Annual Review Prize Lecture

Cytokines – killers in the brain?

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Inflammation in the brain

Interest in neuroimmunology and the actions of cytokines in the brain has grown exponentially over the last decade or so, from a variety of different biological disciplines. Scientists and clinicians studying inflammatory diseases of the CNS, such as multiple sclerosis, began to look to proteins associated with the immune system as potential mediators of these conditions. In parallel, those scientists interested in fever recognized the importance of cytokines as endogenous pyrogens, while others, trying to understand the 'sickness behaviour' which is common to most diseases and injury, also began to study cytokines. My own interest in cytokines also arose from unexpected beginnings. In 1978, my first presentation to The Physiological Society described experiments on body weight regulation (Rothwell & Stock, 1978). Over the next decade our research identified the role of diet-induced thermogenesis and sympathetically mediated thermogenesis in the regulation of energy balance and body weight (Rothwell & Stock, 1984, 1986). The field of energy balance regulation was then, as now, dominated by the goal of understanding the causes of excess fat deposition and obesity and developing possible treatments.

Obesity is a problem of positive energy balance and excess fat deposition. Its antithesis is cachexia, a condition of wasting and weight loss which is usually associated with chronic diseases such as cancer, arthritis and AIDS, and acute disease such as severe injury or sepsis (Tisdale, 1997). In all of these conditions, cachexia contributes to morbidity and mortality and has been proposed as the major cause of death in cancer (Garrattini *et al.* 1980). Cytokines are recognized as primary mediators of cachexia through actions in the periphery and the CNS. Indeed, one of the first cytokines identified was initially named 'cachectin' (now known as tumour necrosis factor, TNFα) because it was believed to be an important mediator of cachexia (Beutler & Cerami, 1989).

During the late 1980s my interest turned towards cachexia and responses to injury, and therefore to cytokines. After studying the involvement of these proteins in CNS and immune responses to peripheral inflammation and tissue injury (Rothwell & Luheshi, 1994; Rothwell & Hopkins, 1995; Rothwell, 1997), we began to address the question of whether pro-inflammatory cytokines such as interleukin-1 (IL-1) might also be involved in host responses (including fever and hypermetabolism) to brain injury, and indeed whether such proteins might actually *mediate* neuronal damage and death.

Early studies on the actions of cytokines in the brain were conducted in the face of a widespread belief that these proteins were associated mainly with immune activation and peripheral inflammation, and therefore unlikely to influence the CNS, and that the brain was an 'immune privileged organ', which failed to exhibit clinical inflammatory or immune responses.

It is now recognized that cytokines have diverse actions in the brain, which modulate and mediate both systemic host responses to disease and local changes caused by CNS inflammation, infection and injury (see Rothwell & Luheshi, 1994; Rothwell & Hopkins, 1995; Rothwell, 1997).

Cytokines

This large and rapidly growing group of polypeptides comprises the interleukins, chemokines, tumour necrosis factors, interferons, and growth and cell stimulating factors; neurotrophins have also been included in this category. Cytokines have diverse actions on cell growth and differentiation, immune and inflammatory responses, and on a number of physiological systems particularly in disease.

Although most cytokines are expressed at low or undetectable levels in the healthy adult brain, many are induced in response to injury or infection (see Beneviste, 1992; Hopkins & Rothwell, 1995). For example, expression in the brain of interleukins (IL) 1, 2, 3, 4, 6, 8 and 10, several chemokines, TNFα, interferons and numerous growth factors is induced rapidly by experimental and clinical insults to the CNS (Table 1). The functions and actions of many of these cytokines in the brain remain to be elucidated, but probably include both beneficial and detrimental effects. However, there is now evidence that IL-1, TNFα, several

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Table 1. Factors which induce cytokine expression in the brain

Systemic or brain infection or inflammation
Brain injury
Stroke
Excitotoxic brain damage
Multiple sclerosis and experimental allergic encephalomyelitis
Scrapie and Creutzfeldt–Jakob Disease
Down's syndrome
Alzheimer's disease
Parkinson's disease

chemokines and interferon-γ may contribute directly to neurodegeneration or impaired neuronal function. This review will focus on IL-1 and its involvement and mechanisms of action in neurodegeneration.

Interleukin-1

The IL-1 family comprises at least three proteins, IL-1α, IL-1β and IL-1ra – the products of separate genes which share a significant homology and probably derived from a common gene ancestor about 350 million years ago (Fig. 1). IL-1α and IL-1β are both agonists which, at least in the periphery, are believed to exert identical actions (Dinarello, 1991, 1998). IL-1 receptor antagonist (IL-1ra) is probably the only current example of a selective and specific *endogenous* receptor antagonist, which blocks the actions of IL-1α and β but has no known agonist activity (Dinarello & Thompson, 1991; Dinarello, 1996, 1998). IL-1ra appears to function as a naturally occurring inhibitor of IL-1 actions in the periphery (Dinarello, 1996, 1998) and in the brain (see below), and also provides a very useful pharmacological tool to study IL-1 functions.

The IL-1 proteins are formed as precursors which lack leader sequences, and are enzymatically cleaved to the mature form by specific cellular proteases. Pro-IL-1α is biologically active but remains mostly within the cell, whereas the IL-1β precursor is inactive, so cleavage is an important regulatory step. Pro-IL-1β is cleaved to release mature, active IL-1β by an enzyme called IL-1β converting enzyme (ICE) (Cerretti *et al.* 1992; Thornberry *et al.* 1992), which has recently gained notoriety because of its potential role in apoptosis (Yuan *et al.* 1993). ICE shares homology with the gene *ced-3*, one of the death genes in the nematode worm *Caenorhabditis elegans*, and was the first identified member of the caspases – a family of cysteine proteases believed to execute apoptosis (Miura *et al.* 1993; Nicholson & Thornberry, 1997).

A third potential member of the IL-1 family has been identified recently. Interferon gamma-inducing factor (IGIF), also known as IL-18, was cloned from mouse liver (Okamura *et al.* 1995) but because of its homology with the IL-1 family, the name IL-1γ was proposed (Bazan *et al.* 1996). Interestingly pro-IL-18, like pro-IL-1β, is cleaved by ICE (Gu *et al.* 1997; Rano *et al.* 1997) and is in fact the *preferred* substrate for the enzyme (Rano *et al.* 1997). We have cloned rat IL-18/IL-1γ from rat brain, shown that it is also cleaved by ICE, and that, in contrast to IL-1α or β, it appears to be expressed constitutively in the rat brain (Culhane *et al.* 1998). The issue of whether IL-18 is a true member of the IL-1 family remains to be resolved, but similarities in its structure, cleavage, receptors and signalling mechanisms suggest a close relationship with IL-1 (Dinarello *et al.* 1998).

