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Contributo Sperimentale Allo Studio Degli Effetti Delle Microonde Sulle Mastcellule Mesenteriali Di Ratto Albino

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EXPERIMENTAL CONTRIBUTION TO THE STUDY OF THE EFFECTS OF MICROWAVES ON THE MESENTERIC MAST CELLS OF THE ALBINO RAT

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INTRODUCTION

The term microwaves or RADAR [sic] waves is used to designate an electromagnetic emission whose spectrum lies between that of short waves and that of infrared rays, whose frequency ranges from 1,000 to 30,000 mc (1 mc = 1,000,000 cycles per second) and wavelength from 1 to 30 cm. Their application to the biological sciences was made possible in 1946 thanks to the perfection of the Gutton Magnetron, and at the Atlantic City Conference in 1948 the fact was brought out that a band between 2,400 and 25,000 mc/sec, equal to 12 cm in wavelength, was useful for medical purposes.

In spite of the publicity that that physiotherapeutic method has received since then, the data resulting from experimental research intended to explain its effect have been scanty and contradictory, and the mechanism of action of the microwaves on living substrata is not very clear. Two theories have been considered to explain the tissue changes following exposure to radar rays: on the one hand, Voser and Hubner (1) interpret the hyperemia that is constantly encountered as due to a stimulation, whose mechanism is not yet well explained, of the vascular neurovegetative system; and on the other, Juliani (2) states that the thermal effect is directly induced by the very high frequency electromagnetic current, considering that the hyperemia encountered by many authors (Busch and Fuchs (3), Cavalot and Einaudi (4), Violanti and al. (5), De Seguin (6), (7), and the changes in the vascular system (Cignolini (8), Fiandesio and Comino (9), Richardson et al. (10), Sacchitelli (11), Stoner (12)) only manifest themselves secondarily to the endogenous hyperthermia. However, substantial experimental data (Van Everdingen (13), De Seguin (6), (7), Terni and Lombardini (14), Dainotto et al. (15), Castagnoli and Fragale (16), Cinti et al. (17), obtained with various research methods, have recently supported the hypothesis that ultrashort waves exert their action through a peculiar wave effect, since following exposure to the radiation very peculiar biological and histological changes which are difficult to reproduce with other sources of heat are observed.

However, there are not available in literature adequate experimental data on the effects of microwaves on the connective tissues for their wide-spread application in the treatment of some diseases of rheumatological interest, nor is there reliable knowledge of their action, as evident from the contradictory results reported by the various authors. In fact, whereas Wise et al. (18) maintain that a large single dose of microwaves may not only retard the growth of a healthy bone, but destroy it, Busch and Fuchs do not confirm such data, and Doyle and Smart (19) directly support the possibility of stimulation of the growth of the bone after exposure to a small dose of ultrashort waves. This last assertion, however, is refuted by Granberry and James (20), who state that doses of radiation so small as to cause no suffering in animals have no effect on the bone tissue.

Recently Valtonen (21) has observed that the mast cells of the mesentery of the rat, following panirradiation with a sublethal dose of microwaves, undergo no changes, as regards their number per mm^2, nor do they exhibit greater degranulation, than in the control animals, with a consequent release of heparin, histamine, or fluorescent amines, a rather strange effect in view of the extreme sensitivity of mastocytes to any irritating stimulus. According to that author, following panirradiation with microwaves only the mast cells of the peritoneal fluid exhibit a statistically significant increase in the degranulation, while it is possible to observe a definite increase in the giant mast cells (Valtonen (22)) normally present in a tiny percentage in the peritoneal fluid.

Considering the substantial value of such data, we wished to conduct careful research in order to analyze the behavior of the mast cells of the mesentery of the rat following panirradiation with ultrashort waves, to show possible changes in the cells under examination, and to attempt in that way to interpret the reaction of the connective substratum to which they belong, under the stimulus of microwaves.

