Research papers

Stress-induced visceral hypersensitivity in female rats is estrogen-dependent and involves tachykinin NK1 receptors

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Abstract

Hormonal cycling may be related to a higher incidence of pain syndrome in female. As tachykinins are pivotal in stress-induced colonic dysfunction, we investigated whether ovarian steroids influence stress-induced visceral hypersensitivity to rectal distension (RD) in female rats and further, whether this influence involves NK1 receptors. Female Wistar rats, either intact or ovariectomized (OVX), were equipped for abdominal muscle electromyography and submitted to 2-h partial restraint stress (PRS) or sham-PRS. First, the effect of PRS was evaluated in intact rats. Second, abdominal response to RD was recorded in OVX rats treated with either, progesterone, 17 β -estradiol, 17 β -estradiol-plus-progesterone, or vehicle, in both basal and PRS conditions. Third, the NK1 receptor-antagonist, SR140333, was tested in PRS-intact and PRS-OVX rats under 17 β -estradiol or 17 β -estradiol-plus-progesterone treatment. PRS induced visceral hypersensitivity to RD and this effect was prevented by ovariectomy. OVX rats treated with 17 β -estradiol or 17 β -estradiol-plus-progesterone, but not progesterone alone, exhibited visceral hypersensitivity after PRS similar to that of intact rats. Both stress-induced visceral hypersensitivity in intact rats and the hormonally-restored visceral hyper-responsiveness of OVX rats were antagonized by SR140333. It is concluded, therefore, that stress-induced visceral hypersensitivity in female rats is estrogens-dependent and mediated through NK1 receptor activation.

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1. Introduction

Both experimental and clinical studies underline the existence of sex-dependent differences in the perception and responsiveness to nociceptive stimuli. Reportedly, women are more likely to experience recurrent pain, have lower pain thresholds, feel pain with higher intensity and less tolerance than men (Unruh, 1996; Berkley, 1997). In this respect, a striking sex-related difference towards a painful pathology regards the prevalence of functional bowel disorders such as irritable bowel syndrome (IBS) and the perception of associated abdominal pain (Naliboff et al., 1999). Indeed, females are more predisposed than males to present IBS symptoms and particularly visceral pain (Taub et al., 1995). Furthermore, the menstrual cycle influences visceral sensitivity in female IBS patients since painful gastrointestinal symptoms are enhanced during the perimenstrual period (Whitehead et al., 1990). Sex-related differences in nociceptive processing and responsiveness have also been documented in animal models of pain. Thus, female rats

exhibit greater pain behavior in response to electric shock (Beatty and Beatty, 1970) or formalin test (Aloisi et al., 1994) than males. In addition, responses to noxious stimuli, as well as responses to analgesic substances (Aloisi et al., 1995) depend on the stage of the estrous cycle and, indeed, on the ovarian steroid priming. Using a rat model of colonic distension, the mean balloon pressure required to induce a visceromotor response was found to be significantly lower during proestrus than at all other stages of the estrous cycle (Sapsed-Byrne et al., 1996). In contrast, rats in diestrus exhibit more pronounced signs of pain behavior associated with cyclophosphamide-induced cystitis than rats in estrus (Bon et al., 1997). Accordingly, findings in relation with the influence of the rat estrous stages on nociception are contradictory. Although, technical differences regarding the use of different nociceptive stimuli and/or methods for assessing pain may account for this apparent inconsistency, all studies emphasized the importance of sex steroids on nociceptive responses (Liu and Gintzler, 2000). Thus, it has been suggested that sex steroids influence nociceptive pathways at multiple levels, including primary afferent nerves and spinal cord projections, and exert complex actions in brain processing (Fillingim and Ness, 2000).

