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Evidence for direct and indirect mechanisms in the potent modulatory action of interleukin-2 on the release of acetylcholine in rat hippocampal slices

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1 The biphasic nature of the potent modulatory action of interleukin-2 (IL-2) on hippocampal acetylcholine (ACh) release was investigated by use of brain slice superfusion.

2 Both the potentiating (10^{-13} M) and inhibitory (10^{-9} M) effects of IL-2 on hippocampal ACh release were stimulation-dependent and were blocked by a neutralizing IL-2 receptor antibody, suggesting the activation of typical IL-2 receptors in both cases.

3 Tetrodotoxin (TTX; 10 μM) failed to block the potentiation of ACh release induced by a very low concentration of IL-2 (10^{-13}M) suggesting a direct effect on cholinergic nerve terminals.

4 In contrast, the inhibitory effect seen at a higher concentration (10^{-9} M) was TTX-sensitive, and hence indicative of an indirect action.

5 To establish the nature of this intermediate mediator, blockers of nitric oxide synthesis, and of opioid and γ-aminobutyric acid (GABA) receptors were used. Only $GABA_A$ and $GABA_B$ receptor antagonists altered the inhibitory action of IL-2, suggesting the participation of GABA as mediator.

6 Taken together, these results provide further evidence for the potent role of IL-2 in the modulation of cholinergic function in the rat hippocampus.

Keywords: Interleukin-2; cytokine; acetylcholine; release; γ-aminobutyric acid (GABA); hippocampus

Introduction

A wealth of evidence supports the genuine existence of interleukin-2 (IL-2) in the mammalian brain (for reviews, see Merrill, 1990; Nisticò & de Sarro, 1991; Plata-Salamán, 1991; Seto *et al*., 1993). IL-2-like immunoreactivities and IL-2 mRNA were found to be widely, but discretely distributed in the rodent brain with greater densities observed in the hippocampus and hypothalamus (Villemain *et al*., 1990; Lapchak *et al*., 1991; Seto *et al*., 1993). In parallel, IL-2 receptor (IL-2R) binding sites or IL-R immunostaining as revealed with an antibody against the α subunit (TAC), and IL-2Rβ mRNA are expressed in the mammalian brain with a pattern mirroring that of the endogenous protein (Araujo *et al*., 1989; Lapchak *et al*., 1991; Petitto & Huang, 1994). Furthermore, recent evidence provided by *in situ* hybridization studies clearly shows that both IL-2 and IL-2R mRNA are expressed by certain neural populations (Villemain *et al*., 1991; Shimojo *et al*., 1993; Petitto & Huang, 1994; 1995; Eizenberg *et al*., 1995; Sawada *et al*., 1995), demonstrating the local synthesis of IL-2 in the rodent brain.

Functionally, IL-2 exerts various biological effects in the normal mammalian brain. In addition to its effects on growth and differentiation of neurones and oligodendrocytes (Benveniste & Merrill, 1986; Haugen & Letourneau, 1990; Eitan *et al*., 1992; Awatsuji *et al*., 1993; Eitan & Schwartz, 1993; Sarder *et al*., 1993; Shimojo *et al*., 1993), IL-2 is able to modulate the release of hypothalamic (Cambronero *et al*., 1993; Karanth *et al*., 1993; Raber & Bloom, 1994; Hillhouse, 1994; Raber *et al*., 1995) and pituitary (Karanth & McCann, 1991) hormones, to block long-term potentiation in the hippocampus (Tancredi *et al*., 1990) and to inhibit electrocorticogram spectrum (de Sarro *et al*., 1990). Moreover, IL-2 has been shown to potentiate scopolamine-induced amnesia (Bianchi & Panerai, 1993), to inhibit Ca^{2+} currents in hippocampal cells (Plata-Salamán & ffrench-Mullen, 1993) and to modulate the release of catecholamines from the hypothalamus *in vitro* (Villemain *et al*., 1992, Lapchak & Araujo, 1993) and dopamine from striatal slices (Lapchak, 1992) and cultured mesencephalic cells (Alonso *et al*., 1993).

