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Research report

Stress-induced dura vascular permeability does not develop in mast cell-deficient and neurokinin-1 receptor knockout mice

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Abstract

Migraine headaches are often precipitated by stress and seem to involve neurogenic inflammation (NI) of the dura mater associated with the sensation of throbbing pain. Trigeminal nerve stimulation had been reported to activate rat dura mast cells and increase vascular permeability, effects inhibited by neonatal pretreatment with capsaicin implicating sensory neuropeptides, such as substance P (SP). The aim of the present study was to investigate NI, assessed by extravasation of ⁹⁹Tc-gluceptate (⁹⁹Tc-G), as well as the role of mast cells, SP and its receptor (NK-1R) in dura mater of mice in response to acute stress. Restraint stress for thirty min significantly increased ⁹⁹Tc-G extravasation in the dura mater of C57BL mice. This effect was absent in W/W^v mast cell-deficient mice and NK-1 receptor knockout mice (NK-1R^{-/-}), but was unaltered in SP knockout mice (SP^{-/-}). Acute restraint stress also resulted in increased dura mast cell activation in C57BL mice, but not in NK-1R^{-/-} mice. These data demonstrate for the first time that acute stress triggers NI and mast cell activation in mouse dura mater through the activation of NK-1 receptors. The fact that SP^{-/-} mice had intact vascular permeability response to stress indicates that some other NK-1 receptor agonist may substitute for SP. These results may help explain initial events in pathogenesis of stress-induced migraines.

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Keywords: Dura; Mast cell; Migraine; Neurokinin-1 receptor; Stress; Vascular permeability

1. Introduction

Neurogenic inflammation (NI) involves vasodilation and plasma protein extravasation in response to neural stimulation. Considerable evidence suggests that NI of the dura mater may be involved in the pathogenesis of vascular headaches and can be used as a relevant 'migraine model' in experimental animals [19,20,33,41]. The leptomeninges, especially the dura mater, are innervated by catecholaminergic (superior cervical ganglion), cholinergic (sphenopalatine ganglion) and sensory neurons (trigeminal ganglion) [1]. Electrical stimulation of the trigeminal [15,31] and the sphenopalatine [12] ganglions leads to

Abbreviations: CGRP, calcitonin gene-related peptide; CRF, corticotropin-releasing factor; NI, neurogenic inflammation; NK-1R, neurokinin-1 receptor; NKB, neurokinin B; NO, nitric oxide; SP, substance P; TNF- α , tumor necrosis factor alpha; VIP, vasoactive intestinal peptide

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increased plasma protein extravasation in the dura mater of rats. Upon stimulation of trigeminal nerve, sensory neuropeptides are released from small unmyelinated C-fibers and activate neurokinin-1 receptors (NK-1R) leading to plasma protein extravasation from postcapillary venules. Sensory neurons containing substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) are often localized in a close contact with small blood vessels and mast cells in dura mater [43,52,55]. Moreover, tachykinins (SP and NKA) and their receptor (NK-1R) have been implicated in pain sensation [8,11,58]. Sensory neuropeptides can activate dura mast cells *ex vivo* to release histamine [40], while electrical stimulation of trigeminal and cervical ganglions can activate dura mast cells *in vivo* [14,25].

Migraines are often precipitated or worsened by stress [13]. The stress response involves release of corticotropin-releasing factor (CRF) from hypothalamic neurons followed by activation of the hypothalamic–pituitary–adrenal (HPA) axis, as well as the sympathetic and parasympathetic nervous systems [9]. Earlier studies had indicated that the central nervous system (CNS) could activate mast cells through classical conditioning [29,45]. We previously reported that acute non-traumatic restraint stress induced degranulation of mast cells in rat dura mater, an effect inhibited by neonatal pretreatment with capsaicin [54]. Subsequently, we found that acute stress increased vascular permeability in the dura mater of rats, and this effect was reduced by pretreatment with the mast cell ‘stabilizer’ disodium cromoglycate [17]. These results suggested that activation of dura mast cells by neuropeptides was necessary for stress-induced vascular permeability to occur in the dura mater.

The aim of the present study was to determine if acute restraint stress could trigger NI in mouse dura mater. Stress-induced vascular permeability and mast cell degranulation were investigated in normal (C57BL and +/+), as well as in mast cell-deficient (W/W^v), NK-1R^{-/-} and SP^{-/-} mice. Our results indicate that stress-induced vascular permeability in the dura mater of mice requires mast cells and NK-1 receptors, but may involve some NK-1R agonist other than SP. Our results might help explain initial events of the pathogenesis of stress-induced migraine headaches.

2. Materials and methods

2.1. Animals

NK-1R^{-/-} breeding mice were a kind gift from Dr. Norma Gerard (Harvard Medical School, Boston, MA, USA) [6]. SP^{-/-} breeding mice were kindly provided Dr. Eva Mezey (NIH); these mice have a deletion of the *Ppta* (preprotachykinin a) gene that encodes SP and NKA [58]. NK-1R^{-/-} and SP^{-/-} mice were bred in the animal

facility of Tufts—New England Medical Center. Both male and female mice were used in these experiments because the knockout mice were in short supply. C57BL mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA) and were used as a control strain since both, NK-1R^{-/-} and SP^{-/-} mice, were derived from them. Mast cell-deficient mice (W/W^v) and their +/+ control mice (WBB6/F₁-Kit⁺/Kit⁺) were also obtained from Jackson Laboratories. Only male C57BL, W/W^v and +/+ mice were used in experiments because they were readily available from Jackson Laboratories and we wished to avoid any possible variability due to the oestrus cycle; however, preliminary findings (not shown) did not indicate any major difference between male and female mice. All animals were housed in plastic cages (three per cage) with a wire top in the animal facility with an automatic 14:10 h dark–light cycle, and were allowed food and water *ad libitum*. Mice weighing 21–27 g (7–20 weeks old) were used in all experiments.

