The systemic inflammatory response is involved in the regulation of K^+ channel expression in brain via TNF-α-dependent and -independent pathways

Rubén Vicente[a,1], Mireia Coma[a,1], Silvia Busquets[b], Rodrigo Moore-Carrasco[b], Francisco J. López-Soriano[b], Josep M. Argilés[b], Antonio Felipe[a,*]

[a] *Molecular Physiology Laboratory, Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain*

[b] *Cancer Research Group, Departament de Bioquímica i Biologia Molecular, Universitat de Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain*

Received 6 June 2004; revised 16 July 2004; accepted 16 July 2004

Available online 22 July 2004

Edited by Ned Mantei

Abstract TNF-α, generated during the systemic inflammatory response, triggers a wide range of biological activities that mediate the neurologic manifestations associated with cancer and infection. Since this cytokine regulates ion channels in vitro (especially Kv1.3 and Kir2.1), we aimed to study Kv1.3 and Kir2.1 expression in brain in response to in vivo systemic inflammation. Cancer-induced cachexia and LPS administration increased plasma TNF-α. Kv1.3 and Kir2.1 expression was impaired in brain during cancer cachexia. However, LPS treatment induced Kv1.3 and downregulated Kir2.1 expression, and TNF-α administration mimicked these results. Experiments using TNF-α double receptor knockout mice demonstrated that the systemic inflammatory response mediates K^+ channel regulation in brain via TNF-α-dependent and -independent redundant pathways. In summary, distinct neurological alterations associated with systemic inflammation may result from the interaction of various cytokine pathways tuning ion channel expression in response to neurophysiological and neuroimmunological processes.

Keywords: K^+ channels; Cancer cachexia; Systemic inflammatory response; Brain; TNF-α; Lipopolysaccharide

1. Introduction

Systemic inflammation represents a pleiotropic mechanism generated in response to a number of body insults. Trauma, AIDS, drug abuse, infection and cancer are often associated with this pathological condition through the production of inflammatory cytokines, TNF-α being one of the most relevant. The administration of lipopolysaccharide (LPS) or pro-inflammatory cytokines to animals mimics several catabolic aspects of cancer-induced cachexia [1,2]. Cytokines act in paracrine, autocrine and intracrine manners, and are often difficult to detect in the circulation. In fact, paracrine interactions are a predominant cytokine mode of action within organs, including the brain. In the central nervous system, the direct action of cytokines in neuronal cells either increases or decreases neuronal activity, while in other nerve cell types, it can activate an immunological/inflammatory response. In certain pathophysiological conditions, cytokine cascades lead to neurotoxicity and neurodegeneration [3].

LPS, a gram-negative bacterial endotoxin, has been extensively used for experimental induction of an inflammatory response; it causes anorexia and fever, and increases TNF-α production in rodents [4,5]. On the other hand, experimentally induced cancer-cachexia is a pathological state also characterized by a significant increase in TNF-α blood levels [6]. This cytokine possesses a wide range of biological activities. In the nervous system, TNF-α exerts cytotoxic effects on oligodendrocytes and affects myelin functions [7]. In addition, the concentration of TNF-α in brain tissues or serum is enhanced in neurodegenerative disorders such as Parkinson's and Alzheimer's diseases [8,9]. Thus, the central nervous system (CNS) is a clear target for cytokines involved in the inflammatory response. In this context, in vivo characterization of the molecular mechanisms responsible for the neurological manifestations triggered by the systemic inflammatory response is a key issue. One such mechanism involves the modulation of ion channels.

Neuronal K^+ channels (KCh) form a large and diverse group that governs many physiological functions, e.g., resting membrane potential, action potential duration and frequency, and neurotransmitter release [10]. Voltage-dependent currents are mainly mediated by *Shaker*-like potassium channels (Kv), whereas inward currents are conducted by inward rectifier K^+ channels (Kir). In CNS excitability, Kv repolarizes the membrane potential following a depolarizing stimulus. However, Kir stabilizes the resting membrane potential near the K^+ equilibrium potential and mediates K^+ transport across membranes [10].

The few in vitro studies that have analyzed the physiological effects of TNF-α on KCh show controversial results [11–14]. In this regard, we have described how Kv1.3 and Kir2.1 are regulated by LPS via TNF-α-dependent and -independent mechanisms in macrophages [15]. We have also reported that

* Corresponding author. Fax: +34-934021559.
E-mail address: afelipe@ub.edu (A. Felipe).

[1] These authors contributed equally to this study.

