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Seizure susceptibility to various convulsant stimuli of knockout interleukin-6 mice

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Abstract

In the present study, the susceptibility of knockout interleukin-6 (IL-6$^{-/-}$) mice to various convulsant stimuli has been evaluated and compared with other three related mice strains. Animals were treated with chemical convulsants impairing the γ-aminobutyric acid neurotransmission [pentylenetetrazole (PTZ), picrotoxin, bicuculline, methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate (DMCM), methyl-β-carboline-3-carboxylate (β-CCM)], enhancing glutamatergic neurotransmission [*N*-methyl-D-aspartate (NMDA), α-amino-3hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid (KA)] or a K^+ channel blocker [4-aminopyridine (4-AP)].

The behavioural changes of such convulsant stimuli on IL-6$^{-/-}$ were observed and compared with those observed in C57, IL-6$^{+/+}$ and DBA/2 mice. The occurrence of clonic and/or tonic seizures was scored and statistically analysed to observe possible differences on seizure susceptibility.

The IL-6$^{-/-}$ mice exhibited significantly higher seizure susceptibility to PTZ, β-CCM, DMCM, NMDA, AMPA and KA than did the other mice strains, with the exception of DBA/2 mice.

This study demonstrates that IL-6$^{-/-}$ mice possess an increased susceptibility to some convulsant stimuli. In particular, the major convulsant effects produced by NMDA, AMPA and KA suggest that the excitatory amino acid system is more active in the central nervous system (CNS) of IL-6$^{-/-}$ mice. The present data suggest that IL-6$^{-/-}$ mice might be a valid novel epileptic model for the study of pathophysiology and pharmacology of epileptic seizures.

Keywords: Interleukin-6; Epilepsy; Seizures; Convulsants; γ-Aminobutyric acid; Excitatory amino acid

1. Introduction

Interleukins belong to the heterogeneous family of cytokines and have been associated with inflammatory responses and the activation of the immune system. More recently, they have been correlated with diverse action on the peripheral and central nervous system (CNS; Hopkins and Rothwell, 1995). Various studies demonstrated that some interleukins are elevated in the cerebrospinal fluid of patients with epileptic seizures (Gidal et al., 1996; Go and Nakamura, 2002; Ichiyama et al., 1998, 2000; Kimura et al., 2002; Peltola et al., 1998, 2000, 2002; Straussberg et al., 2001; Virta et al., 2002).

Interleukin 6 (IL-6) is a multifunctional cytokine that plays a central role in inflammatory responses and in the regulation of cells of the haematopoietic system (Akira et al., 1993). A number of studies have also provided evidence for the expression and action of IL-6 in the nervous system. IL-6 and IL-6 receptor (IL-6R) mRNAs have been detected in some regions of the rat CNS, where both genes are developmentally regulated and localized in specific neuronal subpopulations (Gadient and Otten, 1993, 1994; Schobitz et al., 1993). IL-6 has also been implicated in the regulation of neuronal survival (Hama et al., 1991; Yamada and Hatanaka, 1994; Akaneya et al., 1995) and in the response of the CNS to trauma, inflammation and degenerative disease (Hirohata and Miyamoto, 1990; Blum-Degen et al., 1995; Gijbels et al., 1995; Chai et al., 1996). Other reports suggested that IL-6 appears to be involved in

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excitotoxicity-induced brain damage (Ali et al., 2000; De Bock et al., 1996; Gadient and Otten, 1997; Higuchi et al., 1994; Hopkins and Rothwell, 1995; Minami et al., 1991). In addition, in vitro studies showed that IL-6 can significantly protect against glutamate- and NMDA-induced excitotoxicity (Ali et al., 2000; Carlson et al., 1999; Toulmond et al., 1992; Yamada and Hatanaka, 1994).

In the mouse, a number of single-gene mutations, which cause epilepsy, have been identified (Noebels, 1984; Seyfried and Glaser, 1985; Felix, 2002), and it is hoped that the identification and study of these genes will provide insights into human epilepsy. The laboratory-selected IL-6-deficient IL-$6^{-/-}$ mice may represent an interesting animal model for investigating the role of IL-6 in both the physiological and pathological processes of epilepsy, and the seizure susceptibility of this strain of mice can be used for analysis of the role of IL-6 in ictogenesis.