2.2. Restraint stress

All mice were kept in the animal facility for at least 1 week before use and were handled for 30 min each day in the room where the experiments were performed for 3 days before the experiment. On the fourth day, each control mouse was left in its cage for 30 min. In a separate room, each experimental mouse was stressed for 30 min by placing it in a plexiglass restrainer (Harvard Apparatus, Cambridge, MA, USA). Conscious control and stressed mice were always isolated from the area where perfusion and dissection procedures were performed. All stress experiments were performed between 8.00 and 11.00 a.m. to avoid any non-specific effects due to the changes in circadian rhythms. All procedures performed in this study were approved by the Institutional Animal Care and Use Committee of Tufts—New England Medical Center.

2.3. Extravasation of ⁹⁹Tc-glucaptate in the dura mater

Vascular permeability in the dura mater was evaluated by extravasation of intravenously administered ⁹⁹Tc-glucaptate (⁹⁹Tc-G) as previously described for rat dura [17]. Briefly, the Technetium–D-glycero-D-gluco heptonate complex was prepared by mixing the content of one vial from the Technetium Glucaptate kit (DRAXIMAGE, Kirkland, Canada) with 5 mCi ⁹⁹Tc (DuPont, Billerica, MA, USA) in 10 ml of 0.9% NaCl. While awake, each animal was injected with 75 μCi ⁹⁹Tc-G in a 0.15-ml volume through the tail vein. Mice to be stressed were placed in plexiglass restrainers (Harvard Apparatus) 10 min following the injection of ⁹⁹Tc-G. Control mice were kept in a different room until the time of perfusion. Mice were stressed for 30 min and were anesthetized immediately after stress with an intraperitoneal injection of a mixture (0.15 ml) of ketamine (80 mg/kg) and xylazine

(10 mg/kg). Before perfusion, blood for serum corticosterone measurements was drawn through a 27.5 G needle inserted in the right ventricle of the heart. Then through a blunted needle inserted into the left ventricle of the heart, the circulation was flushed with 1 ml of 0.9% NaCl and intracranial tissues were fixed with an additional 20 ml of 10% formalin. Following the perfusion, mice were decapitated, the whole brain was removed and the supratentorial dura was dissected away from the bone. Dura samples were then counted in a Gamma Well Counter for 1 min (Ludlum Measurements, Sweetwater, TX, USA). In order to account for any error in counts per min due to the decay of the ^{99}Tc in the time interval from perfusion till counting, cpm values were corrected assuming exponential decay of ^{99}Tc using the following formula: $Y_{T_s} = Y_{T_c} \times e^{(T_c - T_s) \times \ln 2 / \tau}$ where Y_{T_s} is cpm at the end of perfusion; Y_{T_c} is cpm at the time of counting; $T_c - T_s$ is the time between counting and perfusion; the half-life of ^{99}Tc $\tau_{1/2} = 6.02$ h.

2.4. Corticosterone measurements

Blood that was collected under anesthesia from the right ventricle before cardiac perfusion was allowed to clot, and serum was separated after centrifugation of blood samples for 15 min at room temperature. Corticosterone levels in stressed and control mice were measured using the ImmunoChem™ Double Antibody Corticosterone ^{125}I -RIA kit (ICN Biomedicals, Costa Mesa, CA, USA). Data are presented as mean \pm S.D.

2.5. Evaluation of mast cell activation

To evaluate the activation of mast cells, mice were killed under ketamine/xylazine anesthesia by perfusion through the ascending aorta with 10 ml phosphate-buffered saline, followed by 30 ml of 10% ethanol-free formaldehyde (EM grade, Polysciences). Whole skulls were postfixed in the same fixative solution overnight; subsequently, the skulls were opened, the whole brain was removed, and the dura carefully dissected away from all areas of the bone. Dura tissue was immersed in freezing medium (Triangle Biomedical Sciences, Durham, NC, USA), and 7 μm serial sections were cut using a cryostat (Jung CM3000, Leica, Deerfield, IL, USA). Every 20th section was then stained with acidified toluidine blue (pH 2.5, 0.05%; Sigma, St. Louis, MO, USA) for 5 min at room temperature. Mast cells were counted using a Nikon inverted light microscope (Diaphot, Don Santo, MA, USA) at magnification $400\times$ in all fields of these sections by two investigators blinded to the experimental conditions. The total number and the number of activated mast cells from these sections was counted. As previously, all mast cells with extruded granules in close proximity, as well as those

with approximately 50% or less of the cell stained with toluidine blue were considered activated [17,54].

2.6. Data presentation and statistics

2.6.1. Extravasation of ^{99}Tc -gluceptate

Total counts (cpm) from the whole dura collected from at least three control animals in each experiment were averaged. Counts from the dura of each stressed animal were expressed as percent change from control using the following equation: percent change from control = $[(\text{Sn} - \text{Mc}) / \text{Mc}] \times 100\%$, where Sn represents counts per min (cpm) of the dura of each stressed animal and Mc is the mean counts of dura of three or more control animals (unstressed). Raw cpm data and percent change over control data is presented as mean \pm S.E. Statistical analysis for comparing stressed and non-stressed mice of different strains was performed using One sample *t*-test which is commonly used to compare one experimental group to baseline set at zero meaning no change observed. Different groups were compared among themselves (e.g., stressed C57BL vs. stressed SP-/-) using the Mann-Whitney *U* ranked sum non-parametric analysis. The differences with $P < 0.05$ were considered statistically significant.

