INT. J. RADIAT. BIOL, OCTOBER, 2003, VOL. 79, NO. 10, 777–785

Taylor & Francis
healthsciences

IL-1β, TNFα and IL-6 induction in the rat brain after partial-body irradiation: role of vagal afferents

C. MARQUETTE†*, C. LINARD†, M. GALONNIER‡, A. VAN UYE‡, J. MATHIEU‡, P. GOURMELON† and D. CLARENÇON‡

(Received 5 August 2002; accepted 14 June 2003)

Abstract.
Purpose: To evaluate the central nervous system neuroimmune and inflammatory responses during the prodromal phase of the acute irradiation syndrome in rat brains after partial-body exposure (head-protected) and to investigate the potential neural signalling pathways from the irradiated periphery to the non-irradiated brain.
Material and methods: The study included four groups of rats: one irradiated group and one sham irradiated group, each containing non-vagotomized and vagotomized rats. In vagotomized rat groups, the subdiaphragmatic vagal section surgery was carried out 45 days before the irradiation exposure. The rats were partial-body irradiated with the head shielded with ^{60}Co γ-rays to a dose of 15 Gy. They were sacrificed 6 h after the end of exposure. The hypothalamus, hippocampus, thalamus and cortex were then collected, and the concentrations of IL-1β, TNFα and IL-6 in each were measured by ELISA assays.
Results: Six hours after irradiation, IL-1β levels had increased in the hypothalamus, thalamus and hippocampus, and TNFα and IL-6 levels had increased significantly in the hypothalamus. Vagotomy before irradiation prevented these responses.
Conclusions: It was concluded that the hypothalamus, hippocampus, thalamus and cortex react rapidly to peripheral irradiation by releasing pro-inflammatory mediators. The results also show that the vagus nerve is one of the major ascending pathways for rapid signalling to the brain with respect to partial body irradiation.

1. Introduction

In cases of accidental irradiation or during radiotherapy, severe haematological and/or digestive clinical manifestations may appear within hours or days of exposure, depending on the radiation dose. Although neurological manifestations are usually considered to be late effects, occurring months or years after exposure, less severe acute neurological symptoms may occur soon after radiation exposure. These include hypothermia, headache, nausea, vomiting, anorexia and lethargy. In addition, experimental irradiation shows that low doses induce neural electrophysiological abnormalities (Court *et al.* 1986). Furthermore, biochemical alterations in neurotransmission have been reported within hours of irradiation. They include changes in dopamine, serotonin and acetylcholine metabolism (Kassayova *et al.* 1995) and in acetylcholinesterase activity (Clarençon *et al.* 1997). Taken together, these observations indicate that the central nervous system (CNS) is functionally radioresponsive.

The molecular and cellular mechanisms underlying these acute and subacute effects of irradiation on the brain are still unclear. A radiation-induced inflammatory response may contribute to the sensitivity of the nervous system. Various non-specific symptoms (i.e. fever, asthenia, anorexia and somnolence) observed during infection are related to the production of pro-inflammatory cytokines in the brain (Dantzer *et al.* 1998). Similarly, the levels of the pro-inflammatory cytokines IL-1β, TNF and IL-6 (messengers and proteins) increase in mouse brain within 24 h of irradiation (Hong *et al.* 1995, Marquette *et al.* 1999). Numerous studies indicate that peripherally generated cytokines mediate both the central and peripheral metabolic response to endotoxins, to changes in electrical activity (Saphier and Ovadia 1990), in monoamine metabolism (Dunn 1992), to immediate early expression of genes such as c-Fos (Brady *et al.* 1994, Ericsson *et al.* 1994) and to the induction of the pro-inflammatory cytokines IL-1β, TNFα and IL-6 in the brain (Layé *et al.* 1994, Van Dam *et al.* 1995). It is now agreed that the immune system communicates with the CNS, although the detailed mechanisms are still uncertain. In view of the general presumption that peripherally produced cytokines cannot enter the CNS, various hypotheses have attempted to explain the presence of those cytokines in the brain. Most focus on cytokine entry into the brain or on signalling at the blood–brain interface. Another possibility, however, is that peripheral cytokines released by activated immune cells in turn activate peripheral afferent nerves and thereby induce cytokine production by brain cells. Other experimental findings are consistent with the involvement of the vagus nerve in communication between the peripheral and central nervous systems. Subdiaphragmatic vagotomy blocks

*Author for correspondence; e-mail: christel.marquette@irsn.fr
†IRSN, BP no. 17, F-92262 Fontenay aux Roses, cedex, France.
‡CRSSA, Department of Radiobiology and Radiopathology, BP 87, F-38702 La Tronche, cedex, France.

International Journal of Radiation Biology ISSN 0955-3002 print/ISSN 1362-3095 online © 2003 Taylor & Francis Ltd
http://www.tandf.co.uk/journals
DOI: 10.1080/09553000310001610998

the CNS expression of IL-1 mRNA (Layé *et al.* 1995, Hansen *et al.* 1998) and c-Fos (Wan *et al.* 1994) that is induced by the peripheral administration of LPS or IL-1β. Similarly, vagus nerve section attenuates the fever induced by cytokines (Fleshner *et al.* 1998).

The aims of this study were: (1) to determine which brain structures are involved in brain activation during early radiation-induced inflammation, and particularly in the production of IL-1β, IL-6 and TNF; (2) to determine if cytokine release in the brain results directly from the effect of ionizing radiation on the CNS or instead from peripheral stimulation; and (3) to determine if the vagus nerve is a likely pathway between the peripheral and central nervous systems that induces the brain to begin producing these pro-inflammatory interleukins soon after irradiation. This experiment involved the partial irradiation (with the head protected) of vagotomized and non-vagotomized rats.

2. Material and methods

2.1. *Animals*

Male Sprague–Dawley rats (Elevage Janvier, St. Islen, France), weighing 300–325 g at the beginning of the experiment, were housed in a facility at $23 \pm 1^{\circ}$C with a 12:12 h light/dark cycle (lights on at 20:00 h). Food and water were available *ad libitum*. The local Ethics Committee for Animal Care and Use approved all experiments.

