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Role of C-fibers during acute and chronic stress on formalin-induced paw edema in rats

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Stimulation of peripheral nociceptors leads to releasing of some mediators such as substance P (SP) and Calcitonin gene-related peptide (CGRP) and contributes to the edema formation by vasodilatation induction. On the other hand glucocorticoids have anti-inflammatory action, and they are elevated in the plasma during stress. This communication reports C-fibers inflammatory role and the effects of chronic and acute stress and/or dexamethasone (as stress mimicry) on paw edema induced by formalin at presence/deficit C-fibers rats. Acute stress and dexamethasone and chronic dexamethasone have shown an anti-inflammatory effect in C-normal groups, but chronic stress had no effect on inflammation. C-fibers reduction (C-lesion) had anti-inflammatory effects. In deficit C-fibers rats, acute and chronic stress had not stronger anti-inflammatory effect, but acute dexamethasone reduced the anti-inflammatory effect of C-fibers reduction while in the same condition, chronic dexamethasone induced stronger anti-inflammatory effect. The results show C-fiber nerve produce and release the peripheral inflammatory mediators, "C-fibers reduction" decreased the paw inflammation. Counter adaptation in C-lesion animals may reduce the modulatory effects of dexamethasone on the remaining C-fibers. Acute dexamethasone diminished the "C-fibers reduction" anti-inflammatory effect, but at chronic treatment, the modulatory effects of dexamethasone aggregated and it augmented the C-fibers reduction anti-inflammatory effect.

Keywords: Capsaicin, C-fiber, Dexamethasone, Inflammation, Stress

Stimulation of peripheral nociceptors terminals (especially C-fibers) leads to release of some mediators such as substance P (SP) and calcitonin gene-related peptide (CGRP) which contribute to axonal reflex. These substances have an important role in edema by acting directly on venules to produce vasodilatation. They also contribute to hyperalgesia through histamine release from mast cells, which decreases the threshold level of activation of nociceptors[1]. Stress activates hypothalamic-pituitary axis (HPA) and increases the cortisol secretion. Glucocorticoids are powerful anti-inflammatory drugs which are widely used for treating inflammatory diseases[2]. The role of glucocorticoids in stress is well known[3]. Inflammation leads to sensitization of nociceptors[4]. Reduction of inflammation leads to reduction of hyperalgesia[4]. The relationship between stress and C-fibers in analgesia and hyperalgesia is well studied[5,6] however only a few reports on the relation of stress and C-fibers in inflammation are available. In dexamethasone-treated animals, pain alleviation occurred and could be related to inhibition of inflammatory reaction[5]. Therefore knowing more about pain to elucidate the physiological role of C-fibers is very important in producing inflammatory or anti-inflammatory effects of stress and dexamethasone during pain and diseases control. Thus the aim of this study is to illustrate the role of C-fibers in acute and chronic stress in inflammation, and for better clarification dexamethasone has been used to show the possible mechanism of C-fibers in inflammation.

Materials and Methods

Animals—Male Wistar rats weighing 180–200 g were used. Animals were kept in a 20±2 °C with a 12:12 h L:D cycle and fed with standard diet and drinking water. The experimental protocol approved by the Ethical Committee of Ferdowsi University and the protocol was following by ethical rules for

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laboratory animals[7]. Animals were randomly divided into following 4 groups of 7 each: Gr.1: acute forced swim stress, Gr.2: chronic forced swim stress, Gr.3: acute dexamethasone, Gr.4: chronic dexamethasone. In each acute and chronic main groups there were three sub-groups; C-normal (intact C-fibers), C-lesion (capsaicin at neonate stage) and sham (capsaicin vehicle at neonate stage). Forced swim stress (10 min/day) in water (18±1 ºC) was considered as acute stress and daily repeated forced swim stress (for 3 constitutive days) as chronic stress[3,4,5,6,8]. Single-dose of dexamethasone (2 mg/kg, ip) was considered as acute and daily injections (for 3 constitutive days), as chronic treatment.

Capsaicin preparation and lesion C-fibers—Capsaicin (Sigma, Germany) was dissolved in a solvent consisting of ethanol, Tween 80 and normal saline in ratio of 1:1:8 (v/v) to prepare a 0.5% solution of capsaicin. Capsaicin (50 mg/kg, ip) treatment within 48 h after birth effectively destroys C-fibers neonatal rats[9,10]. Efficacy of capsaicin treatment in C-fibers lesion was assessed by corneal chemosensitivity test which is principally mediated by C-fibers[11] and describe previously. Briefly, those capsaicin-treated animals in which the number of wipes of their right eye in the first 10 seconds after administration of one drop of 1% ammonium hydroxide was reduced significantly were considered as C-fiber lesion (C-lesion) ones.