0014-5793/$22.00
doi:10.1016/j.febslet.2004.07.030

the activity of both channels is needed for the fine tuning of the immunological response. Kv1.3 is involved in macrophage-induced activation by LPS and TNF-α [15]. It is also present in brain and given its biophysical activity, it has been related to the control of the neuronal firing pattern [16]. Moreover, Kir2.1 is also widely expressed in nerve cells with a relevant role in neuronal excitability and glial K^+ transport [17]. Although experimental cancer-induced cachexia downregulates the expression of Kv channels (i.e., Kv1.3) in brain, there is no information on Kir2.1 regulation or whether impaired Kv expression is directly related to the generation of a systemic inflammatory response [6].

Bearing all this in mind, we aimed to study the expression of the strongly inwardly rectifying potassium channel Kir2.1 in experimental cancer-induced cachexia and to evaluate whether KCh regulation is related to the rise in blood TNF-α levels during the systemic inflammatory response in vivo. In this context, since no in vivo studies have been reported, we followed several related approaches: (i) experimental cancer-induced cachexia by a rapidly growing tumor; (ii) TNF-α chronic administration and (iii) LPS administration using wild-type and TNF-α double receptor knockout (TNF-α RI/II-KO) mice. We show that the systemic inflammatory response regulates Kv1.3 and Kir2.1 channels differentially and that this modulation is mediated via TNF-α-dependent and -independent redundant pathways. Our results suggest that the differential expression of KCh subunits in the brain is crucial to achieving the correct neural activity in the systemic inflammatory response.

2. Materials and methods

2.1. Animals and experimental design

Several experimental groups of at least five animals each were used. Animals from Iffa-Credo (France) were fed ad libitum on a regular chow diet with free access to drinking water.

Experimental cancer-induced cachexia: Female Wistar rats (~200 g) were injected intraperitoneally with a Yoshida AH-130 ascites hepatoma cell suspension (approx. 10^8 cells in 2 ml). Control rats were injected with 0.9% (w/v) NaCl solution (physiological saline) as described [6]. All extractions were performed 7 days after tumor transplantation.

TNF-α treatment: Female Wistar rats (~200 g) were injected twice a day with recombinant-derived human TNF-α (50 μg/kg, i.p.) for 4 days [18]. Control animals followed the same schedule receiving saline.

LPS treatment: C57BL/6 mice of about 20 weeks of age were used. TNF-α RI/II-KO mice kindly provided by Hoffmann–LaRoche were generated and characterized as reported [19,20]. Purified LPS (*Escherichia coli* endotoxin 0111:B4) was dissolved in pyrogen-free saline. Animals were injected intraperitoneally at a dose of 2.5 mg/kg. Animals kept at thermoneutral temperature (30 °C) developed fever 23 h after the LPS injection (data not shown, [21]). Another set of wild type and TNF-α RI/II-KO mice was used to administrate TNF-α (100 μg/kg, i.v.) as previously described [22–24]. In all cases, control mice received sterile saline.

2.2. Samples and TNF-α measurements

Blood was collected from killed animals and the plasma was obtained by centrifugation and quickly frozen. In all cases, tissue samples were removed and weighed at the end of the treatment. The brain and the heart were immediately frozen with liquid nitrogen and maintained at −80 °C until use. Animal handling procedures were approved by the Ethics committee of the University of Barcelona.

Plasma TNF-α was measured by using an ELISA test (Genzyme Corp.) following the manufacturer's instructions.

2.3. RNA extraction, cDNA probes, constructs, reverse transcription-polymerase chain reaction (RT-PCR) and Southern blot

Total RNA from brain and heart was isolated using Tripure (Roche Diagnostics). Samples were further treated with the DNA-free kit from Ambion Inc. to remove DNA. RNA from at least five animals was analyzed per group.

Ready-to-Go RT-PCR Beads (Amersham Pharmacia Biotech) were used in a one-step RT-PCR. Total RNA and Kv1.3, Kir2.1 and 18S primers were added to the beads as described [15]. A range of PCR cycles and RNA dilutions from each independent sample were performed to obtain an exponential phase of amplicon production (data not shown) as described [25]. The same independent RNA aliquot was used to analyze the KCh mRNA expression and the respective amount of 18S rRNA.

Once the exponential phase of the amplicon production had been determined, the specificity of each product was confirmed in test RT-PCRs using the appropriate cDNA probe in a Southern blot analysis [26]. PCR-generated KCh cDNA probes from mouse brain were used as probes as described [15]. At least three filters were prepared from independent samples and representative blots are shown. The densitometric analysis of the blots was performed using Phoretix software (Nonlinear Dynamics). Results are the means ± S.E.M. of each experimental group. Where indicated, statistical analysis by Student's *t* test was performed.

