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Thermoregulatory responses of rats exposed to 9.3-GHz radiofrequency radiation

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Summary. Ketamine-anesthetized Sprague-Dawley rats were exposed in H orientation to far-field 9.3-GHz continuous-wave (CW) and pulsed (2 μ s, 500 pps) radiofrequency radiation (RFR) at average power densities of 30 and 60 mW/cm² (whole-body average specific absorption rates of 9.3 and 18.6 W/kg, respectively). Irradiation was conducted to cyclicly increase colonic temperature from 38.5 to 39.5° C. Colonic, tympanic, and subcutaneous temperatures, ECG, blood pressure, and respiratory rate were continuously recorded during experimentation. At both power densities, the subcutaneous and tympanic temperature increases significantly exceeded the colonic temperature increase. At both exposure levels, heart rate increased significantly during irradiation and returned to baseline when exposure was discontinued. Blood pressure and respiratory rate did not significantly change during irradiation. There were no significant differences between the effects of CW and pulsed RFR exposure. The levels of subcutaneous heating and heart rate change were greater, and the times required to achieve and to recover from a 1° C colonic temperature increase were longer than in previous studies conducted at 2.8 GHz. Results of these studies indicate that the carrier frequency used during irradiation markedly affects the pattern of heat distribution and the physiological responses of RF-irradiated animals.

Introduction

The military and civilian use of gigahertz (GHz) radiofrequency radiation (RFR) has steadily proliferated since the 1940's. Research on the bioeffects of GHz RFR conducted during the last two decades suggests that acute exposure to high levels of RFR may cause morphological and/or functional changes in many biological systems. Since availability of sources emitting RFR in the upper GHz range is limited, most experiments have been conducted in the 1-3 GHz range. Relatively little work has been done in the

9–10 GHz range, yet this frequency is characteristic of several high-peak-power stationary tracking radars, naval ship radars, and the majority of aircraft and small marine craft radars (Stuchly 1977).

RFR may be emitted in two forms, continuous wave (CW) and pulsed, and most radar waves are pulsed. The pulse width and repetition rate are such that the peak power generated during one pulse may be several orders of magnitude greater than that in a continuous wave of the same average power density. The elevated peak power density has led to speculation that pulsed RFR may cause greater biological effects than CW RFR.

The thermoregulatory response in warm-blooded animals, which depends upon the coordination of many physiological systems, is well-suited for the detection of possible differences between the bioeffects of CW and pulsed RFR. The present study was designed to compare the acute effects of CW and pulsed 9.3-GHz RFR upon the thermoregulatory responses and related physiological processes in ketamine-anesthetized rats.

Experimental methods

Animals

Six female Sprague-Dawley rats¹ (Camm Research Lab Animals), weighing between 221 and 239 g (mean \pm SEM, 230 ± 3 g), were used in this study. Animals were housed in Plexiglas cages with free access to food and water, and maintained on a 12/12, light/dark cycle (lights on at 0600) in a climatically controlled environment (ambient temperature of $24 \pm 1^\circ$ C). Prior to surgery, animals were fasted for 18 h (water, ad libitum). An aortic catheter (28 G Teflon) was installed via the left carotid artery. Ketamine HCl (Vetalar), 100–150 mg/kg, I.M., was administered as the anesthetic, and atropine sulfate, 0.04 mg/kg, S.C., was given to prevent excessive salivation. Administration of ketamine at approximately this level has been shown to provide adequate anesthesia in Sprague-Dawley rats (Smith et al. 1980), and produces a stable preparation compatible with physiological monitoring. Ketamine also affects thermoregulatory responses less than most general anesthetics (Hunter et al. 1981). Immediately after surgery, the animal was placed on a holder in the RFR exposure chamber. The holder consisted of seven 0.5-cm (O.D.) Plexiglas rods mounted in a half circle pattern on 4×6 cm Plexiglas plates.

