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Research report

Acute stress increases permeability of the blood–brain-barrier through activation of brain mast cells

Pamela Esposito[a], Daniela Gheorghe[a], Kristiana Kandere[a], Xinzhu Pang[b], Raymond Connolly[c], Stanley Jacobson[d], Theoharis C. Theoharides[a,*]

[a]*Departments of Pharmacology and Experimental Therapeutics, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA*
[b]*Department of Pathology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA*
[c]*Department of Surgical Research, New England Medical Center, 850 Washington Street, Boston, MA 02111, USA*
[d]*Departments of Anatomy and Cell Biology, Tufts University School of Medicine, 136 Harrison Avenue, New England Medical Center, 850 Washington Street, Boston, MA 02111, USA*

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Abstract

Disruption of the blood–brain-barrier (BBB) is important in the pathophysiology of various inflammatory conditions of the central nervous system (CNS), such as multiple sclerosis (MS), in which breakdown of the BBB precedes any clinical or pathological findings. There is some evidence that relapsing–remitting MS attacks may be correlated with certain types of acute stressful episodes. Stress typically activates the hypothalamic–pituitary–adrenal (HPA) axis through the release of corticotropin releasing hormone (CRH), leading to production of glucocorticoids that down regulate immune responses. However, acute stress also has pro-inflammatory effects that appear to be mediated through activation of mast cells. Here we show that acute stress by immobilization increased permeability of rat BBB to intravenous ^{99}Technetium gluceptate (^{99}Tc). This effect was statistically significant in the diencephalon and the cerebellum, while it was absent in the cerebral cortex where there are not mast cells. Immobilization stress also induced activation of mast cells in diencephalon, the site where most mast cells are found in the rat brain. Both BBB permeability and mast cell activation were inhibited by the 'mast cell stabilizer' disodium cromoglycate (cromolyn). These results expand the pathophysiology of mast cells and implicate them in CNS disorders, that may possibly be induced or exacerbated by stress. © 2001 Elsevier Science B.V. All rights reserved.

Theme: Cellular and molecular biology

Topic: Blood–brain barrier

Keywords: Blood–brain-barrier; Corticotropin-releasing hormone; Cromolyn; Diencephalon; Mast cells; Multiple sclerosis; Stress; Technetium

1. Introduction

The BBB is formed by a complex system of endothelial cells [32], astroglia, pericytes, perivascular macrophages and basal lamina [73]. Under normal conditions, the BBB tightly regulates the entry of any compound into the brain [12]. The protective function of the BBB can be altered during various diseases of the CNS, specifically during cerebral inflammation [12] such as that present in multiple sclerosis (MS) [35]. In this case, leukocyte infiltration in the brain parenchyma [65] follows a decrease in the integrity of the BBB [35]. This increase in BBB permeability may possibly be mediated through the action of vasoactive mediators, such as histamine [58], released from perivascular brain mast cells [35,69].

Stress activates the hypothalamic–pituitary–adrenal (HPA) axis through the release of CRH leading to secretion of catecholamines and glucocorticoids [7]. Stress, however, seems to precipitate or worsen a number of neuroinflammatory disorders [55], such as relapsing–remitting multiple sclerosis [24,45,74]. This is a paradoxical finding given the fact that glucocorticoids released during HPA

Abbreviations: BBB, blood–brain-barrier; CNS, central nervous system; CSF, cerebrospinal fluid; 5HT, 5-hydroxytryptamine; CRH, corticotropin-releasing hormone; HPA, hypothalamic–pituitary–adrenal; MS, multiple sclerosis; RMCP, rat mast cell protease; ^{99}Tc, 99-technetium gluceptate

*Corresponding author. Tel.: +1-617-636-6866; fax: +1-617-636-2456.

E-mail address: theoharis.theoharides@tufts.edu (T.C. Theoharides).

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activation are the primary mode of therapy for such conditions [44]. A possible explanation could be that CRH released from the hypothalamus or elsewhere in the brain either affects the BBB directly, or through mast cell activation. Support for this premise comes from the recent evidence that CRH also has proinflammatory effects [33], apparently mediated through mast cell activation [71]. Acute stress by immobilization was shown to induce intracranial rat mast cell degranulation and elevate in the cerebrospinal fluid (CSF) the connective type mast cell marker rat mast cell protease (RMCP); both actions were CRH-dependent [72]. Moreover, CRH [71] and its structurally related urocortin [62] induced mast cell degranulation and Evans blue extravasation in rodent skin, a phenomenon duplicated by acute immobilization stress [63].

Mast cells are ubiquitous in the body and are critical for the development of allergic reactions [21]. In the brain, they are predominantly located perivascularly, especially in the thalamus and hypothalamus [16,17,23,30,51,52] where they have been definitively characterized [50]. Increasing evidence indicates that mast cells may also be involved in neuroimmune interactions [8,20,22,67], especially in the dura [14,56], including the development of neuroinflammatory processes [70]. However, the function of brain mast cells remains unclear [37]. As many mast cell mediators are vasoactive, it has been suggested that mast cells may regulate the permeability of the blood–brain-barrier (BBB) [69]. This proposal was recently supported using a chemical trigger of mast cells, compound 48/80, in the habenula of pigeons which is rich in mast cells [75].