Several putative IL-1 receptors have been characterized, but all actions of IL-1 are believed to be effected through binding to the type 1 (80 kDa) receptor (IL-1RI) (Bomsztyk *et al.* 1989; Sims *et al.* 1993; O'Neill, 1996; Martin & Falk, 1997; Loddick *et al.* 1998), which requires an accessory protein (AcP) for signal transduction (Greenfeder *et al.* 1995). The type II (68 kDa) receptor (RII) has a short intracellular domain and is not thought to initiate signalling, but rather acts as a decoy which is shed from the membrane and binds IL-1 (Colotta *et al.* 1993). However, this receptor may

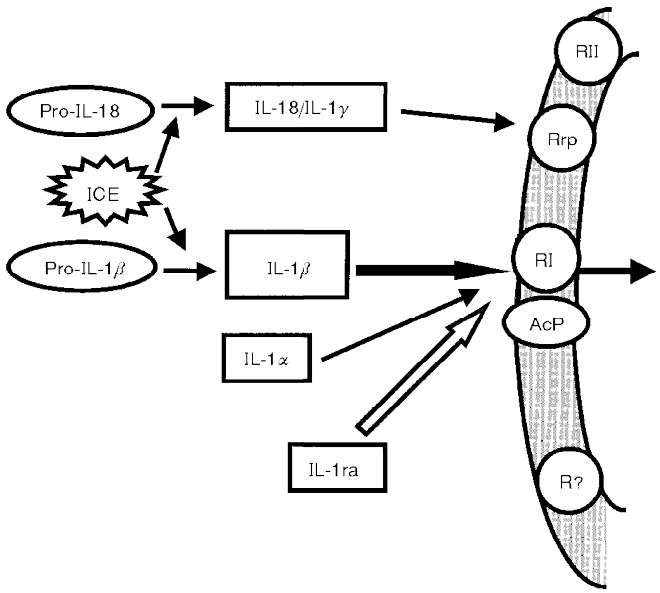

Figure 1. The interleukin-1 (IL-1) family
The IL-1 family comprises the two agonists IL-1α and IL-1β and the IL-1 receptor antagonist IL-1ra. Pro-IL-1β is inactive and must be cleaved by the enzyme interleukin-1β converting enzyme (ICE; also known as caspase 1). Actions of IL-1 are believed to be mediated through an 80 kDa type 1 (RI) receptor which requires an accessory protein (AcP) for signal transduction. IL-18 (also known as interferon gamma-inducing factor or IL-1γ) shares structural homology with the IL-1 family, is activated through cleavage by ICE and acts on an IL-1 receptor-related protein (Rrp), but is not confirmed as a member of the IL-1 family.

mediate responses to IL-1 in the brain because an antibody to RII inhibits febrile responses to IL-1β, but not IL-1α (Luheshi *et al.* 1993; Rothwell *et al.* 1996*b*). There has been much speculation about the presence of novel or atypical IL-1 receptors in the brain, and an IL-1 receptor-related protein (IL-1RrP) was identified on the basis of its homology with known IL-1 receptors (Lovenberg *et al.* 1996; Parnet *et al.* 1996). Although RrP does not bind IL-1α or β it has recently been proposed as a receptor for IL-18 (Torigoe *et al.* 1997).

Expression of IL-1 in the brain

IL-1β seems to be the main IL-1 agonist induced in the brain in response to systemic (e.g. injury, infection) or local (e.g. injury, stroke) insults (see Rothwell & Luheshi, 1994; Hopkins & Rothwell, 1995; Rothwell *et al.* 1997). In rodents, IL-1β mRNA expression is increased within 15–30 min, and protein within 1 h of experimental cerebral ischaemia (stroke), brain injury or infusion of excitotoxins (Minami *et al.* 1992; Liu *et al.* 1993; Buttini *et al.* 1994; Yabuuchi *et al.* 1994), all of which lead to neuronal death. Several groups have also reported increased expression of IL-1β protein after experimental brain damage (e.g. Ianotti *et al.* 1993; Taupin *et al.* 1993).

We have demonstrated by immunocytochemistry using anti-rat IL-1β antibodies and cell-specific markers that in rat brain after ischaemic or excitotoxic insults, early expression (up to 24 h) of IL-1β is predominantly by microglia and meningeal macrophages around and within the emerging infarct (Davies *et al.* 1998; Pearson *et al.* 1998). In contrast, delayed expression (between 24 h and 7 days) is seen, often at distant sites, by astrocytes and invading immune cells (Davies *et al.* 1998). IL-1β expression appears to be associated with increased expression of ICE (Bhat *et al.* 1996) and cleavage of pro-IL-1β, since *bioactive* IL-1 is also markedly increased and is usually maximal 6–8 h after the insult (Fig. 2).

IL-1α expression is increased by stimuli similar to those increasing expression of IL-1β but at lower levels, and slightly after IL-1β in the brain. Expression of IL-1ra, the endogenous inhibitor of IL-1, is also increased by brain damage in rodents, usually slightly (30–60 min) after IL-1β and in different cells, mainly neurones (Toulmond & Rothwell, 1995*a*; Loddick *et al.* 1997).

Actions of IL-1 in the brain

Research into the actions of IL-1 in the brain initially focused on its role in host defence responses to systemic disease. Thus, it has been shown that IL-1 injected into the cerebral ventricles or the brain parenchyma, at doses as little as picomoles, induces responses which mimic those that occur during disease and injury, i.e. fever, anorexia, sickness behaviour, slow-wave sleep, and alterations in neuroendocrine (e.g. activation of the hypothalamic pituitary adrenal axis), cardiovascular and immune system function (see Rothwell & Luheshi, 1994; Rothwell & Hopkins, 1995; Rothwell, 1997). IL-1 appears to act as an *endogenous* mediator of these diverse host defence responses mainly through actions in the hypothalamus, since localized injections of IL-1ra or antibodies to IL-1β attenuate such responses to systemic stimuli (see Rothwell & Luheshi, 1994; Rothwell & Hopkins, 1995; Rothwell, 1997).

IL-1 can also elicit an array of responses which could either inhibit, exacerbate or induce neuronal damage and death. These include induction of fever and oedema, damage to the cerebral vasculature, activation of glia, induction of neurotrophins, growth factors/phospholipase A_2, cyclooxygenase (COX-2), β-amyloid precursor protein, adhesion molecules and corticotrophin-releasing factor (CRF), release of nitric oxide and other free radicals, activation of complement, and modification of calcium homeostasis (see Beneviste, 1992; Giulian *et al.* 1993; Betz *et al.* 1996; Martin *et al.* 1996; Rothwell *et al.* 1996*a*,*c*; Rothwell, 1997; Rothwell *et al.* 1997).

