Electromagnetic Biology and Medicine, 25: 245–258, 2006

ISSN 1536-8378 print
DOI: 10.1080/15368370601044150

informa
healthcare

Electrohypersensitivity: State-of-the-Art of a Functional Impairment

OLLE JOHANSSON

Department of Neuroscience, Karolinska Institute, Stockholm, Sweden

Recently, a new category of persons, claiming to suffer from exposure to electromagnetic fields, has been described in the literature. In Sweden, electrohypersensitivity (EHS) is an officially fully recognized functional impairment (i.e., it is not regarded as a disease). Survey studies show that somewhere between 230,000–290,000 Swedish men and women report a variety of symptoms when being in contact with electromagnetic field (EMF) sources. The aim of our studies has been to investigate possible alterations, in the cellular and neuronal systems of these persons' skin. As controls, age- and sex-matched persons, without any subjective or clinical symptoms or dermatological history, served. Immunohistochemistry using antisera to the previously characterized marker substances of interest has been utilized. In summary, it is evident from our preliminary data that various alterations are present in the electrohypersensitive persons' skin. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequent magnetic fields or microwaves and cancer, our data ought to be taken seriously and further analyzed.

Keywords Dermatoscience; Electrohypersensitivity; Impairment; Immunohistochemistry; Neuroscience.

An ever increasing number of studies has clearly shown various biological effects at the cellular level of electromagnetic fields, including power-frequent and radiofrequent ones as well as microwaves. Such electromagnetic fields are present in your everyday life, at the workplace, in your home, and at places of leisure.

Recently, a new category of persons with a functional impairment (electrohypersensitivity; EHS) has been described in the literature, namely those that claim to suffer from subjective and objective skin- and mucosa-related symptoms, such as itch, smarting, pain, heat sensation, redness, papules, pustles, etc., after exposure to visual display terminals (VDTs), mobile phones, DECT telephones, as well as other

Address correspondence to Olle Johansson, Department of Neuroscience, Karolinska Institute, The Experimental Dermatology Unit, 17177 Stockholm, Sweden; E-mail: olle.johansson@.ki.se

electromagnetic devices. Frequently, symptoms from internal organ systems, such as the heart and the central nervous system, are also encountered.

Persons claiming such adverse skin reactions after having been exposed to computer screens or mobile phones very well could be reacting in a highly specific way and with a completely correct avoidance reaction, especially if the provocative agent was radiation and/or chemical emissions—just as you would do if you had been exposed to, e.g., sun rays, X-rays, radioactivity, or chemical odors. The working hypothesis, thus, early became that they react in a cellularly correct way to the electromagnetic radiation, maybe in concert with chemical emissions such as plastic components, flame retardants, etc., something later focused upon by professor Denis L. Henshaw and his collaborators at the Bristol University (cf. Fews et al., 1999a,b). This is also covered in great depth by Gunni Nordström in her latest book (2004).

Very soon, however, from different clinical colleagues, and in parallel to the above, a large number of other 'explanations' became fashionable, e.g., that the persons claiming electrohypersensitivity were only imagining this, or they were suffering from post-menopausal psychological abberations, or they were old, or having a short school education, or were the victims of classical Pavlovian conditioning, or a journalist-driven mass media psychosis. Strangely enough, most of the, often self-made, 'experts' who proposed these explanations had themselves never met anyone claiming electrohypersensitivity and these 'experts' had never done any investigations of the proposed explanatory models.

The aim of our own studies has been to investigate possible alterations, in the cellular and neuronal systems of these persons' skin. As controls, age- and sex-matched persons, without any subjective or clinical symptoms or dermatological history, have served. Immunohistochemistry using antisera to the previously characterized marker substances of interest has been utilized. Among many discoveries, the following may be mentioned.

We have investigated the presence of intraepidermal nerve fibers in normal human skin from healthy volunteers using the new marker PGP 9.5 (Hilliges et al., 1995; Johansson et al., 1999; Wang et al., 1990). The intraepidermal nerve fibers are found as close as 20–40 μm from the surface, which makes it highly possible that weak electromagnetic fields may affect them.

In facial skin samples of electrohypersensitive persons, the most common finding is a profound increase of mast cells. Nowadays we do not only use histamine, but also other mast cell markers such as chymase and tryptase, but the pattern is still the same as reported previously for other electrohypersensitive persons (Johansson and Liu, 1995). From these studies, it is clear that the number of mast cells in the upper dermis is increased in the electrohypersensitivity group. A different pattern of mast cell distribution also occurred in the electrohypersensitivity group, namely, the normally empty zone between the dermo-epidermal junction and mid-to-upper dermis disappeared in the electrohypersensitivity group and, instead, this zone had a high density of mast cell infiltration. These cells also seemed to have a tendency to migrate towards the epidermis (=epidermiotrophism) and many of them emptied their granular content (=degranulation) in the dermal papillary layer. Furthermore, more degranulated mast cells could be seen in the dermal reticular layer in the electrohypersensitivity group, especially in those cases which had the mast cell epidermiotrophism phenomenon described above. Finally, in the electrohypersensitivity group, the cytoplasmic granules were more densely

distributed and more strongly stained than in the control group, and, generally, the size of the infiltrating mast cells was found to be larger in the electrohypersensitivity group as well. It should be noted that increases of similar nature later on were demonstrated in an experimental situation employing normal healthy volunteers in front of visual display units, including ordinary household television sets (Johansson et al., 2001).

In one of the early papers (Johansson et al., 1994), we made a sensational finding when we exposed two electrically sensitive individuals to a TV monitor. When we looked at their skin under a microscope, we found something that surprised us. In this article, we used an open-field provocation, in front of an ordinary TV set, of persons regarding themselves as suffering from skin problems due to work at video display terminals. Employing immunohistochemistry, in combination with a wide range of antisera directed towards cellular and neurochemical markers, we were able to show a high-to-very high number of somatostatin-immunoreactive dendritic cells as well as histamine-positive mast cells in skin biopsies from the anterior neck taken before the start of the provocation. At the end of the provocation the number of mast cells was unchanged, however, the somatostatin-positive cells had seemingly disappeared. The reason for this latter finding is discussed in terms of loss of immunoreactivity, increase of breakdown, etc. The high number of mast cells present may explain the clinical symptoms of itch, pain, edema, and erythema.

We have compared facial skin from electrohypersensitive persons with corresponding material from normal healthy volunteers (Johansson et al., 1996). The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), neuropeptide tyrosine (NPY), protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5, and phenylethanolamine N-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). In our ongoing investigations, we have also found alterations of the Merkel cell number in the facial skin of electrohypersensitive persons (Yoshimura et al., 2006). However, it has to be pointed out that we cannot, based upon those results, draw any definitive conclusions about the cause of the changes observed. Blind or double-blind provocations in a controlled environment (Johansson et al., 2001) are necessary to elucidate the underlying causes for the changes reported in this particular investigation.

I and my collaborator, Dr. Shabnam Gangi, in two papers of theoretical nature (Gangi and Johansson, 1997, 2000), have put forward a model for how mast cells and substances secreted from them (e.g., histamine, heparin, and serotonin) could explain sensitivity to electromagnetic fields. The model bounces off from known facts in the fields of UV- and ionizing irradiation-related damages, and uses all the new papers dealing with alterations seen after, e.g., power-frequent or microwave electromagnetic fields, to propose a simple summarizing model for how we can understand the phenomenon of electrohypersensitivity.

In the first paper (Gangi and Johansson, 1997), we describe the fact that an increasing number of persons say that they get cutaneous problems as well

as symptoms from certain internal organs, such as the central nervous system and the heart, when being close to electric equipment. A major group of these persons are the users of video display terminals, who claim to have subjective and objective skin- and mucosa-related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The central nervous system-derived symptoms are, e.g., dizziness, tiredness, and headache. Erythema, itch, heat sensation, edema, and pain are also common symptoms of sunburn (UV dermatitis). Alterations have been observed in cell populations of the skin of electrohypersensitive persons similar to those observed in the skin damaged due to ultraviolet light or ionizing radiation. In electrohypersensitive persons a much higher number of mast cells have been observed. It is known that UVB irradiation induces mast cell degranulation and release of TNF-alpha. The high number of mast cells present in the electrohypersensitivity group and the possible release of specific substances, such as histamine, may explain their clinical symptoms of itch, pain, edema, and erythema. The most remarkable change among cutaneous cells, after exposure with the above-mentioned irradiation sources, is the disappearance of the Langerhans' cells. This change has also been observed in electrohypersensitive persons, again pointing to a common cellular and molecular basis. The results of this literature study demonstrate that highly similar changes exist in the skin of electrohypersensitive persons, as regards the clinical manifestations as well as alterations in the cell populations, and in skin damaged by ultraviolet light or ionizing radiation.

In the second publication (Gangi and Johansson, 2000), the relationship between exposure to electromagnetic fields and human health is even more in focus. This is mainly because of the rapidly increasing use of such electromagnetic fields within our modern society. Exposure to electromagnetic fields has been linked to different cancer forms, e.g., leukemia, brain tumours, neurological diseases, such as Alzheimer's disease, asthma, and allergy, and to the phenomenon of electrohypersensitivity/screen dermatitis. There is an increasing number of reports about cutaneous problems as well as symptoms from internal organs, such as the heart, in people exposed to video display terminals. These people suffer from subjective and objective skin and mucosa-related symptoms, such as itch, heat sensation, pain, erythema, papules, and pustules (cf. above). In severe cases, people cannot, for instance, use video display terminals or artificial light at all, or be close to mobile telephones. Mast cells, when activated, release a spectrum of mediators, among them histamine, which is involved in a variety of biological effects with clinical relevance, e.g., allergic hypersensitivity, itch, edema, local erythema, and many types of dermatoses. From the results of recent studies, it is clear that electromagnetic fields affect the mast cell, and also the dendritic cell, population, and may degranulate these cells. The release of inflammatory substances, such as histamine, from mast cells in the skin results in a local erythema, edema, and sensation of itch and pain, and the release of somatostatin from the dendritic cells may give rise to subjective sensations of ongoing inflammation and sensitivity to ordinary light. These are, as mentioned, the common symptoms reported from persons suffering from electrohypersensitivity/screen dermatitis. Mast cells are also present in the heart tissue and their localization is of particular relevance to their function. Data from studies made on interactions of electromagnetic fields with the cardiac function have demonstrated that highly interesting changes are present in the heart after exposure to electromagnetic fields. Some electrically sensitive people have symptoms similar to heart attacks after exposure to electromagnetic fields.

One could speculate that the cardiac mast cells are responsible for these changes due to degranulation after exposure to electromagnetic fields. However, it is still not known how, and through which mechanisms, all these different cells are affected by electromagnetic fields. In this article (Gangi and Johansson, 2000), we present a theoretical model, based upon the above observations of electromagnetic fields and their cellular effects, to explain the proclaimed sensitivity to electric and/or magnetic fields in humans.

In a recent article by Holmboe and Johansson (2005), the functional impairment electrohypersensitivity was investigated with the aim to characterize the complex set of symptoms and to order them according to the WHO's ICQ10 register of diagnoses. Furthermore, we also tested for the presence of increased levels of IgE or signs of a positive Phadiatop Combi (which is a screening test for allergies towards certain articles of food, pollen, insects, and other animals) which both would be indicators of an immune system alert. If such increases would be found, they could then be used in the diagnosis of electrohypersensitivity.

Twenty-two people (5 men, 17 women) participated. The age range was between 25 and 79 years. The symptoms were given in a ranked scale where the symptoms were attributed points according to the following: 0 = no symptoms at all; 1 = occasional, mild symptoms; 2 = occasional, severe symptoms; 3 = regular, mild symptoms; 4 = regular, severe symptoms.

Symptoms of the skin and the nervous system dominated the picture. The most frequent ones were skin redness, eczema and sweating, loss of memory, concentration difficulties, sleep disturbances, dizziness as well as muscular and joint-related pain, and muscular and joint-related weakness. Headache, faintness, nose blockade, and fatigue were also common. In addition, 19 of the people had symptoms from the gastrointestinal tract. All the people with the impairment electrohypersensitivity had tinnitus.

No connection between IgE blood levels and symptoms could be found, all the people with electrohypersensitivity had normal values (<122 kU/l). Only 3 people had a positive Phadiatop Combi.

In summary, it is evident from our preliminary experimental data that various biological alterations are present in the electrohypersensitive persons claiming to suffer from exposure to electromagnetic fields. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequent magnetic fields or microwaves and cancer, our data ought to be taken seriously and further analyzed.

Thus, it is of paramount importance to continue the investigation of persons with the impairment electrohypersensitivity. We would favor studies of electromagnetic fields' interaction with mast cell release of histamine and other biologically active substances, studies of lymphocyte viability, as well as studies of the newly described serotonin-containing melanocytes. Also, continued analysis of the intraepidermal nerve fibers and their relations to these mast cells and serotonin-containing melanocytes are very important. Finally, not to be forgotten, a general investigation—of persons with the impairment electrohypersensitivity versus normal healthy volunteers—regarding the above markers as well as other markers for cell traffic, proliferation, and inflammation, is very much needed. Such scientific work may lay a firm foundation for necessary adjustment of accessibility, thus helping and supporting all persons with the functional impairment electrohypersensitivity.

In addition to the studies in humans, we have also done a series of animal experiments (Rajkovic et al., 2005a,b, 2006). These have been a collaborative effort

between the Department of Biology, Faculty of Sciences, Novi Sad, Serbia and Montenegro, and my own research group at the Karolinska Institute, Stockholm, Sweden.

