Research report

Intracerebral administration of *Mycoplasma fermentans* produces sickness behavior: role of prostaglandins

Raz Yirmiya a,*, Ohr Barak a, Ronit Avitsur a, Ruth Gallily b, Joseph Weidenfeld c

a Department of Psychology, The Hebrew University of Jerusalem, Mount Scopus, Jerusalem 91905, Israel
b The Lautenberg Center for General and Tumor Immunology, Hebrew University-Hadassah Medical School, Jerusalem, Israel
c Department of Neurology, Hadassah University Hospital, Jerusalem, Israel

Accepted 22 October 1996

Abstract

Mycoplasmas are small microorganisms, which cause various diseases in animals and in humans, activate the immune system, and induce the release of various cytokines. Some of the effects of mycoplasmas are mediated by the CNS. Moreover, *Mycoplasma fermentans* (MF) has recently been found in the brain, as well as other tissues of some AIDS patients, who usually display severe neurobehavioral disturbances. The present study was designed to examine the behavioral effects of central administration of MF, and the role of prostaglandins in mediating these effects. In one set of experiments, rats were injected intracerebroventricularly (i.c.v.) with either saline or a dose of MF (5.1–36 μg per rat), and several behavioral parameters were measured. In addition, body temperature and locomotor activity were continuously monitored by a biotelemetric system. MF induced a significant elevation in body temperature and suppression of motor activity levels. MF also significantly reduced the time spent in social exploration, decreased locomotor and exploratory activity in the open field test, suppressed the consumption of food and saccharine solution, and reduced body weight. In a second set of experiments, i.c.v. administration of MF (7.2 μg) was found to produce a significant increase in the production of prostaglandin E2 (PGE2) in hypothalamic, hippocampal, and cortical tissues. This effect was blocked by indomethacin, a prostaglandin synthesis inhibitor. Indomethacin also attenuated the effects of MF on body temperature, motor activity and body weight, suggesting the involvement of prostaglandins in mediating some of the effects of MF. Together, these findings suggest that the presence of MF in the brain may be responsible for some of the neurobehavioral abnormalities in HIV-infected patients.

Keywords: Mycoplasma fermentans; Brain; Sickness; Behavior; Body temperature; Prostaglandins; AIDS

1. Introduction

Infectious diseases are accompanied by many behavioral, emotional, and cognitive changes. Alterations in activity levels, behavior in an open field, startle responses, and learning ability were reported in experimental animals following exposure to several infectious agents [11,17,18,44]. Administration of lipopolysaccharide (LPS), which is a product of the cell walls of gram negative bacteria, has also been shown to produce infection-like sickness behavior symptoms, including anorexia and decreased body weight [21,38], decreased locomotor and exploratory activity [12,56], slow wave sleep [25], suppression of social exploration [5], anhedonia [54], and altered pain sensitivity [31,56].

The behavioral effects of infection are mediated by cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNFα). These cytokines are synthesized both in the periphery and the brain, and affect neural and behavioral functions via specific receptors found in various brain structures [48]. Direct administration of cytokines, particularly TNFα and IL-1, either peripherally or into the cerebral ventricle, also produces marked behavioral effects, including a decrease in appetite [10,39], decreased motor activity [1,39], reduced exploratory behavior [45], depressed social activity [6,10], increased slow wave sleep [25], reduced libido and sexual activity in females [55], general malaise [46], and altered pain sensitivity [49]. Moreover, antibodies against particular cytokines, or cytokine antagonists attenuate or block the behavioral effects of immune activation [5,43].

Mycoplasmas are a group of eubacteria related to gram positive bacteria, and are considered as the smallest organisms capable of self-replication. These microorganisms lost
a marked part of their genome and as a result lost their cell wall in a process of degenerative evolution [41]. Mycoplasmas cause various diseases in animals and in humans, and can contribute to diseases produced by other infectious agents, particularly HIV [4]. Several recent studies demonstrated that products derived from different strains of mycoplasma, which are devoid of LPS, can modulate the activity of several components of the immune system [42]. In particular, exposure to several strains of mycoplasma, as well as components of mycoplasmas’ membrane, induce the production of several cytokines, particularly TNFα, IL-1, and IL-6, by macrophages or monocytes derived from either rodents or humans [13,14,34,35]. Mycoplasmas can also act as superantigens [9], which induce a massive T cell stimulation, and may be involved in autoimmune diseases in humans [53].

