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Central effects of cromoglycate sodium salt in rats treated with lipopolysaccharide

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Abstract

In 24-h water- and food-deprived rats, we have evaluated the effects of cromoglycate sodium salt, an inhibitor of the mast cell degranulation with anti-inflammatory and membrane-stabilizating activity, on the central effects induced by *Escherichia coli* lipopolysaccharide (LPS). Intraperitoneal (i.p.) injection of LPS (0.25, 0.50 and 1 mg/kg) induced a dose-dependent inhibition of water and food intake, fever, reduction in locomotor activity as well as increased anxiety levels. All these LPS effects were antagonized by a prior intracerebroventricular (i.c.v.) injection of cromoglycate sodium salt (100, 150 and 200 μg/rat). Our findings suggest that peripheral LPS administration may activate brain mast cells and indicate an involvement of these cells in brain pathophysiology.

Keywords: Cromoglycate sodium salt; Lipopolysaccharide; Water intake; Food intake; Fever; Locomotor activity; Anxiety; Mast cell

1. Introduction

The activity of the immune system is influenced by specific brain areas such as hypothalamus and limbic system and by different neurotransmitters such as norepinephrine and dopamine (Felten et al., 1991; Zalcman et al., 1991; Black, 1994a; Madden and Felten, 1995). Conversely, the immune system stimulates neurotransmission, possibly via release of several autacoid factors (Black, 1994b; Maier et al., 1994; Linthorst et al., 1995a,b; Leonard and Song, 1996; Linthorst et al., 1996, 1997).

Recent studies have shown that brain mast cells could be involved in neural-immune interactions (Silver et al., 1996). Various authors have indicated that endogenous (e.g., neuropeptides or neurotransmitters) or exogenous agents (e.g., antigens and trauma) (Theoharides, 1990; Johnson and Krenger, 1992; Theoharides et al., 1995) can activate brain mast cells, inducing the release of several classes of mediators that may alter neuronal function: these include histamine, serotonin, pro-inflammatory cytokines, proteases, neuropeptides, prostaglandins, nitric oxide, etc. (Galli and Lichtenstein, 1988; Gordon et al., 1990; Bisonnette et al., 1991; Galli et al., 1991; Johnson and Krenger, 1992; Purcell and Atterwill, 1995; Silver et al., 1996). Moreover, several studies have demonstrated that degranulation of mast cells may initiate and modulate a number of important inflammatory cascades (Burd et al., 1989; Plaut et al., 1989; Wodnar-Filipowicz et al., 1989; Gordon and Galli, 1990; Lambracht-Hall et al., 1990; Gordon and Galli, 1991; Galli et al., 1993; Bebo et al., 1996) that may affect the integrity of the blood–brain barrier (Purcell and Atterwill, 1995; Roznieckl et al., 1995; Zhuang et al., 1996). Interestingly, recent data indicate that mast cells are involved in some neurological diseases such as multiple sclerosis (Theoharides, 1990; Aloe et al., 1994; Roznieckl et al., 1995), autoimmune encephalomyelitis (Bebo et al., 1996) and cluster headache (Dimitriadou et al., 1990).

Lipopolysaccharide (LPS), regarded as a complex glycolipid localised in the outer membrane of gram-negative bacteria, either injected or generated during the course of infections, induces through various mechanisms, several pathophysiological conditions such as fever (Kluger, 1991; Derijk et al., 1993; Klir et al., 1993), sleepiness (Krueger, 1990), inhibition of water (Nava et al., 1996, 1997a,b) and food intake (O'Reilly et al., 1988; Yirmina, 1996), reduction in locomotor activity (Kozak et al., 1994; Yirmina,

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1996) as well as depressive like signs (Yirmina, 1996). Various authors have shown that several of the central LPS effects may be mediated by an overproduction of free radicals, pro-inflammatory cytokines and others autacoid factors (Kent et al., 1993; Hopkins and Rothwell, 1995; Rothwell and Hopkins, 1995; Merril and Benveniste, 1996).

The present study was undertaken to elucidate the role of brain mast cells in relation to the behavioural and pyrogenic responses induced by systemic LPS administration. In particular, we have studied the effects of intracerebroventricular (i.c.v.) administration of cromoglycate sodium salt, an inhibitor of mast cell degranulation with anti-inflammatory and membrane-stabilizating activity (Shapiro and Koning, 1985; Ochoa de Aspuru and Lourdes-Zaton, 1994; Norris, 1996) in 24-h water- and food-deprived rats treated i.p. with LPS.