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Whatever the mechanisms involved, effects of sex hormones on nociception cannot be separated from other variables relevant to pain modulation. The contribution of biological, psychological and socio-cultural factors to sex-related differences in pain experience must be considered (Fillingim, 2000). Human studies bring evidence of men and women coping differently with stress (Wallbott and Scherer, 1991). In addition, data support significant roles of life stress and affective disorders in IBS probably related to both generation and exacerbation of IBS symptoms (Drossman et al., 1988). In addition, it has been shown that significant stressful life events occurring either before or after acute gastroenteritis are the strongest predictors of IBS development with female predominance (Gwee et al., 1999). A combination of stress and neuroendocrine modifications may result in a particular vulnerability of women to numerous pain syndromes (Rollman, 1998). Several models of visceral pain have been developed in animals but few investigations have been carried out to evaluate the influence of stress on abdominal pain. Visceral hypersensitivity to colorectal distension has been described in high-anxiety and partial-restraint stressed rats (Gue et al., 1997; Gunter et al., 2000). Additional data on stress-induced visceral hyperalgesia have been provided by a stress model of neonatal maternal separation (Coutinho et al., 2000). The involvement of glucocorticoids in nociceptive response linked to stress has been investigated. Injection of corticosterone into the amygdaloid nucleus, involved in the expression of anxiety, was found to trigger an exaggerated visceromotor response to colorectal distension in rat (Greenwood-Van Meerveld et al., 2001). Corticotropin releasing factor (CRF) is an additional candidate in the development of visceral hypersensitivity linked to stress (Gue et al., 1997).

Ovarian steroids may, indeed, affect the hypothalamic-pituitary-adrenal (HPA) axis response to stress in female rats by regulating the synthesis of stress mediators such as CRF or adrenocorticotrophic hormone (ACTH) (Roy et al., 1999). Sex steroids also differentially regulate the expression of uterine tachykinin receptors (Pinto et al., 1999), and the implication of intestinal NK1 receptors has been demonstrated in stress-induced bowel dysfunction (Ikeda et al., 1995).

Taken together, these data prompted us to investigate the involvement of ovarian steroids in the altered visceral response to rectal distension in stressed female rat. We further evaluated whether NK1 receptors are involved in the stress-induced visceral hypersensitivity and whether ovarian steroids contribute to NK1 receptor-mediated effects of stress.

2. Methods

2.1. Animal preparation

Female Wistar rats (Harlan, Gannat, France) weighing

200–250 g were surgically prepared for abdominal muscle electromyography according to a previously described technique (Ruckebusch and Fioramonti, 1975). Briefly, rats were anesthetized by intraperitoneal (i.p.) injection of 0.6 mg/kg acepromazine (Calmivet, Vetoquinol, Lure, France) and 120 mg/kg ketamine (Imalgene 1000, Rhône-Mérieux, Lyon, France). Three groups of three NiCr wire electrodes (60 cm length, 80 μ m diameter) were implanted bilaterally into the abdominal external oblique muscle, just superior to the inguinal ligament. Electrodes were exteriorized on the back of the neck and protected by a glass tube attached to the skin. Animals were individually housed in polypropylene cages and kept in a temperature-controlled room (21 °C). They were allowed free access to water and food (UAR pellets, Epinay, France) and were handled daily by the same individuals who performed the experiments.

2.2. Partial restraint stress procedure

All experiments were performed at the same period of the day (between 10:00 and 12:00 h) to avoid the influence of circadian rhythms. Stress effects were studied using the wrap partial restraint stress (PRS) model (Gue et al., 1997). Animals were lightly anesthetized with ethyl-ether and their fore shoulders, upper forelimbs, and thoracic trunk were wrapped in a confining harness of paper tape to restrict, but not impede body movements. Then, rats were placed into their home cage for 2 h. Rats recovered from ethyl-ether anesthesia within 2–3 min and immediately moved around in their cages, but the restricted mobility of their forelimbs prevented grooming behavior. Control sham-PRS animals were anesthetized as above but not wrapped and were allowed to move freely in their cages. PRS at room temperature as used here is considered as a mild and non-ulcerogenic stressor (Gue et al., 1997).

