G.	Giovambattista	De Sarro ^a
D.	Domenicantonio	Rotiroti ^a
M.G.	Maria Giulia	Audino ^a
S.	Santo	Gratteri ^a
G.	Giuseppe	Nisticó ^b

^a Chair of Pharmacology, Department of Experimental and Clinical Medicine, Faculty of Medicine, University of Reggio Calabria, Catanzaro,

^b Chair of Pharmacology, Department of Biology, University of Roma 'Tor Vergata', Rome, Italy

Effects of Interleukin-2 on Various Models of Experimental Epilepsy in DBA/2 Mice

Keywords

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Abstract

The effects of interleukin-2 (IL-2) on various models of experimental epilepsy were studied after intracerebroventricular administration in DBA/2 mice, a strain genetically susceptible to sound-induced seizures. Convulsions were induced by physical stimulus (sound of 109 dB, 12–16 kHz) or by chemical compounds (bicuculline, cephazolin or kainate). The present study demonstrated that human recombinant IL-2 (hr-IL-2) and mouse recombinant IL-2 (mr-IL-2) not only did not antagonize audiogenic seizures in DBA/2 mice but increased the incidence of seizures after the highest doses studied. In addition, hr-IL-2 and mr-IL-2 dose dependently facilitated sound-induced seizures at subthreshold sound exposure (83 dB). Pretreatment with monoclonal rat-anti-mouse IL-2 antibodies significantly affected the changes of occurrence of audiogenic seizures in DBA/2 mice induced by mr-IL-2. In addition, pretreatment with anti-IL-2 receptor monoclonal antibodies (anti-Tac) was able to completely antagonize or reduce the effects of IL-2 on audiogenic seizures. The effects of mr-IL-2 were also studied in two different models of epilepsy: the bicuculline and cephazolin models, due to impairment of GABAergic transmission, and the kainate model, due to an increase in excitatory amino acid transmission. In all models, mr-IL-2 demonstrated to facilitate the seizures induced by these chemoconvulsants. Since the proconvulsant properties of IL-2 were antagonized by specific monoclonal antibodies, we suggest that some epileptic phenomena may be linked to stimulation of IL-2 receptors. Further experiments will be necessary in order to ascertain whether IL-2 acts via the potentialization of the effects of excitatory amino acids or via inhibition of GABA activity, or both in the brain.

Introduction

Interleukin-2 (IL-2) is a small single-chain soluble protein (M_r 15,500). The structure of IL-2 has been characterized by means of X-ray crystallography; the 133 amino

acids in the protein backbone of the molecule appear to be coiled and folded into a roughly spheric shape [1,2]. The structure, organization and amino acid sequence of IL-2 receptors have been characterized on the membrane of T lymphocytes [3,4]. IL-2 enhances T-lymphocyte prolifer-

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Prof. Giovambattista De Sarro, MD

Chair of Pharmacology, Department of Experimental and

Clinical Medicine, Faculty of Medicine

Policiinico Mater Domini, Via T. Campanella

I-88100 Catanzaro (Italy)

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ation after antigenic stimulation [5] accelerates differentiation of primary rat oligodendrocytes, and $IL-2$ may be released from activated glial cells [6,7]. There is also some evidence suggesting the existence of specific binding sites for IL-2 in the central nervous system (CNS) of the rodent brain; higher densities being observed in the hippocampus, median eminence, arcuate nucleus complex and lamina IV of the cerebral cortex [8,9].

In previous studies, it has been demonstrated that IL-2 is able to induce sleep and ECoG synchronization, particularly when administered into the locus ceruleus [10-12]. In addition, IL-2 has been shown to inhibit long-term potentiation in the rat hippocampus [13] and to alter electrical activity in the hippocampus and cerebral cortex [11]. These effects were considered specific since they were antagonized by specific monoclonal antibodies [11, 12]. Taken together these data suggest that IL-2 might play a neuromodulatory role in the CNS.