2. Materials and methods

2.1. Animals

Adult male Sprague–Dawley rats weighing 280–320 g were used. The animals were housed at constant temperature of 23 ± 1°C under a 12/12 light–dark cycle (light on at 0600 h) and had free access to Purina rat chow pellets and tap water, unless otherwise stated.

2.2. Experimental procedure

Our experiment was designed to assess the effect of cromoglycate sodium salt, given i.c.v., immediately prior to an i.p. LPS injection on water and food intake, body temperature, locomotor activity and anxiety levels in 24-h water- and food-deprived rats.

Sixteen groups ($n = 5$) of 24-h water- and food-deprived rats were treated as follows: (1) one group with saline (1 ml/kg, i.p.); (2) three groups with a dose of LPS (0.25, 0.50 or 1 mg/kg, i.p.); (3) one group with saline (3 μg/rat, i.c.v.) immediately prior to a saline dose (1 ml/kg, i.p.); (4) three groups with saline (3 μg/rat, i.c.v.) immediately prior to a LPS dose (0.25, 0.50 or 1 mg/kg, i.p.); (5) three groups with a dose of cromoglycate sodium salt (100, 150 or 200 μg/rat, i.c.v.) immediately prior to saline administration (1 ml/kg, i.p.); (6) three groups with a dose of cromoglycate sodium salt (100, 150 or 200 μg/rat, i.c.v.) immediately prior to LPS (1 mg/kg, i.p.) administration; (7) two groups with a dose of heat-inactivated cromoglycate sodium salt (200 μg/rat, i.c.v.) (at 120°C for 90 min) immediately prior to saline (1 ml/kg, i.p.) or LPS administration (1 mg/kg, i.p.). Treatments were started at 0800 h. Each assessment (i.e., water and food intake, body temperature, locomotory activity and anxiety level determinations) was done in different groups of animals.

2.3. Intracerebroventricular injections

A 23-gauge steel guide cannula was inserted in the left lateral cerebral ventricle (i.c.v.), 7 days before the experiments. The rats were anaesthetised with equitensin (4 mg/kg, i.p.). The coordinates were chosen according to the atlas of Paxinos and Watson (1986) (AP = +1.6 mm respect to the bregma, L = 0.90 mm with respect to the