Subcutaneous (side toward the RFR source), tympanic (side away from the RFR source), and core (5–6 cm post-anus) temperatures were continuously monitored during and after irradiation by use of Vitek 101 electrothermia probes and monitors, and recorded by a Cole-Parmer recorder (model 8373-30). In addition, the ECG, respiratory rate, and arterial blood pressure were continuously monitored and recorded as previously described (Jauchem et al. 1984).

¹ The animals involved in this study were procured, maintained, and used in accordance with the Animal Welfare Act of 1979 and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources – National Research Council

RFR equipment

The CW field was produced by a model 1326 RF power source (Cober Electronics, Inc.) and transmitted by a model 110 X antenna (Struthers Electronic Corp.). The exposures were performed under far-field conditions (animal positioned 150 cm from antenna), and the incident power density was determined with an electromagnetic radiation monitor (model 8616, Narda Microwave Corp., employing a model 8623-B probe). During exposures, the generator power was monitored continuously with a model 432-B power meter (Hewlett-Packard). The pulsed field was produced by a model 2852 power source (Cober Electronics, Inc.) and transmitted by a model 641 standard gain horn (Narda Microwave Corp.). Power density was determined as above and forward power was monitored with a model 436-A power meter (Hewlett-Packard). Pulsed and CW exposures were performed in the same Eccosorb RF-shielded anechoic chamber (Emerson & Cuming, Inc.) at the RFR facility, USAF School of Aerospace Medicine, Brooks Air Force Base, Texas. The chamber temperature ($24 \pm 0.5^\circ \text{C}$) and relative humidity ($50 \pm 5\%$) were monitored during all phases of experimentation.

Exposure conditions

Each animal was exposed individually in an alternating fashion to 9.3-GHz CW and pulsed (2 μs , 500 pps) RFR at average power densities of 30 and 60 mW/cm^2 [whole-body average specific absorption rates (SARs) of 9.3 and 18.6 W/kg , respectively]. The SARs were determined from colonic, tympanic, and subcutaneous temperature curves obtained during exposure, according to the method of Heinmets et al. (1984), and are consistent with theoretical values according to Durney et al. (1978). All animals were exposed in the H orientation (left lateral exposure, long axis of animal parallel to the magnetic field). A stable regimen of colonic temperature (T_c) change was used as the basis for comparing the effects of CW and pulsed RFR. After initial exposure to increase T_c to 39.5°C , irradiation was discontinued. When T_c returned to 38.5°C , irradiation was initiated until T_c again increased to 39.5°C . This procedure was repeated for a total of eight $1^\circ T_c$ cycles in each animal (2 at 60 mW/cm^2 , CW; 2 at 60 mW/cm^2 , pulsed; 2 at 30 mW/cm^2 , CW; 2 at 30 mW/cm^2 , pulsed). The order of stimulus presentation was randomized.

Statistical methods

Student's *t*-test for paired data (two-tailed) was applied to determine if there were significant differences between thermal responses or physiological variables under CW and pulsed exposure conditions. The same test was applied to determine if there were significant differences in heart rates (HR), blood pressures (BP), and respiratory rates (RR) at points of initiation and cessation of irradiation (38.5 and 39.5°C , respectively). Values concerning thermal responses are reported as the group mean *t*, (time required

to accomplish a 1°C T_c increase) and t_d (time required to recover to baseline temperature) \pm SEM. Data regarding physiological parameters are reported as changes from the values obtained at 38.5°C , rather than as absolute values. P values of less than 0.05 were considered to indicate significance in all cases.

Results

Thermal responses

Figure 1 shows the T_c responses of rats exposed in an alternating fashion to 9.3-GHz CW and pulsed RFR at average power densities of 30 and 60 mW/cm^2 . The t_r varied inversely with the average power density used during exposure; however, the t_d was relatively constant and independent of the average power density. The relationship between power density and t_r was nonlinear; t_r at 30 mW/cm^2 was approximately three times longer than at 60 mW/cm^2 . At the two power densities there was no significant difference in colonic heating or cooling times between CW and pulsed RFR exposed animals.