The genetically epilepsy-prone mouse DBA/2J (DBA/2) mouse has an age-dependent susceptibility to audiogenic seizures (AGSs). In fact, between 21 and 26 days of its life, this mouse has generalized clonic-tonic convulsions in response to a loud tone and has been reported to have increased seizure susceptibility to a variety of nonaudio-genic convulsant treatments including chemical and physical stimuli [14-16]. In addition, at 8 weeks, when DBA/2 mice are not susceptible to AGSs, they show a major hyperexcitability to electrically induced convulsions even after developing resistance to sound-induced seizures [14, 16]. The strain DBA/2 mice has been considered an excellent animal model for the study of certain kinds of human epilepsy and for testing new anticonvulsant drugs [15, 17].

The aim of the present study was to investigate the effects of $IL-2$ given intracerebroventricularly (i.c.v.) on AGSs in DBA/2 mice. In addition, the effects of H^2 on bicuculline- and cephazolin-induced seizures as well as on kainate-induced seizures in DBA/2 mice prone to AGSs were examined. Previously, it has been reported that cephazolin given systemically or into some areas of the brain produces epileptogenic effects which are due to impairment of GABAergic transmission [18-21] and that kainic acid given systemically produces epileptogenic limbic seizures [22]. Cephazolin and bicuculline are two chemconvulsants which act as GABA antagonists [23, 24] whilst kainic acid is a glutamate analogue which acts principally at specific kainate/quisqualate receptors [25].

Materials and Methods

Animals and Audiogenic Seizures

DBA/2 mice (6–12 g, 21–26 days old) were purchased from Charles River (Calco, Como, Italy) and were exposed to auditory stimulation, 30 min following i.c.v. administration of vehicle or drugs. IL-2 was given i.c.v. as a freshly prepared solution in 67 mM sodium phosphate buffer containing 5 mg of serum albumin/1 ml. Monoclonal rat anti-mouse IL-2 antibodies and anti-IL-2 monoclonal receptor antibodies were dissolved in sodium phosphate buffer 67 mM and administered 10 min before $IL-2$. In order to examine inhibition or facilitation of AGSs, the mice were exposed to a 12- to 16-kHz sinusoidal tone at either 109 dB (convulsive level) or 83 dB (subconvulsive level) in a hemispheric Plexiglas dome (58 cm diameter). Seizure response (SR), as previously reported [26], was assessed on the following scale: 0 = no response; 1 = wild running; 2 = clonus; 3 = tonus; 4 = respiratory arrest. The maximum response was recorded for each animal.

Rectal temperature was recorded immediately prior to auditory testing using an Elek-trolaboratoriet thermometer type TE 3. Behavioral changes were observed during the period between drug administration and auditory testing.

Testing of Anticonvulsant Activity Against Chemoconvulsants DBA/2 mice (6-12 g, 21-26 days old or 16-24 g, 42-48 days old) were purchased from Charles River (Calco, Como, Italy). Mice were injected intraperitoneally (i.p.) with cephazolin (0.1-2.0 g/kg) dissolved in sterile saline or bicuculline methiodide subcutaneously (s.c.) or i.c.v. dissolved in a minimal quantity 0.2 N HCl and adjusted to pH 5.3 by adding 0.2 N NaOH and then saline. Kainic acid was dissolved in sodium phosphate buffer. Animals were placed in a Plexiglas box (40 x 40 x 30 cm) and observed for 240 min after i.p. or s.c. administration or 120 min after i.c.v. injection of chemoconvulsants. For systemic injections, all compounds were administered with 0.1 ml/10 g body weight of the mouse. For each dose of cephazolin or bicuculline, 10 animals were used. The intensity of SR was scored on the following scale: 0 = no response, 1 = wild running, 2 = clonus, 3 = tonus, 4 = respiratory arrest, which is identical to that observed after auditory stimulation [26]. For each dose of kainate, 8-10 animals were used. A second group of animals was treated i.c.v. with IL-2 at a dose of 1.35, 2.7 pmol/mouse or vehicle and 15 min later the same animals were injected i.p. with various doses of cephazolin, kainate or bicuculline. At the dose of 1.35 pmol injected i.c.v., each IL-2 was unable to elicit seizures in DBA/2 mice and elicited a mild decrease in spontaneous activity and explorative behavior.

