Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats

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Abstract

Here we study the role of a pleiotropic pro-inflammatory cytokine, interleukin-6 (IL-6), in epilepsy. To examine this problem, we used human recombinant IL-6 applied intranasally (400 ng/40 μL) to rats 1 h before seizures induced by systemic injection of pentylenetetrazole (PTZ, 75 mg/kg). Overall, compared to the saline-treated control animals (n = 11 in each group), IL-6-treated rats demonstrated elevated levels of IL-6 in the frontal lobe (measured by ELISA) and increased severity of PTZ-induced seizures (shorter latency, longer duration and higher mortality). Our findings show that IL-6 plays a pro-convulsant role in the brain and suggest that the IL-6 system may be a novel target for the development of anticonvulsant drugs.

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A pleiotropic cytokine interleukin-6 (IL-6) is involved in inflammatory processes as a systemic mediator of the acute phase response in infection [10,18]. IL-6 has been found in the brain, including neuronal, glial and microglial cells [18,27], where it plays an important modulatory and neuroprotective role [5,27]. IL-6 influences mesocorticolimbic dopamine and serotonin neurotransmission and also has activating effects on locomotion [35–37]. Recently IL-6 was reported to have neurotoxic effects and has also been suggested to play a significant role in various diseases of the central nervous system [5,26]. There is a growing body of evidence linking IL-6 to epilepsy [26,27]. Seizures are a common complication in patients with infectious diseases accompanied by elevated plasma and brain IL-6 levels [10,12–14]. Elevated levels of IL-6 occur in the plasma and cerebrospinal fluid of patients with recent epileptic seizures, where the levels of IL-6 generally correlate with the severity of the seizures [16,17,21–23]. These elevated IL-6 levels were observed in patients without any infection or brain trauma, and are most likely a consequence of neuronal epileptic activity associated with seizures [21–23]. In rodents, limbic seizures rapidly and transiently enhanced IL-6 in the hippocampus [6,33]. Elevated IL-6 levels are seen after in vivo administration of excitatory amino acids [20]. Neuronal depolarisation and Ca 2+ influx have been shown to directly activate production of IL-6 in the neuronal cells in vitro [28]. Finally, transgenic mice over-expressing IL-6 in the brain develop spontaneous behavioural seizures, increased hippocampal excitatory activity and increased sensitivity to glutamatergic-induced seizures [4,27,31].

However, the role of IL-6 in epilepsy is still unclear and thus needs further experimental investigation. In the present study, we directly examined the effects of IL-6 on chemically induced seizures. In order to minimise pyrogenic and other unwanted side effects of IL-6, and since this cytokine penetrates the blood–brain barrier poorly, we used intranasal administration shown to be effective in by-passing the blood–brain barrier for several other cytokines and similar polypeptides [7,9,15,19]. Since rat and human IL-6 possess a high degree of homology, we chose to use human recombinant IL-6 (hIL-6) for our experiments. Since our work is the first study to use IL-6 intranasally, we used 400 ng IL-6 for our studies, basing our choice on recent data [36,37] showing that at this dose IL-6 is able to cause central effects in rats following systemic administration. Choosing the pretreatment time for our studies, we...
considered recent findings showing that (i) cytokine-like molecules may appear in the brain within 20–30 min following their intranasal administration [7,19] and (ii) IL-6 can cause its central effects 30–60 min following its systemic administration [36,37]. As such, 1 h pretreatment time seemed appropriate for IL-6 at this dose to reach the brain and affect seizures. Here we demonstrate that intranasally administered hIL-6 appears in the brain and leads to increased severity of seizures and higher mortality in the model of pentylenetetrazole (PTZ)-induced seizures in rats.