In a previous study, we observed that IL-$6^{-/-}$ possesses a marked susceptibility to audiogenic seizures, and this seems to be correlated with a decrease of GABA brain levels (data not published). The present work was directed toward the characterization of the neuropharmacological basis of epileptic excitability in IL-$6^{-/-}$ mice. This was accomplished by the determination of convulsive dose 50s (CD_{50}s) of convulsant drugs with various known mechanisms of action. The convulsant compounds used were antagonists of the GABA–benzodiazepine inhibitory–Cl^- ionophore receptor complex [picrotoxin, bicuculline, pentylenetetrazole (PTZ), methyl-6,7-dimethoxy-4-ethyl-β-3-carboline-3-carboxylate (DMCM) and methyl-β-carboline-3-carboxylate (β-CCM)], selective agonists of ionotropic glutamate receptors [*N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainic acid (KA)], and a K^+ channel blocker, 4-aminopyridine (4-AP).

The convulsant phenomena observed in IL-$6^{-/-}$ mice were correlated with those of C57BL6J nonepileptic mice and with those of audiogenic seizure-sensible DBA/2 mice. These strains of mice were chosen because the chimaeras were obtained from two cell lines, which transmitted the mutation to their progeny derived from the C57BL6J/DBA/2 (B6/D2) hybrid (Poli et al., 1994). The convulsant phenomena observed in IL-$6^{-/-}$ mice were also correlated with those of the IL-$6^{+/+}$ mice. The latter strain of mice was chosen as it represents the normal littermate (Poli et al., 1994).

2. Materials and methods

2.1. Animals

DBA/2 and C57BL/6J mice were purchased from Charles River (Calco, Como, Italy). IL-$6^{+/+}$ and IL-$6^{-/-}$ mice were originally obtained from IRBM P. Angeletti (Pomezia, Italy) and then maintained at the Institute of Pharmacology, University of Messina (Italy). The animals were maintained under environmentally controlled conditions (7 a.m.–7 p.m. light–dark cycle, 22–24 °C, with food and water available ad libitum). The mice used in the present study were from 63 to 80 days old and weighed 24–30 g, either sex. Procedures involving the animals and their care were conducted in conformity with national and international laws and policies [EEC Council Directive of 24 November 1986 (86/609EEC)]. All animal experiments were carried out according to the *NIH Animal Care Guidelines* (NIH Publication Number 80-23). All efforts were made to minimize animal suffering and to use only the number of animals necessary to produce reliable scientific data.

2.2. Seizures induced by chemical convulsants

2.2.1. Experimental design

Seizures were induced by intraperitoneal administration of picrotoxin, PTZ, DMCM and β-CCM, by subcutaneous administration of bicuculline and 4-AP or by intracerebroventricular injection of NMDA, AMPA or KA. For the intracerebroventricular injections, mice were anesthetized with fluothane, and injections were made in the left or right lateral ventricle (coordinates 1 mm posterior and 1 mm lateral to the bregma; depth 2.4 mm) using a 5-μl Hamilton microsyringe, fitted with a nylon cuff on the needle, as previously described; injections of drugs by this procedure led to an uniform distribution throughout the ventricular system within 10 min (De Sarro et al., 1988).

The animals were then placed singly in a 30 × 30 × 30 cm box, and the observation time was 30 min after the administration of all convulsant drugs, except for 4-AP, which was kept under observation for 60 min.

2.2.2. Picrotoxin-, bicuculline- and PTZ-induced seizures

All animals were treated with various doses of picrotoxin (0.5–4 mg/kg), bicuculline (0.5–4 mg/kg) or PTZ (30–90 mg/kg). The doses of these drugs were injected in a volume of 0.1 ml/10 g of body weight of the mouse. The animals were then placed in isolated cages and observed 30 min after the administration of all drugs. A threshold convulsion has been considered as an episode of clonic spasms lasting for at least 5 s. The absence of this threshold convulsion over 30 min indicates that the animal was protected from the convulsant-induced seizures (Swinyard and Woodhead, 1982).

2.2.3. β-Carboline-induced seizures

Seizures were induced in adult mice of all strains by the intraperitoneal injection of DMCM (0.5–8 mg/kg) or β-CCM (0.5–12 mg/kg), dissolved in a minimal amount (< 5% of final volume) of glacial acetic acid, and brought to volume with saline. The final solution showed a pH level of 5–5.5. At least 10 mice were used for each dose of each compound studied. The mice were observed 30 min for the incidence of clonic seizures.