These papers go back to the above-mentioned early observations in people with the impairment electrohypersensitivity where large increases in the cutaneous mast cell count could be demonstrated as compared to normal healthy volunteers. A corresponding effect on cutaneous mast cells from normal healthy volunteers placed in front of ordinary TVs/PCs could also be shown. My working hypothesis since then is that electrohypersensitivity is a kind of irradiation damage, since the observed cellular changes are very much the same as the ones you would find in tissue subjected to UV-light or ionizing radiation (for references, see above).

One very fierce criticism from certain 'opponents' has been that such mast cell alterations in people with electrohypersensitivity (or in normal healthy volunteers) cannot be due to the action of electromagnetic fields (EMFs) and/or airborn chemicals, but must be due to psychological or psychiatric personality disturbances, cognitive malfunction, or likewise.

The aim of these studies has therefore been to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells, parafollicular cells, and nerve fibers in rat skin and thyroid gland, as seen using light and transmission electron microscopy. The experiments were performed on 2-month-old Wistar male rats exposed for 4 h a day, 5 or 7 days a week for 1 month to power-frequent (50 Hz) EMFs (100–300 μT, 54–160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and were then analyzed using the methods of stereology. Antibody markers to serotonin, substance P, calcitonin gene-related peptide (CGRP), and protein gene product 9.5 (PGP) were applied to skin sections and PGP, CGRP, and neuropeptide *Y* (NPY) markers to the thyroid. A significantly increased number of serotonin-positive mast cells in the skin ($p < 0.05$) and NPY-containing nerve fibers in the thyroid ($p < 0.01$) of rats exposed to ELF-EMF was found compared to controls, indicating a direct EMF effect on skin and thyroid vasculature.

After ultrastructural examination, a predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue, and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum, and cell nuclei compared to the control group were also observed. Taken together, the results of this study show the stimulative effect of power-frequency EMFs on thyroid gland at both the light microscopic and the ultrastructural level.

The obtained animal results cannot be understood by psychological or psychiatric theories, but are claimed to be due only to the EMF exposure.

In Sweden, electrohypersensitivity (EHS) is an officially fully recognized functional impairment (i.e., it is not regarded as a disease). Survey studies show that somewhere between 230,000–290,000 Swedish men and women report a variety of symtoms when being in contact with electromagnetic field (EMF) sources.

The electrohypersensitive people have their own handicap organization, The Swedish Association for the ElectroSensitive (http://www.feb.se; the website

has an English version). This organization is included in the Swedish Disability Federation (Handikappförbundens SamarbetsOrgan; HSO). HSO is the unison voice of the Swedish disability associations towards the government, the parliament, and national authorities, and is a cooperative body that today consists of 43 national disability organizations (where The Swedish Association for the ElectroSensitive is 1 of these 43 organizations) with all together about 500,000 individual members. You can read more on http://www.hso.se (the site has an English short version).

Swedish municipalities, of course, have to follow the UN 22 Standard Rules on the equalization of opportunities for people with disabilities ("Standardregler för att tillförsäkra människor med funktionsnedsättning delaktighet och jämlikhet"; about the UN 22 Standard Rules, see website: http://www.un.org/esa/socdev/enable/dissre00.htm). All people with disabilities shall, thus, be given the assistance and service they have the right to according to the Swedish Act concerning Support and Service for Persons with Certain Functional Impairments (LSS-lagen) and the Swedish Social Services Act (Socialtjänstlagen). People with disabilities, thus, have many different rights and can get different kinds of support. The purpose of those rights and the support is to give every person the chance to live like everyone else. Everyone who lives in the Swedish municipalities should be able to lead a normal life and the municipalities must have correct knowledge and be able to reach the people who need support and service. People with disabilities shall be able to get extra support so that they can live, work, study, or do things they enjoy in their free time. The municipalities are responsible for making sure that everyone gets enough support. Everyone shall show respect and remember that such men and women may need different kinds of support.

In Sweden, impairments are viewed from the point of the environment. No human being is in itself impaired, there are instead shortcomings in the environment that cause the impairment (as the lack of ramps for the person in a wheelchair or rooms electrosanitized for the person with electrohypersensitivity). This environment-related impairment view, furthermore, means that even though one does not have a scientifically based complete explanation for the impairment electrohypersensitivity, and in contrast to disagreements in the scientific society, the person with electrohypersensitivity shall always be met in a respectful way and with all necessary support with the goal to eliminate the impairment. This implies that the person with electrohypersensitivity shall have the opportunity to live and work in an electrosanitized environment.

This view can fully be motivated in relation to the present national and international handicap laws and regulations, including the UN 22 Standard Rules and the Swedish action plan for persons with impairments (prop. 1999/2000:79 "Den nationella handlingplanen för handikappolitiken – Från patient till medborgare"). Also, the Human Rights Act in the EU fully applies.

A person is disabled when the environment contains some sort of impediment. It means that in that moment a man or woman in a wheelchair cannot come onto the bus, a train, or into a restaurant, this person has a disability—he or she is disabled. When the bus, train, or restaurant are adjusted for a wheelchair, the person does not suffer from his disability and is consequently not disabled. An electrohypersensitive person suffers when the environment is not properly adapted according to their personal needs. Strategies to enable a person with this disability to attend common rooms such as libraries, churches, and so on, are, for

instance to switch off the high-frequency fluorescent lamps and instead use ordinary light bulbs. Another example is the possibility to switch off—the whole or parts of—the assistive listening systems (persons with electrohypersensitivity are often very sensitive to assistive listening systems).

In the Stockholm municipality—where I live and work as a scientist with the responsibility to investigate comprehensive issues for people with electrohypersensitivity—such persons have the possibility to get their home sanitized for EMFs. This means, for example, that ordinary electricity cables are changed to special cables. Furthermore, the electric stove can be changed to a gas stove and walls, roofs and floors can be covered with special wallpaper or paint with a special shelter to stop EMFs from the outside (from neighbors and mobile telephony base stations). Even the windows can be covered with a thin aluminum foil as an efficient measure to restrain EMFs to get into the room/home. If these alterations turn out not to be optimal they have the possibility to rent small cottages in the countryside that the Stockholm municipality owns. These areas have lower levels of irradiation than others. The Stockholm municipality also intend to build a village with houses that are specially designed for people who are electrohypersensitive. This village will be located in a low-lewel irradiation area. (One of my graduate students, Eva-Rut Lindberg, has in her thesis project studied the "construction of buildings for persons with the impairment electrohypersensitivity". The doctoral thesis will be presented during the spring.)

People with electrohypersensitivity also have a general (legal) right to be supported by their employer so that they can work despite of this impairment. For instance, they can get special equipment such as computers that are of low-emission type, high-frequency fluorescent lamps can be changed to ordinary light bulbs, wireless DECT telephones removed from their rooms, and so on.

Some hospitals in Sweden (e.g., in Umeå, Skellefteå, and Karlskoga) also have built special rooms with very low EMFs so that people who are hypersensitive can get medical care. Another example is the possibility for people who are electrohypersensitive to get a specially designed car so that the person can transport himself/herself between his/her home and their workplace.

Recently, some politicians in the Stockholm municipality even proposed to the politicians responsible for the subway in the Stockholm City that a part of every trainset should be free from mobile phones; that the commuters have to switch of the phones in these selected parts to enable people with electrohypersensitivity to travel with the subway (compare this with people who have an allergy for animal fur whereupon people consequently are prohibited to have animals, such as dogs or cats, in selected parts of the trainset).

In addition, when the impairment electrohypersensitivity is discussed it is also of paramount importance that more general knowledge is needed with the aim to better adapt the society to the specific needs of the persons with this impairment. The Swedish "Miljöbalk" (the Environmental Code) contains an excellent prudence avoidance principle which, of course, must be brought into action also here, together with respect and willingness to listen to the people with electrohypersensitivity.

Naturally, all initiatives for scientific studies of the impairment electrohypersensitivity must be characterized and marked by this respect and willingness to listen, and the investigations shall have the sole aim to help the persons with this particular impairment. Rule 13 in the UN 22 Standard Rules clearly says that scientific investigations of impairments shall, in an unbiased way—and without any

prejudice—focus on cause, occurrence and nature and with the sole and explicit purpose to help and support the person with the impairment. Nothing else!

In addition, it must also be mentioned that quite recently, by the end of 2004, The Irish Doctors' Environmental Association (IDEA) has announced that "they have identified a sub-group of the population who are particularly sensitive to exposure to different types of electromagnetic radiation. The safe levels currently advised for exposure to this non-ionising radiation are based solely on its thermal effects. However, it is clear that this radiation also has non-thermal effects, which need to be taken into consideration when setting these safe levels. The electrosensitivity experienced by some people results in a variety of distressing symptoms which must also be taken into account when setting safe levels for exposure to non-ionising radiation and when planning the siting of masts and transmitters" (IDEA, 2004).

Furthermore, the IDEA also points out the following:

1. An increasing number of people in Ireland are complaining of symptoms which, while they may vary in nature, intensity, and duration, can be demonstrated to be clearly related to exposure to electro-magnetic radiation (EMR).

2. International studies on animals over the last 30 years have shown the potentially harmful effects of exposure to electro-magnetic radiation. In observational studies, animals have shown consistent distress when exposed to EMR. Experiments on tissue cultures and rats have shown an increase in malignancies when exposed to mobile telephone radiation.

3. Studies on mobile telephone users have shown significant levels of discomfort in certain individuals following extensive use or even, in some cases, following regular short-term use.

4. The current safe levels for exposure to microwave radiation were determined based solely on the thermal effects of this radiation. There is now a large body of evidence that clearly shows that this is not appropriate, as many of the effects of this type of radiation are not related to these thermal effects (IDEA, 2004).

Finally, The IDEA "believes that the Irish Government should urgently review the information currently available internationally on the topic of the thermal and non-thermal effects of exposure to electro-magnetic radiation with a view to immediately initiating appropriate research into the adverse health effects of exposure to all forms of non-ionising radiation in this country, and into the forms of treatment available elsewhere. Before the results of this research are available, an epidemiological database should be initiated of individuals suffering from symptoms thought to be related to exposure to non-ionising radiation. Those claiming to be suffering from the effects of exposure to electro-magnetic radiation should have their claims investigated in a sensitive and thorough way, and appropriate treatment provided by the State. The strictest possible safety regulations should be established for the installation of masts and transmitters, and for the acceptable levels of potential exposure of individuals to electro-magnetic radiation, in line with the standards observed in New Zealand." (IDEA, 2004). Of course, these very recent findings must also be taken into serious consideration for any research proposal.

It may also be noted that a unique conference recently was held in Stockholm in May, 2006. The theme for the conference was "The right for persons with the impairment electrohypersensitivity to live in a fully accessible society". The conference was organized by the Stockholm City municipality and the Stockholm County Council and dealt with the most recent measures to make Stockholm fully accessible for persons with the impairment electrohypersensitivity. Among such measures are to offer home equipment adjustments and ban mobile phones from certain underground cars as well as certain public bus seats, and through electrosanitized hospital wards. The conference was documented on film.

The effects of various forms of electromagnetic fields are also discussed within areas of medicine, such as cancer. Cancer is, unfortunately, spreading in the modern society. Nearly all cancer forms are increasing when it comes to incidence, i.e., new cases/year (cf. Hallberg and Johansson, 2002a). It could recently be read in the BBC News that skin cancer is rising in young adults, and Sara Hiom, head of the health information at Cancer Research UK said, when interviewed, that "Non-melanoma cancers are rising at an alarming rate".

More and more research efforts goes into understanding the molecular mechanisms behind these various progressive cancer forms, and much more money is spent on finding new drugs to treat patients. However, oddly enough, very little is spent on understanding the actual causes for cancer. Among such possible causative agents, more and more focus is nowadays put on modern gadgets, such as mobile telephones and computers, and their chemical and physical emissions, including flame retardants and electromagnetic non-ionizing radiation.

Childhood leukemia was early connected to power-frequent magnetic fields already in the pioneering work by Wertheimer and Leeper (1979), and more recently, Scandinavian scientists have identified an increased risk for acoustic neuroma (i.e., a benign tumor of the eighth cranial nerve) in cell phone users, as well as a slightly increased risk of malignant brain tumors such as astrocytoma and meningioma on the same side of the brain as the cell phone was habitually held (Hardell et al., 1999, 2004, 2005; Lonn et al., 2004). In addition, a clear association between adult cancers and FM radio broadcasting radiation has been noticed, both in time and location (Hallberg and Johansson, 2002b, 2004a, 2005a). Initial studies on facial nevi indicates that nowadays young children also can have a substantial amount of these. If it can be shown that radiofrequent radiation is not correlated with child cancers, the current focus on low-frequency electromagnetic fields can continue. If there is also a radiofrequent and/or microwave correlation, then this must be considered in future research as well as in today's preventive work.

Most recently, Dr. Djemal Beniashvili and other scientists at the Edith Wolfson Medical Center in Holon, Israel, have demonstrated a possible link between exposure to power-frequent electromagnetic fields and breast cancer in elderly women (Beniashvili et al., 2005). They compared the breast cancer rates in elderly women from an earlier period (1978–1990) to a more recent period (1991–2003), which has been characterized by a much more extensive use of personal computers (more than three hours a day), mobile telephones, TV sets, and other household electrical appliances. They used available medical records extending over a period of 26 years, involving the analysis of more than 200,000 samples.

Among the elderly women who developed breast cancer in the first time frame, 20% were regularly exposed to power-frequent fields. But in the more modern period, 51% were so exposed, mainly through the use of personal

computers. The authors concluded: "There was a statistically significant influence of electromagnetic fields on the formation of all observed epithelial mammary tumours in the second group." This represented a more than two-fold increase, which was considered highly significant (cf. Beniashvili et al., 2005).

Of course, many other environmental factors have changed during the period 1978–1990, but increased environmental exposure to power-frequent fields is among the more conspicuous changes to have taken place. Naturally, there are many aspects of this question that remain to be clarified, and, from a scientific point of view, it is far from conclusively settled.

