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We have examined experimentally the question of increased vulnerability to the thermalizing effects of MW exposure during febrile illness. In a controlled ambient temperature of 26 degrees C, autonomic mechanisms of heat production and heat loss were measured in febrile squirrel monkeys during 30-min exposures to 450 or 2450 MHz CW MW fields at different phases of the fever cycle (induction, plateau, defervescence). We have shown that MW energy absorbed during a febrile episode spares endogenous energy production, but may augment the fever if deposited deep in the body, as is the case during exposure at the resonant frequency. The fever may also be exacerbated if the MW exposure occurs late in the febrile episode, a condition that may put an organism at some risk, especially if the field strength
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Thermoregulatory Responses of Febrile Monkeys during Microwave Exposure[a]

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INTRODUCTION

Those who set safe exposure guidelines for human exposure to radio frequency (RF) and microwave (MW) fields often assume that the ill or febrile are more vulnerable to these fields than are healthy persons.[1,2] While our knowledge of human and animal thermoregulation during febrile episodes is extensive, no data exist to substantiate or refute the assumption of increased vulnerability during concomitant MW exposure. It is entirely possible that endotherms will use exogenous MW energy to generate a fever, thereby sparing endogenous metabolic energy. It is also possible that MW exposure during defervescence, when active heat loss responses are required, may overwhelm the thermoregulatory system, exacerbating the fever. The studies reported here are a first attempt to explore these possibilities by quantifying, in a nonhuman primate, the changes in autonomic mechanisms of heat production and heat loss when animals, made febrile via a controlled injection of PGE_1 in the medial preoptic nucleus of the hypothalamus (PO/AH), are exposed to controlled MW fields in controlled thermal environments.

The squirrel monkey is an appropriate primate model for the study of the origins and control of human febrile responses because it is susceptible to both the systemic and central administration of agents that cause fever in humans. Early studies[3] showed that these animals would generate high monophasic fevers to both intravenous and intracerebral injections of *S. typhosa* endotoxin; however, systemically induced fevers were characterized by long latencies and development of tolerance. Crawshaw and Stitt[4] generated controlled, reproducible fevers in squirrel monkeys by local microinjections of PGE_1 through cannulae implanted in the PO/AH. The magnitude of the fever, measured by the rise in colonic temperature (T_{co}), was dose dependent. In cool environments (T_a = 22° C), the fever was generated by increases in metabolic heat production (M) while in warmer

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T_a (32° C), the fever was produced by vasoconstriction of the tail and smaller increases in M. Control injections of NaCl produced no fever.

We have adapted Stitt's[5] techniques so as to produce controlled fevers in squirrel monkeys during exposure to MW fields. Nonmetallic substitutes for stainless steel cannula guide tubes, trochars, re-entrant tubes, and skull anchors for brain implants were designed and fabricated in our shops. Methods were devised to make the microinjections following a 90-min equilibration period and with minimal disturbance to the animal. These methods are detailed below.

METHODS

The subjects were four adult male squirrel monkeys (*Saimiri sciureus*). Their ages ranged from 6 to 15 years and their body masses from 900 to 1300 g. They were housed individually in a vivarium at 24 ± 2° C and 40 ± 10% RH. Each animal underwent a sterile surgical procedure during which 1 Delrin injection cannula guide tube and 1 Delrin sealed re-entrant tube were stereotaxically implanted in the PO/AH. The stereotaxic coordinates[6] for the guide tube were A = 13.0, L = 0.5, D = +1.0; the re-entrant tube was centered 3 mm lateral to the guide tube in the contralateral PO/AH. Both tubes were anchored by dental acrylic to 3 Delrin screws in the calvarium. To select the optimal dose for our purposes, dose-response functions were determined on 2 animals for PGE_1 doses from 50 to 300 ng. Subsequent tests of each subject confirmed that a PO/AH injection of 250 ng PGE_1 in 1 μl sterile saline would generate a controlled 80 ± 30 min fever of 0.7 ± 0.3° C, as measured in both the colon and the PO/AH.