2.2.4. Glutamate receptor agonists-induced seizures

Seizures were induced by intracerebroventricular administration of various doses of NMDA (0.5–10 nmol/mouse), AMPA (0.5–10 nmol/mouse) or kainate (KA; 0.01–0.5 nmol/mouse). The mice were administered using a 5-μl Hamilton microsyringe, as described in the Experimental design. The animals were then placed in isolated cages and were observed for 30 min after the administration of each convulsant drug.

2.2.5. 4-AP-induced seizures

The K^+ channel blocker 4-AP was administered subcutaneously to all animals, at doses between 1 and 14 mg/kg, then placed singly in a box and observed for 60 min. The occurrences of clonic and tonic seizure signs were recorded.

2.3. Statistical analysis

Statistical comparison between groups of IL-$6^{-/-}$, C57, IL-$6^{+/+}$ and DBA/2 mice was made using Fisher's Exact Probability Test (incidence of the seizure phases, data not shown in tables). The percentages of animals exhibiting clonic or tonic seizures following various convulsants were plotted against the corresponding doses by a computer construction of the dose–effect curves for calculation of CD_{50} ($\pm$ 95% confidence limits). The CD_{50} values for each compound were calculated using a computer programme of the method of Litchfield and Wilcoxon (1949). At least 32 animals were used to calculate each CD_{50} value.

2.4. Drugs

NMDA, AMPA and KA were purchased from Tocris (Buckhurst Hill, UK) and picrotoxin, bicuculline, PTZ and 4-AP were from Sigma (St. Louis, MO, USA). β-CCM and DMCM were kindly supplied by Schering (Berlin, Germany). For systemic injections, all compounds were given intraperitoneally (0.1 ml/10 g body weight of the mouse) as a freshly prepared solution sterile saline (0.9% NaCl).

All drugs administered intracerebroventricularly were dissolved in sodium phosphate buffer 67 mM, microinjected in a volume of 5 μl/mouse, with the exception of β-CCM and DMCM, which were dissolved in a minimal amount (< 5% of final volume) of glacial acetic acid, and brought to volume with saline. The final solution showed a pH of 5–5.5. The necessary pH was adjusted to 7.3–7.4 by adding 0.2 N HCl or 0.1 NaOH. Due to their light sensitivity, some compounds were weighed and dissolved in semidarkness and were stored in containers wrapped in silver foil to exclude light. Exposure to ambient light during the experiments with these drugs was also minimized.

3. Results

3.1. Involvement of $GABA_A$ receptors

IL-$6^{-/-}$ mice, in general, showed to be more sensible to seizures induced by chemoconvulsants impairing the $GABA_A$ neurotransmission than C57 and IL-$6^{+/+}$ mice did, but not the DBA/2 mice. In particular, the CD_{50}s obtained for IL-$6^{-/-}$ mice were always significantly different from the CD_{50}s obtained for C57 and IL-$6^{+/+}$ mice, except for the convulsion induced by the injection of picrotoxin and bicuculline, even if a slight reduction is noticeable. The only significant difference between the IL-$6^{-/-}$ and DBA/2 mice was encountered for the CD_{50}s obtained after the administration of β-CCM and DMCM (Table 1). In particular, DBA/2 mice were more sensible to β-carbolines, which is in accordance with previously reported data (Chapman et al., 1987).

3.2. Involvement of NMDA receptors

NMDA by itself (0.5–10 nmol/mouse icv) induced generalized seizures in all strains of mice. In particular, tremor and head bobbing, hypermotility, jumping and circling preceded the first clonic episode, which consisted of wild running, jumping and loss of righting. The tonic component of the seizure occurred at the highest dose tested and was occasionally followed by death. The different seizure susceptibility of various strains of mice to NMDA

Table 1
Seizure susceptibility to convulsant drugs acting on the γ-aminobutyric acid–benzodiazepine receptor complex system of IL-$6^{+/+}$, C57, IL-$6^{-/-}$ and DBA/2 mice