Forced swim stress—To avoid the effect of circadian rhythm specially the hypothalamic-pituitary axis (HPA) activity, the experiments were started at 10:00 hrs and finished at 14:00 hrs. The forced swim (10 min/day) was done in a cylindrical plastic container (diameter 35 cm and height 50 cm which was filled with 18±1 ^{o}C water with a depth of 40 cm). After the swimming sessions, rats were immediately dried by a towel[12] and then subsequent assignment was done.

Induction of paw edema and its measurement—Sub-plantar administration of formalin (2.5%, 0.05 mL) induced paw edema and its volume was assessed by digital balance plethysmometer. Briefly, before the injection of formalin the rat's left hind paw was inserted in the mercury plethysmometer column on the sensitive digital balance and after 1 hour of injection of formalin, the measurement repeated. Formalin was injected 30 min after ip administration of dexamethasone (acute and chronic stress groups) or immediately after swimming stress (acute and chronic stress groups). To calculate paw edema volume, the difference of paw volume before and after injection of sub-plantar formalin was measured[13] and the percentage change of edema volume induced by formalin calculated by the formula:

$$\text{Changes of edema volume (\%)} = [(A-B)/B] \times 100$$

where A is the edema volume in treated group and B is the edema volume in control group. It shows anti-inflammatory effect if the value is positive and if it is negative, it shows proinflammatory effect.

Statistical analysis—The data were expressed as mean±SE and ANOVA and Tukey post test were applied to compare the results of control and test groups; $P<0.05$ was considered as significant.

Results

Effect of C-fibers on paw edema induced by formalin—Compare to paw volume before formalin injection, paw volume of the C-fibers intact animals expressed a significant edema after formalin injection ($P<0.001$). The difference between C-normal group and sham group was not significant; therefore using of the solvent (normal saline and capsaicin solvent) for administration of drugs was possible. C-lesion group showed a significant reduction in paw edema compare to C-normal (Fig. 1).

Effect of C-fibers on formalin induced paw edema in acute stress—Acute stress reduced formalin induced paw edema compared to C-normal group ($P<0.001$). This reduction was augmented in acute stress C-lesion group and anti-inflammatory effect was also stronger than acute stress C-normal ($P<0.001$) (Fig. 2).

Effect of C-fibers on formalin induced paw edema in chronic stress—In presence of C-fibers, chronic stress couldn't reduce the paw edema due to formalin administration. Whereas in C-lesion group, chronic stress significantly reduced formalin induced paw edema ($P<0.001$) (Fig. 3).

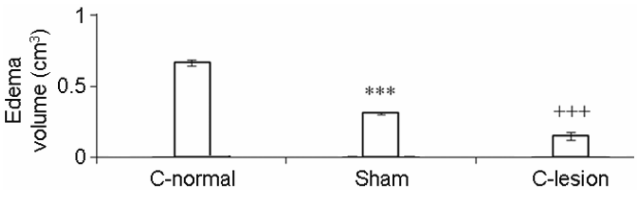

Fig. 1—Comparison of formalin induced paw edema (2.5%, 0.05 mL, sp) in C-normal, sham and C-lesion groups. In C-lesion group paw edema significantly decreased compared to C-normal group (***$P<0.001$), (n=7 in each group).

Effect of acute dexamethasone on paw edema induced by formalin in presence or absence of C-fibers—Paw edema induced by formalin was significantly reduced in acute dexamethasone C-normal group when compared to C-normal group (P<0.001). Acute dexamethasone C-lesion group showed significant reduction in paw edema compared to acute dexamethasone C-normal (P<0.001) (Fig. 4).

Effect of chronic dexamethasone on paw edema induced by formalin in presence or absence of C-fibers—In C-normal animals, administration of chronic dexamethasone (2 mg/kg, ip, repeated for 3 days) reduced paw edema induced by formalin (P<0.01 compared to C-normal). This anti-inflammatory effect has been stronger and almost has diminished edema induced by formalin in C-lesion animals (P<0.001 than chronic dexamethasone C-normal) (Fig. 5).

