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Interleukin-1 receptor antagonist does not reverse lipopolysaccharide-induced inhibition of water intake in rat

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Abstract

The involvement of interleukin-1 in antidipsogenic effects induced by intraperitoneal (i.p.) administration of lipopolysaccharide (0.32, 0.64 and 0.96 mg/kg) in 24-h water-deprived rats, was evaluated by injection of human interleukin-1 receptor antagonist (10, 25 and 50 μg/rat) into the lateral cerebral ventricle (i.c.v.). The effects of either lipopolysacharide or human interleukin-1 receptor antagonist treatment on rectal temperature of 24-h water-deprived rats, were examined. Our date show that human interleukin-1 receptor antagonist administration is able to reverse, dose dependently, fever, but not lipopolysaccharide inhibition of thirst. The reduction of pyrogenic, but not of antidipsogenic, effects of lipopolysaccharide following human interleukin-1 receptor antagonist administration suggests that lipopolysaccharide inhibition of thirst is not dependent on interleukin-1-induced fever and that interleukin-1 is not a direct mediator implicated in inhibition of water intake provoked by peripheral injection of lipopolysaccharide.

Keywords: Water intake; Lipopolysaccharide; Interleukin-1; (Human); Interleukin-1 receptor; Antagonist

1. Introduction

Bacterial endotoxins (lipopolysaccharides) are localized exclusively in the outer membrane of Gram-negative bacteria and their use in a valid model for the study of the pathological events occurring during Gram-negative infections (Morrison and Ryan, 1987).

There is much evidence that demonstrates, in the central nervous system (CNS), the influence of lipopolysaccharide administration on body temperature, somnogenic activity and drinking behaviour (Focà et al., 1983, 1985; Calapai et al., 1990; Krueger, 1990; Wang and Evered, 1993). The injection of lipopolysaccharide in vivo induces a large release of cytokines which are probably responsible for the most important symptoms associated with the activation of host defence responses (Hesse et al., 1988; Henricson et al., 1991; Derijk et al., 1993).

Various studies suggest that interleukin-1, either alone or in combination with other cytokines, is involved in many of the pathophysiological responses observed after lipopolysacharide administration, such as activation of the hypothalamus-pituitary-adrenal axis, fever, hypoglycemia, hypotension, increased somnolence, decreased appetite, inhibition of water intake, shock and death (Rivier et al., 1989; Dinarello, 1991; Kluger, 1991; Cunningham and De Souza, 1993; Derijk et al., 1993; Calapai et al., 1994).

Interleukin-1 is produced by activated mononuclear phagocytes and other cells and is defined, based on its effects on body temperature, as an endogenous pyrogen (Dinarello, 1991). Interleukin-1 exists in two forms: interleukin-1α and interleukin-1β (Dinarello, 1991). Interleukin-1β is the major secreted form and is evident in a high concentration in plasma, tissue fluids and brain (Rothwell, 1991).

Two receptors for interleukin-1 have been characterized, type I known to mediate signalling activity, and type II that acts as a regulated decoy target for interleukin-1 (Colotta et al., 1994). The interleukin-1 receptor type II binds interleukin-1β substantially better than interleukin-1α (Sims et al., 1993).

Recently, a human IL-1 receptor antagonist has been cloned and characterized (Hannum et al., 1990). The human interleukin-1 receptor antagonist is able to block the binding and many of the biological effects of both interleukin-1α and interleukin-1β.

There is important evidence that interleukin-1β exerts

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potent actions on neuronal functions, behavioural patterns and neuroendocrine systems and metabolism (Rothwell, 1991; Cunningham and De Souza, 1993). In particular, it seems to have an antidipsogenic effect on 24-h water deprivation-induced drinking (Masotto et al., 1992).

The principal aims of our work were to study the possible common pathways of antidipsogenic effects of lipopolysaccharide and interleukin-1 on drinking induced by 24-h water deprivation in rats.