2.2. *Surgery*

Surgery was performed after 18 h of food deprivation. All animals were anaesthetized with ketamine (Imalgène®, 150 mg kg^{-1}, intramuscularly) and underwent either sham surgery or subdiaphragmatic vagotomy as described (Bluthé *et al.* 1994). Briefly, after median laparotomy, the two trunks of the vagus nerve were identified and isolated under the microscope. Silk sutures were tied a few millimetres apart around each vagal trunk before the gastric branch bifurcates from the hepatic and celiac branches. The nerve was cut between the sutures. The vagus nerve of the non-vagotomized animals was similarly exposed but neither ligated nor cut. Vagotomized animals lost weight for the first 4–6 days after surgery, and their mortality rate reached 15–20%. One month after surgery, the completeness of the vagotomy was verified by blockading the satiety effect of cholecystokinin (CCK) (Smith *et al.* 1981). Rats were food-deprived for 18 h, then injected with either saline or CCK (4 mg kg^{-1}, intraperitoneally; Sigma-Aldrich, St. Quentin, Fallavier, France) 5 min before being presented with food. Food intake was measured 30 min later.

2.3. *Irradiation procedure*

Animals were anaesthetized with ketamine (150 mg kg^{-1}, i.m.). With the heads protected by 15 cm lead, their bodies were exposed to a 15-Gy dose from a ^{60}Co gamma-radiation source (head protected) at 0.26 Gy min^{-1} at the beginning of the dark period. Non-irradiated animals were anaesthetized at the same time and kept in the same room for the same period and under the same conditions, but without radiation exposure. The vagotomized rats underwent irradiation 7 weeks after the subdiaphragmatic vagotomy.

There were four experimental groups, each containing 12 rats: (1) non-vagotomized and non-irradiated animals (referred to as non-vgx/sham-irr); (2) non-vagotomized and irradiated animals (non-vgx/irr); (3) vagotomized and non-irradiated animals (vgx/sham-irr); and (4) vagotomized and irradiated animals (vgx/irr).

Animals were killed by decapitation 6 h after the end of irradiation. The brains were quickly removed. The hypothalamus, hippocampus, thalamus and cortex were dissected out, frozen on dry ice and stored at -80°C until use. The stomachs were also removed and weighed for confirmation of the section of the vagus nerve in vagotomized rats.

2.4. *Cytokine assay*

IL-1β, IL-6 and TNFα levels were measured in the supernatant of crushed tissue, in a phosphate buffer, with rat enzyme-linked immunosorbent assays (ELISA Quantikine M IL-1β, R&D; Cytoscreen rat TNFα immunoassay and Cytoscreen rat IL-6 immunoassay, Biosource, Montrouge, France). Results were expressed at the mean ($\pm$SEM) as pg mg^{-1} protein after a protein assay with Coomassie plus protein assay reagent (Pierce, Asnière, France).

2.5. *Statistical analysis*

Statistical analysis used the Mann–Whitney rank sum *U*-test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. *Completeness of vagotomy*

The completeness of the subdiaphragmatic vagotomies was confirmed by testing for the expected reduction in CCK-induced satiety. Thirty minutes

Table 1. Stomach weight for confirmation of the completeness of vagotomy surgery.

	Stomach weight (g)		
	Non-vgx/sham-irr	Vgx/sham-irr	*p
Empty stomach	1.87 ± 0.04	$2.16 \pm .01$	<0.02
Filled stomach	4.54 ± 0.6	10.28 ± 1.48	<0.01

Data are weight the mean ($\pm$SEM) weight (g) of empty and filled stomachs of sham-irradiated, non-vagotomized or vagotomized animal. *Six to eight animals per group. Significance differences between non-vagotomiezd and vagotomized animals.

after a CCK dose of $4\,mg\,kg^{-1}$ to sham-irradiated rats, food intake was inhibited by 42.1% in the non-vagotomized and only 9.4% in the vagotomized rats. Moreover, weighing of the rat stomachs filled and then empty indicated stomach distension caused by the vagotomy-induced inhibition of the gastric emptying. Thus, weighing stomachs of the vagotomized and non-vagotomized rats revealed a significant difference between them: the empty stomachs of the vagotomized rats were 13.4% heavier and the filled stomachs 56% heavier (table 1).

The findings of inhibition of CCK-induced satiety and of stomach distension in vagotomized rats together indicate that the surgery successfully inhibited the vagus nerve connection.

3.2. *IL-1β, IL-6 and TNFα levels in the hypothalamus*

3.2.1. *Effect of irradiation on cytokine production in non-vagotomized rats.* The hypothalamus reacted to partial-body 'head-protected' irradiation within 6 h (figure 1). After partial-body irradiation, IL-1β concentrations were 3.6 times increased in the irradiated compared with the non-vgx/sham-irr rats (24.7 ± 5.5 versus $6.8 \pm 2\,pg\,mg^{-1}$ protein, $p<0.01$), 1.66 times increased for IL-6 concentrations (1365.7 ± 111.5 versus $823.2 \pm 84.15\,pg\,mg^{-1}$ protein, $p<0.01$) and 1.56 times increased for TNFα concentrations (235.7 ± 24.7 versus $151.2 \pm 24.2\,pg\,mg^{-1}$ of protein, $p<0.02$).