Subcutaneous and tympanic temperature

Figure 2 shows the mean subcutaneous temperature (T_s) and tympanic temperature (T_t) changes that accompanied the 1°C T_c increase. At both power densities, in all animals, the T_s and T_t increases significantly exceeded the T_c increase. The difference between T_s and T_t increases during CW and pulsed exposure was not significant.

Cardiovascular and respiratory effects

Figure 3 illustrates the mean HR changes that occurred during the 1°C T_c cycles. The mean baseline HRs at 38.5°C were 248 ± 8 , 253 ± 3 , 273 ± 14 , and 276 ± 14 (beats/min \pm SEM) prior to exposure to 30 mW/cm^2 , CW; 30 mW/cm^2 , pulsed; 60 mW/cm^2 , CW; and 60 mW/cm^2 , pulsed RFR, respectively. HR significantly increased during irradiation, and subsequently returned to near baseline levels after exposure. No correlation between power density and degree of HR change was noted. Comparison of the HR effects caused by CW and pulsed RFR showed no significant difference.

The mean baseline BPs at 38.5°C were 92 ± 2 , 92 ± 2 , 92 ± 3 , and 94 ± 3 (mmHg \pm SEM) prior to exposure to 30 mW/cm^2 , CW; 30 mW/cm^2 , pulsed; 60 mW/cm^2 , CW; and 60 mW/cm^2 , pulsed, respectively. Under all exposure conditions, the BP did not significantly change between T_c s of 38.5 and 39.5°C .

Respiratory rates at 38.5°C were 90 ± 3 , 87 ± 4 , 92 ± 4 , and 92 ± 3 (breaths/min \pm SEM) prior to exposure to 30 mW/cm^2 , CW; 30 mW/cm^2 , pulsed; 60 mW/cm^2 , CW; and 60 mW/cm^2 , pulsed, respectively. No significant changes from these rates were observed during the 1°C cycles under any of the exposure conditions.

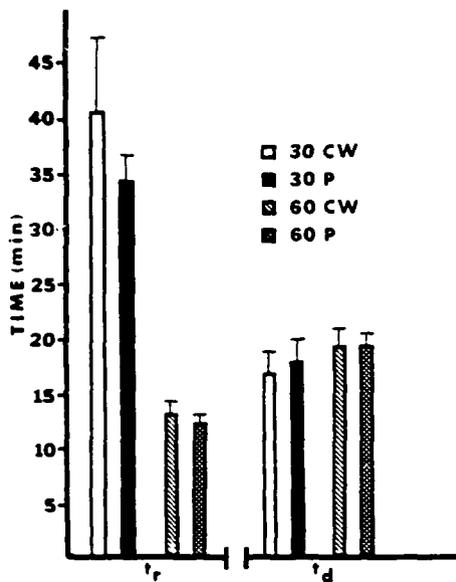


Fig. 1. Relationship between average power density and thermal responses of rats ($n=6$) exposed to 9.3-GHz CW and pulsed ($2 \mu\text{s}$, 500 pps) RFR. t_r = time required to achieve a 1°C colonic temperature increase. t_d = time required to recover to initial temperature upon cessation of irradiation. 30 and 60 refer to average power density in mW/cm^2

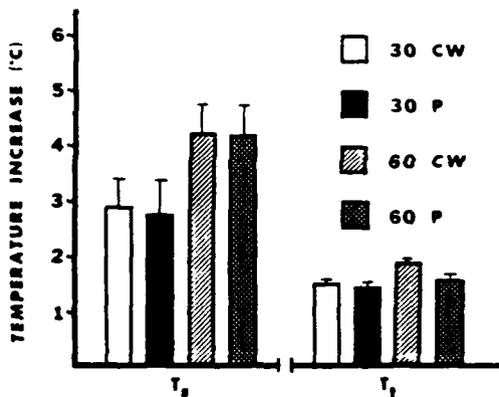


Fig. 2. Subcutaneous (T_s) and tympanic (T_t) temperature increases that accompanied 1°C colonic temperature increases in rats ($n=6$) intermittently exposed to 9.3-GHz CW and pulsed ($2 \mu\text{s}$, 500 pps) RFR. 30 and 60 refer to average power density in mW/cm^2