ECoG Analysis

ECoG activity was recorded (8-channel ECoG machine OTE Biomedica, Florence, Italy) through 4 chronically implanted steel screw electrodes, inserted bilaterally into the fronto-parietal area. The ground electrode was implanted epidurally over nasal bone; at least 3 mice, treated with the largest dose of vehicle + cephazolin, IL-2 + cephazolin, vehicle + bicuculline, or IL-2 + bicuculline, were studied for changes in ECoG activity.

Statistical Analysis

Statistical comparisons among groups of control and drug-treated animals were made using Fisher's exact probability test (incidence of the seizure phases) or ANOVA and Dunnett's t test (rectal tempera-

362

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Proconvulsant Effects of IL-2

Table 1. Proconvulsant effects following the i.c.v. administration of hr-IL-2 and Tai-Rr2 in DBA/2 mice

Drug	Dose pmol	Response, %					Temp., °C mean ± SEM	n
		WR	clonus	tonus	RA	SR		
Vehicle		100	100	100	60	3.6	38.1±0.2	10
hr-IL-2	0.14	100	100	80	50	3.3	37.9±0.2	10
	0.27	100	100	80	60	3.4	38.0±0.2	10
	1.35	100	100	90	50	3.4	38.1±0.2	10
	2.7	100	100	100	60	3.6	38.2±0.3	10
	13.5	100	100	100	100	4	38.1±0.2	14
Vehicle		100	100	100	50	3.5	37.9±0.2	10
mr-IL-2	0.14	100	100	80	40	3.2	37.7±0.2	10
	0.27	100	100	70	50	3.2	37.7±0.2	10
	1.35	100	100	100	40	3.4	37.8±0.2	10
	2.7	100	4JU&	100	70	3.7	37.6±0.2	10

13.5	100	amp;P 100	100	100 0.4	37.7±0.2	16
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Groups of DBA/2 mice were injected i.c.v. with the stated doses of the drugs or vehicle and exposed to auditory stimulation 30 min after drug injection. The incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. WR = Wild running; RA = respiratory arrest; SR = the arithmetic mean of the maximal individual responses for each animal in the group; Temp. = rectal temperature measured immediately before auditory stimulation; n = number of animals used.

tures). The percentage incidence of each phase of the AGS was determined for each dose of compound administered and long dose-response curves were fitted using linear regression analysis of probits. ED₅₀ values (with 95% confidence limits) for each compound and each phase of SR were estimated using the method of probit analysis [27]; the relative convulsant activities were also determined by comparison of respective ED₅₀ values.

Drugs

The sources of the drugs used were: recombinant human interleukin-2 (hr-IL-2), recombinant mouse interleukin-2 (mr-IL-2), kainic acid and bicuculline methiodide were purchased from Sigma (St. Louis, Mo., USA), anti-IL-2 receptor monoclonal antibodies (anti-Tac) and monoclonal rat anti-mouse IL-2 antibodies (mo-Abs-H²) were purchased from Genzyme (Cambridge, Mass., USA), while cephazolin was obtained from Carlo Erba Farmitalia (Milan, Italy). mr-IL-2 was also denaturated at 70 °C for 30 min and used as control.

For i.c.v. injections, mice were anesthetized with ether 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 10- μ l Hamilton microsyringe (type 701N) fitted with a Nylon cuff on the needle, as previously described [28]. Injections of the drugs by this procedure led to a uniform distribution throughout the ventricular system within 10 min [28].