2.3. Electromyographic recording

Electromyographic recordings began 5 days after surgery. The electrical activity of abdominal striated muscles was recorded with an electroencephalograph (Mini VIII; Alvar, Paris, France), using a short time constant (0.03 s) to remove low-frequency signals (<3 Hz) and a paper speed of 3.6 cm/min. Abdominal contractions were measured when spike bursts amplitude was superior to 25 μ V according to a previously described procedure (Morteau et al., 1994).

2.4. Rectal distension procedure

Rats were placed in plastic tunnels (6 cm diameter, 25 cm length) into which they could not move, escape or turn around, in order to prevent damage to the balloon. They were accustomed to this procedure for 3 days before rectal distension to minimize additional stress reactions during experiments. The balloon used for distension was an arterial embolectomy probe (Fogarty; Edwards Laboratories Inc, Santa Ana, CA, USA). Rectal distension was performed

by insertion of the balloon (2 mm diameter, 2 cm long) into the rectum, the catheter being fixed at the tail with adhesive tape. The balloon was inflated progressively by steps of 0.4 ml, from 0 to 1.2 ml, each step of inflation lasting 5 min. The balloon and connected syringe were filled with tepid water in order to avoid temperature contrast. To detect possible leakage, the volume of water introduced into the balloon was checked by complete removal with a syringe at the end of the distension period. This procedure was performed according to a previously described technique (Morteau et al., 1994).

2.5. Ovariectomy procedure

Bilateral ovariectomy (OVX) was performed under deep surgical anesthesia (acepromazine, 0.6 mg/kg i.p.; ketamine, 120 mg/kg i.p.). Briefly, after midline laparotomy, the fallopian tubes were ligated below the ovaries, which were then excised; muscle and skin wounds were closed using silk suture. Sham OVX consisted in externalizing the ovaries from the abdominal cavity and replacing them without being excised. Rats were allowed 2 weeks of post surgical recovery before the beginning of experiments.

2.6. Vaginal smear

The ovarian steroid status of intact and sham ovariectomized rat was evaluated by vaginal smear after Haris–Shorr staining. Only females in proestrus, which corresponds to a combined estradiol and progesterone priming, were used in our study. This procedure was performed before the stress or sham stress session in awake rats. The effectiveness of ovariectomy was controlled through vaginal smear (data not shown).

2.7. Experimental protocol

The study was conducted following three series of experiments.

In a first series, we investigated the effect of sham-PRS and PRS on abdominal response to rectal distension in intact female rats. Rectal distension was performed 20 min after sham-PRS or PRS.

In a second series, groups of ten OVX and ten sham-OVX rats were used. In the first experience, we compared the abdominal muscle responses to rectal distension of OVX and sham-OVX rats in sham-PRS condition. In the following experiments, abdominal response to rectal distension was assessed in OVX and sham-OVX rats submitted to PRS. In order to investigate the effect of ovarian steroids on visceral sensitivity to rectal distension, OVX rats received subcutaneously either vehicle (0.2 ml olive oil/rat), 17 β -estradiol (E; 5 μ g/0.2 ml per rat), progesterone (P; 500 μ g/0.2 ml per rat), or a combination of both 17 β -estradiol and progesterone (E&P; 5 μ g and 500 μ g/0.2 ml per rat, respectively) 1 h before PRS or sham-PRS. As

above, all groups underwent rectal distension 20 min after PRS or sham-PRS.

In a third series of experiments, groups of ten intact rats underwent PRS or sham-PRS and were submitted to rectal distension after a 20-min period of rest. The effect of the tachykinin NK1 receptor antagonist SR 140333 (1 mg/kg, i.p.) or its vehicle on abdominal muscle response to rectal distension was assessed in both sham-PRS and PRS rats. SR 140333 or vehicle was administered 15 min before the onset of rectal distension. Lastly, SR 140333 (1 mg/kg, i.p.) or vehicle was injected 15 min before rectal distension, in OVX + E and OVX + E&P groups submitted to PRS. SR 140333 compound is reported to be a potent and highly selective inhibitor of substance P binding to NK1 receptor from various animal species including rats (Emonds-Alt et al., 1993).