In order to clarify the real role of interleukin-1 in water intake and in the influence of body temperature on thirst, the effects of the block of the receptor for interleukin-1 with human interleukin-1 receptor antagonist were examined.

2. Materials and methods

2.1. Animals

Adult male Sprague-Dawley rats weighing 280–320 g were used. The animals were housed at a constant temperature of 22 ± 2°C under a 12/12 h light-dark cycle (lights on at 06:00 h), with free access to Purina rat chow pellets and tap water, unless otherwise stated.

2.2. Water intake evaluation

Three different groups of animals were used: 24-h water-deprived rats (control), 24-h water-deprived rats with lipopolysaccharide administration and 24-h water-deprived rats with lipopolysaccharide injection and treated with human interleukin-1 receptor antagonist. Drinking was elicited by 24-h water deprivation. The rats had free access to food during this period. Water intake following deprivation was monitored 180 min after saline or lipopolysaccharide administration for a 60 min period and expressed as ml/rat. Water was provided in graduated burettes, with drinking spouts, allowing direct volumetric measurement of intake to the nearest 0.1 ml.

2.3. Rectal temperature evaluation

Rectal temperature was recorded 120, 180 and 240 min from saline or lipopolysaccharide administration using an Elektrolaboratoriet thermometer type T.E.3. Temperatures were allowed to stabilize for 15–30 s before readings were taken. All measurements were made at the subthermoneutral ambient temperature of 24 ± 1°C.

2.4. Intracerebral injections

A 23-gauge stainless steel guide cannula was inserted, 7 days before the experiments, into the lateral ventricle (i.c.v.). The rats were anesthetized with chloral hydrate (400 mg/kg i.p.). The coordinates were chosen according to the atlas of Paxinos and Watson (1986) (AP = +2.5 mm with respect to the bregma, L = 0 mm with respect to the midline, H = −8.0 mm from the surface of the brain). Injections into the lateral ventricle (1 μl) were made with a 30-gauge injector temporarily inserted into the guide cannula and protruding 2 mm beyond the cannula tip. Injections were carried out over a period of 1–2 min.

2.5. Treatments

Lipopolysaccharide at doses of 0.32, 0.64 and 0.96 mg/kg was intraperitoneally (i.p.) injected 180 min before water presentation, in order to obtain its maximum effects (Focà et al., 1983), while naive-deprived animals received saline solution (1 ml/kg).

Finally, a 24-h water-deprived group was treated with human interleukin-1 receptor antagonist at doses 10, 25 and 50 μg/rat intracerebroventricurlarly (i.c.v.), 15 min before, immediately prior to and 90 min after lipopolysaccharide administration.

2.6. Drugs

Escherichia coli lipopolysaccharide (055: B5 phenol extract) was obtained from Sigma (USA); and human interleukin-1 receptor antagonist was purchased from Genzyme (Cinisello Balsamo, Milano, Italy).

Lipopolysaccharide was dissolved in 0.9% NaCl solution and warmed to 37°C before the injection.

2.7. Statistical analysis

The data are expressed as the means ± S.D. Statistical analysis of data was performed by one-way analysis of variance (ANOVA). Statistical significance was set at $P < 0.01$.

3. Results

3.1. Effects of lipopolysaccharide on water intake

The effects of lipopolysaccharide (0.32, 0.64 and 0.96 mg/kg) on water intake, 180 min after i.p. administration to 24-h water-deprived rats, were as other experiments (Focà et al., 1983; Calapai et al., 1990), significantly and dose dependently antidipsogenic (Fig. 1).