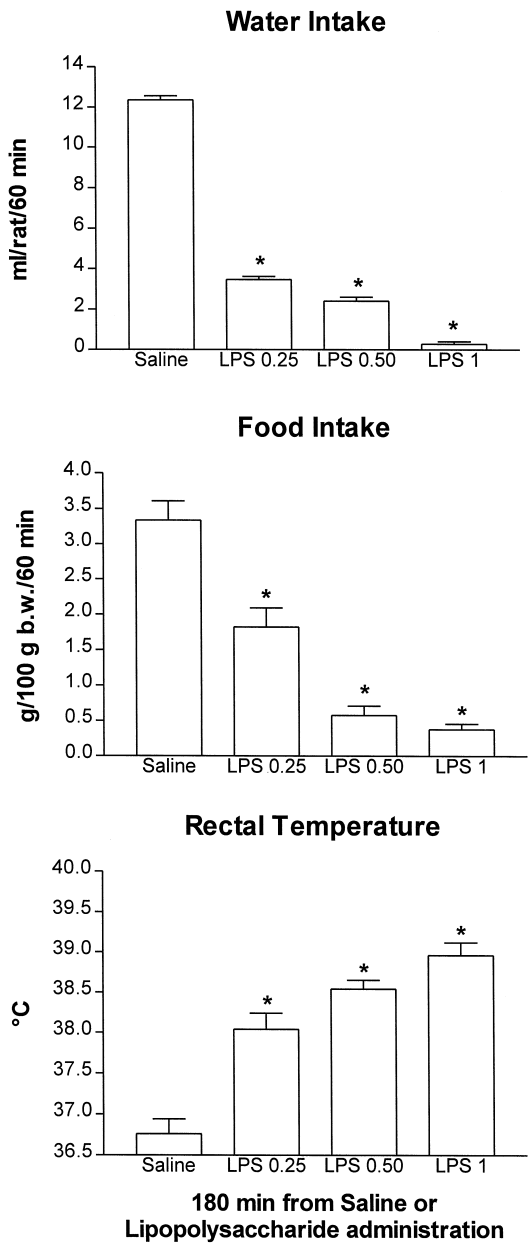

Fig. 1. Effect of i.p. administration of LPS (0.25, 0.50, and 1 mg/kg) or saline (0.3 ml/kg) on water (top) and food intake (middle), and on rectal temperature (bottom) in 24-h water- and food-deprived rats. Each column represents the mean ± S.D. for five animals. The tests were performed 180 min after LPS or saline administration. $^{*}P < 0.05$ vs. controls (ANOVA); $^{a}P < 0.05$ vs. LPS dose of 1 mg/kg (post-hoc Dunnett's test); $^{b}P < 0.05$ vs. LPS dose of 0.25 mg/kg (post-hoc Dunnett's test).

midline, H = −4.80 mm from the surface of the brain). I.c.v. injections of saline (3 μl/rat), cromoglycate sodium salt (3 μl/rat) or heat-inactivated cromoglycate sodium salt (3 μl/rat) were made with a 30-gauge injector temporarily inserted into the guide cannula and protruding 2 mm beyond the cannula tip. Injections were given over a period of 1–2 min.

Post-mortem histological examination (all animals were sacrificed with an overdose of chloral hydrate) confirmed the location of the guide cannula.

2.4. Water intake and food intake evaluation

Thirst and hunger were elicited by 24-h water and food deprivation (Squadrito et al., 1993; Nava et al., 1996). Water intake was tested by measuring the volume of water (ml/rat) taken over a 60-min period. Water was provided in graduated burettes with drinking spouts allowing direct volumetric measurement of intake to the nearest 0.1 ml. In the same period, pre-weighed food was presented to the animals and the amount consumed (g/100 g body wt.) was evaluated by weighing the remaining amounts 60 min after food presentation. The drinking spouts and the food were placed at a height accessible to the experimental animals (5 cm from the floor of the cage), so they did not need to rear up to reach water and food. Water and food intake evaluation started 180 min after i.p. saline or LPS administration.

2.5. Rectal temperature evaluation

Rectal temperature was recorded using an Elektrolaboratoriet thermometer type T.E.3. The thin probe of thermometer was inserted for about 5 mm in the rectum. Temperature was allowed to equilibrate for 15–30 s before reading were taken. All measurements were made at an ambient temperature of 23 ± 1°C. The rectal temperature was recorded 180 min after i.p. saline or LPS treatment.

2.6. Locomotor activity evaluation in the open field apparatus

Locomotor activity was studied with an open-field apparatus (Shagal, 1993) in a lit and a quiet room. The floor of the open field (100 × 100 and 24 cm high) was divided into nine identical squares (32 × 32 cm^2). The rats were placed individually in one corner of the open field and the time elapsed before they started exploring the environment was recorded as the starting latency. They were allowed to freely explore the environment for 6 min. During this period, ambulation was measured by counting the number of times that the rats crossed from one square to another. Into a square entry was counted when all four limbs of the rat were within the given square. The frequency of rearing was also counted. Animals behaviour was recorded on videotape then scored by two observers uninformed about the drug treatment. The data obtained from two scores

