Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 75 (2003) 181–189
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Meal pattern changes associated with temporomandibular joint inflammation/pain in rats; analgesic effects

C.A. Kerins[a], D.S. Carlson[a,b], J.E. McIntosh[a], L.L. Bellinger[a,*]

[a]*Department of Biomedical Sciences, Baylor College of Dentistry, Texas A&M University System Health Science Center, 3302 Gaston Avenue, Dallas, TX 75266-0677, USA*
[b]*Center for Craniofacial Research and Diagnosis, Dallas, TX, USA*

Received 13 August 2002; received in revised form 17 December 2002; accepted 15 March 2003

Abstract

Establishing a valid animal model to study temporomandibular joint (TMJ) pain has proven extremely difficult. Using complete Freund's adjuvant (CFA) to induce TMJ inflammation, we recently showed that meal pattern analysis could be used as a noninvasive biological marker to study TMJ pain in an animal model. The purpose of this study was to further validate our animal model by determining whether aspects of CFA-induced TMJ inflammation/pain are reversed with ibuprofen (IBU) treatment. In the first trial, 48 male rats were used and in the second trial, 32 female ovariectomized rats, given 17β-estradiol replacement, were used. The rats were assigned to one of four groups: control (CON − CON); control + IBU (CON + IBU); CFA − CON; and CFA + IBU. In the male trial, CFA injection ($P < .01$) caused TMJ swelling and chromodacryorrhea (CFA − CON); IBU eliminated these changes in the CFA + IBU group. Meal pattern analysis showed the pertinent CFA-induced change and the IBU effect was that meal duration was increased in the CFA − CON group ($P < .01$), but normal in the CFA + IBU-treated group on the first, but not second, day postinjection. In the female trial, CFA increased TMJ swelling, but did not cause significant chromodacryorrhea (CFA − CON); IBU eliminated swelling in the CFA + IBU group. Meal duration was increased ($P < .01$) in the CFA − CON group, but was normal in the CFA + IBU-treated group on both the first and second days postinjection. In both trials, interleukin-1β (IL-1β) levels were increased similarly in CFA − CON and CFA + IBU groups ($P < .01$). This study shows that CFA-induced TMJ inflammation/pain can cause changes in meal patterns (i.e., meal duration), which may be used as a behavioral marker for TMJ inflammation/pain.

Keywords: Adjuvant; Feeding; IL-1β; Male; Female

1. Introduction

The underlying physiological mechanisms of temporomandibular joint (TMJ) disorders are still poorly understood and difficult to study in humans. TMJ disorders, especially those associated with inflammation, often include biological and behavioral components (Stohler et al., 1988; Sessle, 1999a,b; Sessle and Hu, 1991).

There has been a lack of a noninvasive marker to study TMJ inflammation/pain in an animal model. The study of feeding behavior through detailed meal pattern analysis may provide such a marker (Bellinger and Mendel, 1995; Bellinger and Williams, 1995; Bellinger et al., 1997; Glendinning and Smith, 1994). As the TMJ is the principal joint associated with mastication, it is conceivable that during TMJ inflammation/pain, avoidance behavior will be manifested in altered chewing patterns (Harper et al., 2000a).

We showed that food intake and, more specifically, meal patterns are changed following initiation of inflammation in the TMJ with complete Freund's adjuvant (CFA) (Harper et al., 2000b). Alteration of meal patterns, and in particular meal duration, was correlated to parameters of inflammation such as external swelling, chromodacryorrhea (red eye tearing), histology, retrodiscal tissue interleukin-1β (IL-1β) and trigeminal ganglion calcitonin gene-related peptide and substance P levels, brainstem subnucleus caudalis calcitonin gene-related peptide and substance P levels, and altered diurnal corticosterone (CORT) secretion (Harper et al., 2001; Hutchins et al., 2000).

It was the aim of the present study to further validate this animal model of TMJ inflammation/pain as quantified by

* Corresponding author. Tel.: +1-214-828-8322; fax: +1-214-828-8951.
E-mail address: lbellinger@tambcd.edu (L.L. Bellinger).

0091-3057/03/$ – see front matter © 2003 Elsevier Science Inc. All rights reserved.
doi:10.1016/S0091-3057(03)00072-8

meal pattern analysis by demonstrating that prophylactic administration of an anti-inflammatory drug, ibuprofen (IBU), prior to initiation of inflammation and following experimentation would diminish the inflammatory/pain response and return altered meal patterns toward normal baseline (control) values. IBU has a proven role in modulating inflammation as well as providing analgesia. As nonsteroidal anti-inflammatory drugs are available over the counter and are relatively inexpensive, they are an ideal first choice in medicine for a person experiencing joint inflammation/pain. Moreover, a successful animal model would lend itself to testing various manipulations of the TMJ and allow quantifiable testing of pharmaceutical agents directed at TMJ disorders.

Gender differences exist in the prevalence of TMJ disorders (LeResche, 1997; LeResche et al., 1997; Carlsson, 1999), in meal patterns under normal conditions (Blaustein and Wade, 1977; Tarttelin and Gorski, 1971); following CFA injection (Kerins et al., submitted for publication) in nociception responses (Fillingim and Ness, 2000); in response to opioids (Kest et al., 2000) and in response to IBU (Walker and Carmody, 1998). Therefore, in the present study, both male and female rats were included in the test of the model.

2. Materials and methods

In the first trial of this study, male rats were used and in the second trial, female rats were employed. The Baylor College of Dentistry's Institutional Animal Care and Use Committee approved the experimental protocol. Forty-eight Sprague–Dawley young adult, sexually mature male rats ($n=12$ per group) and 32 ovariectomized young adult, sexually mature female rats ($n=8$ per group) (Harlan Industries, Houston, TX) were caged individually in sound-attenuated modules equipped with photobeam computer-activated pellet feeders and allowed to adjust to the surroundings (Bellinger et al., 1997). The rats were kept on a 12:12-h light–dark cycle with lights on at 1300 h.

A normal and predictable estrous cycle (Butcher et al., 1978) was maintained in the ovariectomized rats by giving each female rat 17β-estradiol (750 ng/day) replacement by a subdermally implanted 14-day Alzet osmotic pump (Durect, Cupertino, CA). This started on the day of arrival, which was 12 days prior to experimentation. The female rats were also injected subcutaneously with 17β-estradiol (800 ng/rat in sesame seed oil) every fourth day to mimic the rat's normal estrous cycle (Butcher et al., 1978). The rationale for using these methods is that total food intake and meal patterns vary during the normal estrous cycle of the rat (Tarttelin and Gorski, 1971) and these differences are attributed to estrous changes in plasma 17β-estradiol concentrations (Blaustein and Wade, 1977; Varma et al., 1999). Within a group of rats, individual animals may be in different stages of their estrous cycle (proestrus, estrus, metestrus, or diestrus) and thus have divergent feeding patterns. Therefore, by using the above paradigm, the estrous cycles and meal patterns of the rats were standardized and it was possible to give the injection of CFA on the day before the rats entered estrus (Butcher et al., 1978). The timing of the CFA injection was chosen because it has been demonstrated that female rats exhibit enhanced pain responses in the proestrus phase and decreased responses during the diestrus phase of their cycles (Fillingim and Ness, 2000).