During the second half of the 20th century an increasing rate of lung cancer was noticed in Sweden. Since the mid-1960's, tobacco smoking has been associated with this cancer and believed to be the main cause. Less noticed, though, is the fact that no connection between smoking and lung cancer was noticed before 1955. Together with my co-worker Örjan Hallberg, we have therefore initiated a project with the intention to review facts that may shed new light on this sudden increase in getting lung cancer after 1955 in Sweden.

A large number of scientific reports point at tobacco smoking as being the main cause of the increasing rate of lung cancer in the world. These reports have mainly been produced during the second half of the 20th century. The Swedish National Board of Health and Welfare ("Socialstyrelsen") states that 80–90% of the lung cancer deaths are caused by smoking. The main part of the victims are also smokers. About 10% of the lung cancer deaths have been non-smokers. This has led to the suspicion that also passive smoking can cause lung cancer. Other environmental factors such as radon and asbestos are believed to cause a number of lung cancer deaths per year, and especially if combined with smoking.

As pointed out above, Hallberg and Johansson have earlier reported about a strong association between body-resonant non-ionizing radiation (FM-radio, 100 MHz) and the existence of malignant melanoma of the skin (Hallberg and Johansson, 2002b, 2004a, 2005a). Since this frequency range has a penetration depth of about 10 cm into the human body, there is a suspicion that resonant currents may affect the immune defense system also when it comes to beating cancer cells in the lungs. Due to that it is well motivated to study in detail how the presence and rate of lung cancer have changed in Sweden, and in other countries, as this new environmental factor was added.

In a yet unpublished report (Hallberg and Johansson, 2006), we have shown how the rate of lung cancer can accelerate in connection with a sudden exposure of a population to such body-resonant radiation. From this work, it can be noticed that people who have been smoking for many years suddenly could get lung cancer relatively short after the introduction of the FM-radio. This abrupt increase was not noticed in counties where the FM-radio still was not rolled out. It is also noticeable that deaths due to asbestosis have not been known until after the 1960's despite the fact that asbestos has been used as a building material since the end of the 19th century. In our work it is also shown how weak the connection is between lung cancer and cigarette consumption in a number of countries. But if the lung cancer mortality is normalized to the melanoma of skin mortality in the same countries, all of a sudden a very strong correlation appears. This indicates that there is a common factor behind the fast increasing mortality of skin and lung cancer that we have noticed, e.g., in Sweden.

An automated computer analysis of the age-specific incidence of lung cancer among men in Sweden points at year 1955 as the starting year for a sudden

environmental change in Sweden and that this disturbance mainly affects men over 60 years of age. This method of analysis has successfully been applied to study the development of melanoma of skin in Sweden, Norway, Denmark, Finland, and the U.S.

Authorities responsible for the health of the general population should have a big interest in causative factors behind such major cancer types. Doctors and specialists should know more about the real causes behind lung cancer. Epidemiologists in general might get inspired to test new methods and to look at population health problems from a new perspective. Only the future, however, will know the answer to these medical hypotheses.

Finally, as already mentioned, one issue that is very much addressed in the public as well as in the scientific literature is the question about the effect(s) of mobile phone radiation on health. Ten years of intensively increasing mobile phone usage have passed. According to some, it has facilitated our lifestyle, but more and more people are nowadays concerned about the lack of knowledge regarding the effects of radiation on health. For instance, it may be noted that mobile and DECT telephones are among the worst sources of problems for electrohypersensitive persons. In addition, it is now a well-known and fully accepted fact that mobile phone usage causes injuries in traffic and during work.

Mobile telephony-related risks may be divided between effects of radiation (microwaves, low-frequency magnetic fields) from the hand-held mobile telephone and radiation (microwaves) from remote base stations mounted on roofs, walls, towers, masts, etc.

Extensive laboratory research on animals, mainly rats, has not revealed premature death, increased cancer risk, or general sickness. However, very little can be drawn from this since rats and other laboratory animals have a maximal life span of approximately two years. The human cancer data point, instead, on an exposure time needed of at least five years, thus data from rats will not be of any real use. In addition, other biologic or metabolic parameters, as well as molecular biology and genetic data, are missing.

Epidemiological research with human case-control methodology suggests an increased risk for highly malignant brain lesions and acoustic neuromas after extended use (>5 years; Hardell et al., 1999, 2004, 2005; Lonn et al., 2004), but additional confirmation is needed. The present epidemiological surveillance thus indicates an increased risk for cancer in humans but observation times are too short. Ecological studies, in addition, suggest an increased general health degradation in areas of high average output power from the hand-held mobile phones (Hallberg and Johansson, 2004b,c,d, 2005b).

There is still insufficient contemporary proof with regard to increased cancer risk to change adult mobile phone usage. However, signs of degrading general health in sparsely populated areas suggest that the use of mobile phones at high output power levels should be avoided. Therefore, it is now of paramount importance that epidemiological research should be supplemented with prospective studies and quality exposure data (standardization). Continuous surveillance is also needed. In the meantime, children and adolescents should definitely be discouraged to use mobile phones.

It is a must that fully financied, truly independent research projects immediately should be initiated to ascertain the public health. They shall be completely devoid of commercial interests of any sort. This is the responsibility of each elected

government in each country, and is of special importance for people with the functional impairment electrohypersensitivity.

Acknowledgments

Supported by the Karolinska Institute, the Cancer and Allergy Foundation (Cancer- och Allergifonden), Sif, and TCO Developments.

References

Beniashvili, D., Avinoach'm, I., Baasov, D., Zusman, I. (2005). The role of household electromagnetic fields in the development of mammary tumors in women: clinical case-record observations. *Med. Sci. Monit.* 11:CR10–13.

Fews, A. P., Henshaw, D. L., Keitch, P. A., Close, J. J., Wilding, R. J. (1999a). Increased exposure to pollutant aerosols under high voltage power lines. *Int. J. Radiat. Biol.* 75:1505–1521.

Fews, A. P., Henshaw, D. L., Wilding, R. J., Keitch, P. A. (1999b). Corona ions from powerlines and increased exposure to pollutant aerosols. *Int. J. Radiat. Biol.* 75:1523–1531.

Gangi, S., Johansson, O. (1997). Skin changes in "screen dermatitis" versus classical UV- and ionizing irradiation-related damage—similarities and differences. Two neuroscientists' speculative review. *Exp. Dermatol.* 6:283–291.

Gangi, S., Johansson, O. (2000). A theoretical model based upon mast cells and histamine to explain the recently proclaimed sensitivity to electric and/or magnetic fields in humans. *Med. Hypotheses* 54:663–671.

Hallberg, Ö., Johansson, O. (2002a). Cancer trends during the 20th century. *J. Aust. Coll. Nutr. Env. Med.* 21:3–8.

Hallberg, Ö., Johansson, O. (2002b). Melanoma incidence and frequency modulation (FM) broadcasting. *Arch. Environ. Health* 57:32–40.

Hallberg, Ö., Johansson, O. (2004a). Malignant melanoma of the skin—not a sunshine story! *Med. Sci. Monit.* 10:CR336–340.

Hallberg, Ö., Johansson, O. (2004b). 1997—A curious year in Sweden. *Eur. J. Cancer Prev.* 13:535–538.

Hallberg, Ö., Johansson, O. (2004c). Long-term sickness and mobile phone use. *J. Aust. Coll. Nutr. Env. Med.* 23:11–12.

Hallberg, Ö., Johansson, O. (2004d). Mobile handset output power and health. *Electromag. Biol. Med.* 23:229–239.

Hallberg, Ö., Johansson, O. (2005a). FM broadcasting exposure time and malignant melanoma incidence. *Electromag. Biol. Med.* 24:1–8.

Hallberg, Ö., Johansson, O. (2005b). Alzheimer mortality—why does it increase so fast in sparsely populated areas? *Eur. Biol. Bioelectromag.* 1:225–246.

Hallberg, Ö., Johansson, O. (2006). Lung cancer may accelerate during exposure to body-resonant FM-broadcasting radiation. To be submitted.

Hardell, L., Näsman, Å., Påhlson, A., Hallquist, A., Hansson Mild, K. (1999). Use of cellular telephones and the risk for brain tumours: a case-control study. *Int. J. Oncol.* 15:113–116.

Hardell, L., Mild, K. H., Carlberg, M., Hallquist, A. (2004). Cellular and cordless telephone use and the association with brain tumors in different age groups. *Arch. Environ. Health* 59:132–137.

Hardell, L., Carlberg, M., Mild, K. H. (2005). Case-control study on cellular and cordless telephones and the risk for acoustic neuroma or meningioma in patients diagnosed 2000–2003. *Neuroepidemiology* 25:120–128.

Hilliges, M., Wang, L., Johansson, O. (1995). Ultrastructural evidence for nerve fibers within all vital layers of the human epidermis. *J. Invest. Dermatol.* 104:134–137.

Holmboe, G., Johansson, O. (2005). Symptombeskrivning samt förekomst av IgE och positiv Phadiatop Combi hos personer med funktionsnedsättningen elöverkänslighet, (= Description of symptoms as well as occurrence of IgE and positive Phadiatop Combi in persons with the physical impairment electrohypersensitivity, in Swedish). *Medicinsk Access* 1:58–63.

IDEA, The Irish Doctors' Environmental Association (2004). IDEA position on electromagnetic radiation. http://www.ideaireland.org/emr.htm.

Johansson, O., Liu, P.-Y. (1995). "Electrosensitivity", "electrosupersensitivity" and "screen dermatitis": preliminary observations from on-going studies in the human skin. In: Simunic, D., ed. *Proceedings of the COST 244: Biomedical Effects of Electromagnetic Fields – Workshop on Electromagnetic Hypersensitivity*. Brussels/Graz: EU/EC (DG XIII), pp. 52–57.

Johansson, O., Hilliges, M., Björnhagen, V., Hall, K. (1994). Skin changes in patients claiming to suffer from "screen dermatitis": a two-case open-field provocation study. *Exp. Dermatol.* 3:234–238.

Johansson, O., Hilliges, M., Han, S. W. (1996). A screening of skin changes, with special emphasis on neurochemical marker antibody evaluation, in patients claiming to suffer from screen dermatitis as compared to normal healthy controls. *Exp. Dermatol.* 5:279–285.

Johansson, O., Wang, L., Hilliges, M., Liang, Y. (1999). Intraepidermal nerves in human skin: PGP 9.5 immunohistochemistry with special reference to the nerve density in skin from different body regions. *J. Peripher. Nerv. Syst.* 4:43–52.

Johansson, O., Gangi, S., Liang, Y., Yoshimura, K., Jing, C., Liu, P.-Y. (2001). Cutaneous mast cells are altered in normal healthy volunteers sitting in front of ordinary TVs/PCs—results from open-field provocation experiments. *J. Cutan. Pathol.* 28:513–519.

Lonn, S., Ahlbom, A., Hall, P., Feychting, M. (2004). Mobile phone use and the risk of acoustic neuroma. *Epidemiology* 15:653–659.

Nordström, G. (2004). *The Invisible Disease—The Dangers of Environmental Illnesses caused by Electromagnetic Fields and Chemical Emissions*. Hants and New York: O Books.

Rajkovic, V., Matavulj, M., Johansson, O. (2005a). Histological characteristics of cutaneous and thyroid mast cell populations in male rats exposed to power-frequency electromagnetic fields. *Int. J. Radiat. Biol.* 81:491–499.

Rajkovic, V., Matavulj, M., Johansson, O. (2005b). The effect of extremely low-frequency electromagnetic fields on skin and thyroid amine- and peptide-containing cells in rats: an immunohistochemical and morphometrical study. *Environ. Res.* 99:369–377.

Rajkovic, V., Matavulj, M., Johansson, O. (2006). Light and electron microscopic study of the thyroid gland in rats exposed to power-frequency electromagnetic fields. *J. Exp. Biol.* 209:3322–3328.

Swedish Association for the Electrosensitive. http://www.feb.se

Swedish Disability Foundation. http://hso.se

United Nations General Assembly. (1993). The standard rules on the equalization of opportunities for persons with disabilities. http://www/un.org/esa/socdev/enable/dissre00.htm

Wang, L., Hilliges, M., Jernberg, T., Wiegleb-Edstrom, D., Johansson, O. (1990). Protein gene product 9.5-immunoreactive nerve fibres and cells in human skin. *Cell Tissue Res.* 261:25–33.

Wertheimer, N., Leeper, E. (1979). Electrical wiring configurations and childhood cancer. *Am. J. Epidemiol.* 109:273–284.

Yoshimura, K., Liang, Y., Kobayashi, K., Johansson, O. (2006). Alteration of the Merkel cell number in the facial skin of electrosensitive persons—a morphological study. To be submitted.

The Biological Effects of Weak Electromagnetic Fields

Andrew Goldsworthy, 2007

What the power and telecoms companies would prefer us not to know

Foreword

There have been many instances of harmful effects of electromagnetic fields from such seemingly innocuous devices as mobile phones, computers, power lines and domestic wiring. They include an increased risk of cancer, loss of fertility and unpleasant physiological symptoms. The power and mobile phone companies, hoping to avoid litigation, often assert that because the energy of the fields is too low to give significant heating, they cannot have any biological effect. However, the evidence that electromagnetic fields can have "non-thermal" biological effects is now overwhelming. In this article, I will explain how these effects arise. I have included key references that should enable the more inquisitive reader to delve deeper. If you do, you will often find contradictory assertions and that the reproducibility of several experiments is only mediocre. As we will see, this is almost certainly because of differences in the genetic and physiological condition of the biological material and its ability to defend itself against electromagnetic insults. Defence mechanisms have evolved by natural selection over countless millions of years of exposure to natural electromagnetic radiation, such as that from thunderstorms. They can often hide the underlying effects of man-made fields so we do not always see them in our experiments. We therefore have to concentrate on the experiments that give positive results if we are to discover the mechanisms. In this context, negative findings (frequently published in work financed by the telecoms and power companies) have no meaning.