Mouse strain	IL-$6^{+/+}$	C57	IL-$6^{-/-}$	DBA/2
Convulsant	CD_{50} values (±95% confidence limits)			
Picrotoxin	2.6 (2.39–2.83)	2.6 (2.12–3.19)	2.2 (1.95–2.51)	2.3 (1.86–2.84)
Bicuculline	2.5 (2.10–2.98)	2.2 (1.9–2.5)	1.83 (1.52–2.20)	1.95 (1.59–2.39)
Pentylenetetrazole	48.5 (40.3–58.3)*	59.13 (45.45–76.93)**	36.8 (32.0–42.2)	38.5 (33.3–44.5)
β-CCM	3.5 (3.2–3.9)**	8.55 (6.07–12.04)**	1.6 (1.3–1.9)	0.76 (0.47–1.13)*
DMCM	3.73 (2.63–5.29)**	3.87 (2.76–5.46)**	2.4 (2.2–2.6)	1.31 (1.18–1.45)*

All data reported in the present table are expressed as mg/kg ip.

* Significant differences between IL-$6^{-/-}$ and the other strains of mice, according to the method of Litchfield and Wilcoxon (1949); $P<.05$.

** Significant differences between IL-$6^{-/-}$ and the other strains of mice, according to the method of Litchfield and Wilcoxon (1949); $P<.01$.

Table 2
Seizure susceptibility to convulsant drug acting on the excitatory amino acid system and 4-AP of IL-$6^{+/+}$, C57, IL-$6^{-/-}$ and DBA/2 mice

Mouse strain	IL-$6^{+/+}$	C57	IL-$6^{-/-}$	DBA/2
Convulsant	CD_{50} values (±95% confidence limits)			
NMDA	1.98 (1.31–3.01)**	2.11 (1.76–2.53)**	0.87 (0.74–1.02)	1.92 (1.25–2.96)**
RS-AMPA	2.24 (1.31–3.83)**	2.41 (1.56–3.74)**	1.03 (0.74–1.43)	2.21 (1.27–3.84)**
Kainic acid	0.048 (0.037–0.062)**	0.047 (0.038–0.057)**	0.030 (0.021–0.043)	0.032 (0.017–0.060)
4-AP	8.83 (6.91–11.28)	8.61 (7.16–10.35)	8.6 (6.8–10.8)	8.11 (6.8–9.65)

All data reported in the present table are expressed as nmol/mouse, with the exception of 4-AP, which is expressed as mg/kg.
** Significant differences between IL-$6^{-/-}$ and the other strains of mice, according to the method of Litchfield and Wilcoxon (1949); $P<.01$.

injection is reported in Table 2. The IL-$6^{-/-}$ mice strain appears to be more sensible to NMDA-induced seizures than the other strains of mice.

3.3. Involvement of AMPA/KA receptors

Injections of AMPA (0.5–10 nmol/mouse icv), an agonist at AMPA/KA receptors, induced generalized seizures, similar with those caused by NMDA administration. Its latency was 1–3 min after the treatment, with 5, 8 and 10 nmol, and it was longer for AMPA at 1 and 3 nmol (up to 5 min). Mice treated with the latter doses showed the characteristic limbic seizures (rearing, jerking, falling down and forelimb clonus), and 9 out of 10 animals died following tonic extension. Similar convulsant effects were observed after intracerebroventricular injection of KA (0.01–0.5 nmol/mouse). The different seizure susceptibility to AMPA or KA of the various strains of mice injection is reported in Table 2. The IL-$6^{-/-}$ mice appear to be more sensible to AMPA and KA than the other strains of mice were, with the exception of DBA/2 mice.

3.4. Involvement of K^+ conductances

4-AP (6–14 mg/kg sc) induced generalized seizures in all strains of mice. Animals showing tonic extension or death were scored as nonprotected, according to Yamaguchi and Rogawski (1992). No significant differences in seizure incidence or latency of clonus, tonus and death were observed among the strains of mice studied when seizures were induced by 4-AP (Table 2).

4. Discussion

The CD_{50} values for PTZ, DMCM and β-CCM were significantly lower in IL-$6^{-/-}$ than in C57 or IL-$6^{+/+}$ mice, but not between IL-$6^{-/-}$ and DBA/2 mice, where the latter strain was more sensible to β-carbolines. Indeed, significant differences between IL-$6^{-/-}$, IL-$6^{+/+}$ and C57 mice were observed when we compared the CD_{50} values calculated following the intracerebroventricular administration of NMDA, AMPA and KA.

