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NALOXONE ANTAGONIZES BEHAVIOURAL AND ECoG EFFECTS INDUCED BY SYSTEMIC OR INTRACEREBRAL ADMINISTRATION OF SOME LYMPHOKINES

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Summary. - *Rat interferon, α-interferon, interleukin-2 and recombinant interleukin-2 microinjected into the third cerebral ventricle produced in rats typical behavioural sedation and/or sleep and ECoG synchronization while β-interferon produced no behavioural sleep and ECoG synchronization. A slight sedation was observed after highest dose of β-interferon only. During sleep induced by these lymphokines a dose-dependent increase in total voltage power as well as in the 0.5-3, 4-7 and 12-16 Hz frequency bands was observed. Much lower doses were required to produce similar behavioural and ECoG spectrum power effects after microinfusion of interferons and interleukin-2 into the* locus coeruleus. *No significant behavioural and ECoG changes were obtained after microinfusion of the same doses of interferons and interleukin-2 into other areas of the brain (*caudate nucleus, dorsal hippocampus, substantia nigra - pars compacta -, ventromedial hypothalamus). *The behavioural and ECoG effects of α-interferon, rat interferon and interleukin-2 were blocked in animals pretreated with naloxone. These results are consistent with the hypothesis that the behavioural and ECoG effects of these lymphokines are mediated at* locus coeruleus *level via a stimulation of opiate receptors.*

Riassunto (Effetti comportamentali e sullo spettro elettrocorticografico delle linfochine ed antagonismo da parte del naloxone). - *La microinfusione di interferone di ratto, α-interferone umano, interleuchina-2 ed interleuchina-2 ricombinante nel terzo ventricolo cerebrale ha prodotto nel ratto uno stato di sedazione comportamentale e/o sonno associato a sincronizzazione elettrocorticografica (ECoG). La somministrazione di β-interferone umano ha prodotto uno stato di lieve sedazione solo alla più alta dose-studiata. Durante lo stato soporifico indotto dalle linfochine è stato osservato un aumento dose-dipendente della potenza totale dello spettro ECoG e delle bande di frequenza comprese tra 0.5-3, 4-7 e 12-16 Hz. Simili effetti comportamentali e sulla potenza dello spettro ECoG sono stati osservati dopo la microinfusione di dosi molto più basse di interferoni e interleuchina-2 nel* locus coeruleus. *La microinfusione delle stesse dosi di interferoni ed interleuchina-2, attive a livello del* locus coeruleus, *a livello del nucleo caudato, ippocampo dorsale,* substantia nigra (pars compacta) *ed ipotalamo ventromediale non ha prodotto alcuna significativa modificazione di tipo soporifico riguardante il comportamento e/o lo spettro ECoG. Un pretrattamento con naloxone è stato in grado di antagonizzare gli effetti comportamenti ed ECoG prodotti dall'α-interferone, interferone di ratto ed interleukina-2. Nell'insieme questi dati fanno supporre che gli effetti comportamentali ed ECoG di tipo soporifico siano mediati a livello del* locus coeruleus *attraverso la stimolazione di recettori oppioidi.*

Introduction

Although the concept of a strict correlation between the central nervous system (CNS), the neuroendocrine and the immune system is quite old, only in recent years convincing evidence has heen accumulated on the occurrence of a complete regulatory loop between these systems. Neurons and certain cells of the immunologic system seem to share some neurotransmitters/neuromodulators, their receptors and hormones, thus making easier to decode the language of their communication [1-3].

Electrolytic lesions or stimulation of various brain structures influence immune responses either directly or through changes in hormone secretion [4-6]. In addition, it has been shown that during an immune challenge there is an exchange of messages between the brain and the circulating immune cells resulting in a modification of brain function, as shown by changes in the firing rate of hypothalamic neurons [7-9]. These findings support reciprocal communication between the brain and the immune system. In particular, cells of the immune system release lymphokines (e.g. interferons, interleukins) which, given systemically or directly applied into some neurons of

the brain, have been shown to affect the bioelectrical activity of the brain [10, 11]. The demonstration of the existence of lymphokines in ameboid microglia cells [12] strengthens the concept that immunologic mediators are able in some ways to exert some direct effects on the CNS.