In light of evidence pointing to the particularly high densities of IL-2 and IL-2R in the hippocampus, we focused on the possible modulatory role of IL-2 on acetylcholine (ACh) release from the septo-hippocampal pathway. Indeed, this is appropriate given the observed effects of IL-2 on the inhibition of long-term potentiation (Tancredi *et al*., 1990) and its facilitation of scopolamine-induced amnesia (Bianchi & Panerai, 1993), two models for which the involvement of this projection is clearly established. Early on, we found that nM concentrations of IL-2 potently inhibited K^+-evoked ACh release from rat hippocampal slices *in vitro* (Araujo *et al*., 1989). In a subsequent study, we observed that IL-2 was among the most potent modulators of ACh release known to date, having a potentiating action at low concentrations (fM–pM) while inhibiting ACh release at nM concentrations (Hanisch *et al*., 1993).

The aim of the present study was thus to explore the nature of the concentration-dependent modulatory actions of IL-2 on hippocampal ACh release. We first determined if the effect of IL-2 was dependent on the nature of the depolarizing agents used before establishing whether the IL-2R subtype mediating these effects was similar to that expressed on T cells using a highly specific anti-rat IL-2Rα antibody. Tetrodotoxin (TTX) was then employed to explore if the potentiating and inhibitory effects of IL-2 were likely to be due to a direct action on cholinergic terminals. Since the inhibitory effect observed at nM concentrations appeared to be indirect (TTX-sensitive), the possible nature of some likely mediators (nitric oxide, opioid peptides, γ-aminobutyric acid (GABA)) was finally investigated.

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Methods

Materials

Recombinant human IL-2, acetylcholine chloride, choline chloride, physostigmine hemisulphate, veratridine, tetrodotoxin, N^G monomethyl-L-arginine acetate (L-NMMA), glycylglycine, choline kinase (ATP: choline phosphotransferase, EC 2.7.1.32) and acetylcholinesterase type V-S (acetylcholine hydrolase, EC 3.1.1.7) were purchased from Sigma Chemical Co. (St-Louis, MO, U.S.A). (−)-Bicuculline methochloride and phaclofen were obtained from Trocris Cookson (Bristol, U.K.), while monoclonal mouse anti-rat IL-2Rα antiserum (NDS-61) was purchased from Serotec/Cedarlane (Hornby, Ontario, Canada). Dithiothreitol and adenosine 5′-triphosphate (ATP) were obtained from Boehringer-Mannheim (Laval, Québec, Canada). Sodium tetraphenylboron was obtained from Fisher Scientific (Montréal, Québec, Canada) while butyronitrile was purchased from Aldrich (Milwaukee, WI, U.S.A.). [γ-^{32}P]-ATP (30 Ci mmol^{-1}) was obtained from New England Nuclear (Boston, MA, U.S.A.). Eco(+)lite scintillating cocktail was purchased from ICN (Montréal, Québec, Canada). The chromatographic resin Duolite was a generous gift from Gerald D. Button (Rohm & Haas Co., Philadelphia, PA, U.S.A.). Other chemical reagents were obtained from either Sigma Chemical Co. or Fisher Scientific Co.

Superfusion of brain slices

Adult male Sprague-Dawley rats (300–325 g) obtained from Charles River (St-Constant, Canada) maintained according to guidelines of the Canadian Council for Animal Care and McGill University, were used in the study. Rats were decapitated and hippocampi dissected out on ice and sliced at 0.4 mm with a McIIwain tissue chopper (Mickle Laboratory Engineering, Gomshall, Surrey, U.K.). Slices of one whole hemispheric region were transferred to one superfusing chamber (12 parallel chambers; Model SF-12, Brandel, Gaithersburg, MD, U.S.A.). The slices were continuously superfused with oxygenated Krebs buffer (mM: NaCl 120, KCl 4.6, $CaCl_2$ 2.4, KH_2PO_4 1.2; $MgCl_2$ 1.2, dextrose 9.9, $NaHCO_3$ 25, choline chloride 0.01, physostigmine 0.03, adjusted to *p*H 7.4) at 37°C at a flow rate of 0.5 ml min^{-1} as described in detail elsewhere (Seto *et al*., 1993). Following a 45-min stabilization period, samples were collected every 20 min during basal efflux and depolarization and/or drug treatment. After one hour of basal efflux, the slices were stimulated with 25 mM K^+ Krebs buffer (with concomitant decrease in Na^+ to maintain isoosmolarity) or veratridine (30 μM) in the absence or presence of IL-2, or in the presence of IL-2 and another drug/agent. At the end of each experiment, protein content of each hemisphere was measured according to Lowry *et al*. (1951). Superfusates collected every 20 min were spun (21800 *g*), and frozen at −70°C until further processing.