All rats were presented with 45-mg rodent pellets (Bioserv, Frenchtown, NJ) in the computerized feeding modules. Removal of a pellet from the trough of the feeder allowed the photobeam to signal the computer to drop another pellet into the trough and to record the time each pellet was released. The record of pellets dropped over time was computer-analyzed with a proprietary computer program to establish the meal patterns (i.e., meal size, meal duration, intermeal interval, and meal frequency) (Bellinger et al., 1997). The rats were given time prior to experimentation to get adjusted to the feeders, the surroundings, and the water system. As the cages have a raised grill-type floor, spillage, droppings, and urine are collected in a pan below, all of which are maintained within the sound-attenuated chamber. After the adjustment period, the rats typically spill less than five pellets per day.

After 5 days of familiarizing themselves with the feeders, the rats were then divided into four groups: Group 1, control (CON − CON); Group 2, control + IBU (CON + IBU); Group 3, bilateral TMJ, CFA-injected (CFA − CON); and Group 4, bilateral TMJ, CFA-injected + IBU (CFA + IBU).

Starting 24 h prior to the day of experimentation and 2 days thereafter, rats in Groups 2 and 4 received two equal doses of IBU (Children's Motrin; McNeil-PPC, Fort Washington, PA) orally by feeding tube at 0730 and 1800 h (total dose, 60 mg/kg/day) (Sharp and LaRegina, 1998; Hawk and Leary, 1995).

At the initiation of the experiment, rats were removed from their cages at 1300 h and anesthesized with a solution of ketamine (52 mg/kg) and Rompun (0.5 mg/kg), which is 60% of the normal surgical dose. The control groups (CON − CON and CON + IBU) were anesthesized, but did not receive TMJ injections. Groups CFA − CON and (CFA + IBU) received a 50-μl (paraffin oil/10 μg *Mycobacterium tuberculosis*) solution of CFA injected bilaterally into the superior joint space of the TMJ (Carleson et al., 1996, 1997; Mazzier et al., 1967). We have previously shown (Harper et al., 2001) that there are no differences between noninjected controls and saline-injected animals in the parameters measured in this study. All rats were mobile within 20 min or less after induction of anesthesia. At the time of injection, the males weighed approximately 190 g and the females weighed approximately 230 g.

Twenty-four hours following injection, external signs of swelling and chromodacryorrhea (red eye) were graded in double-blinded fashion by the following analog scale: 0 = *none*, 1 = *mild*, 2 = *moderate*, and 3 = *severe*.

Forty-eight hours after injection of CFA, the rats were killed by decapitation, within 20 s of removal from their

cages, in order to obtain stress-free blood samples. Blood samples were collected in tubes coated with EDTA and immediately placed on ice. Plasma was separated in a refrigerated centrifuge and stored at −20 °C. The severed heads were placed in plastic bags, submerged in an ice bath, and retrodiscal tissue was dissected and then rapidly frozen in liquid nitrogen and stored at −80 °C for later IL-1 β assay.

Prior to IL-1 β ELISA assay (R&D Systems, Minneapolis, MN), total TMJ protein content was determined for each sample by standard Folin Lowry assay; IL-1β was expressed as picograms per milligram of protein. Plasma 17β-estradiol in the female rats and CORT were determined using radioimmunoassay kits from Diagnostic Systems Laboratories (Webster, TX) and ICN Biomedical, Diagnostics Division (Costa Mesa, CA), respectively.

Food intake, meal parameters, IL-1β, and hormone data were analyzed by one-way and two-way analyses of variance with or without repeated measurements. Data found to be significant were further analyzed by Duncan's multiple range test to determine the location of the significant differences. Swelling and chromodacryorrhea analog scores were analyzed using Kruskal–Wallis one-way analysis of variance and data found to be significant were further analyzed by using Mann–Whitney *U* test.

3. Results

3.1. Trial 1 (males)

The analog score for swelling was significantly increased in the CFA − CON group relative to CON − CON and CON + IBU (Fig. 1). The increase in swelling was diminished significantly with IBU treatment in the CFA + IBU group and the swelling score for this group was not significantly different from the control groups. The analog score for chromodacryorrhea (Fig. 2) was also significantly increased in the CFA − CON group relative to CON − CON and CON + IBU groups. Chromodacryorrhea was significantly ($P<.01$) decreased by administration of IBU to the CFA + IBU and the chromodacryorrhea score for this group was not significantly different from the control groups.

There was no significant difference among groups in plasma CORT levels, but there was a trend ($P<.17$) for

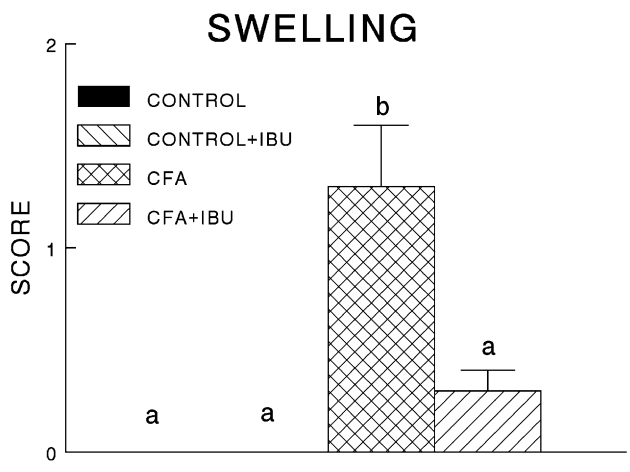

Fig. 1. Visual analog scale score for TMJ area swelling. Mean ± S.E.M. ($n=12$ per group). Swelling was significantly increased in Group 3 (complete Freund's adjuvant [CFA]) relative to controls 24 postinjection. This increase was attenuated in Group 4 (CFA + ibuprofen [IBU]). Significance: a vs. b = $P<.01$.