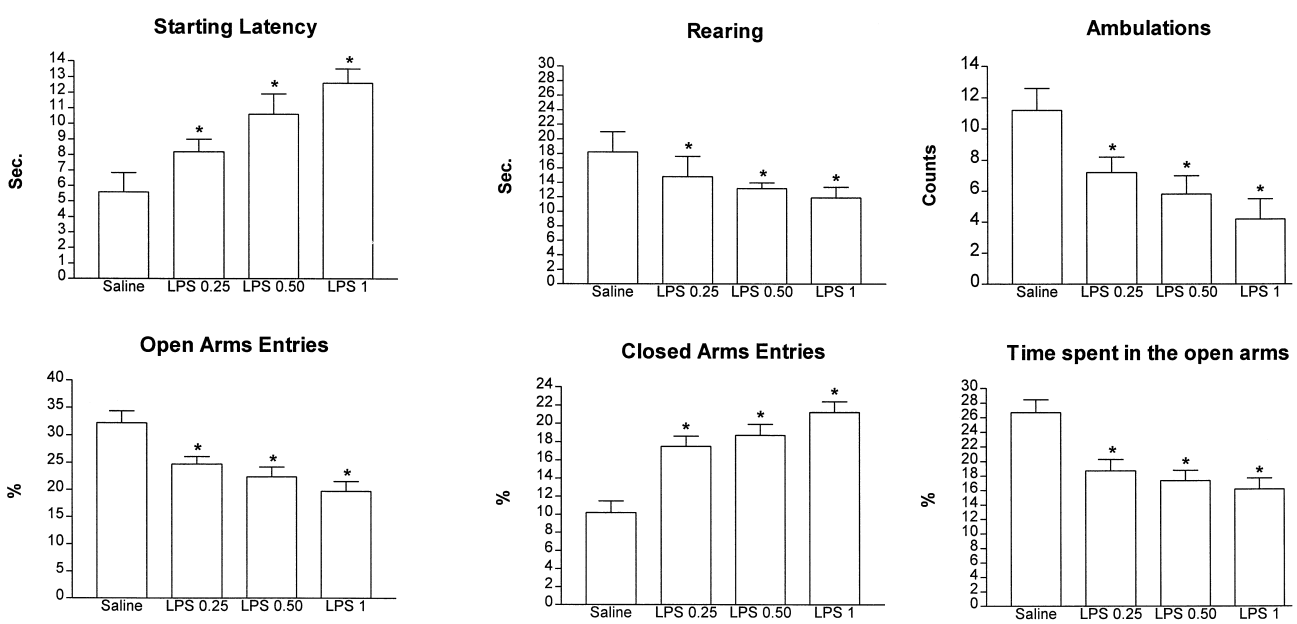

Fig. 2. Effect of i.p. administration of LPS (0.25, 0.50, 1 mg/kg) or saline (0.3 ml/kg) on locomotor activity (top) and anxiety levels (bottom) in 24-h water- and food-deprived rats. Each column represents the mean ± S.D. for five animals. The tests were performed 180 min after LPS or saline administration. * $P < 0.05$ vs. controls (ANOVA); [a] $P < 0.05$ vs. LPS dose of 1 mg/kg (post-hoc Dunnett's test); [b] $P < 0.05$ vs. LPS dose of 0.25 mg/kg (post-hoc Dunnett's test).

were averaged. Locomotor activity evaluation started 180 min after i.p. saline or LPS administration.

2.7. Anxiety levels evaluation in the plus-maze apparatus

Anxiety levels were determined with a plus-maze apparatus (Pellow et al., 1985). The apparatus is in the shape of a plus sign with two open arms (50 × 10 cm) and two arms enclosed by high walls (50 × 10 × 50 cm), extending from a central area (10 × 10 cm). The plus-maze was raised to a height of 50 cm and placed in a lit and quiet room. At the beginning of the experiment, an animal was placed at the centre of the plus-maze, facing the open arm. During a 5-min observation period, the following parameters were measured: the number of open arms entries, the time spent in open arms and the number of closed arm entries. Then, the percentage of entries into the open and closed arms and the percentage of time spent in the open arms were calculated. An arm entry was counted when all four limbs of the rat were within the given arms. The data obtained from two scores were averaged. Anxiety level evaluation started 180 min after saline or LPS administration.

2.8. Drugs

Escherichia coli LPS (055: B5 phenol extract) and cromoglycate sodium salt were obtained from Sigma (USA).

LPS and cromoglycate sodium salt were dissolved in 0.9% NaCl. LPS was warmed to 37°C before the injection.

2.9. Statistical analysis

The data are expressed as means ± S.D. Statistical analysis was performed by omnibus One-way analysis (ANOVA) followed by a post-hoc Dunnett's test. Statistical significance was set at $P < 0.01$.

3. Results

3.1. LPS effects

LPS given at the doses of 0.25, 0.50 and 1 mg/kg induced, in dose dependently and significantly, inhibition of the consumption of water ($F_{(3,16)} = 299.90$, $P < 0.01$) and food ($F_{(3,16)} = 220.19$, $P < 0.01$) and caused fever ($F_{(3,16} = 165.30$, $P < 0.01$) (Fig. 1). LPS at the same doses when tested in the open field reduced locomotor activity ($F_{(3,16)}$ starting latency = 39.15, $P < 0.01$; $F_{(3,16)}$ rearing = 7.98, $P < 0.01$; $F_{(3,16)}$ ambulation = 39.15, $P < 0.01$) and when tested in the elevated plus-maze, increased anxiety levels ($F_{(3,16)}$ open arms entries = 43.44, $P < 0.01$; $F_{(3,16)}$ closed arms entries = 77.25, $P < 0.01$; $F_{(3,16)}$ time spent in the open arms = 44.98, $P < 0.01$) (Fig. 2).