Radioenzymatic analysis of ACh

Samples were processed in triplicate for ACh analysis according to the procedure of Fonnum (1969) and Goldberg and McCaman (1973). Briefly, ACh was extracted by mixing with an equal volume of tetraphenylboron in butyronitrile (30 mM) and recovered from the organic phase by shaking with a half volume of $AgNO_3$ (120 mM). Excess $AgNO_3$ was precipitated by the addition of $MgCl_2$ (1 M) and finally 110 μl of the supernatant was removed and evaporated to dryness. Dried samples were redissolved in 32 μl of a mixture containing ATP (0.8 mM), dithiothreitol (5 mM), $MgCl_2$ (12.5 mM), glycylglycine (25 mM, *p*H 8.0), and choline kinase (0.005 u) and incubated at 30°C for 25 min to phosphorylate the choline and not ACh contained in the samples. Ten microlitres of a solution containing acetylcholinesterase (2 U) and [γ-^{32}P]-ATP (0.45 μCi) were added to each sample during which ACh was hydrolysed and the choline formed phosphorylated to [^{32}P]-phosphorylcholine. The reaction was stopped by the addition of 100 μl of NaOH (50 mM), and radioactive phosphorylcholine was subsequently separated from the radioactive ATP by ionic-exchange chromatography (Duolite) followed by determination of radioactivity with liquid scintillation. For each experiment, known standard amounts of ACh (0–200 pmol) were processed in parallel to monitor recovery (⩾85%; sensitivity of about 5 pmol). Evoked transmitter release was calculated by subtracting the basal efflux from the total release and is expressed as pmol ACh min^{-1} mg^{-1} protein. Replicate determinations for each condition were derived from independent experiments and the data were analysed by one-way ANOVA followed by Fisher's *post-hoc* test and the level of significance was set at $P<0.05$.

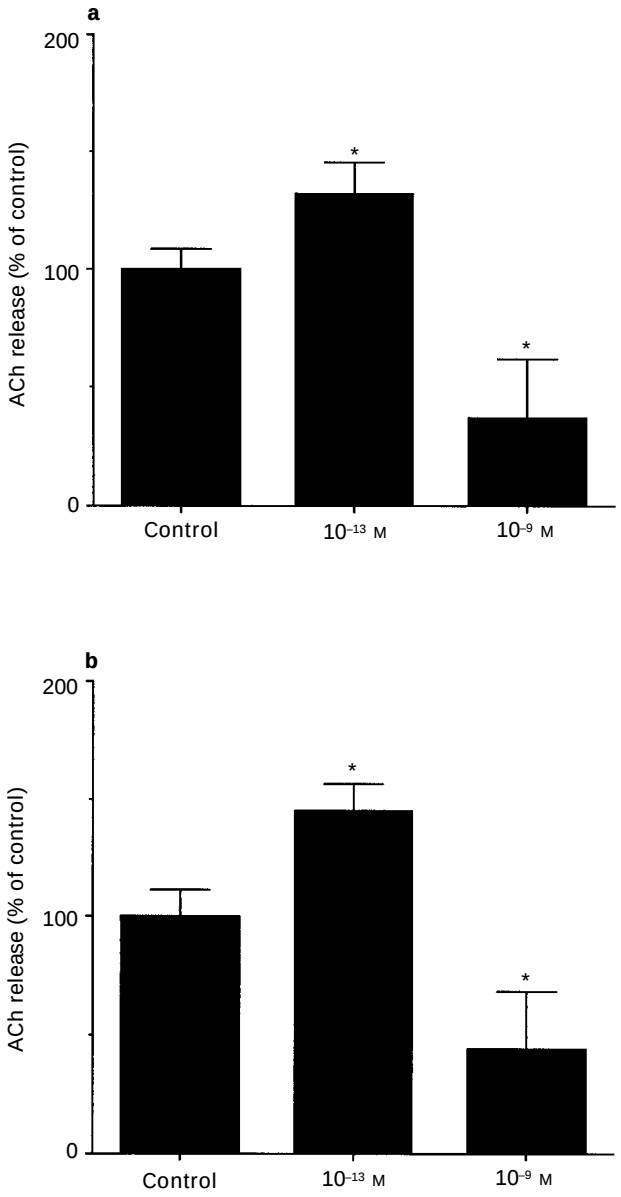

Figure 1 Effect of IL-2 concentrations on KCl (25 mM; a) and veratridine (30 μM; b)-evoked ACh release from hippocampal slices. The slices were stimulated with Krebs buffer containing either 25 mM KCl or 30 μM veratridine in the absence of IL-2 (control) or presence of IL-2 10^{-13} M or 10^{-9} M. Values are expressed as mean±s.e. mean of at least 4 determinations from independent experiments for each condition in reference to control set at 100% (for K^+ stimulation 5.7±0.5 pmol mg^{-1} min^{-1} and veratridine stimulation 3.8±0.5 pmol mg^{-1} min^{-1}). *$P<0.05$ vs control.