Results

Effects of IL-2 on Audiogenic Seizures in DBA/2 Mice hr-H² (0.14, 0.27, 1.35, 2.7 and 13.5 pmol/mouse), mr-IL-2 (0.14, 0.27, 1.35, 2.7 and 13.5 pmol/mouse) administered i.c.v. did not decrease the severity of the AGSs in DBA/2 mice (table 1) but, at the highest doses studied, increased the severity of seizures. In particular, after a latency period of 7-10 min at the doses of 2.7 and 13.5 pmol injected i.c.v., mice showed jumping, increase in locomotor activity, marked tremor, clonus of forelimbs and hindlimbs. Tonic convulsions were observed in 4 out of 14 and 6 out of 16 animals treated with 13.5 pmol of hr-IL-2 and mr-IL-2, respectively. Tonic extension followed by respiratory arrest and death of the animal was seen in 2 out of 14 and 4 out of 16 mice treated with 13.5 pmol of hr-IL-2 and mr-IL-2, respectively.

In previous studies, we demonstrated that a pretreatment (15 min before) with mo-Abs-H² was able to block the effects of H². This was a clear proof that mo-Abs-H² reached the H² receptors and were not degraded by proteases present at the surface of the brain or by peptidases present in the ventricular spaces. In addition, the antagonism with mo-Abs-IL-2 demonstrated that the IL-2 effects were directly mediated [29]. Mo-Abs-IL-2 (10, 50 and

Table 3. Effect of hr-E², mr-IL-2 and mo-Abs-IL-2 alone or in combined treatment in DBA/2 mice after a subconvulsant sound stimulus (83 dB)

Drug	Dose ng	Response, %					Temp., °C mean ± SEM	n
		WR	clonus	tonus	RA	SR		
Vehicle		100	100	100	50	3.5	37.9±0.3	10
mo-Abs-IL-2	10	100	100	100	40	3.4	38.0±0.2	10
	50	100	100	90	50	3.4	38.0 ± 0.3	10
	100	100	100	100	40	3.4	37.9 ± 0.4	10
	50							
mo-Abs-IL-2	50							
+ mr-IL-2	5	100	100	90	50	3.4	38.0±0.2	10
mo-Abs-IL-2	50							
+ mr-IL-2	10	100	100	100	60	3.6	38.0±0.3	10
mo-Abs-IL-2	50							
+ mr-IL-2	50	100	100	100	100	4.0	38.1 ± 0.3	10
Explanations and abbreviations		see footnote to table 1.						
Drug	Dose pmol	Response, %					Temp., °C mean ± SEM	n
		WR	clonus	tonus	RA	SR		
Vehicle		50	20	0	0	0.7	38.0±0.3	10
hr-IL-2	0.27	80	40	30	10	1.6	37.9±0.3	10
	1.35	100	60	50	30	2.4	37.9±0.2	10
	2.7	100	80	80	40	3.0	38.0±0.3	10
	13.5	100	100	100	40	3.4	38.1 ± 0.4	10
mr-IL-2	0.27	70	30	30	10	1.4	38.0±0.2	10
	1.35	100	60	50	30	2.4	37.9 ± 0.3	10
	2.7	100	100	80	50	3.3	37.9±0.3	10
	13.5	100	100	100	70	3.7	38.1 ± 0.3	10
mo-Abs-IL-2	10	50	20	0	0	0.7	38.0±0.2	10
	50	40	30	0	0	0.7	37.9±0.3	10
	100	40	20	0	0	0.6	37.9±0.4	10
mo-Abs-IL-2	100							
+ mr-IL-2	0.27	50	20	0	0	0.7	37.9 ± 0.2	10
mo-Abs-IL-2	100							
+ mr-IL-2	1.35	50	30	10	0	0.9	38.0±0.3	10
mo-Abs-IL-2	100							
+ mr-IL-2	2.7	80	40**	20**	0*	1.4	38.1 ± 0.2	10
mo-Abs-IL-2	100							
+ mr-IL-2	13.5	100	60	40**	20**	2.2	38.1 ± 0.3	10

Groups of DBA/2 mice were injected i.p. with the stated doses of the drugs or vehicle and exposed to auditory stimulation 40 min after mo-Abs-IL-2 injection (ng/mouse). The incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control (mr-IL-2) and drug-treated groups (mo-Abs-IL-2 + mr-IL-2) are denoted: * p < 0.05; ** p < 0.01. The abbreviations used in the headings are explained in the footnote of table 1.

