

VAGAL AFFERENT INPUT FROM THE ACID-CHALLENGED RAT STOMACH TO THE BRAINSTEM: ENHANCEMENT BY INTERLEUKIN-1 β

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Abstract—Exposure of the gastric mucosa to back-diffusing concentrations of HCl (0.25 M, pH 0.51) stimulates vagal afferent input to the brainstem. Here we have examined whether pretreatment of rats with the proinflammatory cytokines interleukin-1 β and tumor necrosis factor- α causes sensitization of vagal afferent pathways to HCl. Rats were pretreated i.p. with interleukin-1 β , tumor necrosis factor- α (10 μ g/kg) or their vehicle (sterile saline) 24, 48 and 96 h before intragastric administration of HCl (0.25 M, 1 ml/100 g). Activation of neurons in the nucleus tractus solitarii was visualized by c-Fos immunohistochemistry 2 h after the HCl challenge. I.p. administration of interleukin-1 β and tumor necrosis factor- α alone induced c-Fos in the brainstem, an effect that was gone after 24 h. At this time, however, the effect of HCl to cause expression of c-Fos in the nucleus tractus solitarii was significantly enhanced by pretreatment with interleukin-1 β and tumor necrosis factor- α . The sensitizing effect of i.p.-administered interleukin-1 β was sustained for more than 48 h and prevented by the interleukin-1 receptor antagonist anakinra. Intracisternal administration of interleukin-1 β and tumor necrosis factor- α (100 ng) failed to amplify the HCl-evoked expression of c-Fos in the brainstem.

These results show that peripheral administration of the proinflammatory cytokines interleukin-1 β and tumor necrosis factor- α induces prolonged sensitization of vagal afferent pathways to gastric HCl challenge. This effect seems to arise from a peripheral action on vagal afferents and may be of relevance to gastric chemoreception. © 2004 Published by Elsevier Ltd on behalf of IBRO.

Key words: acid insult, gastric mucosa, expression of c-Fos, nucleus tractus solitarii, proinflammatory cytokines, sensitization of vagal afferent pathways.

Analysis of the afferent signaling of chemical noxae from the stomach to the brain is of relevance to the understanding of gastric nociception. By visualizing neuronal excitation through expression of the c-fos gene we have found that exposure of the rat gastric mucosa to back-diffusing concentrations of hydrochloric acid is transmitted, via vagal afferents, to the medullary brainstem but not spinal cord (Schuligoi et al., 1998; Michl et al., 2001; Danzer et al., 2004a). The same concentrations of intragastric (IG)

HCl that induce c-Fos in the brainstem also evoke a visceromotor response, i.e. an electromyographic reaction in the neck muscles indicative of gastric pain (Lamb et al., 2003). This correlation, the ability of vagotomy to block both the medullary c-Fos and the visceromotor response to gastric acid challenge and the observation that gastric inflammation and ulceration amplify the gastric acid-evoked pain reaction (Lamb et al., 2003) indicate that vagal afferents play a particular role in gastric chemoreception.

The overall hypothesis tested in the present study was that proinflammatory cytokines such as interleukin-1 β (IL-1 β) and tumor necrosis factor- α (TNF- α) sensitize vagal afferent pathways to gastric acid challenge. This hypothesis was modeled not only by the idea that cytokines cause hypersensitivity of spinal afferent neurons (Fu and Longhurst, 1999; Kreis et al., 2000; Oprea and Kress, 2000; Parada et al., 2003) but also by the concept that vagal sensory neurons participate in the communication between the peripheral immune system and the brain (Goehler et al., 2000; Holzer, 2002; Konsman et al., 2002). As nodose ganglion neurons express IL-1 receptors (Ek et al., 1998), i.v. and i.p. administration of IL-1 β leads to increased firing in vagal afferents and induces c-Fos in the nucleus tractus solitarii (NTS), the central projection area of vagal sensory neurons (Brady et al., 1994; Ericsson et al., 1994; Day and Akil, 1996; Nijima, 1996; Kurosawa et al., 1997; Ek et al., 1998).