2.4. Membrane preparation and Western blot analysis

Brain samples were homogenized in 320 mM sucrose, supplemented with 1 μg/ml aprotinin, 1 μg/ml leupeptin, 86 μg/ml iodoacetamide and 1 mM phenylmethylsulfonyl fluoride as protease inhibitors in a glass homogenizer. An enriched membrane preparation was obtained [27]. Briefly, homogenates were centrifuged at $2000 \times g$ for 5 min and the supernatant was further centrifuged at $\sim 100\,000 \times g$ for 1 h. The pellet was resuspended in the same solution and protein content was determined by Bio-Rad Protein Assay (Bio-Rad). Samples were aliquoted and stored at −80 °C.

Crude membrane protein samples (50 μg) were boiled in Laemmli SDS loading buffer and separated on 10% SDS–PAGE. They were transferred to nitrocellulose membranes (Immobilon-P, Millipore) and blocked in 5% dried milk-supplemented 0.2% Tween 20 PBS before immunoreaction. To monitor Kv1.3 and Kir2.1 expression, rabbit polyclonal antibodies (Alomone Labs) were used. As a loading and transfer control, a monoclonal anti-β-actin antibody (Sigma) was used.

3. Results and discussion

The generation and release of proinflammatory cytokines are among the main features of the systemic inflammatory response. In particular, TNF-α is a pleiotropic cytokine that plays a pivotal role in inflammatory diseases of the central nervous system [3,11,12]. It is generated mostly by macrophages in response to an insult and is a major mediator of the septic shock syndrome induced by either LPS or bacterial superantigens [20]. In addition, high levels of TNF-α have also been detected in neurodegenerative disorders [8,9]. Bearing this in mind, the aim of the present work was to analyze the potential role of TNF-α regulating KCh expression in brain during a systemic inflammatory response.

The results presented in Fig. 1 show plasma TNF-α concentration in two models of systemic inflammatory response, a model of experimental induced cancer-cachexia and in another model involving LPS administration. Rats that efficiently developed the Yoshida AH-130 tumor showed an increase in plasma cytokine concentration. On the other hand, LPS-treated mice also showed high TNF-α levels in plasma, as reported elsewhere [28,29]. During cancer cachexia, the systemic TNF-α concentration increases concomitantly with tumor progression, while LPS rapidly increases plasma TNF-α within the first 2–3 h with a progressive decay over the next 24 h (data not

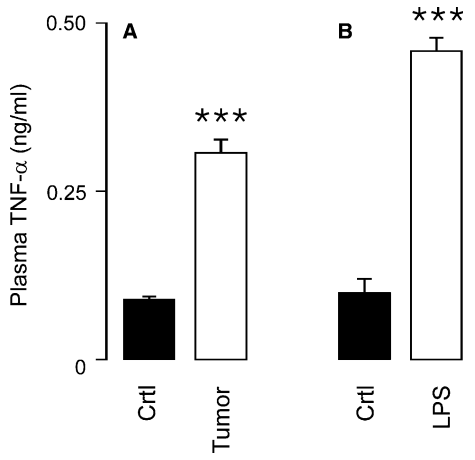

Fig. 1. Experimental cancer cachexia and LPS administration increased plasma TNF-α levels. (A) Wistar rats were injected intraperitoneally with saline (Crtl) or Yoshida AH-130 ascites hepatoma cell suspension (Tumor) as described in Section 2. Samples were collected 7 days after tumor transplantation. (B) Some C57BL/6 mice were injected intraperitoneally (2.5 mg/kg) with purified LPS in saline (LPS). Control mice (Crtl) received an equivalent volume of sterile saline as described in Section 2. Blood samples were collected 3 h after LPS administration. Values are means ± S.E.M. of five animals. *** $P < 0.001$ vs. Crtl (Student's t test).

shown, [2]). These results indicate that both situations share a significant rise in circulating TNF-α triggering the catabolic inflammatory response.

