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IL-1β and TNFα Modulate Δ⁹-Tetrahydrocannabinol-Induced Catalepsy in Mice

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GIBERTINI, M., C. NEWTON, H. FRIEDMAN AND T. W. KLEIN. *IL-1β and TNFα modulate Δ⁹-tetrahydrocannabinol-induced catalepsy in mice.* PHARMACOL BIOCHEM BEHAV **50**(2) 141-146, 1995.—The role of the proinflammatory cytokines interleukin-1α (IL-1α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) in THC-induced catalepsy in mice was examined. Recombinant IL-1β (400 ng/mouse, IV) and TNFα (500 ng/mouse, IV) were effective in potentiating the cataleptic effect of low-dose THC (10 μg/mouse, IV). Recombinant IL-1α and IL-6 did not potentiate catalepsy at any dose tested. Anti-IL-1β and anti-TNFα antibodies were effective in attenuating high-dose (75 μg/mouse) THC-induced catalepsy. Antibodies to IL-1α and IL-6 had no effect on catalepsy. Early onset catalepsy (10 min postinjection) was potentiated by exogenous recombinant IL-1β and TNFα but only later catalepsy (2 h postinjection) was attenuated by antibodies to endogenous IL-1β or TNFα. This divergence of the cytokine effect suggests that these substances regulate, by different mechanisms, the early and late THC-induced cataleptic response.

THC Cytokines Catalepsy Mice IL-1β TNFα

THE MAJOR psychoactive cannabinoid of *Cannabis*, Δ⁹-tetrahydrocannabinol (THC), induces many well-characterized effects, including reductions in spontaneous activity, catalepsy, antinociception, hypothermia, and cognitive changes (26). Some of these effects are believed to be receptor mediated because a cannabiniod receptor has been described (16,28) and the distribution of these receptors in the brain is consistent with the observed behavorial and motor effects (14,27). In addition, an endogenous ligand for the cannabinoid receptor (arachidonylethanolamide or "anandamide") has recently been discovered, synthesized, and shown to have receptor binding, pharmacological, and behavioral activity consistent with cannabinoids (10,12,13).

We have been concerned with the effect of THC on immunity for many years (3,19,20,22,23,35,40,41). In particular, we have been involved in describing the role of drugs of abuse as cofactors in infectious disease susceptibility. During our investigations, we have made both formal and informal observations relevant to this discussion. In particular, we have informally observed that catalepsy appears more intense than usual when THC is injected during the course of a bacterial infection. And, conversely, it appears less intense than usual if sick animals receive anticytokine antibodies prior to THC injection. Also, we have shown THC increases circulating levels of TNFα and IL-6 in mice that were previously infected with gram-negative bacteria (21). Given that: 1) THC may directly influence circulating cytokine levels (21); 2) prostaglandins have been implicated in THC catalepsy (4); 3) prostaglandins are involved in the regulation of IL-1 and TNFα (1);, and 4) proinflammatory cytokines are CNS active and have wide-ranging behavioral effects (2,6,8,11,17,29,31), we wondered whether some part of the cataleptic response to THC is due to THC-induced disturbances in release of cytokines by cells of the immune system. Specifically, we hypothesized that peripherally administered exogenous recombinant IL-1α, IL-1β, IL-6, and TNFα would potentiate, and that administration of monoclonal antibodies to endogenous IL-1α, IL-1β, IL-6, and TNFα would attenuate, THC-induced catalepsy.

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METHOD

Animals

Female BALB/c mice (Harlan, Indianapolis, IN; and Jackson, Bar Harbor, ME) 7–9 weeks old were used in all experiments. Animals weighed 15–21 g and were housed in groups of 10 in an animal room with a normal 12L : 12D cycle and an ambient temperature of 20–22°C. Food and water were continuously available and animals were tested no sooner than 1 week and no later than 3 weeks after their arrival.