This study investigated whether acute non-traumatic stress in rats can alter the permeability of the BBB and whether such an effect involves brain mast cell activation. Extravasation of intravenous ^{99}Tc was used to evaluate BBB permeability in different brain regions and mast cell degranulation was determined histochemically after staining with toluidine blue.

2. Materials and methods

2.1. Mast cell histochemistry

After 99-Technetium gluceptate (^{99}Tc) radioactivity was measured, tissue collected from both control and stressed animals which had been washed intracardially with formalin, was placed in formalin for 2 days. Brain tissue was then immersed in tissue freezing medium and 10 μ sections were cut using a cryostat (Jung CM3000, Leica, Deerfield, IL). Mast cell degranulation was evaluated using a light microscope (Nikon, Don Santo, MA), after toluidine blue (0.5%, pH 2.5) staining of brain sections at room temperature for 5 min. The total number of mast cells and the number of degranulated mast cells were counted in all fields from 9 brain sections from each animal at 400× magnification. Degranulation was defined as the presence of extruded granules close to the surface of the cell in question or staining of less than half of the cell section studied with toluidine blue. Evaluation of mast cells was carried out by two investigators blind to the experimental conditions. Significance was defined as a P-value of ≤ 0.05. It was estimated that 12 animals would be needed to make comparisons with an overall power of 90%.

2.2. Immobilization stress and indicator extravasation

Male Sprague–Dawley 300 g rats (Charles River, NY) were kept on a 14:10 h dark–light cycle and were provided food and water ad libitum. Animals were first anesthetized with one ip injection (0.3 ml) of mixture of ketamine and xylazine anesthesia (1.0 and 0.02 ml, respectively of 100 mg/ml each). They were then cannulated via the jugular vein; in certain cases, intracerebroventricular (icv) cannulas were inserted into the right frontal horn of the lateral ventricle under stereotactic coordinates. The animals were allowed to recover in the animal facility for 5 days prior to use. Animals were handled daily to check on the intravenous catheter and familiarize them with the investigators. The morning of the experiment (9–12 am), animals were injected with either 0.5 ml of 2.0% Evans Blue (Sigma, St. Louis, MO) or 500 μCi of ^{99}Tc which was prepared as follows: Gluceptate (DRAXIMAGE Inc., Kirkland, Quebec, Canada), a D-glycero-D-gluco-heptonate complex was obtained from Synchor Pharmacy (Woburn, MA). The gluceptate was then mixed with ^{99}Tc (Specific activity, DuPont, Billerica, MA) the morning of the experiment. Binding to gluceptate prevents ^{99}Tc from escaping the circulation and constitutes a good marker from extravasation in brain parenchyma [31]. Control animals were left in their cage on the bench top for 120 min, but not in the presence of animals that were being stressed. Rats to be stressed were placed in a plexiglass immobilizer (Harvard Apparatus, Cambridge, MA) immediately following Evans blue or ^{99}Tc injection for 30 min in the laboratory.

In the case of Evans blue, immediately following stress, animals were anesthetized as before and perfused intracardially with 60 ml normal saline to wash out any vascular Evans blue. Brain regions were dissected and the Evans blue was extracted by heating the samples at 55°C in formamide overnight. Following centrifugation at 10,000× g for 15 min at room temperature, Evans blue in the supernatant was assayed fluorometrically and results are reported in arbitrary fluorescent units at 620 nm. This technique had previously been shown to adequately reflect neurogenic inflammation in rat dura [41].

For ^{99}Tc, there were two additional groups. One group of animals to be stressed were injected iv (0.2 ml) via the cannula with (25 mg/kg) cromolyn (Sigma, St. Louis, MO) 60 min prior to their placement in the stress chamber;

alternatively, in another group cromolyn (0.1 mM) was administered (0.01 ml) icv. As a positive control, 0.1 ml of 1 mg/ml of the mast cell secretagogue compound 48/80 was injected via the cannula to another group of animals.

To assess BBB permeability, the animals were anesthetized immediately after, or at the indicated times after stress, with a single iv injection of 0.2 ml ketamine and 0.05 ml xylazine (100 mg/ml each). The heart was then perfused with a 60 ml syringe inserted into the left ventricle. The circulation was flushed with 60 ml of normal saline followed by 120 ml of formalin to remove any tracer (^{99}Tc) trapped in the circulation that may contribute to a high background. Following perfusion, rats were decapitated, the skull was cut open, the whole brain was removed and samples of the brainstem, cortex, cerebellum and diencephalon were collected. After the cerebellum was removed, the spinal cord and brainstem were separated. The brainstem preparation contained pons and medulla. The frontal pole of the cerebral hemispheres was collected and included gray matter and the underlying white matter. The diencephalon was isolated after removal of the midbrain, the cerebral hemispheres, basal ganglia and septum. The block consisted of the internal capsule, thalamus, hypothalamus, subthalamus and epithalamus. Blood was collected at the time of the intracardiac injection for ^{99}Tc and corticosterone measurements. For ^{99}Tc measurements, 100 μl of blood was heparinized and radioactivity was measured in blood and tissue using a Gamma Well Counter. The samples were then weighed and the amount of radioactivity was expressed as counts/100 mg of tissue.