During the experiments, a monkey was lightly restrained in a Plexiglas chair inside a Styrofoam test compartment that was ventilated (v = 0.36 m/sec) with air from a closely-regulated source. The test compartment was located in the far field of a MW antenna inside an anechoic chamber. The air temperature (T_a = 26 ± 0.5° C) and all dependent variables were monitored once/min during 3- to 4-hr test sessions. Luxtron fiberoptic probes measured colonic, PO/AH and 4 representative skin temperatures (abdomen, tail, leg, foot) from which a weighted mean skin temperature was calculated. Also measured was O_2 consumption (from which metabolic heat production, M, was calculated) and, in one animal, sweating rate from the foot. A 22-ga Pyrex cannula, clamped in a threaded Delrin hub, was filled with 1 μl of injectate (PGE_1 in NaCl or the vehicle) and attached, via saline-then-silicone filled tubing to a micrometer-driven microsyringe. The loaded cannula was screwed into the implanted guide tube before the animal was placed in the test chamber.

A continuous-wave (CW) MW signal, at either 450 or 2450 MHz, energized the antenna in one of two anechoic chambers. Field uniformity was determined in each chamber with a Narda broadband isotropic E-field probe in the animal's location. Whole-body energy absorption (SAR), based on temperature increments in saline-filled Styrofoam models exposed to controlled MW fields, was 0.413 or 0.165 (W/kg)/(mW/cm^2) at 450 or 2450 MHz, respectively.[7]

In the experimental tests, T_a was held constant at 26° C, a slightly cool environment for the squirrel monkey. Following a 90-min equilibration period (MW absent), the PGE_1 was injected into the PO/AH to initiate a febrile episode. The connecting tubing was clamped with a polystyrene clamp (to prevent additional fluid from flowing into the cannula), and the metal microliter syringe was disconnected and removed from the test environment. At 5, 35, or 65 min later, the

monkey was exposed to MW at a single power density for 30 min; a subsequent period of up to 90 min (MW absent) allowed conclusion of the febrile episode. Two power densities at each frequency were studied: 5 and 8 mW/cm^2 at 450 MHz and 10 and 20 mW/cm^2 at 2450 MHz. As controls, 4-hr sessions were conducted at each frequency (1) with PGE_1 injection present and MW absent, (2) with MW present and PGE_1 absent, and (3) with both MW and PGE_1 injection absent. In addition, a few experiments were conducted at T_a = 32° C in the 450-MHz chamber at a single power density (5 mW/cm^2), to assess the importance of a warm T_a.

RESULTS

FIGURE 1 shows the generation of a PGE_1-induced fever in one animal and the changes in autonomic responses of heat production and heat loss that occurred throughout the febrile episode. No MW field was present during this experiment. The lower panel shows that as soon as the microinjection was complete, M rose dramatically, forcing the body temperature to rise. Vigorous shivering was usually observed (over closed-circuit TV) during this time. A subsequent slow reduction in M stabilized the fever. Later, a further M reduction and the gradual initiation of tail vasodilation (upper panel) initiated defervescence.

FIGURE 2 superimposes on the data from FIGURE 1 (solid symbols) data from an identical experiment *except* for the addition of a 30-min exposure to a 450-MHz CW MW field, at a power density of 5 mW/cm^2, during the first 30 min of the febrile episode (open symbols). While the rise in colonic temperature is the same during this period, M rises much less while the MW field is present and partial vasodilation of the tail also occurs. In addition, the monkey was not observed to shiver when the MW field was present. It is of interest that, under both conditions, the magnitude of the fever and the induction rate, as measured in the colon, are nearly identical. However, the elevation in M is ~2W/kg less when the MW field is present, a value in agreement with the measured whole-body SAR of 2.06 W/kg at 5 mW/cm^2 for this frequency.

Analyses of other data showed that when the MW exposure occurred during the *second* 30 minutes of the febrile episode, the magnitude of the fever and its induction rate were again identical with the non-MW control. MW exposure initiated a dramatic M reduction (also of ~2 W/kg) and an accelerated vasodilation of the tail in order to hold the fever on its normal course. However, when MW exposure occurred during the *third* 30-min segment of the febrile episode (when defervescence had begun), an additional rise in deep body temperature of about 0.5°C was provoked, despite an M reduction of 2 W/kg and initiation of tail vasodilation.