The first specific aim was to examine whether IG administration of IL-1 β evokes vagal afferent input to the NTS as visualized by c-Fos immunohistochemistry and to compare the activity of IG- and i.p.-administered IL-1 β . In addition, the effect of i.p.-administered IL-1 β was compared with that of two other cytokines, TNF- α and IL-6 (Konsman et al., 2002). Since IL-1 β can sensitize GI afferents to chemical stimuli (Bucinskaite et al., 1997; Fu and Longhurst, 1999; Kreis et al., 2000), the second aim was to explore whether pretreatment of rats with IL-1 β , TNF- α or IL-6 would amplify the responsiveness of the vagal afferent system as determined by an enhanced medullary c-Fos response to gastric acid challenge.

The third aim was to explore whether cytokine-evoked sensitization of the vagal afferent system to gastric acid is brought about by a peripheral or central site of action. Apart from directly acting on vagal afferents (Day and Akil, 1996; Nijima, 1996; Kurosawa et al., 1997; Bucinskaite et al., 1997; Ek et al., 1998), circulating cytokines may enhance c-Fos expression in the NTS via mechanisms that involve the area postrema which is exempt from the blood–brain barrier (Goehler et al., 2000; Konsman et al., 2002). This question was addressed by comparing the activity of

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Abbreviations: ANOVA, analysis of variance; BSA, bovine serum albumin; IC, intracisternal/intracisternally; IG, intragastric/intragastrically; IL, interleukin; NTS, nucleus tractus solitarii; PBS, phosphate-buffered saline; TNF, tumor necrosis factor.

i.p. and intracisternally (IC) injected IL-1 β and TNF- α to amplify the gastric acid-evoked induction of c-Fos in the NTS. The fourth aim was to investigate whether i.p. administration of the IL-1 receptor antagonist anakinra would prevent the effect of peripheral IL-1 β to enhance the activation of NTS neurons elicited by gastric acid challenge.

EXPERIMENTAL PROCEDURES

Animals

The study was approved by an ethics committee at the Federal Ministry of Education, Science and Culture of the Republic of Austria and conducted according to the Directive of the European Communities Council of 24 November 1986 (86/609/EEC). The experiments were designed in such a way that the number of animals used and their suffering was minimized. Female age-matched Sprague–Dawley rats (Institut für Labortierkunde und -genetik, Himberg, Austria) weighing 180–220 g were used. They were housed in groups of three in plastic transparent cages under standard conditions; lights were on from 6:00 AM until 6:00 PM.

Experimental protocols

All experiments took place during the light phase. Four experimental studies with four different protocols were carried out. In study 1 the acute effects of IL-1 β , IL-6 and TNF- α to induce c-Fos in the NTS were examined. Before treatment with these compounds the animals were fasted for 20 h but had free access to water. In one series of experiments, the rats were treated i.p. with 0.5, 1, 5 or 10 μ g/kg IL-1 β , 10 μ g/kg IL-6, 10 μ g/kg TNF- α or their vehicle at a volume of 1 ml/kg. Before treatment, the injection area on the abdomen was sterilized with ethanol. In another series of experiments, the animals were treated IG with 1, 5, 10, or 20 μ g/kg IL-1 β or its vehicle, which were administered through a soft infant feeding tube (outer diameter 2.2 mm; Portex, Hythe, UK) at a volume of 1 ml/kg. Two hours after these acute treatments the rats were deeply anesthetized with an overdose of pentobarbital (200 mg/kg) and transcardially perfused with 150 ml of 0.1 M phosphate-buffered saline (PBS) of pH 7.4, followed by 4% buffered paraformaldehyde (250 ml).

In study 2 non-fasted animals were pretreated i.p. with IL-1 β , IL-6, TNF- α (each at 10 μ g/kg) or their vehicle at a volume of 1 ml/kg. Twenty-four, 48 or 96 h after this i.p. pretreatment the rats received an IG bolus of HCl (0.25 M, pH 0.51) or saline at a volume of 10 ml/kg. Before this IG treatment, the animals were fasted for 20 h but had free access to water. Two hours after the IG treatment the animals were deeply anesthetized and perfused with paraformaldehyde as described above.

In study 3 the effect of IC injected IL-1 β and TNF- α on the medullary expression of c-Fos was examined. To this end, the animals were anesthetized by injection of ketamine (100 mg/kg) and xylazine (8 mg/kg) into the gluteus muscle and placed in a stereotaxic apparatus. The injection area in the neck was sterilized with ethanol. After insertion of a 10 ml Hamilton needle-syringe combination in the cisterna magna, 5 ml of cerebrospinal fluid was withdrawn and IL-1 β , TNF- α (each at 100 ng) or their vehicle injected slowly over a period of 2 min at a volume of 5 ml. Forty-eight hours after this IC pretreatment the rats received an IG bolus of HCl (0.25 M, 10 ml/kg) or saline. Before this IG treatment, the animals were fasted for 20 h but had free access to water. Two hours after the IG treatment the animals were deeply anesthetized and perfused with paraformaldehyde as described above.