Several neurodegenerative disorders, such as Parkinson's and Alzheimer's diseases share a systemic inflammatory response, similar to that we observed [8,9]. In these pathological conditions, nerve KCh are implicated in the neural response. Thus, β-amyloid peptide regulates the expression of KCh in microglia by upregulating Kv1.3 and Kv1.5 with no effects on Kir channels [30]. Kv channels (i.e., Kv1.3) repolarize the membrane potential after depolarization. However, Kir channels stabilize the resting membrane potential near the K^+ equilibrium potential and mediate K^+ transport across membranes. This role is crucial in the case of the strongly inward rectifier Kir2.1, which participates in the intrusion of K^+ ions released from nerve axons. An increase in extracellular K^+ may induce uncontrolled hyperexcitability and abnormal synchronization of neurons [17]. Experimental cancer cachexia specifically downregulated Kir2.1 as observed with Kv1.3 [6, Fig. 2]. Kv1.3 mRNA expression is impaired in the brain of tumor-bearing animals [6]. Here, we confirm and extend these results by reporting a parallel decrease in Kv1.3 protein in crude membrane samples from brain (Fig. 2B). The Kir2.1 downregulation observed in brain seems to be tissue-specific. This result may seem puzzling, since cancer cachexia is char-

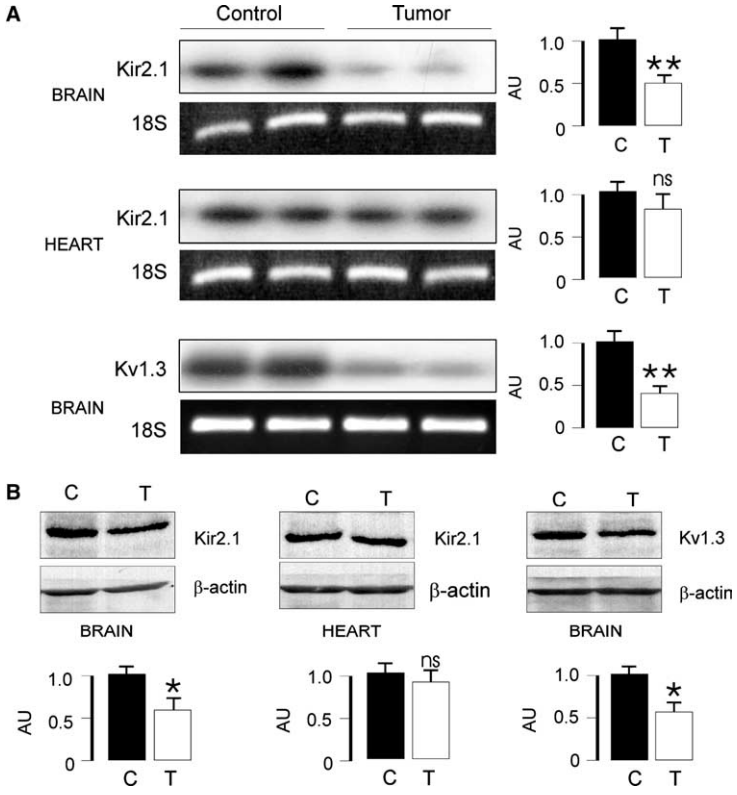

Fig. 2. Expression of Kv1.3 and Kir2.1 in brain and heart from animals under experimental cancer cachexia. At least five animals were inoculated with either saline (Control) or the Yoshida AH-130 ascites hepatoma cell suspension (Tumor) as described in Section 2. Total RNA and membrane protein samples were analyzed for K^+ channel expression after 7 days of tumor growth. (A) Kir2.1 and Kv1.3 mRNA expression. Results from RT-PCR on 0.25 μg and 1 μg for brain and heart, respectively, and 30 cycles for Kir2.1 and Kv1.3, and 0.1 μg and 10 cycles for 18S are shown. These conditions were at the exponential phase of the amplicon production as described in Section 2. (B) Kir2.1 and Kv1.3 Western blot analysis. Representative blots are shown. Arbitrary unit (AU) values the means ± S.E.M. of at least four animals. Closed bars, Control (C); open bars, tumor-bearing animals (T). *$P < 0.05$; **$P < 0.01$; ns, not significant vs. control (Student's t test).