Classical experiments have shown that recombinant leukocyte interferon given systemically produces an increase in the amplitude of the dominant frequency of the ECoG and irregular spiking [13]. In addition, the microiontophoretic application of α-interferon ((α-IFN) both human leukocyte and recombinant) was found to increase in a dose-dependent manner the firing rate of cortical and hippocampal neurons; on the contrary, γ-IFN as well as some fractions of γ-IFN did not alter the bioelectric activity of the same neurons [14].

Evidence exists also that interleukin-1 (IL-1) given systemically or into the cerebral ventricles in several animal species produces fever accompanied by slow-wave sleep [15-16], whereas recombinant interleukin-2 (rIL-2) does not seem to be pyrogenic in rabbits after intravenous administration [17]. However, in man both IFNs and IL-2 have been reported to share some common neurotoxic effects although with a different degreee of frequency, e.g. fatigue, weakness, lethargy and confusion [18].

It has been reported that human α-IFN, but not γ-IFN or β-IFN binds to opiate receptors and produces endorphin-like effects (analgesia and catatonia) reversed or prevented by naloxone, suggesting that some effects of α-IFN in the brain are due to stimulation of opioid receptors [19, 20]. In addition, α-IFN has been shown to suppress the naloxone precipitated withdrawal syndrome in morphine-dependent rats [21-24].

The present experiments were aimed to further characterize the behavioural and ECoG power spectrum effects of IFNs and IL-2. In particular, IFNs were given systemically, into the third cerebral ventricle or into several areas of the rat brain, whereas IL-2 was given directly into the brain. Thus the present experiments could give us further information on the central effects of the lymphokines studied, i.e. to analyse their effects on the ECoG activity continously computerized and quantified in the different bands of the spectral activity as well as to identify the site(s) through which their central effects could be mediated. In addition, a pretreatment with naloxone, an antagonist at opiate receptors could permit us to ascertain whether, similarly to IFNs, some central effects of IL-2 were mediated through endogenous opioid mechanisms.

Methods

Adult male Wistar rats (200-250 g) were purchased from Morini (San Polo d'Enza, Reggio Emilia) and maintained on a 12 h light-dark cycle (lights on 6.00-18.00 h, off 18.00-6.00 h). Animals were stereotaxically implanted with stainless steel guide cannulae under chloral hydrate anaesthesia (400 mg/kg i.p.) according to the atlas coordinates of Paxinos and Watson [22], to permit intracerebroventricular infusion (icv) or an unilateral or a bilateral microinjection into the *locus coeruleus* (LC) or into other brain areas, i.e. *caudate nucleus, dorsal hippocampus, substantia nigra, (pars compacta) ventromedial hypothalamus*. The steel guide cannulae were chronically implanted with the 2 mm away from each brain area studied. After surgery a minimum of 48 h was allowed for recovery before experiments were carried out. All experiments were performed beginning at approximately 10.00 h. Freely moving rats were microinjected via a injector cannula which extended approx. 2 mm below the tip of the guide cannula. *Post-mortem* histological examination confirmed the location of the guide cannulae.

Electrocortical (ECoG) activity was recorded (8 channel ECoG machine, OTE Biomedica, Florence) via 4 chronically implanted steel screw electrodes inserted bilaterally onto the fronto-parietal and the fronto-occipital area. The ground electrode was implanted epidurally over the nasal bone. At least 3 rats for each group were implanted with bipolar hippocampal electrodes (AP = 4.3 posterior to the bregma, L = 3.5, H = 3.5 mm ventral to the skull surface). For statistical purposes, the bipolar signals from each cortical area were integrated by means of a Berg-Fourier analyser (OTE Biomedica, Florence, Italy) according to Bricolo *et al.* [23].

In particular the ECoG changes were continuously (every 5 min) computerized as previously described [27] in order to get continuous information on total voltage power as well as on preselected bands of ECoG frequency (0.25-3; 3-6; 6-9; 9-12 and 12-16 Hz or 0.5-3; 3-7; 8-12; 12-16 and 14-32 Hz). The time constant (0.03) was short enough to reduce the number of artifacts (HF cut-off was 5.3 Hz). The spectrum power was plotted and the integrated energy signals were expressed as μV^2 per second.