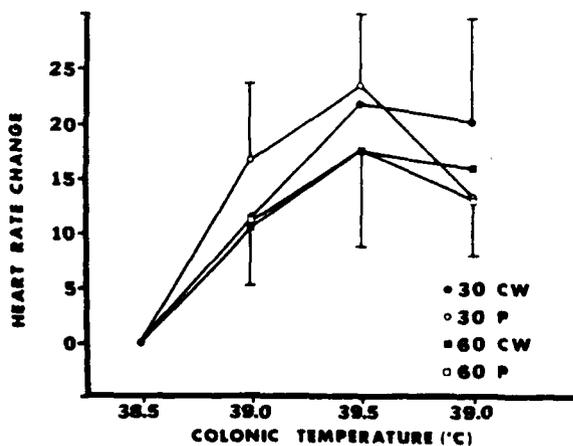


Fig. 3. Heart rate changes (beats/min) during CW and pulsed ($2 \mu\text{s}$, 500 pps) RFR - induced 1°C colonic temperature cycles in rats ($n=6$). Irradiation was initiated at 38.5°C and discontinued at 39.5°C . The data are normalized to the values obtained at 38.5°C . Symbols represent the average power densities used during irradiation

Discussion

Thermal responses

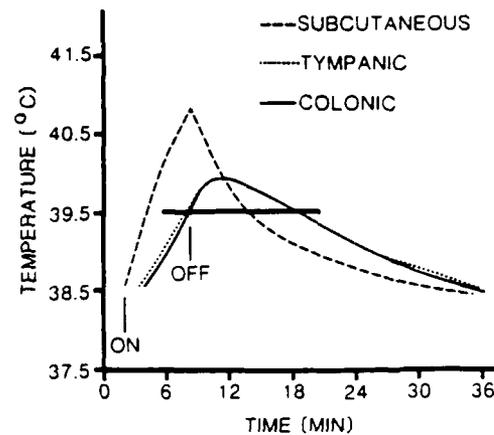
No significant difference in colonic heating or cooling responses occurred between conditions of CW and pulsed RF irradiation at equivalent average power densities of 30 and 60 mW/cm². These results are consistent with those obtained when rats were similarly exposed to 2.8-GHz CW and pulsed RFR (Frei et al. 1988).

It is well established that a threshold temperature change (either central or peripheral) must be achieved before thermoregulatory processes are initiated; however, many reports indicate that the rate of temperature increase also plays an important role in thermoregulation. Reports involving environmental heating (Kenshalo et al. 1968; Dykes 1975; Duclaux and Kenshalo 1980) indicate that certain thermal receptors are highly rate sensitive. Relative to RFR-induced heating, Gordon (1982, 1983) reported that evaporative heat loss and tail vasodilation in mice exposed to 2450-MHz RFR were directly proportional to the heating rate. Our results in anesthetized rats indicated that the colonic cooling rate following RFR-induced heating was independent of the rate of temperature rise. However, other results (Jauchem et al. 1984; Frei et al. 1988) indicated that responses related to thermoregulation, such as HR change, during 2.8 and 5.6-GHz irradiation of rats, were rate dependent.

It is of interest to compare the thermal responses of rats exposed to 9.3-GHz RFR with those from a previous study (Frei et al. 1988) in which rats were exposed under similar conditions to 2.8-GHz RFR. Animals irradiated at 9.3 GHz required longer to achieve a 1° C T_c increase than those exposed to 2.8 GHz. Similarly, D'Andrea et al. (1977) found in rats, that irradiation above the resonant frequency of 600 MHz resulted in slower rates of T_c increase. Lotz (1985) made a comparable observation when rhesus monkeys were exposed to RFR at carrier frequencies of 225 MHz (near resonance) and 1290 MHz (supraresonance). The present and previous studies suggest that during supraresonant irradiation, a great deal of the deep body heating is accomplished through conduction and circulatory heat transfer from the periphery.