To assess BBB at time points following stress, animals were placed back in their cages after immobilization for an additional 45 or 90 min (Note, control animals for each experiment were exposed to ^{99}Tc for the same amount of time).

This study was designed to detect at least a 50% difference in ^{99}Tc uptake in the diencephalon, with a desired power of at least 90%. Five animals per group were needed given the variability observed. Each figure represents data generated from independent experiments.

2.3. Dynamic (real time) permeability studies

With the aid of a Sigma 400 Radioisotope Nuclear Gamma Camera (Ohio Nuclear Inc., Solon, OH), the accumulation of ^{99}Tc in the whole brain was followed after stress. In addition to control and stress animals two other groups of stress animals were injected with cromolyn iv or icv as described above. Rats were injected with ^{99}Tc and then either placed back in their cage or in the immobilizer. Following the stress period, all animals were immediately anesthetized and placed on the camera. A maximum of four animals can be imaged simultaneously on the camera. Images and data were collected each min for 90–120 min. The intensity of radiation (counts per mm^2) of brain were quantified by Macintosh Gamma Camera software. These studies were approved by the University's Animal Research Committee. Four animals per group were used in all dynamic studies.

2.4. Corticosterone RIA

Serum corticosterone levels were measured from blood collected from all animals at the time of perfusion using an ImmuChem Double Antibody Corticosterone ^{125}I-RIA Kit (ICN Biomedicals, Costa Mesa, CA).

2.5. Statistics

Due to the short half-life of ^{99}Tc (about 6 h), it was impossible to assure delivery of exactly the same dose of ^{99}Tc each day the experiment was performed. It was thus necessary to express the difference of counts in stressed animals as a percent of the controls from each day (experimental-control/mean control)×100. Note that a representative graph of raw data is included in results. Values were compared using a One Sample *t*-test which is commonly used to compare one experimental group to baseline which is taken as zero. For camera studies, area under the curve (AUC) for each animal was calculated using the trapezoid rule. Data is expressed as percent change from control AUC. Percent degranulation of mast cells is expressed as mean±standard deviation. Values were compared using the unpaired Student's *t*-test and the non-parametric Mann–Whitney *U*-test. Significance is denoted by the $P \leq 0.05$.

3. Results

3.1. BBB permeability documented by Evans blue extravasation

BBB permeability was first evaluated by extravasation of Evans Blue in the diencephalon (Table 1), the brain region which contains the highest number of mast cells. Immediately following the 30-min acute immobilization stress, dye extravasation measured in arbitrary fluorescence units increased ($P=0.0004$) from 0.019±0.048 (control $n=11$ rats) to 0.187±0.052 (stress $n=12$ rats).

Table 1
Effect of acute stress on diencephalon BBB permeability and mast cell degranulation

Conditions	Evans blue extravasation[c]	Mast cell degranulation (%)
Control ($n=11$)	0.079±0.048	37.8±18.1
Stress ($n=12$)	0.187±0.052[a]	58.8±19.0[b]

[a] Mann–Whitney *U*-test, $P=0.0004$; unpaired *t*-test<0.0001.
[b] Mann–Whitney *U*-test, $P=0.05$; unpaired *t*-test $P<0.02$.
[c] Arbitrary fluorescence units.

3.2. Effect of immobilization stress on serum corticosterone levels

Serum corticosterone levels were increased due to stress, in all experiments. Representative control rats had serum corticosterone levels of 131.1±36.8 ng/ml (n=4 rats). After 30 min of immobilization stress, serum corticosterone levels increased to 222.0±54.9 ng/ml (n=4, $P \leq 0.05$).

3.3. BBB permeability documented by ^{99}Tc extravasation

BBB permeability was then quantitated by extravasation of ^{99}Tc in brain parenchyma because it also permitted fixation of the same tissue for subsequent analysis of mast cell activation by microscopy. Extravasation was studied at 0, 45 and 90 min after animals were subjected to 30 min immobilization stress. Control animals were exposed to ^{99}Tc for the same period. Four different brain areas were evaluated: the (a) brainstem, (b) cerebellum, (c) cerebral cortex, and (d) diencephalon to investigate any regional differences. Extravasation of ^{99}Tc was also investigated in the dura which resides outside the BBB. ^{99}Tc extravasation in response to acute stress in the diencephalon increased for up to 90 min following stress. At 90 min post-stress, counts increased in the diencephalon from 1995.3±563.8 cpm/100 mg to 3578.49± 1199.58 cpm/100 mg (P= 0.005), while in cerebellum they increased from 2442.8±762.6 cpm/100 mg to 4646.2±1425.3 cpm/100 mg (P=0.002). In the brainstem, values increased from 2582.4±806.9 cpm/100 mg to 4568.1± to 1600.9 cpm/100 mg (P=0.012). A similar effect of stress was not evident in the cerebral cortex. It is possible that with more animals the cortex would also be significantly different after stress. Fig. 1 shows the distribution of radioactivity counts in various brain regions.