Chemicals

Δ^9-Tetrahydrocannabinol (THC) was provided in ethanol by the Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD. The ethanol was evaporated with a stream of nitrogen gas and the THC residue was then dissolved in dimethyl sulfoxide (DMSO) to a concentration of 50 mg/ml. This drug solution was further diluted in heat-inactivated mouse serum for injections that were given intravenously in volumes of 0.1 ml. Murine recombinant IL-1α, IL-1β, and TNFα were purchased from Genzyme (Cambridge, MA). IL-6 was purchased from R&D Systems (Minneapolis, MN). Hamster anti-murine IL-1α and IL-1β monoclonal antibodies were purchased from Genzyme. Rat anti-murine IL-6 and anti-murine TNFα monoclonal antibodies were purchased from Pharmingen (San Diego, CA).

Experimental Design

Three series of studies were conducted. In the first series of experiments (dosing series), animals were injected with varying doses of THC and observed for catalepsy over a 4-h period. This series established the dosing and timing parameters for all subsequent studies. In the second series (potentiation series), recombinant cytokines were administered IV to potentiate early onset THC-induced catalepsy. In the third series (attenuation series), monoclonal antibodies to the cytokines were administered IV to attenuate catalepsy over a 4-h period.

For the dosing series, animals were observed for catalepsy at 10, 60, 120, and 240 min after injection with THC. The 10-min time point was included after preliminary studies (data not shown) indicated that mice became cataleptic within a few minutes after IV injection. The inclusion of this measurement in subsequent studies allowed us to assess the effects of cytokines and their antibodies on very early onset catalepsy. The drug preparation was injected IV at four different doses (vehicle only, 10 μg, 50 μg, and 75 μg per mouse). Mice tried on higher doses exhibited catalepsy at a level that consistently exceeded the upper limits of the catalepsy measuring system (data not shown). Therefore, we selected 10 μg/mouse as the minimum and 75 μg/mouse as the maximum dose for subsequent studies.

For the potentiation series, the effect of cytokines on catalepsy was assessed by adding cytokines to the THC (10 μg/mouse) preparation. Animals were injected IV with the THC/cytokine preparation and were observed for catalepsy 10 min later. Cytokines were added to the THC preparation in the following doses: IL-1α and TNFα, 500ng/mouse; IL-1β and IL-6, 400 ng/mouse. IL-1α and TNFα were also tried in combination at doses of 100 and 150 ng/mouse, respectively.

For the attenuation series, the effect of anticytokine antibodies on catalepsy over a 4-h period was assessed by adding the antibodies to the THC (75 μg/mouse) preparation. Animals were injected with the THC/anticytokine preparation and were observed for catalepsy at 10, 60, 120, and 240 min postinjection. Two series of experiments were conducted. In the first series, anticytokines were given at a dose of 20 μg/mouse (except for anti-IL-1α, which was administered at a dose of 10 μg/mouse). In the second series, all anticytokines were given at 30 μg/mouse.

Control Groups

In preliminary studies (data not shown), the cataleptic effect, if any, of the cytokines, the anticytokines, and the DMSO/serum vehicle was tested against mice injected with pyrogen-free saline only. Also, the anticataleptic effect of antibodies of irrelevant specificity (rat or hamster anti-TNP IgG obtained from Pharmingen, San Diego, CA) was tested. No effects were observed for any of these treatments. It should be noted that IL-1 and possibly other cytokines produces behavioral changes generally subsumed under the rubric of "sickness behavior." Decreases in spontaneous locomotion (but not catalepsy) is a common observation. Behavioral changes after administration of exogenous cytokine generally are apparent after 30 min postinjection, peak at 2–4 h, and resolve after 8 h (7). Our measurement of catalepsy occurred once at 10 min postinjection of cytokine/THC. In addition, our catalepsy measuring system monitored nose, whisker, and tail twitches. These movements are notably absent during intense catalepsy but are quite apparent in sickness-related motionlessness. Therefore, cytokine-induced sickness behavior per se probably did not interfere with our measurement of catalepsy. THC plus vehicle then served as the only comparison group for all experiments.

