Blood-borne interleukin-1α is transported across the endothelial blood–spinal cord barrier of mice

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1. Previous work has shown that one mechanism by which blood-borne interleukin-1α (IL-1) may be able to affect the central nervous system (CNS) is by direct transport into the brain across the blood–brain barrier (BBB). The BBB of the brain consists of endothelial (between blood and interstitial fluid) and ependymal (between blood and cerebrospinal fluid) barriers. Which of these barriers IL-1 can cross has not previously been investigated. At the spinal cord, which could be the site of action for some of the effects of IL-1 such as analgesia, the BBB consists only of the endothelial barrier.

2. We show here that IL-1 labelled with ^{125}I (I-IL) is transported across the BBB of the spinal cord by a saturable system similar to the one previously described for the brain. High performance liquid chromatography (HPLC) showed that most of the material entering the spinal cord represented intact I-IL. The BBB of the spinal cord was no more leaky to radioactively labelled albumin than the BBB of the brain and was not disrupted by 50 μg kg^{-1} of IL-1.

3. Capillary depletion showed that most of the I-IL entered the parenchymal–interstitial fluid space of the spinal cord with only a modest amount being sequestered by the endothelial cells of its BBB.

4. I-IL entered the cervical, thoracic and lumbar regions of the spinal cord equally well. I-IL entering at the brain and diffusing caudally was estimated only to account for about 1% of the total radioactivity found in the spinal cord after I.V. injection. These results show that I-IL is able to cross the endothelial part of the BBB and that blood-borne IL-1 has direct access to the spinal cord.

Interleukin-1α (IL-1) and other cytokines play major roles in the co-ordination of the neuroimmune axis (Plata-Salaman, 1991). Several possible mechanisms (Breder, Dinarello & Saper, 1988; Sirko, Bishai & Coceani, 1989; Katsuura, Arimura, Koves & Gottschall, 1990; Hashimoto *et al.* 1991) by which blood-borne cytokines might exert their effects on the central nervous system (CNS) have been identified. One such mechanism is direct transport into the CNS by a saturable transport system located at the blood–brain barrier (BBB). Such systems for transport into the brain have been identified for cytokines related to IL-1 (Banks, Kastin & Durham, 1989; Banks, Ortiz, Plotkin & Kastin, 1991; Banks & Kastin, 1991) and for tumour necrosis factor-α (Gutierrez, Banks & Kastin, 1993).

The BBB, however, can be considered to be composed of two major parts (Davson, Welch & Segal, 1987*a*). The capillary bed of the brain, consisting of endothelial cells modified to restrict entry of serum proteins into the brain, forms one of these parts and is referred to here as the endothelial barrier. The choroid plexus, the major source of production of cerebrospinal fluid (CSF), restricts the entry of serum proteins by modified ependymal cells termed choroid epithelium (Bradbury, 1979) and will be referred to as the ependymal barrier. Both parts of the BBB have saturable systems capable of transporting substances into or out of the CNS. Some of these transport systems are located at both barriers, whereas others are considered to be unique to one particular barrier (Davson *et al.* 1987*a*). Although no physical barrier exists between the CSF and the interstitial fluid surrounding the endothelial barrier in adults, distribution and concentration gradients of regulatory substances within the CNS are probably dependent upon which barrier is crossed (Davson, Welch & Segal, 1987*c*). Intravenously administered IL-1 and tumour necrosis factor-α have been recovered from CSF and from brain interstitial fluid (Gutierrez *et al.* 1993; Banks, Kastin & Gutierrez, 1993).

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The spinal cord differs from the brain in that, although its capillary bed is modified to form an endothelial barrier, it has no choroid plexus and therefore no ependymal barrier (Davson, Welch & Segal, 1987*b*). Since mixing between brain and spinal cord fluids is poor (Bulat & Zivkovic, 1978), any peripherally administered substance recovered from the spinal cord is likely to have crossed at the cord rather than at the brain with subsequent caudad diffusion. This unique arrangement provides an opportunity to determine whether IL-1 can cross the endothelial barrier of the BBB.

The possibility of transport of IL-1 at the spinal cord has potential practical significance. Since peripherally administered IL-1 can produce analgesia (Nakamura, Nakanishi, Kita & Kadokawa, 1988), at least part of this analgesia could be mediated directly at the spinal cord rather than only at the brain. The delivery of cytokines to the CNS for therapeutic reasons may need to include consideration of the spinal cord (Butter, Baker, O'Neill & Turk, 1991), especially for immune diseases that affect the spinal cord first or affect it to a greater extent than the brain. This will require an understanding of how cytokines are handled by the normal and diseased spinal cord BBB. Although it has been suggested that cytokines may disrupt the BBB of the brain (Ellison, Povlishock & Merchant, 1987), this issue has not been examined for the BBB of the spinal cord. For these reasons, we investigated the ability of blood-borne IL-1 to cross the BBB of the spinal cord.