Results

Effects of IL-2 on hippocampal ACh release under stimulated conditions

In keeping with our earlier findings (Hanisch *et al*., 1993), IL-2 at 10^{-13} M potentiated whereas a higher concentration (10^{-9}M) inhibited K^+-evoked ACh release from hippocampal slices (Figure 1a). To assess whether this biphasic response of IL-2 could be mimicked with other depolarizing agents, hippocampal slices were superfused with a submaximal concentration of veratridine (30 μM) either in the presence or absence of IL-2. IL-2 at 10^{-9} M significantly inhibited whereas at 10^{-13} M it potently stimulated veratridine-evoked ACh release from hippocampal slices (Figure 1b). These effects exhibited time-dependency, since both became significant between 20 to 40 min of stimulation. The spontaneous, unstimulated release of ACh from hippocampal slices was not significantly altered by IL-2 in the pM–nM range (throughout the experiment, in the absence or presence of IL-2 at either concentration, basal ACh release was between 0.1 to 1.4 pmol mg^{-1} min^{-1}). Slight variations in the potentiating effect of IL-2 at 10^{-13}M and in the evoked controls (Figures 1, 2, 3 and Tables 1 and 2) are probably attributable to variations in basal release, assay procedures and sample size.

Role of IL-2 receptor in IL-2 effects on ACh release

It is well known that the IL-2 receptor consists of three subunits, α, β, and γ, and that the binding of IL-2 to the α subunit is important for activity (Depper *et al*., 1983; Taniguchi & Minami, 1993). Interestingly, the subunits of the IL-2 receptor are shared by other related cytokines, i.e., IL-4, IL-7, IL-9, IL-13 and IL-15 (Taniguchi & Minami, 1993; Bamford *et al*., 1994; Giri *et al*., 1994; Grabstein *et al*., 1994) such that IL-2 effects may be exerted through other interleukin receptors. To determine the specificity of IL-2 effects on ACh release, hippocampal slices were superfused with 25 mM K^+ Krebs buffer in the presence and absence of an anti-rat IL-2Rα antibody, NDS-61 (1:500) (Wood *et al*., 1992). Figure 2 shows that both the potentiating as well as the inhibitory effects of IL-2 on ACh release were sensitive to NDS-61, suggesting the involvement of a typical IL-2 receptor.

Effects of tetrodotoxin on IL-2 effects on ACh release

To determine whether the IL-2-induced alteration of evoked ACh release was affected by the sodium channel blocker tetrodotoxin (TTX), hippocampal slices were superfused under similar conditions in the presence of 10 μM TTX. By itself TTX failed to alter evoked ACh release (Lapchak *et al*., 1990). However, interestingly, the stimulant effects of pM concentrations of IL-2 on ACh release were unaltered in the presence of TTX (Figure 3). In contrast, the inhibitory effects of a nM concentration of IL-2 on ACh release resulted in enhanced release in the presence of TTX (Figure 3). This indicates that IL-2 probably acts directly on hippocampal cholinergic terminals to potentiate ACh release while the inhibitory response is probably indirect and requires the initiation of impulse activity.

Possible mediators of TTX-sensitive inhibitory action of IL-2 on ACh release

In an attempt to determine the possible neurotransmitter/neuromodulator involved in mediating the TTX-sensitive effects of IL-2 on ACh release, we initially evaluated the effects of opioid receptor blockade, since it has been shown that opioid peptides inhibited hippocampal ACh release (Lapchak *et al*., 1989) and could be involved, at least in part, in mediating the effects of IL-2 in the hippocampus (Araujo *et al*., 1990). Accordingly, hippocampal slices were superfused with or without the opioid antagonist naloxone at a concentration of 10 or 100 μM. Naloxone even at the very high concentration (100 μM) failed to alter IL-2-mediated inhibition of ACh release under our assay conditions (Table 1). It also failed to modulate significantly the stimulant effect of IL-2 on ACh release (not shown), as expected on the basis of the TTX data.