FIGURE 3 shows sample data for the same animal collected during 2 tests when a 30-min exposure to 450 MHz, at a higher power density of 8 mW/cm^2 (SAR = 3.3 W/kg), was imposed during the first 30 min (solid symbols) or the third 30 min (open symbols) of the febrile episode. In general, MW exposures lowered M by substantial amounts whenever it was imposed. During fever induction, M was reduced more than 5 W/kg; later in the fever, the M reduction averaged more than 3 W/kg. Despite vigorous peripheral vasodilation of both tail and foot, MW exposure increased T_{co} to the same level irrespective of the phase of the fever cycle. These sample results hint at the significant role played by field strength, especially when the exposure frequency is at or near whole-body resonance.

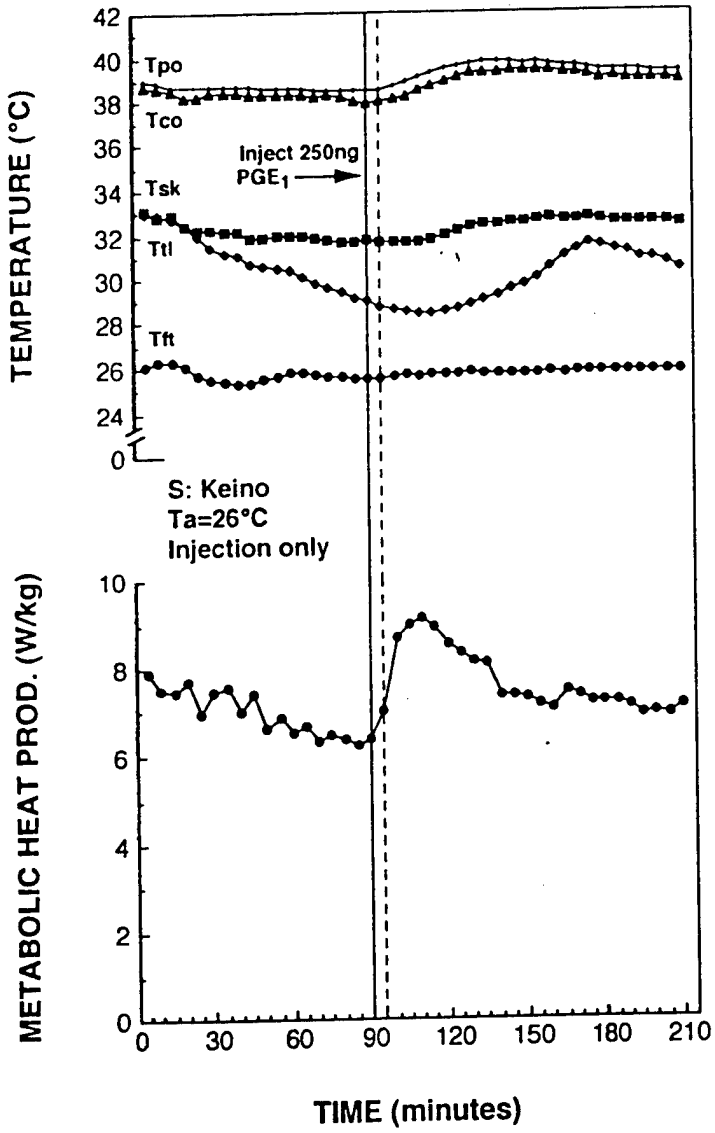

FIGURE 1. Responses of 1 squirrel monkey to an intrahypothalamic injection, via an implanted cannula, of 250 ng prostaglandin E_1 (PGE_1) in 1 μl sterile saline at the end of a 90-min equilibration to a 26° C environment. *Upper panel* shows preoptic temperature (T_{po}), colonic temperature (T_{co}), weighted mean skin temperature (T_{sk}), tail skin temperature (T_{tl}), and foot skin temperature (T_{ft}). *Lower panel* shows metabolic heat production (M) in W/kg.

