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ACCELERATED COMMUNICATION

Immunization against Prostaglandins Reduces Δ^1-Tetrahydrocannabinol-Induced Catalepsy in Mice

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SUMMARY

Immunization of mice with a thyroglobulin-prostaglandin E_2 (PGE_2) conjugate produced animals with measurable blood levels of anti-PGE_2 antibodies. When these mice were challenged with Δ^1-tetrahydrocannabinol (THC) (20 mg/kg), they showed a greatly diminished cataleptic response as compared with control animals. This observation further supports a hypothesis on the mechanism of action of THC in which eicosanoids, such as PGE_2, are early mediators. Based on the likelihood that antibodies were not present in the central nervous system, it is suggested that the initial site of action of THC may be at one or more peripheral locations. The transport of peripheral PGE_2 or other eicosanoids to the brain would result in the eventual manifestation of THC action at this site.

For more than a decade, evidence has been gradually accumulating in support of a hypothesis on the mechanism of action of THC. This mechanism was derived from observations that THC is able to elevate eicosanoid levels in a variety of systems (1, 2). Evidence was reported that this effect seems to be a consequence of an increase in phospholipase activity, after THC exposure, that results in the release of arachidonic acid from its esterified pools (3). Other evidence in support of the mechanism has been reported in which inhibitors of eicosanoid synthesis have been shown to reduce specific effects of THC, such as hypotension in dogs (4) and the cataleptic response in mice (5). Finally, it has been demonstrated that the direct administration of specific eicosanoids such as PGE_2 (5, 6) or PGI_2 (6) to mice results in a cataleptic state resembling that induced by THC.

Although the above reports all point to a role for eicosanoids as mediators of THC action, other aspects of the mechanism have not been elucidated to the same degree. For example, the question of the identity, or even the existence, of a THC receptor remains very much unresolved (2). It has not even been shown whether each of the several effects of THC has either its own mechanism and receptor or whether a single mechanism is responsible for the known pharmacodynamic properties of this drug. There is also a school of thought that holds that THC exerts its major effects by modifying membrane physical properties, especially in regions close to various enzymes and hormone receptors. This latter possibility is, in fact, supported by a substantial body of experimental evidence (2).

As expected, most efforts at identifying the site of action of THC have been focussed on the brain. This is based on the well known psychoactive effects of THC and a number of reports on its ability to affect neurotransmitter systems in the central nervous system (7). In actual fact, only a small fraction of THC administered peripherally reaches the brain and the kinetics of this process do not seem to match the behavioral responses (8), although an explanation for this latter phenomenon based on compartmental models has been reported (9). Moreover, as mentioned above, peripherally administered PGE_2 produces a cataleptic response in the mouse that has a dose-response curve similar to that for THC but showing greater potency (10).

The present report provides further evidence that eicosanoids are mediators of THC action, in particular the cataleptic response in the mouse. An immunological approach has been utilized by us to provide an independent line of evidence from that which has thus far been published. Furthermore, the possibility is raised that the initial site of action in this model of THC action may not be in the central nervous system but may, in fact, be peripheral in nature.

Materials and Methods

Chemicals. THC was supplied by the National Institute on Drug Abuse (Rockville, MD) and its purity was monitored by C-18 reverse phase liquid chromatography. Radiolabeled [^{14}C]THC was obtained from the same source and radiochemical purity was determined by

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ABBREVIATIONS: THC, Δ^1-tetrahydrocannabinol; PG, prostaglandin.

isotopic dilution and radiochromatography. PGE_2 and $PGF_{2\alpha}$ were gifts from the Upjohn Company (Kalamazoo, MI). Water-soluble carbodiimide (1-ethyl-3[3-dimethyl aminopropyl]-carbodiimide) and bovine thyroglobulin were purchased from Sigma Chemicals (St. Louis, MO).

Preparation of the antigens. Type I bovine thyroglobulin (500 mg) was dissolved in 0.1% $NaHCO_3$ solution (50 ml) and then the pH was adjusted to 5.5 with dilute HCl. The carbodiimide (150 mg) was slowly added to the stirred solution of thyroglobulin at room temperature. After 5 min, a dimethylformamide solution of PGE_2 (120 mg in 5 ml) was added dropwise over a period of 30 min while the pH was maintained at 5.5. This mixture was then allowed to react for 18 hr at 5°, at which time it was exhaustively dialyzed against phosphate-buffered saline (pH 7.6) through a collodion membrane. An identical procedure was followed for the preparation of thyroglobulin-$PGF_{2\alpha}$.