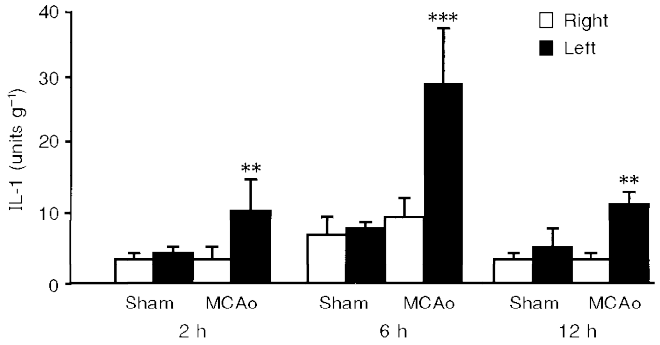

Figure 2. Bioactive IL-1 levels are increased after cerebral ischaemia

The figure shows bioactivity of IL-1 in the left and right cerebral hemispheres of rats subjected to sham surgery or unilateral focal cerebral ischaemia (middle cerebral artery occlusion (MCAo) in the left hemisphere). IL-1 was measured using a D10 thymocyte proliferation assay. Mean values ± S.E.M. are shown ($n = 6$). S. Loddick, S. Hopkins & N. Rothwell, unpublished data.

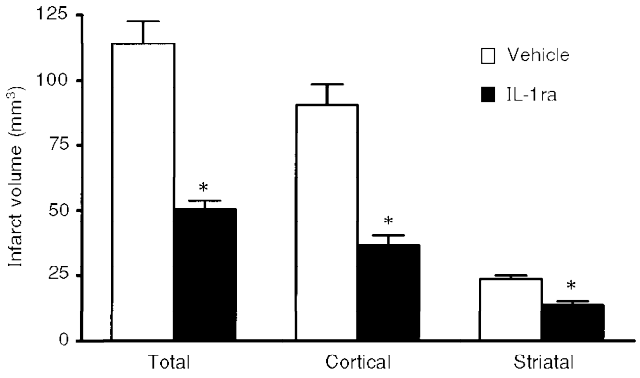

Figure 3. Effect of injection of IL-1ra (10 μg, I.C.V.) or vehicle on ischaemic brain damage

Treatments were given immediately after MCAo and infarct volumes were determined histologically 24 h later. Adapted from Loddick & Rothwell (1996). * $P < 0{\cdot}01$ *vs.* vehicle.

The infusion of IL-1α or β into the brain of normal rodents or the application to neuronal cultures, even at doses in the high nanomolar range, does not result in overt damage or death. However, injection of recombinant IL-1β at picomolar doses into the cerebral ventricles (I.C.V.) or into specific brain regions (see below) of the rat markedly exacerbates neuronal damage induced by cerebral ischaemia, traumatic injury or excitotoxins (Relton & Rothwell, 1992; Loddick & Rothwell, 1996; Lawrence *et al.* 1998; and S. Toulmond, S. M. Allan, R. Grundy & N. J. Rothwell, unpublished data), and IL-1β can enhance cell death in cultures exposed to apoptotic stimuli (Friedlander *et al.* 1996; Troy *et al.* 1996). These data indicate either that IL-1 causes (or exacerbates) damage only to threatened cells, or that it synergizes with, or depends on, other factor(s) or responses present in the damaged brain.

Functional role of IL-1 in neurodegeneration

The most direct and substantial evidence indicating that *endogenous* IL-1 plays a functional role in neurodegeneration derives from *in vivo* studies in which its release or action has been blocked. The majority of such experiments have used recombinant IL-1ra – a highly effective and selective receptor antagonist for IL-1 (see above).

The first report that inhibition of IL-1 reduces brain damage *in vivo* (Relton & Rothwell, 1992) demonstrated that I.C.V. injection of IL-1ra at the time of induction of permanent focal cerebral ischaemia (middle cerebral artery occlusion (MCAo)) in the rat inhibited subsequent brain damage (infarct volume) by almost 70% (Fig. 3). Numerous studies have subsequently verified and extended this observation (e.g. Betz *et al.* 1995, 1996; Garcia *et al.* 1995; Loddick & Rothwell, 1996; Relton *et al.* 1996; Rothwell *et al.* 1996*a*, 1997; Stroemer & Rothwell, 1997). It is now known that even systemic injection of IL-1ra, at considerably higher doses (50–100 mg kg^{-1}), also inhibits ischaemic brain damage (Garcia *et al.* 1995; Relton *et al.* 1996), and indeed both IL-1 and IL-1ra are actively transported into the brain from the circulation (Gutierrez *et al.* 1994). IL-1ra is

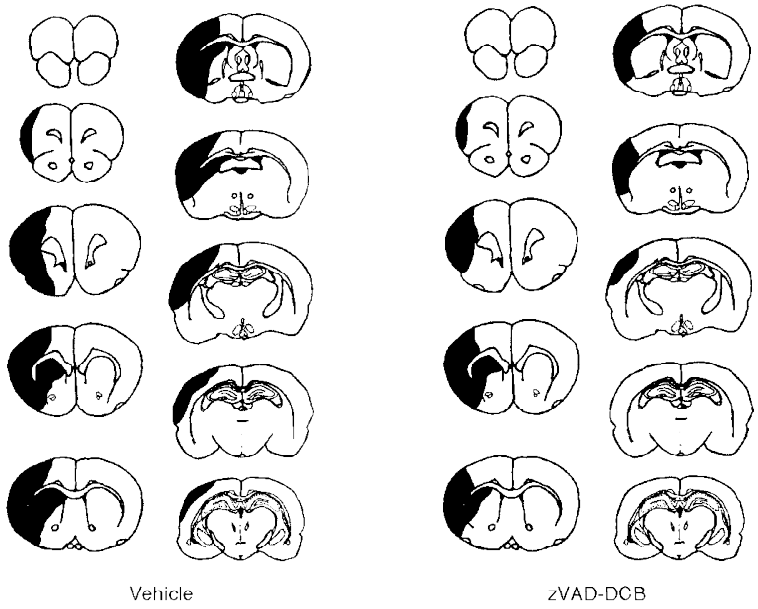

Figure 4. Pattern of brain damage (shown as dark areas) in coronal brain sections of rats measured histologically 24 h after MCAo

Animals were treated with either vehicle (left) or the ICE (caspase) inhibitor zVAD-DCB (right) (I.C.V.). Adapted from Loddick *et al.* (1996).

neuroprotective when administered 30–60 min after focal cerebral ischaemia and reduces not only infarct volume but also oedema, glial activation and neuronal loss, and largely reverses neurological impairment caused by MCAo (Garcia *et al.* 1995; Relton *et al.* 1996). In addition to these effects on MCAo in the adult rat or mouse, IL-1ra also markedly reduces brain damage caused by hypoxia/ischaemia in neonatal rats (Martin *et al.* 1995), global cerebral ischaemia in gerbils (Martin *et al.* 1996), lateral, cortical fluid percussion injury in the rat (Toulmond & Rothwell, 1995*a*) and heat stroke damage in rabbits (Lin *et al.* 1995), and reduces the clinical symptoms of experimental allergic encephalomyelitis (EAE, a rodent model of multiple sclerosis) (Martin & Near, 1995). In several of these paradigms, IL-1ra is effective when administered up to 4 h after the insult, and these actions of IL-1ra are not associated with any changes in body temperature or cardiovascular parameters in normal or brain-damaged rats.