METHODS

Iodination and purification

Recombinant IL-1, a gift from Immunex (Seattle, WA, USA), was iodinated with ^{125}I (I-IL) by the enzymobead method (Biorad, Richmond, VA, USA) and separated from free iodine on a column of G-10 Sephadex (Pharmacia LKB Biotechnology Inc., Piscataway, NJ, USA) previously hydrated with 0·25 M phosphate buffer containing 1% bovine serum albumin (BSA). Before separation, 0·25 M phosphate buffer free of BSA (10 ml) was used to wash the column of excess BSA, and during separation, the 0·25 M phosphate buffer was used to elute the I-IL. About 91% of the ^{125}I that was eluted as I-IL could be precipitated with 30% trifluoroacetic acid (TFA). The specific activity of the I-IL was about 700 Ci mmol^{-1}. Albumin was labelled with ^{99m}Tc (T-Alb) using a kit from Medi+Physics (Paramus, NJ, USA).

Blood to CNS influx

The multiple-time regression analysis (Patlak, Blasberg & Fenstermacher, 1983) as adapted to cytokines (Banks & Kastin, 1993) was used to measure the rate of entry into the brain. Male ICR mice (Charles River Labs Inc., Wilmington, MA, USA) weighing 19–25 g were anaesthetized with urethane (4 g kg^{-1} I.P.) and the left jugular vein and right carotid artery exposed. I-IL ($2{\cdot}0 \times 10^6$ c.p.m. per mouse) was injected into the left jugular vein in 0·2 ml lactated Ringer solution (g l^{-1}): 6 NaCl, 3·1 $C_3H_5O_3Na$, 0·3 KCl, 0·2 $CaCl_2.2H_2O$ and 1% BSA. At 2, 5, 10, 20, 30, 45 or 60 min after injection, blood was collected from the right carotid artery and the mouse decapitated. Only one sample of blood was taken from each mouse. The blood was centrifuged at 5000 *g* for 10 min at 4 °C, and 0·05 ml serum collected. The vertebral column was subsequently excised and the spinal cord easily flushed out intact with 0·9% NaCl. Whole brains without the pineal and pituitary glands were also harvested. Spinal cords and brains were cleansed of excess blood in lactated Ringer solution, dried of excess fluid, and weighed. The levels of radioactivity in the spinal cord, brain and serum samples were measured in a gamma counter. The unidirectional influx rates (K_1 expressed in ml g^{-1} min^{-1}) for spinal cord and brain, and the apparent volume of distribution (V_1 expressed in μl g^{-1}), were determined from the equation (Patlak, Blasberg & Fenstermacher, 1983):

$$A_m/C_{p,t} = K_1[\int_0^t C_p(\tau)d\tau]/C_{p,t} + V_1, \quad (1)$$

where A_m is the c.p.m. (g tissue)$^{-1}$ of spinal cord or brain, $C_{p,t}$ is the c.p.m. ml^{-1} of arterial serum at time t, and $[\int_0^t C_p(\tau)d\tau]/C_{p,t}$ is the exposure time in minutes.

To determine whether the transport was saturable, unlabelled IL-1 (0, 0·5, 2·5, 10 or 50 μg kg^{-1}) was included in the I.V. I-IL injection. These results were expressed as a percentage of the baseline value, and a value for 50% inhibition was calculated. T-Alb was also added to the injection containing 0 or 50 μg kg^{-1} IL-1 to serve as a vascular marker and to measure the integrity of the BBB to albumin. The tissue/blood ratio for T-Alb was expressed in microlitres per gram.

Capillary depletion

Four mice received an I.V. injection of I-IL ($4{\cdot}0 \times 10^6$ c.p.m.) and T-Alb ($1{\cdot}7 \times 10^6$ c.p.m.) in 0·3 ml lactated Ringer solution containing 1% BSA. After 30 min, spinal cords and brains were removed, washed in lactated Ringer solution, weighed and subjected to capillary depletion (Triguero, Buciak & Pardridge, 1990). Both the capillary and supernatant fractions resulting from capillary depletion were corrected for vascular contamination as measured using T-Alb and assayed for levels of protein and for γ-glutamyl transpeptidase (γGT) activity, an enzyme specific for capillaries. Levels of protein were measured using a reagent from Biorad, and γGT activity was measured using a kit from Sigma Chemical Co. (St Louis, MO, USA). Results for levels of radioactivity were expressed as tissue/blood ratios (ml g^{-1}) and for the enzymatic activity of γGT as units (mg protein)$^{-1}$.