Abstract

1. Well-replicated studies have shown that weak electromagnetic fields remove calcium ions bound to the membranes of living cells, making them more likely to tear, develop temporary pores and leak.

2. DNAase (an enzyme that destroys DNA) leaking through the membranes of lysosomes (small bodies in living cells packed with digestive enzymes) explains the fragmentation of DNA seen in cells exposed to mobile phone signals. When this occurs in the germ line (the cells that give rise to eggs and sperm), it reduces fertility and predicts genetic damage in future generations.

3. Leakage of calcium ions into the cytosol (the main part of the cell) acts as a metabolic stimulant, which accounts for reported accelerations of growth and healing, but it also promotes the growth of tumours.
4. Leakage of calcium ions into neurones (brain cells) generates spurious action potentials (nerve impulses) accounting for pain and other neurological symptoms in electro-sensitive individuals. It also degrades the signal to noise ratio of the brain making it less likely to respond adequately to weak stimuli. This may be partially responsible the increased accident rate of drivers using mobile phones.
5. A more detailed examination of the molecular mechanisms explains many of the seemingly weird characteristics of electromagnetic exposure, e.g. why weak fields are more effective than strong ones, why some frequencies such as 16Hz are especially potent and why pulsed fields do more damage.

Introduction

The strange non-thermal biological effects of electromagnetic fields have puzzled scientists for decades and, until now, there has been no clear explanation. In this article, I will outline a new theory, based on experimental evidence gathered over many years, that explains how virtually all of these effects arise.

Firstly, it is not only humans that are affected. Well-researched responses in other organisms include the more rapid growth of higher plants (Smith et al. 1993; Muraji et al. 1998; Stenz et al. 1998), yeast (Mehedintu and Berg 1997) and changes in the locomotion of diatoms (McLeod et al. 1987). The last two are significant because they are both single cells, implying that the effects occur at the cellular level. Furthermore, we can explain virtually all of the electromagnetic effects on humans in terms of changes occurring at the cellular level that may then affect the whole body.

A few basic facts

Field strength: An electromagnetic field consist of an electrical, part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/metre. The magnetic part is generated by any flow of current and is measured in tesla. For example, standing under a power line would expose you to an electrical voltage gradient due to the difference between the voltage of the line (set by the power company) and earth. You would also be exposed to a magnetic field proportional to the current actually flowing through the line, which depends on consumer demand. Both types of field give biological effects, but the magnetic field is more damaging since it penetrates living tissue more easily. Magnetic fields as low as around one microtesla (a millionth of a tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a PDA exposes you to magnetic pulses that peak at several tens of microtesla (Jokela et al. 2004; Sage et al. 2007), which is well over the minimum needed to give harmful effects. Because mobile phones are held

close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses.

Frequency: The fields must vary with time, e.g. those from alternating currents, if they are to have biological effects. Extremely low frequencies (ELF) such as those from power-lines and domestic appliances are more potent than higher frequencies. There is usually little or no biological response to the much higher frequencies of radio waves, unless they are pulsed or amplitude modulated at a biologically active lower frequency (i.e. when the radio signal strength rises and falls in time with the lower frequency). Regular GSM mobile phones and PDAs emit both pulsed radio waves (from the antenna) and ELF (from the battery circuits), and are especially dangerous. So how do these non-thermal effects electromagnetic fields arise?

Weak electromagnetic fields release calcium from cell membranes

The first clue came from Suzanne Bawin, Leonard Kaczmarek and Ross Adey (Bawin et al. 1975), at the University of California. They found that exposing brain tissue to weak VHF radio signals modulated at 16Hz (16 cycles per second) released calcium ions (electrically charged calcium atoms) bound to the surfaces of its cells. Carl Blackman at the U.S. Environmental Protection Agency in North Carolina followed this up with a whole series of experiments testing different field-strengths and frequencies (Blackman et al. 1982) and came to the surprising conclusion that weak fields were often more effective than strong ones. The mechanism was unknown at the time and it was thought to be a trivial scientific curiosity, but as we will see, it has huge significance for us all.

The loss of calcium makes cell membranes leak

Calcium ions bound to the surfaces of cell membranes are important in maintaining their stability. They help hold together the phospholipid molecules that are an essential part of their make-up (see Ha 2001 for a theoretical treatment). Without these ions, cell membranes are weakened and are more likely to tear under the stresses and strains imposed by the moving cell contents (these membranes are only two molecules thick!). Although the resulting holes are normally self-healing they still increase leakage while they are open and this can explain the bulk of the known biological effects of weak electromagnetic fields.

Membrane leakage damages DNA

Leaks in the membranes surrounding lysosomes (tiny particles in living cells that recycle waste) can release digestive enzymes, including DNAase (an enzyme that destroys DNA). This explains the serious damage done to the DNA in cells by mobile phone signals. Panagopoulos et al. (2007) showed that exposing adult Drosophila

melanogaster (an insect widely used in genetic experiments) to a mobile phone signal for just six minutes a day for six days broke into fragments the DNA in the cells that give rise to their eggs and half of the eggs died. Diem et al. (2005) also found significant DNA fragmentation after exposing cultured rat and human cells for 16 hours to a simulated mobile phone signal. See also the 'Reflex Project' in an on-line brochure entitled *Health and Electromagnetic Fields* published by the European Commission. You can find it at http://tinyurl.com/yxy4ld. It shows that exposing human cells for 24 hours to simulated mobile phone signals gave DNA fragmentation similar to that due to the gamma rays from a radioactive isotope! (Gamma rays also make lysosome membranes leak.)

DNA damage may cause cancer

There have been many studies suggesting that exposure to weak electromagnetic fields is associated with a small but significant increase in the risk of getting cancer (Wilson et al. 1990). This could be caused by gene mutations resulting from DNA damage. A gene is a section of DNA containing the information needed to make a particular protein or enzyme. There is also a section that can turn the gene on or off in response to outside signals. The growth of an organism from a fertilised egg involves a hugely complex pattern of switching genes on and off that regulates growth, cell division and differentiation into specific tissues. DNA damage can sometimes give unregulated growth to form tumours. However, the effect may not be immediate. Cancer following exposure to chemical carcinogens such as asbestos may take many years to become rampant. The affected cells seem to go through several stages of ever-increasing genetic and molecular anarchy before they finally reach the point of unstoppable growth and division. When assessing any carcinogenic effects of electromagnetic exposure, we must bear in mind that there may be a similar delay. It may be some years before we know the full carcinogenic effects of the recent explosive growth in the use of mobile phones.

DNA damage reduces fertility

The biological effects of electromagnetically induced DNA fragmentation may not be immediately obvious in the affected cells, since fragments of broken DNA can be rejoined and damaged chromosomes (elongated protein structures that carry the DNA) can be reconstituted. However, there is no guarantee that they will be rejoined exactly as they were. Pieces may be left out (deletions) joined in backwards (inversions) swapped between different parts of the chromosome (translocations) or even attached to the wrong chromosome. In most cases, the new arrangement will work for a while if most of the genes are still present and any metabolic deficiencies can often be made good by the surrounding cells. However, things go badly wrong when it comes to meiosis, which is the process that halves the number of chromosomes during the formation of eggs and sperm.

During meiosis, the chromosomes line up in pairs (one from each original parent) along their entire length so that corresponding parts are adjacent and can be exchanged (this gives each of the daughter cells a unique combination of genes). However, if the arrangement of their genes has been altered by electromagnetic exposure, they cannot align properly and the chromosomes may even tie themselves in knots in the attempt. Such malformed pairs are usually torn apart unequally in the later stages of meiosis so that the eggs or sperm have an incomplete or unbalanced set of genes, may not function properly and so reduce fertility. There is evidence from several independent studies in Australia, Hungary and the United States that this is already occurring. Heavy mobile phone use appears to reduce both the quantity and viability of sperm. The results for the most recent study by Dr Ashok Agarwal and co-workers at the Cleveland Lerner College of Medicine can be seen at http://tinyurl.com/28rm6n. They found that using a mobile phone for more than four hours a day was associated with a reduction in sperm viability and mobility of around 25 percent. The statistical probability of these results being due to chance errors was one in a thousand. There is every reason to believe that human eggs may be similarly affected, but since they are formed in the embryo before the baby is born, the damage will be done during pregnancy but will not become apparent until the child reaches puberty.

There may also be permanent genetic damage

Believe it or not, the electromagnetically induced loss of fertility is the *good news* since it means that badly damaged embryos are less likely to be conceived. The *bad news* is that any damaged genes needed for embryo development but not for normal egg or sperm function will not be weeded out in this way. They can still find their way into the foetus and cause permanent genetic damage. The effect may not be apparent in the first generation since a non-functioning gene from one parent can often be offset if the other parent provides a good version of the same gene. In fact, serious trouble may not arise for many generations until by chance two faulty versions of the same gene end up in the same foetus. What happens then depends on the gene concerned, but it is unlikely to be beneficial and may be lethal.

The overall conclusion is that the genetic damage from exposure to electromagnetic radiation can have an almost immediate effect on fertility, but damage to the offspring may take several generations to show up. If we do nothing to limit our exposure to electromagnetic radiation, we can anticipate a slow decline in the viability of the human genome for many generations to come. It is ironic that having only just discovered the human genome, we have already set about systematically destroying it.

Effects on metabolism

Another major effect of electromagnetic radiation is the leakage of *free* calcium ions, either through the cells' external membranes or those surrounding internal 'calcium stores'. This can have dramatic effects on many aspects of metabolism and explains

most of the mysterious but well-documented physiological effects of electromagnetic fields. These include stimulations of growth, an increased risk of cancer, symptoms suffered by electrosensitive humans and why using a mobile phone while driving makes you four times more likely to have an accident.

How calcium controls metabolism

Apart from its role in maintaining membrane stability, the calcium concentration actually inside cells controls the rate of many metabolic processes, including the activity of many enzyme systems and the expression of genes. The concentration of calcium ions in the cytosol (the main part of the cell) is normally kept about a thousand times lower than that outside by metabolically-driven ion pumps in its membranes. Many metabolic processes are then regulated by letting small amounts of calcium into the cytosol when needed. This is normally under very close metabolic control so that everything works at the right time and speed. However, when electromagnetic exposure increases membrane leakiness, unregulated amounts of extra calcium can flood in. Just what happens then depends on how much gets in and what the cells are currently programmed to do. If they are growing, the rate of growth may be increased. If they are repairing themselves after injury, the rate of healing may be increased but if there is a mutant precancerous cell present, it may promote its growth into a tumour.

Calcium leakage and brain function

Normal brain function in humans depends on the orderly transmission of signals through a mass of about 100 billion *neurones*. Neurones are typically highly branched nerve cells. They usually have one long branch (the *axon*), which carries electrical signals as *action potentials* (nerve impulses) to or from other parts of the body or between relatively distant parts of the brain (a nerve contains many axons bundled together). The shorter branches communicate with other neurones where their ends are adjacent at *synapses*. They transmit information across the synapses using a range of *neurotransmitters*, which are chemicals secreted by one neurone and detected by the other. The exact patterns of transmission through this network of neurones are horrendously complex and determine our thoughts and virtually everything we do.

Calcium plays an essential role in this because a small amount of calcium must enter the neurone every time before it can release its neurotransmitters. Without it, the brain would be effectively dead. But what would happen if electromagnetically induced membrane leakage let in too much calcium? One effect would be to increase the background level of calcium in the neurones so that they release their neuro-transmitters sooner. This improves our reaction time to simple stimuli (which has been experimentally proven). However, it can also trigger the spontaneous release of neurotransmitters to transmit spurious signals have no right to be there. This feeds the brain false information. Similar spurious action potentials may also be triggered in other parts of the neurone if leaks in the membrane temporarily short-circuit the

normal voltage between its inside and outside. These unprogrammed action potentials will degrade the signal to noise ratio of the brain and reduce its ability to make accurate judgements.

It is technically difficult to detect these stray action potentials experimentally since they look like random noise in the measuring system and would in any case be swamped by the relatively strong electromagnetic signals used to induce them. However, similar spurious action potentials should be detectable if we removed some of structural calcium from the membrane by some other means. One way to do this is to lower the concentration of calcium ions in the surrounding medium. For example, Matthews (1986) reported that exposing nerve and muscle cells to calcium concentration about 10–20 percent below normal made them significantly more excitable, which fits with our hypothesis.

These findings also explain many of the symptoms of hypocalcemia (alias hypocalcaemia). Hypocalcemia is a medical condition, usually caused by a hormone imbalance, in which the concentration of ionised calcium in the blood is abnormally low. By removing bound calcium from cell membranes, it should (and does) give similar effects to electromagnetism.

Electrosensitivity and hypocalcemia – a possible cure

Symptoms of hypocalcemia include skin disorders, paresthesias (pins and needles, numbness, sensations of burning etc.) fatigue, muscle cramps, cardiac arrhythmia, gastro-intestinal problems and many others. A more comprehensive list can be found at http://tinyurl.com/2dwwps, which corresponds to the website: www.endotext.org/parathyroid/parathyroid7/parathyroid7.htm.

The symptoms of hypocalcemia are remarkably similar to those of electrosensitivity. If you think you may be electrosensitive, how many of these do you have? If you have any of them, it may be worth having your blood checked for ionised calcium. It is possible that at least some forms of electrosensitivity could be due to the victims having their natural blood calcium levels bordering on hypocalcemia. Electromagnetic exposure would then remove even more calcium from their cell membranes to push them over the edge and give them symptoms of hypocalcemia. If this is correct, conventional treatment for hypocalcaemia may relieve some if not all of these symptoms.