In study 4 it was examined whether the effect of i.p. treatment with IL-1 β to enhance the acid-evoked expression of c-Fos in the NTS is prevented by i.p. pretreatment with the IL-1 receptor antagonist anakinra. For this purpose, rats were pretreated i.p. first

with anakinra (1 mg/kg) or its vehicle and 15 min later with IL-1 β (10 μ g/kg) or its vehicle, all injections being given at a volume of 1 ml/kg. Forty-eight hours after this i.p. pretreatment the rats received an IG bolus of HCl (0.25 M, 10 ml/kg). Before this IG treatment, the animals were fasted for 20 h but had free access to water. Two hours after the IG treatment the animals were deeply anesthetized and perfused with paraformaldehyde as described above.

Immunohistochemistry

Following perfusion of the animals with paraformaldehyde, the brains were removed and postfixed overnight in 4% buffered paraformaldehyde at 4 °C. Then the tissues were cryoprotected for 48 h in 20% sucrose at 4 °C, frozen on dry ice and stored at –70 °C until immunohistochemistry. For this purpose, coronal sections (35 μ m) were cut serially on a cryostat (Microm, Walldorf, Germany) from the medullary region of the brainstem at the rostrocaudal extension of the area postrema, which was identified according to Paxinos and Watson (1997). Every second section was taken and processed free-floating, washed three times in 0.1 M PBS and then incubated in PBS containing 0.3% H₂O₂ for 30 min to block endogenous peroxidase. After rinsing three times with PBS, the sections were incubated for 1.5 h in a blocking solution consisting of 0.3% Triton X-100, 1% bovine serum albumin (BSA) and 5% goat serum in PBS at room temperature, followed by incubation with the primary antibody (rabbit polyclonal anti-c-Fos, 1:20,000) for 48 h at 4 °C. After three washes in PBS the sections were incubated for 1.5 h in a solution containing the secondary antibody (biotinylated goat anti-rabbit). Three other washes with PBS (10 min each) were followed by a 1 h incubation in avidin–biotin complex. The sections were rinsed again and developed with 3,3'-diaminobenzidine substrate intensified with nickel sulfate for about 2 min. Tissues were then mounted on gelatin-covered slides, air-dried overnight, dehydrated through an alcohol series and cover-slipped with Entellan. Preabsorption controls were performed with a c-Fos blocking peptide.

Substances and solutions

The primary antibody (rabbit polyclonal anti-c-Fos) and the c-Fos blocking peptide were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The secondary antibody (goat anti-rabbit), the avidin–biotin complex and 3,3'-diaminobenzidine were part of the Vectastain Elite KIT which was purchased from Vector Laboratories (Burlingame, CA, USA). Entellan and IL-1 β (recombinant rat IL-1 β , solid) came from Sigma (Vienna, Austria), while IL-6 (recombinant rat IL-6) and TNF- α (recombinant rat TNF- α) were provided as solutions by R&D Systems (Minneapolis, MN, USA). Anakinra (Kineret[®], recombinant non-glycosylated form of the human IL-1 receptor antagonist, provided as solution) was a gift of Amgen (Thousand Oaks, CA, USA). IL-1 β was dissolved and diluted with sterile saline (0.15 M NaCl) containing 0.1% pyrogen-free BSA. This vehicle was also used to dilute IL-6 and TNF- α . One milliliter of the anakinra vehicle was made of 5.48 mg sodium chloride, 1.29 mg sodium citrate, 0.12 mg disodium EDTA and 0.7 mg Tween 80.