Spinal cord regions

Transport rates of blood-borne I-IL to the cervical, thoracic and lumbar regions of the spinal cord and to the brain were compared. Seven mice were given an I.V. injection of I-IL ($2{\cdot}0 \times 10^6$ c.p.m.) in 0·2 ml lactated Ringer solution containing 1% BSA. The mice were decapitated in succession 2, 5, 10, 20, 30, 45 and 60 min after I.V. injection and the brains removed, washed, weighed and counted for radioactivity. The vertebral column was excised and dissected into cervical, thoracic and lumbar regions. Each corresponding spinal cord section was flushed from the vertebral column, washed, weighed and counted for radioactivity. K_1 values were computed for each region of the spinal cord as described above. In addition, results were expressed as the radioactivity (c.p.m.) per gram of each spinal cord section relative to the radioactivity (c.p.m.) per gram of the corresponding brain tissue.

Identification of material entering the spinal cord

HPLC was performed on the spinal cord 10 min after I.V. injection of 3×10^7 c.p.m. I-IL. Spinal cords were removed and placed in 2·5 ml of ammonium acetate (10 g (100 ml)$^{-1}$ in distilled H_2O containing 1% BSA) for 10 min and then homogenized in the ammonium acetate with a hand homogenizer. An *in vitro* control for degradation was performed by the addition of spinal cord to a test tube containing I-IL that was processed as described above. Samples were centrifuged at 200000 *g* for 10 min at 4 °C and the supernatants lyophilized. The supernatants were reconstituted with 0·5 ml distilled water containing 1% TFA. The solutions were then centrifuged at 4000 *g* for 30 min. The resulting supernatant was decanted and analysed by HPLC on a VYDAC 214TP54 column (The Separations Group, Hesperia, CA, USA). The acetonitrile gradient was increased from 30 to 90% in a linear fashion in 30 min at a flow rate of 1 ml min^{-1}. The TFA level in the solvent remained constant at 0·1%.

I.C.V. injections

Seven mice received an injection of I-IL ($8·0 \times 10^5$ c.p.m.) into the left lateral ventricle (I.C.V.) by a method previously described (Banks & Kastin, 1989) in 1·0 μl lactated Ringer solution containing 1% BSA. The mice were decapitated in succession 2, 5, 10, 20, 30, 45 and 60 min after I.C.V. injection, and the spinal cord regions and brains removed, washed, weighed and counted for radioactivity. The I-IL used for I.C.V. injections and for the analysis of injected material by HPLC was iodinated on a column washed with distilled H_2O and also eluted from the column with H_2O. The use of H_2O allowed the I-IL to be lyophilized without residual salts or proteins so that it could be reconstituted in lactated Ringer solution containing 1% BSA without producing a hypertonic solution. The specific activity of the I-IL eluted from the column was 700 Ci mmol^{-1}.

Statistics

Values are given as means ± S.E.M. and were compared by analysis of variance (ANOVA) and Duncan's multiple range test. Regression lines were determined by the least-squares method and compared statistically by the use of the BMDP1R program (University of California Press, Berkeley, CA, USA). Regression lines are reported with their correlation coefficients (r), the number of mice per line (n) and the level of statistical significance (P). K_i values (slopes) are reported with the standard error of the estimate.

RESULTS

The relationship between the ratio of cord/blood radioactivity and time of exposure is shown in Fig. 1*A* ($r = 0·843$, $n = 23$, $P < 0·001$); the K_i is $(7·91 \pm 1·10) \times 10^{-4}$ ml g^{-1} min^{-1} and the V_i is 32·3 μl g^{-1}. Figure 1*B* shows the relationship between the ratio of brain/blood and time of exposure ($r = 0·740$, $n = 23$, $P < 0·001$) for the same animals; the K_i is $(8·78 \pm 1·74) \times 10^{-4}$ ml g^{-1} min^{-1} and the V_i is 64·7 μl g^{-1}. There was a significant difference between the lines in Fig. 1*A* and *B*: $F(2,42) = 22·5$, $P < 0·00001$. However, when the respective intercepts were subtracted, the two lines were no longer statistically different: $F(2,42) = 0·3$, $P = 0·76$, demonstrating equivalent slopes, or K_i values.