Fig. 2A shows percent increases in ^{99}Tc extravasation from control in various brain regions immediately after 30 min of stress. While the magnitude of changes was greatest and most significant in the diencephalon and the cerebellum, the brainstem was also significant (P=0.05). The extravasation in the cerebral cortex was not statistically significant. Fig. 2B shows changes 45 min after stress. At this time point, only the diencephalon showed a significant increase. Fig. 2C shows that extravasation in the diencephalon, cerebellum and brainstem is statistically higher than controls 90 min after stress. Extravasation of ^{99}Tc in the dura was significant only immediately after stress.

3.4. Effect of a 'mast cell stabilizer' on ^{99}Tc-extravasation

In order to further investigate the involvement of mast cells, animals were injected with the 'mast cell stabilizer' disodium cromoglycate (cromolyn). Administration of cromolyn iv (n=5 rats per group) reduced ^{99}Tc extravasation in all brain regions (Fig. 3); in the diencephalon and cerebellum, cromolyn reduced the amount of ^{99}Tc extravasation significantly even from control animals (Fig. 3). Similarly, administration of cromolyn icv (n=5 rats) totally inhibited ^{99}Tc extravasation (Fig. 3). As a positive control, the mast cell secretagogue compound 48/80 was injected iv. ^{99}Tc extravasation was statistically increased only in the diencephalon and cerebellum. For example, control counts in diencephalon were increased from 1779.2±130.2 to 2672.3±840.3 cpm/100 mg (n=3).

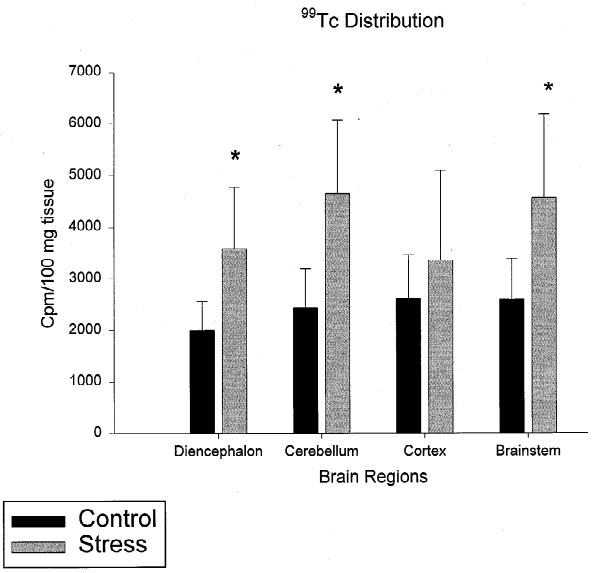

Fig. 1. Effect of acute immobilization stress on actual counts of ^{99}Tc extravasation in different brain regions (n=5 rats per group; representative graph of one of three experiments). *Asterisk indicates P<0.05.

3.5. Real time dynamic study of ^{99}Tc-levels in whole brain

Real time evaluation of ^{99}Tc distribution and extravasation was obtained using a gamma camera. The results show increased uptake in the whole brain following the stress period. Fig. 4A is a representative graph of brain images of 4 animals taken each minute. Following 30 min stress, the whole brain contained more ^{99}Tc than control animals for up to approximately 100 min. This experiment was repeated 3 times with qualitatively similar data (data not shown). Fig. 4B shows calculated percent change from control AUC in animals that were either stressed or stressed and pretreated with iv or icv cromolyn. Pretreatment with cromolyn inhibited stress-induced ^{99}Tc extravasation in the whole brain.