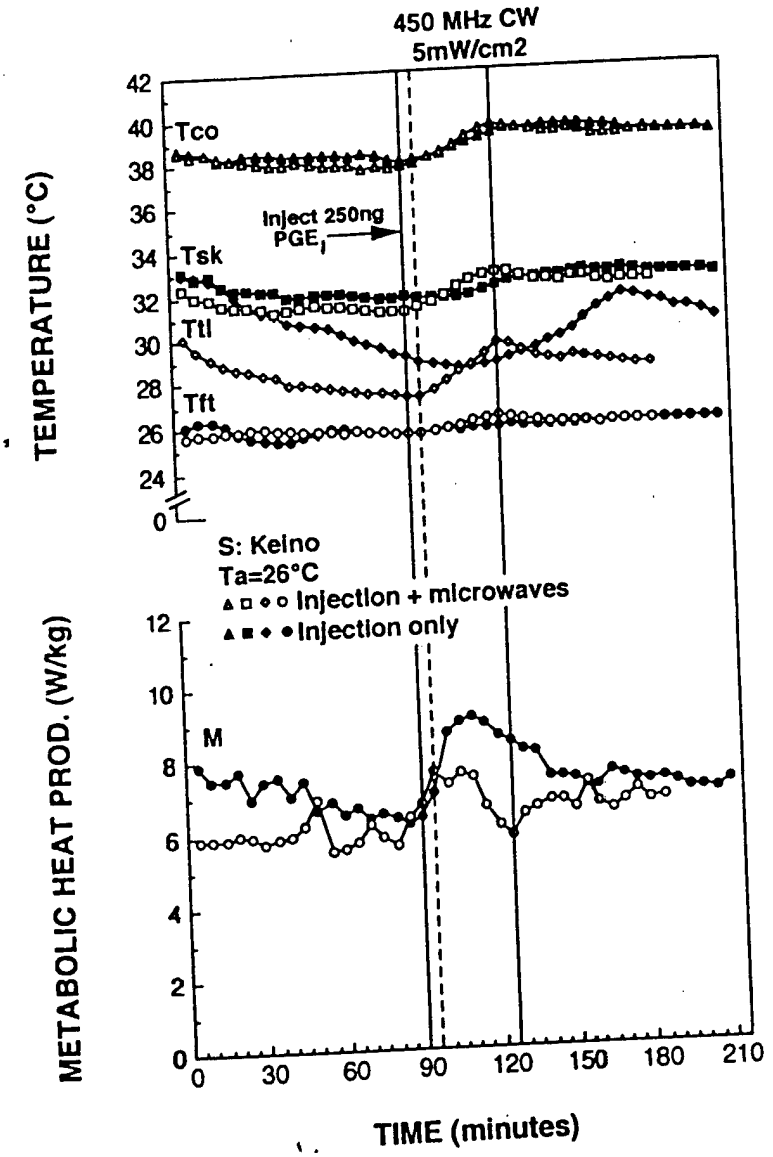

FIGURE 2. Superimposition on FIGURE 1 (*solid symbols*) of data collected on the same monkey when a 30-min exposure to 450 MHz CW microwaves at a power density of 5 mW/cm^2 was imposed at the initiation of the febrile episode (*open symbols*). Ambient temperature (T_a) = 26° C. See legend to FIGURE 1 for key to other symbols.