Since IL-1ra blocks all known actions of both IL-1α and IL-1β, the results described above do not distinguish the relative importance of each of these proteins. There is, however, some evidence to suggest that IL-1β is the primary mediator of neurodegeneration: IL-1β is the predominant form of IL-1 induced by brain insults (see above), and central administration of a neutralizing IL-1β antibody inhibits damage caused by reversible cerebral ischaemia in the rat (Yamasaki *et al.* 1992, 1994, 1995).

We have also demonstrated that injection (I.C.V.) of an irreversible peptide inhibitor of ICE activity (z-VAD), the enzyme required to cleave pro-IL-1β, reduces ischaemic brain damage in a pattern very similar to that of IL-1ra (Loddick *et al.* 1996) (Fig. 4) and this has now been verified by others in focal (Hara *et al.* 1997; Endres *et al.* 1998) and global ischaemia (Cheng *et al.* 1998). However, ICE and related members of the caspase family have also been implicated in apoptosis, and available ICE inhibitors are not selective for ICE (or caspase 1). Nevertheless, several recent studies support the involvement of ICE itself (caspase 1) in ischaemic brain damage. Mice in which the ICE (caspase 1) gene has been deleted (knocked out) or disabled (by over-expression of a dominant negative mutant form of the enzyme) exhibit reduced brain damage in response to MCAo (Friedlander *et al.* 1997; Schielke *et al.* 1998).

These observations further add to the current debate about the importance of apoptosis in neurodegeneration (e.g. Linnick, 1995; Henkart, 1996; Chalmers-Redman *et al.* 1997; Holtzman & Deshmukh, 1997; Barinaga, 1998) and the relationship between IL-1β, ICE and apoptosis. It had been believed that any involvement of ICE in apoptosis was independent of the release or actions of IL-1β. However, at least two studies have now suggested that actions of IL-1β *itself* are required for apoptosis in a variety of cell types, including neurones *in vitro* (Friedlander *et al.* 1996, 1997; Troy *et al.* 1996). Most notably, IL-1ra, or an antibody to IL-1RI, blocks apoptotic cell death (Friedlander *et al.* 1996; 1997). Indeed, the involvement of apoptosis in ischaemic brain damage is now generally accepted, though its quantitative importance is still uncertain (see Linnick, 1995; Henkart, 1996; Barinaga, 1998).

IL-18 is also cleaved by ICE (and signals through an IL-1-like receptor, see above), so caspase inhibitors will block release of active IL-18. However, the involvement of IL-18 in neurodegeneration has not yet been addressed, and it remains possible that further substrates for ICE and/or additional members of the IL-1 family remain to be discovered.

Mechanisms of action of IL-1 in neurodegeneration

Most forms of neuronal death in the adult brain have been ascribed to or associated with excitotoxicity, i.e. excessive release of excitotoxic amino acids (EAAs) such as glutamate, and subsequent activation of NMDA and AMPA receptors (see Doble, 1995; Boxer & Bigge, 1997). Thus, it is likely that IL-1 interacts in some way with this cascade to modify glutamate release, reuptake or actions.

In vitro studies indicate that neither IL-1 nor IL-1ra influences glutamate release or reuptake, at least *in vitro* (e.g. Allan *et al.* 1998; Lawrence *et al.* 1998). Experiments conducted *in vivo* indicate that expression of IL-1β (by immunohistochemistry) is stimulated by pharmacological activation of EAA receptors (Pearson *et al.* 1998), and that IL-1 acts at a point beyond EAA receptor activation to mediate neurodegeneration (Lawrence *et al.* 1998).

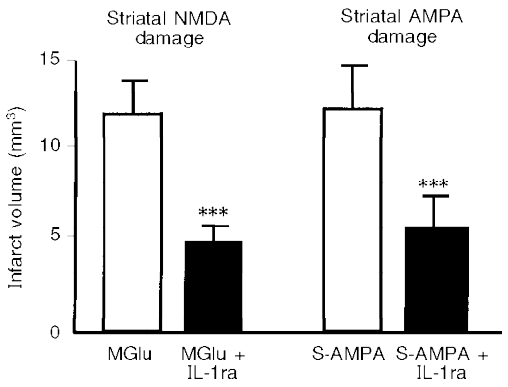

Figure 5. Effect of IL-1ra on excitotoxic brain damage

Infarct volume was measured histologically 24 h after infusion into the striatum of methanoglutamate (MGlu) or S-AMPA. Rats were either co-infused with vehicle (□) or IL-1ra (■; 5 μg). Adapted from Lawrence *et al.* (1998). *** $P < 0{\cdot}001$ *vs.* MGlu or S-AMPA alone.

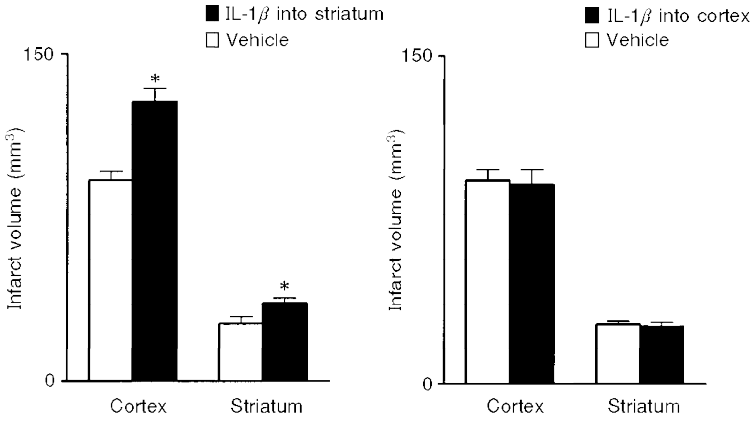

Figure 6. Local effects of IL-1 on ischaemic brain damage
Effects of local infusion of either vehicle or IL-1β (5 ng) into the cortex or striatum on ischaemic brain damage induced by MCAo in the rat, measured as infarct volume. Adapted from Stroemer & Rothwell (1998). * $P < 0{\cdot}05$ *vs.* vehicle.

Excitotoxins such as kainate or selective NMDA receptor agonists induce rapid expression of IL-1β mRNA and protein in the brain (see above), and the distribution and cell source of IL-1β protein induced by NMDA receptor activation in rat brain *in vivo* is remarkably similar to responses which follow cerebral ischaemia or brain trauma (Pearson *et al.* 1998). Co-infusion of IL-1ra with these excitotoxins markedly inhibits brain damage induced by pharmacological activation of either NMDA or AMPA receptors or infusion of kainate in the rat striatum *in vivo* (Lawrence, 1996; Lawrence *et al.* 1998; Panegyres & Hughes, 1998) (Fig. 5), suggesting that IL-1 mediates both of these forms of neuronal death.