Procedures

Animals were transported to the behavioral testing room 30 min prior to testing and were allowed to acclimate to the room undisturbed. The testing room was kept at 25°C and was used only for these experiments so that extraneous sound and changes in lighting could be minimized. A single experimenter administered the drugs and measured the catalepsy for all experiments. A collaborator prepared the drugs in coded vials and delivered them to the experimenter who was therefore unaware of the contents of any injection. Animals were tested in groups of three to five and a control group was included for every trial. All injections were given into a tail vein in volumes of 0.1 ml/mouse.

Animals were naive to THC and had not been used in any other experiments. During the time course experiments (dosing and attenuation series), the mice were kept in heated cages (35–37°C) to prevent changes in catalepsy as a result of THC-induced hypothermia (32). Experiments were conducted between 0800 and 1300 h.

Catalepsy Measurements

The cataleptic response was measured by the ring test (33). The apparatus for the test consisted of a wire ring (5.5 cm in diameter) fixed horizontally to a ring stand at a point 16 cm above the deck. The experimenter placed the mouse across the ring so that it was supported only by its front and rear paws. Its forepaws were placed over the ring and the rear paws were then placed on a diametrically opposite point on the ring. The number of seconds the mouse remained on the ring and motionless (except for movements associated with respiration)

were then recorded. The test was conducted for 5 min and the measure of catalepsy was taken as the percent of total time on the ring in which the mouse was immobile. This procedure is an adaptation of one of the standard tests for catalepsy (33,25) and has been used in many studies (34).

Data Analysis

One-way ANOVA was used to test for differences in catalepsy among THC-only control subjects across experiments. ANOVA results for the potentiation series indicated that no differences existed between the THC control groups from different trials run on different days, $F(5, 17) = 2.38$, NS. Repeated-measures ANOVA for the attenuation series also found no differences, $F(3, 83) = 2.70$, NS, across the THC-only control groups. Control groups were therefore pooled within each of these series.

For the potentiation series, two-tailed independent sample Student's t-test was used to test differences between individual treatments and their respective combined control group. For the time course studies of the attenuation series, two-way repeated-measures ANOVA was used to test differences between each treatment group and the control group over time. The effect of interest in this model is the interaction between group (THC vs. anticytokine) and the linear trend on the repeated factor (catalepsy measurements over time). A statistically significant interaction of this type indicates that the slopes of the linear trend lines are different, which would be the best indication that the rate of attenuation differed between the groups.

RESULTS

The time and dose dependency of THC-induced catalepsy was initially examined. These studies were undertaken because preliminary results suggested our method induced a state of catalepsy that was quantitatively and qualitatively different from that reported in the literature. Also, because the aim of this investigation was to potentiate and attenuate catalepsy through manipulation of circulating cytokines, we wished to establish THC doses that would produce minimal and maximal catalepsy. Figure 1 shows that the DMSO/serum vehicle had no cataleptic effect of its own. Further, there was minimal increase in catalepsy as a result of experience or "training" as evidenced by the consistently low catalepsy scores for vehicle at all time points. Figure 1 also shows that a THC dose of 10 μg/mouse produced a low level of catalepsy (30%) and that 75 μg/mouse produced a near maximal level of catalepsy (93%). The response to all doses was greatest at 10 min and abated slowly over the remaining observation period.