Some of the effects of mycoplasmas may be mediated within the central nervous system. Mycoplasmas have been shown to induce the production of cytokines and prosta-glandins not only by peripheral immune cells, but also by cultured astrocytes [7,8]. Moreover, central administration of heat-inactivated Mycoplasma fermentans (MF) has recently been shown to trigger a dose- and time-dependent increase in serum ACTH and corticosterone in mice. This effect was probably mediated within the brain, as complete deafferentation of the mediobasal hypothalamus inhibited the activation of the HPA by MF [51].

The present study was designed to examine the behavioral effects of central administration of MF. Because many of the central effects of pathogens and cytokines are mediated by prostaglandins [3,10,12,16,32,47] and because a membrane component of MF has previously been shown to induce in vitro production of prostaglandins by macrophages [7,35], we also examined the effects of MF on brain prostaglandins production in vivo, and the effects of a prostaglandins synthesis inhibitor, indomethacin, on MF-induced changes in body temperature and behavior.

2. Materials and methods

2.1. Subjects

Subjects were male Fisher 344 rats (Harlen–Sprague–Dawley, Jerusalem, Israel), 4 (Exp. 1) to 6 (Exp. 2–4) months-old. Animals were housed 3–4/cage in an air-conditioned room (23 ± 1°C), with food and water ad libitum for several weeks before the beginning of the experiment. All manipulations (i.e., injections and initial measurements) were conducted in the first half of the dark phase of a reversed 12 h light/dark cycle (lights off 07:00).

2.2. Mycoplasma fermentans (MF) preparation

MF (KL-4 strain, human isolate) (Batch 4/7/94) obtained from Dr. L. Olseon (FDA, Bethesda, MD, USA) was cultivated in Hayflick medium containing 20% (v/v) horse serum, 10% (v/v) fresh yeast extract, 2% (v/v) glucose, and penicillin-G (1000 U/ml). MF cells were isolated by centrifugation and heat inactivated at 60°C for 30 min. The final preparation was adjusted to contain 3.5 mg of mycoplasmal protein per ml, and was kept at −20°C until use. Before each experiment, the preparation was diluted in saline to get the appropriate protein concentrations for intracerebroventricular (i.c.v.) injections.

2.3. Stereotaxic surgery and i.c.v. injections

Rats were anesthetized with sodium pentobarbital (55 mg/kg, i.p.) and placed in a stereotaxic apparatus. A burr hole was drilled 1 mm posterior to bregma and 1.5 mm lateral to the midline, and a 26-gauge stainless-steel guide cannula (Plastics-One Inc.) was lowered 4 mm below skull surface. The tip of the guide cannula was positioned 1 mm above the lateral ventricle. The guide cannula was secured to the skull with three stainless-steel screws and dental cement, and was closed by a dummy cannula (Plastics-One Inc.). For several days before the experiment, rats were handled and habituated to the i.c.v. injection procedure, to minimize stress and discomfort during the experiment.

MF preparation was infused in a volume of 20 μl into the lateral ventricle through a 33-gauge stainless steel internal cannula (Plastics One Inc.), which was 1 mm longer than the guide cannula. Control animals were infused with saline (same volume). The internal cannula was connected to a micro-syringe pump (Harvard apparatus, South Natic, MA, USA) by a PE20 tube. Solutions were administered at a constant rate for 2 min. The injection cannula was removed 1 min following termination of the injection, to avoid spillage from the guide cannula.

The location of the i.c.v. cannulas was verified by injecting a dye (Trypan Blue) through a cannula at the end of the experiment. Brains were removed, cut with a scalpel, and the spread of the dye within the ventricles was examined. Six animals with a misplaced cannula were excluded from data analysis.

