metabolite of 5HT [15,27]. It has also been reported that quipazine acts presynaptically on the serotonergic neurons of the raphe nucleus to inhibit neuronal firing, which results in an accumulation of 5HT in the neuron [31]. Interestingly, quipazine pretreatment resulted in an attenuation of the ability of PGD_2 to potentiate

sleeping time. This suggests that the mechanism of PGD_2's potentiation could be related to increased release of serotonin, which quipazine inhibits.

PCPA and 5HTP pretreatment did produce significant changes in whole brain serotonin levels as shown in Table 3. Thus, the administration of these serotonergic modulators and the resultant effects on the potentiation of sleeping time produced by PGD_2 can be related to changes in whole brain serotonin levels.

It has been shown that the depressant activity produced by PGE_2 and $PGF\alpha$ can be blocked when animals are pretreated with the relatively specific serotonergic neurotoxin 5,6-dihydroxytryptamine [7]. At the present time, definite conclusions cannot be made about the mechanism of the interaction of PGD_2 with the serotonergic system. However, prostaglandin D_2 does produce a significant depressant effect, both on locomotor activity and on pentobarbital-induced sleep, and the depressant action appears to be linked with serotonergic tone. Similar to the previously cited study utilizing 5,6-dihydroxytryptamine [7], drugs which decrease serotonin levels, synthesis, or neuronal firing also decreased the ability of PGD_2 to potentiate pentobarbital sleeping time. Pretreatment with a drug which increased serotonin levels increased the potentiation of pentobarbital sleeping time by PGD_2. Therefore, prostaglandin-induced depressant activity appears to be correlated with serotonergic neuronal activity, but more studies need to be performed to characterize this interaction.

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