It has also been suggested that some of the modulatory effects of IL-2 are mediated by the synthesis and release of nitric oxide (NO) (Finkel *et al*., 1992; Karanth *et al*., 1993; Raber & Bloom, 1994; Raber *et al*., 1995). Since NO snythase (NOS), the enzyme responsible for NO synthesis, is present in the hippocampus (Bredt *et al*., 1991) and NO is believed to be involved in the regulation of ACh release (Lonart *et al*., 1992),

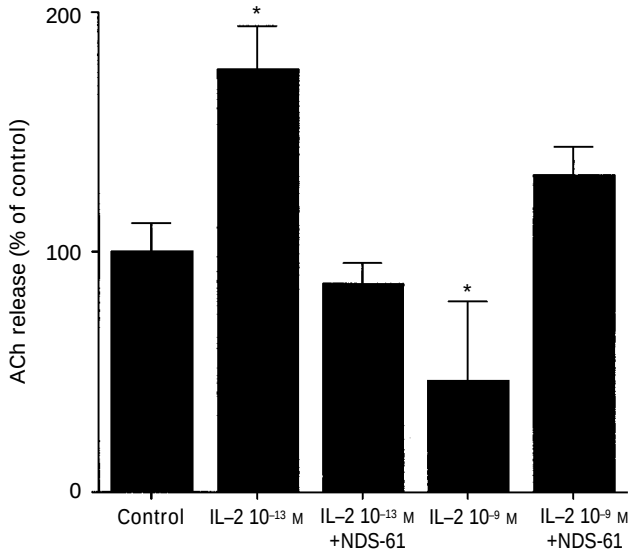

Figure 2 Effect of IL-2 receptor α antibody NDS-61 (1:500) on IL-2-mediated K^+-evoked ACh release from hippocampal slices. Control groups were exposed to NDS-61 alone. Data are expressed as % of control (mean$\pm$s.e.mean) of at least 4 determinations from independent experiments for each condition. *$P<0.05$ vs control (4.0 ± 0.5 pmol mg^{-1} min^{-1}).

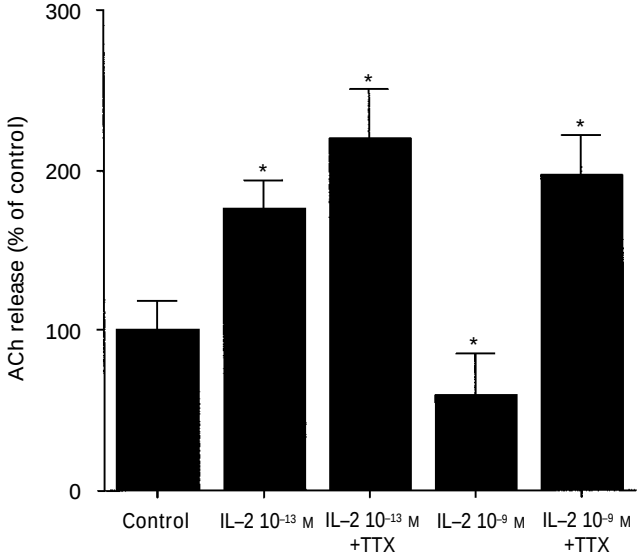

Figure 3 Effect of TTX (10 μM) on IL-2-mediated K^+-evoked ACh release from hippocampal slices. Control groups were exposed to TTX alone. While the potentiating effect of 10^{-13} M IL-2 was not altered by TTX, the inhibition seen at 10^{-9} M IL-2 was reversed in the presence of TTX. Data are expressed as % of control (mean$\pm$s.e.mean) of at least 4 determinations from independent experiments for each condition. *$P<0.05$ vs control (2.7 ± 0.5 pmol mg^{-1} min^{-1}).

we evaluated the role of NO by blocking its production with L-NMMA (10 or 100 μM)) in superfused hippocampal slices. NOS inhibition did not affect either the stimulant or inhibitory action of IL-2 on ACh release (Table 2).