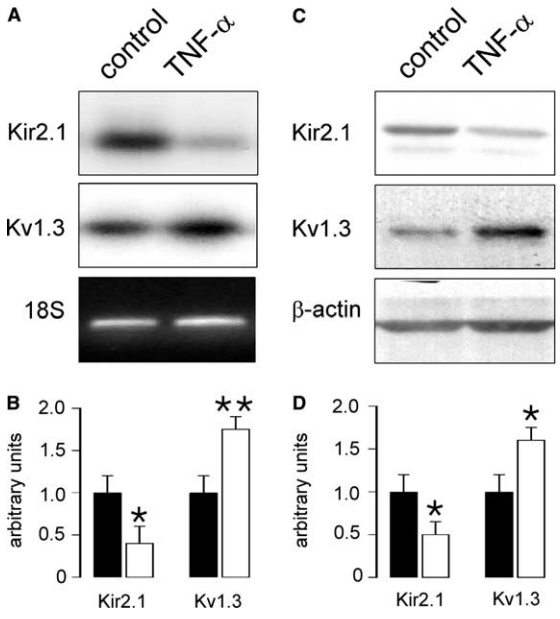

Fig. 3. Expression of Kv1.3 and Kir2.1 in brain from TNF-α-treated animals. At least five animals were administered intraperitoneally with either saline (control) or recombinant-derived human TNF-α (TNF-α) for 4 days. Animals were administered in two daily injections of 50 μg/kg (total dose of100 μg/kg per day) for 4 days as described in Section 2. (A) Kir2.1 and Kv1.3 mRNA expression. Results from RT-PCR on 0.25 μg and 30 cycles for Kir2.1 and Kv1.3, and 0.1 μg and 10 cycles for 18S are shown. These conditions were at the exponential phase of amplicon production as described in Section 2. (B) Kir2.1 and Kv1.3 mRNA expression values derived from panel A. (C) Kir2.1 and Kv1.3 Western blot analysis. Representative blots are shown. (D) Kir2.1 and Kv1.3 protein expression values derived from panel C. In all cases, arbitrary units are standardized to the control value in the absence of TNF-α. Values are means ± S.E.M. of at least four animals. Closed bars, control; open bars, TNF-α-treated animals. $*P < 0.05$; $**P < 0.01$ vs. control (Student's t test).

acterized by muscle wasting [2]. However, the physiological role for Kir2.1 in muscle and brain may differ. In this context, the CNS responds to acute inflammation differently from other body tissues. In fact, calcium channels are regulated differentially in cardiac and neuronal cells [31]. Moreover, evidence indicates that unlike other well-known neurological disorders [3], the heart dysfunction that initiates with the syndrome may be peripheral [1].

Since plasma TNF-α levels increased during experimental cancer cachexia and may be responsible for impaired KCh expression in brain, we performed a set of experiments injecting TNF-α to rats. Previous studies from our group have clearly indicated that this chronic TNF-α administration mimics most of the metabolic alterations formed during experimental cancer cachexia. However, after 4 days of treatment, animals develop tolerance to the cytokine (see [2] for review). TNF-α differentially regulated Kv1.3 and Kir2.1 channels in brain (Fig. 3). In contrast to cancer cachexia, while Kir2.1 expression decreased, Kv1.3 was upregulated. These results may be interpreted taking into consideration certain key differences between the two models. Thus, while experimental cancer cachexia is a non-reversible situation in which brain cells undergo apoptosis [6], the increase in plasma TNF-α by chronic administration of the cytokine may be reversible, as it is during infection. Moreover, K^+ channel expression is impaired in the brain during experimental cancer cachexia, and animals lose weight and develop anorexia [6]. In addition, some K^+ channel gene knockout mice lost body weight and showed altered feeding behavior [32,33]. However, chronic TNF-α administration does not affect food intake or body mass [18]. Other factors may explain the discrepancy between these two models. Thus, during experimental induced cancer-cachexia, the long term increase in plasma TNF-α could desensitize TNF-α receptors or upregulate the soluble forms of the receptor (sTNFR). In fact, high plasma levels of sTNFR have been found in patients with advanced gastrointestinal cancer and sTNFR is an agonist of TNF-α that could prevent some cytokine effects [34–37].

TNF-α is produced by lymphocytes and macrophages in response to an insult [20]. Within the brain, not only microglia but also activated leukocytes generate TNF-α locally [3]. As a result, there is an inflammatory response similar to that described in AIDS patients in which TNF-α expression correlates with the degree of dementia [38]. Few in vitro studies have reported differential K^+ channel regulation by TNF-α in nerve cells, with controversial results, e.g., enhanced, unaltered and reduced outward and inward K^+ currents [11–14]. However, the proteins responsible have not been identified. In addition, a differential Kv1.3 and Kir2.1 regulation by TNF-α in the brain is not surprising. In fact, our results agree with those described in bone marrow and brain macrophages [15,39].

