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PULSE-CHASE STUDIES OF THE POMC/BETA-ENDORPHIN SYSTEM IN THE PITUITARY OF ACUTELY AND CHRONICALLY STRESSED RATS

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Summary

Experiments were carried out to determine whether stress induces biochemical changes in the pro-opiomelanocortin (POMC) system in anterior (AL) and intermediate-posterior lobe (IPL) of rat. In a series of pulse-chase experiments, acute stress led to an increase in POMC biosynthesis and shorter half-life in AL. However, when the animals were chronically stressed, the AL no longer exhibited increased POMC synthesis. On the other hand, in the IPL, acute stress did not produce any biochemical changes, but chronic stress led to an increase in POMC synthesis and shorter half-life. These data suggest that AL and IPL are affected by acute and/or chronic exposure to stress in opposite directions and that the POMC system in AL may play an important role in stress-induced analgesia.

Acute stress is known to release ACTH and B-endorphin (B-END) concomitantly from the AL of the pituitary (1). Behaviorally, acute stress produces analgesia which is naloxone reversible (2) and blockable by hypophysectomy (3) or dexamethasone (4). However, upon chronic stress, the animals become "tolerant", and are no longer rendered analgesic by further acute stress (5). While these results suggest a role of the pituitary in stress-induced analgesia, the biochemical mechanisms underlying the acute and chronic effects of stress are not understood.

The purpose of the present experiments was to investigate the effects of acute stress and chronic stress on the synthesis and the half-life of POMC by the rat AL and IPL.

Materials and Methods

Animals

Male Sprague-Dawley rats weighing 230-250 g were used. Animals were divided into four groups: (i) non-stressed group

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which received no shock (control); (ii) acutely stressed group (30 min intermittent footshock, 5 mA, 1 sec shock every 5 sec) (acute stress); (iii) chronically stressed group which was exposed to footshock for two weeks in the conditions described above, but not stressed on the day of decapitation (chronic stress + rest); (iv) a group which was chronically stressed for two weeks and then exposed to stress acutely prior to sacrifice (chronic stress + acute stress).

Biosynthetic Studies

The animals were sacrificed between 9:00 and 10:00 AM by decapitation (immediately after exposure to footshock for group (ii) and (iv)). The pituitary gland was carefully divided into the AL and the IPL and then immediately immersed into Krebs-Ringer-bicarbonate medium (KRB) containing 3mg/ml crude collagenase (Sigma, Type I) and 5mg/ml bovine serum albumin (BSA; Sigma, fraction V). Cell-suspensions were prepared by the method of Mulder and Smelik (6). Al and IPL cells from each group of rats were then transfered to incubation vials (AL or IPL cells taken from 2 rats/vial) containing 200ul KRB and 5mg/ml BSA. After pre-incubation for 1.5 hrs at 37°C in a gaseous atmosphere at 95% O_2 + 5% CO_2, each sample was incubated with 0.5mCi ^{3}H-L-lysine (NEN, specific activity 65 Ci/mmol). ^{3}H-lysine was added in a volume of 50ul KRB aliquot. After 15 min incubation, each vials received 50ul of 50mM unlabeled L-lysine and further incubated for 15, 30, 60, and 90 min (chase). Following the chase period, each sample was washed with KRB containing unlabelled L-lysine (20mM) and extracted with ice cold 5N acetic acid containing 0.3mg/ml phenylmethylsulfonyl fluoride and 0.3mg/ml of iodoacetamide. The extract was purified with B-END antibody (Brenda) affinity column and then applied SDS-polyacrylamide disc gel electrophoresis (SDS-PAGE; 5% cross linkage, 12.5% gel) by the method of Laemmli (7). The gels were cut into 2mm disc and gel fragments were incubated for 16 hrs at 37°C in 0.5ml of 50mM sodium-phosphate/0.06% SDS/0.2mg/ml BSA/pH7.6. The radioactivity was counted in a Beckman LS9000 liquid scintillation counter. On the affinity column, B-END antibody Brenda captured the POMC-, B-LPH- and B-END-Sized materials with no evidence of any peaks of other molecular weights, as determined by SDS-PAGE. Recovery was 92%.

Results

In the steady labeling experiments, protein synthesis, as judged by the incorporation of labeled amino acid into trichloroacetic acid (TCA) precipitable material was linear for at least 4 hrs. In the pulse-chase experiments, unlabeled lysine which was added to the incubation medium at the end of the labeling period, produced a decrease in the incorporation of ^{3}H-lysine into TCA-insoluble protein within 10 min.

In the AL preparations, acute stress induced an increase in incorporation of ^{3}H-lysine into total proteins (137%) and POMC (128%), as compared with those of the control group. POMC half-life was also altered ($t_{1/2}$=35.0 min control, $t_{1/2}$=18.8 min acute stress), suggesting more rapid processing from precursor to products. When the animals were chronically stressed, these

effects were reversed back to a normal pattern in both chronic stress + rest and chronic stress + acute stress group. In the IPL preparations acute stress did not affect POMC biosynthesis or half-life. However chronic stress for 2 weeks induced an increase in ^{3}H-lysine incorporation into total proteins (187%) and POMC (143%), and accelerated the POMC half-life, but these effects were not elevated further by additional exposure to stress prior to sacrifice.

TABLE I

Effect of Acute and Chronic Stress on Rate of Maturation of POMC to B-END ($t_{1/2}$ in minutes)

	Anterior Lobe	Intermediate Lobe
Control	35.0	27.3
Acute stress	18.8	30.0
Chronic Stress + Rest	41.1	21.4
Chronic Stress + Acute Stress	36.2	16.7

Discussion

The present investigations show that acute stress led to an increase in incorporation of labeled amino acid into POMC and a shortening of POMC half-life in AL. These results may suggest that, in AL, POMC biosynthesis is activated by acute exposure to footshock stress and that the maturation of POMC to B-LPH and B-END is accelerated. Furthermore, these effects were reversed by chronic exposure to stress. The IPL processing was also affected by footshock stress, but only upon chronic exposure, and in the opposite direction to AL.

The changes in the anterior pituitary are consistent with the observed behavioral effects, whereby analgesia is evident with acute stress, but no longer evident after chronic exposure. The adaptive changes in the anterior lobe POMC-B-END biosynthesis may represent part of the biochemical mechanims underlying this "tolerance" phenomenon. Thus, the acute and chronic stress paradigm may prove a valuable tool for elucidating the mechanism regulating pituitary POMC-B-END system and their relation to behavioral events.

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