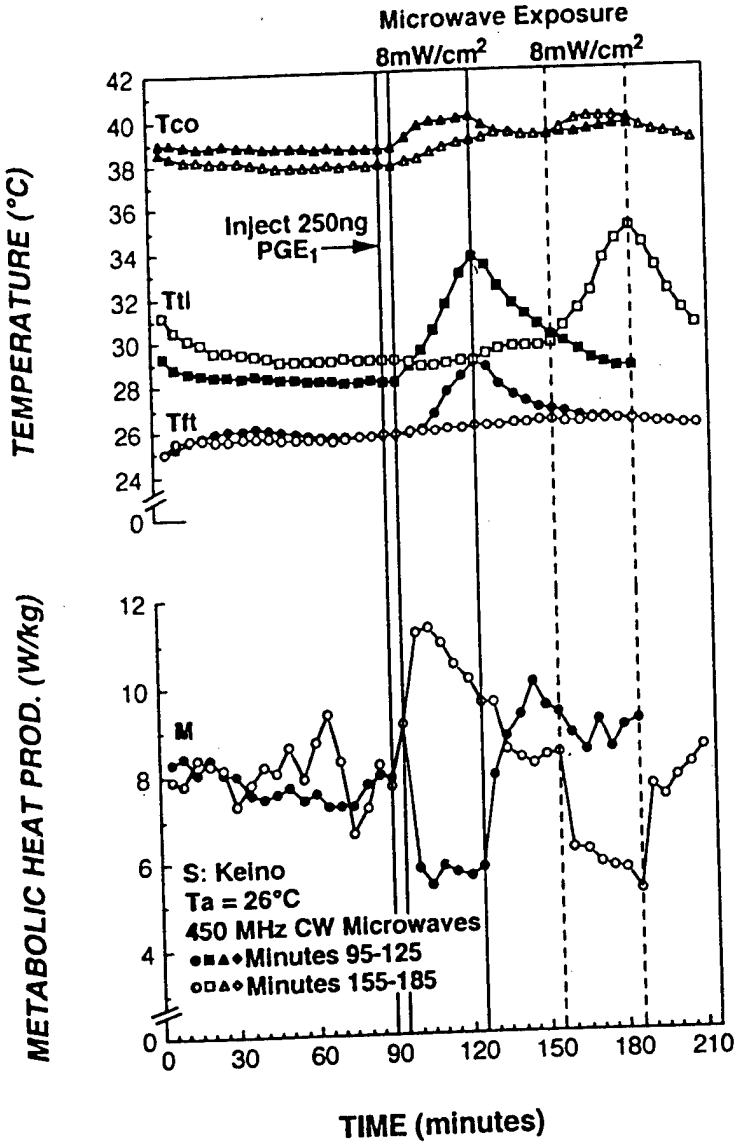

FIGURE 3. Superimposed responses of 1 febrile monkey exposed to 450 MHz CW microwaves at a power density of 8 mW/cm² (SAR = 3.3 W/kg) during different parts of the fever cycle in 2 separate experiments. *Solid symbols* show experiment in which MW exposure occurred during minutes 95–125; *open symbols* show experiment in which MW exposure occurred during minutes 155–185. Ambient temperature (T_a) = 26° C. See legend to FIGURE 1 for key to other symbols.

Not only were the thermoregulatory responses of febrile squirrel monkeys complex when MW exposure occurred at the resonant frequency, but other data showed that these responses were also frequency dependent. FIGURE 4 shows data for the same animal during 2 tests when a 30-min exposure to 450 MHz (solid symbols) or 2450 MHz (open symbols) was imposed 30 min into the febrile episode. In each case, the MW power density was set so as to yield a SAR of 3.3 W/kg. At both frequencies, the reductions in M were nearly identical, averaging about 3.2 W/kg. However, at resonance (450 MHz), MW exposure stimulated a greater rise in T_{co}, by about 1.0° C, than did 2450 MHz, despite the induction of robust tail vasodilation as an aid to heat loss.

For all animals, mean values of each dependent variable (*e.g.*, M, T_{co}, T_{tail}, etc.) were calculated for the final 10 min of the 90-min equilibration period and the final 10 min of each 30-min MW exposure period (*i.e.*, min, 25–35, 55–65, and 85–95 following PGE_1 injection). A summary of all data collected at 450 MHz (18 experiments) as a function of exposure condition and MW exposure phase appears in FIGURE 5 for the major dependent variables, T_{co}, M, T_{tail}, and T_{foot}. A comparable summary of all data collected at 2450 MHz (12 experiments) appears in FIGURE 6. Control data for NaCl injection only (4 experiments) and PGE_1 injection only (5 experiments) are included in both figures.

FIGURES 5 and 6 each display changes in T_{co} in panel A, changes in M in panel B, changes in tail temperature (T_{tl}) in panel C, and changes in foot temperature (T_{ft}) in panel D. In general, the magnitude of change in any particular response (*e.g.*, T_{co}) was usually greater when the animals were irradiated at the resonant frequency (450 MHz), the sole exception being the M reduction at both frequencies when MW exposure occurred during the second 30 min of the febrile episode (shown for 1 animal in FIG. 4). A greater rise in T_{co} always accompanied exposure at resonance, and a greater mobilization of vasomotion in the extremities. In addition, at both frequencies, the magnitude of response change was nearly always dependent on the MW field strength.