Electromagnetic exposure and motor accidents

Only a small proportion of the population is electrosensitive in that they show obvious symptoms from electromagnetic exposure. However, everyone may affected without being aware of it, e.g. when using a mobile phone. According to the Royal Society for the Prevention of Accidents, you are four times more likely to have an accident if you use a mobile phone while driving. This is not due to holding the phone since using a hands-free type makes no difference. It is also not due to the distraction

of holding a conversation, since talking to a passenger does not have the same effect. This leads us to the conclusion that the electromagnetic radiation from the phone is the most likely culprit.

This fits with the notion that spurious action potentials triggered by electromagnetic radiation creates a sort of 'mental fog' of false information that makes it harder for the brain to recognise weak but real stimuli. For example, a driver using a mobile phone may still see the road ahead using the strong images from the central part of the eye but may be less aware of weaker but still important images coming from the side. He may also be less able to conduct relatively complex tasks such as judging speed and distance in relation to other moving vehicles. This needs a lot of 'computing power' and will therefore be more susceptible to random interference. Although an experienced driver may do much of his driving automatically, his brain still has to do just as much work as if he were still learning; it is just that he is unaware of it. Therefore, an old hand at driving is just as likely to be forced into making a mistake when using a mobile while driving as a novice, so don't imagine you can get away with it just because you have been driving for years. Another important point is that, if this theory is correct, and the electromagnetic signal is mainly to blame, not only is it inadvisable to use a mobile yourself while driving, but your passengers should not use them either since their radiation may still affect *your own* driving.

The theory behind it all

We have seen that weak electromagnetic fields can remove calcium from cell membranes and make them leak. If we theorise about the mechanism, we can explain many of the seemingly weird characteristics of bioelectromagnetic responses. These include why weak fields can be more effective than strong ones, why low frequencies are more potent, why pulses do more damage than sine waves and what is special about 16Hz. The following hypothesis was proposed by Goldsworthy (2006).

The role of eddy currents

Before they can give biological effects, the electromagnetic fields must generate electrical 'eddy currents' flowing in and around the cells or tissues. Both the electrical and magnetic components of the fields can induce them and they tend to follow low impedance pathways. These can be quite extensive; for example in the human body, the blood system forms an excellent low resistance pathway for DC and low frequency AC. It is an all-pervading system of tubes filled with a highly conductive salty fluid. Even ordinary tissues carry signals well *at high frequencies* since they cross membranes easily via their capacitance. In effect, the whole body can act as an efficient antenna to pick up electromagnetic radiation. If you need convincing, try a simple experiment. Tune in a portable radio to a weak station and see by how much you can improve reception by simply grasping the antenna. There is little doubt that signals transmitted by a mobile phone, even if it is a hands-free type, will reach all parts of the body, including the sex organs.

How calcium is released

The membrane: Most biological membranes are negatively charged, which makes them attract and adsorb positive ions. However, these ions are not stuck permanently to the membrane but are in dynamic equilibrium with the free ions in the environment. The relative amounts of each kind of ion attached at any one time depends mainly on its availability in the surroundings, the number of positive charges it carries and its chemical affinity for the membrane. Calcium normally predominates since it has a double positive charge that binds it firmly to the negative membrane. Potassium is also important since, despite having only one charge, its sheer abundance ensures it a good representation (potassium is by far the most abundant positive ion in virtually all living cells and outnumbers calcium by about ten thousand to one in the cytosol).

The signal: When an alternating electrical field from an eddy current hits a membrane, it will tug the bound positive ions away during the negative half-cycle and drive them back in the positive half-cycle. If the field is weak, strongly charged ions (such as calcium with its double charge) will be preferentially dislodged. Potassium (which has only one charge) will be less attracted by the field and mostly stay in position. Also, the less affected free potassium will tend to replace the lost calcium. In this way, weak fields increase the proportion of potassium ions bound to the membrane, and release the surplus calcium into the surroundings.

Why there are amplitude windows

The main effect, electromagnetic treatment is to change the normal chemical equilibrium between bound calcium and potassium in favour of potassium. Even very weak fields should have at least some effect. This effect should increase with increasing field-strength, but only up to a point. If the field were strong enough to dislodge large quantities of potassium too, there will be less discrimination in favour of calcium. This gives an *amplitude window* for the *selective* release of calcium, above and below which there is little or no observable effect.

The field strength corresponding to the amplitude window may vary with the ease with which eddy currents are induced and the nature and physiological condition of the tissue. There may also be more than one in any given tissue. Blackman et al. (1982) discovered at least two for brain slices, perhaps because the brain contains two main types of cell; the neurones and the glial cells, each of which have different membrane compositions.

Why low frequencies and pulses work better

The hypothesis also explains why only frequencies from the low end of the spectrum give biological effects and why pulses and square waves are more effective than sine waves. Only if the frequency is low will the calcium ions have time to be pulled clear

of the membrane and replaced by potassium ions before the field reverses and drives them back. Pulses and square waves work best because they give very rapid changes in voltage that catapult the calcium ions well away from the membrane and then allow more time for potassium to fill the vacated sites. Sine waves are smoother, spend less time at maximum voltage, and so allow less time for ion exchange.

Frequency windows

The hypothesis also explains the curiosity that some frequencies are especially effective, with 16Hz being the most obvious. This is because 16Hz is the ion cyclotron resonance frequency for potassium in the Earth's magnetic field (see Box). When exposed to an electromagnetic field at this frequency, potassium ions resonate, absorb the field's energy and convert it to energy of motion. This increases their ability to replace calcium on cell membranes. Although the extra energy gained by each potassium ion may be small, the fact that there are about ten thousand of them competing with just one calcium ion for each place on the membrane means that even a slight increase in their energies due to resonance will have a significant effect.

Ion Cyclotron Resonance

Abraham Liboff, in the mid 1980s, developed the idea that the frequency windows for the biological effects of electromagnetic fields were in some way due to ion cyclotron resonance, but he didn't link it to membrane stability (Liboff et al.1990). Ion cyclotron resonance occurs when ions move in a steady magnetic field such as that of the Earth. The field deflects them sideways and they go into orbit around its lines of force at a characteristic 'resonant' frequency, which depends on the charge/mass ratio of the ion and the strength of the steady field. Exposing them to an oscillating electric or a magnetic field at their resonant frequency lets them absorb its energy and they gradually increase the size of their orbits and their energy of motion. The resonant frequency for potassium in the Earth's magnetic field is close to 16Hz. According to my hypothesis, electromagnetic fields at this frequency specifically increase the ability of potassium ions to bombard cell membranes and replace bound calcium. This increases the biological hazards of electromagnetic exposure near 16Hz and has already caused concern about the safety of the TETRA mobile telecommunications system, which transmits pulses at 17.6Hz.

Amplitude modulated and pulsed radio waves also work

Amplitude modulated and pulsed radio waves consist of a high frequency 'carrier' wave whose strength rises and falls in time with a lower frequency signal. This is the basis of AM radio transmissions, where the low frequency signal comes from an audio source. The receiver demodulates the signal to regenerate the audio. Unmodulated carrier waves usually have little or no biological effect, but if modulated at a biologically-active low frequency (such as 16Hz) they give marked effects (Bawin et al. 1975). This has posed problems for scientists trying to work out how living cells

could demodulate radio signals to regenerate the low frequency and elicit a biological response.

However, we can now explain it easily. Imagine a child bouncing a ball continuously against the ground. The harder he hits it, the higher it bounces and the greater its average height. The layer of free positive ions that congregate near but are not bound to the negatively charged surface of a cell membrane will behave in the same way. They bounce against the membrane in time with the radio wave, and the average distance of the electrical centre of the layer from the membrane rises and falls with any amplitude modulation. For example, modulating the signal at 16Hz makes the centre of the layer rise and fall at 16Hz. It does not have to move very far at this frequency, since any free potassium ions in the vicinity will resonate, gradually gain energy from the oscillations and become more able to bombard and displace calcium ions bound to the membrane. Non-resonant frequencies need a stronger signal but can give a similar effect.

Continuous waves can also work

This is probably because living cells can introduce their own time variation in field strength. The membrane systems in active living cells are constantly on the move, e.g. from the Brownian motion of membrane-bound particles (a purely physical process due to molecular bombardment) and physiological processes such as their active transport. This exposes any given section of their membranes to a full frontal attack by the field in one orientation followed by a much quieter period if it rotates through 90 degrees and receives the signal edge-on. This means that it experiences what looks (to it) like a time-varying field and may therefore give a physiological response even to a constant radio signal. However, because these are random changes and are not sharply pulsed, we might expect them to need stronger fields and/or longer exposure times if they are to give effects. This may explain the unpleasant symptoms experienced by many electrosensitive individuals when using UMTS (3G) handsets or living close to high power TETRA base stations. Although neither signal is pulsed, the sheer proximity of the UMTS handset to the user and the raw power of a nearby TETRA base station may give the necessary signal strength. In addition, the lack of any quiet gaps in the signal increases the net exposure time, which may more than compensate for the lack of pulses.

How calcium loss makes holes in membranes

Cell membranes are made of sheets of fatty materials called phospholipids surrounding islands of protein. The proteins have a variety of metabolic functions, but the main role of the phospholipids is to fill the spaces between them and act as a barrier to prevent leakage. Calcium loss weakens the phospholipid sheet and makes it more likely to leak; but how does it do this?

The membrane phospholipids are long molecules. One end consists of hydrophobic (water hating) hydrocarbon chains. The other end has a negatively charged phosphate group and is hydrophilic (water loving). In a watery medium, they arrange themselves spontaneously to form double-layered membranes with a central core made from their water hating ends. Their water loving phosphate ends face outwards towards the water. The affinity that the central hydrophobic parts have for one another helps hold the membrane together but the negatively charged phosphate groups on the outside repel each other and try to tear it apart. Normally, the membrane is stabilised by positive ions that fit in between the negative phosphate groups, so that they do not repel each other. They act as a kind of cement that helps to hold the membrane together.

However, not all positive ions stabilise the membrane equally well. Calcium ions are particularly good because of their double positive charge, but monovalent potassium, with just one charge, is only mediocre. Therefore, when electromagnetic fields swap membrane-bound calcium for potassium, it weakens the membrane (These membranes are only a hundred thousandth of a millimetre thick) and it becomes more prone to accidental tearing and the formation of transient pores. This happens to some degree all the time, even in stationary artificial membranes (Melikov et al. 2001), but the membranes of living cells are often stressed by the cells' moving contents, so the effects should be much greater. Fortunately, these pores are usually self-healing and the damage to the membrane is not permanent. However, during electromagnetic exposure there will be more tears, slower repair and consequently more overall leakage. The metabolic effects of even a brief period of leakage may be much longer lasting (e.g. if dormant genes are activated) and perhaps (as in the case of DNA damage) permanent.

Defence mechanisms

Calcium pumps: Cells have to be able to pump out any extra calcium that has entered their cytosols to reset the low cytosolic calcium level every time it is disturbed by a programmed calcium influx. They should therefore be able to respond to unprogrammed calcium influx due to electromagnetic exposure. This should minimise any unwanted metabolic effects, but the scope to do this is limited. If it were too effective, it would also prevent legitimate cell signalling.

Gap junction closure: If calcium extrusion fails and there is a large rise in internal calcium, it triggers the isolation of the cell concerned by the closure of its gap junctions (tiny strands of cytoplasm that normally connect adjacent cells) (Alberts et al. 2002). This also limits the flow of eddy currents through the tissue and so reduces the effects of radiation.

Heat shock proteins: These were first discovered after exposing cells to heat, but they are also produced in response to a wide variety of other stresses, including weak electromagnetic fields. They are normally produced within minutes of the onset of the stress and combine with the cell's enzymes to protect them from damage and shut

down non-essential metabolism (the equivalent of running a computer in 'safe mode'). When the production of heat shock proteins is triggered electromagnetically it needs 100 million million times less energy than when triggered by heat, so the effect is truly non thermal (Blank & Goodman 2000). Their production in response to electromagnetic fields is activated by special base sequences (the nCTCTn motif) in the DNA of their genes. When exposed to electromagnetic fields, they initiate the gene's transcription to form RNA, which is the first stage in the synthesis of the protein (Lin et al. 2001).

As we can see, there are several defence mechanisms against damage by electromagnetic fields and there may be more we do not know about. They probably evolved in response to natural electromagnetic fields such as those generated by thunderstorms but are now having their work cut out to respond to the continuous and all-pervading fields associated with modern living. How well they perform will depend on many factors, including environmental conditions, the physiological condition of the cells and how much energy they have to spare. Consequently, they do not always succeed. When the defences fail, we may get visible symptoms from the radiation, but when they succeed, there may be little obvious effect.

The power and mobile phone companies have seized upon this characteristic variability to discredit work on the non-thermal effects of electromagnetic fields as being due to the experimental error. Nothing could be further from the truth. Many of these experiments are highly reproducible, especially the fundamental and all-important ones on the effects of the radiation on the release of calcium from cell membranes. Secondary effects further down the line may be less reproducible since they are more likely to be mitigated by the intervention of cellular defence mechanisms. Therefore, we cannot expect rigidly reproducible results in all circumstances any more than we can expect everyone to experience exactly the same side effects from taking a medicinal drug. However, that does not mean that they can be safely ignored!