Table 4. The effect of denaturated mr-IL-2 and anti-IL-2 receptor Abs-IL-2 (anti-Tac) alone or in combined treatment in DBA/2 mice after a subthreshold convulsant sound stimulus (83 dB)

Drug	Dose pmol	Response, %					Temp., °C mean ± SEM	n
		WR	clonus	tonus	RA	SR		
Vehicle		50	20	0	0	0.7	38.0±0.3	10
Den-mr-IL-2	0.27	50	20	0	0	0.7	37.9±0.2	10
	1.35	40	20	0	0	0.6	38.0±0.3	10
	2.7	50	20	0	0	0.7	37.8±0.3	10
	13.5	40	20	10	0	0.7	38.7±0.3	10
Anti-Tac	50	40	20	0	0	0.6	37.8±0.3	10
	100	40	20	0	0	0.6	37.8±0.4	10
Anti-Tac	100							
+ mr-IL-2	0.27	40	20	0	0	0.6	37.9±0.2	10
anti-Tac	100							
+ mr-IL-2	1.35	50	30	10	0	0.9	38.0±0.3	10
anti-Tac	100							
+ mr-IL-2	2.7	70	40**	20**	0*	1.3	38.0±0.2	10
Anti-Tac	100							
+ mr-IL-2	13.5	90	50	40**	20**	2.1	38.0±0.3	10

Groups of DBA/2 mice were injected i.c.v. with the stated doses of the drugs or vehicle and exposed to auditory stimulation 30 min after denaturated mr-IL-2 or 40 min after anti-Tac injection (ng/mouse). The incidence of each seizure phase is expressed as the percentage of mice in each group displaying that phase. Significant differences in the incidence of seizure phases between concurrent control (mr-IL-2, table 3) and drug-treated group (anti-Tac + mr-VU-2) are denoted: * p < 0.05; ** p < 0.01. Den-mr-IL-2 = denaturated mr-IL-2. The abbreviations used in the headings are explained in the footnote to table 1.

100 ng/mouse i.c.v.) did not significantly modify the occurrence of AGSs in DBA/2 mice (table 2). A pretreatment (10 min before) with mo-Abs-H² (50 and 100 ng/mouse) microinjected into a lateral ventricle did not significantly affect the incidence of the AGSs observed following the microinjection by the same route of mr-IL-2 (table 2). In addition, hr-IL-2 and mr-IL-2 facilitated dose-dependently sound-induced seizures at a subthreshold sound exposure (83 dB) with an ED₅₀ for tonus of 1.35 for both mr-IL-2 and hr-IL-2 (table 3). A treatment with denaturated mr-IL-2 did not significantly affect the incidence of the audiogenic seizures elicited by a subthreshold sound exposure (83 dB) (table 4). Mo-Abs-ILr2 (10, 50 and 100 ng/mouse, 5 µl i.c.v.) did not significantly affect the occurrence of AGSs in DBA/2 mice after exposure to a subthreshold stimulus of 83 dB. In addition, a pretreatment 10 min before with mo-Abs-IL2 (50 and 100 ng) microinjected into a lateral ventricle protected against the proconvulsant effects of mr-IL-2 observed with a subthreshold sound stimulus (83 dB) (table 3). A pretreatment, 10 min before, with anti-Tac (50 and 100 ng/mouse i.c.v.) was able to antagonize or to reduce the effects of mr-IL-2 on AGSs (table 4).