Twenty two adult male Wistar rats (220–240 g; University of Tampere, Finland) were maintained in a standard virus/parasite-free facility and exposed to a 12h/12h light-dark cycle. Lights were turned off at 18:00 and on at 06:00 h. The animals were experimentally naive and housed in pairs, with food and water freely available. The testing was always conducted between 14:00 and 18:00 h. On the days of the experiments animals were brought individually into the experiment room and immobilised in a vertical position, head up, for 5 min in a specially designed plastic restraint box. HIL-6 purchased from R&D Systems (USA; 10 μg diluted in 1 ml of sterile isotonic saline) was used in this study. Intranasal administration was performed in immobilised anaesthetised animals using a 40 μl micropipette (Lab systems, Finland). Each animal received 10 μl of hIL-6 or isotonic saline at once in each nostril. This treatment was repeated after a 3-min interval (total amount administered was 400 ng/40 μl/rat). After the intranasal administration procedure the animals were returned to their cages for recovery. One hour later, each animal received a bolus of intraperitoneal injection of PTZ (Sigma, UK, 75 mg/kg) and was placed in a new clean cage for observation of seizure profile. Seizures and seizure latency times were observed visually over a 30-min observation period by a trained experimenter blind to the treatment groups. The following parameters were recorded: the time to onset of the first twitch, the number of rats having motor seizures; the duration of the oro-facial, clonic and tonic components of seizures; the mortality rate. The intensity of seizures was registered using a modified Racine’s scoring system [25]: 0 (no response), 1 (freezing), 2 (head nodding or isolated twitches), 3 (oro-facial seizure), 4 (clonic seizure), 5 (tonic seizure), 6 (death). Clonic seizures consisted of rhythmic contractions of forelimbs and/or hindlimbs. Tonic seizures consisted of rigid extension of the fore- and/or hindlimbs with or without posture loss. An animal was considered dead if the heart was not beating upon manual check. Following decapitation, brains were removed and dissected on a cold plate. Frontal lobes (anterior to optic chiasma) were isolated and stored at −70 °C until the time of assay. Samples were weighted and homogenised in 50 mM PBS (pH = 7.5) using a Potter homogeniser. Thereafter, they were centrifuged for 15 min (5000 rpm) and supernatant was removed and stored. Commercial ELISA kit (R&D Systems, USA) was used to measure the level of hIL-6 in the frontal lobe of the brain. Animal care and experimental procedures were conducted in accordance with the European legislation and the guidelines of the National Institutes of Health. All animal experiments reported here were approved by the Ethical Committee of the University of Tampere. All results are expressed as means ± S.E.M. Data were analysed by Student’s t-test for independent samples. A probability of less than 0.05 was considered statistically significant.

The results of the present study are summarised in Table 1. Overall, hIL-6 produces a dramatic increase in seizure severity in PTZ-treated rats. While the duration of relatively mild oro-facial seizures was similar in both groups (Table 1), the duration of more severe clonic/tonic components and the total duration of seizures were almost double in rats treated with hIL-6. Also, there was a significantly shorter latency to the first twitch (Table 1). Mortality rate (55%) was almost 3-fold higher in the hIL-6-treated group (18%), although not significantly. Analysis of ELISA data shows that intranasal administration of hIL-6 results in a significant increase in IL-6 levels in frontal lobe (18.6 ± 5.4) compared to (2.2 ± 0.6) in saline-treated controls (P < 0.05), thus, correlating with an increased severity of seizures. Together, these data clearly indicate a pro-convulsant effect of exogenously applied hIL-6 in the brain following intranasal administration in a model of PTZ-induced seizures.

In general, our findings are the first data that directly link exogenous IL-6 to increased seizure severity in rats. The chemoconvulsant drug PTZ used in the present study to induce seizures, is known to act in the brain through inhibition of gamma-aminobutyric acid (GABA) [29]. Many drugs that act on the GABAergic system are able to affect IL-6. For example, the antiepileptic drug sodium valproate, a stimulator of GABAergic transmission, inhibits IL-6
In summary, here we have demonstrated a clear pro-convulsant action of exogenously applied IL-6 in rats. Using the intranasal route for IL-6 delivery to the brain, we minimised possible peripheral side effects of IL-6, resolved the problem of by-passing the blood–brain barrier, and assessed the central effects of IL-6 during seizures induced by inhibition of the central GABAergic system. Our data show that intranasally administered hIL-6 (400 ng) leads to a rise in IL-6 levels in the frontal lobe and increases the susceptibility of rats to PTZ-induced seizures. IL-6 affected predominantly the more severe stages of seizures (Table 1), showing the pro-convulsant profile which is clinically relevant and may be of interest for potential application. Importantly, since behavioural activation and epilepsy are based on the same arousal-related neural pathways, the pro-convulsant profile of IL-6 in our study is consistent with earlier data on the general pro-activatory action of IL-6 on behaviour in rats [35–37]. The results of our studies also confirm recent data showing increased sensitivity to glutamatergic-induced seizures in mice with elevated IL-6 levels in the brain [27]. Taken together, these data and those reported here prove that IL-6 has a marked pro-convulsant effect in several experimental models of epilepsy, and may therefore act in the brain as a pro-convulsant cytokine. These findings may point to a new area for the search of novel anti-epileptic drugs based on targeting the central IL-6 system.
D. Lawrence, Intranasal delivery could be used to administer drugs


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