An obvious potential mechanism of IL-1 action on neurodegeneration is the induction of fever. IL-1 is a potent pyrogen (see Rothwell & Luheshi, 1994) and raised body temperature exacerbates some forms of neurodegeneration (Ridenour *et al.* 1992). However, IL-1ra does not affect body temperature in normal or ischaemic rats (Loddick & Rothwell, 1996). Furthermore we have found that the sites of action of IL-1 in the brain in fever differ from those involved in neurodegeneration, that cyclo-oxygenase inhibitors block IL-1-induced fever but do not affect ischaemic or excitotoxic brain damage (Relton & Rothwell, 1992; R. Grundy, N. Rothwell & S. M. Allan, unpublished data), and that the cytokine IL-6, which is a potent pyrogen, inhibits rather than enhances neural damage (see below).

Investigation of the mechanisms of neurodegeneration and IL-1 action should be facilitated by the use of *in vitro* approaches such as neuronal cell cultures. However, such approaches have yielded conflicting data. In primary neuronal cultures from the rat cortex or striatum, IL-1ra does not inhibit, and IL-1 does not enhance, excitotoxic cell death induced by glutamate or by selective NMDA or AMPA receptor agonists (Strijbos & Rothwell, 1995). Indeed, IL-1 applied in low (picomolar) concentrations (i.e. within the uncertain range which activates known IL-1 receptors) *protects* against excitotoxic damage, probably via induction of nerve growth factor (NGF) (Strijbos & Rothwell, 1995). Much higher concentrations (5–50 nM) of IL-1 are neurotoxic, but this far exceeds the amounts of IL-1 found even in injured brain. Similarly in neuronal cell lines, IL-1 fails to induce cell death, though it has been reported to exacerbate apoptosis (see above). Thus it has not been possible to mimic effects obtained reproducibly *in vivo* in cell culture systems.

In contrast, in mixed cultures of glia and neurones, IL-1 can be toxic even in the absence of other insults at low (picomolar) concentrations, and this has been ascribed to the release of secondary mediators, including nitric oxide and superoxides (e.g. Araujo, 1992; Beneviste, 1992; Giulian *et*

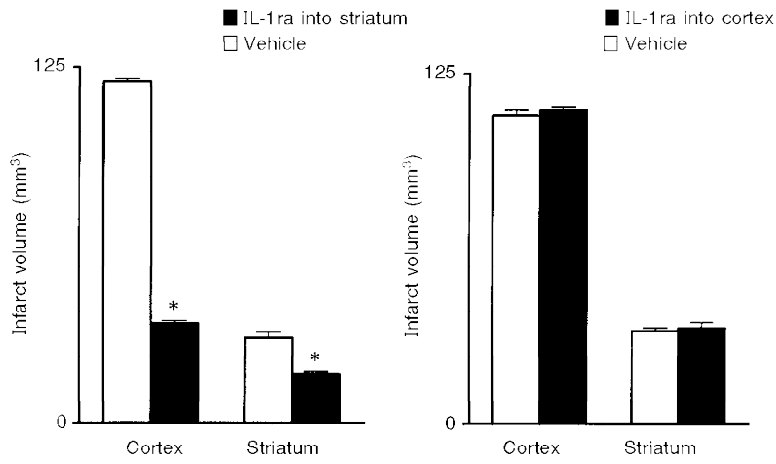

Figure 7. Local effects of IL-1ra on ischaemic brain damage
Effects of local infusion of vehicle or IL-1ra (5 μg) into the cortex or striatum on ischaemic brain damage induced by MCAo in the rat, measured as infarct volume. Adapted from Stroemer & Rothwell (1997). * $P < 0{\cdot}01$ *vs.* vehicle.

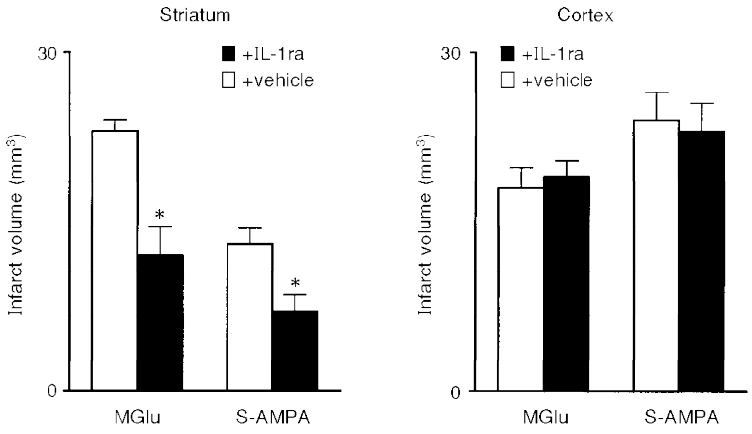

Figure 8. Effects of IL-1ra on excitotoxic damage induced by methanoglutamate or S-AMPA in the striatum or cortex

Infarct volume was measured 72 h after co-infusion of either methanoglutamate (MGlu) or S-AMPA plus vehicle or IL-1ra into the striatum (left) or cortex (right). Adapted from Lawrence (1996), Lawrence *et al.* (1998). * $P < 0{\cdot}01$ *vs.* vehicle.

al. 1993; Banati *et al.* 1993; Chao *et al.* 1995, 1996; Hu *et al.* 1995; and J. Relton, personal communication). Nevertheless, some caution must be exercised in interpreting these results because, unlike in the mixed cultures, IL-1 is not toxic to the *normal* adult brain *in vivo* (see above). Furthermore, these cultures comprise immature neurones without complex synaptic connections, and IL-1 actions may involve the activation of multiple neuronal pathways and several distinct brain regions (see below).

Studies both *in vivo* and *in vitro* have revealed diverse activities of IL-1, which could be either neuroprotective – e.g. through induction of NGF, inhibition of calcium concentrations and calcium entry into neurones and enhanced GABA activity (Spranger *et al.* 1990; Plata-Salaman & Ffrench-Mullen, 1991; Coogan & O'Connor, 1997) or neurotoxic – such as enhanced cell calcium entry, induction of other pro-inflammatory cytokines, eicosanoids, adhesion molecules, corticotrophin-releasing factor (CRF), acute proteins such as β-amyloid precursor protein, release of nitric oxide and other free radicals, activation of complement and neutrophins and blood–brain barrier damage (see Hartung *et al.* 1989; Moser *et al.* 1989; Sullivan *et al.* 1989; Quagliarello *et al.* 1991; Lee *et al.* 1993; Simmons & Murphy, 1993; Shrikant *et al.* 1994; Rothwell *et al.* 1997). Any number of these responses could contribute to the overall effects of endogenous and exogenous IL-1, which may depend on the concentration of the cytokine, the environment, and other factors present at specific sites within the brain.