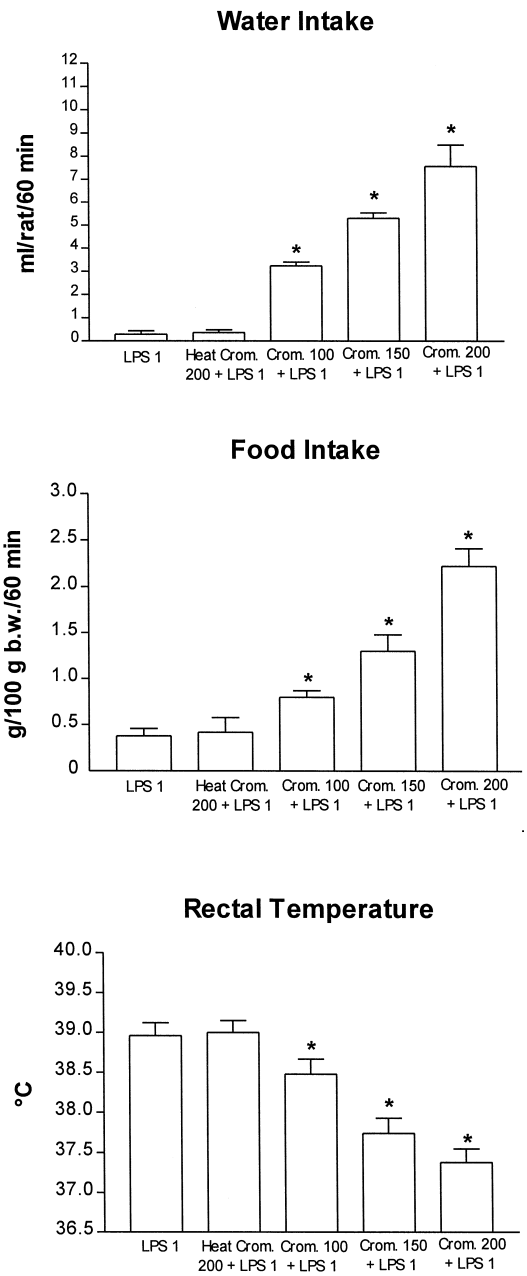

Fig. 3. Effect of i.p. administration of LPS (1 mg/kg), LPS (1 mg/kg)+ heat cromoglycate sodium salt (200 μg/rat) or LPS (1 mg/kg)+ cromoglycate sodium salt (100, 150 and 200 μg/rat) on water (top) and food intake (middle) and on rectal temperature (bottom) in 24-h water- and food-deprived rats. Each column represents the mean ± S.D. for five animals. The tests were performed 180 min after LPS administration. * $P < 0.05$ vs. controls (ANOVA); [a] $P < 0.05$ vs. LPS dose of 1 mg/kg (post-hoc Dunnett's test); [b] $P < 0.05$ vs. LPS dose of 0.25 mg/kg (post-hoc Dunnett's test).