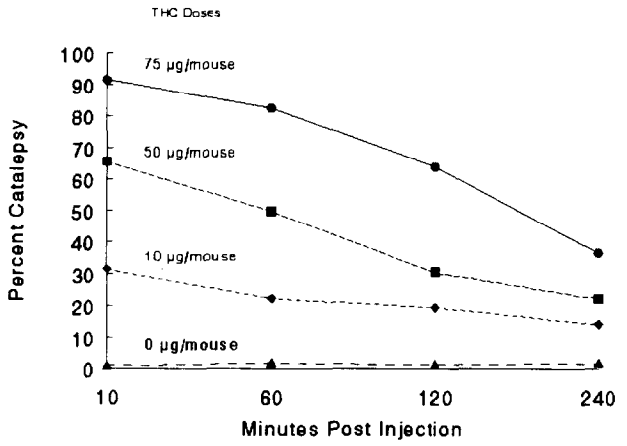

FIG. 1. Catalepsy over 4 h at varying doses of THC. Vehicle-only group (THC = 0 μg/mouse) received injection vehicle (DMSO and normal mouse serum) as described in the Method section. Percent catalepsy is the percent of total test time (5 min) during which the mouse was immobile.

TABLE 1

POTENTIATION OF EARLY ONSET THC-INDUCED CATALEPSY BY EXOGENOUS CYTOKINES

Cytokine	Dose	N	Mean*	SD	t
THC only	–	39	36.7	16.0	–
IL-1α	500 ng	4	39.1	7.9	0.3
IL-1β	400 ng	4	69.8	13.1	4.0†
IL-6	400 ng	6	21.6	10.8	−2.2
TNFα	500 ng	4	72.6	8.9	4.4†
IL-1β+ TNFα	100 ng 150 ng	10	61.6	12.1	4.5†

Data were evaluated by independent samples t-tests comparing the control group to the respective treatment group. Mice were injected IV with a combination of THC (10 μg/mouse) and the relevant cytokine as described in the Method section and were observed for catalepsy 10 min later.

*Percent of total test time (5 min) spent immobile.
†$p < 0.001$.

From the results in Fig. 1, a dose of 10 μg/mouse was selected to test the potentiating effect of cytokines on THC-induced catalepsy. Contrary to our initial hypothesis, only IL-1β and TNFα potentiated the cataleptic effect of THC at the 10-min time point (Table 1). IL-1α and IL-6 did not potentiate THC catalepsy. Also, synergy between IL-1β and TNFα was suggested by the finding that combining cytokines at low doses increased catalepsy at a level comparable to that produced by higher doses of either one administered singly. When these lower doses of IL-1β and TNFα were tried singly, no potentiation was observed (data not shown).

The results of experiments designed to test the attenuating effects of low-dose anticytokine antibodies on high-dose (75 μg/mouse) THC-induced catalepsy are presented in Table 2. This table shows that at the doses tested, only anti-IL-1β significantly decreased catalepsy, and then only after 2 h postinjection. Anti-IL-1α, anti-IL-6, and anti-TNFα had no effect on THC-induced catalepsy through the 240-min observation period. The highly significant F-value for IL-1β indicates that the rate of attenuation was significantly faster for the IL-1β group than that for the THC-only group.

Table 3 shows that with a higher dose (30 μg/mouse) of antibody, both anti-IL-1β and anti-TNFα were effective in attenuating catalepsy. Further, the trend lines for these two antibodies over the observation period were significantly different, $F(1, 55) = 30.21$, $p < 0.001$, indicating that anti-TNFα may be associated with more pronounced attenuation of THC catalepsy than is anti-IL-1β at this dose. Neither anti-IL-1α nor anti-IL-6 affected catalepsy (Table 3).