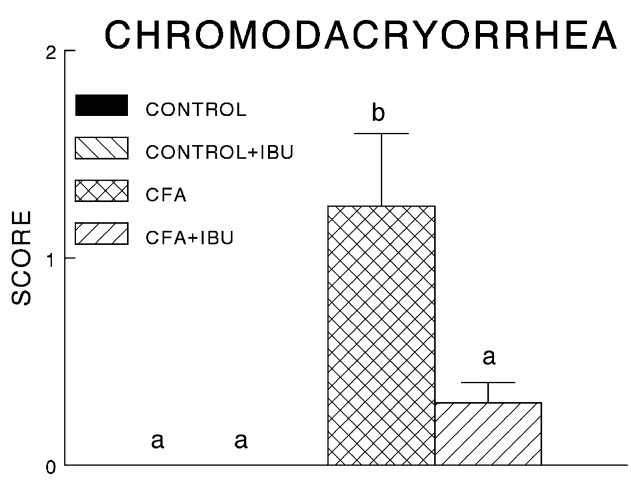

Fig. 2. Visual analog scale score for chromodacryorrhea. Mean ± S.E.M. ($n=12$ per group). Chromodacryorrhea was significantly increased in Group 3 (CFA-treated) relative to control (CON) Groups 1 and 2 (CON and CON + IBU) 24 h postinjection. Chromodacryorrhea was significantly decreased by administration of IBU to Group 4. Significance: a vs. b = $P<.01$ (see Fig. 1 legend for additional abbreviation).

Table 1
Twenty-four-hour food intake the day prior to treatment (Preday) and 2 days (Post 1 and Post 2) thereafter, in male ($n=12$ per group) and female ($n=8$ per group) rats

	Preday	Post 1	Post 2
Male rats			
CON − CON	20.6 ± 1.1	15.8 ± 0.8	20.6 ± 0.9
CON + IBU	19.2 ± 1.0	14.4 ± 0.7	18.9 ± 0.8
CFA − CON	19.4 ± 0.5	12.1 ± 1.6 *	20.0 ± 1.5
CFA + IBU	17.6 ± 1.0	11.1 ± 1.6 **	16.1 ± 1.2
Female rats			
CON − CON	14.3 ± 1.7	15.1 ± 0.9	15.7 ± 1.4
CON + IBU	14.6 ± 1.3	10.9 ± 0.9***	11.3 ± 1.3***
CFA − CON	13.9 ± 1.8	12.2 ± 1.2	14.4 ± 0.6
CFA + IBU	11.4 ± 1.6	9.0 ± 1.3****,†	9.2 ± 1.6 **,****,‡

The data are expressed as mean ± S.E.M.
Group 1, noninjected control; Group 2, noninjected control + ibuprofen (IBU); Group 3, CFA injected bilaterally into the TMJ; and Group 4, CFA injected bilaterally into the TMJ + IBU.
* Group 3 vs. Group 1, $P<.05$.
** Group 4 vs. Group 2, $P<.05$.
*** Group 2 vs. Group 1, $P<.05$.
**** Group 3 vs. Group 4, $P<.01$.
† Group 3 vs. Groups 2, $P<.01$.
‡ Group 4 vs. Group 1, $P<.01$.

Table 2
Meal size (grams) the day prior to treatment (Preday) and 2 days (Post 1 and Post 2) thereafter, in male ($n=12$ per group) and female ($n=8$ per group) rats

	Preday	Post 1	Post 2
Male rats			
CON – CON	1.6 ± 0.1	1.5 ± 0.1	1.8 ± 0.1
CON + IBU	1.8 ± 0.3	1.4 ± 0.8	1.5 ± 0.1
CFA – CON	1.4 ± 0.1	1.5 ± 0.6	1.9 ± 0.2
CFA + IBU	1.5 ± 0.1	1.3 ± 0.1	1.7 ± 0.1
Female rats			
CON – CON	1.2 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
CON + IBU	1.3 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
CFA – CON	1.3 ± 0.1	1.2 ± 0.2	1.6 ± 0.1
CFA + IBU	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1 *

The data are expressed as mean ± S.E.M.
Group 1, noninjected control; Group 2, noninjected control + ibuprofen (IBU); Group 3, CFA injected bilaterally into the TMJ; and Group 4, CFA injected bilaterally into the TMJ + IBU.
* Group 3 vs. Group 4, $P<.01$.

Table 4
Twenty-four-hour meal number the day prior to treatment (Preday) and 2 days (Post 1 and Post 2) thereafter, in male ($n=12$ per group) and female ($n=8$ per group) rats

	Preday	Post 1	Post 2
Male rats			
CON – CON	13.0 ± 1.0	10.8 ± 0.7	11.8 ± 0.8
CON + IBU	13.0 ± 0.9	10.5 ± 0.7	12.8 ± 0.8
CFA – CON	14.3 ± 0.9	7.6 ± 0.7 *	11.0 ± 0.6
CFA + IBU	11.9 ± 0.6	8.9 ± 0.9	10.0 ± 0.8
Female rats			
CON – CON	12.3 ± 0.6	14.3 ± 1.3	12.1 ± 1.2
CON + IBU	11.6 ± 0.8	11.0 ± 1.4	11.1 ± 1.0
CFA – CON	11.0 ± 1.4	9.5 ± 1.0	9.4 ± 1.1
CFA + IBU	11.1 ± 1.6	10.9 ± 1.3	10.5 ± 1.3

The data are expressed as mean ± S.E.M.
Group 1, noninjected control; Group 2, noninjected control + ibuprofen (IBU); Group 3, CFA injected bilaterally into the TMJ; and Group 4, CFA injected bilaterally into the TMJ + IBU.
* Group 3 vs. Group 1, $P<.05$.

plasma CORT to be elevated in the CFA – CON group (27.2 ± 5.8 ng/ml) relative to CON – CON (15.3 ± 3.3 ng/ml) and CON + IBU (15.9 ± 2.0 ng/ml). Compared to the CFA – CON, the CORT group was slightly lower than the CFA + IBU group (20.1 ± 4.5 ng/ml).

Total food intake on the days prior to experimentation was not significantly different among groups (Table 1). The 24-h intake of CON – CON and CON + IBU did not differ at any time; thus, IBU alone did not affect the 24-h food intake (Table 1). Compared to the CON – CON group, the 24-h food intake of the CFA – CON group was significantly ($P<.05$) attenuated on the first, but not the second, day postinjection. Similarly, the 24-h food intake of CFA + IBU was significantly ($P<.05$) less than CON + IBU group on the first, but not the second, day postinjection.

Meal pattern analysis comparing Day −1 with Day +2 demonstrated no effect of IBU or CFA treatment on the group's Meal Size [$F(3,44)=0.59$, ns] (Table 2), or Intermeal Interval [$F(3,44)=2.32$, ns] (Table 3). CFA – CON took significantly ($P<.05$) fewer meals (Table 4) than CON – CON on the first day postinjection, but no other comparisons were significant on Day +1 or +2. Notably, Meal Duration (Fig. 3) was significantly [$F(3,44)=5.84$, $P<.01$] increased on Postinjection Day 1 in CFA – CON relative to the CON – CON and CON + IBU control groups. Administration of IBU to CFA + IBU was found to normalize the increased meal duration found in Group CFA – CON on Postinjection Day 1, but not Postinjection Day 2.