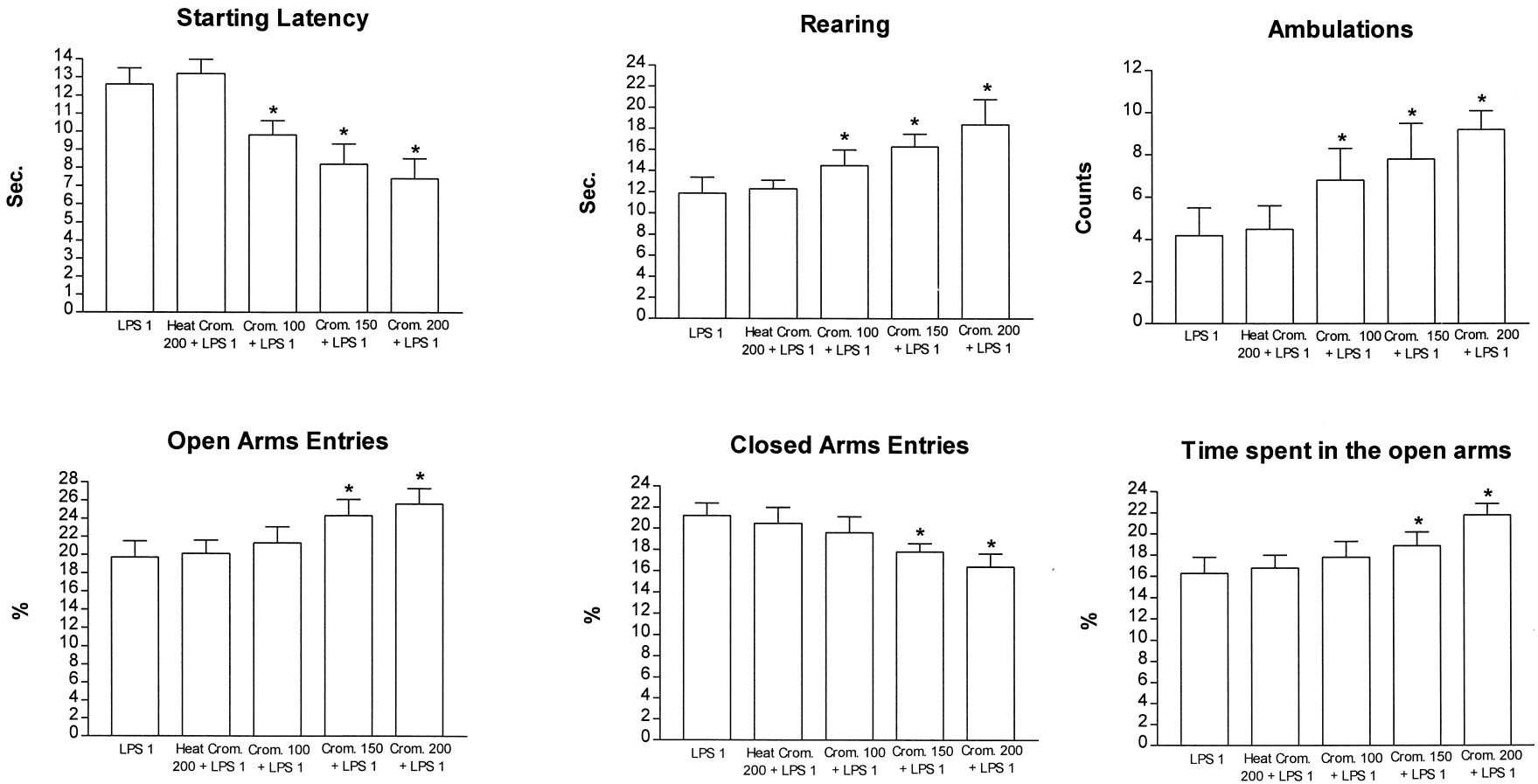

Fig. 4. Effect of i.p. administration of LPS (1 mg/kg), LPS (1 mg/kg)+heat cromoglycate sodium salt (200 μg/rat) or LPS (1 mg/kg)+cromoglycate sodium salt (100, 150 and 200 μg/rat) on locomotor activity (top) and anxiety levels (bottom) in 24-h water- and food-deprived rats. Each column represents the mean±S.D. for five animals. The tests were performed 180 min after LPS administration. * $P < 0.05$ vs. controls (ANOVA); [a] $P < 0.05$ vs. LPS dose of 1 mg/kg (post-hoc Dunnett's test); [b] $P < 0.05$ vs. LPS dose of 1 mg/kg (post-hoc Dunnett's test).