Analysis and statistics

The brainstem sections were examined in a coded manner with a light microscope (Axiophot; Zeiss, Oberkochen, Germany). At least four sections per animal were analyzed, the c-Fos-positive cells counted on one side of the NTS, and the number of immunopositive cells in all sections of each animal averaged. These average values were then used to calculate the mean number of c-Fos-positive cells in the NTS of each experimental group. The data are presented as means \pm S.E.M., *n* referring to the number of rats in the respective group. Statistical evaluation of the results

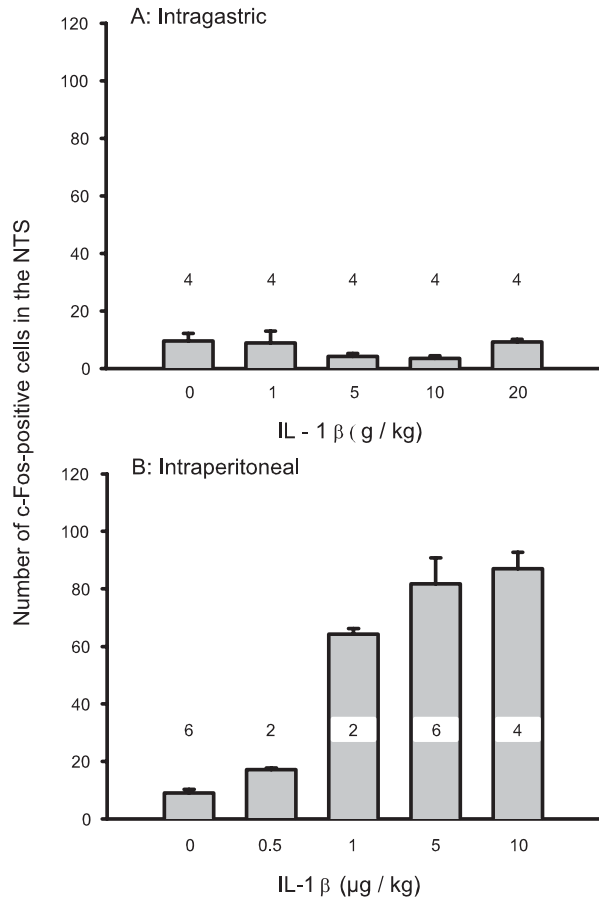


Fig. 1. Effect of IG (A) and i.p. (B) administration of IL-1 β or its vehicle (indicated by the dose 0) to induce c-Fos in the NTS. The neurons expressing c-Fos were visualized by immunohistochemistry 2 h post-treatment. The values represent means \pm S.E.M., *n* as indicated.

was performed on Sigma-Stat with Student's *t*-test or one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test. Probability values of $P < 0.05$ were regarded as significant.

RESULTS

Effect of peripheral administration of IL-1 β , IL-6 and TNF- α to induce c-Fos in the NTS

Acute exposure of the rat gastric mucosa to IL-1 β (1–20 μ g/kg), relative to vehicle (saline), failed to cause any expression of c-Fos in the NTS as examined 2 h post-treatment (Fig. 1A). However, when IL-1 β (0.5–10 μ g/kg) was administered i.p., the number of c-Fos-positive cells in the NTS increased in a dose-dependent manner, the maximal effect being seen at a dose of 5 μ g/kg (Fig. 1B). I.p. administration of TNF- α (10 μ g/kg) likewise enhanced the expression of c-Fos in the NTS as investigated 2 h post-treatment. While in rats treated with vehicle 9.0 ± 1.4 ($n=6$) cells in the NTS stained for c-Fos, 57.1 ± 6.7 ($n=3$) cells expressed c-Fos following treatment with TNF- α . In contrast, i.p. administration of IL-6 (10 μ g/kg) was unable to induce c-Fos in the NTS 2 h post-treatment, the number of c-Fos-positive cells in the animals treated with IL-6 being

14.5 ± 0.9 ($n=6$) compared with 14.0 ± 2.5 ($n=6$) in vehicle-treated animals.

Effect of i.p. administration of IL-1 β , IL-6 and TNF- α on the afferent input from the acid-challenged stomach to the NTS