The present results clearly show that the absence of IL-6 is responsible for a higher seizure susceptibility to some chemoconvulsant agents.

4.1. γ-Aminobutyric acid–benzodiazepine receptor complex system

$GABA_A$ receptors are the most important inhibitory receptors of the CNS. They are constituted of five subunits, which express binding sites that can modulate the Cl-induced conductance. Numerous studies have shown that an insufficient synaptic inhibition by GABA on $GABA_A$ receptors may generate and/or contribute to the propagation of seizures.

Up to date, data regarding a possible variation in the expression of the subunits of $GABA_A$ receptor in IL-$6^{-/-}$ mice are not present. Picrotoxin, bicuculline, PTZ, β-CCM and DMCM act at different sites of GABA–benzodiazepine–Cl^- ionophore receptor complex.

A number of evidence of a role for GABA in audiogenic seizure susceptibility of DBA/2 mice has been previously provided (Chapman et al., 1987; Engstrom and Woodbury, 1988), and, recently, De Luca et al. (data not published) showed that IL-$6^{-/-}$ mice have a deficiency on GABA neurotransmission, which might be responsible for the audiogenic seizure susceptibility of this strain. Furthermore, DMCM and β-CCM were more potent as convulsant agents in DBA/2 mice than the other strains of mice, and this is likely due to the altered GABA–benzodiazepine receptor complex in such a seizure-susceptible strain of mice (see Chapman et al., 1987).

The effects produced by the convulsant agents used in this study suggest that GABA–benzodiazepine inhibitory inputs are impaired in the CNS of IL-$6^{-/-}$ mice. IL-$6^{-/-}$ mice showed an intermediate sensitivity to β-carbolines between DBA/2 mice and the other two strains of mice studied. Furthermore, IL-$6^{-/-}$ and DBA/2 mice showed a higher sensitivity to PTZ-induced seizures than did the other strains of mice used in the present study, whereas there was no difference for picrotoxin- and bicuculline-induced seizures. The similarity between the CD_{50} values of IL-$6^{-/-}$ and those of DBA/2 suggests that decreased GABA–benzodiazepine-mediated inhibition may have a role in seizure susceptibility in both strains of mice.

In conclusion, we suggest that IL-6 may be important for the expression and/or combination of some specific subunits in the constitution of the GABA–benzodiazepine receptor complex, and this could explain the variability in the response to the different chemoconvulsants acting on the same receptor complex.

4.2. Excitatory amino acid system

NMDA, AMPA and KA act at different excitatory amino acid ionotropic receptors. The AMPA/KA receptors are highly concentrated in the hippocampus and play a critical role in mediating signals from the dentate granule cells to the CA3 pyramidal neurones via mossy fibers (see Takumi et al., 1999). The increased sensitivity of IL-6$^{-/-}$ mice to AMPA-, KA- and NMDA-induced seizures may be partly due to an enhanced neurotransmission via glutamate receptors. The major convulsant effects produced by these agents, in comparison with all strains of mice studied, suggest that excitatory amino acid inputs are more active in the CNS of IL-6$^{-/-}$ mice. These data also suggest that the excessive excitatory amino-acid-mediated synaptic driving may lead to a hyperexcitable condition, which is responsible for the epileptic manifestations occurring in the IL-6$^{-/-}$ mice. Although differences in drug metabolism and pharmacokinetics between strains have been previously described following intraperitoneal administration, possible pharmacokinetic differences in drug distribution were not taken in account for the increased convulsant sensitivity of IL-6$^{-/-}$ and DBA/2 mice to excitatory amino acid agonists because the present results were obtained following intracerebroventricular injections.

4.3. Involvement of K^+ conductance

No significant differences in CD_{50} values between IL-6$^{-/-}$ mice and other strains were found. The present data are in agreement with those recently described by our group in the maximal electroshock test (De Luca et al., data not published) and suggest that the voltage-dependent ionic mechanisms are not significantly affected in the IL-6$^{-/-}$ mice.

Additional studies could be done in IL-6$^{-/-}$ mice to better characterize whether the seizure susceptibility observed in IL-6$^{-/-}$ depends on similar mechanisms already described in DBA/2 mice.

In conclusion, our findings indicate that IL-6-deficient mice show a range of functional neurological abnormalities. In particular, a major seizure susceptibility was found to excitatory amino acids and β-carbolines.

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