2.4. Biotelemetric measurements of activity and body temperature

Activity and body temperature (BT) were measured using battery-operated biotelemetric transmitters (model VM-FH, Mini Mitter Co. Inc., Sunriver, OR, USA) implanted in the peritoneal cavity, as previously described [57]. Output was monitored by a receiver board (model RA-1010) placed under each animal’s cage and fed into a peripheral processor (BCM100) connected to a personal computer. Activity was measured by detecting changes in signal strength that occur as the animal moves about its cage. The number of pulses that were generated by the transmitter was proportional to the distance the animal moved. The cumulative number of pulses generated every
10 min of measuring was recorded. BT was detected by a sensor imbedded in the transmitter and recorded at 10 min intervals.

2.5. Determination of prostaglandin \( E_2 \) (PGE\(_2\)) production

Ex vivo PGE\(_2\) production was determined as previously described [50]. In brief, rats were killed by decapitation 3 h following administration of either indomethacin (4 mg/kg) or vehicle, and their brains were rapidly removed and placed on ice-cold petri dishes. Slices were taken from the frontal cortex, hippocampus and hypothalamus (10–20 mg/slice). Two slices from each region were each placed in a tube containing 1 ml ice-cold oxygenated (95% \( O_2 \)-5% \( CO_2 \)) Krebs-Ringer buffer, pH 7.4. The supernatant was immediately decanted and replaced by 1 ml fresh buffer, and the tubes were tightly closed and placed in an incubator (37°C, 5% \( CO_2 \)) for 1 h. The supernatants were then removed and kept at –80°C until assayed for PGE\(_2\). The tissue was homogenized in 1 ml water, and protein was determined on an aliquot of the homogenate. The assay for determination of PGE\(_2\) was carried out in 0.01 M phosphate buffer, pH 7.4, using specific antibody against PGE\(_2\). (Bio-Yeda, Rehovot, Israel), which had cross-reactivity with other PGs of less than 1%. Tritiated PGE\(_2\) (100 Ci/mmol) was purchased from New England Nuclear (Boston, MA, USA). Bound and free fractions were separated by dextran-coated charcoal (Notir, Sigma), and radioactivity was counted in a liquid scintillation counter.

2.6. Procedure

2.6.1. Experiment 1: effects of MF on ingestive behavior, social exploration and behavior in the open field test

Baseline consumption of food, water and saccharin solution was measured 2 days before the experiment. Food consumption was measured by giving each rat 100.0 g of food pellets and weighing the remaining food 24 h later. Preliminary experiments showed that food spillage was negligible (less than 1% of the food consumed).

One day before the experiment, rats were weighed and baseline social exploration was assessed. Each rat was placed in a semicircular, transparent observation box and allowed 15 min for habituation, following which a male juvenile rat (26–30 days-old) was introduced. The time spent by the experimental rat in social exploration, consisting of ano-genital sniffing, body sniffing, and grooming of the juvenile, was measured during a 3-min period, using a computerized event recorder. Following this test, rats were divided into three subgroups \((n = 7–8)\) matched for mean baseline social exploration time, food consumption and body weight.

On the morning of the behavioral experiment day, rats were weighed and injected i.c.v. with MF at a dose of either 5.1 or 36 \( \mu \)g protein/rat (dissolved in 20 \( \mu \)l of saline), or with saline. Immediately following the injection, food was replaced with 100.0 g of fresh pellets for measurement of food consumption. Social exploration was assessed 2, 6 and 24 h after the injections, as described earlier. Activity in the Open Field test was assessed in the same rats 4 h after the injections. Each rat was placed in the corner of an open field (95 × 95 × 60 cm) divided into 25 identical squares. The incidence of line crossing with both hind paws, rearing, and grooming episodes was recorded by an observer blind to the treatment received by the animal, over a period of 3 min. Body weight and food consumption were measured 24 h after the injection. The results were analyzed by ANOVAs followed by post-hoc tests with the Fisher PLSD procedure.