In the absence of MW irradiation, injection of PGE_1 into the PO/AH stimulated an *increase* in M that generated a fever of about 0.7° C; this fever was maintained when M returned to its characteristic level and slowly decreased thereafter. In the presence of MW irradiation at 2450 MHz, the change in T_{co} at each phase was very similar, but was always accompanied by a *reduction* in M and strong tail vasodilation (FIG. 6). In the presence of MW irradiation at 450 MHz, the pattern of responses was similar, even exaggerated, but the rise in T_{co} was much greater than that produced by PGE_1 injection alone or when accompanied by 2450 MHz MW exposure. The fact that MW energy penetrates maximally into the body's tissues during exposure at resonance facilitates heat storage and precludes efficient heat loss except in a favorable (cool) environment. Three pilot experiments conducted on one monkey at T_a = 32° C, featuring resonant MW exposure at 5 mW/cm^2, confirmed a doubling of the fever's magnitude in all phases. At this T_a, M is at the resting level and vasodilation is nearly complete; thus, unless the animal can mobilize robust sweating, heat loss capacity will be minimal and continued hyperthermia must result.

DISCUSSION

We have shown that a 250-ng dose of PGE_1, injected in the PO/AH of adult squirrel monkeys, initiates a fever (indexed by a rise in T_{co}) of 0.7 ± 0.3° C.

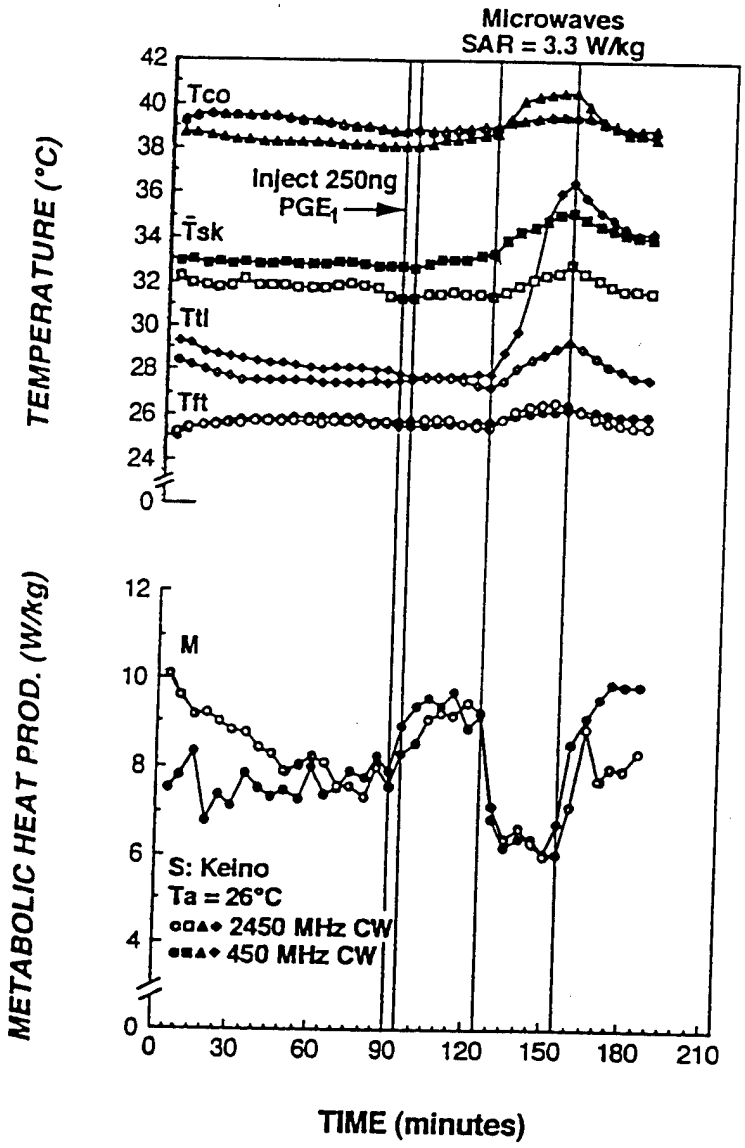

FIGURE 4. Superimposition of data from 2 experiments to show thermoregulatory responses of 1 febrile squirrel monkey, exposed for 30 min to either 450 (*solid circles*) or 2450 (*open symbols*) MHz CW microwaves (SAR = 3.3 W/kg) midway in the fever cycle (minutes 125–155). Ambient temperature (T_a) = 26° C. See legend to FIGURE 1 for key to other symbols.

