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Free tryptophan/large neutral amino acids ratios in blood plasma do not predict cerebral spinal fluid tryptophan concentrations in interleukin-1-induced anorexia

Alessio Molfino [a], Maurizio Muscaritoli [a], Antonia Cascino [a], Francesca Fanfarillo [a], Alessandra Fava [a], Giuseppe Bertini [b], Gennaro Citro [b], Filippo Rossi Fanelli [a], Alessandro Laviano [a,*]

[a] *Department of Clinical Medicine, Sapienza - University of Rome, 00185 Rome, ITALY*
[b] *SSD SAFU IFO, Rome, Italy*

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Abstract

Peripheral administration of interleukin-1 (IL-1) reduces food intake and affects brain serotonergic activity, suggesting a causal relationship. Furthermore, IL-1 increases the brain concentrations of the serotonin precursor, tryptophan (TRP), by unclear mechanism(s). We aimed at confirming the link between IL-1 administration, raised brain TRP concentrations and the development of anorexia, and at investigating the mechanisms of TRP entry into the brain. Thirty adult, overnight fasted Sprague–Dawley rats were randomly assigned to i.p. injections of 1 μg/kg BW of IL-1α ($n=10$) or vehicle ($n=10$), or to pair-feeding with IL-1 animals ($n=10$). After 2 h, food intake, blood plasma concentrations of total TRP, free TRP, large neutral amino acids (LNAA; competing with TRP for brain entry) were measured. Cerebral spinal fluid (CSF) TRP concentrations were also measured. TRP brain availability was assessed by calculating the plasma ratio free TRP/LNAA. Following IL-1 injection, food intake significantly declined in IL-1 rats, which was paralleled by decreased plasma free TRP and increased plasma LNAA. Despite a decrease in the free TRP/LNAA ratios in plasma, IL-1 significantly increased concentrations of TRP in CSF. These data show that the acute peripheral administration of IL-1 induces anorexia and raises CSF TRP levels. Considering the possible role of the raised CSF TRP in influencing brain serotonin activity, it is postulated that increased serotonergic neurotransmission could be involved in IL-1 induced anorexia.

Keywords: Interleukin-1; Anorexia; Tryptophan; Serotonin; Brain; Large neutral amino acids

1. Introduction

Anorexia and reduced food intake are frequently observed in the clinical course of acute and chronic diseases. The onset of anorexia worsens the prognosis of the underlying disease and contributes to the development of cachexia, further exacerbating the detrimental effects on nutritional status (Laviano et al., 2003). Thus, the investigation of the pathogenic mechanisms of anorexia has important clinical implications, since it may help in devising effective therapeutic strategies.

The pathogenesis of disease-associated anorexia appears to be related to the development of the inflammatory response (Kalantar-Zadeh et al., 2004), whose central effects derange brain neurochemistry. Indeed, anorexia could be considered as a clinical marker of the activation of the immune response. Pro-inflammatory cytokines, including interleukin-1 (IL-1), are involved in mediating the behavioral effects of the inflammatory response triggered by acute or chronic diseases (Plata-Salaman, 2000). In anorectic tumor-bearing rats, hypothalamic IL-1 mRNA is increased (Turrin et al., 2004), while anorexia is ameliorated by the intra-hypothalamic injection of the IL-1 receptor antagonist (Laviano et al., 2000). Some evidence show that the anorectic effects of IL-1 are centrally mediated, at least in

* Corresponding author. Tel.: +39 0649973902; fax: +39 064440806.
E-mail address: alessandro.laviano@uniroma1.it (A. Laviano).

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part, by the serotonergic system (El-Haj et al., 2002). Indeed, the peripheral injection of IL-1 induces anorexia and increases brain serotonin concentrations (Mohankumar et al., 1998). Also, the block of brain serotonin receptors ameliorates the anorectic effects of centrally administered IL-1 (von Meyenburg et al., 2003). However, it should be acknowledged that IL-1-induced hypophagia is likely mediated by more than a single system (Swiergiel et al., 1999; Swiergiel and Dunn, 2002).