2.6.2. Experiment 2: effects of MF on body temperature and activity measured with a biotelemetric system

Three weeks before the experiment, 24 rats were implanted with cannulae for i.c.v. injections and with intra-abdominal biotelemetric transmitters. One week following the operation, each rat was placed in an individual cage. During the next 6 days rats received daily handling and habituation to an i.c.v. injection procedure. On the next day, rats were transferred to the individual cages, and were habituated to saccharin drinking by allowing them to choose from two graduated tubes containing either water or 10 mM saccharin solution (Sigma, St. Louis, MO, USA). The amount of saccharin and water was recorded every 24 h for 2 days. Saccharin was not available during the day preceding the experiment (to prevent later association between its taste and the administration of MF).

On the experimental day, rats were divided into two groups, equated for mean body weight and consumption of food, water, and saccharin solution during the last 24 h of baseline measurement. Rats were injected with either saline or MF (7.2 \( \mu \)g/rat). Based on the results of Exp. 1, we expected that this dose will produce maximal behavioral responses (i.e., would be more effective than the lower dose in Exp. 1), without producing floor effects (which might have been caused by the higher dose in Exp. 1). Body weight was measured, and food (100 g) and drinking solutions (two tubes containing water and 10 mM saccharin solutions) were added before placing the cage on the receiver.

Body weight, the weight of food and the volume of the solutions consumed was measured 24 h later, and again 48 h after the i.c.v. administration. Body temperature and activity were monitored continuously as described above for 48 h following the injection. The results were analyzed by 2-way ANOVAs with the injection as a between subjects factor and the time as a repeated measures, within subject factor.

2.6.3. Experiment 3: effects of MF on PGE\(_2\) production in the brain

Three weeks before the experiment, rats were implanted with cannulae for i.c.v. injections. On the experiment day,
rats were divided into two groups (n = 7–9), injected with either saline or MF (7.2 μg), as described above. Three hours following the injection, rats were sacrificed by decapitation, brains were removed, and PGE₂ production in the hypothalamus, hippocampus and frontal cortex was determined, as described above.

In another experiment, we tested the inhibitory effect of indomethacin, a prostaglandins synthesis inhibitor, on MF-induced PGE₂ production in the brain. Two groups of rats (n = 5) were injected i.p. with indomethacin (4 mg/kg in a solution of 0.4 ml propylene glycol (PG)). Fifteen minutes later, rats were administered i.c.v. with either saline or MF (7.2 μg/kg). PGE₂ production in the hypothalamus, hippocampus, and frontal cortex was determined, as described above.

2.6.4. Experiment 4: effects of indomethacin on MF-induced fever and sickness behavior

The procedure for this experiment was similar to the procedure of Exp. 2, except for the following details. One day before the experimental day, the rats in their individual cages were placed on the receivers, and baseline body temperature and activity levels were measured. On the experiment day, rats were divided into four groups (n = 10–13), matched for mean body temperature, activity levels, body weight and consumption of food, water and saccharin solution. Rats within two of the groups were injected i.p. with indomethacin (4 mg/kg in a solution of 0.4 ml PG); the others with the vehicle (PG) only. Fifteen minutes later, rats within each one of these groups were administered i.c.v. with either MF (7.2 μg/rat) or saline. Body weight, the weight of the food and the volume of the solutions consumed were measured before, and 24 h later. Body temperature and activity were monitored continuously for 8 h following the injection, as described above.

The results of body temperature and activity levels were analyzed by 3-way ANOVAs with the first (PG/indomethacin) and second (saline/MF) injections as between subjects factors, and the time as a repeated measures, within subject factor. In addition, planned comparisons were used to examine separately the effect of MF in vehicle- and indomethacin-injected rats. The results of the body weight, and food and fluid consumption were analyzed by 2-way ANOVAs followed by post-hoc tests with the Fisher PLSD procedure.

