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BRIEF COMMUNICATION

Brain and Adrenal Tyrosine Hydroxylase Activity after Chronic Footshock Stress

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STONE, E. A., L. S. FREEDMAN AND L. E. MORGANO. *Brain and adrenal tyrosine hydroxylase activity after chronic footshock stress.* PHARMAC. BIOCHEM. BEHAV. 9(4) 551–553, 1978.—Rats subjected to 9 daily sessions of electric footshock stress showed marked increases in tyrosine hydroxylase activity in various brain regions and in the adrenal gland. The activity of the brain enzyme was elevated in the cerebral cortex, hypothalamus, locus coeruleus and the pons-medulla indicating a widespread effect of stress throughout the brain. Anatomical specificity of the response was indicated by a greater percent increase in the locus coeruleus, a nucleus containing noradrenergic cell bodies, than in the hypothalamus, cortex and pons-medulla, areas that contain noradrenergic terminals.

Stress Footshock Tyrosine hydroxylase Adrenal Brain regions Norepinephrine

IN SEVERAL recent experiments we have employed chronic electric footshock to test the effect of emotional stress on the responsiveness of the norepinephrine (NE) sensitive cyclic AMP generating system in the rat hypothalamus and cerebral cortex. It was reasoned that footshock which increases the release of brain NE [14] should reduce sensitivity as do other procedures which increase the content or release of this brain amine [19]. In our initial experiments we failed to find an effect of footshock on the cyclic AMP response to NE [15] however in more recent studies using improved and more sensitive methodology we have found that the stress produces a significant reduction of the response of cortical tissue (Stone, unpublished observations). Despite the latter finding, the initial negative results led us to question the severity of the stress and to determine if the shock procedure is of sufficient intensity to chronically activate central noradrenergic neurons. It is known that prolonged activation of peripheral or central noradrenergic neurons or chromaffin cells of the adrenal medulla by either electrical stimulation [13,22], drugs [17] or stressors (cited below) results in an increased in vitro activity of tyrosine hydroxylase (TH). In the present experiment therefore we have examined the effectiveness of our footshock procedure in elevating TH activity in several brain regions as well as the adrenal gland. Our results show that chronic footshock is a highly potent stimulus for increasing brain and adrenal TH activity in rats.

METHOD

Eight male albino Sprague Dawley rats, 250–300 g were used. Four animals were randomly assigned to the stressed group and the remaining 4 to the control group. All animals were housed in pairs by group and were maintained on a 12 hr light-dark cycle with food and water ad lib. Footshock stress was applied for one hr on each of 9 consecutive days as described in detail previously [15]. In brief, rats were given shocks of 1 sec duration on a 20 sec variable interval schedule with the shock intensity at 2 mA on days 1–3, 3.5 mA on days 4–6 and 5 mA on days 7–9. Twenty four hr after the last shock period, the animals were killed by decapitation and the brain and adrenal glands rapidly removed. The hypothalamus and pons-medulla were dissected using previously described methods [5]. A circular area of cerebral cortex, 6 mm in dia., located 1 mm lateral to the midline, 1 mm rostral to the caudal border of the occipital cortex and 2 mm dorsal to the rhinal fissure was removed and dissected free of subcortical tissue. This area is referred to as posterior cerebral cortex. A region of pons tissue containing the locus coeruleus (LC) was obtained by making a transverse anterior cut at the level of the caudal border of the posterior colliculi, a transverse posterior cut just caudal to the cerebellar peduncles, two lateral cuts at the medial borders of the cerebellar peduncles, and a horizontal cut removing the pes pedunculi. Mean wet weights in mg ± SD of the dissected tissues were as follows: hypothalamus, 80.5 ± 4.4; cortex,

68.8 ± 5.7; pons-medulla, 170 ± 16; region containing LC, 48.6 ± 16.1. Tissues were homogenized in 5 to 10 volumes of buffer (5.0 mM Tris-HCl, pH 6.8, containing 0.2% Triton X-100) with a Polytron homogenizer. Homogenates were centrifuged at 4°C for 10 min at 10,000×*g* and aliquots of the supernatants were taken for determination of TH activity by a micro-modification of the method of Nagatsu *et al.* [10]. The assay mixture contained 50 μl of enzyme and 100 μl of a cocktail containing 1.1 μmoles of ferrous sulfate, 675 units of catalase, 0.7 μmoles of 6-methyl-5,6,7,8-tetrahydropterine, 12.8 μmoles of 2-mercaptoethanol, 25 μmoles of Trismaleate buffer, pH 5.9 and 7.5 mμmoles of L-tyrosine containing 500,000 cpm of L-tyrosine-3,5-^{3}H. The assay mixture was incubated for 15 min at 37°C. The reaction was stopped by addition of 100 μl of glacial acetic acid. The entire incubation mixture was passed over a 3.0×0.5 cm Dowex 50WX4 (H^+) column and washed 3 times with 500 μl of distilled water. The combined effluent and washes were mixed with 15 ml of ACS scintillation fluid and ^3H_2O radioactivity counted in a liquid scintillation spectrometer. TH activity was calculated as nanomole per hr per pair of LC or adrenal glands, or per g tissue for other brain regions. Enzyme activity in the LC was expressed in this manner since it is the most accurate and reproducible method for assessing TH activity in this brain area [8]. Data were analyzed by a 2×5 (stress×tissue) ANOVA for repeated measures on one factor (tissue) according to Winer [21]