Of the radioactivity extracted from the spinal cord, 66·1% eluted at the position of I-IL by HPLC; 88·9% of the radioactivity from the processing control eluted as intact I-IL. Corrected for processing, it was calculated that 74·3% of the radioactivity in the spinal cord was intact I-IL.

Entry of I-IL into both the spinal cord and brain was inhibited by unlabelled IL-1. Figure 2 expresses this inhibition as a percentage of the baseline value in relation

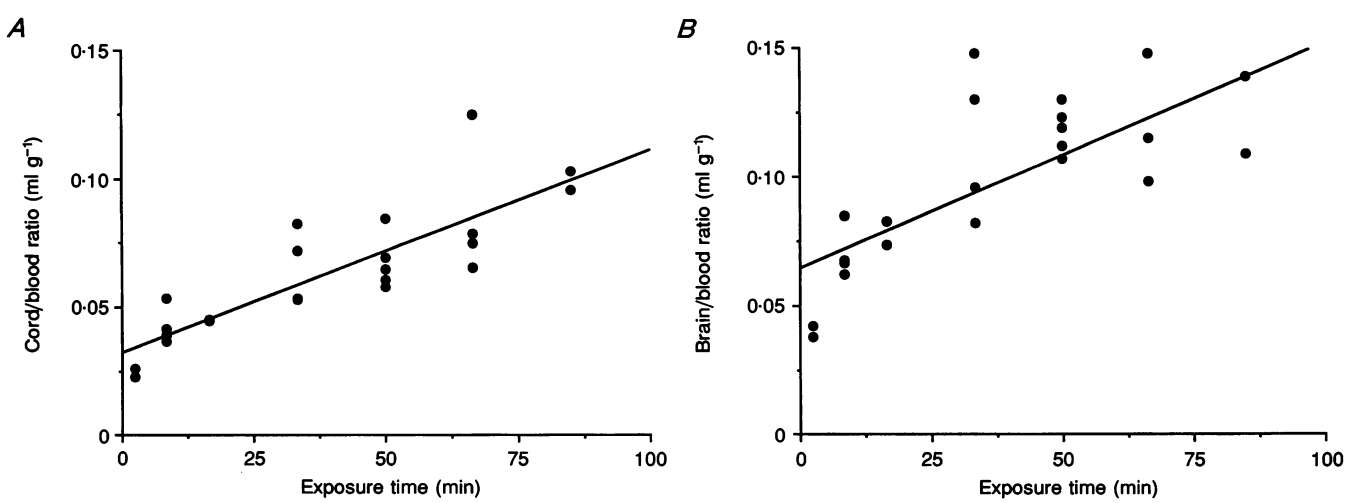

Figure 1. Entry of blood-borne I-IL into the spinal cord and brain
The relationship of time of exposure to ratios of radioactivity for cord/blood (*A*) and for brain/blood (*B*) after I.V. injection of I-IL is shown. The unidirectional influx constant was determined by multiple-time regression analysis. The lines in *A* and *B* were statistically different until corrected for intercepts.

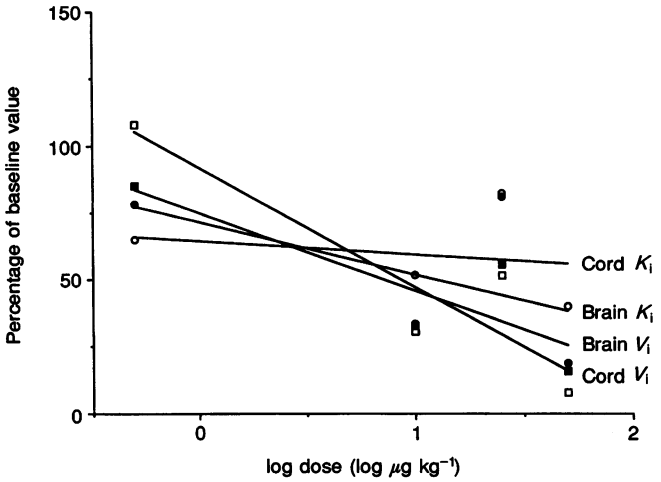

Figure 2. Inhibitory effect of various doses of IL-1 on spinal cord K_i, brain K_i, brain V_i and spinal cord V_i
Percentage of baseline transport was plotted against the log dose of IL-1. The dose needed to inhibit K_i by 50% was 19·6 μg (kg body weight)$^{-1}$ for spinal cord (○) and 6·11 μg kg^{-1} for brain (●). The dose needed to inhibit V_i by 50% was 4·9 μg kg^{-1} for spinal cord (□) and 4·2 μg kg^{-1} for brain (■).

to the log dose of unlabelled material. The dose needed to produce a 50% inhibition of the K_i was 19·6 μg kg^{-1} for the spinal cord and 6·11 μg kg^{-1} for the brain. The dose needed to produce a 50% inhibition of the V_i was 4·9 μg kg^{-1} for the spinal cord and 4·2 μg kg^{-1} for the brain.