During an infection, LPS is one of the strongest activators of macrophages and leads to the secretion of TNF-α. The generation of this and other cytokines such as IL-1β, IL-6 and IL-12 leads to a rapid systemic inflammatory response in the host. As mentioned above, LPS administration increases the systemic TNF-α concentration (Fig. 1). Despite the amount of information on how LPS affects KCh in in vitro studies [15,40,41], there are few in vivo studies. Exposure to sub-lethal doses of LPS evokes a systemic inflammatory response that includes fever and increased oxygen consumption similar to that described in certain types of cancer [21]. In acute inflammatory response, KCh were differentially regulated (Fig. 4). Kv1.3 expression was induced, whereas Kir2.1 was downregulated as observed with TNF-α. Several LPS effects are mediated by the production of TNF-α. Bearing this in mind, we aimed to test whether this modulation depended on the cytokine. For this purpose, we used TNF-α RI/II-KO mice, which received an acute LPS injection as mentioned above. While Kv1.3 upregulation was TNF-α-dependent, Kir2.1 modulation was independent (Fig. 4). These results are in agreement with those described using macrophages, which indicate that the LPS modulation of Kir2.1 is independent of TNF-α, whereas Kv1.3 regulation could be partially dependent [15]. However, TNF-α may also promote physiological effects by pathways independent of TNF-α receptors via its lectin binding domain [42,43]. This possibility was tested by injecting TNF-α into wild-type and TNF-α RI/II-KO mice. This treatment, which generates important metabolic alterations [22–24], did not modify KCh expression in the brain of TNF-α RI/II-KO mice. However, the effects in wild-type animals were similar to those found with LPS (data not shown). Taken together, these data suggest that LPS involves TNF-α-dependent and -independent redundant mechanisms in a number of cases. In fact, not only KCh but also nucleoside transport regulation may require this mode of action [44]. In addition, LPS-induced apoptosis involves TNF-α as an early step followed by induction of nitric oxide synthase [20]. In this regard, cancer may

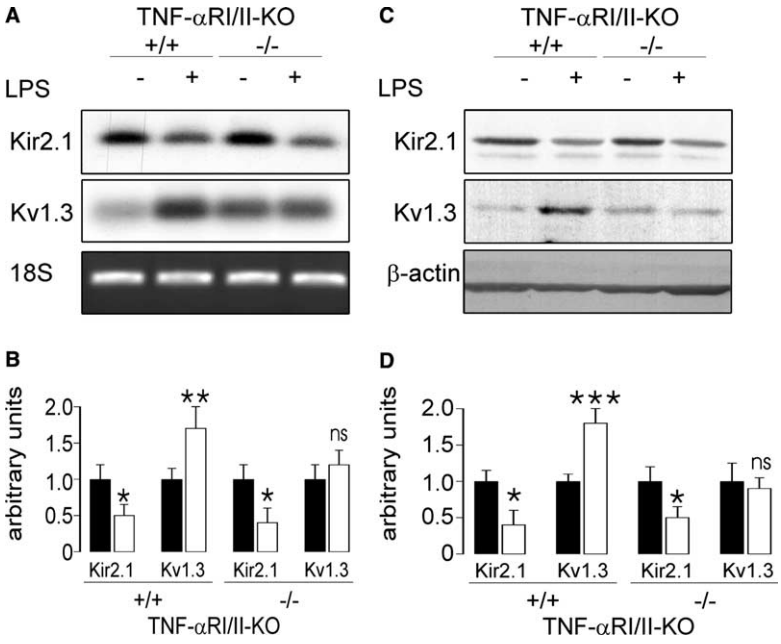

Fig. 4. Expression of Kv1.3 and Kir2.1 in brain from LPS-treated animals. At least five C57BL/6 wild type (+/+) or TNF-α receptor I/II double knockout (−/−) mice were administered intraperitoneally with either saline (−) or 2.5 mg/kg LPS (+). Animals were kept at thermoneutral temperature (30 °C) and samples were collected 23 h after LPS injection. (A) Kir2.1 and Kv1.3 mRNA expression. Results from RT-PCR on 0.25 μg and 30 cycles for Kir2.1 and Kv1.3, and 0.1 μg and 10 cycles for 18S are shown. These conditions were at the exponential phase of the amplicon production as described in Section 2. Representative blots are shown. (B) Kir2.1 and Kv1.3 mRNA expression values derived from panel A. (C) Kir2.1 and Kv1.3 Western blot analysis. Representative blots are shown. (D) Kir2.1 and Kv1.3 protein expression values derived from panel C. In all cases, arbitrary units are standardized to the control value in the absence of LPS (−). Values are means ± S.E.M. of at least four animals. Closed bars, control; open bars, LPS-treated animals. No significant differences were found between wild type (+/+) and TNF-α receptor I/II double knockout (−/−) mice in the absence of TNF-α (−). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant vs. control (Student's t test).

induce oxidative stress within the brain, which has been related to the appearance of neurological disorders [45]. Once cytokine production is activated within the brain, paracrine and autocrine interactions can sustain local cytokine production through positive-feedback systems [3].