TABLE 2

ATTENUATION OF THC-INDUCED CATALEPSY OVER 4 H BY LOW-DOSE EXOGENOUS ANTICYTOKINES

Anticytokine	N	10 Min	60 Min	120 Min	240 Min	
THC only	27	91.5 (8.2)	82.6 (12.3)	64.0 (15.0)	36.7 (17.8)	–
Anti-IL-1α	8	94.2 (3.1)	86.8 (9.5)	66.7 (12.3)	23.5 (9.9)	5.07
Anti-IL-1β	9	92.9 (2.4)	79.5 (14.8)	43.7 (16.8)	12.7 (10.4)	19.77*
Anti-IL-6	8	94.6 (6.3)	82.2 (8.9)	56.6 (10.0)	32.8 (13.8)	1.54
Anti-TNFα	8	95.8 (3.4)	88.8 (9.5)	66.3 (11.9)	30.1 (15.1)	2.51

Values are mean with SD in parentheses. Mice were injected IV with a combination of THC (75 μg/mouse) and the relevant anticytokine as described in the Method section and were observed for catalepsy at 10, 60, 120, and 240 min postinjection. Doses: anti-IL1α, 10 μg/mouse; anit-IL1β, anti-IL6, and anti-TNFα; 20 μg/mouse. Means represent the percent of total test time (5 min) spent immobile.

*$p < 0.001$.

DISCUSSION

In this study, we observed catalepsy earlier and at lower doses than has been previously reported (5). It is possible that our technique of using DMSO as a drug vehicle and then mixing the THC/DMSO preparation with mouse serum for injection improves the bioavailability of THC and, therefore, penetration into central areas; however, this effect was not directly tested. The establishment of a THC dose that produced minimal catalepsy allowed us to test the possibility that coinjection of cytokines and THC potentiated catalepsy. Our results showed that IL-1α and IL-6 were not active in this regard; however, IL-1β and TNFα were capable of potentiating the THC effect. Furthermore, these two cytokines displayed synergy of potentiation, in that, a low-dose, combination injection of IL-1β and TNFα was much more effective than injection of either cytokine alone.

The mechanism of cytokine potentiation is not clear at this time. However, the rapid effect (i.e., within 10 min) suggests it possibly directly affects the blood-brain barrier (BBB), allowing for increased movement of THC from blood to brain and a quantitative increase in the amount of the drug reaching targets in the basal ganglia (15). Some reports have shown that cytokines or agents that induce them increase BBB permeability following systemic injection into rodents (18,24); however, other reports tend to discount this effect of circulating cytokines, emphasizing instead the importance of centrally produced cytokines (36). Cytokine effects on arachidonic acid metabolism by cells in the periphery, CNS, or BBB might also be involved because THC-induced catalepsy is attenuated by cyclooxygenase inhibitors (4), and prostaglandins and cytokines are known to interact in the periphery (1,38) and to influence CNS function (6,37).

We also examined the attenuation of THC-induced catalepsy by anticytokine antibodies. Neutralizing blood and tissue levels of either IL-1β or TNFα by the administration of monoclonal antibodies was shown to hasten the recovery from catalepsy, although it did not prevent early (10 min) catalepsy. Presumably, these antibodies do not easily cross the BBB, indicating that cytokines in the periphery may be more important in the persistence of the cataleptic effect rather than the initiation of catalepsy. The source and mechanism of action of the peripheral cytokine is not clear at this time. THC injections at the doses used in this study do not cause a detectable increase in serum cytokine levels (unpublished), but drug-induced increases in local areas such as the BBB cannot be ruled out. Increases of cytokines in the BBB would be expected to develop within hours, to be susceptible to neutralizing antibody in the blood, and to effect BBB permeability (18,29).

Although the current results tend to implicate IL-1β and TNFα in the cataleptic response to THC, caution must be exercised in interpreting the results. We do not know whether the cytokines act at the level of extrapyramidal motor system

TABLE 3

ATTENUATION OF THC-INDUCED CATALEPSY OVER 4 H BY HIGH-DOSE EXOGENOUS ANTICYTOKINES

Anticytokine	N	10 Min	60 Min	120 Min	240 Min	
THC only	11	97.0 (2.6)	87.6 (5.2)	60.3 (13.0)	27.1 (15.4)	–
Anti-IL-1α	7	98.7 (2.5)	89.8 (5.9)	65.8 (16.8)	25.7 (9.3)	0.08
Anti-IL-1β	7	95.9 (5.9)	81.4 (12.6)	50.0 (8.5)	11.1 (9.3)	4.92*
Anti-IL-6	7	97.8 (1.4)	96.0 (6.0)	63.0 (17.8)	33.0 (16.1)	0.12
Anti-TNFα	7	90.3 (6.1)	46.0 (20.7)	22.6 (13.6)	11.0 (9.3)	43.02*