Comparison of anti-inflammatory effect in C-lesion groups: acute and chronic stress, acute and chronic dexamethasone—All these groups are the C-fibers depleted and showed anti-inflammatory effect because positive value showed anti-inflammatory and negative proinflammatory effect but in comparison of C-lesion group, they have variable anti-inflammatory effect. Therefore stress did not show stronger anti-inflammatory effect but acute dexamethasone reduced the anti-inflammatory effect of C-fibers reduction (P<0.001) while in the same condition, chronic dexamethasone induced stronger anti-inflammatory effect (P<0.01) (Fig. 6).

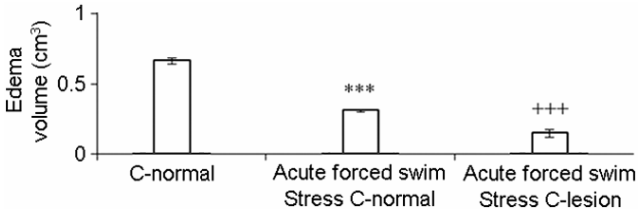

Fig. 2—Effects of acute forced swim stress on formalin induced paw edema (2.5%, 0.05 mL, sp) in C-normal and C-lesion fibers. Paw edema reduced significantly in acute forced swim stress C-normal compare to C-normal group (***P<0.001), Acute forced swim C-lesion group showed more reduction in paw edema compare to acute forced swim stress C-normal group (+++P<0.001), (n=7 in each group).

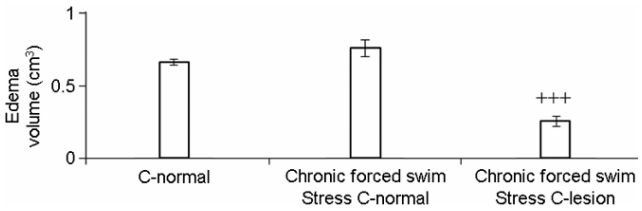

Fig. 3—Effects of chronic forced swim stress on formalin induced paw edema (2.5%, 0.05 mL, sp) in C-normal and C-lesion fibers. Chronic stress has not any effect on paw edema in C-normal animals but it reduced paw edema in C-lesion fibers (+++P<0.001 compare to chronic stress C- normal), (n=7 in each group).

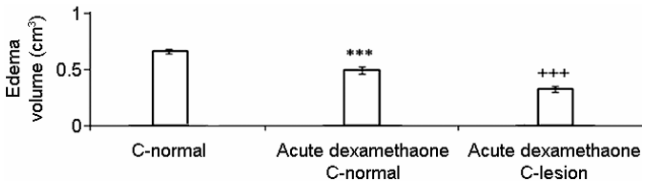

Fig. 4—Effect of acute administration of dexamethasone on formalin induced paw edema (2.5%, 0.05 mL, sp) in C-normal and C-lesion fibers. Paw edema was reduced by acute administration of dexamethasone in C-normal animals (***P<0.001 Compare to C-normal group), and in C-lesion fibers (+++ P<0.001 compare to acute dexamethasone administration C-normal group), (n=7 in each group).

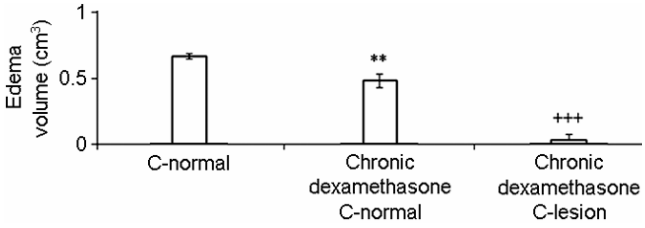

Fig. 5—Effect of chronic administration of dexamethasone on formalin induced paw edema (2.5%, 0.05 mL, sp) in C-normal and C-lesion fibers. Paw edema was reduced by administration of chronic dexamethasone in C-normal animals (**P<0.01 compare to C-normal group). Paw edema by chronic administration of dexamethasone was reduced in C-lesion fibers group compare to chronic administration of dexamethasone C-normal group (+++ P<0.001), (n=7 in each group).