Figure 3 presents the relationship between the ratios for cord/blood *vs.* brain/blood for mice receiving I-IL only ($r = 0{\cdot}9781$, $n = 8$, $P < 0{\cdot}00005$), and for mice also receiving 50 μg kg^{-1} unlabelled IL-1 ($r = 0{\cdot}9835$, $n = 8$, $P < 0{\cdot}00005$). The two lines in Fig. 3 show a statistically significant difference: $F(2,12) = 30{\cdot}7$, $P < 0{\cdot}0001$. When the lines in Fig. 3 are corrected for their respective V_i values, the lines are still significantly different: $F(2,12) = 24{\cdot}1$, $P < 0{\cdot}0001$. By contrast, the ratios for neither cord/blood nor brain/blood for the T-Alb controls (7·12 ± 0·4 μl g^{-1}, $n = 9$; 10·7 ± 0·282 μl g^{-1}, $n = 9$, respectively) were significantly different from the ratios of mice receiving T-Alb with unlabelled IL-1 at 50 μg kg^{-1} (7·42 ± 0·262 μl g^{-1}, $n = 9$; 11·15 ± 0·385 μl g^{-1}, $n = 9$, respectively).

The capillary depletion method has not been previously applied to the spinal cord. The results of the γGT assay showed that the separation of parenchyma and capillaries was successful. For capillaries and parenchyma there were 39·6 ± 13·2 and 3·14 ± 0·42 units mg^{-1} respectively ($n = 3$ for both), which gave a ratio of 12·6 : 1. This is similar to the ratio obtained for mouse whole brain in previous studies (Gutierrez *et al.* 1993; Banks & Broadwell, 1994; Banks *et al.* 1993). Figure 4 shows that I-IL enters the capillaries and parenchyma of both spinal cord and brain, but with entry into the parenchyma predominating, especially in the spinal cord. ANOVA showed differences among these four tissues for entry of I-IL: $F(3,12) = 10{\cdot}9$, $P < 0{\cdot}001$. The range test showed that the ratio in spinal cord parenchyma differed from that in spinal cord capillaries ($P < 0{\cdot}0005$) and from that in brain parenchyma ($P < 0{\cdot}01$). The differences between brain capillaries, spinal cord capillaries and brain parenchyma were not statistically significant in the range test.

Figure 5 shows the entry of I-IL into the cervical, thoracic and lumbar regions of the spinal cord after I.V. injection. There were no statistically significant differences among the lines for the spinal cord regions: $F(4,15) = 0{\cdot}7$,

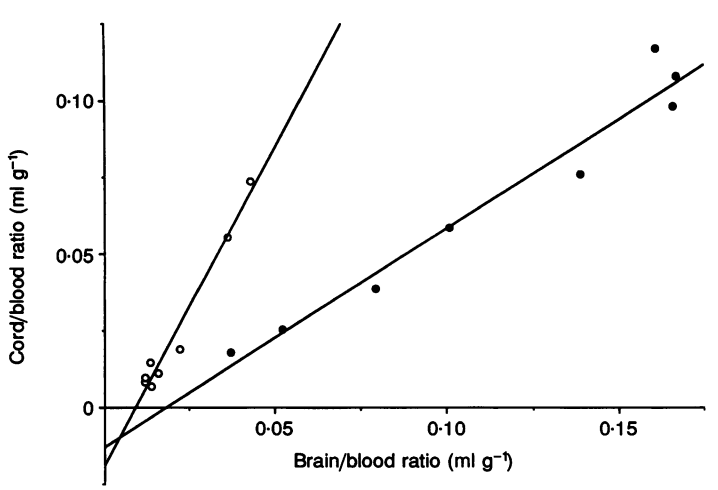

Figure 3. Self inhibition of I-IL entry into the spinal cord and brain
The effect of 50 μg kg^{-1} unlabelled IL-1 on the relationship of the ratios of cord/blood and brain/blood for I-IL (●) from corresponding mice is shown. The presence of unlabelled IL-1 (I-IL + IL-1; ○) shifted the line to the left demonstrating that accumulation by the brain was inhibited to a greater extent than accumulation by the spinal cord.