Conclusion

In the latter part of this article, I have explained how weak electromagnetic fields can interact with cell membranes to weaken them and make them more permeable. As with all theories, it will be subject to modification and refinement as time goes by, but some facts are already inescapable. There is undeniable experimental proof that weak electromagnetic fields can remove bound calcium ions from cell membranes. There is also no doubt that bound calcium ions are essential for the stability of these membranes. Consequently, their loss will increase temporary pore formation under the mechanical stresses from pressure differences within the cell and abrasion by its moving contents. This very simple conclusion can account for virtually all of the known biological effects of electromagnetic fields, including changes in metabolism, the promotion of cancer, genetic damage, loss of fertility, deleterious effects on brain function and the unpleasant symptoms experienced by electrosensitive individuals.

However, it seems possible that at least some cases electrosensitivity could be due to low levels of ionised calcium in the blood exacerbating the electromagnetic effects. If so, it may be possible to relieve some or all of the symptoms by conventional treatment for hypocalcemia.

Footnote

Andrew Goldsworthy is an Honorary Lecturer at Imperial College London.

References

Adey WR (1990) 'Electromagnetic fields, cell membrane amplification, and cancer promotion'. In: Wilson BW, Stevens RG, Anderson LE (eds) *Extremely Low Frequency Electromagnetic Fields: the Question of Cancer*. Battelle Press, Columbus, Ohio, pp 211-249

Alberts et al. (2002) *Molecular Biology of the Cell*. (Garland Science, New York)

Bawin SM, Kaczmarek KL, Adey WR (1975) *Effects of modulated VHF fields on the central nervous system*. Ann. N.Y. Acad Sci 247: 74-81

Bawin SM, Adey WR (1976) 'Sensitivity of calcium binding in cerebral tissue to weak environmental electric fields oscillating at low frequency'. *Proc Nat Acad Sci* USA 73: 1999-2003

Blackman CF (1990) 'ELF effects on calcium homeostasis'. In: Wilson BW, Stevens RG, Anderson LE (eds) *Extremely Low Frequency Electromagnetic Fields: the Question of Cancer*. Battelle Press, Columbus, Ohio, pp 189-208

Blackman CF, Benane SG, Kinney LS, House DE, Joines WT (1982) 'Effects of ELF fields on calcium-ion efflux from brain tissue in vitro'. *Radiat. Res.* 92: 510-520

Blank M, Goodman R (2000) 'Stimulation of stress response by low frequency electromagnetic fields: possibility of direct interaction with DNA'. *IEEE Trans Plasma Sci* 28: 168-172

Diem E, Schwarz C, Aldkofer F, Jahn O, Rudiger H (2005) 'Non-thermal DNA breakage by mobile phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro'. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* 583: 178-183

Goldsworthy A (2006) 'Effects of electrical and electromagnetic fields on plants and related topics'. In: Volkov AG (ed.) *Plant Electrophysiology – Theory & Methods* . Springer-Verlag Berlin Heidelberg 2006. Pp 247-267.

Ha B-Y (2001) 'Stabilization and destabilization of cell membranes by multivalent ions'. *Phys. Rev*. E. 64: 051902 (5 pages)

Jokela K, Puranen L, Sihvonen A-P (2004) 'Assessment of the magnetic field exposure due to the battery current of digital mobile phones'. *Health Physics* 86: 56-66.

Liboff AR, McLeod BR, Smith SD (1990) 'Ion cyclotron resonance effects of ELF fields in biological systems'. In: Wilson BW, Stevens RG, Anderson LE (eds) *Extremely Low Frequency Electromagnetic Fields: the Question of Cancer*. Battelle Press, Columbus, Ohio, pp 251-289

Lin H, Blank M, Rossol-Haseroth K, Goodman R (2001) 'Regulating genes with electromagnetic response elements'. *J Cellular Biochem* 81: 143-148

Matthews EK (1986) 'Calcium and membrane permeability'. *British Medical Bulletin* 42: 391-397

McLeod BR, Smith SD, Liboff AR (1987) 'Potassium and calcium cyclotron resonance curves and harmonics in diatoms (A. coffeaeformis)'. *J Bioelectr* 6: 153-168

Mehedintu M, Berg H (1997) 'Proliferation response of yeast Saccharomyces cerevisiae on electromagnetic field parameters'. *Bioelectrochem Bioenerg* 43: 67-70

Melikov KC, Frolov VA, Shcherbakov A, Samsonov AV, Chizmadzhev YA, Chernomordik LV (2001) 'Voltage-induced nonconductive pre-pores and metastable single pores in unmodified planar lipid bilayer'. *Biophys J* 80: 1829-1836

Muraji M, Asai T, Wataru T (1998) 'Primary root growth rate of Zea mays seedlings grown in an alternating magnetic field of different frequencies'. *Bioelectrochem Bioenerg* 44: 271-273

Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH (2007) 'Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation'. *Mutation Research* 626: 69-78

Sage C, Johansson O, Sage SA (2007) 'Personal digital assistant (PDA) cell phone units produce elevated extremely low frequency electromagnetic field emissions'. *Bioelectromagnetics*. DOI 10.1002/bem.20315 Published online in Wiley InterScience (www.interscience.wiley.com)

Smith SD. McLeod BR, Liboff AR (1993) 'Effects of SR tuning 60Hz magnetic fields on sprouting and early growth of Raphanus sativus'. *Bioelectrochem Bioenerg* 32: 67-76

Stenz H-G, Wohlwend B, Weisenseel MH (1998) 'Weak AC electric fields promote root growth and ER abundance of root cap cells'. *Bioelectrochem Bioenerg* 44: 261-269

Wilson BW, Stevens RG, Anderson LE eds (1990) *Extremely low frequency electromagnetic fields: the question of cancer*. Battelle Press, Columbus, Ohio

© Med Sci Monit, 2006; 12(10): RA235-240
PMID: 17006416

www.MedSciMonit.com
Review Article

Received: 2006.06.19
Accepted: 2006.08.24
Published: 2006.10.01

Pesticides may be altering constitutive nitric oxide release, thereby compromising health

Federico M. Casares[1], Kirk J. Mantione[2]

[1] Marine Sciences Research Center, Stony Brook University, Stony Brook, New York, U.S.A.
[2] Neuroscience Research Institute, State University of New York at Old Westbury, Old Westbury, NY, U.S.A.

Source of support: Dr. Casares is supported by a Turner Postdoctoral Faculty Traineeship Program of Stony Brook University

Summary

Numerous studies, focusing on the effects of environmental pollutants such as pyrethroids, organochlorines, organophosphate pesticides, etc, appear to affect intracellular ion balance, particularly calcium. We speculate that these chemicals may specifically target constitutive nitric oxide synthase (cNOS)-mediated processes, e.g., immune. The alteration of intracellular Ca^{2+} transients by these pollutants may represent an important common mechanism responsible for the abrogation of cNOS activation. Moreover, one can hypothesize that exposure to sublethal levels of pesticides that alter calcium transients, could potentially lead to immune, neural and vascular dysfunction in animals. This may be especially true for marine organisms that can be found close to shore and in estuaries, which are more likely to be exposed to these compounds resulting from riverine and other inputs.

key words: **environmental pollutants • pesticides • calcium • nitric oxide • constitutive nitric oxide synthase • basal NO levels • lobster • nervous system • immune system • pyrethroids • organochlorines • organophosphates**

Full-text PDF: http://www.medscimonit.com/fulltxt.php?IDMAN=9436
Word count: 2315
Tables: –
Figures: 1
References: 82

Author's address: Dr. Federico M. Casares, Marine Sciences Research Center, Stony Brook University, Stony Brook, New York, U.S.A., e-mail: fcasares@sunynri.org

BACKGROUND

In October 1999, a massive lobster die-off was observed in western Long Island Sound that collapsed the local lobster industry in New York and Connecticut. Many of these lobsters exhibited a paramoebic infection that mainly targeted the nervous system [1–5]. Scientists from several universities and research institutions along with state and federal environmental agencies gathered to study this phenomenon (i.e., NY-CT Sea Grant's Long Island Sound Lobster Research Initiative). Six years later, most of the scientists agree that the die-off was probably due to a combination of observed high temperature anomalies, driven in part by rapid water column destratification following hurricane Floyd, and low dissolved oxygen levels (promoting high sediment ammonia and sulfide) stressing lobsters, leaving them susceptible to disease [1–5].

Another factor, namely, the enhanced used of pesticide spraying (e.g., resmethrin, malathion) that took place in response to the discovery of mosquitoes carrying the West Nile virus that summer, could not be ruled out [3,4,6]. In fact, local lobstermen first pointed to pesticide spraying as the major culprit in the die-off [2]. Thus, any combination of these prevailing phenomena may have promoted impairment in the lobsters' ability to cope with additional environmental challenges, resulting in opportunistic infections (e.g., *Paramoeba* spp.) and death.

In a recent study endogenous morphine was shown to be involved in the stress response of lobsters and that it modulates immunocyte activation via induction of nitric oxide (NO) production by the Ca^{2+}-dependent constitutive nitric oxide synthase enzyme (cNOS) [7]. Based on numerous studies focusing on the effects of environmental pollutants such as pyrethroids, organochlorines, organophosphate pesticides, etc, [8–17] shown to affect intracellular ion balance, particularly calcium homeostasis, we surmise that commonly used pesticides specifically target constitutive nitric oxide-mediated protective mechanisms, specifically immune and cardiovascular [18], compromising the ability of marine animals to deal with additional stressors, such as hypoxia, high temperatures, pollutants, and pathogens.

CRUSTACEAN IMMUNE PROCESSES

Crustacean immunity, as observed in most invertebrates, relies largely on non-specific actions of circulating hemocytes for coping with immune associated stress (e.g., microbes) and, thus, for maintaining homeostasis [19–26]. Damaged cells and foreign materials represent a stimulus that can be regarded as stress, which under normal events can be dealt with by a fully functioning immune system. Specific immune actions are those that involve highly selective elements, such as memory molecules or antibodies, which are designed for non-self antigenic recognition. This highly specific type of action, for the moment, is typically observed only in vertebrates [21,22]. Non-specific hemocyte actions include phagocytosis, nodule formation, encapsulation, lysosome formation, exocytosis, pseudopodia formation, chemotaxis, as well as the synthesis of different factors, some of which (e.g., humoral) are secreted into the hemolymph (e.g., agglutinins) [21,22]. These factors can act as a primitive non-self recognition system; since they are activated by compounds found on diverse cell surfaces, such as microbial carbohydrates (e.g, beta-glucans, peptidoglycans, lipopolysaccharides), inducing particle aggregation and thus, enhancing phagocytic activity [21,22].

Many hemocyte actions require a great deal of cell membrane plasticity to allow vesicles to be formed and to be fused to different membranes, and pseudopodia to be shaped. These rearrangements involve, in part, polymerization and depolymerization of microtubules and microfilaments of cytoskeletal peptides (e.g., F-actin) [9,27,28]. Thus, any factor affecting membrane plasticity (e.g., NO, which can interfere with actin polymerization), will likely affect immune processes involving cell membrane mobilization (see [9,27,28]). This will be particularly important in organisms that rely largely on cellular (i.e., macrophage-like cell) actions for immunity.

Although little is known about the nature or mode of action of many antimicrobial factors in crustaceans, the evidence reviewed here demonstrates that different species utilize similar biochemical mechanisms (e.g., NO, melanin, enkelytin, penaeidin-like compounds, etc.) for direct microbial killing or for hemocyte activity regulation (e.g., NO, Met-enkephalin, lectins, interleukins, etc.), as it is generally observed in other invertebrates and vertebrates as well [21,22,25,29–31]. The same principle applies to humoral factors produced by cells other than hemocytes (e.g., nerve cells) or for regulatory factors produced by more than one cell type, depending on the specific signal transduction pathway being triggered.

In this scenario, NO certainly stands as one of the most widely used biochemical signals across taxa. Nitric oxide is known for possessing direct antimicrobial and antiviral properties [24,32–35]. It may also be involved in the regulation of different immunological activities, including hemocyte activation, up- or down-regulation of functional enzymatic systems (e.g., phenoloxidase) via its interaction with Cu and/or Fe in the center of enzymes [34,36], and/or by interference with cytoskeletal peptides [27,28,37–40].

NITRIC OXIDE AND INTERNAL HOMEOSTASIS

Nitric oxide is produced from L-arginine, intracellularly, by the enzyme nitric oxide synthase (NOS), using oxygen and NADPH-derived electrons in this reaction, and yielding L-citrulline as a byproduct [33,41–43]. Nitric oxide synthase is found in three main isoforms in mammals. Two of them (neuronal and endothelial NOS) are found constitutively in cells and collectively referred to as cNOS, and are involved in immediate responses to stress. The third isoform, inducible nitric oxide synthase (iNOS), requires induction by cytokines [33,41,42,44–46].

These three isoforms share structural and catalytic similarities, depending on a number of co-factors and prosthetic groups to which they associate. However, only the constitutive isoforms are known to depend on calcium-calmodulin co-factor binding for activation. In contrast, iNOS activation is independent of Ca^{2+} concentrations [33,41,42]. The Ca^{2+} dependency of cNOS denotes an important link with cell receptor types (e.g., opiate, estrogen, etc) that are coupled to Ca^{2+} fluxes. For example, as a typical opiate

receptor, μ_3, is linked to a trimeric G protein system. This membrane protein has the ability to modulate Ca^{2+} and K^+ channels, as well as adenylate cyclase (among other signal transduction systems) [31,35,40,47,48].

Nitric oxide modulates several functions in many physiological systems, including, neural, immune and cardiovascular. In addition, NO has been reported to act as free radical, second messenger, neurotransmitter, hormone, etc, depending on the particular site and/or microenvironment where it occurs [33,34,38,41,42,49–52]. This short-lived molecule ($t_{1/2}$ = seconds), capable of diffusing across membranes, is also thought to be a direct effector in the activation of regulatory proteins, proteases and kinases that are controlled by reactive oxygen intermediates [33,34,36-38,41,42,49–52]. For example, NO has been shown to affect cytochrome P-450 activity (i.e., inactivation of hepatic cytochrome P-450 in humans [49,51]. In this regard, NO has been implicated in the regulation of Cu and Cu/Fe proteins, in general (e.g., hemocyanin, tyrosinases, cytochrome c oxidase, etc) [34,36].