Animals irradiated at 9.3 GHz also consistently required longer to recover from the 1° C T_c increase than did those irradiated at 2.8 GHz. Examination of the temperature curves obtained during irradiation revealed the cause of the longer recovery time; a representative set of curves is seen in Fig. 4. During 2.8-GHz RFR-induced 1° C T_c cycles, the temperatures at all three monitoring sites (subcutaneous, tympanic and colonic) immediately decreased when irradiation was discontinued. However, at 9.3 GHz, when irradiation was discontinued, only the T_c began to decrease immediately. The T_c and T_i continued to increase (overshoot) for a period of 3–4 min. Following the overshoot, colonic and tympanic cooling continued at a rate comparable to that found during 2.8-GHz irradiation. These data indicate that heat dissipation is related to the pattern of heat distribution.

Fig. 4. Colonic, tympanic, and subcutaneous temperature curves obtained during intermittent exposure of a rat to 9.3-GHz CW RFR at an average power density of 60 mW/cm^2 . "On" indicates when power was applied; "off" indicates termination of irradiation. Portion of colonic temperature curve above solid horizontal line indicates degree of overshoot



Subcutaneous and tympanic temperature changes

The depth of penetration and absorption of RFR energy depend upon the electrical properties of the absorbing medium and the carrier frequency of the applied radiation. Comparison of data from a previous study (Frei et al. 1988) conducted at 2.8 GHz with those from the present study at 9.3 GHz very dramatically shows the effect of frequency on depth of energy penetration. During irradiation of rats at 30 and 60 mW/cm^2 , the T_s increase was greater at 9.3 GHz than at 2.8 GHz (by almost a factor of two). At both power levels, the T_s increase was also greater at the higher frequency. However, during irradiation at 2.8 GHz, pulsed exposure produced significantly greater T_s and T_t increases than did CW exposure. This difference between CW and pulsed exposure was not seen during 9.3-GHz irradiation. At lower frequencies, pulsed irradiation may have resulted in more reflection or standing wave production as it passed through dissimilar tissues underlying the skin than did CW RFR. This condition may not have existed at 9.3 GHz since most energy was deposited in the outer few millimeters and absorbed primarily in the skin, regardless of whether the RFR was CW or pulsed.

Cardiovascular and respiratory effects

The reported effects of RFR upon the cardiovascular system are extremely diverse, ranging from tachycardia and hypertension, to no effect, to bradycardia and hypotension. In the present study, HR increased significantly (8–10%) during irradiation; however, there was no correlation between rate of T_c change and degree of HR change. This finding contrasts with previous work in which the degree of HR increase (2–6%) was directly related to the rate of T_c increase. However, the earlier investigations were performed at 2.8 GHz (Frei et al. 1988) and 5.6 GHz (Jauchem et al. 1984). At these frequencies, the depth of RFR energy deposition was considerably greater and more uniform than at 9.3 GHz and the level of T_s heating was signifi-

cantly less. Although the exact mechanism responsible for the greater HR increase during 9.3-GHz irradiation than during lower frequency exposure is unclear, it is probable that the difference was primarily related to the level or rate of peripheral heating. The higher T_s increase during 9.3-GHz exposure could have enhanced peripheral thermoreceptor discharge which influenced the degree of HR change. Skin temperature increase has previously been shown to cause increased HR (Cooper and Kerslake 1955; Rowell et al. 1969a, b). The elevated T_s may also have resulted in pacemaker heating via warmed blood from the cutaneous area. This possibility was considered by Cooper et al. (1962) when microwave-induced hyperthermia (T_c of 40.5° C) in rats was accompanied by HR increase. Later studies by Phillips et al. (1975) and Adair and Adams (1980) showed cutaneous vasodilation under RF irradiation conditions in which the T_c increased only slightly.

The rate of T_s increase could also have influenced the degree of HR change. In earlier investigations at 2.8 (Frei et al. 1988) and 5.6 GHz (Jauchem et al. 1984), the rate of T_s increase was 2 times greater than the rate of T_c increase. In the present study, the rate of T_s increase was 5 times greater than the rate of T_c increase, and was accompanied by a greater HR increase. Candas et al. (1985) concluded that in squirrel monkeys, the rate of skin temperature change, in addition to central temperature change, affects the magnitude of thermoregulatory responses.