Rats were pretreated i.p. with IL-1 β , IL-6, TNF- α (each at 10 μ g/kg) or their vehicle 24, 48 or 96 h before IG administration of 0.25 M HCl (pH 0.51) or saline. It has previously been shown that exposure of the rat gastric mucosa to 0.25 M HCl enhances the expression of c-Fos in the NTS to a significant extent (Danzer et al., 2004a). Immunohistochemical photomicrographs of the NTS displaying c-Fos-positive neurons in response to gastric acid challenge are presented in Danzer et al. (2004b). The acute effect of IL-1 β and TNF- α to induce c-Fos in the NTS, as described in the preceding paragraph, was completely gone 24 h later, since the expression of c-Fos in the NTS of rats subjected to IG saline was not different when the animals had been pretreated with vehicle, IL-1 β or TNF- α (Fig. 2A, B). In contrast, both IL-1 β and TNF- α enhanced the expression of c-Fos in the NTS of rats challenged with IG HCl in a time-dependent manner (Fig. 2A, B). Thus, the amplification of c-Fos-positive cells was most prominent 24 h after cytokine pretreatment, largely maintained 48 h after cytokine pretreatment but no longer statistically significant 96 h after cytokine pretreatment (Fig. 2A, B). It appeared, however, that the sensitizing effect of IL-1 β was sustained for a longer time than that of TNF- α . Unlike IL-1 β and TNF- α , IL-6 failed to modify the expression of c-Fos in the NTS of rats challenged with IG HCl, given that the number of c-Fos-positive cells in the NTS of IL-6-pretreated rats was 31.8 ± 1.6 ($n=4$) compared with 33.1 ± 2.9 ($n=4$) in vehicle-pretreated rats.

Effect of IC administration of IL-1 β and TNF- α on the afferent input from the acid-challenged stomach to the NTS

Rats were pretreated IC with IL-1 β , TNF- α (100 ng) or their vehicle 48 h before IG administration of 0.25 M HCl or saline. As shown in Fig. 3, IC pretreatment with saline did not change the number of c-Fos-positive cells in the NTS of rats subjected to IG saline or HCl when compared with that in the NTS of sham-treated rats in which a 10 ml Hamilton needle–syringe combination was inserted in the cisterna magna but no fluid was withdrawn or injected. IC pretreatment with IL-1 β and TNF- α likewise failed to modify the expression of c-Fos in the NTS seen after IG administration of saline or HCl (Fig. 3).

Effect of anakinra on the IL-1 β -evoked enhancement of the afferent input from the acid-challenged stomach to the NTS

Rats were pretreated i.p. with anakinra or its vehicle, followed by IL-1 β or its vehicle, 48 h before IG administration of 0.25 M HCl. Relative to vehicle, IL-1 β (10 μ g/kg) enhanced the HCl-evoked expression of c-Fos in the NTS of rats pretreated with the vehicle for anakinra. As shown in

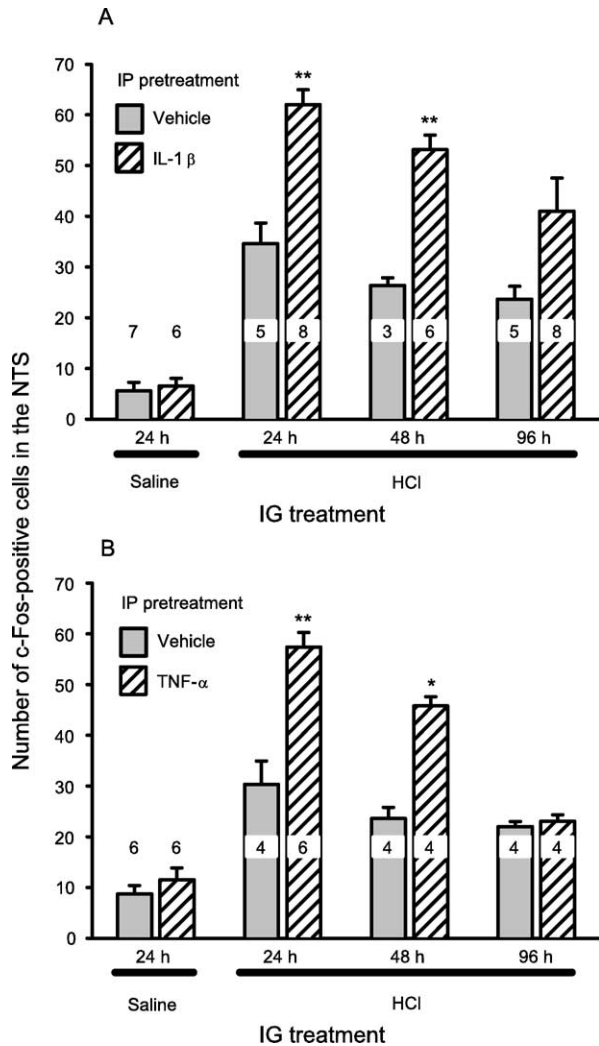


Fig. 2. Effect of i.p. pretreatment with IL-1β (A) and TNF-α (B) on the expression of c-Fos in the NTS evoked by IG administration of HCl or saline. The rats were pretreated i.p. with IL-1β, TNF-α (each at 10 μg/kg) or their vehicle 24, 48 or 96 h before IG treatment with HCl (0.25 M) or saline. The neurons expressing c-Fos were visualized by immunohistochemistry 2 h after IG treatment with HCl or saline. The values represent means ± S.E.M., n as indicated. * $P < 0.05$, ** $P < 0.01$ vs. i.p. pretreatment with vehicle at the same time point (one-way ANOVA).