Values are mean with SD in parentheses. Mice were injected IV with a combination of THC (75 μg/mouse) and the relevant anticytokine (30 μg/mouse) as described in the Method section and were observed for catalepsy at 10, 60, 120, and 240 min postinjection. Means represent the percent of total test time (5 min) spent immobile.

*$p < 0.001$.

or at some other point. It is possible, for example, that the different cytokines interacted with THC's hypothermic effect in different ways and the measurement of catalepsy was thereby differentially affected (39). We did not examine this possibility directly and it remains open as an alternative explanation of the results. Also, it should be stated that our results are limited to THC-induced catalepsy and may not be relevant to other types of catalepsy, any of which may have different neural substrates (9).

From a different perspective, the current results do suggest that cytokine mobilization can influence a highly specific, receptor-mediated motor disturbance. We believe this finding extends the literature on immune system/CNS communication as well as the literature on THC's many physiological effects.

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REFERENCES

1. Badger, A. M.; Olivera, D.; Talmadge, J. E.; Hanna, N. Protective effect of SK&F 86002, a novel dual inhibitor of arachidonic acid metabolism, in murine models of endotoxin shock: Inhibition of tumor necrosis factor as a possible mechanism of action. Circ. Shock 27:51-61; 1989.
2. Bianchi, M.; Sacerdote, P.; Locatelli, L.; Mantegazza, P.; Panerai, A. E. Corticotropin releasing hormone, interleukin-1-alpha, and tumor necrosis factor-alpha share characteristics of stress mediators. Brain Res. 546:139-142; 1991.
3. Blanchard, D. K.; Newton, C.; Klein, T. W.; Stewart, W. E.; Friedman, H. In vitro and in vivo suppressive effects of delta-9-tetrahydrocannabinol on interferon production by murine spleen cells. Int. J. Immunopharmacol. 8:819-824; 1986.
4. Burstein, S. H.; Hull, K.; Hunter, S. A.; Shilstone, J. Immunization against prostaglandins reduces delta-1-tetrahydrocannabinol-induced catalepsy in mice. Mol. Pharmacol. 35:6-9; 1989.
5. Compton, D. R.; Johnson, M. R.; Melvin, L. S.; Martin, B. R. Pharmacological profile of a series of bicyclic cannabinoid analogs: Classification as cannabimimetic agents. J. Pharmacol. Exp. Ther. 260:201-209; 1992.
6. Dantzer, R.; Bluthe, R.; Kelley, K. W. Androgen-dependent vasopressinergic neurotransmission attenuates interleukin-1-induced sickness behavior. Brain Res. 557:115-120; 1991.
7. Dantzer, R.; Bluthe, R. M.; Kent, S.; Kelly, K. W. Behavioral effects of cytokines. In: Rothwell, N.; Dantzer, R., eds. Interleukin-1 in the brain. New York: Pergamon Press; 1992.
8. del Cerro, S.; Borrell, J. Interleukin-1 affects the behavioral despair response in rats by an independent mechanism which requires endogenous CRF. Brain Res. 528:162-164; 1990.
9. De Ryck, M.; Schallert, T.; Teitelbaum, P. Morphine versus haloperidol catalepsy in the rat: A behavioral analysis of postural support mechanisms. Brain Res. 201:143-172; 1980.
10. Devane, W. A.; Hands, L.; Brewer, A.; Partway, R. G.; Stevenson, L. A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A.; Mechoulam, R. Isolation and structure of a brain constituent that binds to the cannabinoid receptor [see comments]. Science 258:1946-1949; 1992.
11. Dunn, A.; Antoon, M.; Chapman, Y. Reduction of exploratory behavior by intraperitoneal injection of interleukin-1 involves brain corticotropin-releasing factor. Brain Res. Bull. 26:539-542; 1991.
12. Felder, C. C.; Briley, E. M.; Axelrod, J.; Simpson, J. T.; Mackie, K.; Devane, W. A. Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. Proc. Natl. Acad. Sci. USA 90:7656-7660; 1993.