2.6.2. Mast cell activation

The percent of mast cells activated for each mouse was calculated as follows: $[(\text{number of activated mast cells}) / (\text{number of total mast cells})] \times 100\%$. Total number of mast cells was obtained by counting mast cells in every 20th section. The degree of mast cell activation in each group is reported as mean of percent mast cell activation \pm S.E. Different groups were compared using the Mann-Whitney *U* ranked sum *t*-test; differences with $P < 0.05$ were considered statistically significant.

3. Results

3.1. Acute restraint stress increases vascular permeability in C57BL mice

C57BL mice stressed for 30 min displayed increased vascular permeability when compared to unstressed control mice. Representative data from two experiments for control and stress groups, respectively, were as follows: (experiment 1) 888 ± 246 and 1881 ± 440 cpm; (experiment 2) 2436 ± 779 and 4062 ± 292 cpm (Fig. 1A). It is obvious that the raw cpm for control and stress groups varied considerably from experiment to experiment, most likely due to the short half-life of ^{99}Tc and the use of ^{99}Tc of slightly different specific activity in each experiment. In order to normalize the values from different experiments, the results were expressed as percent change from control (see Materials and methods section) with zero meaning no change observed (0% change). The mean \pm S.E. increase in

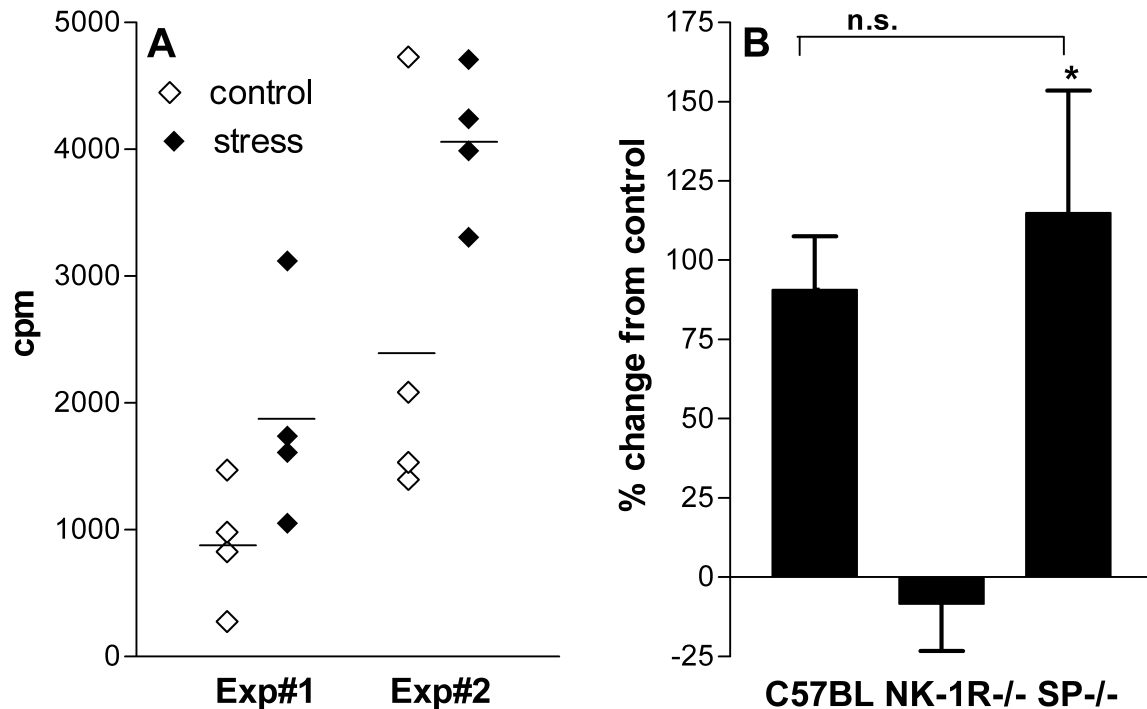


Fig. 1. Stress-induced extravasation of ^{99}Tc -glucaptate in the dura mater. (A) Scattergram of raw cpm recorded in two different experiments using C57BL mice; each diamond represents one control (empty diamonds) or stressed (filled diamonds) animal, and horizontal bars represent the respective means. (B) Percent change from control in vascular permeability of stressed mice in C57BL, NK-1R^{-/-} and SP^{-/-} mice ($n=7-12$; mean \pm S.E.; * $P<0.05$, one sample t -test); n.s.=not significant.

vascular permeability using this approach was $90.8\pm 16.8\%$ for stressed C57BL mice ($n=12$, $P<0.05$, Fig. 1B).

3.2. Neurokinin-1 receptor knockout mice do not display increased vascular permeability

Further experiments evaluated the role of NK-1 receptors and SP in stress-induced vascular permeability of dura mater by using NK-1 receptor (NK-1R^{-/-}) and SP (SP^{-/-}) knockout mice. NK-1R^{-/-} mice stressed for 30 min did not show increased vascular permeability ($-8.4\pm 14.2\%$, $n=8$; Fig. 1B). In contrast, stressed SP^{-/-} mice showed increased vascular permeability ($114.9\pm 38.8\%$, $n=7$, $P<0.05$; Fig. 1B) comparable to that seen in stressed C57BL mice ($90.8\pm 16.8\%$, $n=12$, $P<0.05$; Fig. 1B).

3.3. Mast cell-deficient mice do not display increased vascular permeability

To assess the involvement of mast cells in stress-induced vascular permeability of dura mater directly, mast cell-deficient mice WBB6/F₁-Kit^w/Kit^{wv} (W/W^v) and their congenic wild type WBB6/F₁-Kit⁺/Kit⁺ (+/+) littermates were restrained for 30 min (stress group) or kept in their cages in a different room (control group). The extent of ^{99}Tc -G extravasation in dura mater of stressed

+/+ mice ($157.5\pm 17.4\%$; $n=8$, $P<0.05$) was somewhat higher but not statistically different from what was seen in stressed C57BL mice ($90.8\pm 16.8\%$; $n=12$, $P<0.05$). In contrast, there was no increase in ^{99}Tc -G extravasation observed in stressed W/W^v mice ($0.2\pm 18.6\%$; $n=7$; Fig. 2).