3.2.2. *Effect of vagotomy on cytokine response to irradiation*
3.2.2.1. *Effect of vagotomy in sham-irradiated rats.* Among the sham-irradiated rats, IL-1β, IL-6 and TNFα concentrations in the hypothalamus did not

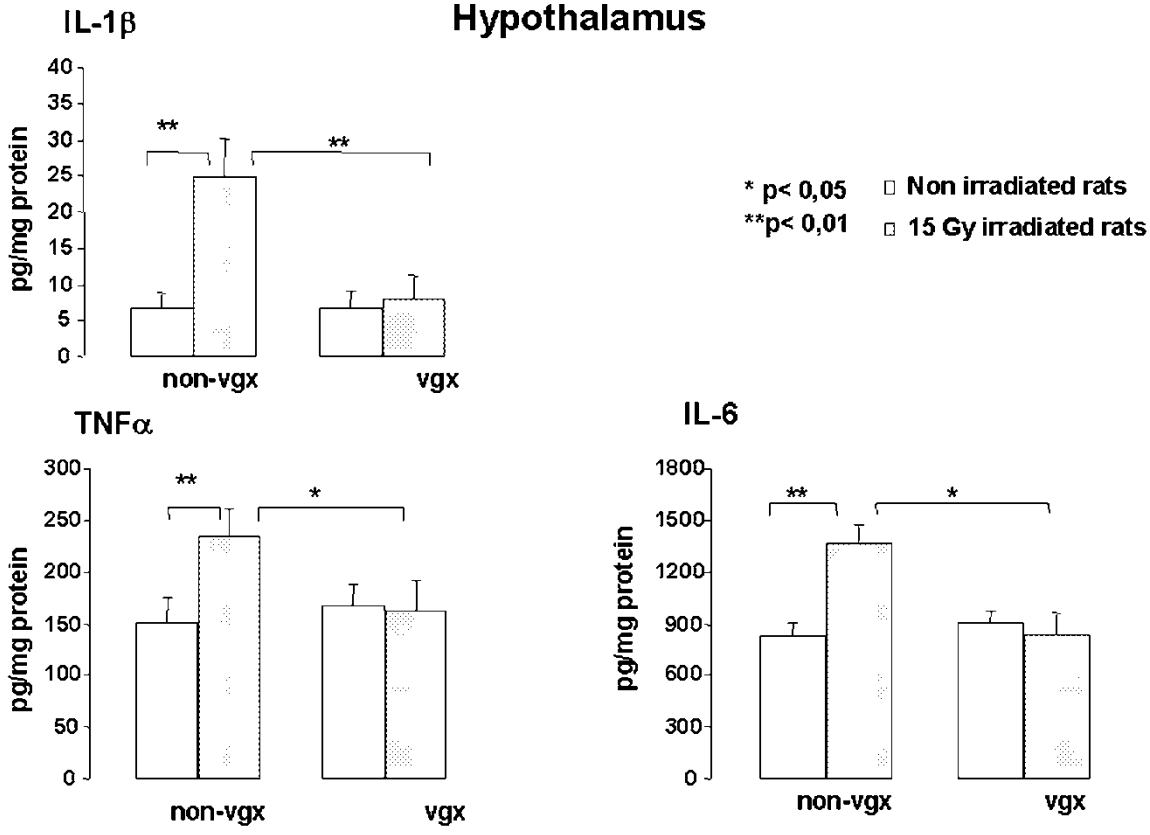

Figure 1. Concentrations of IL-1β, IL-6 and TNFα in the hypothalamus 6 h after partial-body irradiation with a 15-Gy dose with the head shielded in non-vagotomized or vagotomized rats. Data are the mean ($\pm$SEM) of the concentrations of cytokines expressed as $pg\,mg^{-1}$ protein contained in the tissue for $n=12$ animals.

differ significantly between those vagotomized and non-vagotomized. These results indicate that the vagotomy did not modify basal cytokine levels in that structure.

3.2.2.2. *Effect of vagotomy on radiation-induced cytokine production.* The increases in the cytokine concentrations in the hypothalamus after partial-body 'head-protected' irradiation were significantly lower among the rats that had, compared with those that did not have, vagal surgery: 67.5% ($p<0.01$) for IL-1β, 38.7% for IL-6 ($p<0.02$) and 31% ($p=0.02$) for TNFα.

3.3. *IL-1β levels in the hippocampus, thalamus and cortex*

3.3.1. *Effect of irradiation on IL-1β production in non-vagotomized rats*

In the non-vagotomized rats that underwent partial-body 'head-protected' irradiation, IL-1 levels were 2.33 times higher than in those sham-irradiated in the hippocampus (12.8 ± 3.2 versus $5.5\pm0.7\,\mathrm{pg\,mg^{-1}}$ protein, $p<0.05$) and two times higher in the thalamus (2.3 ± 0.5 versus $1.2\pm0.2\,\mathrm{pg\,mg^{-1}}$ protein, $p=0.05$). The levels were unchanged in the cortex (figure 2).

3.3.2. *Effect of vagotomy on radiation-induced IL-1β production.*

3.3.2.1. *Effect of vagotomy in sham-irradiated animals.* Comparison of non-vagotomized and vagotomized rats in the sham-irradiated group indicated that the vagotomy did not cause any significant changes in IL-1β concentrations in the hippocampus, thalamus or cortex (figure 2).

3.3.2.2. *Effect of vagotomy on IL-1β production.* Vagotomy inhibited radiation-induced IL-1β increases in the hippocampus by 2.5 times (5.1 ± 1 in vagotomized versus $12.8\pm3.2\,\mathrm{pg\,mg^{-1}}$ protein in non-vagotomized, $p<0.05$) and by 30%, albeit not significantly, in the thalamus. On the other hand, IL-1β production in the cortex was 1.46 times higher among the vagotomized irradiated rats than among their non-vagotomized counterparts (5.7 ± 1.3 versus $3.9\pm1.2\,\mathrm{pg\,mg^{-1}}$ protein, $p<0.05$) (figure 2).

3.4. *IL-6 and TNFα levels in the hippocampus, thalamus and cortex (table 2)*

3.4.1. *Hippocampus.* IL-6 was not detected in the hippocampus of any of these rats, and hippocampal TNFα levels did not differ significantly between groups.