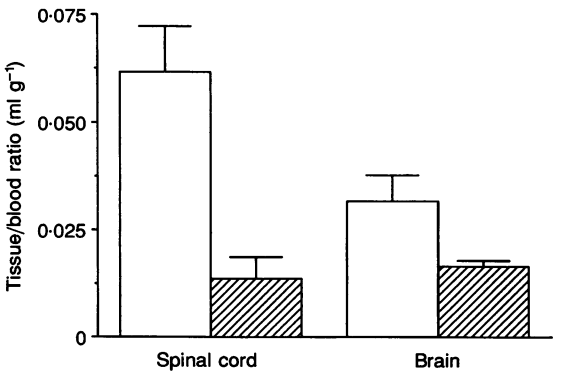

Figure 4. Blood-borne I-IL enters the parenchymal–interstitial fluid space of the spinal cord and brain
Distribution of I-IL between capillary (▨) and parenchymal (□) fractions for the spinal cord and brain was determined by the capillary depletion method. The value for the spinal cord parenchyma was statistically different from that for the spinal cord capillaries ($P < 0{\cdot}0005$) and for brain parenchyma ($P < 0{\cdot}01$). The differences between values for brain capillaries, spinal cord capillaries, and brain parenchyma were not statistically significant.

$P = 0{\cdot}61$. The relationship for cord/blood *vs.* exposure time was statistically significant when all regions were considered together ($r = 0{\cdot}601$, $n = 21$, $P < 0{\cdot}01$) with a K_1 of $(4{\cdot}57 \pm 1{\cdot}40) \times 10^{-4}$ ml g^{-1} min^{-1} and a V_i of 37·9 μl g^{-1}.

The relationship between the ratio for cord/brain and time after I.V. injection is shown in Fig. 6*A*. There was no significant difference among the spinal cord regions, and the line for all regions did not vary significantly with time ($r = -0{\cdot}043$, $n = 21$, $P = 0{\cdot}85$). The mean ratio for cord/brain was $0{\cdot}857 \pm 0{\cdot}044$ ($n = 21$). After I.C.V. injection (Fig. 6*B*), there was a statistical difference between the lines for the cervical and lumbar regions consistent with entry into the cord from the brain: $F(2,10) = 4{\cdot}12$, $P < 0{\cdot}05$. The mean cord/brain ratio after I.C.V. injection was $0{\cdot}007 \pm 0{\cdot}002$ ($n = 21$), with the cervical cord having the highest mean ratio of $0{\cdot}011 \pm 0{\cdot}005$ and the lumbar cord having the lowest mean ratio of $0{\cdot}003 \pm 0{\cdot}001$. All of the ratios for cord/brain after I.V. injection (Fig. 6*A*) were significantly different from all of the ratios for cord/brain after I.C.V. injection (Fig. 6*B*): $F(5,36) = 84{\cdot}3$, $P < 0{\cdot}001$.

DISCUSSION

The results show that blood-borne IL-1 enters the spinal cord by a saturable system. This shows that IL-1 can be transported by the endothelial barrier of the BBB. Transport occurred throughout the spinal cord without gross regional differences, although uptake by the brain may exceed uptake by the spinal cord.

The uptake of IL-1 by both the spinal cord and brain was saturable, showing similar K_1 values, the unidirectional uptake constants (Fig. 1). Capillary depletion showed that I-IL gained access to the interstitial/parenchymal spaces of the spinal cord and the brain as well as being sequestered by their endothelial cells. HPLC showed that the radioactivity entering the spinal cord mainly represented intact cytokine, as was previously found for brain (Banks *et al.* 1991; Banks & Kastin, 1991). These results confirm that blood-borne I-IL can cross the endothelial part of the BBB to enter the spinal cord. However, this does not rule out the possibility that I-IL might also be transported by the choroid plexus.

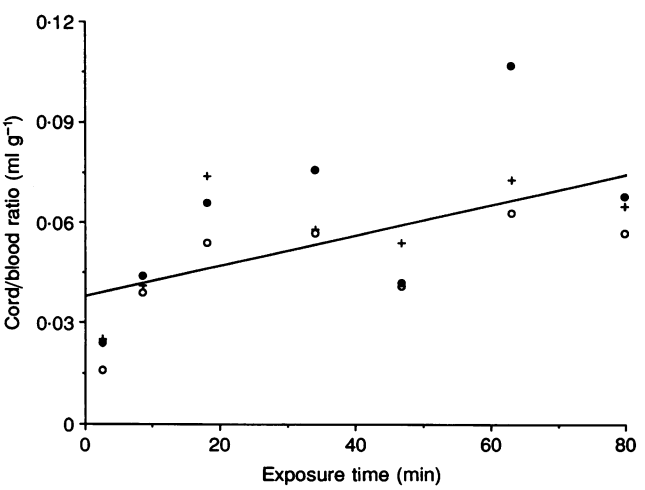

Figure 5. Regional permeability of the spinal cord to I-IL
The relationship of ratios of cord/blood for radioactivity after I.V. injection of I-IL for the cervical (●), thoracic (○) and lumbar (+) regions of the spinal cord is shown; no regional differences existed among the lines for the three regions.