Fig. 4, this sensitizing effect of IL-1β was significantly attenuated by pretreatment with anakinra (1 mg/kg).

DISCUSSION

This study has shown that i.p.-administered IL-1β and TNF-α elicit a transient stimulation of NTS neurons and cause a sustained facilitation of the afferent input from the acid-challenged stomach. The cytokine- and HCl-evoked activation of NTS neurons was visualized by c-Fos immunohistochemistry 2 h post-treatment, a widely used method of functional neuroanatomy. Being an immediate-early gene, c-fos is rapidly and transiently transcribed so that expression of the c-Fos protein reaches a maximum between 1 and 3 h post-stimulus and then quickly ceases (Traub et al., 1996; Kovacs, 1998). Previous work has shown that IG concentrations of 0.15–

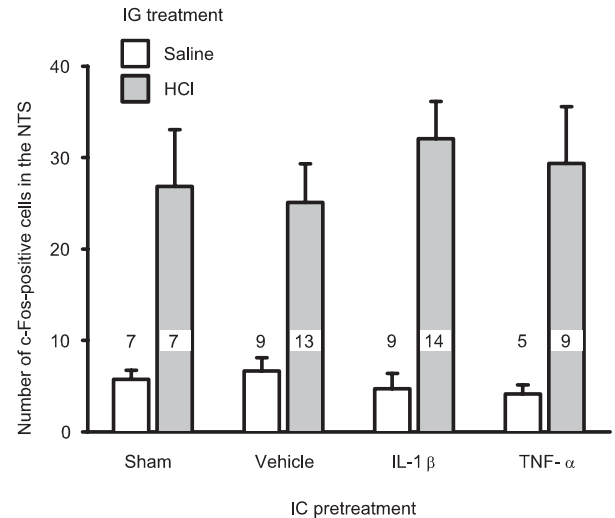


Fig. 3. Effect of IC pretreatment with IL-1β and TNF-α on the expression of c-Fos in the NTS evoked by IG administration of HCl or saline. The rats were sham-pretreated or injected IC with IL-1β, TNF-α (each at 10 ng) or their vehicle 48 h before IG treatment with HCl (0.25 M) or saline. The neurons expressing c-Fos were visualized by immunohistochemistry 2 h after IG treatment with HCl or saline. The values represent means ± S.E.M., n as indicated.

0.35 M HCl are needed to induce c-Fos in the NTS (Danzer et al., 2004a). As these acid concentrations are supraphysiological, but do not cause appreciable damage of the gastric mucosa (Schuligoi et al., 1998), it appears as if only a substantial increase of the H⁺ gradient across the gastric mucosal barrier is able to drive enough H⁺ ions into the lamina