Immunization procedure. The antigen (1.0 ml containing approximately 10 mg of conjugate) was diluted with Freund's complete adjuvant (9.0 ml) for the initial injection or Freund's incomplete adjuvant (9.0 ml) for the booster injections. Groups of 10 female CD-1 mice (Charles River) were injected with the three antigens (total volume, 0.1 ml) at three subcutaneous sites, followed by booster injections at biweekly intervals. Two days after each booster injection, blood was collected from the orbital sinus under ether anesthesia and 0.1 ml of plasma harvested for binding studies.

Binding was determined by diluting the plasma to 1.0 ml with radioimmunoassay buffer and adding [^{3}H]-PGE_2 (5000 dpm; specific activity, 160 Ci/mmol). The radioimmunoassay buffer consists of 0.04 M Trizma base (Sigma), pH 7.3, containing 9% NaCl, 1% gelatin, and 2% sodium azide. After equilibration for 3 hr, the free and bound fractions were separated by treatment with dextran-coated charcoal and duplicate samples of the bound were assayed by liquid scintillation counting. By this method, it was found that two or three booster injections were required to reach maximum blood levels of antibodies.

Catalepsy measurements. The cataleptic response was measured by means of the so-called "ring test" described by Pertwee (11). Mice are placed on a horizontal wire ring 5.5 cm in diameter, which is attached to a 16-cm vertical rod. The animals are placed so that the hind paws and fore paws are at opposite sides of the ring. It is important that the ambient temperature is maintained at 30° and the environment is free of auditory stimuli and bright lights. The criteria for immobility are detailed in Ref. 11. The response is calculated as the fraction of time the mouse is immobile over a 5-min test period. Measurements were always done between 1 and 4 p.m. and the animals were used once. The observers were not aware of the immunization history of the mice, to prevent biasing of the data.

Results

Mice were actively immunized against eicosanoids and plasma samples obtained during the course of immunization were analyzed for their ability to bind PGE_2; the results are shown in Table 1. Whereas mice immunized with thyroglobulin, the protein carrier, showed little binding, those injected with the thyroglobulin-PGE_2 complex showed over 50% binding under identical conditions. Mice given a thyroglobulin-$PGF_{2\alpha}$ antigen displayed intermediate binding, suggesting that a considerable degree of cross-reactivity exists between prostaglandins. It was impossible to obtain complete antibody titer data because of the very limited amount of blood available.

Table 2 shows the effects on the cataleptic response of immunization with the three antigens followed by a THC challenge. Mice immunized against PGE_2 responded only half as well as control animals, which were immunized with thyroglobulin. Interestingly, the mice treated with the thyroglobulin-$PGF_{2\alpha}$ antigen also showed a 50% decrease in catalepsy when challenged with THC, which may reflect the cross-reactivity seen in the binding data shown in Table 1. Previous studies in our laboratory on THC-induced catalepsy, measured exactly as in the present experiments, gave immobility indices of 32% for THC in naive mice; the vehicle control was 7% in this earlier study (6).

The effect of a PGE_2 challenge on catalepsy was also examined and the results are given in Table 3. As with the THC challenge shown in Table 2, the anti-PGE_2 mice responded only about half as well as the thyroglobulin controls. In this case, however, $PGF_{2\alpha}$-immunized animals did not show a statistically significant decrease in catalepsy. Our earlier study demonstrated that naive mice show 26% immobility when given PGE_2 under similar conditions (6). Radiolabeled PGE_2 was injected to determine whether significant amounts of this eicosanoid reach the brain. Tail vein injection of 1 μCi of [^{3}H] PGE_2 resulted in the uptake of 10,840 dpm, which appeared mainly as radioactivity tightly bound to brain tissue and which represents about 0.5% of the dose.

The plasma levels of PGE_2 and PGI_2 were measured in nonimmunized mice after THC exposure under conditions very similar to those used for the catalepsy measurements. Table 4 shows that PGI_2 levels are more responsive to THC than are

TABLE 1

Binding of PGE_2 to plasma from immunized mice

Plasma samples were obtained from mice 2 days after the third booster injection of antigen and their binding capacity for [^{3}H] PGE_2 was measured as described in Materials and Methods. Values are the means of 10 measurements ± standard deviation.