In order to quantify changes of total voltage power and of preselected bands of frequency induced by lymphokines, the area (expressed in mm^2) under the curve corresponding to plotted total voltage values during 3 min periods after each compound was integrated by means of Commodore Computer and percentage changes of the integrated area in comparison to the same interval during pretreatment period were calculated according to the "trapezoidal rule" [28]. In addition, in order to reduce the possible inter-animal variations of baseline electrocortical activity and of a single frequency bands existing into the same group, the percentage changes following drug-treatment were compared to the values of corresponding period before treatment using paired Student's t-test. In addition, statistical analysis among groups treated with IFNs or IL-2 or vehicle

(in the same volume) was performed using the Man-n-Whitney U-test.

Microinfusion of the same volume of the vehicle (0.5 µl or 2.0 µl for specific brain area studied or icv injection, respectively) lacked effects on behaviour and electrocortical activity. In brackets the number of experiments is reported.

Drugs: the following compounds were used: α-interferon (Roche, Milan, Italy), β-interferon (Serono, Roma, Italy), rat-interferon (Sigma St. Louis, Mo. USA), interleukin-2 (Sigma St. Louis, Mo. USA), recombinant interleukin-2 (Janssen, Beerse, Belgium), naloxone hydrochloride (Sigma St. Louis, Mo. USA). All drugs were easily dissolved in 67 mM sodium phosphate buffer containing 5 mg of serum albumin for 1 ml. The drugs were infused into the LC or other areas of the brain in a volume of 0.5 µl, into the third cerebral ventricle in a volume of 2 µl at a constant rate of 0.2 µl/min and the injector cannula left *in situ* for a further 1 min.

Results

Interferons

Intravenous administration. - Human α-IFN and rat-IFN given intravenously (1,000, 5,000 and 10,000 units/kg) produced within 1 min wet-dog shake episodes which were followed (approx. 10 min later) by a decrease in locomotor activity, drowsiness or sleep, the latter phenomena were depending on the dose (at least 5 rats for each dose). This symptomatology was accompanied by an increase in ECoG slow wave activity, sometimes associated with high voltage sharp waves, which lasted from 20 to 80 min depending on the dose. The highest doses of α-IFN and rat-IFN induced a cataleptic and analgesic state (i.e. animals were less responsive to touch or auditory stimuli). The ECoG changes firstly appeared in the fronto-parietal cortex and then involved the *dorsal hippocampus*. They were characterized on the cortical recordings by an increase in total voltage power as well as in the 4-7, 8-13 and 12-16 and sometimes also in the 0.5-3 Hz frequency bands (Fig. 1), and on the hippocampal recording by an increase in total voltage power and in particular in the 4-7 Hz frequency.

No clear synchronization of ECoG changes was recorded among these two brain structures. No significant effects of both IFNs on the ECoG activity in comparison to vehicle treated animals could be detected 3 h after administration.

β-IFN up to 1,000 units produced no significant behavioural and ECoG changes, while the highest doses (5,000 and 10,000 units/kg) induced respectively a drowsy state or sleep (at least 4 rats for each dose). The behavioural and ECoG slow waves sleep observed after β-interferon 10,000 units/kg lasted approx. 35 min. No analgesic or cataleptic activity were observed after β-IFN.

A pretreatment with naloxone (1 mg/kg i.v., 15 min before), was able to reduce or to antagonize