The higher T_s , or the greater rate of T_s increase, during high frequency exposure could have resulted in greater stress hormone release which is known to cause increased HR. Lotz (1985) showed that the level of stress hormone release in rhesus monkeys was significantly greater during suprarsonance irradiation than during near-resonance exposure.

In general, stimuli that cause increased HR also cause increased BP, while those that decrease HR lower BP. In the study by Cooper et al. (1962), whole body exposure to RFR at a power density of 80 mW/cm² caused both tachycardia and hypertension. Other investigators have reported that during hyperthermia in humans, increased HR was accompanied by decreased BP (Kim et al. 1979; Tonnesen et al. 1987). In our present and previous studies in anesthetized rats (Jauchem et al. 1984; Frei et al. 1988), no significant changes in BP were noted even though HR consistently increased as the T_c increased from 38.5 to 39.5° C. The stability of mean BP during RFR exposure may have been related to changes in the rate of blood flow to the skin (cutaneous dilation) that accompanied the HR increase.

Earlier reports showed that exposure of animals to high-level RFR caused increased RR in conjunction with increased body temperature (Michaelson et al. 1961; Subbota 1967). Kaplan et al. (1971) observed increased RR in rabbits exposed to 2400-MHz RFR for 20 min at power densities of 40 mW/cm² or greater. Dorsal irradiation of rabbits at 2400 MHz for 60 min at a power density of 20 mW/cm² also caused increased RR (Birenbaum et al. 1975). In these studies, rates were determined prior to and after, but not during, irradiation. Criborn and Clemenson (1980) monitored

RR during exposure of unanesthetized mice to 2.45-GHz RFR. Irradiation at 100 mW/cm² caused an initial decrease in respiratory minute volume that was followed by a rapid increase until exposure was discontinued. The increase in RR accompanied a 2° C T_c increase. A similar increase in RR was noted by Jauchem et al. (1983) when the T_c of RFR-exposed anesthetized rats exceeded 41–41.5° C. Gordon and Ali (1984) found a two-fold increase in ventilatory rate in mice exposed to 2450-MHz RFR at an SAR of 18.2 W/kg; however, no change occurred in mice irradiated at an SAR of 8.9 W/kg. Gordon and Long (1984) showed that above a threshold SAR there was a direct relationship between SAR and ventilatory frequency in mice. In the last two reports, core temperature changes were not mentioned. In the present and previous studies (Jauchem et al. 1984, 1985a, b; Frei et al. 1988) no significant changes in RR were observed under any of the irradiation conditions that resulted in a 1° C T_c change (38.5 to 39.5° C).

In contrast to animals such as canines, changes in RR and depth do not play a major role in heat dissipation in rodents. It is probable that the animals in our studies were not thermally stressed beyond the point where changes in cardiovascular function were sufficient to maintain thermal balance. A similar situation has been observed in humans. Saxton (1975) showed that RR increased in man only if the body temperature increased by ~1.5° C or more. Thus, it is possible that in animals in which the respiratory system does not play a major role in thermoregulation, there is a higher thermal set point for the onset of increased respiratory function.

In summary, our results show no significant difference between the effects of CW or pulsed (2 μ s, 500 pps) 9.3-GHz RFR upon the ketamine-anesthetized rat's thermal responses, HR, BP, or RR. During RFR exposure that increased T_c from 38.5 to 39.5° C, HR increased significantly and returned to baseline when irradiation was discontinued. BP and RR did not significantly change during irradiation. The level of subcutaneous heating was greater and the time required to achieve and recover from a 1° C T_c increase was longer than in previous studies at 2.8 GHz. The degree of HR increase during irradiation was greater at 9.3 GHz than at 2.8 GHz. Our present and previous studies indicate that the pattern of heat distribution within RF-irradiated animals is extremely frequency dependent and that the animal's thermoregulatory responses are affected by differences in heat distribution patterns.

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