Effects of IL-2 on Kainate-Induced Seizures Intraperitoneal administration of kainic acid induced characteristic limbic seizures. A previous (15 min before) i.c.v. administration of mr-IL-2 (1.35 pmol/mouse) was able to significantly increase the incidence and severity of seizures induced by kainic acid in DBA/2 mice. In particular, the percentage of animals showing clonic seizures was significantly increased in animals receiving mr-IL-2 and kainate in comparison to the group treated with vehicle + kainate (table 5).

Effects of IL-2 on Bicuculline-Induced Seizures The convulsive response to i.c.v. administration of bicuculline in mice consisted of wild running, clonic seizures and loss of righting reflex immediately after the administration of high doses of the drug. The CD₅₀ value for producing clonic and tonic convulsions following i.c.v. administration of vehicle + bicuculline in mice were 80.2 (64.7-99.4) and 94.7 (85-105.9) ng/mouse, respectively (table 6). A pretreatment with mr-IL-2 (1.35 pmol/mouse i.c.v.) 15 min prior the microinjection by the same route of bicuculline induced an increase in seizure incidence

Table 5. Effects of i.c.v. mr-IL-2 administration on seizures induced by i.p. administration of kainate

Drug mg/kg	Dose	Clonus	Tonus	Death
Vehicle + kainate	10 15 20 30	0/8 2/10 8/8	0/8 2/10 4/10 7/8	0/8 2/10 4/10 6/8
mr-IL2 + kainate	5 10 15 20	0/8 6/8* 10/10* 10/10	0/8 2/8 7/10* 10/10*	0/8 0/8 4/10 6/10

Significant differences among concurrent control groups are expressed as * p < 0.05 using Fisher's test.

Table 6. Convulsant doses (CD50) values (with 95% confidence limits) of 50 i.c.v. or s.c. bicuculline administered after an intracerebroventricular pretreatment with vehicle or mr-IL-2

Treatment (route)	Clonic phase	Tonic phase
Vehicle + bicuculline (i.c.v.)	80.2 (64.7-99.4)	94.7 (85.1-105.9)
mr-IL2 + bicuculline (i.c.v.)	46.1 (34.6-61.3)*	55 (43.8-67.7)*
Vehicle + bicuculline (s.c.)	2.4 (2.11-2.74)	2.6 (2.39-2.83)
mr-IL2 + bicuculline (s.c.)	2.2 (1.95-2.51)	2.4 (2.20-2.70)

Significant differences between CD₅₀ values of concurrent groups treated with vehicle + bicuculline and mr-IL-2 + bicuculline are denoted: * p < 0.01. The data observed following i.c.v. injection are expressed as ng/mouse whilst those observed following s.c. administration are expressed as mg/kg.

Table 7. Convulsant doses (CD50) values (with 95% confidence limits) of intraperitoneal cephalozin after i.c.v. pretreatment with vehicle or mr-IL-2 in DBA/2 mice

Compounds	Clonic phase	Tonic phase
Vehicle + cephalozin	0.91 (0.72-1.15)	1.27 (1.03-1.55)
mr-IL-2 (1.35 pmol) + cephalozin	0.57 (0.34-0.94)*	0.86 (0.56-1.25)*
mr-IL-2 (2.7 pmol) + cephalozin	0.43 (0.23-0.66)*	0.69 (0.44-1.09)*

All data are expressed as g/kg and were calculated according to the method of probits [27]. Significant differences between CD₅₀ values of group treated with vehicle + cephalozin and groups treated with mr-IL-2 + cephalozin are denoted: * p < 0.01,

with a CD50 value for clonic convulsions in mice of 46.1 (34.6-61.3), whilst that for tonic convulsions was 55 (43.8-67.7) ng/mouse (table 6). In addition, s.c. administration of larger doses of bicuculline consistently led to clonitonic seizures in mice. The CD50 value for producing clonus following s.c. administration of vehicle + bicuculline was 2.4 (2.11-2.74) mg/kg, while that for triggering tonus was 2.6 (2.39-2.83) mg/kg (table 6). Furthermore systemic administration of bicuculline induced lethality with a LD₅₀ of 2.85 (2.56-3.18) mg/kg.