Antigen	PGE_2 bound	
	dpm	%
Thyroglobulin	364 ± 52	7.3
PGE_2-Thyroglobulin	2600 ± 788	53.2
$PGF_{2\alpha}$-Thyroglobulin	970 ± 704	19.4

TABLE 2

Effect of immunization on THC-induced catalepsy

Female, CD-1 mice that had been immunized as indicated were given THC (20 mg/kg) in peanut oil (50 μl) orally. One hour later, the cataleptic effect was measured using the procedure described in Materials and Methods. The number of mice in each group is shown in parentheses. Values are mean ± standard deviation.

	Cataleptic response		
	Thyroglobulin	Thyroglobulin-PGE_2	Thyroglobulin-$PGF_{2\alpha}$
	% immobility		
Experiment 1	30.9 ± 7.9 (8)	14.8 ± 11 (9)	14.7 ± 12 (8)
Experiment 2	41.5 ± 21 (6)	23.8 ± 10 (6)	14.8 ± 10 (6)
Mean	34.7 ± 16 (14)	18.4 ± 11 (15)[a]	14.7 ± 11 (15)[a]

[a] One-way ANOVA gave a p value of 0.0002 when compared with the thyroglobulin control value. Post hoc Fisher and Scheffe tests gave significance levels of 95% in each case.

TABLE 3

The effect of immunization on PGE_2-induced catalepsy

Female CD-1 mice that had been immunized as indicated were injected via the tail vein with PGE_2 (0.4 mg/kg) in saline (20 μl). Thirty minutes later, the cataleptic effect was measured using the procedure described in Materials and Methods. The number of mice in each group are shown in brackets. Values are mean ± standard deviation).

Cataleptic Response		
Thyroglobulin	Thyroglobulin-PGE_2	Thyroglobulin-$PGF_{2\alpha}$
	% immobility	
29.1 ± 7.5 (10)	13.0 ± 13 (10)[a]	21.4 ± 13 (9)[b]

[a] One-way ANOVA gave a p value of 0.022 when compared with the thyroglobulin control value. Post hoc Fisher and Scheffe tests gave a significance level of 95%.

[b] Not significantly different from the thyroglobulin control value.

TABLE 4

Plasma PG levels after THC

Female CD-1 mice, 20–25 g, were given vehicle (50 μl of peanut oil) or drugs in oil by mouth 15 min before bleeding from the orbital sinus. Blood (0.2 ml) was collected in tubes moistened with indomethacin solution and 0.1 ml of plasma was obtained for direct assay. Duplicate unextracted samples were analyzed using [^{125}I] radioimmunoassay kits (NEN Products) by the procedures included with the kits. The results are expressed as ng/ml of plasma, mean ± standard deviation. Numbers in parentheses are the number of mice in each group.

Treatment	PGE_2	PGI_2
	ng/ml of plasma	
Vehicle	6.5 ± 0.69 (10)	12.0 ± 0.73 (10)
THC (20 mg/kg)	7.0 ± 0.79 (10)	17.5 ± 1.7 (10)[a]
THC (40 mg/kg)	10.5 ± 1.4 (10)[b]	15.0 ± 1.5 (9)[a]
Indomethacin (10 mg/kg) then THC (40 mg/kg)	6.0 ± 0.56 (10)	12.0 ± 1.0 (10)

[a] $p = 0.01$ by ANOVA.
[b] $p = 0.0011$ by ANOVA.

TABLE 5

Tissue distribution of orally administered [^{14}C]THC in the mouse

Four female CD-1 mice weighing 20–25 g were given [^{14}C]THC (1 mg, 1.7 μCi) in peanut oil (50 μl) orally. The mice were killed at 1 hr and tissues were collected, homogenized, and extracted with ethyl acetate. Carbon-14 was analyzed by liquid scintillation counting. Values shown are the percentage of the dose given in the entire organ.

Tissue	Carbon-14 content
	% of dose
Liver	2.63 ± 0.54
Stomach	30.45 ± 8.32
Duodenum	2.99 ± 1.09
Jejunum	9.16 ± 3.23
Ileum	6.85 ± 4.35
Colon	0.15 ± 0.04
Kidney	0.19 ± 0.06
Lung	0.05 ± 0.01
Brain	0.04 ± 0.01

PGE_2 levels, exhibiting a 45.8% rise after the administration of 20 mg/kg THC. When the mice were pretreated with indomethacin followed by THC 30 min later, blood levels of PGs remained at the values of the vehicle controls.