3.4. Effects of restraint stress on the activation of mast cells in C57BL and NK-1R^{-/-} mice

The number of activated mast cells in the dura of C57BL mice stressed for 30 min was higher ($60.7\pm 2.2\%$, $n=4$, $P<0.05$) than in control C57BL mice ($39.9\pm 3.0\%$, $n=4$, $P<0.05$; Fig. 3). No significant increase of mast cell activation was observed in stressed NK-1R^{-/-} mice ($43.2\pm 2.0\%$, $n=5$, $P<0.05$) as compared to control NK-1R^{-/-} mice ($41.2\pm 1.4\%$, $n=6$, $P<0.05$; Fig. 3).

3.5. HPA axis is functional in NK-1R^{-/-}, SP^{-/-} and W/W^v mice

In order to exclude the possibility that the differences in vascular permeability we observed were due to a dysfunctional HPA axis, we compared serum corticosterone levels in control and stressed mice. In response to 30 min restraint stress, there was a similar increase in serum corticosterone levels in all strains of mice (C57BL, +/+, SP^{-/-}, NK-1R^{-/-} and W/W^v) used in this study (Fig.

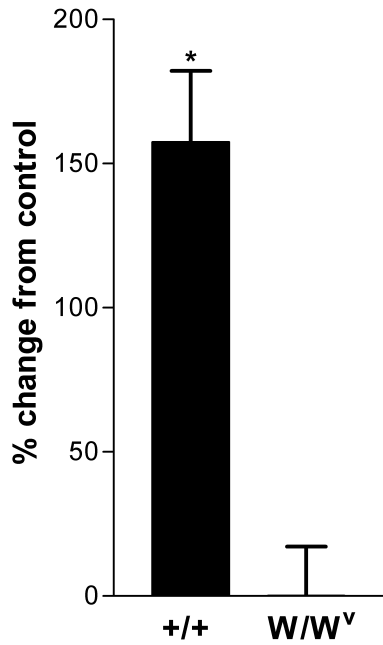


Fig. 2. Stress-induced extravasation of 99-Technetium-glucaptate in the dura mater of +/+ and W/W^v mast cell-deficient mice. Data are expressed as percent change from control ($n=6-9$; mean \pm S.E.; * $P<0.05$, one sample t -test).

4). The serum corticosterone levels of control and stress groups, respectively, were 135.9 ± 35.5 and 370.7 ± 2.6 ng/ml in C57BL mice; 188.9 ± 90.2 and 461.5 ± 14.7 ng/ml in NK-1R^{-/-} mice; 44.2 ± 53.2 and 461.5 ± 14.7 ng/ml in

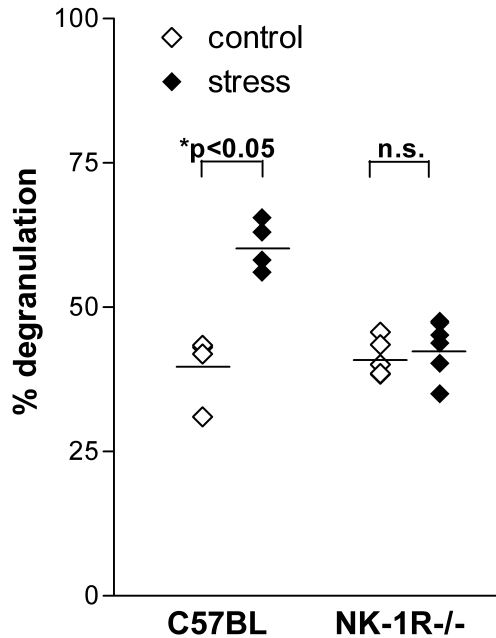


Fig. 3. Stress-induced activation of dura mast cells in C57BL and NK-1R^{-/-} mice. Scattergram showing degranulation of mast cells in dura of control (empty diamonds) and stressed (filled diamonds) mice ($n=4-5$; * $P<0.05$, Student's t -test). Each diamond represents one animal and horizontal bars represent respective means; n.s.=not significant.

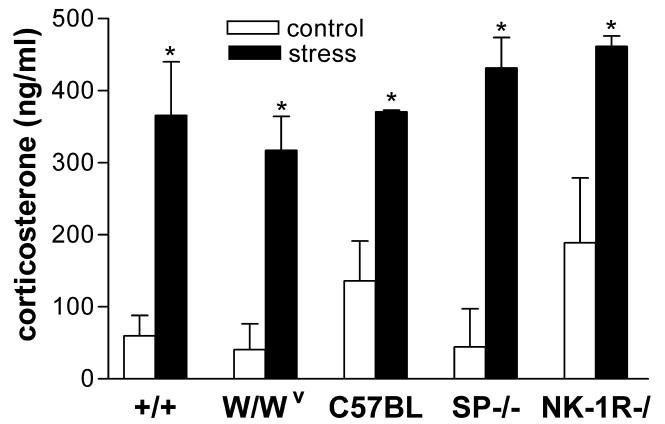


Fig. 4. Stress-induced activation of HPA axis. Serum corticosterone levels (ng/ml) in control (white bars) and stressed (black bars) mice ($n=4-5$; mean \pm S.D.; * $P<0.05$, Student's t -test).