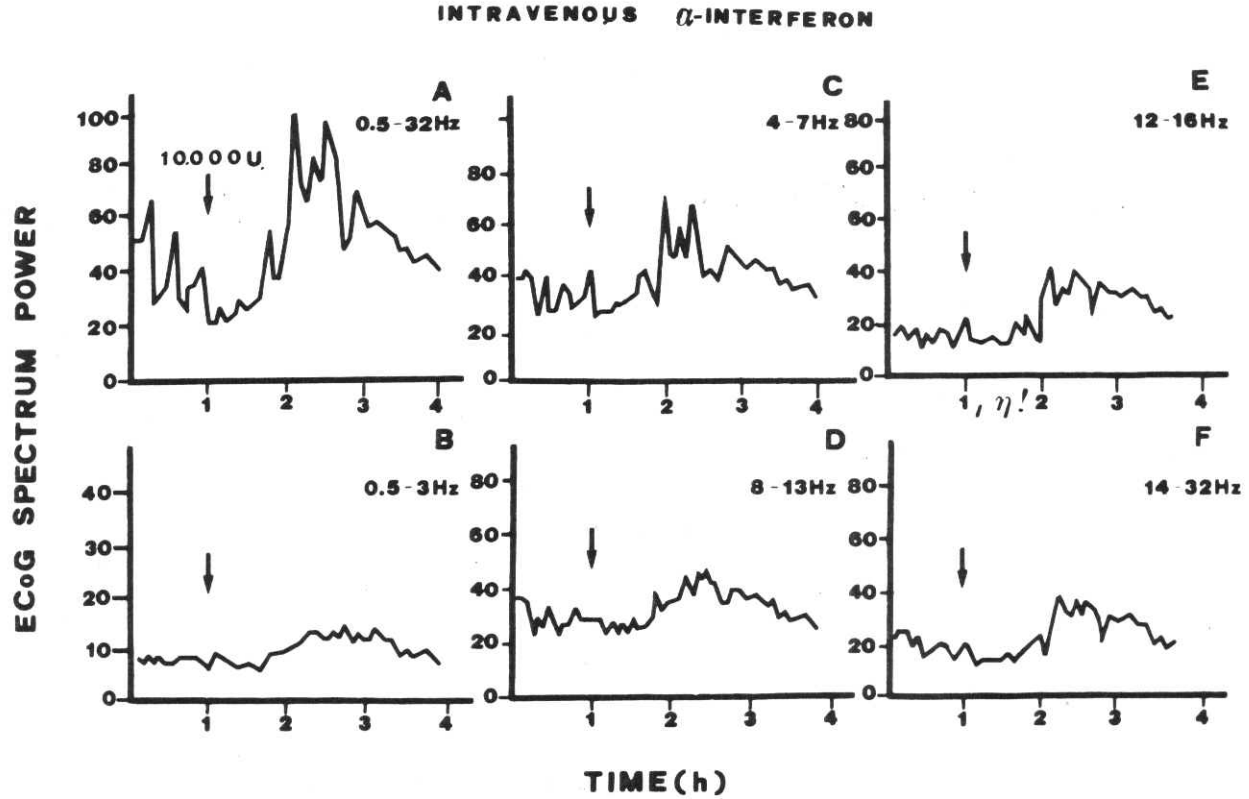

Fig. 1. - Effects of a single intravenous administration of α-IFN (10,000 units/kg) on electrocortical spectrum power. Ordinates show the voltage power expressed in μV^2 per s, abscissae show the time (h). Note the significant ($p < 0.01$) increase in total and 4-7, 8-13 and 12-16 Hz voltage power. The slight increase in 0.5-3 and 14-32 Hz frequency bands was not statistically significant.

both behavioural and electrocortical changes induced by α-IFN, β-IFN or rat-IFN (at least 4 rats for each dose and IFN studied).

Intracerebroventricular administration. - Both α-IFN (500, 1,000 and 4,000 units) and rat-IFN (500, 1,000 and 4,000 units) induced during the microinjection several wet dog shakes episodes and within 5 to 15 min after the administration a typical behavioural sedation, and/or sleep associated with ECoG synchronization lasting from 25 to 100 min depending on the dose (n = 5 for each dose). In addition, a dose-dependent increase in ECoG total voltage power as well as in the 0.5-3, 4-7, 8-13 and 12-16 Hz frequency bands was evident (Fig. 2).

With the highest dose used (i.e. 4,000 units) both interferons initially elicited cortical and hippocampal spiking activity which was sometimes related to wet dog shake episodes. This ECoG activity completely disappeared within 5 min after the administration.

However, the microinfusion of β-IFN (500 and 1,000 units) did not affect significantly both behavioural and ECoG activity (n = 4).

Microinjection in the locus coeruleus. - Lower doses were required after unilateral microinjection of α-IFN (100 and 500 units) or rat-IFN (100 and 500 units) into the LC to induce a dose-dependent behavioural and ECoG soporific activity similar to that observed after intraventricular injection (at least 5 animals for each dose). The sedative effects appeared within 1-3 min after the microinfusion and lasting from 25 to 100 min depending on the dose (Fig. 3). During this period the animals showed a significant ($p < 0.01$) increase in total voltage power as well as in the 0.5-3, 4-7, 8-13 and 12-16 Hz frequency bands (Fig. 4).