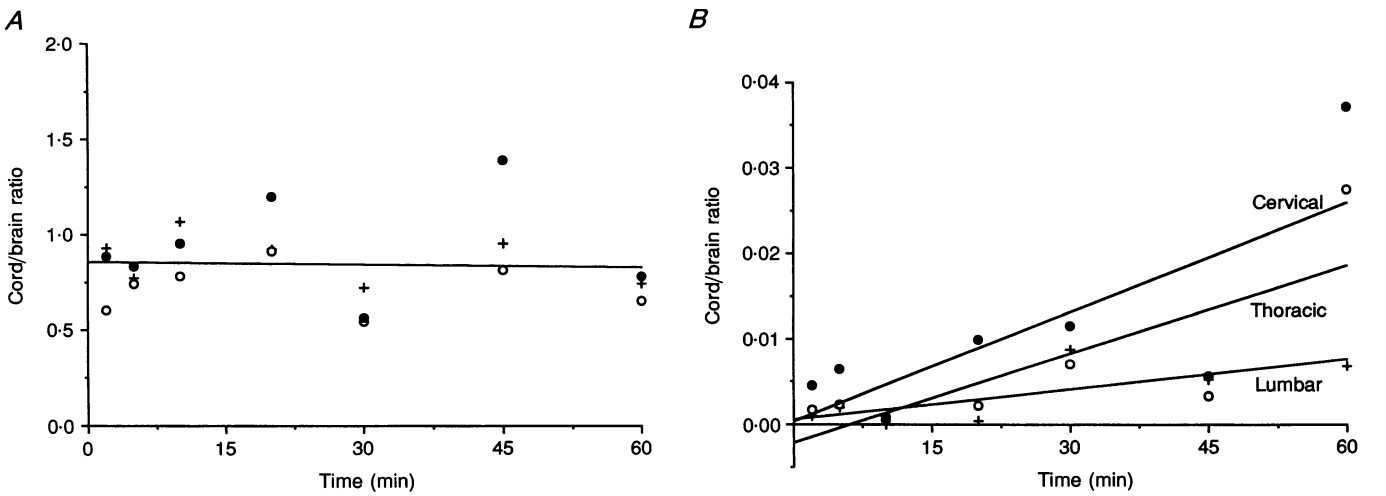

Figure 6. Brain *vs.* blood as source of spinal cord I-IL
Ratios of cord/brain for radioactivity at various times after I.V. (*A*) or I.C.V. (*B*) injection of I-IL is shown. ●, cervical cord; ○, thoracic; +, lumbar. After I.V. injection, no differences occurred among the lines or cord/brain ratios over time. After I.C.V. injection, the lines for cervical and lumbar regions were statistically significant; the cord/brain ratio never exceeded 0·04. Taken together, these results show that the entry of IL-1 into the spinal cord cannot be accounted for by caudad diffusion from the brain.

The similar K_i values for T-Alb in the spinal cord and the brain show that these two CNS tissues have BBBs that are equally tight to albumin. However, the vascular space of the spinal cord may be smaller than that of the brain as shown by its smaller V_i. Even the highest dose of IL-1 given (50 μg kg^{-1}) did not disrupt the BBB of either the spinal cord or the brain to T-Alb. Others have indicated that cytokines such as IL-2 can disrupt the BBB of the brain (Ellison *et al.* 1987). Although such disruption now appears to have been caused by contamination with sodium dodecylsulphate (Kobiler, Lustig, Gozes, Ben-Nathan & Akov, 1989) rather than by IL-2 (Banks & Kastin, 1992), cytokines do increase the leakiness of peripheral tissue vascular beds (Rosenstein, Ettinghausen & Rosenberg, 1986). Furthermore, the BBB of the spinal cord is disrupted by substances (Jacques & Couture, 1990; Long, Rigamonti, Dosaka, Kraimer & Martinez-Arizala, 1992) that do not typically affect the BBB of the brain (Banks, Kastin, Radulovic, Conley, Johnson & Schally, 1992). However, our results with albumin suggest that the blood–spinal cord and blood–brain barriers are not disrupted by IL-1.