3. Results

3.1. Experiment 1: effects of MF on ingestive behavior, social exploration and activity in the open field test

MF produced significant suppressive effects on both food consumption and body weight gain (F(2,18) = 62.04 and 20.97, respectively, P < 0.0001) (Fig. 1A,B, respectively). Post-hoc tests showed that compared to the saline-injected group, both the low and the high doses of MF significantly reduced food consumption and body weight. Significant differences between the groups were also found with respect to social exploration (F(2,19) = 5.92, P <
3.2. Experiment 2: effects of MF on body temperature, motor activity, and the consumption of food, water and saccharin solution

Body temperatures following either MF or saline administration are presented in Fig. 4A. Body temperature was elevated in rats injected with MF compared to saline-injected rats 2–5 h after the injection, and again 26–30 h following the injection (i.e., during the middle portion of the dark phase of the circadian cycle) (Fig. 4A). The febrile response was statistically significant only during the latter time period ($F(1,4) = 4.98, P < 0.05$). Motor activity was reduced in MF-injected rats throughout the measurement period (i.e., 1–48 h following the injection; $F(1,47) = 12.64, P < 0.01$) (Fig. 4B). MF-induced suppression of motor activity was particularly evident during the dark phase of the cycle both during the first day (1–8 h following the injection; $F(1,7) = 5.55, P < 0.05$), and the second day (20–32 h following the injection; $F(1,11) = 12.17, P < 0.01$) after the injection.

Fig. 3. Effects of MF on behavior of rats in the open field test. Rats were administered i.c.v. with either saline (Sal) or MF (5.1 or 36 μg/rat) and line crossing (A), rearing (B) and grooming (C) were assessed in the open field test 4 h later. Significantly different from saline-injected rats ($P < 0.05$). **Significantly different from rats injected with either saline or with the low dose of MF ($P < 0.05$).

Fig. 4. Effects of MF on body temperature and motor activity, measured continuously using a biotelemetric system. Five hours following lights off, rats were administered i.c.v. with either MF (7.2 μg/rat) or saline (Sal). (A) Mean (± S.E.M.) core body temperature (°C). Measurements were taken every 10 min, and averaged every 60 min, for clarity of presentation. (B) Mean (± S.E.M.) activity levels, measured by monitoring the cumulative activity counts in 60-min intervals.
**Fig. 5. Effects of MF on food consumption and body weight change.** (A) Mean (±S.E.M.) consumption of food during two consecutive 24 h periods following i.c.v. administration of either saline (Sal) or MF (7.2 μg/rat). (B) Mean (±S.E.M.) change in body weight during two consecutive 24 h periods following i.c.v. administration of either saline (Sal) or MF (5.1 or 36 μg/rat). *Significantly different from saline-injected controls (P < 0.05).

*MF* produced an overall significant reduction in food consumption (*F*(1,18) = 10.66, *P* < 0.01) (Fig. 5A). The interaction between the injection and time was also significant (*F*(1,18) = 4.68, *P* < 0.05), reflecting the recovery of food consumption during the second day after the injection. Post-hoc tests revealed that *MF*-induced suppression of food consumption was significant on the first day, but not the second day, after *MF* administration. Analysis of body weight changes revealed a significant interaction between the injection and the days (*F*(1,18) = 5.65, *P* < 0.05), reflecting the suppressive effects of *MF* on the first day after the injection and the full recovery of body weight on the following day (Fig. 5B). Post-hoc tests revealed that *MF*-induced reduction in body weight was significant on the first day, but not on the second day, after *MF* administration.

Saccharin consumption was significantly suppressed by *MF* (*F*(1,18) = 7.89, *P* < 0.05) (Fig. 6). Post-hoc tests showed that this suppression was significant on the first, but not on the second day after the injection. Water consumption was significantly increased by *MF* (*F*(1,18) = 6.47, *P* < 0.05). Post-hoc tests showed that this increase was significant both on the first and the second days after the injection. Analysis of the same data using saccharin preference (i.e., the amount of saccharin consumed/the amount of saccharin + water consumed) as the dependent measure, also revealed a significant suppressive effect of *MF* (*F*(1,18) = 7.96, *P* < 0.05). Post-hoc tests showed that *MF*-induced suppression of saccharin preference was significant both on the first and the second day after the injection.