MATERIALS AND METHODS

Our research was conducted on the mesenteric membrane of Wistar Albini rats, with an average weight of 150 g and 4 weeks old. The animals were divided into two batches - the first one composed of three rats which were exposed to no radiation and were used as controls, and the second batch, of four animals, which were exposed to microwave radiation. For that treatment, immobilized in the supine position, each animal was exposed to a single dose of panirradiation using an ultrashort wave generator operating on a frequency of 2,425 MHz, with a wavelength of 12.4 cm, fitted with a conveyor with a round field 17 cm in diameter, maintaining a distance of 15 cm from the radiation generator to the skin of the animal.

Each dose of radiation lasted 5 minutes, with a power of 80 watts, a sublethal dose for the rat (Valtonen (22)).

Three hours after the application, the animals were killed, and from each of them were taken strips of mesentery, which were washed repeatedly and carefully in physiological solution, laid on glass slides which had been polished and degreased in advance, dried in the air at room temperature and protected from possible contamination, and dyed by the May-Grunwald-Giensa method used for hematology specimens.

The specimens obtained, allowed to dry in the air, were examined under the microscope without preparing slides.

For the mast cell count, for the division of the degranulated cells into types, for the exact evaluation of the dimensions of the unaffected cells, and for their subsequent differentiation by type, we used the method described by Crespi et al. (23).

RESULTS

The results that we obtained using the technique described above have been given in four tables, which contain the data relating to the two batches under examination:

The first one indicates the average number of mast cells present in the surface unit of the membrane of the mesentery;

The second table gives the mean percentage values for the unaffected mast cells and those undergoing degranulation;

The third table gives the percentage values relating to the three types of mast cells undergoing degranulation;

The fourth table, finally, contains the mean percentage data relative to the four types into which the unaffected mast cells have been divided.

We should mention that in each table the number of observations refers to the number of animals used for our investigation; we wish to recall to mind, however, that for each animal 10 microscopic fields were examined each time, while the numerical values considered from time to time for each animal are the average of 10 observations.

DISCUSSION

As indicated by the values given in table I, the average number of mast cells present in the surface unit of mesenteric membrane (1 mm^2) showed no changes in the animals treated with ultrashort

waves as compared with the control animals; however, the examination of the mean percentage values for unaffected and degranulated mastocytes and the observation of the average diameter of the unaffected mast cells furnished interesting parameters for evaluating the reaction of the mesenteric connective tissue to electromagnetic stimulation. It can be observed, in fact (table II), how the total proportion of degranulated mast cells in rats exposed to panirradiation shows an increase which cannot be attributed in particular to one of the three types into which we had divided such mast cells (table III); even if that increase was not significant for the statistical investigation, we feel it is worthy of being considered, since it appears to be not only in agreement with what was found by Valtonen in research similar to ours (Valtonen (22)), but also in accordance with what was stated by Hill (24), by Calzavara et al. (25), and by Sanyal (26); those last-mentioned authors state that heat applied percutaneously causes in the laboratory animal either a rapid degranulation of the local mast cells (Sanyal) or a vacuolation (Hill) and degranulation (Hill and Calzavara) of the peritoneal mast cells of the mesentery and the omentum, interpreting that reaction at a distance as an specific expression of the alarm reaction. However, in our case it seems that the greater degranulation observed in the rats subjected to panirradiation, although within the limits of acceptability in regard to the absence of statistical significance, may on the other hand be interpreted as being associated with the action of the microwaves on the structure of the tissues subjected to radiation, taking into consideration the fact that the endogeous heat developed by them occurs at a depth of 7 cm in the tissues, a level at which the mesentery of the rat subjected to radiation in the supine position is largely situated.

Degranulation, which must be interpreted as the maximum expression of the reactive capacity of the mast cells to stimuli capable of inducing biochemical changes within the connective tissue, proves the biosynthetic activity of those mesenchymal cells, as manifested by the growth and increase in the dimensions and number of granules contained in them (Radden) (27).

The exogenic stimulus is able to cause activation of the "mast cell cycle" (Riley) (28), and then the rapid increase in their dimensions until rupture with consequent degranulation, release of mucopolysacchrarides, 5HT, histamine, actually acting, besides directly on the mast cells, also on the composition of the connective tissue. In our observations, in fact, we noted that while in the control animals, associated with their young age, there was a population of unaffected mesenteric mast cells composed only of types A and B, i.e. of a maximum diameter of 15 microns, in the rats subjected to radiation it was possible to demonstrate a significant increase in the number of mast cells between 11 and 15 microns in diameter, and the appearance of cells 20 microns in diameter.