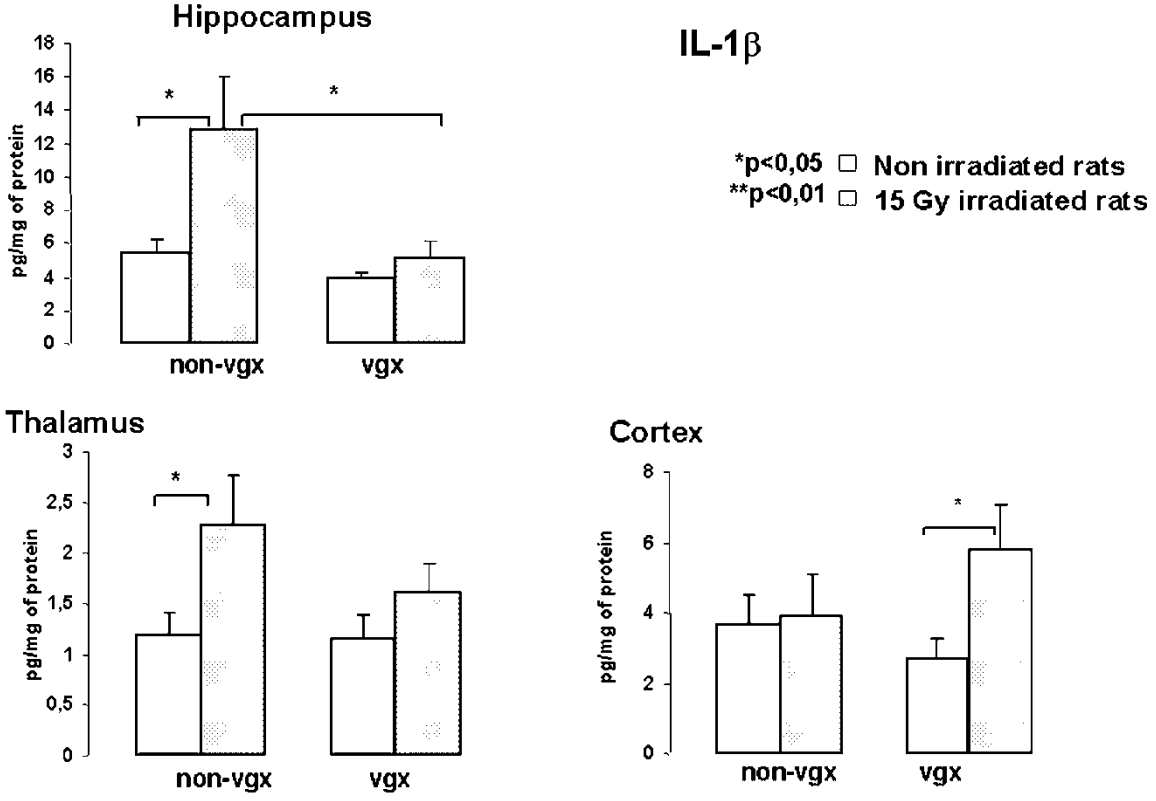

Figure 2. Concentrations of IL-1β in the hippocampus, thalamus and cortex 6 h after partial-body irradiation with a 15-Gy dose with the head shielded in non-vagotomized or vagotomized rats. Data are the mean ($\pm$SEM) of the concentrations of IL-1β expressed as $\mathrm{pg\,mg^{-1}}$ protein contained in each tissue for $n=12$ animals.

Table 2. Concentrations of IL-6 and TNFα in the hippocampus, the thalamus and the cortex 6 h after 15 Gy partial-body irradiation with the head shielded in non-vagotomized or vagotomized rats.

(pg mg^{-1} protein)	Non-vgx/sham-irr	Non-vgx/irr	Vgx/sham-irr	Vgx/irr
Hippocampus				
IL-6	nd	nd	nd	nd
TNFα	37.2 ± 2.6	45.8 ± 4.7	40.3 ± 1.9	39.2 ± 2.7
Thalamus				
IL-6	212.7 ± 17.1	210.3 ± 13.6	196.4 ± 10.5	185.8 ± 13.6
TNFα	29.1 ± 2.4	32.2 ± 3.8	22.4 ± 2.1	29.3 ± 4.8
Cortex				
IL-6	9.8 ± 0.6	8.3 ± 0.5*	8.3 ± 0.3#	8.4 ± 0.5
TNFα	11.6 ± 1	10.3 ± 1.4	14.6 ± 1.4	11.5 ± 1.1

Data are the mean (± SEM) of the concentrations of IL-6 or TNFα expressed as pg mg^{-1} protein contained in each tissue for $n = 12$ animals.

3.4.2. *Thalamus*. At 6 h after irradiation, neither IL-6 nor TNFα levels in the thalamus had changed significantly. Vagotomy did not modify cytokine concentrations in sham- or irradiated animals.

3.4.3. *Cortex*. In the non-vagotomized rats, irradiation resulted in a significant decrease (15%, $p = 0.01$) in IL-6 levels. In addition, among the sham-irradiated rats, IL-6 levels were 15% lower in those who had been vagotomized ($p = 0.02$). In vagotomized animals, however, irradiation did not modify the IL-6 levels in the cortex.

TNFα concentrations in the cortex did not change after irradiation in vagotomized or non-vagotomized rats.

4. Discussion

The aim was to evaluate the neuroimmune and inflammatory responses of the CNS during the prodromal phase of the acute irradiation syndrome and, in particular, the role of the vagal nerve pathway in this response.

Because most of the CNS is constituted of non-proliferating cells, it is usually considered less radiosensitive than tissues that renew themselves more rapidly, such as haematopoietic and gastrointestinal tissues. While histological modifications, characterized by radiation-induced necrosis, appear several months after exposure (Hopewell 1998), functional aspects of the CNS respond in the early phase after irradiation. Electroencephalograms demonstrate changes in electrophysiological biorhythms (Bassant and Court 1978) and in cerebral temperature (Mestries *et al.* 1987) soon after exposure to 4-Gy exposure. Modifications in acetylcholinesterase activity in rat brains have been reported within hours of irradiation with 10 Gy (Clarençon *et al.* 1997). Recent data suggest that radiation might activate secondary reactive processes that generate persistent oxidative stress and inflammatory reactions in the CNS (Tofilon and Fike 2000). These responses occur rapidly, since increases in mRNA expression and in pro-inflammatory cytokine (e.g. IL-1α, IL-1β and TNFα) protein levels are observed in the brains of mice 4–6 h after either total-body or mid-brain irradiation of 5- and 7-Gy gamma doses (Hong *et al.* 1995, Marquette *et al.* 1999). Taken together, these data strongly indicate that the brain is highly responsive to direct irradiation of the head and that this irradiation rapidly induces inflammation in CNS tissues.