All protocols were approved by the Local Animal Care and Use Committee of the Institut National de la Recherche Agronomique.

2.8. Chemicals

Progesterone (4-Pregnene-3, 20-dione) and 17 β -estradiol (1,3,5[10]-estratriene-3, 17 β -diol, 3-benzoate) were purchased from Sigma Chemical (St Quentin Fallavier, France). The nonpeptide tachykinin NK1 receptor antagonist SR140333 (S)-1-[2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenylacetyl) piperidin-3-yl] ethyl] -4-phenyl-1-azoniabicyclo[2,2,2]octane chloride was a gift from Dr. Emonds-Alt (Sanofi Research, Montpellier, France). Doses used for each compound were selected according to relevant references in the literature.

2.9. Statistical analysis

Statistical analysis of the number of abdominal muscle contractions for each 5-min period during rectal distension was performed by one-way analysis of variance followed by Student's unpaired or paired *t*-test where relevant. Values were expressed as mean $\pm$ SEM. Differences were considered significant for $P < 0.05$.

3. Results

3.1. Stress-induced visceral hypersensitivity

Gradual rectal distension increased the frequency of abdominal muscle contractions in a volume-dependent manner (Fig. 1). In sham-PRS rats, this increase became significant ($P < 0.05$) when the volume of distension reached 0.8 ml. PRS applied to female rats significantly enhanced the number of abdominal contractions for all volumes of distension from 0.4 to 1.2 ml. Thus, the first volume of distension inducing a significant increase of abdominal contractions was lowered from 0.8 ml (in control sham-PRS) to 0.4 ml in PRS rats (Fig. 2).

Fig. 1. Electromyographic recording in intact female rats for the different volume of rectal distension.

3.2. Ovariectomy, hormonal treatments and visceral sensitivity

OVX and sham-OVX rats exhibited similar increases in the number of abdominal muscle contractions in response to rectal distension for all volumes of distension (Table 1). However, in contrast to sham-OVX rats (data not shown), OVX rats did not exhibit abdominal muscle hyperresponsiveness to rectal distension after PRS. Indeed, the number of abdominal contractions in response to gradual rectal distension recorded in OVX rats submitted to PRS was similar to that observed in OVX rats submitted to sham-PRS (Fig. 3).

In control situation (sham-PRS), treatment of OVX rats with progesterone (P) failed to modify the abdominal response to rectal distension compared with vehicle-treated OVX rats (Fig. 3A). Similarly, OVX rats treated with estradiol (E) exhibited a similar response than vehicle-treated sham-PRS rats (Fig. 3B). Furthermore, when OVX rats were given a combination of 17β -estradiol and progesterone, no change in visceral sensitivity was observed compared with rats treated with vehicle, in basal situation (sham-PRS) (Fig. 3C).

PRS applied to OVX + P rats did not induce any change in the abdominal response to rectal distension compared with OVX + P sham-PRS rats (Fig. 3A). In contrast, PRS triggered an increase of the number of abdominal contractions in response to rectal distension in OVX rats treated with E. This increase was significant ($P < 0.05$) for all

volumes of distension (Fig. 3B). Moreover, PRS significantly enhanced the abdominal response to rectal distension in OVX + E&P-treated rats compared with sham-PRS rats given the same hormonal treatment (Fig. 3C).

3.3. Stress-induced visceral hypersensitivity and NK1 receptor antagonism

SR 140333 (1 mg/kg) injected to intact rats had no effect per se on the abdominal response to rectal distension in sham-PRS conditions (Table 2). However, when given to rats submitted to PRS, SR 140333 significantly counteracted the stress-induced increase of abdominal contractions in response to all volumes of distension (Fig. 4).