Site-specific effects of IL-1

In vivo studies described above have revealed that IL-1 enhances and IL-1ra inhibits various forms of neuronal damage when injected into rodents either I.C.V. or peripherally, but both of these molecules appear to have highly site-specific effects in the brain on neurodegeneration.

We have found that injection of IL-1β (2 ng) into the striatum of rats exacerbates both striatal and cortical damage caused by cerebral ischaemia (MCAo), but is ineffective when administered into the cortex, even at a higher dose (Stroemer & Rothwell, 1998) (Fig. 6). Injection of low doses of IL-1ra into the striatum of rats subjected to MCAo inhibits subsequent damage in both the striatum *and* the cortex, whereas injection of the same, or higher, doses into the cortex fails to protect either region (Stroemer & Rothwell, 1997) (Fig. 7). Surprisingly, IL-1ra injected into the *contralateral* striatum also offers some protection against MCAo damage (Stroemer & Rothwell, 1997).

Similarly in excitotoxic damage, IL-1ra infused into the striatum reduces the volume of lesions caused by NMDA or AMPA agonists administered into the striatum, but infusion of IL-1ra together with these toxins into the cortex does not inhibit damage (Lawrence, 1996; Lawrence *et al.* 1998) (Fig. 8). Recombinant IL-1 also has surprising and

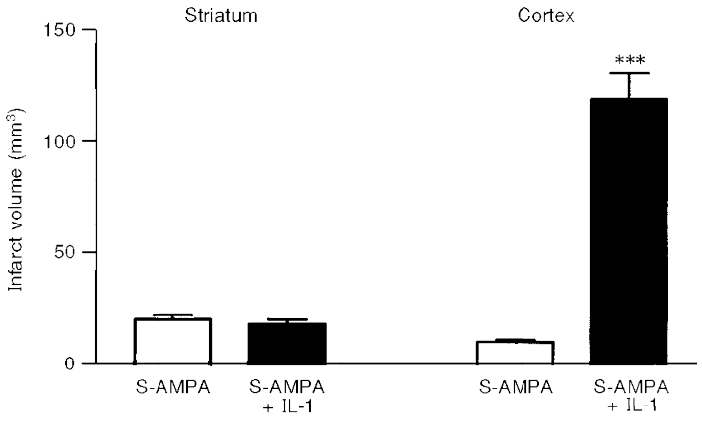

Figure 9. Effects of IL-1 on striatal and cortical damage caused by S-AMPA

Effects of co-infusion of either vehicle or IL-1 (5 ng) with S-AMPA into the striatum on infarct volume in the striatum and cortex. Adapted from Lawrence *et al.* (1998). *** $P < 0{\cdot}001$ *vs.* S-AMPA.

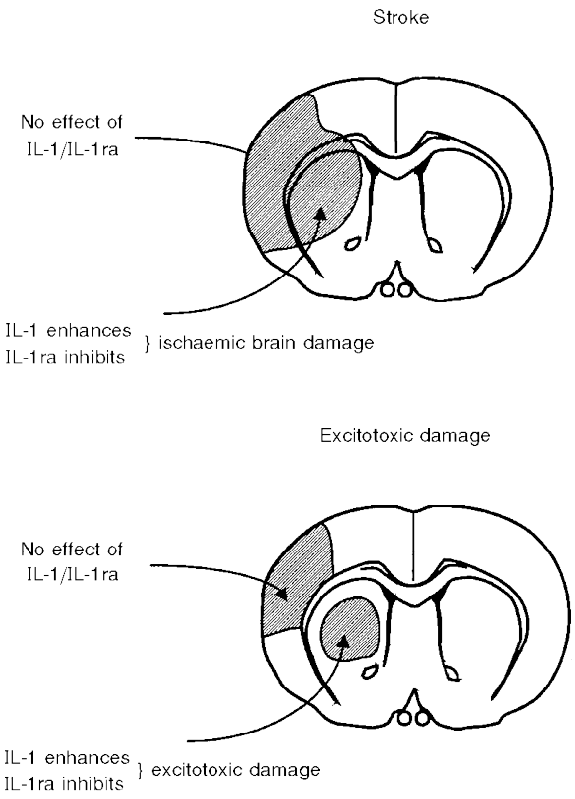

Figure 10. Site-specific actions of IL-1/IL-1ra

Diagram depicting site-specific effects of IL-1 and IL-1ra on ischaemic (stroke, top panel) and excitotoxic brain damage (bottom panel). For details, see text and Figs 7–9.

dramatic effects on excitotoxic damage, since *again* it has no effect when administered into the cortex with an excitotoxin, but when co-infused into the striatum with S-AMPA, it causes extensive damage throughout the cortex, which cannot simply be ascribed to the diffusion of IL-1 (Lawrence *et al.* 1998) (Fig. 9). The resulting initial infarct (which is more than five times greater than the primary striatal damage) is associated with marked oedema, which resembles the effects of a major stroke.

These effects of IL-1 in the striatum do not correlate with its effects on body temperature (see above), and the sites of action are not consistent with the reported distribution of the IL-1 type I receptor, which is believed to mediate all actions of IL-1 (Sims *et al.* 1993). Published reports indicate that IL-1RI mRNA is localized mainly in the hippocampus, with low levels of expression in the cortex but little or none reported in the striatum (Cunningham *et al.* 1992; Ericsson *et al.* 1995), although the accessory protein, required for full binding of the ligand and signal transduction (Huang *et al.* 1997), is found in this region (see Loddick *et al.* 1998). These data might suggest that the effects of exogenous and endogenous IL-1 in the striatum may be dependent on novel or atypical IL-1 receptor(s). However, we have detected low levels of IL-1RI in the rat striatum by reverse transcriptase-polymerase chain reaction (RT-PCR) (L. Parker,

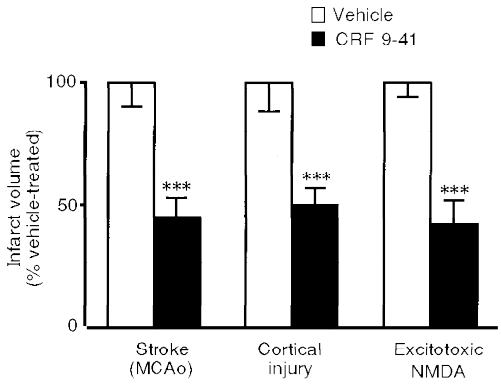

Figure 11. Inhibition of corticotrophin-releasing factor (CRF) reduces brain damage

Effects of injection of a CRF receptor antagonist (α-helical CRF 9-41; 25 μg I.C.V.) on neuronal damage induced by cerebral ischaemia (left), lateral fluid percussion injury (centre) or striatal infusion of an NMDA agonist (right). Open bars show values for vehicle-treated rats depicted as 100%. *** $P < 0{\cdot}001$.