The present results support the hypothesis that after partial-body irradiation with the head shielded, brain non-irradiated also triggers an early radiation-induced inflammatory response in different brain structures. It consists of the secretion of the pro-inflammatory cytokines IL-1β, IL-6 and TNFα and depends on peripheral inputs through a neural pathway. Moreover, it is shown that some of the specific cerebral areas are involved in this early (6 h afterwards) regulatory response to peripheral irradiation: radiation-induced pro-inflammatory cytokines are produced mainly in the hypothalamus (IL-1β, IL-6 and TNFα), in the hippocampus (IL-1β) and in the thalamus (IL-1β and TNFα). However, it cannot be excluded that this cytokine production occurs in other brain areas at time points not investigated in this study, since radiation-induced responses on a dynamic process with continuous although varying cytokine production (Rubin *et al.* 1995).

The increase of cytokines in the hypothalamus after partial-body irradiation with the head shielded might be related to their regulatory role there in the adaptive response to peripheral inflammation. The hypothalamus is involved in the activation of the hypothalamo-pituitary-adrenal (HPA) axis and in autonomic functions (appetite, sleep, thermogenesis, emotion, sexual behaviour) that regulate homeostasis.

Moreover, Layé *et al.* (1995) showed that the hypothalamus expresses mRNA of IL-1β IL-6 and TNFα in response to LPS-induced systemic inflammation.

Many clinical symptoms observed during the prodromal phase of acute radiation syndrome (anorexia, fever, weakness or somnolence) (Anno *et al.* 1989) are similar to the non-specific sickness manifestations and behaviour observed after systemic inflammation (Bluthé *et al.* 1994). Thus, the radiation-induced cytokine production observed by the present authors in the hypothalamus may be involved in the CNS pathophysiological reaction to radiation. In other irradiation configurations that included head exposure, early and delayed radiation effects on the metabolism of the hypothalamus have been already described. First, 24 h after whole-body X-irradiation, catecholamines are depleted (Varagic *et al.* 1967) and cholecystokinin release increases dose dependently (Kandasamy 1998), possibly related to radiation-induced reduction in food intake. Late effects on regulation of the HPA axis are usually reported 2–10 years following radiotherapy to the head: alteration of hypothalamic endocrine function causes the development of endocrine disorders associated with hypopituitarism (Mechanick *et al.* 1986, Pai *et al.* 2001). Finally, studies have showed morphological modifications 1–2 years after irradiation of the head, associated with necrotic areas, degenerative changes, calcified deposits and dilatation of vessels (Arnold 1954, Ibrahim *et al.* 1967). All together, these data indicate the sensitivity of the hypothalamus to direct effect of irradiation.

The impairment of the hypothalamus observed in the present experiments might also be related to hippocampal damage after direct radiation exposure (Huang *et al.* 1994, Roozendaal *et al.* 1998) since hypothalamic functions are regulated by hippocampal activity, through direct connections between the hypothalamus and the dentate gyrus (Wayner *et al.* 1997). The hippocampus is also highly sensitive to radiation. In the hours after whole-body or head irradiation, apoptotic neurons have been detected in the granular cell layer of the dentate gyrus and, to a lesser extent, in the pyramidal cells layer (Gueneau *et al.* 1979, Peissner *et al.* 1999). The increased IL-1β concentrations observed in the hippocampus 6 h after partial-body irradiation with the head shielded are consistent with these apoptosis and cognitive impairments observed after irradiation, since IL-1β secretion in the hippocampus during the inflammatory phase has been shown to be involved in neuron apoptosis there, via the activation of caspase 1. Neuronal apoptosis also decreases long-term potentiation (LTP) of the perforant granule cells of the dentate gyrus (Vereker *et al.* 2000), and impaired ability to sustain LTP causes cognitive deficiencies (Lynch 1998). Thus, the impairment of new neuron production and the LTP alteration associated with it 3 weeks after gamma-irradiation of the brain (Snyder *et al.* 2001) suggest a causal relation between neurogenesis and the hippocampal functions of learning and memory. Moreover, increased hippocampal IL-1β appears to be a common feature of impaired LTP, with the IL-1β concentration increasing as LTP is decreased (Bellinger *et al.* 1993, Cunningham *et al.* 1996). Intracerebroventricular administration of IL-1β or of agents that induce its production impairs the consolidation of hippocampal memories (Pugh *et al.* 2001). IL-1β activity in the hippocampus is supported by the presence of IL-1 receptors (IL-1R) located on granular cells of the dentate gyrus (Ban *et al.* 1991, Parnet *et al.* 1994). The hippocampal IL-1β/IL-1R system may therefore be directly involved in the radiation symptoms observed after irradiation.

Investigating the pathways from the periphery involved in brain cytokine induction should help us understand the mechanisms that underlie radiation-induced inflammation in cerebral tissues when the brain was not irradiated. The vagus nerve is one of these pathways and is widely known to be involved in mediating inflammatory peripheral signals to the CNS (Bluthé *et al.* 1996). Therefore, the hypothesis was investigated that hypothalamic cytokine production after partial-body irradiation with the head shielded is stimulated by information from the body. To do so, surgical vagotomy was performed on rats that were subsequently irradiated. It was found, as R.-M. Bluthé observed (personal communication), that the subdiaphragmatic section of the nerve did not modify the basal cytokine levels in the brain. The results showed that IL-1β, IL-6 and TNFα production in the hypothalamus and the hippocampus are under vagal control. They therefore suggest that these cytokines could be responsible for the effects and sickness-like manifestations observed after irradiation.