3.4. Hormonal treatment, stress-induced visceral hypersensitivity and NK1 receptor antagonist

We investigated the effect of SR 140333 in OVX + E and OVX + E&P groups where stress-induced visceral hypersensitivity to rectal distension was previously described. The increase of abdominal contraction numbers observed in OVX + E and OVX + E&P animals submitted to PRS was abolished by a treatment with the NK1 receptor antagonist SR 140333 for all volumes of distension (Fig. 5).

4. Discussion

The present study provides novel evidence indicating that partial restraint stress-induced visceral hypersensitivity depends upon ovarian steroids and is linked to the activation of tachykinin NK1 receptors. We first showed that restraint stress in female rats enhanced visceral sensitivity to rectal distension and lowered the nociceptive threshold volume. These findings are in agreement with previous studies reporting visceral hypersensitivity in rat models of restraint (Gue et al., 1997), high anxiety (Gunter et al., 2000), or neonatal stress (Coutinho et al., 2000). Among other mediators, central CRF has been proposed to be involved in stress-induced visceral hypersensitivity, since centrally administered CRF was found to mimic the effects of restraint stress (Gue et al., 1997). Visceral hypersensitivity generated by stress has also been connected with the release of mediators from cells within the gut wall, such as mast cells, which in

Fig. 2. Abdominal muscle response to rectal distension in female rats submitted to partial restraint stress (PRS) or sham-PRS. Values are means $\pm$ SEM, $n = 8$. * $P < 0.05$ compared with sham-PRS (control).

Table 1
Lack of effect of ovariectomy on the abdominal muscle response to rectal distension of rats in control (sham-PRS) conditions^a

	Volume of distension (ml)			
	0	0.4	0.8	1.2
OVX	4.5 $\pm$ 1.2	5.3 $\pm$ 0.7	24.2 $\pm$ 3.62	31.0 $\pm$ 2.8
Sham-OVX	5.1 $\pm$ 0.9	7.0 $\pm$ 1.3	24.9 $\pm$ 2.6	29.0 $\pm$ 4.1

^a Values (mean $\pm$ SEM; $n = 8$) are the number of abdominal contractions occurring during 5-min periods for each volume of distension. OVX, ovariectomized rats; PRS, partial restraint stress.

Fig. 3. Effect of pretreatment of ovariectomized (OVX) rats with progesterone (P) (panel A), 17 β -estradiol (E) (panel B) or a combination of 17 β -estradiol (E) and progesterone (P) (panel C) on the abdominal muscle response to rectal distension in partial restraint stress (PRS) and sham-PRS (control) conditions. Values are means $\pm$ SEM, $n = 8$. * $P < 0.05$ compared with OVX treated rats submitted to sham-PRS.

turn sensitize visceral afferent terminals (Gue et al., 1997). The modulatory role of ovarian steroids on stress-induced visceral hypersensitivity was first evidenced by our data showing that ovariectomy prevented stress-induced visceral hyper-responsiveness to rectal distension. Interestingly, ovariectomy did not affect the nociceptive response to rectal distension in basal conditions. Abundant data support the hypothesis of a close relationship between ovarian steroids and pain sensitivity. However, published results are somewhat contradictory that may be linked to the influence of

Table 2

Lack of effect of SR 140333 on the abdominal muscle response to rectal distension of rats submitted to control (sham-PRS) conditions^a

	Volume of distension (ml)			
	0	0.4	0.8	1.2
Vehicle	5.3 $\pm$ 0.6	10.3 $\pm$ 2.1	23.8 $\pm$ 2.5	28.0 $\pm$ 2.1
SR140333	8.3 $\pm$ 1.6	8.0 $\pm$ 2.6	29.8 $\pm$ 7.6	30.2 $\pm$ 2.5

^a Values are the number of abdominal contractions occurring during 5-min periods for each volume of distension (mean $\pm$ SEM; $n = 8$). PRS, partial restraint stress.