3.2. Effects of lipopolysaccharide administration on rectal temperature

I.p. injection of lipopolysaccharide (0.32, 0.64 and 0.96 mg/kg), increased significantly and dose dependently, rectal temperature compared with values obtained from saline-treated controls (Fig. 2). Rectal temperature was

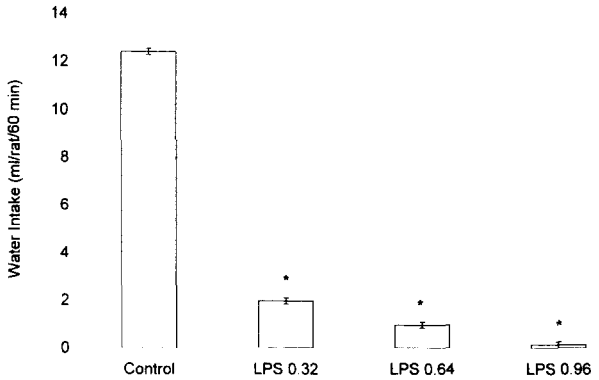

Fig. 1. Effects of i.p. administration of saline (control) or lipopolysaccharide (LPS) at different concentrations on drinking behaviour induced by 24-h water deprivation. The lipopolysaccharide doses are expressed as mg/kg. Each column represents the mean ± S.D. for 5 animals. * $P < 0.001$ vs. control.

evaluated 120, 180 and 240 min after saline or lipopolysaccharide treatment.

3.3. Effect of human-interleukin-1 receptor antagonist on lipopolysaccharide inhibition of thirst

The i.c.v. administration of human interleukin-1 receptor antagonist at doses 10, 25 and 50 μg/rat, 15 min before, immediately prior to and 90 min after lipopolysaccharide given i.p. (0.32, 0.64 and 0.96 mg/kg) was unable to reverse antidipsogenic effects induced by i.p. injection of endotoxin (Table 1).

Table 1
Effects of highest doses of human IL-1 receptor antagonist (IL-1ra) or saline (control) given i.c.v. at −15, 0 and 90 min after i.p. lipopolysaccharide (LPS) or saline administration on 60 min water intake (ml/rat) stimulated by 24-h water deprivation in rat

Animals (n)	IL-1ra treatment (μg/rat i.c.v.)			LPS treatment (mg/kg i.p.)	Water intake (ml/rat)
	−15 min	0 min	90 min		
5	Saline	-	-	Saline	12.70 ± 1.00
5	Saline	-	-	0.32	1.94 ± 0.16 *
5	50	-	-	0.32	1.96 ± 0.11 *
5	Saline	-	-	0.64	0.93 ± 0.11 *
5	50	-	-	0.64	0.92 ± 0.16 *
5	Saline	-	-	0.96	0.14 ± 0.05 *
5	50	-	-	0.96	0.12 ± 0.04 *
5	-	Saline	-	Saline	12.64 ± 0.20
5	-	Saline	-	0.32	1.96 ± 0.15 *
5	-	50	-	0.32	1.92 ± 0.13 *
5	-	Saline	-	0.64	0.93 ± 0.11 *
5	-	50	-	0.64	0.90 ± 0.10 *
5	-	Saline	-	0.96	0.12 ± 0.04 *
5		50	-	0.96	0.12 ± 0.04 *
5	-	-	Saline	Saline	12.58 + 0.19
5		-	Saline	0.32	1.96 ± 0.16 *
5	-	-	50	0.32	1.90 ± 0.14 *
5	-	-	Saline	0.64	0.93 ± 0.11 *
5	-	-	50	0.64	0.92 ± 0.13 *
5	-	-	Saline	0.96	0.10 ± 0.07 *
5	-	-	50	0.96	0.12 ± 0.04 *

Water presentation was 180 min after lipopolysaccharide administration.
* $P < 0.001$ vs. control.