Afferent terminations of the vagus nerve are localized in the dorsal vagal complex of the caudal medulla, which is composed of the area postrema, the nucleus of the solitary tract (nTS) and the dorsal motor nucleus of the vagus. This complex is considered an entry point for visceral sensory information coming from the vagal nerve. The nTS regulates hypothalamic functions by direct projections to different hypothalamic nuclei (e.g. paraventricular and dorsomedial nuclei) and throughout the parabrachial nucleus (Saper 1995). Direct electrical stimulation of vagal afferents has demonstrated that the vagus nerve activates IL-1β mRNA and protein expression in the hippocampus and the hypothalamus (Hosoi *et al.* 2000). IL-1β is also a potent stimulant of these

primary afferent neurons, via IL-1R located on the abdominal paraganglia (Goehler *et al.* 1997). Thus, the immediate early gene product c-Fos is induced in vagal sensory neurons 90 min after direct intraperitoneal administration of IL-1β (Goehler *et al.* 1998). The neural transduction of the peripheral signal has been demonstrated by detection of increased c-fos mRNA expression between 1 and 3 h afterwards in the area postrema and in the nucleus of the nTS (Brady *et al.* 1994). This vagal pathway has already been demonstrated to be the major route for emesis induction after radiation exposure (Makale and King 1993). Yamada *et al.* (2000) showed that X-radiation induces early emesis by activating 5-HT_3 receptors on the terminations of the vagus nerve: c-Fos is thus expressed in the afferents and in the nTS.

What, however, is the radio-induced peripheral signal that caused this vagal stimulation and brain cytokine secretion? One hypothesis involves the perivagal immune cells, composed of the glomus cells of the paraganglia, the dendritic cells within and around the vagal nerve, and the macrophages located within vagal nerve connective tissue. These different cells represent the so-called 'sentinel cells of the immune system', which induce the rapid release of various inflammatory factors, such as IL-1β. This model is supported by experiments showing that peritoneal macrophages express IL-1 and IL-6 mRNA as early as 1 h after exposure to doses of 0.1 and 2 Gy, respectively, and up to 10 Gy (Hosoi *et al.* 2001). This local inflammatory response may thus function as a paracrine-type signal to the vagus nerve. This would indicate that the vagal afferents can activate specific and distinct forebrain structures via the terminations in the nTS (Goehler *et al.* 1999).

Partial-body irradiation of vagotomized rats caused inconsistent effects in the cortex: in the group of vagotomized and irradiated rats, IL-1β increased significantly, while IL-6 decreased. It is nonetheless unclear whether this statistically significant difference is also clinically significant whether there is a real biological effect. Note that an irradiation-induced inflammatory response in the cortex could also have occurred at a time other than that studied here and may produce responses more similar to the hypothalamus and hippocampus responses observed here.

5. Conclusion

The originality of the present study lies in the finding that cytokine production occurred in the brain when the head was not irradiated. Comparing vagotomized and non-vagotomized rats, it was shown that partial irradiation of the body with the head shielded induced this early pro-inflammatory cytokine release in the brain, via the vagal afferent pathway. However, the possibility that other pathways are involved in cytokine secretion in the brain cannot be ruled out. Humoral pathways might also play a role: they may alter the blood–brain barrier by a parenchymal inflammatory reaction or at the level of the endothelial cells (Olschowka *et al.* 1997). In any case, vagal communication may constitute the immediate physiological response against radiation-induced stress-stimuli (Watkins *et al.* 1995). In addition, this early inflammatory response underlines the major role that the hypothalamus plays in the alteration of the neuroimmunoendocrine regulation of the HPA axis described in delayed radiation syndrome in both accidental (Ivanov *et al.* 2000) and therapeutic radiation exposure (Mechanick *et al.* 1986). This early neural response, which consists in the release of brain cytokines, is therefore likely to be involved in the regulatory loop between the body and the brain that occurs during the acute phase of radiation syndrome and may be implicated in the genesis of these late radiation symptoms.

Acknowledgements

The authors thank R.-M. Bluthé for technical vagotomy recommendations, J. D. Laval for expert technical and dosimetry assistance, and Dr E. Ban for improving the manuscript.

References

Anno, G. H., Baum, S. J., Withers, H. R. and Young, R. W., 1989, Symptomatology of acute radiation effects in humans after exposure to doses of 0.5–30 Gy. *Health Physics*, **56,** 821–838.

Arnold, A., 1954, Effect of X-irradiation on the hypothalamus: a possible explanation for the therapeutic benefits following X-irradiation of the hypophyseal region for pituitary dysfunction. *Journal of Clinical Endocrinology*, **14,** 859–868.

Ban, E., Milon, G., Prudhomme, N., Fillion, G. and Haour, F., 1991, Receptors for interleukin-1 (alpha and beta) in mouse brain: mapping and neuronal localization in hippocampus. *Neuroscience*, **43,** 21–30.

Bassant, M. H. and Court, L., 1978, Effects of whole-body γ irradiation on the activity of rabbit hippocampal neurons. *Radiation Research*, **75,** 593–606.

Bellinger, F. P., Madamba, S. and Siggins, G. R., 1993, Interleukin 1 beta inhibits synaptic strength and long-term potentiation in the rat CA1 hippocampus. *Brain Research*, **628,** 227–234.

Bluthé, R. M., Michaud, B., Kelley, K. W. and Dantzer, R., 1996, Vagotomy attenuates behavioural effects of interleukin-1 injected peripherally but not centrally. *Neuroreport*, **7,** 1485–1488.

Bluthé, R. M., Walter, V., Parnet, P., Laye, S., Lestage, J., Verrier, D., Poole, S., Stenning, B. E., Kelley, K. W. and Dantzer, R., 1994, Lipopolysaccharide induces sickness behaviour in rats by a vagal mediated mechanism. *Comptes Rendus de l'Académie des Sciences, Paris*, **317,** 499–503.