gonadal steroids on nociceptive pathways which concern different interacting levels including cross-reactivity with the opioid system (Danzewitz et al., 1995), the direct modulation of afferent fiber activity (Frye et al., 1990) and the regulation of neurotransmitter release at central level (Loscher et al., 1992). Sex hormones may also influence the contractile response and myoelectric activity of the gastrointestinal smooth muscle (Heitkemper and Bond, 1995; Chen et al., 1995) that may in turn, affect the visceral sensitivity. However, the putative regulation of the gastrointestinal motility and transit by ovarian steroids is not related to altered visceral sensitivity in our study since hormonal treatments do not affect basal abdominal response to rectal distension in ovariectomized rats. Nevertheless, regulation of CRF synthesis by sex steroids can be proposed as a prominent mechanism by which stress-induced visceral hypersensitivity could be modulated in females. Activation of the hypothalamic-pituitary-adrenal (HPA) axis during stress is more pronounced in females (Young, 1995) and was suggested to be modulated by ovarian steroids. Interestingly, the CRF gene contains the estrogen response element allowing estrogens to directly affect CRF expression (Vamvakopoulos and Chrousos, 1993). However, the modulating influence of sex steroids on the HPA response includes complex regulation loops and its involvement in

Fig. 4. Effect of the tachykinin NK1 receptor antagonist, SR 140333, on the abdominal muscle response to rectal distension in female rats submitted to partial restraint stress (PRS). Values are means $\pm$ SEM, $n = 8$. * $P < 0.05$ compared with sham-PRS + vehicle; + $P < 0.05$ compared with PRS + vehicle.

Fig. 5. Effect of the tachykinin NK1 receptor antagonist, SR 140333, on increased abdominal muscle response to rectal distension in ovariectomized (OVX) rats treated with 17 β -estradiol (E) (panel A) or a combination of 17 β -estradiol (E) and progesterone (P) (panel B) submitted to partial restraint stress (PRS). Values are means $\pm$ SEM, $n = 8$. * $P < 0.05$ compared with vehicle.

nociceptive processes obviously needs further investigations.

Sex steroid replacement strategies were undertaken in ovariectomized rats to determine how specific hormones affect the abdominal muscle response to stress. Progesterone-primed rats failed to exhibit the visceral hypersensitivity which was observed in response to PRS in intact animals. Therefore, progesterone by itself does not seem to have a major regulatory role in the development of stress-linked visceral hypersensitivity. In contrast, 17 β -estradiol- or 17 β -estradiol-plus-progesterone-primed OVX rats exhibit similar PRS-induced visceral hypersensitivity to rectal distension as that seen in intact controls, suggesting a major role of estrogens in this effect. However, the permissive influence of progesterone cannot be totally ruled out in the effect of combined treatment and we can expect a cooperative effect of both steroids in the resulting visceral hypersensitivity after stress.

Furthermore, the efficacy of SR 140333 in our study to prevent the PRS-induced increase of abdominal muscle response to rectal distension, suggests that NK1 receptor activation during stress may be an additional mechanism

by which stress enhances visceral sensitivity. In contrast, SR 140333 failed to modify the abdominal response to rectal distension in basal condition. These findings are supported by previous studies showing that mice depleted for the tachykinin NK1 receptor gene (NK1 $-/-$), failed to develop visceral hyperalgesia in reaction to an acute chemical stimulus (Laird et al., 2000). In addition, NK1 $-/-$ mice showed normal response to visceral mechanical stimuli (Laird et al., 2000). Moreover, recent studies showed that stress-induced colonic dysfunction in rats involves activation of NK1 receptors by substance P released from enteric neurons (Ikeda et al., 1995). Taken together, these data suggest that NK1 receptor sensitivity, functionality or expression may be modulated by stress as previously shown in inflamed tissues (Mantyh et al., 1995; Evangelista et al., 1996). However, NK1 receptor antagonists were shown to be ineffective to inhibit visceral hypersensitivity related to inflammatory stimulation in rats (Julia and Bueno, 1997). In addition, increased levels of substance P have been detected in the peritoneal fluid of stressed mice (Chancellor-Freeland et al., 1995). Then, the expression and/or release of tachykinins may directly or indirectly affect tachykinin receptor function or expression, which could further affect the nociceptive response during stress.