Pretreatment with mr-IL-2 (1.35 pmol/mouse i.c.v.) 15 min prior the s.c. injection of bicuculline induced an increase in seizure incidence in mice. In particular, the CD50 values of combined treatment of mr-IL-2 and bicuculline given systemically was 2.2 (1.95-2.51) mg/kg for clonus (table 6). In addition, the CD50 values of combined treatment of mr-IL-2 and bicuculline after systemic administration was 2.4 (2.2-2.7) mg/kg for tonus and 2.45 (2.2-2.7) mg/kg for the LD₅₀ (table 6).

Effects of IL-2 on Cephazolin-Induced Seizures The relative CD50 values (with 95% confidence limits) of vehicle + cephazolin and different doses of mr-IL-2 + cephazolin are reported in table 7. The CD50 for producing clonus was 0.91 (0.72-1.15) g/kg and for tonus was 1.27 (1.03-1.55) g/kg. The concentration of cephazolin which produced a higher incidence of seizures was lower prior pretreatment with mr-IL-2 (1.35 or 2.7 pmol/mouse i.c.v.) in comparison to mice receiving a pretreatment with vehicle. The percentage of DBA/2 mice showing seizures when pretreated (15 min before) with mr-IL-2 (1.35 pmol or 2.7 pmol/mouse i.c.v.) and then receiving cephazolin i.p. was significantly increased ($p < 0.05$) in comparison to the group of mice receiving vehicle (i.c.v.) and then cephazolin (table 7).

Electrocortical Activity in Mice

Electrocortical epileptic discharges appeared after a shorter latency period in mice pretreated with mr-IL-2 in comparison to those receiving vehicle + cephazolin or bicuculline (fig. 1, 2). In addition the animals pretreated with mr-IL-2 showed ECoG epileptic discharges of a longer duration than those treated with vehicle + cephazolin or bicuculline (data not shown). However, the ECoG epileptic features were similar in DBA/2 mice which received vehicle + bicuculline or mr-IL-2 + bicuculline and in animals which received vehicle + cephazolin or mr-IL-2 + cephazolin (fig. 1, 2).

Fig. 1. ECoG patterns after vehicle + cephazolin (0.6 g/kg, i.p.) and mr-IL-2 (1.35 pmol i.c.v.) + cephazolin (0.6 g/kg, i.p.) administration in DBA/2 mice. ECoG patterns from right (rCX) and left (lCX) frontoparietal cortex, illustrating the effects of either vehicle or mr-IL-2 on the ECoG activity induced by i.p. administration of a subconvulsant dose of cephazolin (0.6 g/kg). Vehicle or IL-2 (1.35 pmol) were injected i.c.v. (A and Al). ECoG recordings, 12 min after the injection of vehicle or IL-2 (B and Bl) recordings 12 min after the i.p. administration of cephazolin (0.6 g/kg). C, Cl, D, Dl: ECoG recordings 60 and 120 min after the administration of cephazolin.

Control rCX

B 12 min after cephalosporin rCX U^A***U^A.Xj.<<>>.UJu4JUvUUJaJU CX M^MMIUAMJiMUM^AOIUUA

C 60 min

rC
X
IC

D 120 min rCX /A^AV^{*}A^HUHUVWM-^Av^AV»v^{*} CX A^{*}Mt^{*}»A^Jvvnv^AA\^W^AvV⁻A^H