GABA is a major inhibitory neurotransmitter in the hippocampus (for a recent review, Thompson, 1994). Accordingly, its potential relevance in mediating the indirect effects of nM concentrations of IL-2 on ACh release was undertaken by use of prototypical $GABA_A$ and $GABA_B$ receptor antagonists. As shown in Figure 4, both the $GABA_A$ receptor antagonist bicuculline (10 μM) and the $GABA_B$ receptor blocker phaclofen (10 μM) alone or in combination reversed the inhibitory effects of IL-2 on hippocampal ACh release, suggesting that the release of GABA and the activation of its receptors are involved in mediating the indirect inhibitory action of IL-2 on ACh release. Bicuculline or phaclofen alone or in combination, at 10 μM each, had no effect on the IL-2-mediated potentiation of ACh release (not shown).

Discussion

The present study provides the first detailed evidence regarding the potent modulatory action of IL-2 on hippocampal ACh release. Data obtained with non-stimulated and veratridine-evoked release in comparison to K^+ stimulation suggest that depolarization is required for IL-2 to exert its effects on ACh release. The use of the anti-rat IL-2Rα subunit antibody revealed that both the potentiating and inhibitory effects of IL-2 on hippocampal ACh release result from an action via the same component/subunit of the prototypical IL-2 receptor. Interestingly, the potentiating and inhibitory effects of IL-2 on ACh release can be dissociated on the basis of their sensitivity to TTX which suggests that the stimulation of ACh release by a low concentration of IL-2 results from a direct action of the cytokine on cholinergic nerve terminals of the septo-hippocampal pathway. As to the nature of the TTX-sensitive inhibitory effects of a higher concentration of IL-2 on ACh release, it is unlikely to be mediated by either nitric oxide or opioid peptides but may involve GABA as it was blocked by $GABA_A$ and $GABA_B$ receptor antagonists. Hence the potent, biphasic action of IL-2 on hippocampal ACh release is complex and involves a direct effect on cholinergic nerve terminals and most likely the release of an inhibitory transmitter at higher concentrations.

Previous investigations of IL-2 effects on hippocampal ACh release were solely based on K^+ stimulation (Araujo *et al*., 1989; Hanisch *et al*., 1993; Seto *et al*., 1993). Results obtained under veratridine-evoked conditions indicate that the biphasic effect of IL-2 on ACh release does not depend on the nature of the depolarizing agent, but that a depolarizing stimulus is necessary, since no effects were observed under basal unstimulated conditions. This observation is significant as effects on transmitter release under non-stimulated conditions are often not specific and associated with passive transmitter leakage, not genuine vesicular release. However, in contrast to hippocampal ACh release, corticotrophin and vasopressin release from hypothalamic and amygdalar slices (Cambronero *et al*., 1992; Karanth *et al*., 1993; Hillhouse, 1994; Raber & Bloom, 1994; Raber *et al*., 1995) revealed that IL-2 can evoke peptide release under non-stimulated conditions. Accordingly, additional studies are necessary to establish if the neuromodulatory action of IL-2 varies between classical transmitter and neuropeptides.

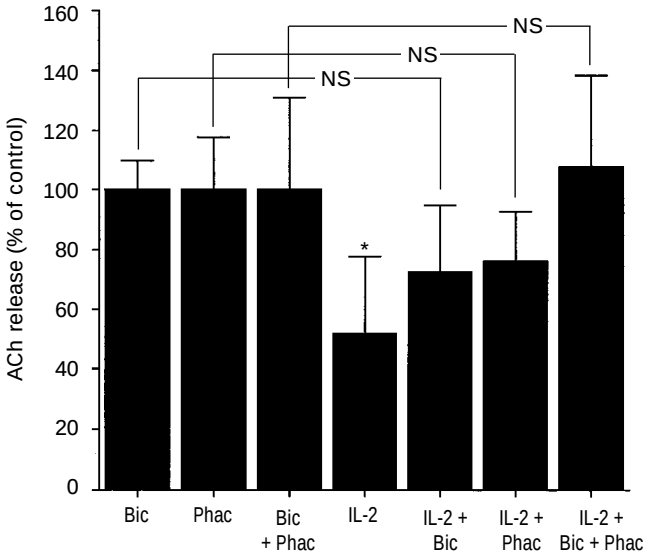

Figure 4 Effects of a $GABA_A$ antagonist (bicuculline, Bic) and a $GABA_B$ antagonist (phaclofen, Phac) alone or in combination on IL-2 inhibition of K^+-evoked ACh release from hippocampal slices. Control groups were exposed to either bicuculline or phaclofen alone or in combination at 10 μM. IL-2 10^{-9} M inhibited ACh release ($P<0.05$), this effect being reversed by GABA antagonists alone or in combination. Data are expressed as % of control (mean$\pm$s.e.mean) of at least 4 determinations from independent experiments for each condition. *$P<0.05$ vs control (bicuculline 4.0 ± 0.4 pmol mg^{-1} min^{-1}; phaclofen 3.7 ± 0.6 pmol mg^{-1} min^{-1}; bicuculline and phaclofen 1.5 ± 0.5 pmol mg^{-1} min^{-1}).