Considering the regulatory potency of ovarian steroids on tachykinin receptor expression (Villablanca and Hanley, 1997; Pinto et al., 1999), we further investigated the contribution of NK1 receptor activation during stress-induced visceral hypersensitivity in estrogen- or combined estrogen and progesterone-primed ovariectomized rats. Increased abdominal muscle response after stress in these two groups was found to be abolished by prior treatment with the NK1 receptor antagonist, SR 140333. These results suggest a key role of endogenous NK1 receptor ligand, most likely substance P, in the visceral hyper-responsiveness linked to stress, depending on the availability of estrogen in the milieu.

Concurrently, a positive correlation between plasma 17 β -estradiol and substance P concentrations has been reported in female rats (Duval et al., 1996), and estradiol benzoate was found to enhance the substance P content in the hypothalamus (Duval et al., 1998). In addition, increasing evidence suggests that NK1 receptor gene expression is under hormonal control. In rat pancreatic acinar cells, 17 β -estradiol treatment led to a 2.5-fold increase in substance P receptor mRNA levels (Villablanca and Hanley, 1997). In the rat uterus, NK1 receptor mRNA was significantly increased in rats treated with 17 β -estradiol (Pinto et al., 1999). Additional data suggested that estrogens might modulate substance P responsiveness in nodose ganglion neurons in guinea pigs (Oh et al., 2000). The contribution of sex hormones to the transcriptional regulation of genes encoding for different components has largely been described (Madeddu et al., 1997) and we cannot exclude that estrogens may act as a transcriptional factor modulating the synthesis of specific mRNAs and proteins for NK1

receptors in various tissues, especially the gut. A direct genomic effect of estrogens can not be excluded after a short-term treatment. Indeed, an acute steroid replacement in ovariectomized rats was previously shown to affect mRNA and proteins levels for other factor such as brain-derived neurotrophic factor (Gibbs, 1999) or benzodiazepine receptors (Bitran et al., 1998). Considering these data, the restored visceral hypersensitivity observed in estradiol-treated rats submitted to stress could be explained by the sensitization of visceral afferent nerve endings following an increased release of substance P interacting with NK1 receptors. Furthermore, exposure to acute stress was reported to significantly enhance serum estradiol concentrations in intact female rats (Shors et al., 1999). Then, this process may be involved in the stress-induced visceral hypersensitivity first evidenced in our study in intact female rats. Substance P receptors were found to be expressed in different cell types including nerves (Bowden et al., 1994), endothelial cells (Portbury et al., 1996), and mast cells (Cooke et al., 1998). The binding of substance P to visceral afferent nerves could induce the release of neurotransmitters, stimulating mast cells to further release several mediators, which in turn could sensitize afferent nerve endings, resulting in an increased response to painful stimuli. In addition, direct activation of mast cells by substance P is possible and may lead to an amplifying loop involving substance P, histamine, and nerve growth factor. This hypothesis is supported by previous results from our group showing an effect of substance P directly toward mast cells in colonic samples of stressed female rats (Bradesi et al., 2001).

In conclusion, the present study provides evidence indicating that stress-induced visceral hypersensitivity to a mechanical stimulus depends upon the hormonal status of female rats affecting gut responsiveness through activation of NK1 receptors and probably substance P release. Estradiol seems to be responsible of stress-induced visceral hyperalgesia by favoring the functionality of NK1 receptors triggered by stress. These results are supported by our previous data showing the regulatory potency of ovarian hormones in stress-induced induction of NK1 receptor expression or functionality, evidenced by the *in vitro* ability of NK1 receptor antagonist to inhibit substance P-induced histamine release from colonic samples of stressed female rats (Bradesi et al., 2001). These observations are consistent with the concept that pain sensitivity to noxious stimuli depends on a complex regulatory system involving the interaction of sex steroids and psychological factors, and could be part of the explanation for female predominance in the occurrence of altered pain sensitivity in functional gastrointestinal disorders such as IBS.

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