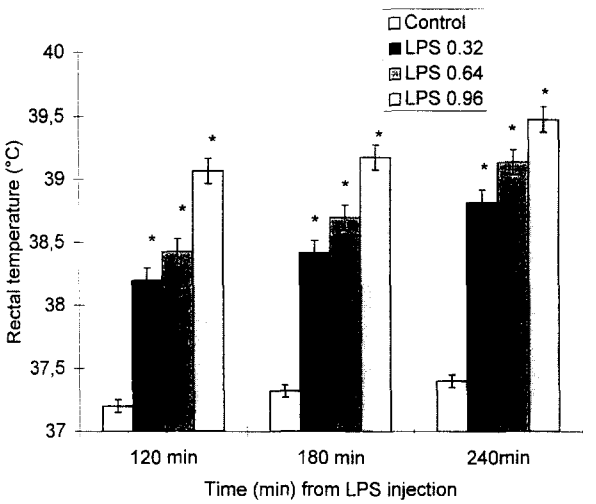

Fig. 2. Rectal temperature of rats 120, 180 and 240 min after i.p. injection of saline (control) or lipopolysaccharide (LPS) at different doses. The lipopolysaccharide doses are expressed as mg/kg. Each column represents the mean ± S.D. for 5 animals. * $P < 0.001$ vs. control.

3.4. Effects of human interleukin-1 receptor antagonist on rectal temperature after lipopolysaccharide administration

Human interleukin-1 receptor antagonist (10, 25 and 50 μg/rat) given i.c.v. 15 min before, immediately prior to and 90 min after lipopolysaccharide i.p. treatment (0.32, 0.64 and 0.96 mg/kg), greatly reduced, in a dose-depen-

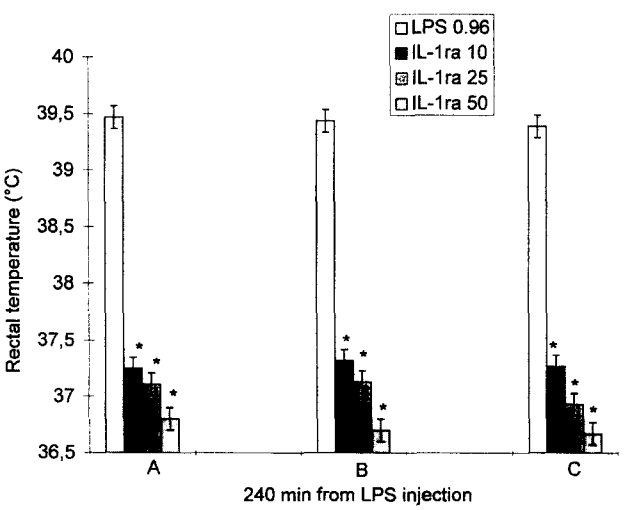

Fig. 3. Effects of i.c.v. administration of human interleukin-1 receptor antagonist (IL-1ra) at −15 (A), 0 (B) and 90 min (C) after pyrogen administration on rectal temperature 240 min after i.p. injection of the highest doses of lipopolysaccharide (LPS). The human interleukin-1 receptor antagonist doses are expressed as μg/rat and those of lipopolysaccharide as mg/kg. Each column represents the mean ± S.D. for 5 animals. * $P < 0.001$ vs. group treated with lipopolysaccharide.

dent manner, the magnitude of the lipopolysaccharide-induced fever (Long et al., 1990; Rothwell et al., 1990; Rothwell, 1991) (Fig. 3). Body temperature was evaluated 240 min after lipopolysaccharide treatment.

4. Discussion

Various studies have demonstrated that the systemic administration of lipolysaccharide induces, with an unclear mechanism (Banks et al., 1991; Hopkins and Rothwell, 1995; Rothwell and Hopkins, 1995), high concentrations of interleukin-1 and other cytokines, such as interleukin-6, and tumor necrosis factor-α in the brain (Klir et al., 1993). These cytokines, and in particular interleukin-6, cause the release of prostaglandins, particularly prostaglandin E_2 and prostaglandin $F_{2\alpha}$, responsible for the induction of fever elicited by peripheral injection of lipopolysaccharide (Kluger, 1991; Coceani et al., 1992; Klir et al., 1993). Previous data suggest that prostaglandins within the preoptic area may mediate the andipsogenic effects of *Escherichia coli* endotoxin (Focà et al., 1985) and that inhibitors of cyclooxygenase block the lipopolysaccharide inhibition of water intake (Calapai et al., 1990). However, no direct evidence of prostaglandin involvement in the inhibitory effects of interleukin-1 on water intake is available (Masotto et al., 1992).