The fact thus reveals a change in the mesenteric mast cell population after panirradiation with ultrashort waves appreciably greater than could be deduced only from an examination of the mast cells undergoing degranulation, and seems to confirm what was observed by Valtonen in the mast cells of the peritoneal fluid (Valtonen (22)).

CONCLUSIONS

Our observations have made it possible to show how panirradiation with microwaves can cause a change in the mast cell population in the mesentery of the rat, characterized by an increase in the average diameter and by the degranulation of those cells; it remains to be established whether that change is caused primarily by a direct action exerted by the microwaves on the mast cells and therefore representing an immediate response of those cells to the exogenous stimulus, or rather the expression of heightened mast cell activity associated with biochemical changes caused by the panirradiation in the mesenteric connective tissue.

The experimental observations of mast cells subjected to stimuli capable of acting directly on them, as for example the intraperitoneal injection of compound 48/80 which is capable of causing their degranulation, actually through an enzymatic mechanism (Borens (29), Hogberg and Uvnas (30), Val Arsdel and Bray (31), demonstrate how those cells react to a similar type of stimulus with rapid, total degranulation: following intraperitoneal injection of sufficient doses of compound 48/80, in fact, all the mesenteric mast cells of the rat appear degranulated, with the exception of a few which, on the other hand, should be considered as fibroblasts filled with metachromatic granules incorporated subsequent to the mast cell degranulation and not yet digested and therefore rendered orthochromatic (Gustaffson and Gromberg (32)).

Therefore, it seems reasonable to consider that microwaves are able to cause a biochemical change in the mesentery, and that the morphological changes observed in the mast cell population represent the expression of the activity of those cells heightened by the above-mentioned changes, thus constituting an initial stage in the more complex homeostatic mechanism for maintaining the integrity of the connective tissue and of its fundamental substances described by Riley.

SUMMARY

The authors reveal how panirradiation with microwaves caused a change in the mast cells population of the mesentery of the rat, characterized by an increase in the mean diameter and the de-

granulation of those cells. The data are interpreted as having been caused by a biochemical change in the mesenteric connective tissue induced by treatment with ultrashort waves.

Table I. Number of mast cells present in the surface unit of the mesenteric membrane of rats exposed to radiation

Treatment	Number of observations	Average number of mast cells per mm	Standard deviation	Standard error
Control	3	159.90	5.109	± 2.94
Microwaves 80 watts/5'	4	159.99	3.102	± 1.36

Table II. Mean percentage values of unaffected mast cells and those undergoing degranulation in theperitoneal membrane of rats exposed to radiation.

Treatment	Number of observations	Mean percentage values for mast cells		Standard deviation	Standard error
		unaffected	degranulated		
Control	3	63.40	36.60	5.25	+ 2.14
Microwaves 80 watts/5'	4	42.00	58.00	5.36	+ 2.66

Table III. Percentage values relating to the three types of mast cells undergoing degranulation

Treatment	Number of observations	Mean percentage values of mast cells undergoing degranulation								
		Type I			Type II			Type III		
		Mean value	Standard deviation	Standard error	Mean value	Standard deviation	Standard error	Mean value	Standard deviation	Standard error
Control	3	66.66	5.79	± 3.34	19.00	5.75	± 3.32	14.34	0.50	± 0.28
Microwaves 80 watts/5'	4	67.95	4.91	± 2.45	19.55	2.01	± 1.00	12.50	2.94	± 1.47

Table IV. Mean percentage values for the three types of mast cells

Treatment	Number of observations	Unaffected mast cells								
		Type A			Type B			Type C		
		Mean value	Standard deviation	Standard error	Mean value	Standard deviation	Standard error	Mean value	Standard deviation	Standard error
Control	3	52.80	7.24	± 4.16	47.20	9.00	± 5.17	-	-	-
Microwaves 80 watts/5'	4	8.90	3.59	± 1.74	85.00	9.71	± 2.85	6.10	0.41	± 0.24

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