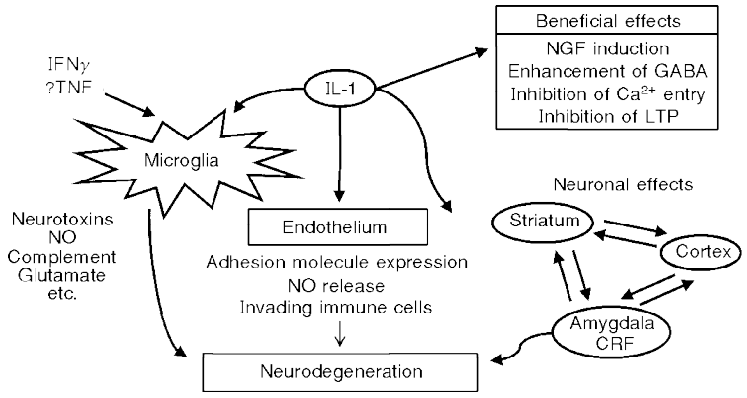

Figure 12. Scheme depicting potential actions of IL-1 in neurodegeneration
IFNγ, interferon γ. TNF, tumour necrosis factor.

G. N. Luheshi & N. Rothwell, unpublished data) which could mediate these actions of IL-1.

The effects described above indicate that IL-1 or IL-1ra infused into the striatum can have effects at distant brain sites to cause or inhibit damage in the cortex (see Fig. 10). The mechanisms of these distant effects are unknown, but there are several possibilities. IL-1 could induce expression or release of molecule(s) in the striatum (but not the cortex), which can then diffuse to, or indirectly influence, the cortex to cause neuronal death. Secondly, IL-1 could influence the cortex via activation of neuronal pathways. Since few, if any, striato-cortical afferent pathways have been identified, IL-1 would have to induce damage via retrograde neuronal pathways or by stimulating complex multi-synaptic pathways involving several other brain regions. It seems that glutamatergic pathways and NMDA receptors are involved in the distant cortical damage caused by striatal IL-1, because AMPA-induced damage in the striatum is not affected by NMDA antagonists, but cortical damage resulting from co-infusion of IL-1 and AMPA in the striatum appears to be inhibited by NMDA antagonists (S. M. Allan & N. Rothwell, unpublished data).

It also seems likely that other brain regions are associated with these distant effects of IL-1, since damage often occurs in the thalamus as well as the cortex (Lawrence, 1996). A possible mediator of these complex actions of IL-1 is the neuropeptide, corticotrophin-releasing factor (CRF), best known for its involvement in stress and activation of the hypothalamic pituitary adrenal axis. Several years ago it was reported that a CRF receptor antagonist reduced the damage caused by global ischaemia in the gerbil (Lyons *et al.* 1991). We have now shown that CRF mRNA is increased by cerebral ischaemia or traumatic brain damage in the rat cortex associated with the primary injury, but more specifically in the amygdala unilateral to the insult, where no neuronal loss occurs in response to damage in the rat (Wong *et al.* 1995). Injection of a CRF receptor antagonist (I.C.V.) significantly reduces subsequent damage in the cortex (Wong *et al.* 1995; Roe *et al.* 1998) (Fig. 11). Our preliminary data suggest that unilateral lesions of the amygdala reduce subsequent cortical injury (S. Roe & N. Rothwell, unpublished data). The mechanisms of action of CRF on neuronal death are not known, and indeed it remains to be proven that CRF mediates directly by the actions of IL-1.

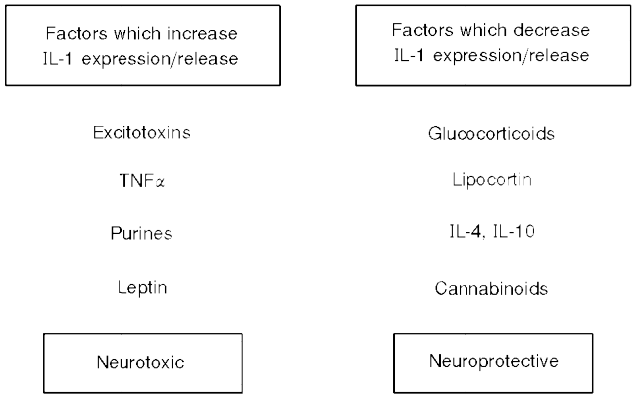

Figure 13. Regulation of IL-1 expression and action
The factors shown may regulate expression and/or release of IL-1 and therefore modify neurodegeneration.

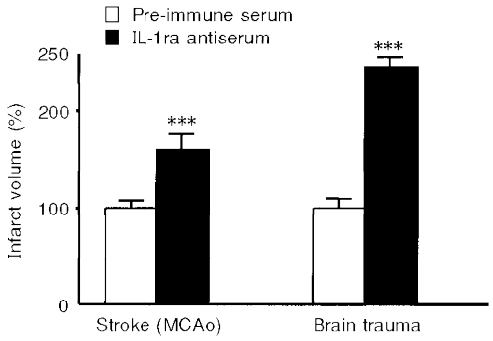

Figure 14. Effects of inhibition of endogenous IL-1ra
The effects of inhibiting endogenous IL-1ra by I.C.V. injection of an anti-rat IL-1ra antiserum or pre-immune serum on infarct volume (expressed as a percentage of pre-immune serum-treated groups) induced by cerebral ischaemia (left) or lateral fluid percussion injury (right). *** $P < 0{\cdot}001$.

At present the mechanism(s) of IL-1 actions on neurodegeneration remain unknown. It is likely that IL-1 exerts diverse effects on a variety of cell types, including neurones, glia and endothelial cells (Fig. 12) to influence neurodegeneration. However, as yet the precise sites, receptors, signalling pathways and mediators of IL-1 actions have not been identified.

Endogenous inhibitors of cytokines

A number of endogenous inhibitors of cytokine synthesis or action have been identified in the periphery, and several appear to be functionally active in the brain (Fig. 13). For IL-1, the most notable of these is IL-1ra, the naturally occurring receptor antagonist (Dinarello, 1996, 1998). IL-1ra mRNA and protein are induced in the brain by the same stimuli which upregulate IL-1 expression, i.e. excitotoxic, ischaemic and traumatic brain injury (Toulmond & Rothwell, 1995*b*; Loddick *et al.* 1997; Wang *et al.* 1997). Although the spatial patterns of expression of IL-1ra and IL-1 in the brain after injury are similar, these molecules are expressed by different cells (IL-1ra by neurones, IL-1 by glia) and IL-1ra is upregulated slightly later than IL-1 (Toulmond & Rothwell, 1995*b*; Loddick *et al.* 1997). We have suggested that IL-1ra is a functional inhibitor of IL-1 action and of neuronal death, since administration of anti-IL-1ra antibodies (injected I.C.V. into the rat) markedly exacerbates ischaemic and brain damage (Toulmond & Rothwell, 1995*b*; Loddick *et al.* 1997) (Fig. 14).