A slight analgesic effect occurred concomitantly with the sedative behavioural correlates of the highest doses of α-IFN and rat-IFN. Bilateral microinjection of α-IFN (20 units) or rat-IFN (20 units) into the LC was as effective as unilateral injection of 100 units of both IFNs in producing behavioural and ECoG slow-wave (n = 5). Only the highest dose of β-IFN (500 units) was able to produce behavioural and ECoG sedative effect lasting approx. 30 min (n = 4).

Microinjection into other brain areas. - In comparison to the LC site, equimolar doses of α-IFN and rat-IFN (100 and 500 units) given into the *dorsal hippocampus, caudate nucleus, substantia nigra (pars compacta)* and *ventromedial hypothalamus* were ineffective in inducing behavioural and ECoG slow-wave sleep (at least 3 experiments for each dose and brain area studied). However, α-IFN and rat-IFN, microinjected into the *caudate nucleus* or *substantia*

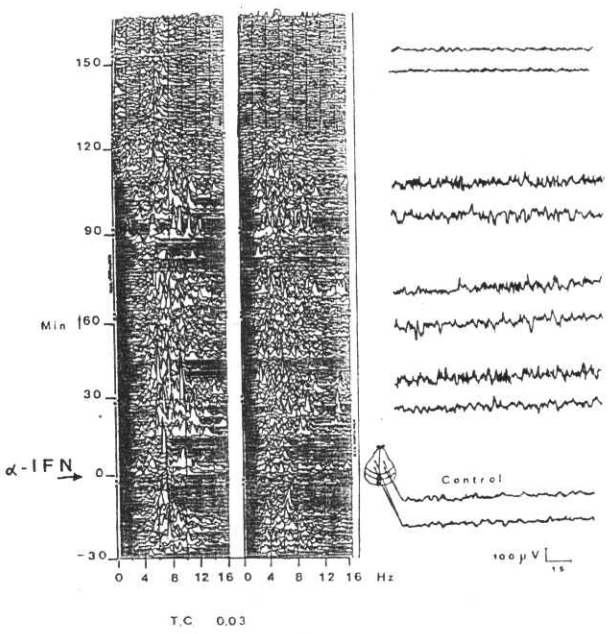

Fig. 3. - Sequential spectral analysis and electrographic recordings illustrating the effects of α-IFN (100 units) on electrocortical activity in rats. The ECoG activity, evaluated at various times after α-IFN administration, shows ECoG synchronization lasting approx. 110 min. No major alterations in the cortical recordings were observed 120 min after α-IFN.

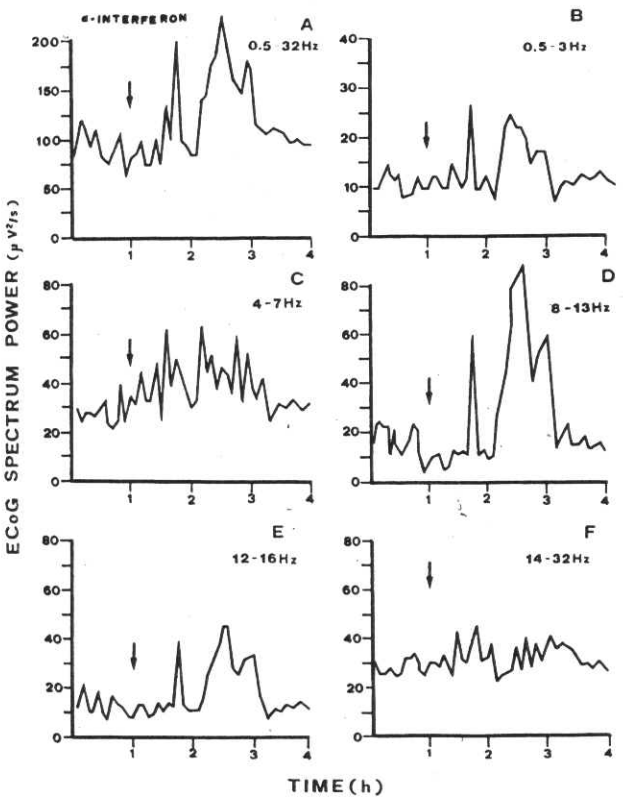

Fig. 2. - Effects of a single intracerebroventricular injection of rat α-IFN (1,000 units) on electrocortical spectrum power. Ordinates show the voltage power expressed in μV^2 per s, abscissae show the time (h). Note the significant increase ($p < 0.05$) in total and 0.5-3, 4-7, 8-13 and 12-16 Hz voltage power.