Brady, L. S., Lynn, A. B., Herkenham, M. and Gottesfeld, Z., 1994, Systemic interleukin-1 induces early and late patterns of c-fos mRNA expression in brain. *Journal of Neuroscience*, **14,** 4951–4964.

Clarençon, D., Gourmelon, P., Multon, E., Galonnier, M., Fornier, C. and Fatome, M., 1997, Modulation of the brain acetycholinesterase activity after gamma irradiation or cytokine administration. *Radioprotection*, **32,** C1–31/C31–33.

Court, L., Bassant, M. H., Gourmelon, P., Gueneau, G. and Pasquier, C., 1986, Impairment of electrical functions of CNS and alterations in cell populations associated with irradiation. *British Journal of Cancer*, **53(Suppl. VII),** 230–231.

Cunningham, A. J., Murray, C. A., O'Neill, L. A., Lynch, M. A. and O'Connor, J. J., 1996, Interleukin-1 beta (IL-1 beta) and tumour necrosis factor (TNF) inhibit long-term potentiation in the rat dentate gyrus *in vitro*. *Neuroscience Letters*, **203,** 17–20.

Dantzer, R., Bluthé, R. M., Gheusi, G., Crémona, S., Layé, S., Parnet, P. and Kelley, K. W., 1998, Molecular basis of sickness behavior. *Annals of the New York Academy of Sciences*, **856,** 132–138.

Dunn, A. J., 1992, Endotoxin-induced activation of cerebral catecholamine and serotonin metabolism: comparison with interleukin-1. *Journal of Pharmacology and Experimental Therapeutics*, **261,** 964–969.

Ericsson, A., Kovacs, K. and Sawchenko, P., 1994, A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *Journal of Neuroscience*, **14,** 897–913.

Fleshner, M., Goehler, L., Schwartz, B., McGorry, M., Martin, D., Maier, S. and Watkins, L., 1998, Thermogenic and corticosterone responses to intravenous cytokines (IL-1beta and TNF-alpha) are attenuated by sub-diaphragmatic vagotomy. *Journal of Neuroimmunology*, **86,** 134–141.

Goehler, L. E., Gaykema, R. P., Hammack, S. E., Maier, S. F. and Watkins, L. R., 1998, Interleukin-1 induces c-Fos immunoreactivity in primary afferent neurons of the vagus nerve. *Brain Research*, **804,** 306–310.

Goehler, L. E., Gaykema, R. P., Nguyen, K. T., Lee, J. E., Tilders, F. J., Maier, S. F. and Watkins, L. R., 1999, Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? *Journal of Neuroscience*, **19,** 2799–2806.

Goehler, L. E., Relton, J. K., Dripps, D., Kiechle, R., Tartaglia, N., Maier, S. F. and Watkins, L. R., 1997, Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Research Bulletin*, **43,** 357–364.

Gueneau, G., Drouet, J., Privat, A. and Court, L., 1979, Differential radiosensitivity of neurons and neuroglia of the hippocampus in the adult rabbit. *Acta Neuropathology*, **48,** 199–209.

Hansen, M. K., Taishi, P., Chen, Z. and Krueger, J. M., 1998, Vagotomy blocks the induction of interleukin-1beta (IL-1beta) mRNA in the brain of rats in response to systemic IL-1beta. *Journal of Neuroscience*, **18,** 2247–2253.

Hong, J.-H., Chiang, C.-S., Campbell, I. L., Sun, J.-R., Withers, H. R. and Mcbride, W. H., 1995, Induction of acute phase gene expression by brain irradiation. *International Journal of Radiation Oncology Biology and Physics*, **33,** 619–626.

Hopewell, J. W., 1998, Radiation injury to the central nervous system. *Medical and Pediatric Oncology*, **Suppl. 1,** 1–9.

Hosoi, T., Okuma, Y. and Nomura, Y., 2000, Electrical stimulation of afferent vagus nerve induces IL-1beta expression in the brain and activates HPA axis. *American Journal of Physiology*, **279,** R141–147.

Hosoi, Y., Miyachi, H., Matsumoto, Y., Enomoto, A., Nakagawa, K., Suzuki, N. and Ono, T., 2001, Induction of interleukin-1beta and interleukin-6 mRNA by low doses of ionizing radiation in macrophages. *International Journal of Cancer*, **96,** 270–276.

Huang, T. S., Huang, S. C. and Hsu, M. M., 1994, A prospective study of hypothalamus pituitary function after cranial irradiation with or without radiosensitizing chemotherapy. *Journal of Endocrinology Investigation*, **17,** 615–623.

Ibrahim, M. Z., Haymaker, W., Miquel, J. and Riopelle, A. J., 1967, Effects of radiation on the hypothalamus in monkeys. *Archiv für Psychiatrie und Nervenkrankheiten*, **210,** 1–15.

Ivanov, V. K., Maksioutov, M. A., Chekin, S., Kruglova, Z. G., Petrov, A. V. and Tsyb, A. F., 2000, Radiation-epidemiological analysis of incidence of non-cancer diseases among the Chernobyl liquidators. *Health Physics*, **78,** 495–501.

Kandasamy, S. B., 1998, Effect of ionizing radiation on the release of cholecystokinin in the hypothalamus of the rat. *Radiation Research*, **150,** 298–303.

Kassayova, M., Ahlersova, E., Pastorova, B. and Ahlers, I., 1995, The early response of pineal N-acetyltransferase activity, melatonin and catecholamine levels in rats irradiated with gamma rays. *Physiological Research*, **44,** 315–320.

Layé, S., Bluthé, R. M., Kent, S., Combe, C., Médina, C., Parnet, P., Kelley, K. and Dantzer, R., 1995, Subdiaphragmatic vagotomy blocks induction of IL-1β mRNA in mice brain in response to peripheral LPS. *American Journal of Physiology*, **268,** R1327–R1331.

Layé, S., Parnet, P., Goujon, E. and Dantzer, R., 1994, Peripheral administration of lipopolysaccharide induces the expression of cytokine transcripts in the brain and pituitary of mice. *Molecular Brain Research*, **27,** 157–162.