RESULTS

There was a highly significant effect of stress on TH activity, $F(1,6)=72.17$, $p<0.0005$, and a significant interaction of stress×tissue, $F(4,24)=4.61$, $p<0.01$. Tests on simple main effects revealed that footshock stress significantly elevated TH activity in all tissues examined (Table 1). The significant interaction reflected the fact that the absolute increase in TH activity was much greater for the adrenal gland than for the brain tissues. It is interesting to note, however, that in terms of percent of control, the greatest increase in enzyme activity was elicited in the LC, the region containing cell bodies of the dorsal noradrenergic bundle. Changes in noradrenergic terminal regions (i.e., the hypothalamus, cortex and pons-medulla) were less marked.

DISCUSSION

The present results are in agreement with previous research by others demonstrating increased brain and/or adrenal TH activity after such diverse stressors as restraint [4, 6, 7], cold [16,23], tail shock with partial restraint [20], social stimulation [1], forced swimming [11], and electroconvulsive shocks [9]. Since TH is located in central dopaminergic as well as noradrenergic neurons it may be argued that the above changes occurred in terminals of the former but not latter neurons. This appears highly unlikely however in view of the fact that the hypothalamus and pons-medulla have a preponderance of noradrenergic terminals [18]. Furthermore, Hokfelt *et al.* [3], using a sensitive histochemical method, were unable to demonstrate the presence of dopaminergic terminals in the portion of posterior cerebral cortex used in the present study.

The present data do not provide information as to the mechanism of the footshock-induced increase in TH activity. However previous experiments using immunotitration of TH in the rat LC and superior cervical ganglion after chronic reserpine administration [12] and preganglionic electrical stimulation [22] respectively, indicate that prolonged activation of noradrenergic neurons leads to an increased accumulation of specific enzyme protein rather than an activation of preexisting enzyme molecules. In view of the chronic nature of the stress used it is likely that a similar accumulation of enzyme protein was responsible for the increases in TH activity observed in the present study. The present data also show that there is a greater percent increase in enzyme activity in noradrenergic cell bodies located within the LC than in areas rich in nerve terminals. Similar results have been reported in the rat brain after chronic reserpine [2,12] and cold stress [23]. As suggested by Reis *et al.* [12] this may result from an apparent high degree of arborization of central noradrenergic neurons and a consequent dilution of newly formed TH as the latter is transported from cell bodies in the LC to widely divergent terminal areas. Alternatively, the differential response of cell bodies and terminals may reflect differences in the time course of changes in enzymatic activity and/or differences in the turnover of the enzyme [2].

In summary our results indicate that the present foot-

TABLE 1

EFFECT OF CHRONIC FOOTSHOCK ON TYROSINE HYDROXYLASE ACTIVITY

Tissue	Control	Footshock	% Increase	*p*-value
	nmol/pair/hr			
Adrenal gland	62.5 ± 6.2*	166.4 ± 9.6	166	< 0.0001†
Locus coeruleus	1.17 ± 0.13	3.88 ± 0.46	232	< 0.05
	nmol/g/hr			
Hypothalamus	103.4 ± 6.8	146.4 ± 4.4	42	< 0.05
Pons-medulla	30.0 ± 4.5	68.0 ± 15.2	127	< 0.001
Posterior cerebral cortex	4.08 ± 0.89	9.12 ± 1.31	124	< 0.05

* Values represent means ± SEM for 4 rats.

† F ratios for simple main effects (1, 17): adrenal, 129.7; locus coeruleus, 5.35; hypothalamus, 6.78; pons-medulla, 23.5; cerebral cortex, 5.66.

shock paradigm is a potent long term activator of central noradrenergic neurons and can be a useful method for studies of pre- and postsynaptic functional changes during prolonged central noradrenergic hyperactivity.

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