IL-1β (Fig. 4) was significantly ($P<.001$) elevated in both CFA-treated groups relative to controls; therefore, the

Table 3
Twenty-four-hour intermeal interval (in seconds) the day prior to treatment (Preday) and 2 days (Post 1 and Post 2) thereafter, in male ($n=12$ per group) and female ($n=8$ per group) rats

	Preday	Post 1	Post 2
Male rats			
CON – CON	5566.4 ± 287.6	6918.3 ± 437.7	6728.7 ± 460.4
CON + IBU	5780.2 ± 939.2	7614.6 ± 484.1	6237.4 ± 397.0
CFA + CON	5177.6 ± 404.6	8659.0 ± 936.0	6817.9 ± 251.2
CFA + IBU	5607.4 ± 267.2	10180.0 ± 1410.9	8413.4 ± 1248.0
Female rats			
CON – CON	6659.6 ± 547.8	5239.6 ± 474.1	6109.3 ± 620.9
CON + IBU	6630.2 ± 657.4	6178.4 ± 547.5	6156.7 ± 412.1
CFA + CON	8830.1 ± 1908.3	6410.4 ± 493.4	6193.9 ± 939.5
CFA + IBU	8701.8 ± 2607.1	6758.5 ± 1245.0	6170.9 ± 620.4

The data are expressed as mean ± S.E.M.
Group 1, noninjected control; Group 2, noninjected control + ibuprofen (IBU); Group 3, CFA injected bilaterally into the TMJ; and Group 4, CFA injected bilaterally into the TMJ + IBU.

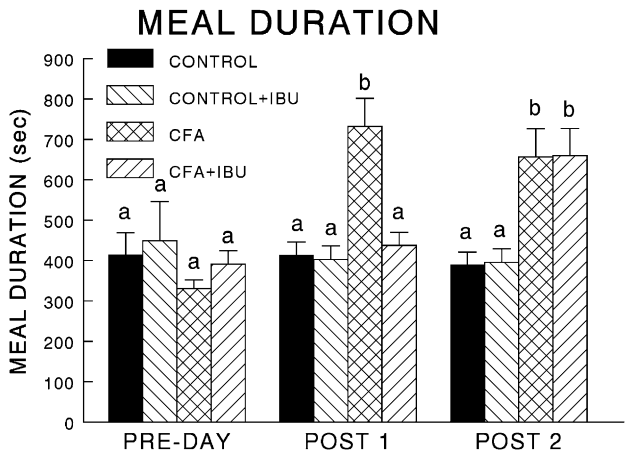

Fig. 3. Meal duration (in seconds) on the day before injections (Preday) and 2 days thereafter (Post 1, Post 2). Mean ± S.E.M. ($n=12$ per group). Meal duration was significantly increased on Post 1 day in Experimental Group 3 relative to control Groups 1 and 2 (CON and CON + IBU). Administration of IBU to Group 4 was found to normalize the increased meal duration found in Group 3 (CFA) on Post 1, but not Post 2. Significance: a vs. b = $P<.05$ (see Figs. 1 and 2 legends for additional abbreviations).

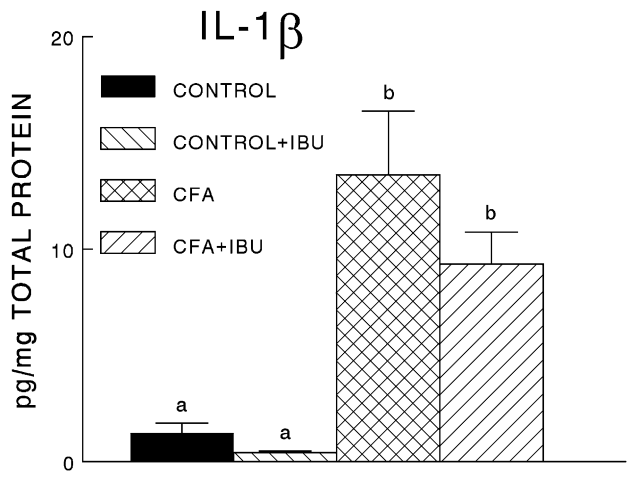

Fig. 4. TMJ tissue IL-1β (in pg/mg TMJ protein) 2 days after CFA injections. Mean ± S.E.M. ($n = 12$ per group). IL-1β levels were significantly elevated in both CFA-treated groups (Groups 3 and 4) relative to controls 48 h postinjection. Administration of IBU to Group 4 did not significantly reverse elevated IL-1β levels. Significance: a vs. b = $P < .01$ (see Figs. 1 and 2 legends for additional abbreviations).

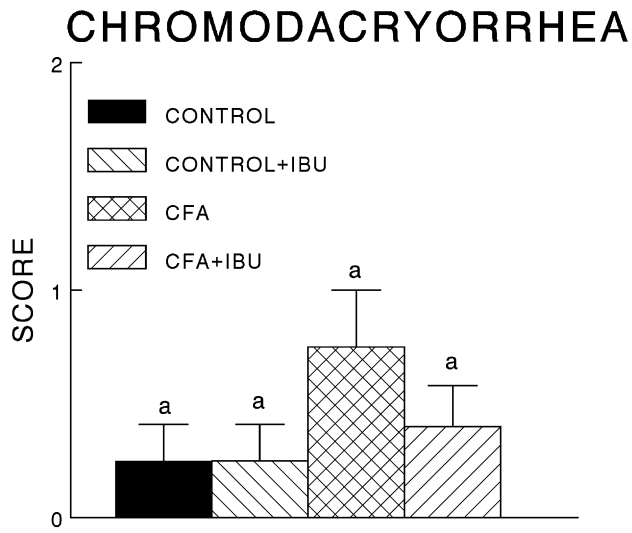

Fig. 6. Visual analog scale score for chromodacryorrhea. Mean ± S.E.M. ($n = 8$ per group). Chromodacryorrhea did not differ significantly among groups 24 h postinjection (see Figs. 1 and 2 legends for additional abbreviations).

administration of IBU to CFA + IBU did not significantly reverse the CFA-induced increase in TMJ IL-1β.

3.2. Trial 2 (females)

The analog score for swelling was significantly increased in the CFA-treated CFA − CON relative to the CON − CON and CON + IBU control groups (Fig. 5). This increase was attenuated with IBU treatment in CFA + IBU and the swelling score of CFA + IBU was not significantly different from the control groups. The analog score for chromodacryorrhea (Fig. 6) did not differ significantly among the groups; there was a trend toward an increase in red eye in CFA − CON group; however, this was not significant ($P = .24$).