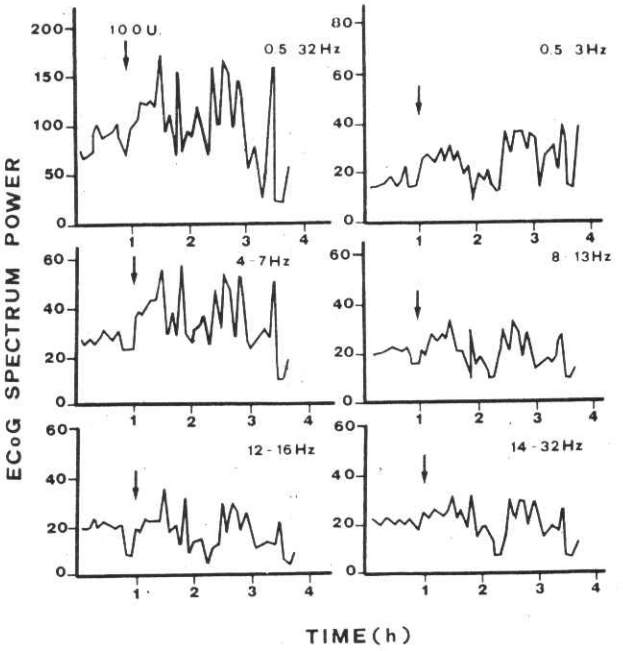

Fig. 4. - Effects of a single injection into the *locus coeruleus* of rat-IFN (100 units) on electrocortical spectrum power. Ordinates show the voltage power expressed in μV^2 per s, abscissae show the time (h). Note the significant increase ($p < 0.05$) in total and 0.5-3, 4-7, 8-13 and 12-16 Hz voltage power.

nigra (pars compacta) elicited asymmetrical body posture with ipsilateral turning behaviour and periodic ipsilateral circling lasting from 30 to 70 min depending on the dose. In addition, these effects were longer-lasting (from 1,5 to 2 times) after microinfusion of the same doses into the *substantia nigra (pars compacta)* that into the *caudate nucleus.*

Animals treated with α-IFN or rat-IFN into the dorsal hippocampus or ventromedial hipothalamus showed an increase in locomotor and exploratory activity lasting 25-70 min depending on the dose.

Interleukin-2

IL-2 (5-50 units) and rIL-2 (5-20 units) after microinjection into the third cerebral ventricle typical behavioural sedation and/or sleep associated with ECoG synchronization (at least 5 animals for each dose). The sedative effects appeared within 5-10 min depending on the dose and with the highest dose of IL-2 were preceded by spiking in cortical and hippocampal activity sometimes associated with wet dog shake episodes. No spiking activity was observed in animal-treated with rIL-2. The soporific effects induced by IL-2 or rIL-2 lasted between 25 and 140 min depending on the dose. Lower doses of IL-2 (1-10 units) and rIL-2 (1-5 units) were infused into the LC (n = 6) and induced a behavioural and ECoG sedative activity (Fig. 5) similar to that observed after intraventricular injection. No spiking activity was observed when IL-2 or rIL-2 was infused into the LC.

Bilateral microinjection of IL-2 (1 and 2 units) or rIL-2 (1 unit) into the LC was as effective as unilateral injection of 5 units of both ILs-2 in producing behavioural and ECoG, slow wave sleep (n = 4). In comparison to the LC route, equimolar doses of IL-2 (5-50 units) and rIL-2 (20 units) given into the *dorsal hippocampus, caudate nucleus, substantia nigra* and *ventromedial hypothalamus* were ineffective in inducing behavioural and ECoG slow-ware sleep (at least 3 experiments for each dose and brain area studied). However, IL-2 and rIL-2 microinjected into the *caudate nucleus* and *substantia nigra (pars compacta)* elicited asymmetric body posture with ipsilateral turning behaviour and periodic ipsilateral circling lasting from 25 to 60 min depending on the dose. These effects were longer lasting in animals injected into the *substantia nigra* than in those injected into the *caudate nucleus.*

Animals treated with IL-2 or rIL-2 into the *dorsal hippocampus* or *ventromedial hypothalamus* showed an increase in locomotor and exploratory activity lasting 20-65 min depending on the dose.