**3.3. Experiment 3: effects of MF on PGE₂ production in the brain**

*MF* produced a significant increase in PGE₂ production (*F*(2, 69) = 9.74, *P* < 0.001) (Fig. 7). Planned comparisons demonstrated that this effect was significant in all three brain locations (*P* < 0.05). In contrast, there were no differences between *MF*- and saline-injected rats that were pretreated with indomethacin (Table 1), indicating that indomethacin blocked the increase in PGE₂ production following *MF* administration.

**Fig. 6. Effects of MF on consumption of saccharin solution and water.** Rats were administered i.c.v. with either saline or *MF* (7.2 μg/rat), and were immediately presented with two tubes, one containing saccharin solution (10 mM) and the other containing water. Fluid consumption (ml/24 h) was measured during two consecutive 24 h periods. * Significantly different from saline-injected controls (*P* < 0.05).

**Fig. 7. Effects of MF on prostaglandin E₂ (PGE-2) production by cortical, hippocampal and hypothalamic tissues.** Mean (±S.E.M.) levels of PGE₂ (pg/mg protein) secreted ex vivo over a period of 60 min, beginning 3 h following i.c.v. administration of either saline or *MF* (7.2 μg/rat). * Significantly different from saline-injected controls (*P* < 0.05).
Table 1
Effect of MF on prostaglandin E$_2$ (PGE$_2$) production in the brains of indomethacin-treated rats. Mean (± S.E.M.) levels of PGE$_2$ (pg/mg protein) secreted ex vivo by the hypothalamus, hippocampus and frontal cortex over a period of 60 min, beginning 3 h following i.c.v. administration of either saline MF (7.2 μg/rat)

<table>
<thead>
<tr>
<th></th>
<th>Hypothalamus</th>
<th>Hippocampus</th>
<th>Cortex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>42.8 (6.45)</td>
<td>53.4 (6.19)</td>
<td>49.5 (7.39)</td>
</tr>
<tr>
<td>MF</td>
<td>42.8 (6.45)</td>
<td>45.9 (3.68)</td>
<td>46.0 (6.37)</td>
</tr>
</tbody>
</table>

3.4. Experiment 4: effects of indomethacin on MF-induced fever and sickness behavior

Body temperature was significantly affected by MF administration over the period of 1.5–8 h after the injection ($F(1,32) = 4.47, P < 0.05$) (Fig. 8A). Planned comparisons revealed that MF significantly elevated body temperature in rats injected with vehicle ($F(1,32) = 7.42, P < 0.05$), but not in rats injected with indomethacin ($P > 0.1$). Activity levels during the same period were also significantly affected by MF ($F(1,33) = 8.03, P < 0.01$) (Fig. 8B). Planned comparisons showed that MF significantly reduced motor activity in rats injected with vehicle ($F(1,33) = 7.03, P < 0.05$), but not in rats injected with indomethacin ($P > 0.1$).

MF produced a significant reduction in both food consumption and body weight ($F(1,43) = 43.67$ and 32.22, Fig. 10).
respectively, $P < 0.0001$) (Fig. 9). A significant interaction between indomethacin and MF administration was found with respect to body weight change ($F(1,43) = 8.14$, $P < 0.01$), but not food consumption. Post-hoc tests showed that the groups injected with MF showed significant reduction of food consumption and body weight compared to the two control groups that were not injected with MF. The latter two groups were not significantly different from each other. Rats injected with indomethacin and MF lost significantly less weight than rats injected with vehicle only.

Consumption of saccharin solution was significantly reduced by MF ($F(1,42) = 12.65$, $P < 0.001$) (Fig. 10). There were no significant interactions between indomethacin and MF administration, and no significant effect of MF on water consumption. Post-hoc tests revealed that both vehicle- and indomethacin-injected rats that were injected with MF consumed less saccharin solution than the two control groups, that were not injected with MF.

4. Discussion

The results indicate that intracerebral administration of MF produces substantial behavioral alterations. The effects of MF on line crossing and rearing in the open field test may be viewed as a general depressive effect on locomotion as well as suppression of exploratory behavior. Motor activity was also found to be suppressed when measured by the biotelemetric system. The suppression of grooming by MF may reflect the reduction in self-care behaviors that is usually associated with illness [15]. Other symptoms of sickness produced by MF include the suppression of food and saccharin solution consumption, loss of body weight, and reduced social activity. The findings of decreased consumption of and preference for saccharin solution, but not water, indicate that the effects of MF are specific to the intake of palatable solutions, and do not result from a general depressive effect on drinking.