The brain interleukin-1 receptors controlling pyrogenic actions are sensitive to the human interleukin-1 receptor antagonist (Coceani et al., 1992). In particular, pretreatment i.c.v. with human interleukin-1 receptor antagonist is unable to antagonize the effects induced by an i.c.v. bolus of lipopolysaccharide, while it can reverse the actions induced by i.c.v. interleukin-1 admininstration (Coceani et al., 1992). Also, human interleukin-1 receptor antagonist given i.c.v. is completely ineffective against systemic interleukin-1 or lipopolysaccharide administration (Coceani et al., 1992). This supports the idea that peripheral production of interleukin-1, induced by systemic endotoxin administration, is able to induce febrile effects with mediation by brain regions, such as organum vasculosum laminae terminalis, and can, with unclear mechanism, translate the interleukin-1 stimulus into thermoregulatory areas (Coceani et al., 1992). Central regions regulating temperature are directly affected by interleukin-1 formed in brain in response to a local noxious stimulus (Coceani et al., 1992). This evidence suggests the existence of two different pathways in the central mechanisms of fever depending on the nature of the initial insult (Coceani et al., 1992). There is recent evidence demonstrating that many of central antidipsogenic effects induced by lipopolysaccharide are mediated by an overproduction of nitric oxide (NO) (Calapai et al., 1992, 1994). It has also been shown that NO synthase-like activity can be induced in microglia and astrocytes by lipopolysaccharide (Simmons and Murphy, 1992).

Since our data showed that human interleukin-1 receptor antagonist administration is able to reverse lipopolysaccharide-induced fever, but not endotoxin inhibition of thirst, we can conclude that interleukin-1 is not a crucial factor for lipopolysaccharide inhibition of water intake, and perhaps it has no direct effect on central brain areas controlling water intake. These results are in accord with results of recent in situ hybridization studies in the mouse that confirm the presence of interleukin-1 receptors in central regions that are not directly involved in thirst mechanisms (Cunningham and De Souza, 1993). In particular, the distribution of neurons synthesizing interleukin-1 receptor are expressed in dentate gyrus, CA3 pyramidal neurons, anterior dorsal nucleus of the thalamus, dorsal raphe, mesencephalic nucleus of the trigeminal nerve, Purkinje cells of the cerebellum, anterior pituitary gland, although interleukin-1 receptors may be transported to axon terminals in the other CNS regions (Colotta et al., 1994) including thirst centers (e.g. preoptic area) (Masotto et al., 1992). Therefore, we believe that interleukin-1 is only one of the most important mediators (e.g. NO), implicated in antidipsogenic effects caused by lipopolysaccharide peripheral administration. This is in accord with our earlier data suggesting that N^G-nitro-L-arginine methyl ester (L-NAME), an inhibitor of NO synthase, and Methylene blue, an inhibitor of guanylate cyclase activation, given into the preoptic area, antagonize, in a specific manner, the inhibition of drinking induced by lipopolysaccharide (Calapai et al., 1994).

In the light of the above findings, we can conclude that the effects of lipopolysaccharide on drinking behaviour, in 24-h water-deprived rats, are not principally and directly mediated by interleukin-1. In particular, we suppose that interleukin-1 could be an indirect mediator implicated in lipopolysaccharide inhibition of thirst, activating transducer mechanisms or various substances which carry the antidipsogenic stimulus to the central areas controlling water intake. For example, it could be possible that interleukin-1 increases the expression of NO synthase in brain (Nussler et al., 1992).

Our studies on body temperature show that human interleukin-1 receptor antagonist is able to control lipopolyssaccharide fever (Long et al., 1990; Rothwell et al., 1990; Rothwell, 1991), but does not reverse the antidipsogenic effects of lipopolysaccharide treatment, suggesting, therefore, that interleukin-1-induced fever is not a true impediment to drinking behaviour.

Finally, we can conclude that interleukin-1 is not the single and or a direct mediator involved in antidipsogenic effects of lipopolysaccharide treatment and that fever does not hinder water intake.

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