A number of other endogenous regulators of cytokine action have been identified including, for example, soluble IL-1 receptors, which are released and bind cytokines to inhibit their action – though the functions of these are not yet established in the brain.

Glucocorticoids are potent inhibitors of the synthesis and actions of pro-inflammatory cytokines such as IL-1 and TNFα, but have complex actions on neurodegeneration (Sapolsky & Pulsinelli, 1985; Reagan & McEwen, 1997). Lipocortin-1 (annexin-1) is a mediator of glucocorticoid action (Rothwell & Flower, 1992), that inhibits cytokine synthesis and actions and is a very potent neuroprotective agent. Lipocortin expression is upregulated in response to focal cerebral ischaemia in the rat brain (Relton *et al.* 1991). We have further shown that intracerebroventricular injection of recombinant lipocortin-1 markedly inhibits ischaemic and excitotoxic damage in the rat brain, while injection of a blocking antibody to lipocortin-1 enhances damage (Relton *et al.* 1991; Strijbos *et al.* 1994) (Fig. 15). The mechanisms by which lipocortin exerts its neuroprotective effects are unclear, but it has a number of actions which may contribute to these effects (see Rothwell & Flower, 1992).

Another, somewhat surprising inhibitor of ischaemic and excitotoxic brain damage is the cytokine IL-6, which shares many actions with IL-1, including induction of fever (Rothwell *et al.* 1991). IL-6 protects against excitotoxic (Toulmond *et al.* 1992) and ischaemic (Loddick *et al.* 1998) brain damage in the rat *in vivo* (Fig. 16). A recent report suggests that IL-6 induces lipocortin-1 expression and causes translocation to the cell surface (Solito *et al.* 1998), which may contribute to its neuroprotective effects.

Several anti-inflammatory cytokines have been identified, such as IL-4, IL-10, IL-13 and transforming growth factor β

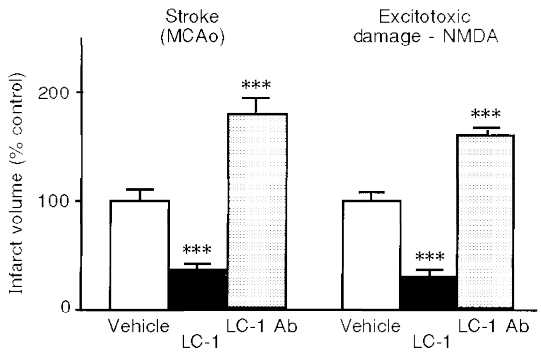

Figure 15. Lipocortin-1 is an endogenous neuroprotectant
Effects of injection (I.C.V.) of vehicle, 1 μg recombinant lipocortin-1 (LC-1) or 3 μl anti-rat lipocortin-1 antibody (LC-1 Ab) on infarct volume (expressed as a percentage of vehicle-treated rats) induced by cerebral ischaemia (MCAo, left) or striatal infusion of an NMDA agonist (right). *** $P < 0{\cdot}001$ *vs.* vehicle.

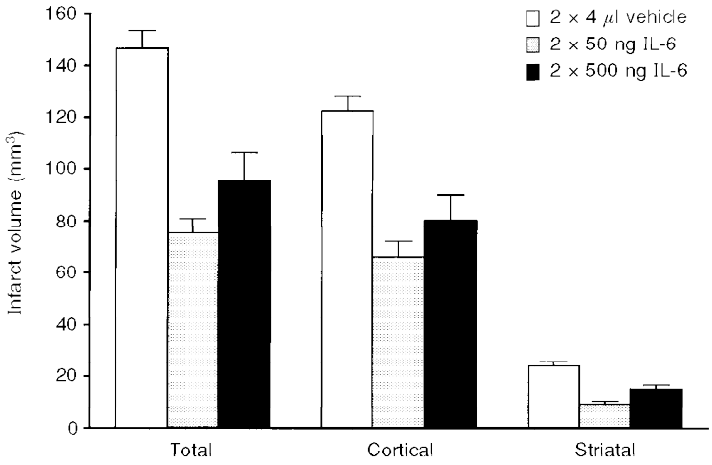

Figure 16. Effect of IL-6 on ischaemic brain damage
Effects of injection (I.C.V.) of saline vehicle or two doses of recombinant IL-6 (each given twice) on infarct volume induced by focal cerebral ischaemia (MCAo). Adapted from Loddick *et al.* (1998).

(TGFβ), which can inhibit the release or actions of IL-1 and TNFα, and may induce IL-1ra (e.g. Vannier *et al.* 1992; Chao *et al.* 1993; Ohmori *et al.* 1996) (Fig. 17). Several of these molecules are induced by brain injury and are neuroprotective.

The pharmacological induction of these molecules which inhibit IL-1 activity, or the development of new agents which mimic their actions, may be of therapeutic value. It is likely that the *balance* between IL-1 and its inhibitors (particularly IL-1ra) determines the fate of injured neurones and inflammatory events in the brain.

Summary and implications

There is now considerable evidence to suggest that specific cytokines, particularly IL-1, are involved directly in neuronal death resulting from diverse insults and diseases. The mechanisms of these effects are not yet fully understood, but probably include complex actions on several types of brain cells and physiological systems. In addition to the involvement of IL-1 in acute neurodegeneration, circumstantial evidence also implicates this cytokine in chronic conditions such as Alzheimer's and Parkinson's disease (Royston *et al.* 1992; Sheng *et al.* 1996). Inhibition of the synthesis or actions of cytokines therefore provides an attractive therapeutic approach to the treatment of both acute and chronic neurodegenerative conditions.

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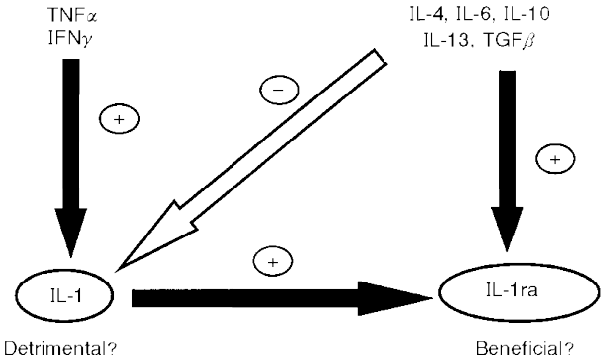

Figure 17. Diagram depicting cytokine modification of IL-1 and IL-1ra
TNFα, tumour necrosis factor α; IFNγ, interferon γ; TGFβ, transforming growth factor β.

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