Brain serotonin synthesis depends on the brain availability of its precursor, the amino acid tryptophan (TRP) (Diksic and Young, 2001). Tryptophan binds to albumin, but only unbound TRP, i.e., free TRP (FTRP), enters into the brain by competing with the other large neutral amino acids (LNAA; i.e., valine, leucine, isoleucine, tyrosine and phenylalanine) for the same transport system located on the blood-brain barrier (Cangiano et al., 1990). In previous clinical studies, it has been shown that the oral administration of pharmacological doses of 5-hydroxy-TRP, the immediate precursor of serotonin, reduces food intake while increasing the urinary levels of 5-hydroxy-indoleacetic acid, the final metabolite of serotonin (Cangiano et al., 1992). Also, consistent evidence shows that the anorexia associated to cancer, end-stage renal disease or liver cirrhosis can be ameliorated by reducing brain TRP entry achieved via increased plasma concentrations of LNAA (Laviano et al., 2005; Fernstrom, 2005).

The role of plasma and CSF TRP concentrations in influencing brain serotonin synthesis and neurotransmission is still unclear. Supporting results show that in healthy volunteers, acute TRP depletion decreases plasma and CSF TRP levels and CSF concentrations of 5-hydroxy-indoleacetic acid (Williams et al., 1999). Using in vivo microdialysis in rats, Sharp et al. (1992) demonstrated that acute i.p. administration of TRP increases brain release of serotonin, which in turn is dependent not only on TRP levels but on neuronal activity as well (Schaechter and Wurtman, 1989).

We previously demonstrated that IL-1 i.p. administration for 2 consecutive days reduces food intake and body weight in normal rats (Sato et al., 2003). These effects were paralleled by an increase of plasma FTRP concentrations, which may lead to an increase in brain TRP and possibly an increase in serotonin synthesis. To confirm our previous inference, the aim of this study was to measure CSF TRP concentrations following IL-1

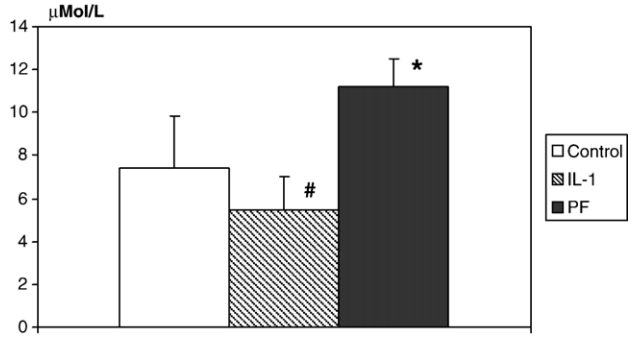

Fig. 1. Plasma free tryptophan levels (µMol/L) in the three groups studied ($^{\#}p=0.05$ vs Control group; $^{*}p<0.01$ vs Control and IL-1 groups).

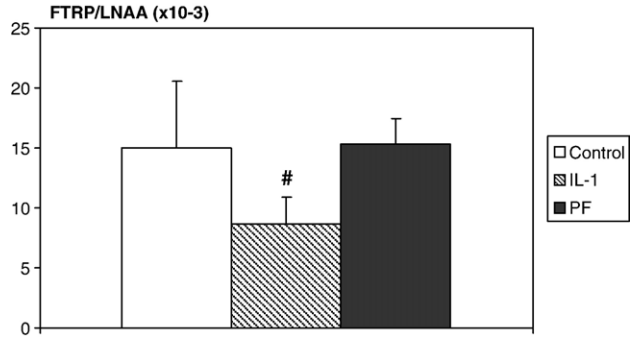

Fig. 2. The ratio in plasma FTRP/LNAA ($\times 10^{-3}$), by which brain TRP availability is inferred, is significantly lower ($^{\#}p<0.01$) in IL-1 rats when compared with controls and PF animals.

administration. Also, our goal was to further detail the acute effects of IL-1 i.p. injection on plasma amino acid concentrations, to elucidate the role of TRP, and particularly the mechanisms of its entry into the brain, in IL-1-induced anorexia.

2. Materials and methods

After approval from the Animal Care Reviewing Board at our Institution, 30 adult, male Sprague–Dawley rats from our rat colony and weighing approximately 300 g were studied. Rats were housed in holding wire cages for 10 days to habituate them to the study surrounding: 12 h light/dark cycle (light on 06:00–18:00), room temperature of 26±1 °C, and 45% relative humidity. After the habituation period, rats were individually caged and overnight fasted. The next morning, rats were randomly assigned to i.p. injections of either IL-1α (1 µg/kg BW; Sigma, Milan, Italy) dissolved in 200 µL of saline (IL-1 group; $n=10$) or the vehicle (Control group; $n=10$). Then, rats were placed in their individual cage and food was offered *ad libitum*. After 2 h, food intake was measured by weighing leftovers and under halothane anesthesia blood and CSF samples were collected via cardiac and cisterna magna punctures, respectively. After completion of the experiment in IL-1 group, on the next day an additional group of 10 rats was overnight fasted, pair-fed with IL-1 rats (i.e., given the amount of food eaten by the IL-1-treated rats on the previous day) and blood and CFS samples collected (PF group; $n=10$). After sampling, all rats were sacrificed.

