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STRESS AT THE SYNAPSE. THE SYNAPTIC ACTION OF ACUTE BEHAVIOURAL STRESS AND THE PROTECTIVE EFFECT OF PSYCHIATRIC DRUGS.

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INTRODUCTION

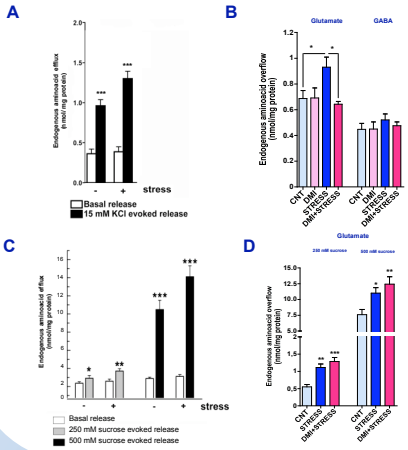
Accumulating evidence suggests that repeated exposure to different stressful events represents a risk factor for neuropsychiatric diseases. Acute and chronic stressors can have lasting consequences on the brain; indeed, stress has been shown to cause structural changes, such as dendritic atrophy and loss of dendritic spines in neuronal populations. Although different neurotransmitter and neuromodulatory systems in various brain regions have been shown to contribute to the stress response, several studies suggest a critical role of glutamatergic neurotransmission, in particular in the prefrontal cortex, in mediating some of the major effects of stress on psychopathology and cognition [1]. Indeed, in previous studies we found that Footshock (FS)-stress induced a marked increase of circulating corticosterone (CORT) and a rapid (non genomic) increase of glutamate release from synaptosomes of prefrontal/frontal cortex (P/FC) via selective activation of glucocorticoid receptor and rapid accumulation of SNARE complexes in synaptic membranes. The increase of glutamate release was prevented by chronic antidepressant (AD) treatments [2]. Therefore, aim of the present work was to study if FS-stress induces a change in glutamate release by increasing the number of vesicles anchored to the presynaptic membrane and modifying the distribution of vesicles among the presynaptic pools, in particular if the readily releasable pool (RRP) is increased by stress or CORT.

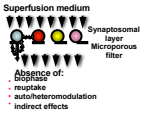
METHODS

Rats were chronically (2 weeks) treated with vehicle or desipramine (DMI) 10 mg/Kg and then subjected to a standard Footshock (FS)-stress protocol [3]. Immediately after FS-stress, P/FC was dissected and synaptosomes were purified on Percoll gradients. Glutamate release was measured in freshly purified synaptosomes by using superfusion technique [4]. The number of docked vesicles in P/FC synaptosomes was measured using electron microscopy [5]. Changes in vesicles mobilization were measured in P/FC synaptosomes from control rats loaded with 4 μM FM1-43 and incubated or not with corticosterone (10 μM, 10 min) by Total Internal Reflection Fluorescence Microscopy (TIRFM) [6, 7].

RESULTS

FIGURE 1 FS-stress increased glutamate release from P/FC synaptosomes in superfusion, evoked by both depolarization and hypertonic sucrose. Glutamate release induced by hypertonic sucrose is a measure of RRP [8]. In FS-stressed animals the RRP was significantly higher. Chronic desipramine prevented the increase of glutamate release evoked by depolarization but not by hypertonic sucrose.





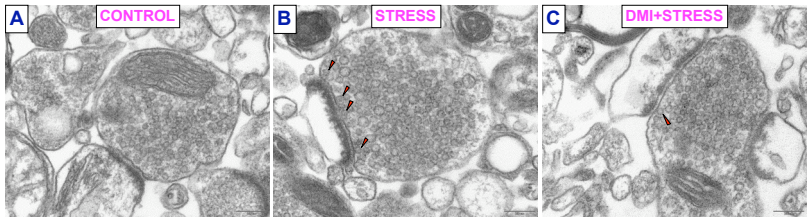
A Basal and 15 mM KCl evoked glutamate release from P/FC synaptosomes of rats subjected or not to FS-stress (-/+ Stress). Data are expressed as means±SEM. *** p<0.001 vs. basal release, Two-Way ANOVA followed by Bonferroni post-hoc test. n = 4–6 rats/group. From ref. 2.