SP^{-/-} mice; 59.8 ± 28.3 and 365.5 ± 74.5 ng/ml in +/+ mice; 40.5 ± 35.9 and 317.3 ± 47.0 ng/ml in W/W^v mice ($n=3-6$ in all groups). Tail vein injection did not cause any additional stress, as indicated by similar corticosterone levels in mice in which tail-vein injection was omitted (data not shown).

4. Discussion

To our knowledge, this is a first demonstration of acute restraint stress increasing vascular permeability in the dura mater of mice. Moreover, our results indicate that this effect depends on mast cells and NK-1 receptors, but does not involve SP. The lack of any increase in vascular permeability in response to acute stress in W/W^v mast cell-deficient mice definitively implicates mast cells and complements our previous studies showing that pretreatment of rats with the mast cell 'stabilizer' disodium cromoglycate (cromolyn) inhibits increased vascular permeability of the dura mater in rat [17].

Studies using pharmacological receptor antagonists have shown that the NK-1 receptor, but not NK-2 or NK-3 receptors, is essential for the development of NI in the dura mater and other tissues in response to electrical stimulation of nerves in guinea pigs [36,49] and rats [3,48]. Furthermore, findings of the lack of microvascular permeability in immunologically or chemically stimulated peripheral tissues of NK-1R^{-/-} mice [6,7] suggest that NK-1R is the sole most important tachykinin receptor involved in regulation of vascular permeability. It, therefore, appears that stress-induced NI of the dura mater as shown herein is similar to chemically or electrically induced vascular permeability in its dependence on NK-1 receptors. A series of independent studies with NK-1R^{-/-} mice and W/W^v mast cell-deficient mice have recently reported the absence of an inflammatory response. For instance, intradermal injection of SP resulted in edema in dorsal skin of wild

type mice, but not in NK-1R^{-/-} mice [7] and in the ear of +/+ , but not W/W^v mice [56]. Plasma extravasation was not observed in the bladders of W/W^v mice that were infused with SP or LPS [5], or had CNS infection of pseudorabies virus [24]. Inflammation also did not develop in the bladders of NK-1R^{-/-} mice during antigen-induced cystitis, even though, mast cells in this study were found to be degranulated [47]. Taken together, these and our present findings suggest that a complex interplay between mast cells and NK-1R regulates vascular permeability. Anatomical and functional associations of mast cells and neurons making such an interaction possible have been demonstrated in the dura mater [16,43].

The present findings using mice are consistent with previous reports showing that stress-induced intracranial mast cell degranulation in rats is dependent on sensory neuropeptides [54]. SP stimulates release of histamine from rat dura ex vivo in NK-1R-dependent manner [40], and induces plasma protein extravasation in rat dura mater when injected IV [31]. It was, therefore, surprising to find that SP^{-/-} mice, unlike NK-1R^{-/-} mice, exhibited increased vascular permeability during acute restraint stress. This finding suggests that unlike in normal rodents where SP stimulates dura mast cells [43], NK-1R agonists other than SP could substitute for SP to stimulate ⁹⁹Tc-G extravasation in SP^{-/-} mice. Possible candidates include Neurokinin B, as well as the recently discovered hemokinin-1 [32,57]. The possibility of NK-1R subtypes [4] may explain the findings showing that NK-1R antagonists failed to block migraine pain in human patients [21].

The location of NK-1 receptors involved in stress-induced NI, however, cannot be deduced from the present findings. Evidence from receptor binding studies and histamine release assays suggest their expression by mast cells of some strains of rats [27,37]. Data from functional assays support this evidence. For instance, SP stimulated production of TNF- α in a murine mast cell line [2] and induced calcium transients in rat basophilic leukemia mast cells (RBL-2H3) cocultured with sensory neurons [51]; both effects were dependent on NK-1 receptors. Low concentrations of neuropeptides were sufficient for ‘priming’ of RBL cells via NK-1 receptors for their activation by subsequent stimuli [23]. Activation of mast cells in dura mater was greater with electrical stimulation of the trigeminal ganglion at lower frequencies as opposed to higher frequencies [14]. Moreover, injection of lower, but not higher, concentrations of SP led to mast cell activation and subsequent granulocyte infiltration in the ear of mice [56]. Such mast cell activation via NK-1R would be consistent with low concentrations (pM–nM) of sensory neuropeptides found at tissue sites. Alternatively, high concentrations of SP (μ M) could bypass receptors to directly activate G proteins resulting in mast cell degranulation [34,35]. Our results do not exclude the possibility that NK-1 receptors required for stress-induced vascular permeability are expressed by other cells than mast cells.

For instance, it was recently reported that mast cells and NK-1 receptors were required for bladder inflammation [47]; however, reconstitution of W/W^v mice with bone marrow mast cell progenitors from NK-1 receptor knock-out mice showed that NK-1 receptors did not have to be present on mast cells [46]. In our study, basal mast cell degranulation in control mice was about 40%. Similar high baseline mast cell degranulation (>25%) was previously observed in dura mater [17,54], skin [26], and heart [22] suggesting a role of baseline mast cell activation in maintenance of homeostasis possibly through nitric oxide [53] or histamine-3-receptors [16,44]. In our experiments, the actual mast cell activation, however, might be overestimated. Both partially and totally degranulated mast cells were counted to obtain percent of degranulated mast cells, which may not be the same as percent of total mediators secreted.