Plasma was immediately obtained by centrifugation. CSF samples were also centrifuged and samples showing blood contamination were discarded. Then, plasma and CSF were stored at −80 °C until assay. Plasma total and FTRP concentrations, as well as CSF TRP concentrations, were measured using the spectrofluorimetric technique described by Denckla and Dewey (1967) as revised by Bloxam and Warren (1974). The plasma concentrations of the amino acids competing with TRP for brain entry (valine, leucine, isoleucine, tyrosine and phenylalanine) were measured by HPLC using orthophtaldialdehyde for derivatization and fluorescence for detection, as described by Araujo et al. (2001). Then, TRP brain availability was assessed by calculating the ratio between the plasma

concentrations of FTRP and the competing LNAA. This ratio (FTRP/LNAA $\times 10^{-3}$) has been demonstrated to predict CSF TRP levels (Cangiano et al., 1990).

Data were statistically analyzed using the Student's *t*-test for unpaired data. A $p<0.05$ was considered of statistical significance. Data are presented as mean±SD.

3. Results

On experimental day, mean body weight was not significantly different between Control, IL-1 and pair-fed (PF) rats (295.0±12.6 g, 302.5±19.3 g and 300.0±16.1 g, respectively). Following IL-1 i.p. injection, food intake (grams) significantly declined in IL-1 rats when compared to Control (0.9±1.0 vs 6.4±1.5; $p<0.01$). Consequently, food intake in PF rats was also significantly different from control rats.

After saline or IL-1 injections, no statistically significant difference was found in plasma total TRP concentrations (μMol/L) between Control, IL-1 and PF rats (57.7±18.8, 64.2±18.7 and 71.7±9.2; $p>0.05$).

In contrast, plasma FTRP concentrations (μMol/L) showed marked differences in the 3 groups studied. As illustrated in Fig. 1, plasma FTRP declined in IL-1 rats when compared with control animals (5.5±1.5 vs 7.4±2.4; $p=0.05$), but increased in PF rats (11.2±1.3; $p<0.01$ vs Control and IL-1 rats).

The molar sum in plasma of the LNAA (μMol/L) competing with TRP for brain entry was affected by IL-1 i.p. injection. It significantly increased in IL-1 and PF groups when compared with control animals (630.8±97.3, 759.7±92.0 and 515.3±140.8; $p<0.05$), the difference between IL-1 and PF rats being also statistically significant ($p<0.05$). As shown in Fig. 2, brain TRP availability as assessed by the molar ratio in plasma between FTRP concentrations and the molar sum of the competing LNAA significantly decreased in IL-1 rats when compared with Control and PF rats (8.7±2.2 vs 15.0±5.6 and 15.1±2.2; $p<0.01$).

Only 7 CSF samples from the Control group, 7 samples from the IL-1 group and 8 samples from the PF group were not contaminated during sampling and were therefore assayed for TRP concentrations. As shown in Fig. 3, CSF TRP concentrations (μMol/L) significantly increased in IL-1 rats when compared with Control and PF groups (7.0±1.8 vs 4.3±1.2 and 4.0±1.2; $p<0.01$).

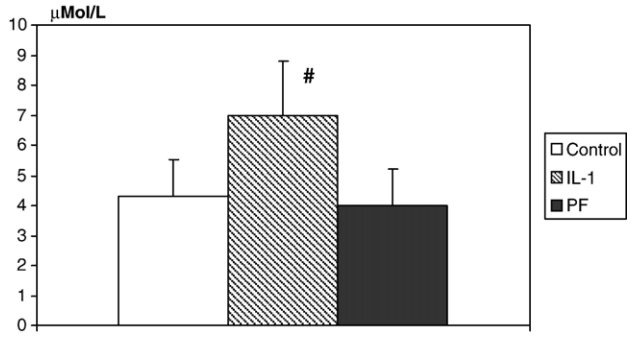

Fig. 3. CSF tryptophan levels (μMol/L) in the three groups studied ($^{\#}p=0.01$ vs Control and PF groups).