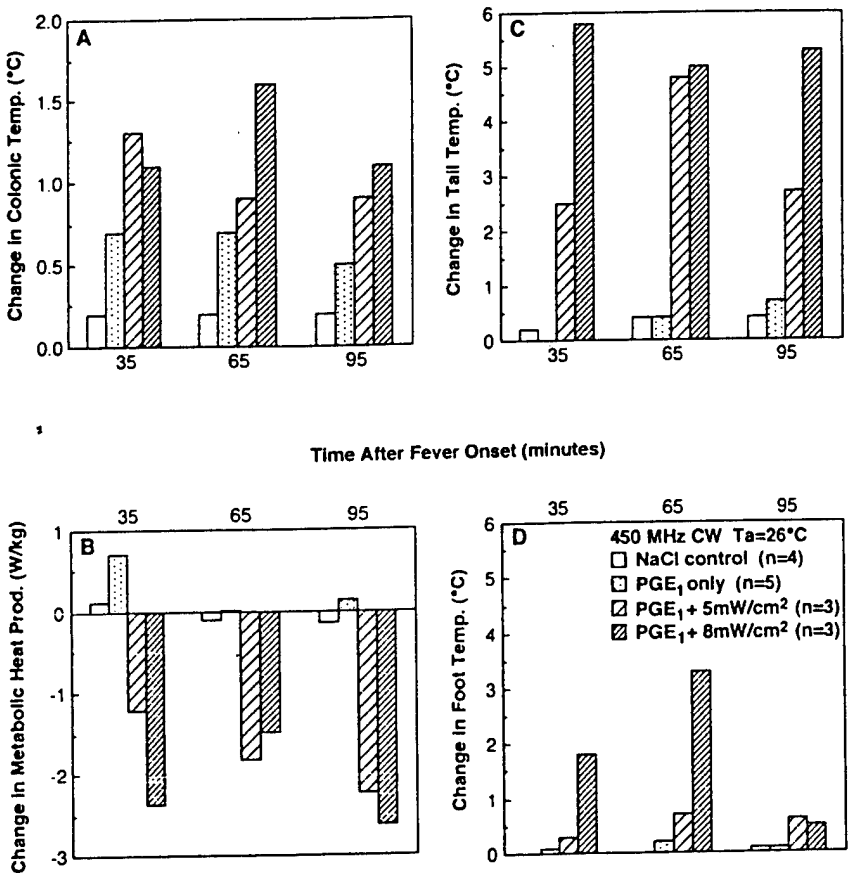

FIGURE 5. Mean changes in thermoregulatory responses of 3 squirrel monkeys exposed to 450 MHz CW microwaves at 2 power densities (5 and 8 mW/cm^2) for 30-min periods during fever induction, plateau, and defervescence (*hatched bars*). **(A)** Mean change in colonic temperature; **(B)** mean change in metabolic heat production; **(C)** mean change in tail temperature, and **(D)** mean change in foot temperature. Mean responses for NaCl injection (n = 4) and PGE_1 injection (n = 5) are also shown. T_a = 26° C.

This value is similar in magnitude to the steady-state T_{co} rise that occurs during prolonged MW exposure (T_a = 26° C) at a whole-body SAR of about 3.3 W/kg.[7] However, the changes in autonomic responses in the two cases are vastly different. The fever is generated by an increase in M and peripheral vasoconstriction, while the hyperthermia resulting from absorbed MW energy is accompanied by a decrease in M and peripheral vasodilation, responses similar to those occurring during exercise. Data from the present study show that exogenous MW energy, absorbed during the induction phase of a fever, reliably spares endogenous energy production, M, but may augment the fever somewhat if the field strength is excessive or if the energy is deposited deep in the body, as is the case during exposure at the resonant frequency. Further, under the specific conditions studied here,

