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Short communication

Inhibition of interleukin-1β-induced pyresis in the rabbit by peptide 204–212 of lipocortin 5

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Abstract

The intracerebroventricular administration of interleukin-1β (12.5 ng/kg) in rabbits caused a prompt rise of prostaglandin E_2 concentration (+632.6 ± 243.9%) in the cerebrospinal fluid followed by hyperthermia (+1.61 ± 0.14Δ°C). The intracerebroventricular administration of an anti-inflammatory nonapeptide (amino acids 204–212, SHLRKVFDK) derived from lipocortin 5, thereafter referred to as lipocortin 5-(204–212)-peptide, inhibited in a significant manner both the increase in cerebrospinal fluid [prostaglandin E_2] and the febrile response induced by the cytokine. This inhibitory effect is probably due to interference by the peptide with phospholipase A_2 activity. A control peptide (FKRVHDLKS) formed by the same amino acids in a randomly shuffled sequence had no effect. These results show that, in addition to the anti-inflammatory effect previously reported, the peptide 204–212 of lipocortin 5 possesses, like glucocorticoids, anti-pyretic activity. The research on lipocortin-derived peptides may lead to the development of novel anti-inflammatory and anti-pyretic compounds.

Keywords: Lipocortin/annexin; Anti-inflammatory peptide; Interleukin-1β; Fever; Prostaglandin E_2

1. Introduction

Lipocortins (annexins) are a family of calcium- and phospholipid-binding proteins which may mediate some of the anti-inflammatory effects of glucocorticoids. To date anti-inflammatory activity has been described for lipocortin 1, lipocortin 2, and lipocortin 5 (Cirino et al., 1989; Parente et al., 1990; Becherucci et al., 1993). Moreover and of some importance for the present paper both lipocortin 1 and a lipocortin 1 fragment exhibited antipyretic activity in rats (Carey et al., 1990) and in rabbits (Davidson et al., 1991). Since lipocortins are large proteins of 35–40 kDa, research has been focused on the attempt to identify smaller peptides which may mimic the action of the whole proteins in order to overcome stability and delivery problems (reviewed by Perretti, 1994). We have previously described a nonapeptide (residues 204–212) from lipocortin 5, thereafter referred to as lipocortin 5-(204–212)-peptide, able to reproduce some of the anti-inflammatory effects of glucocorticoids such as inhibition of both eicosanoid formation in vitro and carrageenin-induced rat paw edema (Perretti et al., 1991; Douglas et al., 1992). In the present paper we report that lipocortin 5-(204–212)-peptide, like dexamethasone, inhibited the increase of prostaglandin E_2 concentration in the cerebrospinal fluid and the hyperthermia induced by intracerebroventricular injection of interleukin-1β in rabbits.

2. Materials and methods

2.1. Experimental protocol

The animal surgery and the methodology for assessment of rectal temperature and prostaglandin E_2 con-

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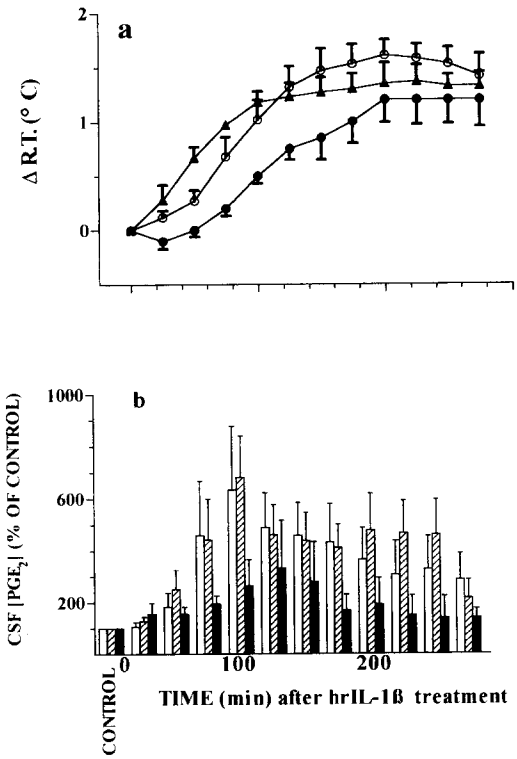

Fig. 1. Effects of peptide 204–212 of lipocortin 5 and a control peptide on rectal temperature (R.T.) (panel a) and prostaglandin E_2 concentration ([PGE_2]) (panel b) in rabbit cerebrospinal fluid (CSF) after intracerebroventricular injection of interleukin-1β (IL-1β). (○) and open columns indicate IL-1β (12.5 ng/kg at time 0); (▲) and hatched columns indicate control peptide; (●) and solid columns indicate lipocortin 5-(204–212)-peptide. Three separate intracerebroventricular injections of peptides were carried out at −50 min (100 μg/kg), −25 min (50 μg/kg), and +100 min (50 μg/kg). Panel (a) shows the mean Δ rectal temperature ±S.E.M. from 4–7 rabbits. Baseline values of rectal temperature were: IL-1β, 38.6±0.2°C (n = 7); control peptide, 39.05±0.19°C (n = 4); lipocortin 5-(204–212)-peptide, 38.6±0.14°C (n = 5). In panel (b) columns (mean±S.E.M. of triplicate determinations of cerebrospinal fluid fractions from 4–7 animals) show percentage deviations in [PGE_2] over controls. Controls (100%) represent the average concentration from 4 fractions collected over the period −100–0 min. Group data of rectal temperature and [PGE_2] were compared statistically by ANOVA across all treatment conditions. IL-1β vs. (IL-1β + control peptide): R.T. and [PGE_2], not significant. IL-1β vs. (IL-1β + lipocortin 5-(204–212)-peptide): R.T. and [PGE_2], $P < 0.01$. (IL-1β + control peptide) vs. (IL-1β + lipocortin 5-(204–212)-peptide): R.T. and [PGE_2], $P < 0.01$.