In summary, the systemic inflammatory response may be responsible for KCh regulation in brain. Blood TNF-α levels during the endotoxic shock may trigger a finely tuned modulation of KCh in the neurological response. Moreover, redundant TNF-α-dependent and -independent mechanisms are probably involved. The apparent discrepancy between infection and experimental cancer cachexia is not an irrelevant issue. On the contrary, it is indicative of quite different catabolic conditions. Upon experimental cancer cachexia, brain cells undergo apoptosis [6], which leads to neurotoxicity and neurodegeneration [3]. In contrast, acute inflammation caused by LPS may induce various responses within the brain that may lead to distinct neurological disfunctions.

Acknowledgements: This study was supported by grants from the Ministerio de Ciencia y Tecnología, Spain (BFI2002-00764 to AF and BFI2002-02186 to J.M.A.) and the Ministerio de Sanidad, Spain (FIS 03/0100 to F.J.L.-S.), Generalitat de Catalunya, Catalonia, Spain (SGR00108 to J.M.A.) and the Universitat de Barcelona (to A.F.). R.V. and R.M.-C. hold a fellowship from the Universitat de Barcelona.

References

[1] Sharma, R. and Anker, S.D. (2002) Int. J. Cardiol. 85, 161–171.
[2] Argilés, J.M., Alvarez, B. and López-Soriano, F.J. (1997) Medicinal Res. Rev. 17, 477–498.
[3] Turrin, N.P. and Plata-Salamán, C.R. (2000) Brain Res. Bulletin 51, 3–9.
[4] Li, S., Wang, Y., Matsumura, K., Ballou, L.R., Morham, S.G. and Blatteis, C.M. (1999) Brain Res. 825, 86–94.
[5] Porter, M.H., Hrupka, B.J., Altreuther, G., Arnold, M. and Langhans, W. (2000) Am. J. Physiol. Regul. Integr. Comp. Physiol. 279, R2113–R2120.
[6] Coma, M., Vicente, R., Busquets, S., Carbó, N., Tamkun, M.M., López-Soriano, F.J., Argilés, J.M. and Felipe, A. (2003) FEBS Lett. 536, 45–50.
[7] Selmaj, K.W. and Raine, C.S. (1988) Ann. Neurol. 23, 339–346.
[8] Boka, G., Anglade, P., Wallach, D., Javoy-Agid, F., Agid, Y. and Hirsch, E.C. (1994) Neurosci. Lett. 172, 151–154.
[9] Fillit, H., Ding, W.H., Buee, L., Kalman, J., Altstiel, L., Lawlor, B. and Wolf-Klein, G. (1991) Neurosci. Lett. 129, 318–320.
[10] Hille, B. (2001) Ion channels of excitable membranes. Sinauer associates Inc, Sunderland, MA.
[11] Houzen, H., Kikuchi, S., Kanno, M., Shinpo, K. and Tashiro, K. (1997) J. Neurosci. Res. 50, 990–999.
[12] Köller, H., Allert, N., Oel, D., Stoll, G. and Siebler, M. (1998) Neuro Report 9, 1375–1378.
[13] Wu, S.N., Lo, Y.K., Kuo, B.I. and Chiang, H.T. (2001) Endocrinology 142, 4785–4794.
[14] Diem, R., Meyer, R., Weishaupt, J.H. and Barhr, M. (2001) J. Neurosci. 21, 2058–2066.
[15] Vicente, R., Escalada, A., Coma, M., Fuster, G., Sanchez-Tillo, E., Lopez-Iglesias, C., Soler, C., Solsona, C., Celada, A. and Felipe, A. (2003) J. Biol. Chem. 278, 46307–46320.
[16] Kupper, J., Prinz, A.A. and Fromberg, P. (2002) Plügers Arch.-Eur. J. Physiol. 443, 541–547.
[17] Horio, Y. (2001) Jpn. J. Pharmacol. 87, 1–6.
[18] Llovera, M., García-Martínez, C., López- Soriano, F.J. and Argilés, J.M. (1994) Biochem. Mol. Biol. Int. 33, 681–689.