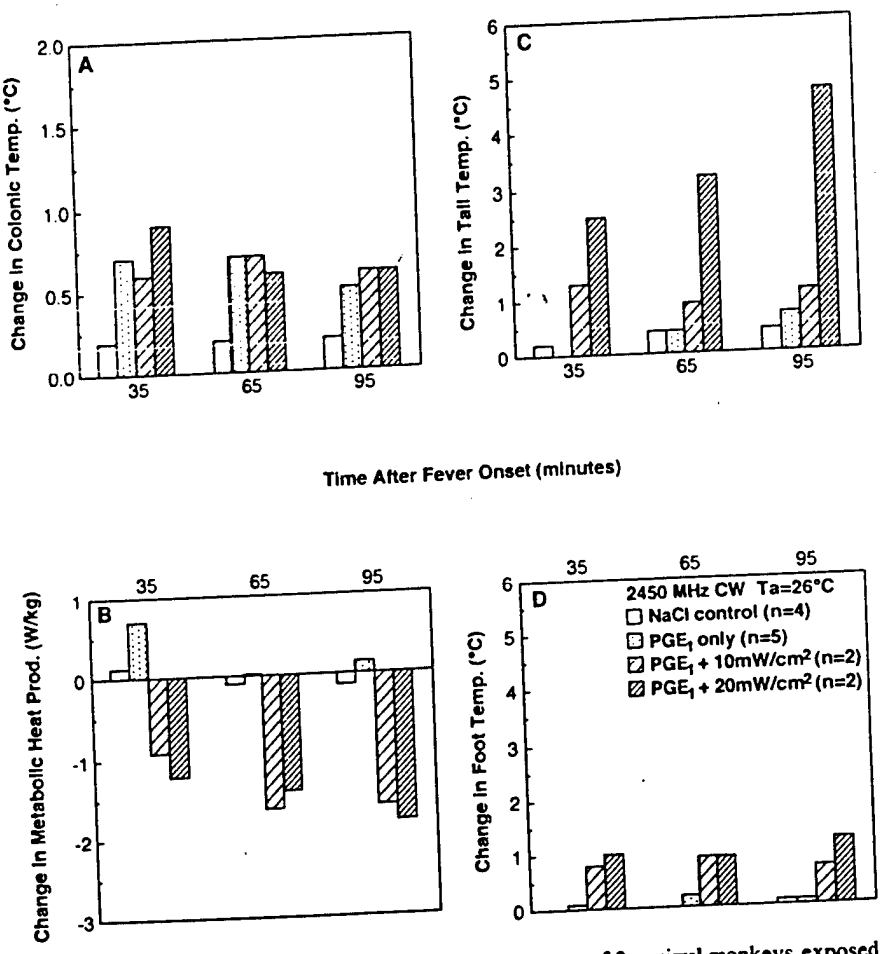

FIGURE 6. Mean changes in thermoregulatory responses of 2 squirrel monkeys exposed to 2450 MHz CW microwaves at two power densities (10 and 20 mW/cm^2) for 30-min periods during fever induction, plateau and defervescence (*hatched bars*). For panel designations, control data and T_a, see legend to FIGURE 5.

there is suggestive evidence that MW exposure later in the febrile episode (*e.g.*, during early defervescence) may compromise the efficiency of autonomic control of the body temperature.

It is important to put the results of this study in proper perspective, however. The field strengths, and associated SARs, studied here are about an order of magnitude greater than the current safety guidelines for human exposure to RF and MW fields.[1,2] Furthermore, the ambient RF and MW levels to which 99% of the U.S. population may be exposed are ≤ 1 μW/cm^2, more than 2 orders of magnitude below the safety guidelines.[8] At such low levels, absorbed RF and MW energy is neither perceiveable nor thermogenic, even at human resonant frequencies of 30 to 300 MHz. While the results of our experiments suggest that

absorbed MW energy may substitute for metabolic energy to generate and maintain a fever, ambient levels will be truly irrelevant in this context.

SUMMARY

We have examined experimentally the question of increased vulnerability to the thermalizing effects of MW exposure during febrile illness. In a controlled ambient temperature of 26° C, autonomic mechanisms of heat production and heat loss were measured in febrile squirrel monkeys during 30-min exposures to 450 or 2450 MHz CW MW fields at different phases of the fever cycle (induction, plateau, defervescence). We have shown that MW energy absorbed during a febrile episode spares endogenous energy production, but may augment the fever if deposited deep in the body, as is the case during exposure at the resonant frequency. The fever may also be exacerbated if the MW exposure occurs late in the febrile episode, a condition that may put an organism at some risk, especially if the field strength exceeds safety guidelines.

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