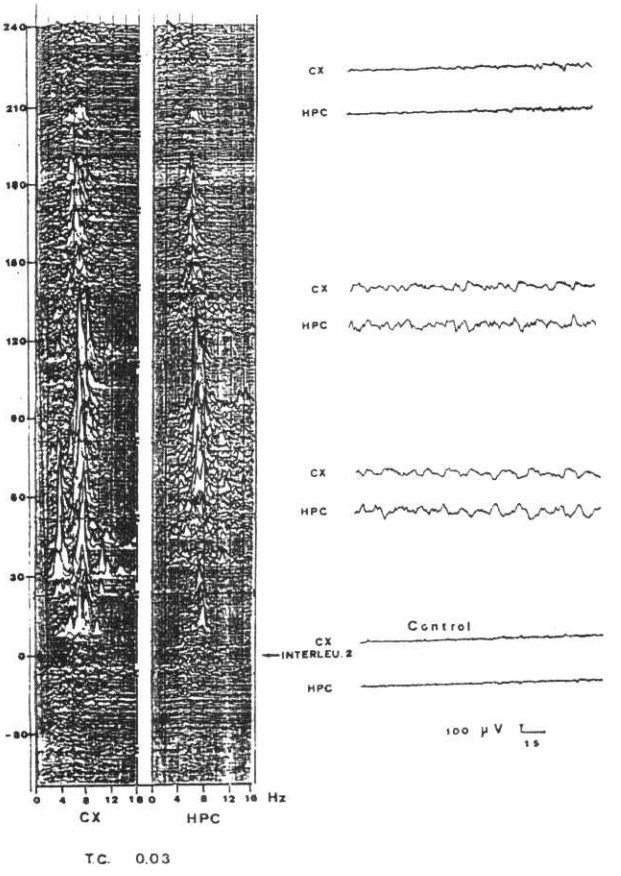

Fig. 5. - Sequential spectral analysis and electrographic recordings illustrating the EEG effects after into the LC microinjection of IL-2 (10 units) on cortical and hippocampal activity in rats. The EEG activity, evaluated at various times after IL-2, shows cortical and hippocampal synchronization. The EEG activity was characterized by an increase of 3-6 and 6-9 Hz frequency bands at cortical level and by an increase of 6-9 Hz frequency band at hippocampal level. No major alterations in both cortical and hippocampal recordings were observed 150 min after IL-2 infusion.

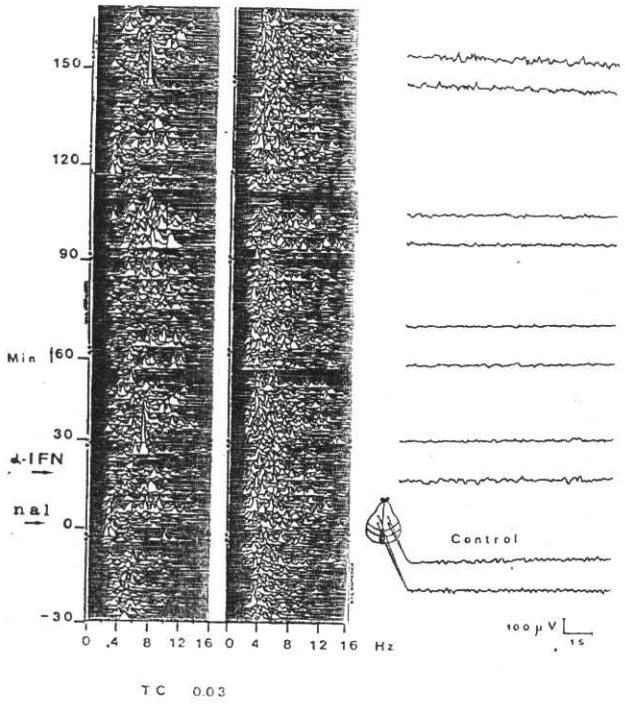

Fig. 6. - Sequential spectral analysis and electrocorticographic recordings illustrating the effects of a pretreatment (15 min before) with naloxone (10 pmol) on the ECoG changes induced by into the *locus coeruleus* administration of α- IFN (100 units). No major alteration of ECoG activity were recorded in animal pretreated with naloxone.