13. Fride, E.; Mechoulam, R. Pharmacological activity of the cannabinoid receptor agonist, anandamide, a brain constituent. Eur. J. Pharmacol. 231:313-314; 1993.
14. Herkenham, M. Cannabinoid receptor localization in brain: Relationship to motor and reward systems. Ann. NY Acad. Sci. 654: 19-32; 1992.
15. Herkenham, M.; Lynn, A. B.; De Costa, B. R.; Richfield, E. K. Neuronal localization of cannabinoid receptors in the basal ganglia of the rat. Brain Res. 547:267-274; 1991.
16. Howlett, A. C.; Bidaut-Russell, M.; Devane, W. A.; Melvin, L. S.; Johnson, M. R.; Herkenham, M. The cannabinoid receptor: Biochemical, anatomical and behavioral characterization. Trends Neurosci. 13:420-423; 1990.
17. Janz, L. J.; Brown, R.; Zuo, L.; Falk, J.; Greenberg, A. H.; Dyck, D. G. Conditioned taste aversion but not adrenal activity develops to ICV administration of interleukin-1 in rats. Physiol. Behav. 49:691-694; 1991.
18. Kim, K. S.; Wass, C. A.; Cross, A. S.; Opal, S. M. Modulation of blood-brain barrier permeability by tumor necrosis factor and antibody to tumor necrosis factor in the rat. Lymphokine Cyto. Res. 11:293-298; 1992.
19. Klein, T. W.; Kawakami, Y.; Newton, C.; Friedman, H. Marijuana components suppress induction and cytolytic function of murine cytotoxic T cells in vitro and in vivo. J. Toxicol. Environ. Health 32:465-477; 1991.
20. Klein, T. W.; Newton, C.; Friedman, H. Inhibition of natural killer cell function by marijuana components. J. Toxicol. Environ. Health 20:321-332; 1987.
21. Klein, T. W.; Newton, C.; Widen, R.; Friedman, H. Delta-9-THC injection induces cytokine-mediated mortality of mice infected with *Legionella pneumophila*. J. Pharmacol. Exp. Ther. 267:635-640; 1993.
22. Klein, T. W.; Newton, C.; Widen, R.; Friedman, H. Marijuana and bacterial infections. Adv. Exp. Med. Biol. 335:67-72; 1993.
23. Klein, T. W.; Newton, C. A.; Widen, R.; Friedman, H. The effect of delta-9-tetrahydrocannabinol and 11-hydroxy-delta-9-tetrahydrocannabinol on T-lymphocyte and B-lymphocyte mitogen responses. J. Immunopharmacol. 7:451-466; 1985.
24. Lustig, S.; Danenberg, H. D.; Kafri, Y.; Kobiler, D.; Ben-Nathan, D. Viral neuroinvasion and encephalitis induced by lipopolysaccharide and its mediators. J. Exp. Med. 176:707-712; 1992.
25. Martin, B. R.; Prescott, W. R.; Zhu, M. Quantitation of rodent catalepsy by a computer-imaging technique. Pharmacol. Biochem. Behav. 43:381-386; 1992.
26. Martin, B. R.; Compton, D. R.; Thomas, B. F.; Presecott, W. R.; Little, P. J.; Razdon, R. K.; Johnson, M. R.; Melvin, L. S.; Mechoulam, R.; Ward, S. J. Behavioral, biochemical, and molecular modeling evaluations of cannabinoid analogs. Pharmacol. Biochem. Behav. 40:471-475; 1991.
27. Matsuda, L. A.; Bonner, T. I.; Lolait, S. J. Localization of cannabinoid receptor mRNA in rat brain. J. Comp. Neurol. 327: 535-550; 1993.
28. Matsuda, L. A.; Lolait, S. J.; Brownstein, M. J.; Young, A. C.; Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA [see comments]. Nature 346:561-564; 1990.
29. Megyeri, P.; Abraham, C. S.; Temesvari, P.; Kovacs, J.; Vas, T.; Speer, C. P. Recombinant human tumor necrosis factor alpha constricts pial arterioles and increases blood-brain barrier permeability in newborn piglets. Neurosci. Lett. 148:137-140; 1992.
30. Opp, M. K.; Kapas, L.; Toth, L. A. Cytokine involvement in the regulation of sleep. Proc. Soc. Exp. Biol. Med. 201:16-27; 1992.
31. Otterness, I. G.; Seymour, P. A.; Golden, H. W.; Reynolds, J. A.; Daumy, G. O. The effects of continuous administration of murine interleukin-1-alpha in the rat. Physiol. Behav. 43:797-804; 1988.