4. Discussion

Data obtained in the present study confirm that IL-1 induces anorexia, whose onset is paralleled by the acute rise of CSF TRP levels. Considering that serotonin synthesis is influenced by brain TRP levels (Esteban et al., 2004), our data suggest that increases in brain serotonergic activity may contribute to the reduction of food intake triggered by IL-1 administration. On the other hand, the results obtained in the present study question the role of plasma FTRP and the ratio FTRP/LNAA as markers of TRP entry into the brain, since the acute rise of CSF TRP levels was not related to increased plasma FTRP levels nor to increased ratio FTRP/LNAA in plasma.

In previous studies both in humans and in animals, it has been demonstrated that increased plasma FTRP levels and increased ratio FTRP/LNAA in plasma are related to increased CSF TRP levels (Cangiano et al., 1990; Diksic and Young, 2001; Sato et al., 2003). In the present study, these logically consequent biochemical events are dissociated. It should be acknowledged that the acute experimental setting described in this study is different from our previous report involving repeated IL-1 administration. In the present study, plasma and CSF amino acid levels were measured 2 h after a single IL-1 challenge, in order to more precisely monitor the response of plasma amino acid profile to the effects of the cytokine which peak approximately 1 h after administration (Lenard and Dunn, 2005). From our data, it can be excluded that the changes in plasma and CSF TRP concentrations occurred as a result of an increase of the circulating TRP pool, since total TRP did not significantly change following IL-1 injection. This suggests that the bound and the unbound pools of TRP were redistributed by IL-1 injection.

The changes induced by IL-1 administration on intermediary TRP-related metabolism occur much faster than those induced by other compounds including lypopolysaccharide. Therefore, it could be speculated that IL-1 injection prompts an early response leading to acute increase of plasma FTRP concentrations by mechanisms yet to be determined, but possibly including increased protein degradation, although the observed significant rise of plasma LNAA following IL-1 challenge may actually reduce TRP entry into the brain (Joseph and Kennett, 1983). However, 2 h after IL-1 challenge, when animals were sampled, a large proportion of TRP could have already crossed the blood-brain barrier and entered into the brain, thereby reducing plasma FTRP concentrations and consequently the ratio FTRP/LNAA. Although the balance in plasma between albumin-bound TRP and FTRP is dynamic, it cannot be excluded that FTRP and the other amino acids were sampled and measured before circulating albumin-bound TRP could compensate for the amount of FTRP entered into the brain. Bearing this in mind, if the ratio in plasma FTRP/LNAA is involved in the increase of CSF TRP after IL-1 administration, then pre-treatment of animals with any competitor amino acid (i.e., valine) would prevent the rise of CSF TRP and the increase of serotonin synthesis (Joseph and Kennett, 1983). Indirect supporting evidence comes from clinical studies showing that disease-associated anorexia, whose pathogenesis involves the

effects of chronic IL-1 exposure, is effectively counteracted by the administration of LNAA, and in particular of branched chain amino acids (Fernstrom, 2005; Laviano et al., 2005).

The dissociation between plasma TRP levels or FTRP/LNAA ratio and CSF TRP concentrations has been previously reported under specific experimental conditions (Fernstrom and Fernstrom, 1993; Lenard and Dunn, 2004). Indeed, our study shows that pair-feeding and sham operation are experimental procedures in which the ratio FTRP/LNAA predicts CSF TRP concentrations more precisely than plasma FTRP levels, while IL-1 challenge appears to involve different pathways. Therefore, it could be argued that the results obtained in the present study are secondary to the activity of a specific transport system located at the blood-brain barrier which is activated by IL-1 administration. This explanation seems to be supported by a number of experimental data. The administration of clenbuterol, a β_2-adrenoceptor agonist, has been shown to raise brain tryptophan levels, also determining decreased plasma tryptophan concentrations (Edwards et al., 1989). This effect is blocked by the administration of a β_2-adrenoceptor antagonist. Even if the increase in brain tryptophan was similar to the increase of the plasma ratio between tryptophan and the other LNAA, still the sympathetic nervous system appears to exert a direct action in brain to regulate levels of aromatic amino acids (Edwards et al., 1989). Supporting the inference that a specific amine precursor amino acid transport exists at the blood-brain barrier, Takao et al. showed that different beta-adrenoceptor agonists increase brain concentrations of aromatic amino acids, including tryptophan (Takao et al., 1992). Further detailing the biochemical mechanisms mediating the adrenergic control of tryptophan entry into the brain, Lenard et al. (2003) showed that β_2- and β_3-adrenergic receptors but not β_1-adrenoceptors increase brain tryptophan concentrations, while vagotomy has a minor role in attenuating the responses to IL-1 challenge (Laviano et al., 1995; Wieczorek et al., 2005). On the other hand, consistent data indicate that peripheral IL-1 administration increases sympathetic activity (Kannan et al., 1996) by activating forebrain neural circuits (Kenney et al., 2002). In particular, IL-1 has been shown to enhance β_2-adrenergic receptor expression at least in human airway epithelial cells (Bin et al., 2001), while IL-1-induced increase of brain tryptophan content is prevented by adrenoceptor antagonists (Lenard and Dunn, 2005).