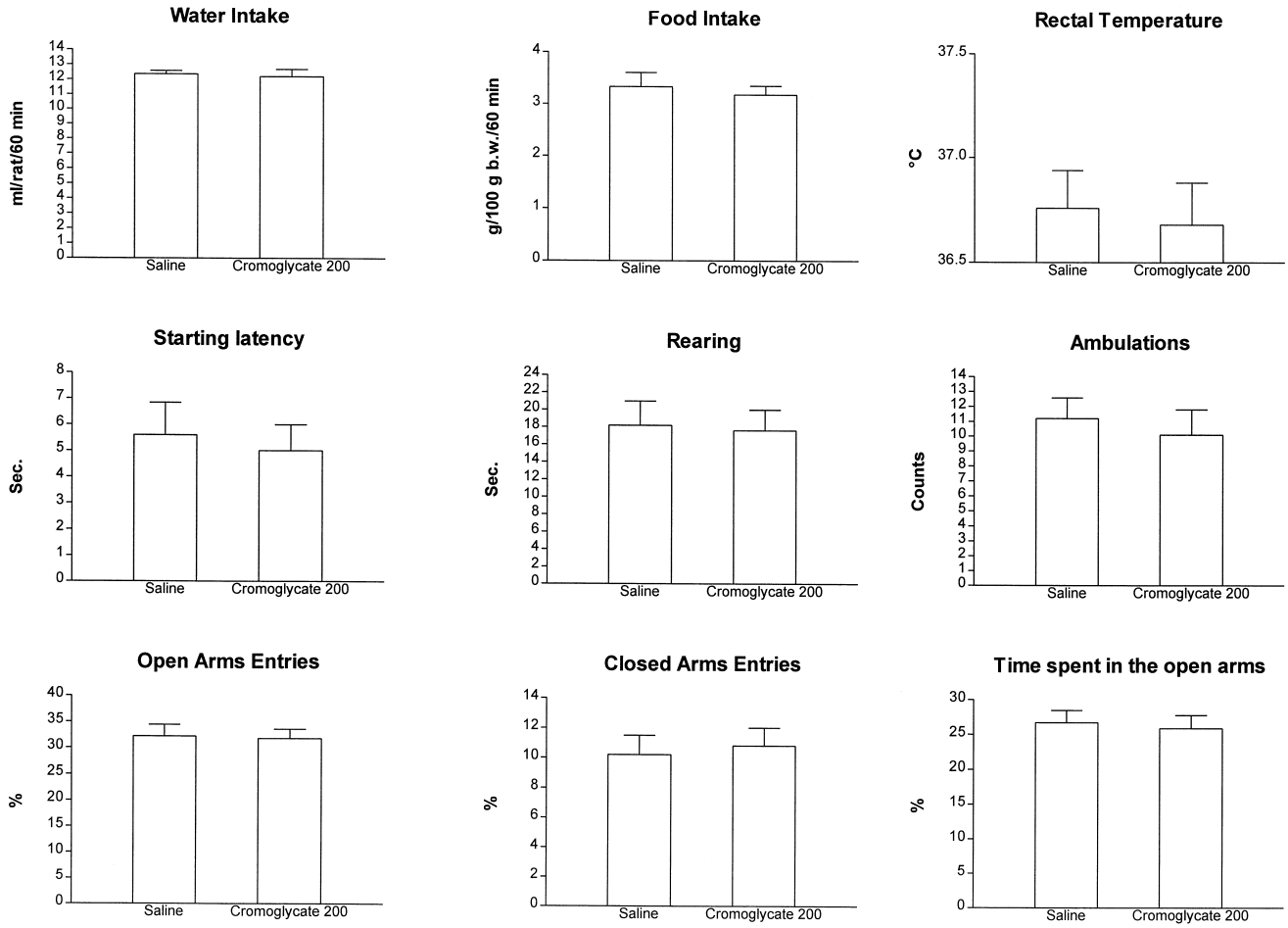

Fig. 5. Effect of i.c.v. injection of saline (3 μl/rat) or cromoglycate sodium salt (200 μg/rat) on water and food intake and rectal temperature (top), locomotor activity (middle) and anxiety levels (bottom) in 24 h water- and food-deprived rats. Each column represents the mean ± S.D. for five animals. The tests were performed 180 min after LPS or saline administration.

3.2. Cromoglycate sodium salt effects on LPS administration

Cromoglycate sodium salt (100, 150, and 200 μg/rat) given i.c.v., immediately prior the highest i.p. LPS dose (1 mg/kg) significantly reduced the inhibition of water ($F_{(3,16)} = 197.43$, $P < 0.01$) and food intake ($F_{(3,16)} = 157.08$, $P < 0.01$) as well as fever ($F_{(3,16)} = 80.27$, $P < 0.01$) caused by LPS (Fig. 3). Interestingly, the cromoglycate sodium salt injection antagonised when tested in the open field, the reduction of locomotor activity ($F_{(3,16)}$ starting latency = 27.22, $P < 0.01$; $F_{(3,16)}$ rearing = 27.22, $P < 0.01$; $F_{(3,16)}$ ambulation = 11.26, $P < 0.01$) and when tested in the elevated plus-maze, the increase in anxiety levels ($F_{(3,16)}$ open arms entries = 11.59, $P < 0.01$; $F_{(3,16)}$ closed arms entries = 15.19, $P < 0.01$; $F_{(3,16)}$ time spent in the open arms = 15.05, $P < 0.01$) observed after LPS treatment (Fig. 4).