The tissue distribution of cannabinoids in the mouse was examined with particular regard to the conditions of the other experiments done in this study. Table 5 shows that, when [^{14}C]THC was orally administered, the largest concentrations of radioactivity, as might be expected, are found in the gastrointestinal tract. Brain and lung contained very low levels of carbon-14, which were near the limits of detection. The values in the table represent total organ content of THC and its metabolites; however, chromatographic analysis showed that a large proportion of each extract was unchanged THC.

Discussion

The data presented in this report demonstrate that mice with elevated blood levels of antibodies to PGs show a reduced cataleptic response to THC. The specific nature of the effect was evidenced by the fact that mice immunized against the carrier, bovine thyroglobulin, gave a normal response to THC. The control thyroglobulin antigen was prepared by subjecting a sample of commercially available material to the conditions of the coupling procedure used to prepare the PG-thyroglobulin antigens. Thus, any modifications of the protein structure should be present in the control antigen as well.

Greater specificity, namely determination of a PG that might be shown to be the unique cataleptic mediator, could not be attained in these studies. Apparently there was sufficient cross-reactivity between the two classes of PGs, the E series and F series, that both groups of mice displayed a similar reduction in their responses to THC. Although some of the prior evidence suggests that PGE_2 could serve as the mediator of catalepsy (5), this point is not firmly established. Direct injection of PGs into mice peripherally showed that, whereas $PGF_{2\alpha}$ and PGE_1 were not active, both PGE_2 and PGI_2 were potent effectors of a cataleptic response (5, 6). In the present report, it has also been demonstrated that blood concentrations of both PGE_2 and PGI_2 are increased following THC exposure (Table 4). These measurements were made 15 min after THC administration because preliminary experiments showed that PGE_2 levels decline by 60 min, which is when the cataleptic effect reaches its maximum. These observations are all consistent with a mechanism in which THC causes an increase in free arachidonic acid, which could then lead to increased synthesis of one or more eicosanoids.

In addition to providing further support for the "prostaglandin hypothesis" on THC action, the data reported here raise the possibility that the initial site of action for THC may not be located in the central nervous system. This suggestion is based primarily on the fact that both thyroglobulins and immunoglobulins are generally believed not to be able to cross the blood-brain barrier. Therefore, if THC acts to stimulate eicosanoid synthesis in the central nervous system, the presence of antibodies in peripheral areas should have no significant effect on its pharmacological properties. The finding that immunized mice exhibit a decreased response to THC implies that the PGs originate from a peripheral site(s) and therefore that the drug is also acting outside of the central nervous system.

In connection with this question of peripheral versus central sites of action, it was of interest to determine how [^{14}C]THC is distributed in the mouse under the conditions used in the present study. The data in Table 5 show that very little of the drug reaches the brain; moreover, part of that found in brain may be due to vascular tissue, which would be included in this measurement. As expected, most of the radioactivity is found in the gastrointestinal tract, with lesser amounts at other peripheral sites. Gastric mucosal cells are certainly capable of producing PGs (12) and are thus a potential site for THC action. It is interesting to note in this connection that Fairbairn and Pickens (5) have suggested this possibility, based on other considerations.

THC may also stimulate eicosanoid synthesis in the central nervous system and, in fact, there is *in vitro* evidence that could support such a possibility (13, 14). However, it has not been demonstrated that this is an important process *in vivo*. Bhattacharya (15) reported that brain concentrations of PGE_2 in rats were increased following THC given intraperitoneally. It is, of course, entirely possible that the PGE_2 was of peripheral origin.

The conclusions that may be drawn from the data presented in this report are as follows. Eicosanoids, such as PGE_2 or PGI_2, are likely mediators of the THC-induced cataleptic response in mice. Also, it is conceivable, in light of the present evidence, that the initial site of action for THC in this model may not be in the central nervous system, as has been generally assumed. When combined with earlier reports, the evidence is now reasonably strong for a mechanism in which THC acts at

some peripheral site(s) to release eicosanoids, which then are transported to the brain where they interact with neurotransmitter systems to produce catalepsy and, perhaps, other pharmacological effects as well.

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