3.6. Effect of acute stress on mast cell degranulation in the diencephalon

Degranulation of diencephalic mast cells in response to

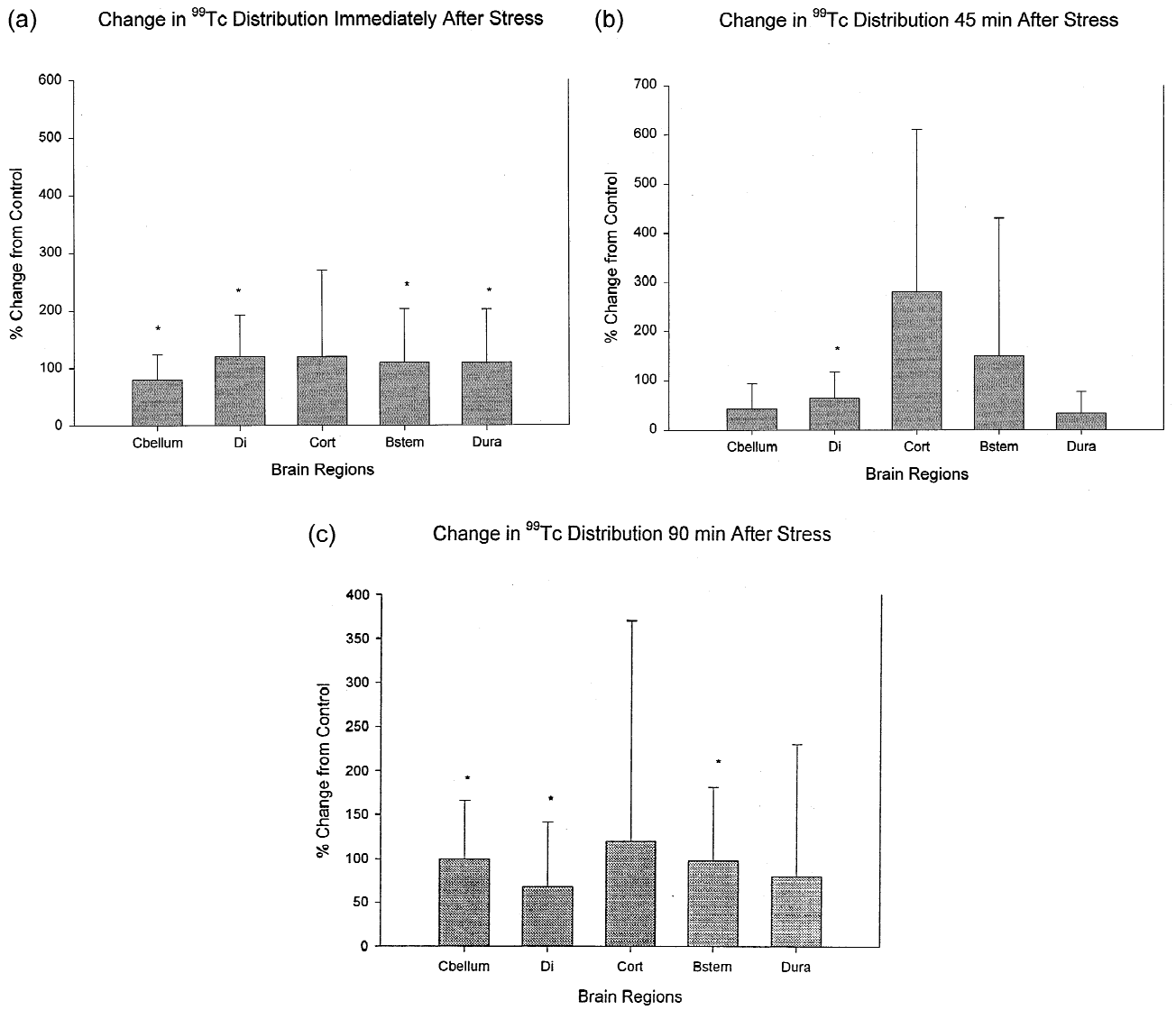

Fig. 2. Effects of acute immobilization stress on ^{99}Tc extravasation reported as percent change in various brain regions: (a) immediately; (b) 45 min; (c) 90 min following stress. Statistical significance ($P<0.05$) is denoted by an asterisk ($n=5$ rats per group; representative graph of one of three experiments). Cbellum=cerebellum, Di=diencephalon, Cort=cerebral cortex, Bstem=brain stem.

30 min immobilization stress was documented by partial toluidine blue staining of mast cells and presence of extruded granule contents (Fig. 5) close to the cell surface. A representative picture of an intact mast cell from the hypothalamus of a control, non-stressed animal is shown (Fig. 5A) and appears fairly round with numerous metachromatic (violet) granules that obscure the nucleus. This normal mast cell is compared to one from an animal that was stressed for 30 min (Fig. 5B). Note that the mast cell is flat, the nucleus is now seen and the cytoplasm has 'empty' looking areas where secretory granules may have been present. The content of numerous granules is seen at or around the surface of the cell and nearby. Degranulation in control animals (Table 1) was 37.8 ±18.1% ($n=11$) and increased to 58.8±19.0% ($n=12$) with 30 min immobilization stress ($P=0.194$).

4. Discussion

Acute stress by immobilization induced a significant increase in BBB permeability as evidenced by Evans blue and ^{99}Tc extravasation in the diencephalon, cerebellum and brainstem. The cerebral cortex showed an increase that was not statistically significant due to the large standard deviation. This may require further investigation using a different study design (and power) and ultimately a larger sample size (to avoid a possible type 2 error).

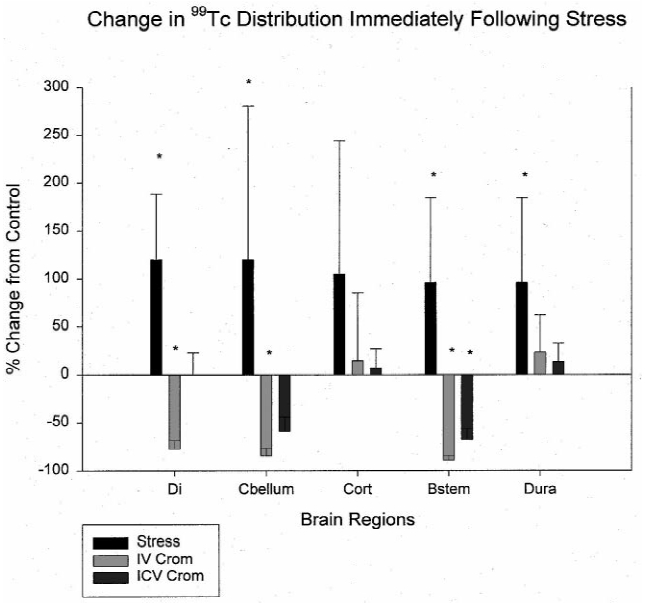

Fig. 3. Effect of cromolyn on ^{99}Tc extravasation in brain regions after 30 min stress with either 60 min iv pretreatment with 0.1 mM cromolyn (0.1 ml) before stress or 60 min icv pretreatment with 0.1 mM cromolyn (0.005 ml) before stress (n=5 rats per group; representative graph of one of two experiments). Di=diencephalon, Cbellum=cerebellum, Cort=cerebral cortex, Bstem=brain stem.