The relevance of stress-induced mast cell activation in migraines comes from studies showing that migraine headaches in children are associated with urine elevations of the unique mast cell enzyme tryptase, both of which were decreased when the patients were taught to relax [39]. We have shown that restraint stress-induced degranulation of skin mast cells was blocked by pretreatment with neutralizing antiserum to CRF [50]. Stress-induced activa-

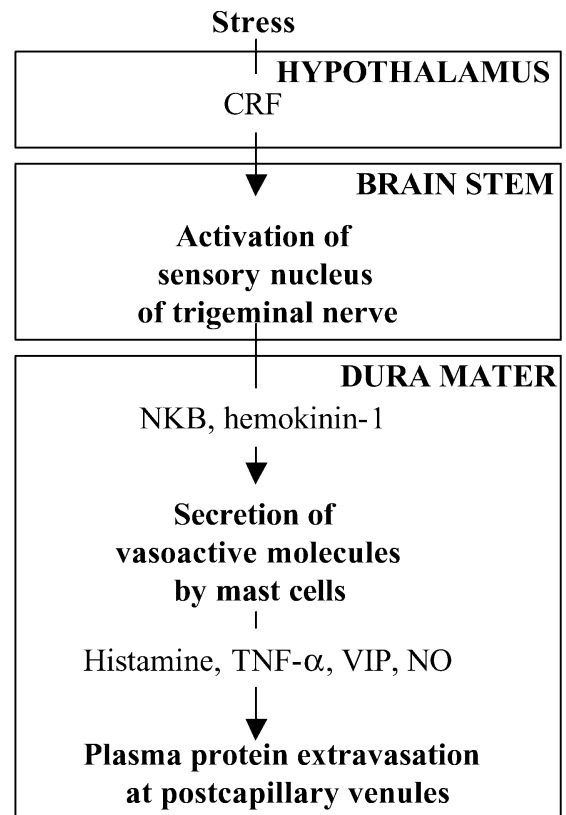


Fig. 5. Schematic representation of proposed events contributing to stress-induced vascular permeability in mouse dura mater. CRF—Corticotropin releasing factor, NKB—neurokinin B, VIP—vasoactive intestinal peptide, NO—nitric oxide, TNF- α —tumor necrosis factor alpha.

tion of rat dura mast cells was blocked by a CRF receptor antagonist [43]. Moreover, CRF could trigger vascular permeability in the skin [53] of rodents and humans [10]. Taken together this evidence suggests that either hypothalamic or local CRF could initiate pro-inflammatory events during stress, including mast cell activation and increase in vascular permeability. With respect to migraines, we hypothesize that stress-induced release of CRF from the hypothalamus stimulates the trigeminal nerve to secrete sensory neuropeptides, which subsequently activate mast cells to release vasoactive molecules; these include histamine, TNF- α [52], NO [30] and vasoactive intestinal peptide (VIP) [28], which have been implicated in migraines [18,38], leading to NI (Fig. 5). In keeping with this hypothesis, high levels of CRF receptor expression were recently demonstrated on the principal sensory nucleus of the trigeminal nerve [42]. Further studies elucidating the mechanism of stress-induced activation of sensory nerves and mast cells leading to NI might provide additional insights. Nevertheless, our study demonstrates that acute stress in mice might be a relevant animal ‘migraine model’ for testing novel therapeutics and for addressing further questions of how stress exacerbates migraines and other neuroinflammatory processes.