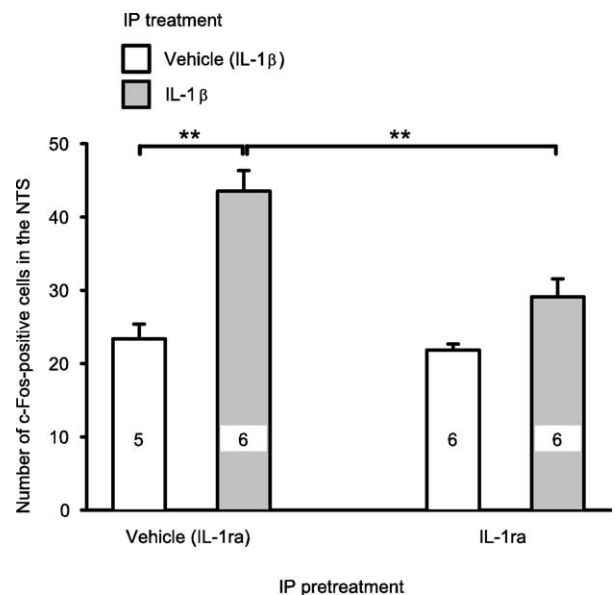


Fig. 4. Antagonism by the IL-1 receptor antagonist (IL-1ra; anakinra) of the effect of IL-1β to enhance the gastric acid-evoked expression of c-Fos in the NTS. The rats were pretreated i.p. with IL-1ra (1 mg/kg) or its vehicle followed, 15 min later, by IL-1β (10 μg/kg) or its vehicle. HCl (0.25 M) was administered IG 48 h after this pretreatment. The neurons expressing c-Fos were visualized by immunohistochemistry 2 h after IG treatment with HCl. The values represent means ± S.E.M., n as indicated. ** $P < 0.01$ as indicated (one-way ANOVA).

propria (Danzer et al., 2004a) where they can excite vagal afferents either directly (Clarke and Davison, 1978; Hillsley and Grundy, 1998) or indirectly via neuroactive factors released in the tissue. Our experimental model thus reflects pathophysiological conditions where back-diffusion of luminal acid leads to activation of sensory nerve fibers in the gastric mucosa (Danzer et al., 2004a).

The results of the present study confirm previous reports that i.v. and i.p. administration of IL-1 β induces c-Fos in the NTS (Brady et al., 1994; Ericsson et al., 1994; Day and Akil, 1996), the major central projection area of vagal afferents (Norgren and Smith, 1988; Altschuler et al., 1989). In addition, we found that the effect of IL-1 β is shared by TNF- α , another proinflammatory cytokine known to participate in the communication between the immune system and the brain (Goehler et al., 2000; Konsman et al., 2002). In contrast, IG administration of IL-1 β doses higher than those effective after i.p. administration failed to stimulate neurons in the NTS, which may be explained by rapid inactivation of the cytokine in the gastric juice and/or its inability to penetrate the gastric mucosal barrier. Proinflammatory cytokines are released from the rat gastric mucosa under conditions of gastric acid secretion and mucosal injury (Gislason et al., 1996; Montuschi et al., 1996), and it remains to be investigated whether formation of IL-1 β and TNF- α within the gastric mucosa activates vagal afferents. This possibility may be envisaged from the presence of IL-1 receptors of type I on nodose ganglion neurons (Ek et al., 1998) and from the ability of peripheral administration of IL-1 β to enhance both the firing of vagal afferents (Nijijima, 1996; Kurosawa et al., 1997; Ek et al., 1998) and the expression of c-Fos in the NTS (Brady et al., 1994; Ericsson et al., 1994; Day and Akil, 1996).

The major discovery of this study was that i.p. pretreatment of rats with IL-1 β and TNF- α (10 μ g/kg) enhances the expression of c-Fos in the NTS induced by gastric acid challenge. This facilitation of NTS activation was most prominently seen 1 day post-treatment, when the effect of the cytokines to induce per se c-Fos in the brainstem had been gone, and was sustained for more than 2 days. Our observation is in overall accordance with reports that the responsiveness of mesenteric afferent nerve fibers to prostaglandin E₂ is enhanced 20 h after pretreatment with 5 μ g/kg IL-1 β (Kreiss et al., 2000) and that acute administration of IL-1 β sensitizes vagal afferents to cholecystokinin (Bucinskaite et al., 1997) and spinal afferents to ischemia and histamine (Fu and Longhurst, 1999).

Although these findings suggest that i.p. pretreatment of rats with IL-1 β and TNF- α amplifies the gastric acid-evoked stimulation of NTS neurons by sensitizing vagal afferents, it could also be hypothesized that the cytokines act directly on the brainstem to sensitize NTS neurons to vagal afferent input. This scenario is conceivable because circulating cytokines are known to activate brain neurons via mechanisms that involve circumventricular organs such as the area postrema which is exempt from the blood–brain barrier (Konsman et al., 2002). Thus, circulating IL-1 β induces macrophage-like cells in the area postrema to synthesize IL-1 β which, in turn, stimulates IL-1 receptors on area postrema neurons (Ericsson et al., 1995; Quan et al., 1998; Konsman

et al., 1999, 2002), some of which project to the NTS (Bonham and Hassler, 1993). In addition, i.v. IL-1 β causes endothelial cells of cerebral blood vessels and perivascular macrophages to release prostaglandins which then diffuse into the brain parenchyma to activate specific neural pathways via prostaglandin EP₃ or EP₄ receptors (Quan et al., 1998; Ek et al., 2000; Konsman et al., 2002). This pathway may be of relevance to the effect under study, given that NTS neurons expressing c-Fos in response to circulating IL-1 β bear EP₃ receptors (Ek et al., 2000) but not IL-1 receptors (Ericsson et al., 1995).