^{99}Technetium-gluceptate was used because the technetium gluceptate complexes [47] remain in the vasculature long enough to permit studies of induced extravasation [31]. In contrast, technetium-99m-pertechnetate is appropriate for first pass circulation in the brain, but over 20% becomes free ^{99m}Tc that leaks out of the circulation by 2 h [36].

This is the first time that the BBB permeability and brain mast cell activation were both shown to be increased simply by acute stress. The findings using the gamma camera support the results obtained with the gamma counter at specific time points. This approach allowed minute to minute documentation of ^{99}Tc levels in the brain following stress and permitted estimation of: (a) the time of maximal activity/the peak level and (b) how long the ^{99}Tc stays in the brain. The gamma camera shows that the brain of both control and stressed animals contain ^{99}Tc. But, following a 30-min stress, animals contain more ^{99}Tc than control animals. The magnitude of the difference is greater than that observed in isolated brain regions studied with a gamma counter. It is unlikely that a change in cerebral blood flow is contributing to this discrepancy as it has been shown that during immobilization regional cerebral blood flow actually decreases [48]. This discrepancy could be explained by the presence of the vasculature, meninges and skin when using the gamma camera.

A surprising finding was the apparent degranulation of 37% of brain mast cells in control animals. At least part of this effect could be due to the transport of the animals out of the animal facility and into a special laboratory where radioactivity could be used. We had previously shown that when such stress experiments were performed inside the animal facility, degranulation in control animals was about 40% less [49]. The diencephalon is the only brain area with a substantial number of mast cells [16,17,23,30, 39,40,52], while the cerebellum also contains a smaller number of mast cells [40,53]. Mast cells have been specifically localized around the cerebral microvasculature [2,34,54]. The involvement of mast cells in BBB permeability is supported by our present findings that iv administration of the mast cell secretagogue compound 48/80 induced marker extravasation only in the diencephalon and cerebellum. This compound had previously been shown to stimulate brain mast cells [13]. Somewhat similar findings had previously been reported with intramuscular administration of 48/80 to pigeons [75]. A recent paper also reported that local application of 48/80 to pia, induced BBB permeability to fluorescein-labeled dextran, thus implicating mast cells [43]. Moreover, ^{99}Tc extravasation was completely eliminated with icv introduction of the 'mast cell stabilizer' drug disodium cromoglycate (cromolyn). The fact that iv cromolyn also reduced ^{99}Tc extravasation in the diencephalon implies that some drug gets into the brain, especially anterograde through the portal system, although the possible contribution of vasoactive mediators released from peripheral mast cells can not be excluded. Icv administration of 48/80 reduced thalamic histamine content and stimulated corticosterone levels [5]. These results imply that brain mast cells respond to 48/80 and that some brain mast cell mediator could stimulate the HPA axis. A possible candidate could be interleukin-6 [38] which is a potent stimulus of CRH release [46].

Mast cells release a variety of vasoactive mediators [21] that could affect BBB permeability [73], such as histamine [58]. Using ^{99m}Tc-sodium pertechnetate or ^{131}I-serum albumin as markers, histamine was shown to increase BBB permeability investigated in the whole brain [4]. This effect was blocked by the histamine-2 receptor antagonist cimetidine [4]. An inhibitory effect of cimetidine was also shown on histamine-induced BBB permeability measured by transendothelial electrical resistance in brain microvessels [6].

Brain histamine had previously been shown to be decreased in the hypothalamus of electrically stressed guinea pigs [42] and in rats subjected to restraint stress [66]. Acute immobilization stress decreased the *hypothalamic* histamine content [3], while another study reported that acute immobilization increased *plasma* histamine concentration about 3-fold as compared to that in freely moving rats [29]; the cromolyn analogue nedocromil inhibited this increase [29]. Elsewhere, rats exposed to water immersion stress, had a 4-fold transient increase in plasma histamine levels that peaked at 30 min and was absent in Ws/Ws mast cell deficient rats [28]. Our results of maximal ^{99}Tc extravasation in response to both stress and 48/80 in the diencephalon and cerebellum, where