Therefore, it could be hypothesized that acutely i.p. administered IL-1 increases sympathetic activity, which in turn results in the upregulation of an amine precursor amino acid transport across the blood-brain barrier, leading to increased CSF tryptophan concentrations.

In our study, IL-1 administration reduced food intake while simultaneously increasing CSF TRP levels. We acknowledge that in our study we measured CSF and not brain TRP, and thus we may only infer the effects of CSF TRP on brain serotonin synthesis. However, experimental and clinical data support the relationship existing between CSF and brain TRP levels and serotonin synthesis and release (Young and Gauthier, 1981; Schaechter and Wurtman, 1990; Sarna et al., 1991; Sharp et al., 1992; Williams et al., 1999). It is therefore conceivable that increased brain serotonin synthesis and serotonergic activity may mediate at least in part IL-1-induced anorexia. Although this inference is supported by previous reports (Yang et al., 1999; El-Haj et al., 2002; von Meyenburg et al., 2003), it should be acknowledged that controversy still exists on the involvement of brain serotonin in IL-1-induced anorexia (Swiergiel and Dunn, 2000). In line with this reasoning, we could not demonstrate a role for brain serotonin in the initial phase of anorexia development in a well established model of sepsis, in which the contribution of the cytokine network is critical (Torelli et al., 2000). These controversial results could be related to the different methodologies used in the different studies and/or to species differences.

However, an additional explanation could be related to the immunomodulating effects of tryptophan (Platten et al., 2005). At least during the initial phase, increased brain TRP concentrations following peripheral IL-1 challenge could be diverted to sustain the mounting cerebral myelin-specific T-cell response, which includes production of proinflammatory T helper-1 cytokines (Platten et al., 2005) rather than to promote serotonin synthesis and serotonergic activity. Thus, initial anorexia following IL-1 injection could be secondary to the direct action of the cytokine on hypothalamic anorexigenic neurons and/or the activation of yet to be determined other anorexigenic pathways. In a later phase, brain TRP could be mainly used to increase serotonin synthesis which in turn may maintain hypophagia and optimize immune modulation (Leon-Ponte et al., 2007). Supporting the critical role of brain serotonin in the regulation of different biochemical and molecular pathways, a specific brain TRP hydroxylase, which is different from peripheral TRP hydroxylase, has been shown to regulate its synthesis (Walther et al., 2003).

We acknowledge that translation of our results to the interpretation of the complex clinical syndrome of anorexia could be difficult. However, if supported by further studies, the hypothesis of the temporal changes in brain neurochemistry following IL-1 challenge will open new avenues in the developments of effective therapeutic strategies to counteract the detrimental effects of neuroinflammation on the nutritional status of patients affected by acute and chronic diseases.

In summary, our study confirms that the peripheral administration of IL-1 reduces food intake and increases CSF TRP levels, suggesting a role for brain serotonin in mediating hypophagia. The observed absolute and relative changes of circulating FTRP and LNAA suggest that TRP entry into the brain after IL-1 challenge could be mediated at least in part by a specific aromatic acid transport system located at the blood-brain barrier, as already shown in other experimental conditions. Considering that brain TRP and serotonin regulate a number of functional and biochemical pathways, it could be speculated that these effects are secondary to the specific ability of TRP to be involved in different and time-dependent synthetic and/or degradation pathways.

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