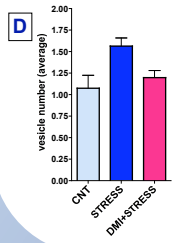
B 15 mM KCl evoked glutamate and GABA release from P/FC synaptosomes of vehicle-treated (CNT), chronically treated with desipramine (DMI), subjected to FS-stress (STRESS) or chronically treated with DMI and then subjected to FS-stress (DMI+STRESS) rats. Data are as in A. *p<0.05, One-way ANOVA followed by Newman-Keuls post-hoc test (n = 6–9 rats/group). From ref. 2.

C Basal and 250 mM or 500 mM sucrose evoked glutamate release from P/FC synaptosomes of rats subjected or not to FS-stress (-/+ Stress). Data are as in A. * p<0.05, ** p<0.01, *** p<0.001 vs. basal release, Two-Way ANOVA followed by Bonferroni post-hoc test. n = 4–6 rats/group.

D 250 mM and 500 mM sucrose evoked glutamate release from P/FC synaptosomes of vehicle-treated (CNT), subjected to FS-stress (STRESS) or chronically treated with DMI and then subjected to FS-stress (DMI+STRESS) rats. Data are as in A. *p<0.05, ** p<0.01, *** p<0.001 One-way ANOVA followed by Newman-Keuls post-hoc test (n = 6–9 rats/group).

FIGURE 2 Preliminary results suggested that FS-stress increased the number of docked vesicles in P/FC synaptosomes. Desipramine only partly prevented this change.



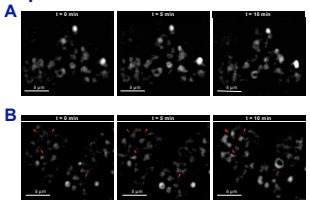


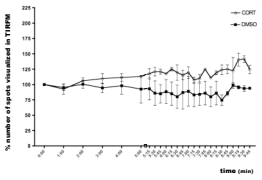
To investigate if FS-stress induced an increase of the number of vesicles docked to the presynaptic membrane, we measured the number of docked vesicles in P/FC synaptosomes of FS-stressed rats (vehicle- and DMI-treated), using electron microscopy (EM). Only the vesicles in contact with the membrane were counted.

Representative EM images (magnification 46000 X) of synaptosomes from: **A** Vehicle-treated rats (CONTROL). **B** Rats subjected to FS-stress (STRESS). **C** Rats chronically treated with DMI and then subjected to FS-stress (DMI+STRESS).
The arrows show docked vesicles: n = 0 in A, n = 4 in B, n = 1 in C.

D Average number of vesicles docked to the presynaptic membrane per synaptosome of vehicle-treated (CNT), subjected to FS-stress (STRESS) or chronically treated with DMI and then subjected to FS-stress (DMI+STRESS) rats (8 rats/group; 20-24 synaptosomes analyzed/rat).

FIGURE 3 Preliminary TIRFM experiments suggested that the incubation in vitro of P/FC synaptosomes with corticosterone increased the size of the readily releasable pool.





Since the effects of FS-stress on glutamate release are mediated by corticosterone [2], we incubated synaptosomes in vitro with corticosterone [9] and visualized by TIRFM the changes induced by the hormone on mobilization of vesicles labeled with FM1-43.

Representative TIRFM images (magnification 100 X): **A** Synaptosomes incubated with DMSO (0.01 %). **B** Synaptosomes incubated with 10 μM CORT [9]. The arrows in B show the fluorescent spots appearing in TIRF field compared with number of spots at t = 0.
C Number of spots visualized in TIRFM (% vs t = 0) during 10 min of in vitro incubation with DMSO (0.01 %) or CORT (10 μM). Data are expressed as means±SEM. Two-Way ANOVA showed a significant effect of CORT (p<0.001). n = 3 fields from 2 independent experiments/group.

CONCLUSIONS

- Acute FS-stress increased depolarization-evoked glutamate release from P/FC synaptosomes. Previous chronic treatment with antidepressants prevented this effect [2].
- Glutamate release evoked by hypertonic sucrose (which mobilizes exclusively the RRP) was increased by FS-stress. Desipramine did not block the increase of glutamate release induced by hypertonic sucrose, most likely because this is not a "physiological" mechanism of vesicle fusion.
- Both EM and TIRFM studies suggested that acute stress/corticosterone induced an increase of the number of docked vesicles. EM showed that DMI only partly prevented this change.

Together, these results suggest that acute stress upregulates glutamate release by increasing the size of RRP and that the dampening effect of antidepressants involves downstream mechanisms (probably affecting the priming of vesicles and the probability of release).

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No potential conflict of interest