Control

A₁ 12 min after IL-2

Bi 12 min after cephalosin

d 60 min

AXWMkXAAA

1 s

Fig. 2. ECoG patterns after vehicle + bi-cuculline (1.8 mg/kg s.c.) and mr-IL-2 (1.35 pmol i.c.v.) + bicuculline (1.8 mg/kg s.c.) administration in DBA/2 mice. ECoG patterns from right (rCX) and left (lCX) frontoparietal cortex, illustrating the effects of combined treatment of either vehicle or mr-IL-2 administration on the ECoG activity induced by a s.c. administration of sub-convulsant dose of bicuculline (1.8 mg/kg). Vehicle or IL-2 (1.35 pmol i.c.v.) were injected i.c.v. (A and Al). ECoG recordings, 12 min after the injection of vehicle or IL-2. (B and Bl) EEG correlates recordings 12 min after the s.c. administration of bicuculline (1.8 mg/kg). C, Cl, D, Dl: ECoG recordings 60 and 120 min after the administration of bicuculline.

Control

A 12 min after vehicle rCX

The results of the present study demonstrate that IL-2 given into the cerebral ventricles possesses a proconvulsant activity. This was an unexpected effect since in previous experiments we have shown that IL-2 microinjected cerebrally possesses marked behavioral and ECoG sedative effects [11, 19, 29, 30]. However, large doses of IL-2 infused into the third cerebral ventricle or the dorsal hippocampus elicited electrical spiking activity associated with a frozen state or with wet dog shake episodes and rarely with clonic convulsions [11, 30]. These observations taken together with the present data suggest that IL-2 possesses different effects according to the dose and the site of microinjection, i.e. low doses are soporific and large doses are epileptogenic. The most sensitive area through which soporific effects were elicited was the locus ceruleus [11, 29, 30], whereas the most sensitive area for the epileptogenic properties of IL-2 was the hippocampus [30]. Some other peptides applied in the hippocampus induce seizures [31], whereas in other structures such as the locus ceruleus they are soporific [32]. The present study also confirms that the AGSs in DBA/2 mice represent a sensitive test for discriminating compounds with anticonvulsant or proconvulsant activity [16, 33]. Since the proconvulsant or convulsant properties of IL-2 were antagonized by mo-Abs-IL-2, it is possible to suppose that IL-2 acts directly through stimulation of specific receptors. The findings that anti-IL-2 receptor monoclonal antibodies antagonized or reduced the effects of IL-2 on audiogenic seizures, seem to further support the previous hypothesis. It is also likely that IL-2 may interact indirectly with some neurotransmitters, but at the moment data are not available. In previous experiments we have demonstrated that the monoclonal antibodies used were able to block the soporific effects elicited by the microinfusion of interleukins into the locus ceruleus [11, 12, 19, 29, 30], suggesting that this technical approach is quite correct to

block the effects of interleukins. Thus the findings of the effects of these antibodies tell us that some of epileptogenic effects of IL-2 are directly mediated. It is of interest that proconvulsant effects of IL-2 were obtained in two different models of epilepsy, the cephazolin and bicuculline model due to impairment of GABAergic transmission [19,24] and the kainate model, due to an increase in excitatory amino acid transmission [25]. In addition, the fact that the proconvulsant activity of mr-IL-2 was more evident when bicuculline was administered directly into the brain than when it was given peripherally clearly suggests the presence of important interactions between the two compounds. In fact, the potentiation of convulsant properties of bicuculline was more easily elicited when both agents were present principally at the liquor of the ventricular spaces. Whereas it was less evident when IL-2 was present prevalently at the liquor of the ventricular spaces and bicuculline, administered peripherally, was present in all brain. Furthermore, it appeared clear that the brain sites where IL-2 acts are close to ventricular spaces. Further experiments will be necessary in order to ascertain whether IL-2 preferentially acts potentiating the effects of excitatory amino acids or inhibiting GABAergic neurotransmission in the brain. The epileptogenic properties of IL-2 seem specifically linked to the stimulation of IL-2 receptors since the specific mo-Abs-ILr2 or anti-Tac were able to prevent them. This indicates that the mechanisms underlying the increased excitability are very likely linked to IL-2 receptors, however an interference with other neurotransmitters cannot be excluded.

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