Effects of naloxone on overt sedation and ECoG spectrum power effects induced by lymphokines

A pretreatment (15 min before) with naloxone (40 pmol intracerebroventricularly) was able to antagonize or to significantly reduce the effects of α-IFN and rat-IFN (1,000 and 4,000 units, icv). The antagonistic effects of a pretreatement (15 min before) with naloxone (10 pmol), given into the LC on behavioural and ECoG spectrum power changes induced by α-IFN (100 units) microinjected into the same site are shown in Fig. 6.

Naloxone (10 pmol into the LC) was able to prevent or reduce the behavioural and electrocortical soporific effects induced by α-IFN (500 units), rat-IFN (500 units) and IL-2 (5 units). In addition, a pretreatment with naloxone (10 pmol) significantly decreased or completely antagonized the effects of recombinant interleukin-2 (5 units).

Discussion

The present experiments show that α-IFN, rat-IFN and IL-2 produce specific and powerful behavioural and ECoG changes. Thus it is not surprising that more frequently or occasionally according to the lymphokine used after systemic administration in man several CNS disorders, including confusion, fatigue and lethargy, have been described to occur (see refs in [18]).

The soporific effects elicited after systemic or intracerebroventricular administration of IFNs or IL-2 confirm previous results showing that ILs and IFNs possess sleep-promoting effets [17, 21]. In addition in the present experiments we have provided evidence showing that sleep-induced effects of both types of lymphokines are very likely mediated through the *locus coeruleus*. In fact, this area was the only sensitive one in mediating behavioural and ECoG sleep and sleep was not obtained after direct microinfusion of these lymphokines into other brain structures. The LC is the area containing the largest clusters of noradrenaline-containing cell bodies in the CNS [29]. Evidence exists that α_2-adrenoceptors and opiate receptors are located at the noradrenaline somata and dendrites in the LC and that clonidine and opioid peptides produce behavioural and ECoG sleep when injected directly into this area in rats [27, 30]. The finding that IL-2 and IFNs reproduce the same behavioural and ECoG effects after their microinfusion into the LC indicates that these lymphokines may influence the firing activity of LC neurons, through an increase in potassium conductance as with α_2-adrenoceptor agonists [31, 32] or opioid peptides [33]. The present experiments show that only α-IFN possesses marked central nervous system effects and shares with β-endorphin some pharmacological actions such as analgesia and catatonia, and indirectly this is supported by the affinity for [^{3}H] morphine binding sites in mouse brain membranes [20]. In fact, in agreement with previous studies [6, 13, 14] we observed that α-IFN influences CNS activity much more than β-IFN. This may be due to the fact that IFNs often exhibit maximal biological activity on cells of homologous species, and α-IFN exhibits a great degree of cross-species activity [21, 35, 36]. This may explain in part our results which demonstrated that β-IFN failed to induce marked behavioural and ECoG spectrum power changes in rats, while α-IFN and rat-IFN did not. The finding that naloxone pretreatment prevented or reversed behavioural soporific and ECoG effects of the lymphokines suggests that these effects seem to be mediated through stimulation of opiate receptors. Similar hypothesis could be postulated for the analgesic and cataleptic activity of rat-IFN and α-IFN which were also antagonized with naloxone (1 mg/kg, iv). Similar evidence linking α-IFN with opiates was provided by Dafny [36].

The body postural asymmetry with ipsilateral turning and circling after direct application of lymphokines into the *substantia nigra (pars compacta)* or into the *caudate nucleus* suggest that they inhibit ipsilaterally to the microinfusion the nigro-striatal dopaminergic pathway, thus allowing the predominance of the contralateral dopaminergic mechan-

isms. On the other hand it is known that morphine and opioid peptides given unilaterally into the *pars reticulata* of the *substantia nigra* produce contralateral turning and circling through inhibition of nigral non-dopaminergic output neurons [37, 38]. The spiking activity associated with the intracerebroventricular injection of IL-2, did not develop in animals receiving rIL-2; this suggests that such phenomena may be related to some contaminating compounds which are not present in the rIL-2 preparation.

In conclusion, the present experiments show that IL-2 and some IFNs produce marked behavioural and electrocortical soporific effects and these are most likely mediated by an action at the *locus coeruleus* neurons. In addition, they suggest that lymphokines may share some common mechanism of action with opioid peptides.

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