Table 1 Effect of naloxone (10 μM and 100 μM) on IL-2 inhibition of K^+-evoked ACh release from hippocampal slices

Treatment	*ACh release* (% of control)
Naloxone 10 μM (Control)	100$\pm$18
IL-2 10^{-9} M	58$\pm$17*
IL-2 + naloxone	47$\pm$27*
Naloxone 10 μM (Control)	100$\pm$20
IL-2 10^{-9} M	53$\pm$3*
IL-2 + naloxone	42$\pm$21*

The slices were stimulated with K^+ Krebs buffer containing naloxone alone (control), 10^{-9} M IL-2 alone or 10^{-9} M IL-2 plus naloxone. Data are expressed as % of control (mean$\pm$s.e.mean) of at least 4 determinations from independent experiments for each condition. *$P<0.05$ vs control (10.7 ± 1.9 pmol mg^{-1} min^{-1} for naloxone 10 μM and 7.0 ± 1.1 pmol mg^{-1} min^{-1} naloxone 100 μM).

Table 2 Effect of the nitric oxide synthase inhibitor, L-NMMA (10 μM), on IL-2-mediated K^+-evoked ACh release from hippocampal slices

Treatment	*ACh release* (% of control)
Control	100$\pm$44
IL-2 10^{-13} M	242$\pm$18*
IL-2 10^{-13} M + NMMA	244$\pm$14*
IL-2 10^{-9} M + NMMA	58$\pm$19*
IL-2 10^{-9} M + NMMA	46$\pm$18*

Control groups were exposed to L-NMMA alone. Other groups shown are 10^{-13} M IL-2 alone, 10^{-13} M IL-2 plus L-NMMA, 10^{-9} M IL-2 alone and 10^{-9} M IL-2 plus L-NMMA. L-NMMA failed to alter either the inhibiting or potentiating effects of IL-2 on ACh release. Data are expressed as % of control (mean$\pm$s.e.mean) of at least 4 determinations from independent experiments for each condition. *$P<0.05$ vs control (2.8 ± 0.6 pmol mg^{-1} min^{-1}).

Using a highly specific antibody against the rat α subunit of the rat IL-2 receptor (Wood *et al*., 1992), we observed that both the stimulant and inhibitory effects of IL-2 on ACh release are mediated by a typical IL-2R and that the α subunit is required to produce an action (Depper *et al*., 1983). It is also likely that other subunits (β and γ) of the IL-2 receptor are involved in the observed effects as these subunits are usually essential in mediating various actions of IL-2. In any case, our results suggest that the integrity of the receptor complex with a fully available α subunit is required for the effect of IL-2 on ACh release. It could also be of interest to establish if other cytokines such as IL-4, IL-7, IL-9, IL-13 and IL-15, which all apparently share various subunits of the IL-2 receptor complex (Bamford *et al*., 1994; Giri *et al*., 1994; Grabstein *et al*., 1994; Kishimoto *et al*., 1994) can also modulate ACh release. Thus far, only IL-4 which shares the γ subunit of the IL-2 receptor, has been studied in this regard, and it failed to modulate hippocampal ACh release (Araujo *et al*., 1989).

The use of TTX allowed us to distinguish between the stimulant and inhibitory actions of IL-2 on hippocampal ACh release. The TTX-insensitive nature of the potentiating effect of IL-2 on ACh release suggests an action directly on or in very close proximity to the septo-hippocampal cholinergic nerve terminals. Accordingly, it is likely that IL-2 receptors postively modulating ACh release are directly located on cholinergic terminals. In support of this hypothesis, we have recently shown that IL-2 can stimulate choline acetyltransferase (ChAT) activity in primary septal cultures enriched in cholinergic neurones (Mennicken & Quirion, 1996). In contrast, the inhibitory effect of higher concentrations of IL-2 on hippocampal ACh release is most likely indirect as it is fully reversed by TTX. In fact, in the presence of TTX, a nM concentration of IL-2 demonstrated a potent stimulating action on ACh release (Figure 3).