centration ([prostaglandin E_2]) in the cerebrospinal fluid has been recently described in detail (Palmi et al., 1994). Briefly, male New Zealand rabbits (2.0–2.5 kg) under anesthesia were implanted with cannulae in one lateral ventricle and the cisterna magna. Cerebrospinal fluid samples were collected every 25 min up to 275 min from cisterna magna in restrained conscious rabbits. Human recombinant interleukin-1β (12.5 ng/kg) was administered intracerebroventricularly at time 0 (see Fig. 1) according to previous experiments (Palmi et al., 1994). The peptides were administered intracerebroventricularly 3 times according to the following schedule: at −50 min (100 μg/kg), at −25 min (50 μg/kg), and at +100 min (50 μg/kg). The doses of peptides were chosen on the basis of their efficacy in carrageenin-induced edema experiments as previously reported (Perretti et al., 1991). Rectal temperature was measured every 25 min up to 275 min by a thermocouple thermometer connected to a personal computer with an iso-thermex software (Columbus, OH, USA). [Prostaglandin E_2] in cerebrospinal fluid was determined by radioimmunoassay (NEN-Du Pont, Dreieich, Germany). Reference values for cerebrospinal fluid [prostaglandin E_2] and rectal temperature were collected over the period −100–0 min (4 fractions) of each experiment.

2.2. Materials

Dexamethasone acetate was purchased from Sigma (St. Louis, MO, USA). Human recombinant interleukin-1β (specific activity 10^9 units/mg) was prepared at the Immunobiology Research Institute, Siena, Italy. Lipocortin 5-(204–212)-peptide (SHLRKVFDK) and the control shuffled peptide (FKRVHDLKS) were purchased from Peptide & Protein Research, Exeter Devon, UK. Purity was always >95% analysed by high-performance liquid chromatography; amino acid composition and M_r were confirmed by mass spectrometry (data from the manufacturer).

2.3. Statistical analysis

Values are expressed as mean ± S.E.M. Data were compared by one-way analysis of variance with $P < 0.05$ considered significant.

3. Results

Fig. 1 shows that an intracerebroventricular injection of interleukin-1β (12.5 ng/kg) at 0 time caused a prompt and significant rise in rectal temperature as well as an increase in cerebrospinal fluid [prostaglandin E_2]. The peak of [prostaglandin E_2] (+632.6 ± 243.9%) was observed at 100 min followed by the peak rise in temperature (+1.61 ± 0.14°C) at 200 min after cytokine injection. The intracerebroventricular administration of lipocortin 5-(204–212)-peptide significantly counteracted both the hyperthermia and the increase in cerebrospinal fluid [prostaglandin E_2] induced by interleukin-1β. The control peptide had no effect on either parameter. In addition the values obtained with the active peptide were significantly lower than those obtained with the control peptide. The baseline values of the rectal temperature did not change significantly among the three experimental groups (see the legend to Fig. 1).

4. Discussion

These results demonstrate that lipocortin 5-(204–212)-peptide inhibited the rise in body temperature and in cerebrospinal fluid [prostaglandin E_2] induced by an intracerebroventricular injection of interleukin-1β. A control peptide formed by the same amino acids in a randomly shuffled sequence was devoid of inhibitory effect. We have recently reported that these actions of interleukin-1β are likely due to an increase of the calcium concentration in the cerebrospinal fluid which in turn may activate extracellular phospholipase A_2 leading to enhanced prostaglandin E_2 synthesis which is the final effector of the hyperthermia. We have also shown that the interleukin-1β effects are inhibited by the previous administration of dexamethasone which could interfere with both calcium increase and phospholipase A_2 activation (Palmi et al., 1994). It is conceivable that the inhibitory effects of lipocortin 5-(204–212)-peptide are due to inhibition of phospholipase A_2 activity. This peptide has been shown to reduce the release of eicosanoids from cultured cells and from arterial preparations, to block phospholipase A_2-induced, but not arachidonate-induced, contractions of rat stomach strips and to reduce carrageenin-induced rat paw edema when given locally (Perretti et al., 1991; Douglas et al., 1992). On the other hand, it is unlikely that lipocortin 5-(204–212)-peptide may interfere with cerebrospinal fluid calcium increase since structural studies have shown that the peptide is not part of the calcium binding site of lipocortin 5. These studies (Huber et al., 1990) have also shown that the peptide falls in the third repeat of the parent protein and it is exposed on the convex surface of the molecule where it may potentially interact with biological membranes.

In conclusion, these data confirm in vivo the in vitro inhibition of prostanoid release by lipocortin 5-(204–212)-peptide previously reported (Perretti et al., 1991; Douglas et al., 1992). They also demonstrate that, in addition to the anti-inflammatory effect, the peptide possesses, like glucocorticoids, anti-pyretic properties. The research on lipocortin-derived peptides may lead to the development of novel anti-inflammatory and anti-pyretic compounds.

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