32. Pertwee, R. G. The central neuropharmacology of psychotropic cannabinoids. Pharmacol. Ther. 36:189–261; 1988.
33. Pertwee, R. G. The ring test: A quantitative method for assessing the cataleptic effect of cannabis in mice. Br. J. Pharmacol. 46: 753–763; 1972.
34. Pertwee, R. G.; Ross, T. M. Drugs which stimulate or facilitate central cholinergic transmission interact synergistically with delta-9-THC to produce marked catalepsy in mice. Neuropharmacology 30:67–71; 1991.
35. Pross, S. H.; Nakano, Y.; Widen, R.; McHugh, S.; Newton, C. A.; Klein, T. W.; Friedman, H. Differing effects of delta-9-tetrahydrocannabinol (THC) on murine spleen cell populations dependent upon stimulators. Int. J. Immunopharmacol. 14:1019–1027; 1992.
36. Quagliarello, J. V.; WispElwey, B.; Long, W. J.; Scheld, W. M. Recombinant human interleukin-1 induces meningitis and blood-brain barrier injury in the rat. Characterization and comparison with tumor necrosis factor. J. Clin. Invest. 87:1360–1366; 1991.
37. Saper, C. B.; Breder, C. D. Endogenous pyrogens in the CNS: Role in the febrile response. Prog. Brain Res. 93:419–428; 1992.
38. Scales, W. E.; Chensue, S. W.; Otterness, I.; Kunkel, S. L. Regulation of monokine gene expression: Prostaglandin E2 suppresses tumor necrosis factor but not interleukin-1 alpha or beta mRNA and cell-associated bioactivity. J. Leukoc. Biol. 45:416–421; 1989.
39. Schallert, T.; Whishaw, I. Q.; De Ryck, M.; Teitelbaum, P. The postures of catecholamine-depletion catalepsy: Their possible adaptive value in thermoregulation. Physiol. Behav. 21:817–820; 1978.
40. Specter, S. C.; Klein, T. W.; Newton, C.; Mondragon, M.; Widen, R.; Friedman, H. Marijuana effects on immunity: Suppression of human natural killer cell activity of delta-9-tetrahydrocannabinol. Int. J. Immunopharmacol. 8:741–745; 1986.
41. Zhu, W.; Igarashi, T.; Qi, Z. T.; Newton, C.; Widen, R. E.; Friedman, H.; Klein, T. W. Delta-9-Tetrahydrocannabinol (THC) decreases the number of high and intermediate affinity IL-2 receptors of the IL-2 dependent cell line NKB61A2. Int. J. Immunopharmacol. 15:401–408; 1993.