The immune down-regulatory effect of constitutive NO has been reported in a broad range of evolutionarily divergent organisms (e.g., marine mussels, nematodes, rats, humans, and lobsters) [35,45,46,53–62]. Constitutive NOS-derived NO inhibits the expression of different adhesion molecules on immune and endothelial cells in both, vertebrates and invertebrates [35,45,46,60,63,64]. This molecule can interfere with actin polymerization by way of ADP-ribosylation (i.e., causing actin depolymerization) [9,27,28], making the cells round and non-adherent, preventing immunocytes from migrating to the site of trauma or immune challenge and suppressing phagocytic activity [18,27,28,37,38, 40,48,61]. This can be observed after the activation of opiate or estrogen receptors [33,34,37,38,40–42,65]. Once in the cytosol, NO can activate guanylate cyclase (Gc), which increases the levels of cGMP that, in turn, induces intracellular calcium sequestration and extrusion [9,31,40,41,66]. This results in a reduction in intracellular Ca^{2+} concentrations, which induces cell rounding, since high concentrations of Ca^{2+} are needed for immunocyte morphological changes (e.g., pseudopodia formation) [9,40,67]. In similar way, NO would also affect vesicle (e.g., lysosomal) formation and thus, result in further reduction of immunocyte activity (i.e., by way of inhibition of particle immobilization in vesicles, processing and extrusion).

Moreover, cNOS-derived NO – at nanomolar concentrations – can concomitantly inhibit the activity of cytokine-stimulated inducible nitric oxide synthase (iNOS) through inhibition of the transcriptional factor nuclear factor κB (NF-κB), by stabilization of its precursor, IκB [52,64,68]. In this regard, NF-κB can bind to the promoter region of the gene that encodes for iNOS, inducing its transcription [69–71]. The higher levels of NO typically observed during iNOS activation are associated with pro-inflammatory processes, such as immunocyte up-regulation, as well as with other cytotoxic effects of NO [31,33,38,40,41,46,64]. For this reason, it has been further speculated that a 'basal' or 'tonal' level of NO would act as a filtering mechanism for 'noise' or 'background' stimulatory cytokine levels (which are always present in hemolymph) [18,31]. This biphasic effect [38], dependent on concentration of a compound (e.g., NO, micronutrient, hormone, etc), appears to be a common occurrence in biological systems.

Nitric Oxide in *Homarus americanus*

In 1994, Barnhart and Orona demonstrated the presence of NOS in the olfactory system of adult lobsters [72]. Interestingly, two types of soluble guanylate cyclases (an NO-sensitive Gc and a novel NO-insensitive Gc) have been later identified in *Homarus americanus* [73]. Here, higher levels of cytoplasmic cyclase activity were observed in *Homarus* nerve cord than were observed in other lobster tissues or even in mammalian cells. This latter study demonstrated that only a small subset of neurons expressed NO-sensitive Gc, a first indication of a highly selective signaling system. In 1998, a study by Scholz and colleagues showed that in postembryonic *Homarus americanus*, NOS and the NO-sensitive soluble Gc were present in the central nervous system (CNS) at hatching, and that some neurons became responsive to NO during metamorphosis, suggesting a role for NO in the development of the lobster nervous system [74]. The authors speculate that NO may be serving as a modulatory neurotransmitter for diverse neurons in *H. americanus*. This specialized subset of nerve cells (subject to NO signaling regulation) may, in turn, be locally modulating the actions of a larger group of cells (e.g., without NO-sensitive Gc), the same occurs with those subsets of neurons expressing NOS, evidencing a highly selective and highly controlled role for NO, as a modulatory neurotransmitter. Taken together, we surmise that a relatively small amount of NO (i.e., at nM concentrations), would have the ability to trigger – or cascade into – a larger modulatory response.

Overall, the findings by Barnhart and Orona (1994) and by Prabhakar and colleagues (1997) seem to be consistent with those of Scholz et al. (1998), by way of Gc and NOS immunocytochemistry, supporting the idea of the involvement of a few number of cells in the modulation of neural development and functioning in lobsters (i.e., via NO signaling). These findings are also in agreement with studies showing similar distributional patterns of NOS and Gc in cells of the CNS of other invertebrates (e.g., starfishes, squids, pond snails) [75–77].

Moreover, in lobsters, constitutive NOS-derived NO release determined in real-time via amperometric means, was linked to opiate-alkaloid action in neural and immune cells, via coupling to the μ_3 opiate receptor [7]. The demonstration of endogenous morphine and the highly specific μ_3 opiate receptor in *Homarus* tissue (e.g., neural, immune) represented the first evidence for the existence of this type of chemical messenger coupling in lobsters [7]. This also evidences not only a neuromodulatory but also an immunomodulatory role for NO, in this case, via endogenous opiate signaling. In addition, recent studies have demonstrated a cardioregulatory role for cNOS-derived NO, in a retrofeedback mechanism resulting in decreased cardiac ganglion burst frequency and myocyte contractility in lobsters [78].

Hypothesis

Given the biomedical and physiological significance of NO and NOS (both, constitutive and inducible isoforms) we hypothesize that common environmental pollutants, e.g., pesticides, capable of altering calcium homeostasis, may affect

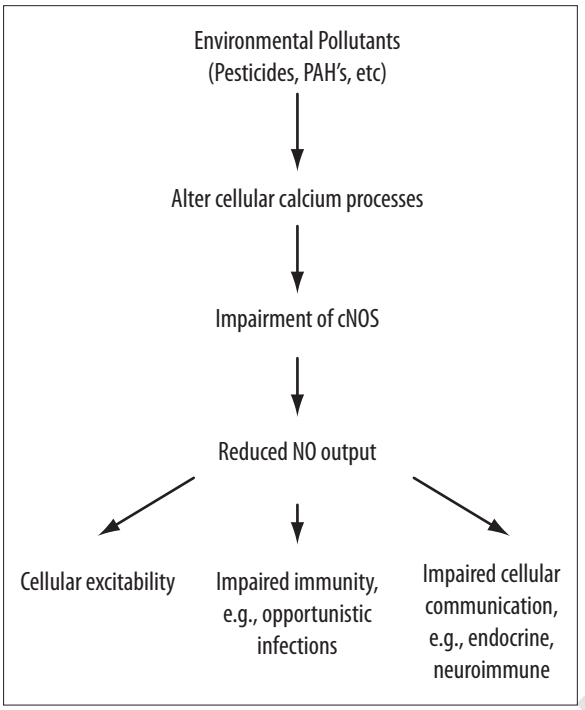

Figure 1. Common environmental pollutants, including widely used pesticides, have been shown to alter calcium homeostasis, which results in reduced constitutive NO production. This, in turn, will have deleterious effects that may include cellular excitability (e.g., neural, immune, vascular), impairment of immunity (e.g., chemotaxis) and impairment of neuroimmune communication (i.e., via uncoupling between cNOS and cell surface receptors such as opiate or estrogen, among others).

the production of cNOS-derived NO and thus, compromise these animals' ability to respond to additional negative environmental factors, e.g., pathogens.

There appears to be a common point of alteration in pesticide action, namely, with ion modifications, specifically calcium. For example, hexachlorobenzene, a typical organochlorine, can disrupt neuronal repolarization by interfering with Na^+ and K^+ fluxes, as well as by impairing calcium-calmodulin co-factor binding, causing hyperexcitation and seizures [8,10,11]. In rats, the pyrethroid pesticide permethrin inhibits Ca^{2+}-ATPase activity, which disrupts Ca^{2+} homeostasis [13]. In human tissues, voltage-sensitive calcium channels (VSCC) are also targets of pyrethroid action [14]. Moreover, although not a pesticide, the environmental pollutant benzo(*a*)pyrene, which is a polycyclic aromatic hydrocarbon (PAH) derived from the incomplete combustion of, for example, fossil fuels, exhibited deleterious effects on endocytosis and integrity of the actin cytoskeleton of hemocytes from mussels [12,79]. In these studies, the authors suggested that these effects might be directly linked to reactive oxygen species (ROS)-induced damage to cytoskeletal proteins involved in calcium homeostasis [12,79]. Another non-pesticide environmental contaminant, tributyltin (TBT), used as a powerful antibiofouling agent in marine paints, was shown to affect Ca^{2+}-calmodulin coupling at sublethal concentrations and, indirectly, Ca^{2+}-ATPase, resulting in cytoskeletal disorganization in hemocytes of a colonial tunicate (star ascidian) [9]. Taken together, alteration of Ca^{2+} homeostasis seems to be a common and important effect shared by some widely used pesticides and other common environmental pollutants.

The alteration of intracellular Ca^{2+} transients and/or of its binding to co-factors (e.g., calmodulin) by these pollutants may represent an important mechanism responsible for the abrogation of cNOS activation with a concomitant alteration of basal NO levels. This hypothesis is further supported by the fact that cNOS-derived NO release is Ca^{2+}-dependent and that intracellular calcium transients precede constitutive NO production (see Figure 1) [18,37,48,65,80].

Conclusions

Based on the knowledge that cNOS activation can lead to immune and nervous down-regulation as well as hypotension [18,78,81,82], one can hypothesize that exposure to the compounds previously discussed (i.e., capable of altering Ca^{2+} homeostasis) at low concentrations (e.g., parts per billion), could potentially lead to a state of hyper stimulation leading to conditions such as hemocyte hyperactivity, tachycardia, etc. This type of response would render an organism's immune system dysfunctional, impairing a specific response to an immune challenge. Furthermore, we surmise this immune impaired response would allow opportunistic infections to occur, compromising the organism's health. Therefore, exposure to these types of environmental contaminants, at sublethal concentrations, may lead to their demise if multiple stressors are present.

Acknowledgements

We wish to express our gratitude to Dr. George B. Stefano for his thoughtful comments.

References:

1. Russell S, Hobbie K, Burrage T et al: Identification of a protozoan parasite in the American lobster Homarus americanus. J Shellfish Res, 2000; 19: 581
2. Long Island Sound Lobster Research Initiative. 2004
3. Pearce J, Balcom N: The 1999 Long Island Sound lobster mortality event: Findings of the comprehensive research initiative. J Shellfish Res, 2005; 24: 691–97
4. Cuomo C, Valente R, Dogru D: Seasonal variations in sediment and bottom water chemistry of western Long Island Sound: Implication for lobster mortality. J Shellfish Res, 2005; 24: 805–14
5. Mullen T, Russell S, Tucker M et al: Paramoebiasis associated mass mortality of American lobster (Homarus americanus) in Long Island Sound. J Aquat Anim Health, 2004; 16: 29–38
6. Zulkosky A, Ruggieri J, Terracciano S et al: Acute toxicity of resmethrin, malathion and methoprene to larval and juvenile american lobsters (Homarus americanus) and analysis of pesticide levels in surface waters after Scourge, Anvil and Altosid application. J Shellfish Res, 2005; 24: 795–804
7. Casares FM, McElroy A, Mantione KJ et al: The American lobster, Homarus americanus, contains morphine that is coupled to nitric oxide release in its nervous and immune tissues: Evidence for neurotransmitter and hormonal signaling. Neuroendocrinology Letters, 2005; 26: 89–97
8. ATSDR – Agency for Toxic Substances and Disease Registry. Toxicological Profiles, 2002
9. Cima F, Ballarin L: Tributyltin induces cytoskeletal alterations in the colonial ascidian Botryllus schlosseri phagocytes via interaction with calmodulin. Aquatic Toxicol, 2000; 48: 419–29
10. Andrews JE, Courtney KD, Donaldson WE: Impairment of calcium homeostasis by hexachlorobenzene (HCB) exposure in Fischer 344 rats. J Toxicol Environ Health, 1988; 23: 311–20