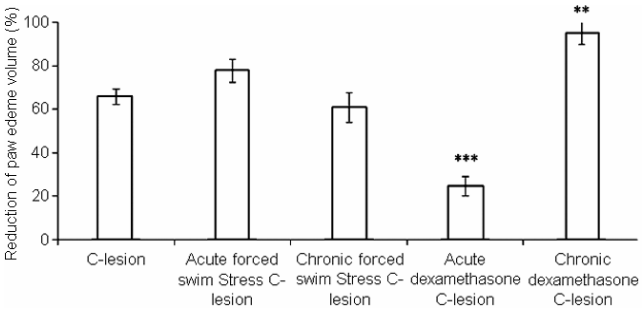

Fig. 6—Comparison the percent reduction of paw edema volume in C-lesion groups. Acute and chronic forced swim stress showed no difference with C-lesion saline group. Acute dexamethasone administration significantly reduced the percent reduction of paw edema volume and chronic dexamethasone administration increased the percent reduction of paw edema volume compare to C-lesion saline group (***P<0.001, **P<0.01), (n=7 in each group).

Discussion

Nociceptors are responsible for inflammation progression and reciprocally inflammation can lead to sensitization of nociceptors[4]. Stimulation of peripheral nociceptors specially peripheral terminals of C-fiber nerves in tissues, leads to release of some inflammatory mediators such as substance P (SP) and calcitonin gene-related peptide (CGRP) which spread the edema by directly acting on venules to produce vasodilatation[1]. Therefore it can be hypothesized that just decreasing the number of the C-fibers can diminish the paw edema induced by formalin as reported (Fig 1). Acute stress diminished edema due to formalin administration, in C-normal animals (Fig 2). It is suggested that related neurotransmitters in stress-induced analgesia are endocannabinoids that mediate their antinociceptive effects via CB1 and CB2 receptors[15]. CB1 is expressed on central and peripheral neurons. Activation of the CB1 receptor is negatively coupled to adenylate cyclase and blocks excitability and activation of primary afferents[16]. On the other hand, neuropeptides such as substance P and CGRP have a broad spectrum of effects within peripheral tissues and have an important role to produce plasma extravasation and vasodilatation which also causes nociceptor activity during inflammation[17]. Therefore it could be inferable that "reduction of C-fibers action" due to cannabinoids action on CB1 receptors plus cannabinoids effects via immune cell CB2 receptors can decrease the inflammation which this assigns the anti-inflammatory effects of acute stress. Chronic stress had no anti-inflammatory effect on inflammation due to formalin administration, in C-normal animals (Fig. 3), it can suggest a new series of investigations to question the hypothesis of endocannabinoids tolerance responses due to chronic stress. Also the results showed, both the acute and chronic stress in the C-lesion animals have anti-inflammatory effects and even more for acute stress in contrast to the same in C-normal animals (Fig. 2, 3), not only can confirm the C-fibers importance in the peripheral induction of inflammation but also suggest that stress can act on C-fibers to reduce the inflammation, at least in part.

Edema reduction potency of acute or chronic stress in C-lesion animals doesn't show any significant difference in comparison with the same effect for C-lesion alone (Fig. 6), this can confirm again that stress may act on C-fibers to reduce the inflammation, as hypothesized in the present study. Stress physiologically eliminates the effect of C-fibers in inflammation and edema induction, therefore physical elimination of C-fibers have to show the same effect as we had seen.

Glucocorticoids (GCs) can prevent the inflammation and are used to treat painful inflammatory rheumatic diseases[18]. They are also secreted during the stress[4]. Acute and repeated dexamethasone administration decreased inflammation in C-normal animals (Fig. 4, 5). Dexamethasone as a synthetic glucocorticoid (GCs) has anti-inflammatory and immunosuppressive effects[3]. Therefore it can reduce inflammation by effect on macrophages and decreasing release of cytokines, can reduce the C-fibers excitations and thus the role of C-fibers in the inflammation progression.

On the other hand the present data also showed that acute dexamethasone contradicting to the results of C-fibers reduction in C-lesion animals (Fig. 6), it is shown that GCs facilitate the N-methyl-d-aspartate (NMDA) receptors play a significant role in pain[6]. Facilitation of NMDA receptor increases the neurons excitability in the dorsal horns lead to excessive release of neuropeptides such as SP from the remained C-fibers in the central and peripheral regions of C-lesion animals which in turn it can lead to alleviate the anti-inflammatory effect of GCs as we have seen here[6].

However in C-fibers-lesion state, chronic dexamethasone decreased inflammation significantly (Fig. 6). NMDA receptors facilitation by GCs is not enough to address the effect. To clarify, another mechanisms such as involvement of peripheral endorphin receptors or any other possible mechanism for potentiating of GCs anti-inflammatory effect after chronic usage, may be involved which require further investigation.

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