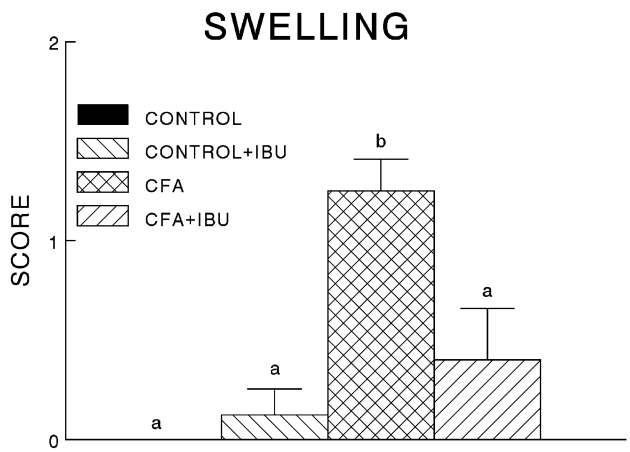

Fig. 5. Visual analog scale score for TMJ area swelling. Mean ± S.E.M. ($n = 8$ per group). Swelling was significantly increased in Group 3 (CFA) relative to controls 24 h postinjection. This increase was attenuated in Group 4 (CFA + IBU). Significance: a vs. b = $P < .01$ (see Figs. 1 and 2 legends for additional abbreviations).

The plasma concentrations of 17β-estradiol were similar in all the groups: CON − CON, 7.3 ± 1.0 pg/ml; CON + IBU, 9.5 ± 1.8 pg/ml; CFA − CON, 7.0 ± 0.5 pg/ml; and CFA + IBU, 5.6 ± 0.4 pg/ml. Administration of CFA and/or IBU did not significantly alter CORT levels: CON − CON, 256.9 ± 70.9 ng/ml; CON + IBU, 202.3 ± 56.9 ng/ml; CFA − CON, 193.8 ± 59.6 ng/ml; and CFA + IBU, 287.8 ± 59.3 pg/ml.

As in the male trial, on the days prior to experimentation, the 24-h food intake of all the groups was similar (Table 1). On the first day postinjection, the 24-h food intake was decreased in both the IBU-treated groups (CON + IBU and

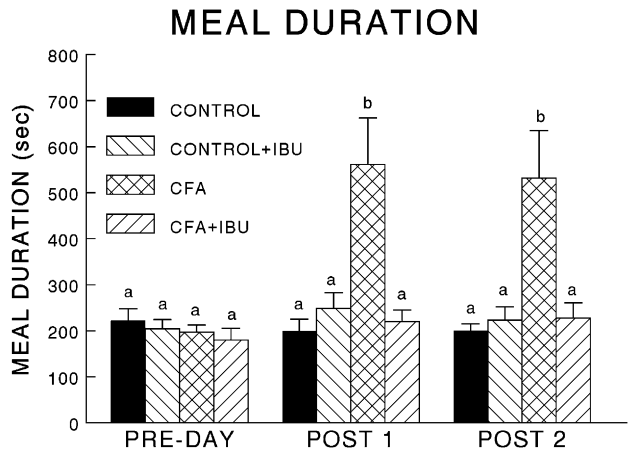

Fig. 7. Meal duration (in seconds) on Preday and on Post 1 and Post 2. Mean ± S.E.M. ($n = 8$ per group). Meal duration was significantly increased on Post 1 and Post 2 days in Groups 3 (CFA) and 4 (CFA + IBU) relative to Groups 1 and 2 (CON, CON + IBU). Administration of IBU to Group 4 normalized the increased meal duration on both Post 1 and Post 2 days. Significance: a vs. b = $P < .01$ (see Figs. 1 and 2 legends for additional abbreviations).

CFA + IBU) when compared to the CON – CON group, whereas CFA – CON's 24-h intake did not differ significantly from CON – CON. Meal pattern analysis revealed that CFA or IBU did not affect Meal Number [$F(3,28) = 0.24$, ns] (Table 4) or Intermeal Interval [$F(3,28) = 0.66$, ns] (Table 3). When compared to the controls, CFA or IBU treatment did not significantly alter meal size on Day +1 (Table 2). However, on Day +2, the meal size of CFA – CON group was greater than the CON – IBU group (Table 2); no other comparisons were significant. Despite the normal 24-h food intake of CFA – CON, the meal duration of these rats (Fig. 7) was significantly increased on Postinjection Days +1 and +2 relative to both control groups. When meal duration of the male and female rats is compared as a percentage change from control values, distinct differences are noted. On Experimental Day +1, meal duration, after CFA treatment, is increased by 283.7 ± 51.0% in the female rats and 176.9 ± 16.8% in the males rats ($P < .05$). This same difference was manifested on Experimental Day 2 (268.6 ± 52.0% vs. 168.4 ± 17.8%, $P < .05$). The data show that both CFA-injected groups increased their meal duration, but when normalized to their control group, the female CFA-injected group took much longer to eat a meal than the CFA-injected male rats. In partial contrast to the previous male trial, wherein CFA + IBU administration of IBU normalized meal duration only on Day +1, meal duration of CFA + IBU in this female trial was normalized on both Days +1 and +2.

IL-1β (Fig. 8) was significantly ($P < .001$) elevated in both CFA-treated groups relative to controls; thus, as in the male trial, the administration of IBU to CFA + IBU did not significantly reverse the CFA-induced increase in TMJ IL-1β.

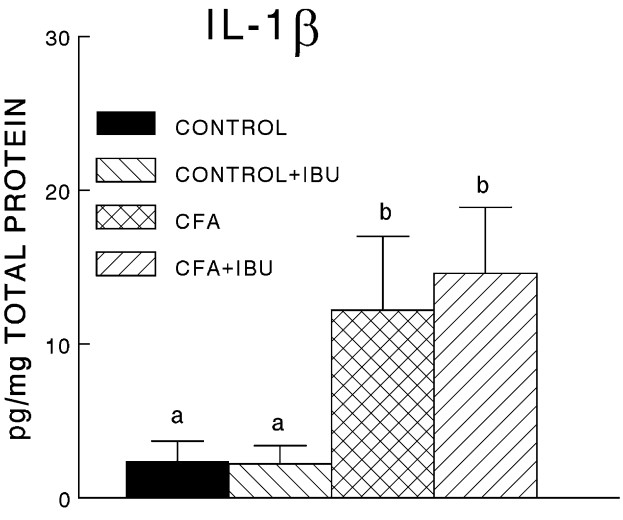

Fig. 8. TMJ tissue IL-1β (in pg/mg TMJ protein) 2 days after CFA injections. Mean ± S.E.M. ($n = 8$ per group). IL-1β levels were significantly elevated in both CFA-treated groups (Groups 3 and 4) relative to controls 48 h after injection. Administration of IBU to Group 4 did not significantly reverse elevated IL-1β levels. Significance: a vs. b = $P < .01$ (see Figs. 1 and 2 legends for additional abbreviations).