In addition to its behavioral effects, MF also produced fever. The results of Exp. 2 indicate that this effect was particularly evident during the dark, but not the light, period of the circadian cycle. In that experiment, MF produced only a small, and non-significant elevation of body temperature during the first few hours following the injection, and fever was fully developed only 24 h post-administration. In contrast, administration of MF in Exp. 4 did produce a significant elevation of body temperature during the first 8 h post-injection. The difference between the two experiments may have resulted from small differences in the injection time, 5 vs. 2 h following light off in Exp. 2 and 4, respectively. Another possibility is that the addition of a second injection in Exp. 4 (i.e., propylene glycol (PG) as the vehicle for indomethacin) somehow interacted with the MF administration and potentiated its effect on body temperature. The febrile response following MF administration is consistent with a previous report that MDHM, a macrophage-stimulatory product of MF, is pyrogenic in rabbits [35]. Analysis of the febrile and behavioral effects of MF indicates that these effects are not always correlated. Whereas fever was significantly increased only during the dark phase of the second day after the injection, activity levels were attenuated during the dark and the light periods of both the first and the second day. Moreover, the reduced consumption of food and saccharin solution induced by MF was evident during the first, but not the second day after the injection. Furthermore, indomethacin attenuated the fever, but not the suppression of ingestive behavior induced by MF. These findings demonstrate a dissociation of the febrile and behavioral effects of MF.

The behavioral and febrile effects obtained in the present study are similar to the effects observed with other pathogens and pathogen products, particularly endotoxin (LPS) [5,12,38,54,56]. LPS levels, measured by the LAL reagent (with a sensitivity of 0.1 pg/ml) indicated that a suspension of 10 μg MF protein/1 ml of various batches contained 30–99.8 pg LPS. Thus, an injection of 36 μg MF (the highest dose used in these experiments) would contain a maximum of 360 pg of LPS. We assessed that effects of i.c.v. administration of this dose of LPS in five rats, compared to a group of five rats injected with saline. Using the same methods described above, we found no differences between the two groups with respect to food consumption, body weight, social exploration and open field activity. These findings indicate that the results reported above are not influenced by endotoxin found within the MF preparate. This conclusion is further supported by a previous report, showing that i.c.v. administration of MF to LPS-resistant mice activated the adrenocortical response, while LPS was ineffective [51].

Recently we argued that exposure to pathogens and activation of the immune system produces a depressive-like episode in rats. This episode is characterized by anhedonia, i.e., a diminished capacity to experience pleasure [54], suppression of goal-motivated behaviors, including sexual activity, exploration and social activity [54], and other depressive-like behavioral symptoms, including deficits in escape in the learned helplessness paradigm [30]. The consumption of and preference for saccharin solutions provides a model for hedonic behavior, since animals will perform operant tasks to obtain saccharin as a rewarding stimulus, and will increase the consumption of and preference for solutions containing this non-nutritive compound [23,40]. Indeed, several previous studies have used this paradigm, describing a decrease in the consumption of and preference for saccharin and sucrose solutions in an animal model of depression (exposure to chronic mild unpredictable stress) [20,40]. We have recently demonstrated that LPS-induced suppression of saccharin preference was abolished by chronic, but not acute treatment with the tricyclic antidepressant imipramine [54]. Moreover, several other depressive-like symptoms produced by LPS, includ-
Various pathogen products. Syndrome is a general characteristic of illness produced by various pathogen products.

Central administration of MF produced a marked increase in PGE₂ production in the frontal cortex, hippocampus and hypothalamus. This finding is consistent with previous reports that addition of MF [7] or MF-derived high molecular weight material (MDHM) [35], increase the production of prostaglandins in cultured macrophages and astrocytes. Since cytokines, particularly IL-1, were previously found to increase the production of PGE₂ in the brain [22], it is likely that the effect of MF on PGE₂ production is mediated by MF-induced cytokine secretion.