The transport of I-IL by the spinal cord is similar to its transport by the brain. Subtle differences may exist, however, between the uptake of I-IL in the spinal cord and the brain regarding endothelial cell sequestration and saturability. Figure 1 shows that the V_i for spinal cord (Fig. 1*A*) is smaller than the V_i for brain (Fig. 1*B*). V_i represents the vascular space of the tissue and those spaces that can rapidly equilibrate with it. The smaller extravascular aspect of V_i for the spinal cord may be due to less sequestration by the endothelial cells of its BBB. This is suggested by the results with capillary depletion, which showed less I-IL associated with the endothelial cells derived from the spinal cord than from the brain (Fig. 4). It is not known whether this sequestration by the endothelium is part of the transport system or part of an unrelated receptor system.

For both spinal cord and brain, the experiments involving capillary depletion showed that most of the I-IL was found in the parenchymal fraction. However, relatively more of the I-IL was sequestered by brain endothelial cells (48% of the total recovered from brain) than by spinal cord endothelial cells (18% of the total recovered from the spinal cord). This is consistent with a previous study showing that 10 min after injection most of the I-IL in the whole brain was associated with the capillaries, although significant amounts had also entered the parenchyma and CSF by a saturable mechanism (Banks *et al.* 1993). Saturability may also differ between spinal cord and brain since the dose of unlabelled IL-1 needed to inhibit K_i was about three times greater for the spinal cord than for the brain. The analysis illustrated in Fig. 3 shows that 50 μg kg^{-1} unlabelled IL-1 produced a greater degree of inhibition in the uptake of I-IL by the brain than by the spinal cord. This differential inhibition could be due to variation between the transport rates of brain and spinal cord endothelia. The choroid plexus, if it also transports IL-1, could contribute to differences between brain and spinal cord accumulation. By contrast, the dose needed to inhibit the V_i values for spinal cord and brain by 50% differed little. Overall, the similarities between spinal cord and brain for the transport of IL-1 were more striking than their differences.

Differences did not exist in transport of I-IL into the varying regions of the spinal cord (Fig. 5). The cervical, thoracic and lumbar regions of the spinal cord were not distinguishable in their transport rates of I-IL. However, in some diseases, such as experimental allergic encephalomyelitis (EAE), which has been proposed as an animal model for multiple sclerosis, disruption begins in the lumbar area and extends cephalad as the disease progresses (Butter *et al.* 1991). The concentrations of some cytokines are elevated in serum during the course of EAE and may play a role in exacerbation and recovery from the disease (Jacobs *et al.* 1991; Kennedy, Torrance, Picha & Mohler, 1992; Sharief, Noori, Ciardi, Cirelli & Thompson, 1993). This raises the question of the effect of diseases of the spinal cord on cytokine transport. Whether cytokine transport across the BBB of the spinal cord and brain is disturbed during EAE remains to be investigated.

The possibility that the I-IL found in the spinal cord originated in the brain was eliminated. When mice were injected I.C.V. with I-IL to simulate this possibility, characteristic patterns were found. As would be predicted from the slow exchange between spinal and brain fluids (Grundy, 1962), cord/brain ratios for radioactivity after I.C.V. injection of I-IL were very low (less than 0·01), increased over time, and were significantly higher in the cervical cord than in the lumbar cord (Fig. 6*B*). After I.V. injection, cord/brain ratios were high (about 0·85), did not increase over time, and did not differ among the regions of the spinal cord (Fig. 6*A*). This shows that diffusion of I-IL from brain to spinal cord is very limited and could only account for 0·01/0·85, or about 1%, of the I-IL found in the spinal cord after I.V. injection.

These results show that I-IL can cross the endothelial BBB of the spinal cord, as an intact substance, by means of a saturable transport system. The rate of entry of I-IL does not differ among the cervical, thoracic and lumbar regions of the spinal cord and is similar to its rate of entry into the brain. Although the capillaries are capable of sequestering I-IL, most of the I-IL completely crosses the BBB to reach the parenchymal–interstitial fluid space of the spinal cord and brain. It is not known whether endothelial sequestration is a process related to transport. Unlabelled IL-1 (50 μg kg^{-1}) did not disrupt the BBB of the spinal cord to radioactively labelled albumin. Thus, IL-1 from the periphery can enter the spinal cord of the CNS in intact form by a saturable transport system.

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