11. Andrews JE, Jackson LD, Stead AG, Donaldson WE: Morphometric analysis of osteosclerotic bone resulting from hexachlorobenzene exposure. J Toxicol Environ Health, 1990; 31: 193–201
12. Gomez-Mendikute A, Cajaraville MP: Comparative effects of cadmium, copper, paraquat and benzo[a]pyrene on the actin cytoskeleton and production of reactive oxygen species (ROS) in mussel haemocytes. Toxicol In Vitro, 2003; 17: 539–46
13. Grosman N, Diel F: Influence of pyrethroids and piperonyl butoxide on the Ca(2+)-ATPase activity of rat brain synaptosomes and leukocyte membranes. Int Immunopharmacol, 2005; 5: 263–70
14. Shafer TJ, Meyer DA: Effects of pyrethroids on voltage-sensitive calcium channels: a critical evaluation of strengths, weaknesses, data needs, and relationship to assessment of cumulative neurotoxicity. Toxicol Appl Pharmacol, 2004; 196: 303–18
15. Rao MR, Kanji VK, Sekhar V: Pesticide induced changes of nitric oxide synthase in rat brain *in vitro*. Drug Chem Toxicol, 1999; 22: 411–20
16. Pala I, Vig PJ, Desaiah D, Srinivasan A: *In vitro* effects of organophosphorus compounds on calmodulin activity. J Appl Toxicol, 1991; 11: 391–95
17. Liu PS, Kao LS, Lin MK: Organophosphates inhibit catecholamine secretion and calcium influx in bovine adrenal chromaffin cells. Toxicology, 1994; 90: 81–91
18. Stefano GB, Goumon Y, Bilfinger TV et al: Basal nitric oxide limits immune, nervous and cardiovascular excitation: Human endothelia express a mu opiate receptor. Progress in Neurobiology, 2000; 60: 531–44
19. Hose JE, Martin GG: Defense functions of granulocytes in the ridgeback prawn Sicyonia ingentis. J Invertebr Pathol, 1989; 53: 335–46
20. Johansson MW, Keyser P, Sritunyalucksana K, Soderhall K: Crustacean hemocytes and haematopoiesis. Aquaculture, 2000; 191: 45–52
21. Lee SY, Soderhall K: Early events in crustacean innate immunity. Fish Shellfish Immunol, 2002; 12: 421–37
22. Soderhall K: Invertebrate immunity. Dev Comp Immunol, 1999; 23: 263–66
23. Soderhall K, Cerenius L: Role of the prophenoloxidase-activating system in invertebrate immunity. Curr Opin Immunol, 1998; 10: 23–28
24. Chisholm JR, Smith VJ: Comparison of antibacterial activity in the hemocytes of different crustacean species. Comp Biochem Physiol A Physiol, 1995; 110: 39–45
25. Freire Marques MR, Barracco MA: Lectins, as non-self-recognition factors, in crustaceans. Aquaculture, 2000; 191: 23–44
26. Sritunyalucksana K, Soderhall K: The proPO and clotting system in crustaceans. Aquaculture, 2000; 191: 53–69
27. Clancy RM, Leszczynska-Piziak J, Abramson SB: Nitric Oxide stimulates the ADP-ribosylation of actin in human neutrophils. Biochem Biophys Res Commun, 1993; 191: 847–52
28. Jun CD, Han MK, Kim UH, Chung HT: Nitric oxide stimulates the ADP-ribosylation of actin in murine macrophages: association with the inhibition of pseudopodia formation, phagocytic activity, and adherence on a laminal substratum. Cell Immunol, 1996; 174: 25–31
29. Bachere E, Destoumieux D, Bulet P: Penaeidins, antimicrobial peptides of shrimp: A comparison with other effectors of innate immunity. Aquaculture, 2000; 191: 71–88
30. Destoumieux D, Bulet P, Loew D et al: Penaeidins, a new family of antimicrobial peptides isolated from the shrimp Penaeus vannamei (Decapoda). J Biol Chem, 1997; 272: 28398–406
31. Stefano GB, Cadet P, Zhu W et al: The blueprint for stress can be found in invertebrates. Neuroendocrinology Letters, 2002; 23: 85–93
32. Croen KD: Evidence for antiviral effect of nitric oxide. Inhibition of herpes simplex virus type 1 replication. J Clin Invest, 1993; 91: 2446–52
33. Murad F: Nitric oxide signaling: would you believe that a simple free radical could be a second messenger, autacoid, paracrine substance, neurotransmitter, and hormone?. Recent Progress in Hormone Research, 1998; 53: 43–59
34. Torres J, Wilson MT: The reactions of copper proteins with nitric oxide. Biochim Biophys Acta, 1999; 1411: 310–22
35. Stefano GB: Autoimmunovascular regulation: Morphine and anandamide stimulated nitric oxide release. J Neuroimmunol, 1998; 83: 70–76
36. Gorren AC, de Boer E, Wever R: The reaction of nitric oxide with copper proteins and the photodissociation of copper-NO complexes. Biochim Biophys Acta, 1987; 916: 38–47
37. Nieto-Fernandez FE, Mattocks DW, Cavani F et al: Morphine coupling to invertebrate immunocyte nitric oxide release is dependent on intracellular calcium transients. Comp Biochem Physiol, 1999; 123: 295–99
38. Magazine HI, Chang J, Goumon Y, Stefano GB: Rebound from nitric oxide inhibition triggers enhanced monocyte activation and chemotaxis. J Immunol, 2000; 165: 102–7
39. Magazine HI, Liu Y, Bilfinger TV et al: Morphine-induced conformational changes in human monocytes,granulocytes, and endothelial cells and in invertebrate immunocytes and microglia are mediated by nitric oxide. J Immunol, 1996; 156: 4845–50
40. Stefano GB, Benz D: Nitric oxide modulates cell shape. Current Opinions in European Medicine, 2002; 3: 32–39
41. Moncada S, Palmer RMJ, Higgs EA: Nitric oxide: physiology, pathophysiology, and pharmacology. Pharmacological Reviews, 1991; 43: 109–42
42. Moncada S, Higgs A: The L-arginine-nitric oxide pathway. New Eng J Med, 1993; 329: 2002–12
43. Szabo C, Day BJ, Salzman AL: Evaluation of the relative contribution of nitric oxide and peroxynitrite to the suppression of mitochondrial respiration in immunostimulated macrophages using a manganese mesoporphyrin superoxide dismutase mimetic and peroxynitrite scavenger. FEBS Letters, 1996; 381: 82–86
44. Stefano GB, Bilfinger TV: Human neutrophil and macrophage chemokinesis induced by cardiopulmonary bypass: Loss of DAME and IL-1 chemotaxis. J Neuroimmunol, 1993; 47: 189–98
45. Szabo C: Alterations in nitric oxide production in various forms of circulatory shock. New Horizons, 1995; 3: 2–32
46. Szabo C: Physiological and pathophysiological roles of nitric oxide in the central nervous system. Brain Res Bull, 1996; 41: 131–41
47. Pasternak GW: The Opiate Receptors. New Jersey: Humana Press, 1988
48. Stefano GB, Prevot V, Beauvillain JC et al: Acute exposure of estrogen to human endothelia results in nitric oxide release mediated by an estrogen surface receptor coupled to intracellular calcium transients. Circulation, 2000; 101: 1594–97
49. Takehara Y, Kanno T, Yoshioka T et al: Oxygen-dependent regulation of mitochondrial energy metabolism by nitric oxide. Archives of Biochemistry & Biophysics, 1995; 323: 27–32
50. Brown GC: Reversible binding and inhibition of catalase by nitric oxide. Eur J Biochem, 1995; 232: 188–91
51. Takemura S, Minamiyama Y, Imaoka S et al: Hepatic cytochrome P450 is directly inactivated by nitric oxide, not by inflammatory cytokines, in the early phase of endotoxemia. J Hepatol, 1999; 30: 1035–44
52. Welters ID, Menzebach A, Goumon Y et al: Morphine inhibits NF-kB nuclear binding in human neutrophils and monocytes by a nitric oxide dependent mechanism. Anesthesiol, 2000; 92: 1677–84
53. Tubaro E, Borelli G, Croce C et al: Effect of morphine on resistance to infection. J Infect Dis, 1983; 148: 656–66
54. Weber RJ, Band LC, DeCosta B et al: Neural control of immune function: Opioids, opioid receptors and immunosuppression. NIDA Res Monogr, 1991; 105: 96–102
55. Perez-Castrillon JP, Perez-Arellano JP, Garcia-Palomo JD et al: Opioids depress *in vitro* human monocyte chemotaxis. Immunopharmacol, 1992; 23: 57–61
56. Stefano GB, Cadet P, Dokun A, Scharrer B: A neuroimmunoregulatory-like mechanism responding to electrical shock in the marine bivalve Mytilus edulis. Brain Behav Immun, 1990; 4: 323–29
57. Stefano GB, Hartman A, Bilfinger TV et al: Presence of the mu3 opiate receptor in endothelial cells: Coupling to nitric oxide production and vasodilation. J Biol Chem, 1995; 270: 30290–93
58. Stefano GB, Leung MK, Bilfinger TV, Scharrer B: Effect of prolonged exposure to morphine on responsiveness of human and invertebrate immunocytes to stimulatory molecules. J Neuroimmunol, 1995; 63: 175–81
59. Stefano GB, Scharrer B, Smith EM et al: Opioid and opiate immunoregulatory processes. Crit Rev in Immunol, 1996; 16: 109–44
60. Rojavin M, Szabo I, Bussiere JL et al: Morphine treatment *in vitro* or *in vivo* decreases phagocytic functions of murine macrophages. Life Sci, 1993; 53: 997–1006
61. Makman MH, Bilfinger TV, Stefano GB: Human granulocytes contain an opiate receptor mediating inhibition of cytokine-induced activation and chemotaxis. J Immunol, 1995; 154: 1323–30
62. Sonetti D, Mola L, Casares F et al: Endogenous morphine levels increase in molluscan neural and immune tissues after physical trauma. Brain Res, 1999; 835: 137–47
63. Stefano GB, Scharrer B: Endogenous morphine and related opiates, a new class of chemical messengers. Adv Neuroimmunol, 1994; 4: 57–68
64. Stefano GB, Salzet M, Magazine HI, Bilfinger TV: Antagonist of LPS and IFN-g induction of iNOS in human saphenous vein endothelium by morphine and anandamide by nitric oxide inhibition of adenylate cyclase. J Cardiovasc Pharmacol, 1998; 31: 813–20

65. Stefano GB, Cadet P, Breton C et al: Estradiol-stimulated nitric oxide release in human granulocytes is dependent on intracellular calcium transients: Evidence for a cell surface estrogen receptor. Blood, 2000; 95: 3951–58

66. Moncada S: Nitric oxide. J Hypertens Suppl, 1994; 12: S35–39

67. Kruskal BA, Shak S, Maxfield FR: Spreading of human neutrophils is immediately preceded by a large increase in cytoplasmic free calcium. Proc Natl Acad Sci USA, 1986; 83: 2919–23

68. Welters ID, Fimiani C, Bilfinger TV, Stefano GB: NF-kB, nitric oxide and opiate signaling. Medical Hypotheses, 1999; 54: 263–68

69. Xie QW, Kashiwahara Y, Nathan C: Role of transcription factor NF-kB/Rel in induction of nitric oxide synthase. J Biol Chem, 1994; 269: 4705–8

70. Nathan C, Xie QW: Regulation of biosynthesis of nitric oxide. J Biol Chem, 1994; 269: 13725–28

71. You HJ, Choi CY, Jeon YJ et al: Suppression of inducible nitric oxide synthase and tumor necrosis factor-alpha expression by 4-nonylphenol in macrophages. Biochem Biophys Res Commun, 2002; 294: 753–59

72. Barnhart A, Orona E: Nitric oxide synthase staining in lobster olfactory system. Chem Senses, 1994; 19: 438–42

73. Prabhakar S, Short DB, Scholz NL, Goy MF: Identification of nitric oxide-sensitive and -insensitive forms of cytoplasmic guanylate cyclase. J Neurochem, 1997; 69: 1650–60

74. Scholz NL, Chang ES, Graubard K, Truman JW: The NO/cGMP pathway and the development of neural networks in postembryonic lobsters. J Neurobiol, 1998; 34: 208–26

75. Martinez A, Riveros-Moreno V, Polak JM et al: Nitric oxide (NO) synthase immunoreactivity in the starfish Marthasterias glacialis. Cell Tissue Res, 1994; 275: 599–603

76. Adam-Vizi V, Allen TJ, Baker PF: The effects of nitroprusside and putative agonists on guanylate cyclase activity in squid giant axons. Biochimica et Biophysica Acta, 1988; 938: 461–68

77. Cooke IR, Edwards SL, Anderson CR: The distribution of NADPH diaphorase activity and immunoreactivity to nitric oxide synthase in the nervous system of the pulmonate mollusc Helix aspersa. Cell Tiss Res, 1994; 277: 565–72

78. Mahadevan A, Lappe J, Rhyne RT et al: Nitric oxide inhibits the rate and strength of cardiac contractions in the lobster Homarus americanus by acting on the cardiac ganglion. J Neurosci, 2004; 24: 2813–24

79. Gomez-Mendikute A, Etxeberria A, Olabarrieta I, Cajaraville MP: Oxygen radicals production and actin filament disruption in bivalve haemocytes treated with benzo(a)pyrene. Mar Environ Res, 2002; 54: 431–36

80. Stefano GB, Prevot V, Beauvillain JC et al: Estradiol coupling to human monocyte nitric oxide release is dependent on intracellular calcium transients: Evidence for an estrogen surface receptor. J Immunol, 1999; 163: 3758–63

81. Stefano GB, Goumon Y, Casares F et al: Endogenous morphine. Trends in Neurosciences, 2000; 9: 436–42

82. Scholz NL, Labenia JS, de Vente J et al: Expression of nitric oxide synthase and nitric oxide-sensitive guanylate cyclase in the crustacean cardiac ganglion. J Comp Neurol, 2002; 454: 158–67

Index Copernicus

Global Scientific Information Systems for Scientists by Scientists

www.IndexCopernicus.com

TM
INDEX COPERNICUS INTERNATIONAL

EVALUATION & BENCHMARKING

PROFILED INFORMATION

NETWORKING & COOPERATION

VIRTUAL RESEARCH GROUPS

GRANTS

PATENTS

CLINICAL TRIALS

JOBS

STRATEGIC & FINANCIAL DECISIONS

Index Copernicus integrates

IC Scientists

Effective search tool for collaborators worldwide. Provides easy global networking for scientists. C.V.'s and dossiers on selected scientists available. Increase your professional visibility.

IC Virtual Research Groups [VRC]

Web-based complete research environment which enables researchers to work on one project from distant locations. VRG provides:

- customizable and individually self-tailored electronic research protocols and data capture tools,
- statistical analysis and report creation tools,
- profiled information on literature, publications, grants and patents related to the research project,
- administration tools.

IC Journal Master List

Scientific literature database, including abstracts, full text, and journal ranking. Instructions for authors available from selected journals.

IC Patents

Provides information on patent registration process, patent offices and other legal issues. Provides links to companies that may want to license or purchase a patent.

IC Grant Awareness

Need grant assistance? Step-by-step information on how to apply for a grant. Provides a list of grant institutions and their requirements.

IC Conferences

Effective search tool for worldwide medical conferences and local meetings.

IC Lab & Clinical Trial Register

Provides list of on-going laboratory or clinical trials, including research summaries and calls for co-investigators.