Lynch, M. A., 1998, Age-related impairment in long-term potentiation in hippocampus: a role for the cytokine, interleukin-1 beta? *Progress in Neurobiology*, **56,** 571–589.

Makale, M. T. and King, G. L., 1993, Plasticity of autonomic control of emesis in irradiated ferrets. *American Journal of Physiology*, **265,** R1092–1099.

Marquette, C., Weber, P., Gourmelon, P., Tsiang, H. and Ceccaldi, P.-E., 1999, Modulation of brain inflammation during rabies infection by immunosuppressive treatments: ionizing radiation, cyclosporine and cyclophosphamide. *Recent Research Developments in Virology*, **1,** 735–751.

Mechanick, J., Hochberg, F. and Laroque, A., 1986, Hypothalamic dysfunction following whole-brain irradiation. *Journal of Neurosurgery*, **65,** 490–494.

Mestries, J. C., Gourmelon, P. and Court, L., 1987, Modifications précoces du débit sanguin cortical, de la température cérébrale et de l'activité électrique cérébrale après irradiation globale chez le macaque cynomolgus (doses de 3 à 20 Gy). Rapport CEA, R-5386.

Olschowka, J. A., Kyrkanides, S., Harvey, B. K., O'Banion, M. K., Williams, J. P., Rubin, P. and Hansen, J. T., 1997, ICAM-1 induction in the mouse CNS following irradiation. *Brain, Behavior, and Immunity*, **11,** 273–285.

Pai, H. H., Thornton, A., Katznelson, L., Finkelstein, D. M., Adams, J. A., Fullerton, B. C., Loeffler, J. S., Leibsch, N. J., Klibanski, A. and Munzenrider, J. E., 2001, Hypothalamic/pituitary function following high-dose

conformal radiotherapy to the base of skull: demonstration of a dose–effect relationship using dose–volume histogram analysis. *International Journal of Radiation Oncology Biology and Physics*, **49,** 1079–1092.

Parnet, P., Amindari, S., Wu, C., Brunke-Reese, D., Goujon, E., Weyhenmeyer, J. A., Dantzer, R. and Kelley, K. W., 1994, Expression of type I and type II interleukin-1 receptors in mouse brain. *Molecular Brain Research*, **27,** 63–70.

Peissner, W., Kocher, M., Treuer, H. and Gillardon, F., 1999, Ionizing radiation-induced apoptosis of proliferating stem cells in the dentate gyrus of the adult rat hippocampus. *Molecular Brain Research*, **71,** 61–68.

Pugh, R. C., Fleshner, M., Watkins, L. R., Maier, S. F. and Rudy, J. W., 2001, The immune system and memory consolidation: a role for the cytokine IL-1beta. *Neuroscience Biobehavioral Reviews*, **25,** 29–41.

Roozendaal, B., Sapolsky, R. M. and McGaugh, J. L., 1998, Basolateral amygdala lesions block the disruptive effects of long-term adrenalectomy on spatial memory. *Neuroscience*, **84,** 453–465.

Rubin, P., Johnston, C. J., Williams, J. P., McDonald, S. and Finkelstein, J. N., 1995, A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. *International Journal of Radiation Oncology Biology and Physics*, **33,** 99–109.

Saper, C. B., 1995, Central autonomic system. In G. Paxinos (ed.), *The Rat Nervous System* (San Diego: Academic Press), pp. 107–135.

Saphier, D. and Ovadia, H., 1990, Selective facilitation of putative corticotropin-releasing factor-secreting neurones by interleukin-1. *Neuroscience Letters*, **114,** 283–288.

Smith, G. P., Jerome, C., Cushin, B. J., Eterno, R. and Simansky, K. J., 1981, Abdominal vagotomy blocks the satiety effect of cholecystokinin in the rat. *Science*, **213,** 1036–1037.

Snyder, J. S., Kee, N. and Wojtowicz, J. M., 2001, Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *Journal of Neurophysiology*, **85,** 2423–2431.

Tofilon, P. J. and Fike, J. R., 2000, The radioresponse of the central nervous system: a dynamic process. *Radiation Research*, **153,** 357–370.

Van Dam, A. M., Bauer, J., Tilders, J. H. and Berkenbosch, F., 1995, Endotoxin-induced appearance of immunoreactive interleukin-1β in ramified microglia in rat brain: a light and electron microscopic study. *Neuroscience*, **65,** 815–826.

Varagic, V., Stepanovic, S., Svencenski, N. and Hajdukovic, S., 1967, The effect of X-irradiation on the amount of catecholamines in heart atria and hypothalamus of the rabbit, and in brain and heart of the rat. *International Journal of Radiation Biology*, **12,** 113–119.

Vereker, E., Campbell, V., Roche, E., Mcentee, E. and Lynch, M. A., 2000, Lipopolysaccharide inhibits long term potentiation in the rat dentate gyrus by activating caspase-1. *Journal of Biological Chemistry*, **275,** 26 252–26 258.

Wan, W., Wetmore, L., Sorensen, C. M., Greenberg, A. H. and Nance, D. M., 1994, Neural and biochemical mediators of endotoxin and stress-induced c-fos expression in the rat brain. *Brain Research Bulletin*, **34,** 7–14.

Watkins, L. R., Maier, S. F. and Goehler, L. E., 1995, Cytokine-to-brain communication : a review and analysis of alternative mechanisms. *Life Sciences*, **57,** 1011–1026.

Wayner, M. J., Phelix, C. F. and Amstrong, D. L., 1997, Lateral hypothalamic stimulation inhibits dentate gyrus granule cell LTP: direct connections. *Brain Research Bulletin*, **43,** 5–15.

Yamada, Y., Tsukamoto, G., Kobashi, M., Sasaki, A. and Matsumura, T., 2000, Abdominal vagi mediate c-Fos expression induced by X-ray irradiation in the nucleus tractus solitarii of the rat. *Autonomic Neuroscience*, **83,** 29–36.