Despite these considerations, our data negate the possibility that peripheral IL-1 β and TNF- α enhanced the gastric acid-evoked stimulation of NTS neurons by a central site of action, because IC pretreatment of rats with IL-1 β and TNF- α failed to reproduce the sensitizing effect of the i.p.-administered cytokines. A similar conclusion has been reached with regard to the effect of IL-1 β to induce per se c-Fos in the NTS, as i.p. administration of the cytokine is more effective than i.c.v. administration (Day and Akil, 1996). We consider our data conclusive because the dose of IL-1 β and TNF- α administered IC (100 ng) has been found effective in other studies of central cytokine actions (Kent et al., 1992; Rivest et al., 1992; Day and Akil, 1996; Ek et al., 2000).

In view of the IL-1 β -evoked sensitization of spinal afferents (Bucinskaite et al., 1997; Fu and Longhurst, 1999; Kreiss et al., 2000) we propose that i.p. pretreatment of rats with IL-1 β and TNF- α amplifies gastric acid-evoked stimulation of NTS neurons by a peripheral action on acid-sensitive vagal afferents. The sensitizing effect of IL-1 β is mediated by IL-1 receptors, since it is inhibited by i.p. administration of anakinra, a non-glycosylated form of the human IL-1 receptor antagonist (Kent et al., 1992; Bluthé et al., 1995). Further experiments are needed to explore whether anakinra is able not only to prevent IL-1 β from sensitizing vagal afferents to noxious chemicals but also to reverse cytokine-induced hypersensitivity, which would be of therapeutic relevance. Since the cytokines were administered intraperitoneally, we can only speculate on the site of their sensitizing action which may take place at the level of the gastric wall, of the vagal paraganglia, of the vagal afferent nerve fibers or of their somata in the nodose ganglion. In addition, cytokines may facilitate the access of acid from the lumen to the lamina propria of the gastric mucosa, given that IL-1 β and TNF- α increase gastrointestinal epithelial permeability *in vitro* (Taylor et al., 1998; Matysiak-Budnik et al., 2001). If cytokines are assumed to act on vagal afferents, the question arises as to which populations of vagal afferents are sensitized. As vagal afferents innervating gastrointestinal and non-gastrointestinal tissues converge in the NTS (Paton and Kasparov, 2000), it is conceivable that intraperitoneally administered cytokines enhance gastric acid-induced c-Fos expression in the NTS because gastric mucosal afferents converge with sensitized afferents that innervate territories other than the gastric mucosa.

IL-6 is a cytokine with pro- and antiinflammatory activity that likewise participates in the behavioral manifestations of peripheral immune challenge and hyperalgesia (Bluthé et al.,

2000; Oprea and Kress, 2000). Although i.v. IL-6 has been found to induce c-Fos in the NTS (Niimi et al., 1997), our findings indicate that i.p.-administered IL-6 is neither able to induce per se c-Fos in the NTS nor to amplify the gastric acid-evoked expression of c-Fos. It remains to be explored whether this lack of effect of IL-6 is due to the absence of IL-6 receptors on the vagal afferent system.

In summary, our work indicates that the proinflammatory cytokines IL-1 β and TNF- α sensitize vagal afferent neurons to gastric acid challenge. This finding is of pathophysiological and therapeutic relevance, given that vagal afferents play an important role in gastric chemoreception and gastritis-induced chemical hypersensitivity (Lamb et al., 2003). Furthermore, our observations emphasize that vagal sensory neurons participate in the communication between the peripheral immune system and brain (Goehler et al., 2000; Konsman et al., 2002). Following infection or inflammation, IL-1 β may be presented to vagal afferents by Kupffer cells in the liver and by macrophages and dendritic cells in the paraganglia and connective tissue associated with the abdominal vagus (Goehler et al., 2000; Konsman et al., 2002). Being activated in this way, vagal afferents contribute to the behavioral depression induced by peripheral immune signals (Goehler et al., 2000; Konsman et al., 2002).

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