

SEROTONIN EFFLUX FROM RAT DORSAL RAPHE NUCLEUS SLICES: MODULATION BY CORTICOTROPIN RELEASING FACTOR

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Corticotropin releasing factor (CRF) is an important modulator of autonomic and behavioral responses to stressful stimuli and anxiety. Although evidence exists that CRF plays a role in regulating serotonergic transmission, neurochemical *in vitro* studies are still lacking. Serotonin (5-hydroxytryptamine, 5-HT) efflux was studied in rat dorsal raphe nucleus (DRN) slices preloaded with [³H]5-HT (50 nM for 30 min), superfused with Krebs solution and electrically stimulated for 2 min. Basal tritium efflux at the 50th min was 0.061±0.005 pmoles; electrical stimulation (50 mA, 2 msec), applied at the 55th (St₁) and the 85th (St₂) min of superfusion, evoked a frequency-dependent peak of tritium efflux (171± 8.3% of the basal efflux at a 3 Hz frequency, close to the physiological firing rate of serotonergic neurons). The St₂/St₁ ratio in control slices was 0.85±0.03 (n=30). The electrically-evoked [³H]5-HT efflux from rat DRN slices proved to be sodium and calcium-dependent: St₂/St₁ was reduced to 0.14±0.08 in the presence of 0.5 μM tetrodotoxin, added at the 60th min (n=5, P<0.05), and to 0.15±0.08 by reducing to 0.2 mM the concentration of calcium ions in the superfusion fluid (n=4, P<0.05). Conversely, under our experimental conditions, serotonin release did not appear to be subjected to a tonic auto-feedback inhibition, since in the presence of the non-selective 5-HT antagonist methiothepin (0.1 μM) the efflux of [³H]5-HT was not modified (95±7.4% of the controls, n=7).

CRF (1-100 nM), added 5 min before St₂, inhibited the stimulation evoked [³H]5-HT efflux; the effect was concentration-dependent, reached a maximum (to 64±5.7% of the controls, n=14, P<0.01) at 10 nM, and was mediated by CRF₁ receptors, being prevented both by the non selective antagonist alpha-helical CRF(9-41), 300 nM (100±8%, n=4, P<0.05 vs. CRF alone), and by the CRF₁ receptor antagonist antalarmin, 100 nM (114±6.4%, n=4, P<0.05 vs. CRF alone). Since it is well known that CRF receptors are linked to G_s proteins, it has been postulated that the observed inhibitory effect on serotonin release was actually due to a facilitation of an inhibitory signal. Bicuculline, a GABA_A antagonist (10 μM), per se ineffective on [³H]5-HT efflux, prevented the CRF-mediated inhibitory action (91±6.9%, n=6, P<0.05 vs. CRF alone) thus supporting the hypothesis that it was mediated by an increase in GABA release. Further studies are in progress to directly verify the hypothesized GABA-releasing action of CRF, and to test the effects of anxiolytic drugs.