4. Discussion

When tissue damage occurs within the construct of a joint, functional changes typically occur initially due to the perception and response to pain. An objective measure of masticatory efficiency can readily be determined by computer measurement and analysis of meal patterns. In a typical limb joint such as the ankle or knee, pain due to tissue damage normally results in an adaptive behavioral response classified as a limp, which represents in part an attempt to guard against the damage and pain during locomotion (Stohler et al., 1988). The joint associated with the locomotion of the jaw, principally during mastication, is the TMJ. Consequently, one might hypothesize that CFA-induced TMJ inflammation/pain would alter masticatory function. Further rationale for suggesting meal pattern analysis as a pain marker stems from a clinical study of juvenile rheumatoid arthritic children (Harper et al., 2000b). That study looked at chewing performance as an objective measure of masticatory function, and showed that the juvenile rheumatoid arthritic children with TMJ disease symptoms changed their chewing habits to presumably guard against pain (Harper et al., 2000b). Also in support of TMJ, pain-altering meal patterns are the findings by Westberg et al. (1997), who showed that nociceptive mechanisms slowed the rhythm of fictive mastication after chemical stimulation of masseter muscle nociceptors of decerebrate, paralyzed rabbits. It is also clear that ingestion, mastication, and deglutition of food are essential for survival. Since the mechanical aspect of feeding is widespread among animals, the use of meal pattern analysis to study TMJ pain may prove to be a useful technique.

In this study, we tested the validity of the animal model of meal pattern analysis in establishing a potential non-invasive marker of TMJ pain induced by CFA inflammation and its sequelae. Blocking one of the CFA-induced inflammatory pathways (i.e., arachidonic acid) by administration of IBU further tested this model.

With regard to the use of IBU, it should be noted that there are other more potent anti-inflammatory agents (e.g., dexamethasone or methotrexate) that could be used; however, these have more significant systemic side effects. Previously published dose–response studies suggested that the analgesic effect of IBU followed a dose-dependent relationship that plateaued at the 50- and 100-mg/kg levels (Price et al., 1996). Lictenberger et al. (2001) similarly found the analgesic activity of IBU to occur at the 50 mg/kg level as opposed to lower levels. A dose of 60 mg/kg IBU was chosen in this study as it falls between the anti-inflammatory dose and the maximum analgesic dose.

In the present study, CFA caused increased swelling, increased chromodacryorrhea (males only), and increased retrodiscal tissue IL-1β levels. All of these variables are direct indicators of inflammation and indirect indicators of pain (Kopp, 1998; Nordahl et al., 1998; Harper et al., 2000b). However, CFA treatment increased the pain neuro-

transmitters calcitonin gene-related protein and substance P in the trigeminal ganglia and brainstem subnucleus caudalis, suggesting that hyperalgesia potentially occurs following CFA TMJ injection (Hutchins et al., 2000). These earlier data lend themselves to the possibility that meal pattern differences seen in the present study are most likely not due to a simple mechanical alteration of joint function by tissue swelling, but rather are induced by pain. Preliminary data from our laboratory (Marr et. al., submitted for publication), in which an analgesic agent (butorphanol) was used, support this hypothesis.

In support of a CFA-induced stress response is the finding of chromodacryorrhea or red tears and tissue swelling. Chromodacryorrhea is due to a stress-induced excessive production of porphyrins by the Harderian gland in the rat (Donnelly, 1997). The nasolacrimal duct releases a porphyrin, which dries around the eyes and external nares. Chromodacryorrhea can be produced following acute stress induced by limb restraint, pain, or illness (Harkness and Ridgway, 1980). Additionally, chromodacryorrhea has been suggested to be an indicator of a chronic underlying disease (Donnelly, 1997). The present data suggest that stress associated with CFA-induced TMJ inflammation/pain can also induce chromodacryorrhea and tissue swelling. These were associated with an increase in meal duration in male rats. IBU treatment reversed these changes in male rats. The IBU treatment reversal of chromodacryorrhea and tissue swelling was associated with a normalization of meal duration. Interestingly, the same dose of CFA given to female rats produced significant swelling, but did not produce significant chromodacryorrhea. Nevertheless, CFA treatment still lengthened meal duration. Treatment with IBU reversed the tissue swelling and normalized meal duration. These data suggest that while gender differences do exist in some of the animal responses to CFA, meal duration appears to be a valid noninvasive marker in both male and female rats.

Following CFA injection, the 24-h food intake was decreased in the male rats, while it was unchanged in female animals. This may be attributed to the fact that females defend their body weight better than males (Nance et al., 1977; Westerterp, 1994). However, differences became readily apparent when the microstructure of the food intake was explored using meal patterns analysis. That is, inflammation/pain of the TMJ significantly increased meal duration in both the male and female rats. We hypothesize that the TMJ-induced inflammation/pain affected the rat, such that when a hungry animal initiated a meal, the meal was of at least normal size. However, the rat would eat slowly due to the pain associated with movement of the mandible during the chewing process. This would result in longer meal duration. Previously (Harper et al., 2000b), a much larger dose of CFA was used to induce TMJ inflammation/pain. In that case, the pain associated with eating not only increased meal duration, but also affected the next time the rat initiated a meal. This was reflected in a significant increase in the intermeal interval. In that study, the chewing process associated with the previous meal probably aggravated the inflammation/pain and when the rat again became hungry, it hesitated to further exacerbate the pain by eating. Much later, hunger drove the rat to eat a normal meal size, but this reaggravated the pain process and a vicious cycle was established. In the present study, a much smaller dose of CFA was used and we did not see a lengthening of the intermeal interval. This was most likely due to a lower level of inflammation/pain. This suggests that the model may be able to indirectly differentiate the magnitude of inflammation/pain present.

CORT is often considered to be the "stress hormone" (Lundberg and Frankenhauser, 1980; Hargreaves, 1990). Previously, Harper et al., (2000b) demonstrated that TMJ injection of a larger dose of CFA disrupted the normal diurnal patterning of CORT in male rats and this effect was not anorexia-induced (Moberg et al., 1975). In the present experiments using a 30-fold lower dose of CFA, which presumably produced less inflammation/pain, only trends were observed in altering CORT secretion. In the present study, a lesser degree of inflammation resulting from the 10-μg CFA dose is supported by the fact that the TMJ IL-1β concentration was significantly less (i.e., about one-fifth) than that found after using a 50-μg dose of CFA (Harper et al., 2001) and presumably much less than would occur after a 300-μg dose. Additionally, it is important to mention that CORT may or may not be a reliable marker for CFA-induced stress in female rats as estrogen can influence CORT secretion (Carey et al., 1995). Female rats have been shown to have higher plasma CORT levels than male rats (Jezova et al., 1996) and this difference was also observed in the present study. Endogenous estrogen-driven increases in CORT secretion may confound any changes in rhythmic CORT secretion induced by TMJ CFA induced inflammation/pain.