Thus it would appear that IL-2 receptors are located both on hippocampal cholinergic nerve terminals as well as on other neuronal populations distal to them; the resulting effect on ACh release depending upon the concentration of IL-2 available in the intercellular space, higher concentrations leading to the release of inhibitory sustance(s), counteracting the direct, stimulating effect of IL-2 on cholinergic nerve terminals. To our knowledge, this is the first example of a cytokine having such a complex profile on the release of a given neurotransmitter in the brain.

The nature of the inhibitory substance possibly involved in mediating the TTX-sensitive effects of IL-2 was investigated next. Some studies have suggested the possible involvement of endogenous opioid peptides in certain CNS effects of IL-2 (Araujo *et al*., 1990; Lapchak & Araujo, 1993; Jiang *et al*., 1995). It has recently been proposed that IL-2 could directly act on opioid receptors to induce its effects (Jiang *et al*., 1995). Moreover, since various opioid peptides have shown to modulate ACh release (Lapchak *et al*., 1989), naloxone, a potent opioid receptor antagonist was tested in our model. It failed to alter the TTX-sensitive inhibitory action of IL-2 on ACh release suggesting that opioid peptides and their receptors are unlikely to be involved, at least under our assay conditions. An inhibition of nitric oxide synthase, L-NMMA (Bredt *et al*., 1991; Karanth *et al*., 1993; Raber *et al*., 1995) was investigated next since recent data have shown that some effects of IL-2 in the CNS, especially at the hypothalamus, probably involve the participation of NO or NO-related mechanisms (Karanth *et al*., 1993; Raber & Bloom, 1994; Raber *et al*., 1995). There is also evidence that NO may potentiate hippocampal ACh release (Lonart *et al*., 1992). However, the inhibition of NO synthase failed to alter the inhibitory effect of a nM concentration of IL-2 on ACh release. Similarly, the potentiating action of a lower concentration of IL-2 failed to be modulated by the inhibition of the production of NO. Accordingly, it seems unlikely that NO plays a critical role in the modulation of the biphasic effects of IL-2 on hippocampal ACh release.

GABA is a major inhibitory neurotransmitter in the hippocampus (for a recent review, Thompson, 1994) and the existence of GABA receptor sub-types in the brain is well established (Sivilotti & Nistri, 1991). Accordingly, the possible role of GABA and its receptors in the inhibitory action of IL-2 on ACh release was investigated by use of $GABA_A$ (bicuculline: Olpe *et al*., 1988; Arenas *et al*., 1990) and $GABA_B$ (phaclofen: Soltesz *et al*., 1988; Arenas *et al*., 1990) receptor antagonists. Interestingly and rather surprisingly, both antagonists blocked the inhibitory effects of IL-2. In addition, the combination of both antagonists was also effective in blocking the IL-2-induced inhibition of ACh release. These results suggest that the release of the inhibitory transmitter GABA induced by a nM concentration of IL-2 acts on both $GABA_A$ and $GABA_B$ receptor sub-types to dampen hippocampal ACh release. The simultaneous participation of both the $GABA_A$ and $GABA_B$ receptor sub-types in the inhibitory action of GABA has already been shown to occur in the human cortex on the basis of electrophysiological data (McCormick, 1989). To our knowledge, the present results are the first to suggest possible interactions between GABAergic neurotransmission and IL-2 in the CNS and studies are currently under way to establish further the nature of this association.

In summary, IL-2 is among the very most potent modulators of ACh release in the rat hippocampus. The stimulant effect observed at a very low (10^{-13} M) concentration is TTX-resistant suggesting a direct action on cholinergic nerve terminals. In contrast, the inhibitory action observed at a nM concentration is TTX-sensitive and is probably mediated by the GABAergic sytem. In view of the well established role of hippocampal cholinergic innervation in learning and memory (see Björklund & Dunnett, 1995 for a recent discussion), and the marked alteration of this structure and cholinergic markers in neurodegenerative disorders such as Alzheimer's disease (AD; Bartus *et al*., 1982; Selkoe, 1993), it is of interest that earlier studies have shown an increased expression of IL-2 and IL-2 receptors in AD (Luber-Narod & Rogers, 1988; Araujo & Lapchak, 1994). On the basis of the present results, it is tempting to speculate that heightened IL-2 activity could exacerbate cholinergic deficits in AD by inhibiting ACh release. Further studies in this respect are certainly warranted in view of the recent surge of interest on the possible involvement of a neuro-immune component in AD (McGeer *et al*., 1994).

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