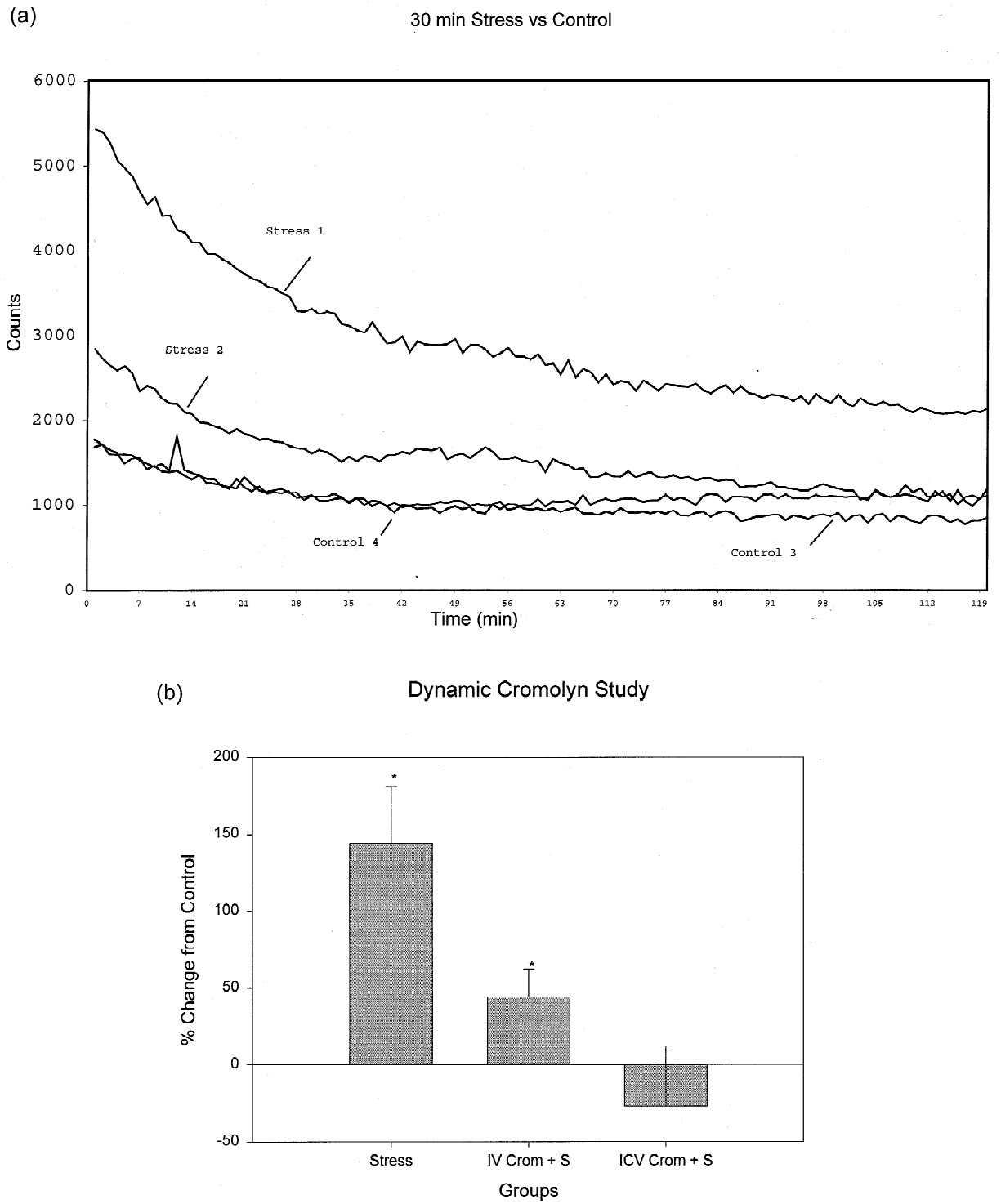

Fig. 4. Real time dynamic study of ^{99}Tc levels in the brain using a gamma camera (n=4). (A) an actual graph of ^{99}Tc levels for 120 min following a 30 min stress. (B) AUC for 30 min immobilization stress(s), iv cromolyn for 60 min followed by stress and icv cromolyn for 60 min followed by stress with the amounts and concentrations indicated for Fig. 3 (n=4 rats per group; representative graph of one of three experiments). Crom=cromolyn, S=stress, IV=intravenous, ICV=intracerebroventricular.

most of the brain mast cell reside, implicate mast cell involvement. Acute immobilization stress was previously shown to result in highest increased endogenous albumin extravasation, measured by immunoelectrophoresis, in the hypothalamus with lower levels in the cerebellum and brainstem [64]. Preliminary studies using W/W^v mast cell-deficient mice indicate that there was no ^{99}Tc-extravasation in these mice, while it was normal in +/+ controls