On the contrary, the heat-inactivated cromoglycate sodium salt (200 μg/rat) modified none of the LPS effects (Figs. 3 and 4).

I.c.v. administration of cromoglycate sodium salt (200 μg/rat) or saline (3 μl/rat) per se did not influence water and food intake, rectal temperature, locomotor activity and anxiety levels (Fig. 5).

4. Discussion

Our results confirmed that systemic LPS administration inhibits the consumption of water (Nava et al., 1996, 1997a,b) and food (O'Reilly et al., 1988; Yirmina, 1996), reduces locomotor activity (Yirmina, 1996; Nava et al., 1997b), increases anxiety levels (Maier et al., 1994; Leonard and Song, 1996) and has pyrogenic properties (Kluger, 1991; Derijk et al., 1993; Klir et al., 1993; Elmquist et al., 1997). These findings are in accord with results of previous experiments demonstrating that LPS treatment induces a response in brain neurotransmission (Linthorst et al., 1995a,b, 1996, 1997) and activation of the hypothalamic–pituitary–adrenocortical axis (Linthorst et al., 1995a). Despite extensive study, the mechanism of

action of LPS in the brain has yet to be fully elucidated (Sugino et al., 1989; Ulevitch and Tobias, 1995). Several lines of investigations now suggest that its primary action in the brain may be mediated by an increase in the concentrations of pro-inflammatory cytokines and several autacoid factors (Kent et al., 1993; Layé et al., 1994; Hopkins and Rothwell, 1995; Rothwell and Hopkins, 1995; Merril and Benveniste, 1996). Moreover, it has also been proposed that LPS may stimulate cerebral lipid peroxidation and oxidative damage through an increased production of reactive oxygen intermediates (Sakaguchi et al., 1981; Sugino et al., 1989; Yoshikawa et al., 1994). Based on this evidence, many of the tissue injuries induced by LPS could be mediated by an overproduction of reactive oxygen, free radicals, proteases and pro-inflammatory cytokines (Nathan, 1982; Batuista and Spitzer, 1990; Lipton et al., 1993; Breder et al., 1994; Yirmina, 1996).

Since treatment with cromoglycate sodium salt, an agent with anti-inflammatory and membrane-stabilizating activity and able to block mast cell degranulation, antagonised the behavioural and pyrogenic effects induced by LPS we could believe in an interaction between LPS administration and brain mast cells. Several in vitro studies have shown that chemokines and growth factors can induce mast cell degranulation (Valent, 1995; Metcalfe et al., 1997). On this basis, it is believable that systemic LPS administration may affects brain mast cells through a cytokine- and autacoid-mediated process. On the other hand, after i.p. LPS administration, mast cell degranulation may contribute to affect water and food intake, body temperature, locomotion and anxiety by increasing brain release of cytokines, autacoid factors and free radicals. Moreover, LPS could induce direct activation of brain mast cells through a receptor-dependent mechanism (Ulevitch and Tobias, 1995).

The exact mechanism of action of sodium cromoglycate salt in brain still remains unclear. Cromoglycate sodium salt produces a stabilising action on the cell membrane and blocks mast cell degranulation, but it is still not known exactly how this drug acts in the brain. Several of the cromoglycate sodium salt effects observed after LPS administration could be due to a block of the release of mast cells mediators. Several authors have proposed that the cromoglycate sodium salt may induce neurotransmission changes and may affect neurons that mediate analgesia, locomotor activity and opiate abstinence (San-Martin-Clark et al., 1993, 1995). However, since the cromoglycate sodium salt is only active after local administration and does not easily cross the blood–brain barrier (Shapiro and Koning, 1985; Leone Bay et al., 1996; Norris, 1996), it is clear that its effects on LPS injuries are not due to a systemic action of the drug.

Further studies are necessary to make clear the cromoglycate sodium salt actions on central LPS effects. Moreover, the lack of a way to measure in vivo cerebral mast cell activity and the release of their mediators does not permit the elucidation of the mechanisms that could be involved in the brain cromoglycate sodium salt effects. However, although many facets of the central actions of cromoglycate sodium salt remain to be clarified a possible role of the mast cells in central LPS actions appears to be likely.

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