[19] Bruce, A.J., Boling, W., Kindy, M.S., Peschon, J., Kraemer, P.J., Carpenter, M.K., Holtsberg, F.W. and Mattson, M.P. (1996) Nat. Med. 2, 788–794.
[20] Xaus, J., Comalada, M., Valledor, A.F., Lloberas, J., López-Soriano, F.J., Argilés, J.M., Bogdan, C. and Celada, A. (2000) Blood 95, 3823–3831.
[21] Busquets, S., Alvarez, B., Van Royen, M., Figueras, M.T., López-Soriano, F.J. and Argilés, J.M. (2001) Biochim. Biophys. Acta 1499, 249–256.
[22] Garcia-Martinez, C., Agell, N., Llovera, M., Lopez-Soriano, F.J. and Argiles, J.M. (1993) FEBS Lett. 323, 211–214.
[23] Garcia-Martinez, C., Llovera, M., Agell, N., Lopez-Soriano, F.J. and Argiles, J.M. (1994) Biochem. Biophys. Res. Commun. 201, 682–686.
[24] Busquets, S., Sanchis, D., Alvarez, B., Ricquier, D., Lopez-Soriano, F.J. and Argiles, J.M. (1998) FEBS Lett. 440, 348–350.
[25] Fuster, G., Vicente, R., Coma, M., Grande, M. and Felipe, A. (2002) Methods Find. Exp. Clin. Pharmacol. 24, 253–259.
[26] Grande, M., Suàrez, E., Vicente, R., Cantó, C., Coma, M., Tamkun, M.M., Zorzano, A., Gumà, A. and Felipe, A. (2003) J. Cell. Physiol. 195, 187–193.
[27] Coma, M., Vicente, R., Tsevi, I., Grande, M., Tamkun, M.M. and Felipe, A. (2002) J. Physiol. Biochem. 58, 195–203.
[28] Frost, R.A., Nystrom, G.J. and Lang, C.H. (2003) Endocrinology 144, 1770–1779.
[29] Madiehe, A.M., Mitchell, T.D. and Harris, R.B. (2003) Am. J. Physiol. Regul. Integr. Comp. Physiol., R763–R770.
[30] Chung, S., Lee, J., Joe, E.H. and Uhm, D.Y. (2001) Neurosci. Lett. 300, 67–70.
[31] Callewaert, G., Hanbauer, I. and Morad, M. (1989) Science 243, 663–666.
[32] Espinosa, F., McMahon, A., Chan, E., Wang, S., Ho, C.S., Hantz, N. and Joho, R.H. (2001) J. Neurosci. 21, 6657–6665.
[33] Giese, P.K., Storn, J.F., Reuter, D., Fedorov, N.B., Shao, L.-R., Leicher, T., Pongs, O. and Siva, A.J. (1998) Learning and Memory 5, 257–273.
[34] Slotwinski, R., Olszewski, W.L., Chaber, A., Slodkowski, M., Zaleska, M. and Krasnodebski, I.W. (2002) J. Clin. Immunol. 22, 289–296.
[35] Muc-Wierzgon, M., Nowakowska-Zajdel, E., Zubelewicz, B., Wierzgon, J., Kokot, T., Klakla, K., Szkilnik, R. and Wiczkowski, A. (2003) J. Exp. Clin. Cancer Res. 22, 171–178.
[36] Sugano, M., Tsuchida, K., Hata, T. and Makino, N. (2004) FASEB. J. 18, 911–913.
[37] Sugano, M., Tsuchida, K. and Makino, N. (2004) Mol. Cell. Biochem. 258, 57–63.
[38] Wesselingh, S.L., Power, C., Glass, J.D., Tyor, W.R., McArthur, J.C., Farber, J.M., Griffin, J.W. and Griffin, D.E. (1993) Ann. Neurol. 33, 576–582.
[39] Eder, C. (1998) Am. J. Physiol. Cell. Physiol. 275, C327–C342.
[40] Draheim, H.J., Prinz, M., Weber, J.R., Weiser, T., Kettenmann, H. and Hanisch, U.K. (1999) Neuroscience 89, 1379–1390.
[41] Nelson, D.J., Jow, B. and Jow, F. (1992) J. Membr. Biol. 125, 207–218.
[42] Lucas, R., Magez, S., De Leys, R., Fransen, L., Scheerlinck, J.P., Rampelberg, M., Sablon, E. and De Baetselier, P. (1994) Science 263, 814–817.
[43] Hribar, M., Bloc, A., van der Goot, F.G., Fransen, L., De Baetselier, P., Grau, G.E., Bluethmann, H., Matthay, M.A., Dunant, Y., Pugin, J. and Lucas, R. (1999) Eur. J. Immunol. 29, 3105–3111.
[44] Soler, C., Valdés, R., García-Manteiga, J., Xaus, J., Comalada, M., Casado, F.J., Modolell, M., Nicholson, B., MacLeod, C., Felipe, A., Celada, A. and Pastor-Anglada, M. (2001) J. Biol. Chem. 276, 30043–30049.
[45] Freitas, J.J.S., Pompéia, C., Miyasaka, C.K. and Curi, R. (2001) J. Neurochem. 77, 655–663.