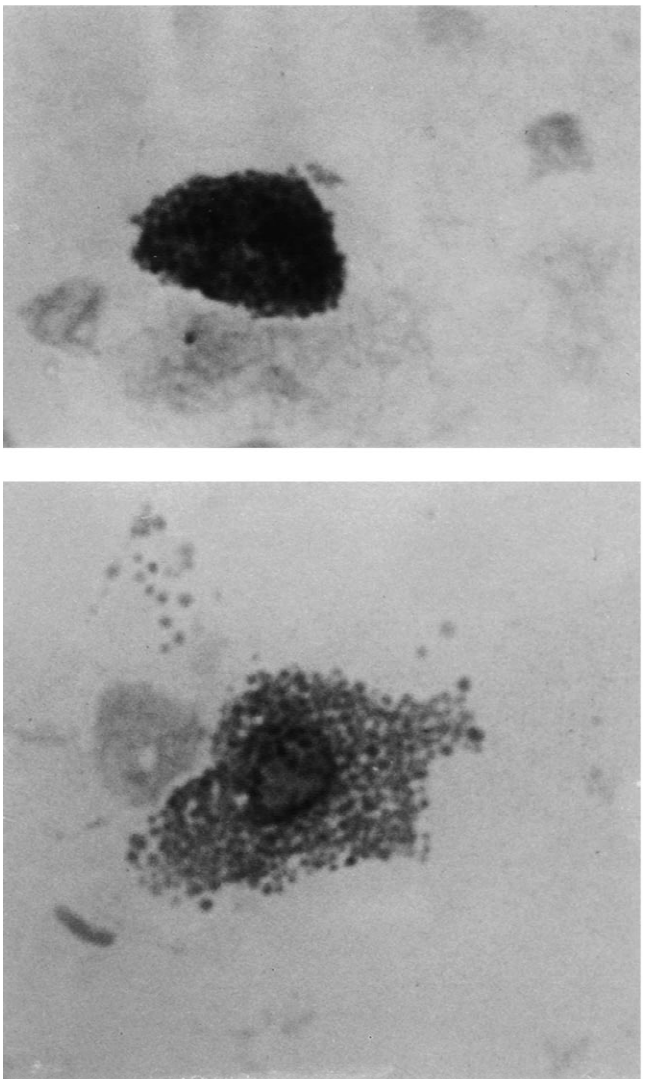

Fig. 5. Photomicrographs showing two hypothalamic mast cells from different rats: (a) control, non-stressed animal; note the round shape of the cell with numerous, intact, metachromatic secretory granules obscuring the nucleus; (b) following 30 min immobilization stress; note the cell is flat with a prominent nucleus, cytoplasmic areas with no secretory granules, while the content of numerous secretory granules is seen close to the surface of the cell and nearby. Magnification ×1000.

(results not shown). These findings are supported by the recent publication that experimental allergic encephalomyelitis can not be induced in W/W^{v} mice [59].

The actual degree of mast cell activation or the type and/or amount of mediators released under stress may be higher than we observed. For instance, many studies have shown that activated brain mast cells contain secretory granules with intragranular alteration of their electron dense content consistent with secretion, but recognizable only by electron microscopy [72]. Change in BBB permeability may not directly parallel the number of mast cells present or the extent of their activation, as involvement of a small number of mast cells may be sufficient to alter BBB permeability. Nevertheless, the present results are supported by the fact that rat mast cell protease I, a marker for connective tissue mast cells, was elevated in the cerebrospinal fluid of rats following acute immobilization stress [50].

Increase in BBB permeability was also shown in response to short-term forced swimming in the cerebellum, but also thalamus and hypothalamus using Evans blue albumin or ^{131}I-sodium [60]. In this study, the plasma and brain 5-hydroxytryptamine (5HT) content was shown to be increased as much as 250% and pretreatment with the mixed histamine and 5HT receptor antagonist cyproheptadine inhibited BBB permeability [60]. These findings imply that both histamine and serotonin may be involved, especially since both amines are known to regulate vascular permeability in rodents [1]. Another vasodilatory and proinflammatory mediator released from mast cells that could be involved is tumor necrosis factor (TNF) [21]. In fact, this molecule was recently shown to be released along with histamine from rat hypothalamic mast cells [10]. TNF has been shown to be increased in the CSF of MS patients [25].

CRH had previously been shown to be involved in dura mast cell degranulation in response to immobilization stress, an effect blocked by the nonpeptide CRH receptor antagonist Antalarmin [72]. Mast cells have often been localized close to neurons [11] and mast cell–neuron interactions [14,56] are increasingly invoked in the context of disease [15]. Mast cells were actually identified close to CRH positive neurons, suggesting that CRH may be acting directly on mast cells [72]. Moreover, CRH [71] and its structural analog urocortin [62] were recently shown to induce mast cell degranulation and vascular permeability in rodent skin, a process also induced by acute immobilization stress [63]. Some direct action of CRH on endothelial cells can not be precluded, however. For instance, CRH was shown to bind to endothelial cells [19] and inhibit their synthesis of prostaglandins [18], as well as and to induce vasodilation in the human fetal–placental circulation [9]; in fact, cultured human umbilical vein endothelial cells secreted CRH and expressed CRH type 2 receptor mRNA [61].

The present results could further our understanding of the regulation of BBB permeability [73] and its involvement in neuroinflammatory diseases [12]. For instance, breakdown of BBB integrity has been documented to precede any clinical symptoms or pathological findings in MS [35,68] is of interest that symptoms in relapsing–remitting MS patients occasionally appear to worsen by psychological stress [24,45,74]. In fact, the unique mast cell enzyme tryptase was shown to be elevated in the CSF of MS patients [57] and this enzyme has recently been shown to induce micro-vascular leakage of Evans blue [26]. However, it is too premature to suggest that immobilization stress in rodents would reflect psychological stress in humans. It is also somewhat confusing that BBB permeability did not increase in the cortex where most MS

lesions typically occur. However, the diencephalon (where we saw a maximal effect) is involved in MS [27] and could be a sufficient starting point through which vasodilatory molecules could eventually affect the cerebral vasculature or through which leukocytes could slowly enter the brain.

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