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References

- [1] K.H. Andres, M. VonDoring, K. Muszynski, R.T. Schmidt, Nerve fibers and their terminals of the dura mater encephali of the rat, *Anat. Embryol. (Berl.)* 175 (1987) 289–301.
- [2] J.C. Ansel, J.R. Brown, D.G. Payan, M.A. Brown, Substance P selectively activates TNF- α gene expression in murine mast cells, *J. Immunol.* 150 (1993) 4478–4485.
- [3] D.T. Beattie, I.J. Beresford, H.E. Connor, F.H. Marshall, A.B. Hawcock, R.M. Hagan, J. Bowers, P.J. Birch, P. Ward, The pharmacology of GR203040, a novel, potent and selective non-peptide tachykinin NK1 receptor antagonist, *Br. J. Pharmacol.* 116 (1995) 3149–3157.
- [4] J.C. Beaujouan, M. Saffroy, Y. Torrens, J. Glowinski, Different subtypes of tachykinin NK(1) receptor binding sites are present in the rat brain, *J. Neurochem.* 75 (2000) 1015–1026.
- [5] D.E. Bjorling, T.J. Jerde, M.J. Zine, B.W. Busser, M.R. Saban, R. Saban, Mast cells mediate the severity of experimental cystitis in mice, *J. Urol.* 162 (1999) 231–236.
- [6] C.R. Bozic, B. Lu, U.E. Höpken, C. Gerard, N.P. Gerard, Neurogenic amplification of immune complex inflammation, *Science* 273 (1996) 1722–1725.
- [7] T. Cao, N.P. Gerard, S.D. Brain, Use of NK1 knockout mice to analyze substance P-induced edema formation, *Am. J. Physiol.* 277 (1999) R476–R481.
- [8] Y.Q. Cao, P.W. Manthly, E.J. Carlson, A. Gillespie, C.J. Epstein, A.I. Basbaum, Primary afferent tachykinins are required to experience moderate to intense pain, *Nature* 392 (1998) 390–393.
- [9] G.P. Chrousos, Stressors, stress, and neuroendocrine integration of the adaptive response: The 1997 Hans Selye Memorial Lecture, *Ann. N. Y. Acad. Sci.* 851 (1998) 311–335.
- [10] V.L. Clifton, R. Crompton, R. Smith, I.M. Wright, Microvascular effects of CRH in human skin vary in relation to gender, *J. Clin. Endocrinol. Metab.* 87 (2002) 267–270.
- [11] C. De Felipe, J.F. Herrero, J.A. O’Brien, J.A. Palmer, C.A. Doyle, A.J.H. Smith, J.M.A. Laird, C. Belmonte, F. Cervero, S.P. Hunt, Altered nociception, analgesia and aggression in mice lacking the receptor for substance P, *Nature* 392 (1998) 394–397.
- [12] L. Delepine, P. Aubineau, Plasma protein extravasation induced in the rat dura mater by stimulation of the parasympathetic sphenopalatine ganglion, *Exp. Neurol.* 147 (1997) 389–400.
- [13] D. Deleu, Y. Hanssens, E.A. Worthing, Symptomatic and prophylactic treatment of migraine: a critical reappraisal, *Clin. Neuropharmacol.* 21 (1998) 267–279.
- [14] V. Dimitriadou, M.G. Buzzi, M.A. Moskowitz, T.C. Theoharides, Trigeminal sensory fiber stimulation induces morphologic changes reflecting secretion in rat dura mast cells, *Neuroscience* 44 (1991) 97–112.
- [15] V. Dimitriadou, M.G. Buzzi, T.C. Theoharides, M.A. Moskowitz, Ultrastructural evidence for neurogenically mediated changes in blood vessels of the rat dura mater and tongue following antidromic trigeminal stimulation, *Neuroscience* 48 (1992) 187–203.
- [16] V. Dimitriadou, A. Rouleau, M.D. Trung Tuong, G.J.F. Newlands, H.R.P. Miller, G. Luffau, J.-C. Schwartz, M. Garbarg, Functional relationships between sensory nerve fibers and mast cells of dura mater in normal and inflammatory conditions, *Neuroscience* 77 (1997) 829–839.
- [17] P. Esposito, D. Gheorghe, K. Kandere, X. Pang, R. Conally, S. Jacobson, T.C. Theoharides, Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells, *Brain Res.* 888 (2001) 117–127.
- [18] P.J. Goadsby, L. Edvinsson, R. Ekman, Vasoactive peptide release in the extracerebral circulation of humans during migraine headache, *Ann. Neurol.* 28 (1990) 183–187.
- [19] H. Goebel, N. Czech, A. Heinze-Kuhn, Evidence of regional plasma extravasation in cluster headache using Tc-99m albumin SPECT, *Cephalalgia* 20 (2001) 287.
- [20] J.E. Hardebo, The involvement of trigeminal substance P neurons in cluster headache. A hypothesis, *Headache* 24 (1984) 294–304.
- [21] R. Hill, NK₁ (substance P) receptor antagonists—why are they not analgesic in humans?, *Trends Pharmacol. Sci.* 21 (2000) 244–246.
- [22] M. Huang, X. Pang, L. Letourneau, W. Boucher, T.C. Theoharides, Acute stress induces cardiac mast cell activation and histamine release, effects that are increased in apolipoprotein E knockout mice, *Cardiovasc. Res.* 55 (2002) 150–160.
- [23] J. Janiszewski, J. Bienenstock, M.G. Blennerhassett, Picomolar doses of substance P trigger electrical responses in mast cells without degranulation, *Am. J. Physiol.* 267 (1994) C138–C145.
- [24] L. Jasmin, G. Janni, P.T. Ohara, S.D. Rabkin, CNS induced neurogenic cystitis is associated with bladder mast cell degranulation in the rat, *J. Urol.* 164 (2000) 852–855.