Administration of indomethacin effectively blocked the effect of MF on brain PGE₂ production. Indomethacin also attenuated the effects of MF on body temperature, motor activity and body weight loss, but had no effect on MF-induced reduction in the consumption of food and saccharin solution. Prostaglandins have been implicated in mediating many of the central effects of pathogens and cytokines. In particular, the febrile response to endotoxin, IL-1 and to other cytokines is blocked by inhibitors of prostaglandin synthesis, including indomethacin [33]. Prostaglandins, particularly PGE₂, are also involved in mediating some, but not all, behavioral actions of cytokines, particularly IL-1. However, in contrast to the results of the present study, the anorexic effects of IL-1 were completely blocked by cyclooxygenase inhibitors, including indomethacin and ibuprofen [16,47], whereas the effects of IL-1 on exploratory behavior in the multicomartment chamber were not affected by indomethacin [12,45]. These differential findings related to indomethacin suggest that some of the behavioral changes induced by MF are not mediated by IL-1. The effects of indomethacin in the present study also indicate that the behavioral effects were not mediated by LPS, which may be present in the MF preparation, since the anorexic effect of LPS was blocked by cyclooxygenase inhibitors [3,32], whereas the effect of LPS on locomotor activity was not influenced by indomethacin [12].

Indomethacin partly attenuated the loss of body weight induced by MF. However, there was no effect of MF on food and fluid consumption. Thus, the attenuated body weight loss cannot be attributed to the lower ingestive behavior. The production of fever is associated with a dramatic increase in metabolic rate; the overall mean percentage increase in metabolism per 1°C of fever was estimated at 13% [15]. Therefore, the decrease in body weight may reflect the reduction of MF-induced fever by indomethacin. This hypothesis is supported by a recent study reporting that mice injected with LPS showed a greater loss of body weight than control mice, which were pair-fed the same amount of food consumed by the LPS-injected mice [24]. Together, these findings argue that prostaglandins are involved in mediating the effects of MF on body temperature, and therefore also affect indirectly the effect of MF on body weight. However, prostaglandins are not involved in mediating the effects of food and fluid consumption.

Several species of mycoplasma, including MF, M. penetrans, and M. pirum, have recently been isolated from many tissues, including the brain, of AIDS patients [4,26]. MF and M. penetrans have been suggested to be cofactors in the progression of HIV infection, possibly by enhancing the cytocidal effects of HIV-1 in CD4 + lymphocytes [4,27]. Infection with HIV is associated with many neurobehavioral changes, including anorexia and loss of body weight [52], psychiatric symptoms, particularly depression and anxiety [19], and cognitive impairments, e.g., psychomotor slowing and problems in attention and memory [2], which may progress to severe dementia [37]. The results of the present study suggest that some of the behavioral problems associated with AIDS may be produced by mycoplasmas, rather than by the HIV itself. The findings of severe neuropsychiatric abnormalities, in patients with very limited HIV burden [36], supports this hypothesis. Moreover, the findings that inoculation of MF in monkeys produced a wasting syndrome [28,29], and that MF is associated with other diseases [4], including an AIDS-like syndrome in a HIV-seronegative patient [29], suggest that MF can produce neuropsychiatric disturbances also in illnesses not related to HIV infection.

In conclusion, intracerebral administration of MF produces marked physiological and behavioral alterations, including an elevation of body temperature, decrease of body weight and ingestive behavior, and suppression of locomotor, exploratory, and social activity. MF also elevates brain PGE₂ levels, which mediate the febrile and some of the behavioral effects. These findings suggest that the presence of MF in the brain may be responsible for some of the neurobehavioral disturbances found in mycoplasma-associated diseases.

Acknowledgements

The authors thank Edna Cohen, Dorey Friedman, Roee Canaan, Yehuda Polak, and Porat Rosenberg, for their help in running the experiments. This work was supported by a grant from the Volkswagen Foundation (R.Y.).

References