Tissue swelling in acute inflammation is due to increased capillary permeability. Prostaglandins, leukotrienes, IL-1β, histamine (via H1 receptors), and bradykinin (via B2 receptors) released during inflammation can directly activate vasoactive afferent nerve fibers to release neuropeptides (such as CGRP and SP) that cause hyperemia. (Rosloniec et al., 1999). Both prostaglandins and leukotrienes are products of the arachidonic acid cascade, but by two different routes: the cyclooxygenase pathway forms prostaglandins, whereas the lipooxygenase pathway forms leukotrienes. IBU does not affect the lipooxygenase pathway, but does inhibit the cyclooxygenase pathway. Therefore, in the present study, IBU administration inhibited one component of the inflammatory processes, while not eliminating other inflammatory mediators such as IL-1β, tumor necrosis factor, free radicals, etc. IL-1β is a potent inducer of inflammatory hyperalgesia and, as such, has a complex role in both pain and inflammation pathways. Local release of IL-1β causes an escalated production of nerve growth factor by increasing the expression of nerve growth factor recep-

tors and also raising the numbers of bradykinin receptors, both of which are involved in the pain pathway. Moreover, IL-1β, along with PGE2 and bradykinin, can directly stimulate afferent nerve endings (Dray, 1995). Bradykinin in turn facilitates the additional release of prostanoids, cytokines, and oxygen free radicals. These free radicals are generated following activation of the inductive form of nitric oxide synthase. Advancing this cascade is the role inducible nitric oxide synthase has in regulating the inducible form of cyclooxygenase (i.e., cyclooxygenase II) and leading to the concomitant synthesis of prostaglandins (Dray, 1995). Therefore, cyclooxygenase inhibition offers one window of opportunity in down-regulating this complexly interactive inflammation cascade. Importantly, administration of IBU to the CFA-treated group was found to decrease swelling, chromodacryorrhea, and, theoretically, pain. As mentioned above, the result of this drug intervention was normalization of meal duration in both sexes. Notably, these IBU-treated male and female rats still had elevated TMJ IL-1β. These findings, and in particular the latter finding, suggest that IBU treatment reduced TMJ pain without totally eliminating TMJ inflammation. This opens the possibility that the animal model may allow for a biological distinction between TMJ pain and inflammation.

With regard to the gender differences: (a) the sexually mature young male rats were lighter on the day prior to experimentation than the sexually mature young female rats, yet the total food intake of the male rats was greater than the female rats. This was attributed to the larger meal size taken by the males. Baseline meal duration was greater in the males than in the females. However, CFA injection in the female rats caused a much greater percentage increase in meal duration when compared to the male rats. (b) CFA treatment attenuated 24-h food intake more in male than in female rats. There are inherent gender differences in baseline food intake, feeding responses after drug treatment (Walker and Carmody, 1998), as well as food intake changes with the estrous cycle (Varma et al., 1999; Clifton, 2000). These factors may explain the decrease in food intake on Day +1 in males and not in the female. (c) Administration of IBU attenuated 24-h food intake more in the female rats than in the male animals, whereas treatment with IBU normalized the CFA-induced increase in meal duration over the 2-day measurement period in the female rats, whereas in male rats, meal duration was normalized only on Experimental Day +1. The reason for this is uncertain, but may be due to well-noted differences in drug efficacies in males and females (Walker and Carmody, 1998). (d) On Day +2, the meal size of the CFA TMJ-injected female rats was increased, whereas it was normal in similarly treated male rats. This may have been the result of an interaction of estrus cycle-induced changes in feeding behavior and their response to CFA.

The above noted sex dissimilarities suggest that the animal model may also prove useful in studying gender differences in their response to TMJ pain and drug treatment. These data suggest that both intersubject and intrasubject meal pattern analyses may be used as a noninvasive method for analysis of TMJ pain. Using this approach, the animal model may be a useful tool in studying TMJ pain since it is difficult to translate subjective pain into meaningful data through clinical observation alone. As noted, noninvasive biologic markers would be useful as objective indicators of pain and stress. Development of a valid animal model for TMJ inflammation/pain is important, as there is currently no noninvasive model available. Furthermore, human studies often give only radiographic data or data from other joints (Nordahl et al., 1998).

In summary, the present study further validated our animal model by demonstrating that treatment with the anti-inflammatory/analgesic agent, IBU, normalized TMJ swelling and chromodacryorrhea, and meal duration validated the model. The IBU effects also indicate that the animal model may be useful in quantifying the efficacy of pharmacological treatment of TMJ inflammation/pain. This model was able to inferentially measure TMJ inflammation/pain. The model also detected some gender differences in the rats' responses to TMJ inflammation/pain and their response to IBU treatment. Additionally, while IBU treatment attenuated pain and inflammation, it did not totally eliminate inflammation as shown by the persistent elevation in TMJ IL-1β. This latter finding opens the additional possibility that the animal model may allow for a biological distinction between TMJ pain and inflammation per se.

Acknowledgements

This study was funded by NIH grants F30DE057-01 to L.L.B. and C.A.K., NIHR03 DE12657-01 to L.L.B., and BCD/TAMUHSC Research Funds, by the Baylor College of Dentistry Center for Craniofacial Research and Diagnosis. The authors thank Connie Tillberg, Gerald Hill, and Priscilla Gillespie for their technical support.

References

Bellinger LL, Mendel VE. Blood profile and balance study of rats given the putative anorectic agent satietin. Am J Physiol 1995;268:R1–7.

Bellinger LL, Williams FE. Meal patterns and plasma liver enzymes and metabolites after total liver denervations. Physiol Behav 1995;58:625–8.

Bellinger LL, Fabia R, Husberg BS. Meal patterns prior to and following liver transplantation in rats. Physiol Behav 1997;62:525–9.

Blaustein JD, Wade GN. Ovarian hormones and meal patterns in rats: effects of progesterone and role of gastrointestinal transit. Physiol Behav 1977;19:23–7.

Butcher RL, Inskeep EK, Pope RS. Plasma concentrations of estradiol produced with two delivery systems in ovariectomized rats. Proc Soc Exp Biol Med 1978;158:475–7.

Carey MP, Deterd CH, de Koning J, Helmerhorst F, de Kloet ER. The influence of ovarian steroids on hypothalamic–pituitary–adrenal regulation in the female rat. J Endocrinol 1995;144:311–21.

Carleson J, Alstergren P, Appelgren A, Appelgren B, Kopp S, Srinivasan GR,

et al. Effects of adjuvant on neuropeptide-like immunoreactivity in experimentally induced temporomandibular arthritis in rats. Arch Oral Biol 1996;41:705–12.