- [25] J.T. Keller, R.V. Dimlich, M. Zuccarello, L. Lanker, T.A. Strauss, M.J. Fritts, Influence of the sympathetic nervous system as well as trigeminal sensory fibres on rat dural mast cells, *Cephalalgia* 11 (1991) 215–221.
- [26] M.L. Kowalski, M.A. Kaliner, Neurogenic inflammation, vascular permeability, and mast cells, *J. Immunol.* 140 (11) (1988) 3905–3911.
- [27] S.A. Krumins, C.A. Broomfield, Evidence of NK1 and NK2 tachykinin receptors and their involvement in histamine release in a murine mast cell line, *Neuropeptides* 21 (1992) 65–72.
- [28] E. Lutz, W. Chan, N.S. Track, A. Goth, S.I. Said, Release of vasoactive intestinal polypeptide in mast cells by histamine liberators, *Nature* 275 (1978) 661–662.
- [29] G. MacQueen, J. Marshall, M. Perdue, S. Siegel, J. Bienenstock, Pavlovian conditioning of rat mucosal mast cells to secrete rat mast cell protease II, *Science* 243 (1989) 83–85.
- [30] P.F. Mannaioni, E. Masini, A. Pistelli, D. Salvemini, J.R. Vane, Mast cells as a source of superoxide anions and nitric oxide-like factor: relevance to histamine release, *Int. J. Tissue React.* 13 (1991) 271–278.
- [31] S. Markowitz, K. Saito, M.A. Moskowitz, Neurogenically mediated leakage of plasma protein occurs from blood vessels in dura mater but not brain, *J. Neurosci.* 7 (1987) 4129–4136.
- [32] O. Morteau, B. Lu, C. Gerard, N.P. Gerard, Hemokinin 1 is a full agonist at the substance P receptor, *Nat. Immunol.* 2 (2001) 1088.
- [33] M.A. Moskowitz, Neurogenic inflammation in the pathophysiology and treatment of migraine, *Neurology* 43 (1993) S16–S20.
- [34] M. Mousli, C. Bronner, Y. Landry, J. Bockaert, B. Rouot, Direct activation of GTP-binding regulatory proteins (G-proteins) by substance P and compound 48/80, *FEBS Lett.* 259 (1990) 260–262.
- [35] M. Mousli, J.-L. Bueb, C. Bronner, B. Rouot, Y. Landry, G protein activation: a receptor-independent mode of action for cationic amphiphilic neuropeptides and venom peptides, *Trends Pharmacol. Sci.* 11 (1990) 358–362.
- [36] C.T. O’Shaughnessy, H.E. Connor, Investigation of the role of tachykinin NK1, NK2 receptors and CGRP receptors in neurogenic plasma protein extravasation in dura mater, *Eur. J. Pharmacol.* 263 (1994) 193–198.
- [37] T. Okada, Y. Hirayama, S. Kishi, K. Miyayasu, J. Hiroi, T. Fujii, Functional neurokinin NK-1 receptor expression in rat peritoneal mast cells, *Inflamm. Res.* 48 (1999) 274–279.
- [38] J. Olesen, L.L. Thomsen, H. Iversen, Nitric oxide is a key molecule in migraine and other vascular headaches, *Trends Pharmacol. Sci.* 15 (1994) 149–153.
- [39] K. Olness, H. Hall, J.J. Rozniecki, W. Schmidt, W. Boucher, T.C. Theoharides, Mast cell activation in children with migraine before and after training in self-regulation, *Headache* 39 (1999) 101–107.
- [40] A. Ottosson, L. Edvinsson, Release of histamine from dural mast cells by substance P and calcitonin gene-related peptide, *Cephalalgia* 17 (1997) 166–174.
- [41] M. Papagallo, Z. Szabo, G. Esposito, Imaging neurogenic inflammation in patients with migraine headaches, *Neurology* 52 (1999) A274–A275.
- [42] S. Rivest, N. Laflamme, R.E. Nappi, Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus, *J. Neurosci.* 15 (1995) 2680–2695.
- [43] J.J. Rozniecki, V. Dimitriadou, M. Lambracht-Hall, X. Pang, T.C. Theoharides, Morphological and functional demonstration of rat dura mast cell–neuron interactions in vitro and in vivo, *Brain Res.* 849 (1999) 1–15.
- [44] J.J. Rozniecki, R. Letourneau, M. Sugiultozglu, C. Spanos, J. Gorbach, T.C. Theoharides, Differential effect of histamine-3 receptor active agents on brain but not peritoneal mast cell activation, *J. Pharmacol. Exp. Ther.* 290 (1999) 1–9.
- [45] M. Russell, K.A. Dark, R.W. Cummins, G. Ellman, E. Callaway, H.V. Peeke, Learned histamine release, *Science* 225 (1984) 733–734.
- [46] R. Saban, N.P. Gerard, M.R. Saban, N.B. Nguyen, D.J. DeBoer, B.K. Wershil, Mast cells mediate substance P-induced bladder inflammation through an NK(1) receptor-independent mechanism, *Am. J. Physiol. Renal Physiol.* 283 (2002) F616–629.
- [47] R. Saban, M.R. Saban, N.B. Nguyen, B. Lu, C. Gerard, N.P. Gerard, T.G. Hammond, Neurokinin-1 (NK-1) receptor is required in antigen-induced cystitis, *Am. J. Pathol.* 156 (2000) 775–780.
- [48] G.R. Seabrook, S.L. Shephard, D.J. Williamson, P. Tyrer, M. Rigby, M.A. Cascieri, T. Harrison, R.J. Hargreaves, R.G. Hill, L-733,060, a novel tachykinin NK1 receptor antagonist; effects in [Ca²⁺]_i mobilisation, cardiovascular and dural extravasation assays, *Eur. J. Pharmacol.* 317 (1996) 129–135.
- [49] S.L. Shephard, D.J. Williamson, R.G. Hill, R.J. Hargreaves, The non-peptide neurokinin1 receptor antagonist, RP 67580, blocks neurogenic plasma extravasation in the dura mater of rats, *Br. J. Pharmacol.* 108 (1993) 11–12.
- [50] L.K. Singh, X. Pang, N. Alexacos, R. Letourneau, T.C. Theoharides, Acute immobilization stress triggers skin mast cell degranulation via corticotropin releasing hormone, neurotensin and substance P: a link to neurogenic skin disorders, *Brain Behav. Immunol.* 13 (1999) 225–239.
- [51] R. Suzuki, T. Furuno, D.M. McKay, D. Wolvers, R. Teshima, M. Nakanishi, J. Bienenstock, Direct neurite–mast cell communication in vitro occurs via the neuropeptide substance P, *J. Immunol.* 163 (1999) 2410–2415.
- [52] T.C. Theoharides, The mast cell: a neuroimmunoendocrine master player, *Int. J. Tissue React.* 18 (1996) 1–21.
- [53] T.C. Theoharides, L.K. Singh, W. Boucher, X. Pang, R. Letourneau, E. Webster, G. Chrousos, Corticotropin-releasing hormone induces skin mast cell degranulation and increased vascular permeability, a possible explanation for its pro-inflammatory effects, *Endocrinology* 139 (1998) 403–413.
- [54] T.C. Theoharides, C.P. Spanos, X. Pang, L. Alferes, K. Ligris, R. Letourneau, J.J. Rozniecki, E. Webster, G. Chrousos, Stress-induced intracranial mast cell degranulation. A corticotropin releasing hormone-mediated effect, *Endocrinology* 136 (1995) 5745–5750.
- [55] R.M. Williams, J. Bienenstock, R.H. Stead, Mast cells: the neuroimmune connection, *Chem. Immunol.* 61 (1995) 208–235.
- [56] H. Yano, B.K. Wershil, N. Arizono, S.J. Galli, Substance P-induced augmentation of cutaneous vascular permeability and granulocyte infiltration in mice is mast cell dependent, *J. Clin. Immunol.* 84 (1989) 1276–1286.
- [57] Y. Zhang, L. Lu, C. Furlonger, G.E. Wu, C.J. Paige, Hemokinin is a hematopoietic-specific tachykinin that regulates B lymphopoiesis, *Nat. Immunol.* 1 (2000) 392–397.
- [58] A. Zimmer, A.M. Zimmer, J. Baffi, K. Reynolds, T. Usdin, M. Palkovits, E. Mezey, Hypoalgesia in mice with a targeted deletion of the tachykinin 1 gene, *Proc. Natl. Acad. Sci. USA* 95 (1998) 2630–2635.