Carleson J, Bileviciute I, Theodorsson E, Appelgren B, Appelgren A, Yousef N, et al. Effects of adjuvant on neuropeptide-like immunoreactivity in the temporomandibular joint and trigeminal ganglia. J Orofac Pain 1997;11:195–9.

Carlsson GE. Epidemiology and treatment need for temporomandibular disorders. J Orofac Pain 1999;13:232–7.

Clifton PG. Meal patterning in rodents: psychopharmacological and neuroanatomical studies. Neurosci Biobehav Rev 2000;24:213–22.

Donnelly TM. Blood-caked staining around the eyes in Sprague–Dawley rats. Lab Anim Sci 1997;47:17–8.

Dray A. Inflammatory mediators of pain. Br J Anaesth 1995;75:125–31.

Fillingim RB, Ness TJ. Sex-related hormonal influences on pain and analgesic responses. Neurosci Biobehav Rev 2000;24:485–501.

Glendinning JI, Smith J. Consistency of meal patterns in laboratory rats. Physiol Behav 1994;56:7–16.

Hargreaves KM. Neuroendocrine markers of stress. Anesth Prog 1990;37:99–105.

Harkness JE, Ridgway MD. Chromodacryorrhea in laboratory rats (*Rattus norvegicus*): etiologic considerations. Lab Anim Sci 1980;30:841–4.

Harper RP, Brown CM, Triplett MM, Villasenor A, Gatchel RJ. Masticatory function in patients with juvenile rheumatoid arthritis. Pediatr Dent 2000a;22:200–6.

Harper RP, Kerins CA, Talwar R, Spears R, Carlson DS, McIntosh JE, et al. Meal pattern analysis in response to temporomandibular joint inflammation in the rat. J Dent Res 2000b;79:1704–11.

Harper RP, Kerins CA, McIntosh JE, Spears R, Bellinger LL. Modulation of the inflammatory response in the rat TMJ with increasing doses of complete Freund's adjuvant. Osteoarthr Cartil 2001;9:619–24.

Hawk CT, Leary SL. Formulary for animals. Ames (IA): Iowa State University Press; 1995.

Hutchins B, Spears R, Hinton RJ, Harper RP. Calcitonin gene-related peptide and substance P immunoreactivity in rat trigeminal ganglia and brainstem following adjuvant-induced inflammation of the temporomandibular joint. Arch Oral Biol 2000;45:335–45.

Jezova D, Jurankova E, Mosnarova A, Kriska M, Skultetyova I. Neuroendocrine response during stress with relation to gender differences. Acta Neurobiol 1996;56:779–85.

Kest B, Sarton E, Dahan A. Gender differences in opioid mediated analgesia. Anesthesiology 2000;93(2):539–47.

Kopp S. The influence of neuropeptides, serotonin, and interleukin-1β on temporomandibular joint pain and inflammation. J Oral Maxillofac Surg 1998;56:189–91.

LeResche L. Epidemiology of temporomandibular disorders: implications for the investigation of etiologic factors. Crit Rev Oral Biol Med 1997;8:291–305.

LeResche L, Saunders K, Von Korff MR, Barlow W, Dworkin SF. Use of exogenous hormones and risk of temporomandibular disorder pain. Pain 1997;69:153–60.

Lictenberger LM, Romero JJ, de Ruijter WMJ, Behbod F, Darling R, Ashraf AQ, et al. Phosphatidylcholine association increases the anti-inflammatory and analgesic activity of ibuprofen in acute and chronic rodent models of joint inflammation: relationship to alterations in bioavailability and cyclooxygenase inhibitory potency. J Pharmacol Exp Ther 2001;298:279–87.

Lundberg U, Frankenhauser M. Pituitary–adrenal and sympathetic–adrenal correlates of distress and effort. J Psychosom Res 1980;24:125–30.

Mazzier AG, Laskin DM, Catchpole HR. Adjuvant induced arthritis in the temporomandibular joint of the rat. Arch Pathol Lab Med 1967;83:543–9.

Moberg GP, Bellinger LL, Mendel VE. Effect of meal feeding on daily rhythms of plasma corticosterone and growth hormone in the rat. Neuroendocrinology 1975;19:160–9.

Nance DM, Bromley B, Barnard RJ, Gorski RA. Sexual dimorphic effects of forced exercise on food intake and body weight in the rat. Physiol Behav 1977;19:155–8.

Nordahl S, Alstergren P, Eliasson S, Kopp S. Interleukin-1β in plasma and synovial fluid in relation to radiographic changes in arthritic temporomandibular joints. Eur J Oral Sci 1998;106:559–63.

Price DD, Mao J, Lu J, Caruso FS, Frenk H, Mayer DJ. Effects of the combined oral administration of NSAIDs and dextromethorphan on behavioral symptoms indicative of arthritic pain in rats. Pain 1996;68:119–27.

Rosloniec EF, Ballou LR, Raghow R, Hasty KA, Kang AH. Molecular biology of autoimmune arthritis. In: Serhan CN, Ward PA, editors. Molecular and Cellular Basis of Inflammation. Totowa, NJ: Humana Press; 1999. p. 289–307.

Sessle BJ. Neural mechanisms and pathways in craniofacial pain. Can J Neurol Sci 1999a;26(Suppl 3):S7–S11.

Sessle BJ. The neural basis of temporomandibular joint and masticatory muscle pain. J Orofac Pain 1999b;13:238–45.

Sessle BJ, Hu JW. Mechanisms of pain arising from articular tissues. Can J Physiol Pharmacol 1991;69:617–26.

Sharp P, LaRegina M. In: Suckow MA, editor. The laboratory rat: a volume in the laboratory animal pocket reference series. New York: CRC Press; 1998.

Stohler CS, Ashton-Miller JA, Carlson DS. The effects of pain from the mandibular joint and muscles on masticatory motor behaviour in man. Arch Oral Biol 1988;33:175–82.

Tarttelin M, Gorski RA. Variations in food and water intake in the normal and acyclic female rat. Physiol Behav 1971;7:847–52.

Varma M, Chai J-K, Meguid MM, Laviano A, Gleason JR, Yang Z-J, et al. Effect of estradiol and progesterone on daily rhythm in food intake and feeding patterns in Fischer rats. Physiol Behav 1999;68:99–107.

Walker JS, Carmody JJ. Experimental pain in healthy human subjects: gender differences in nociception and in response to ibuprofen. Anesth Analg 1998;86:1257–62.

Westberg K-G, Clavelou P, Schwartz G, Lund JP. Effects of chemical stimulation of masseter muscle nociceptors on trigeminal motoneuron and interneuron activities during fictive mastication in the rabbit. Pain 1997;73:295–308.

Westerterp KR. Body composition. In: Westerterp-Plantenga MS, Fredrix EWHM, Steffens AB, editors. Food intake and energy expenditure. Boca Raton (FL): CRC Press; 1994. p. 259–77.