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Inhibition of Catecholamine Synthesis With α-Methyl-*p*-Tyrosine Apparently Increases Brain Serotoninergic Activity in the Rat: No Influence of Previous Chronic Immobilization Stress

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POL, O., L. CAMPMANY AND A. ARMARIO. *Inhibition of catecholamine synthesis with α-methyl-*p*-tyrosine apparently increases brain serotoninergic activity in the rat: No influence of previous chronic immobilization stress.* PHARMACOL BIOCHEM BEHAV **52**(1) 107–112, 1995.—The functional relationship between brain catecholamines and serotoninergic function was studied in stress-naive and chronically immobilized rats after blockade of catecholamine synthesis with α-methyl-*p*-tyrosine (αMpT) . The levels of noradrenaline (NA), serotonin, and 5-hydroxyindole acetic acid (5-HIAA) in pons plus medulla, brainstem, hypothalamus, hippocampus, and frontal cortex, and those of 3-methoxy, 4-hydroxyphenileteneglicol sulphate (MHPG-SO_4) in the hypothalamus were measured by HPLC. Chronic immobilization (IMO) resulted in higher NA levels in pons plus medulla and hypothalamus, the latter area (the only one in which the NA metabolite was determined) also showing slightly elevated MHPG-SO_4 levels as compared to stress-naive rats. Chronic IMO did not alter either serotonin or 5-HIAA levels, but acute stress consistently increased 5-HIAA levels in all areas, independently of previous chronic stress. Administration of α-MpT drastically reduced NA and increased 5-HIAA levels in all brain regions excepting the frontal cortex. The effect of the drug on serotoninergic function was not altered by previous chronic exposure to IMO. These data suggest that the noradrenergic system appears to exert a tonic inhibitory effect on serotoninergic activity in the brain, with the intensity of the effect depending on the brain area studied. In addition, chronic stress does not appear to alter the functional relationship between noradrenergic and serotoninergic activities, although interactions might exist in more restricted brain areas; this deserves further study.

Chronic stress Noradrenergic system Serotoninergic system Immobilization α-Methyl-*p*-tyrosine

THERE IS extensive experimental evidence for an interaction between central noradrenergic and serotoninergic systems. Thus, blockade of serotoninergic function by administration of 5,6-dihydroxytryptamine or PCPA, or by electrolytic ablation of raphe nuclei has resulted in increased tyrosine hydroxylase (TH) activity in the locus coeruleus (7,34,37,42), one of the brain stem areas containing perikarya of noradrenergic neurons, where serotoninergic terminals have also been visualized (36,47). Apparently, serotoninergic terminals in the locus coeruleus exert a tonic inhibitory effect on noradrenergic neurons (14), probably through 5-HT_2 receptors localized in noradrenergic neurons (25). However, in terminals the effect of serotonin on noradrenergic neurons could depend on the area studied and the drug used (14,15,16,50,59). Also, the various types of adrenergic receptors have been found to change differentially after functional serotoninergic denervation (22,49,53). Particularly relevant is the finding that the serotoninergic system is necessary for chronic desipramine adminis-

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tration to cause downregulation of β-adrenergic receptors (30).

Although there are noradrenergic terminals in the raphe nuclei (4), the influence of the noradrenergic system on serotoninergic function is not clear. Yohimbine, an α_2 antagonist, has been reported to decrease 5-hydroxyindoleacetic acid (5-HIAA) accumulation after probenecid (46), which might be a reflection of reduced brain serotoninergic activity. Administration of clonidine at a dose that presumably only stimulates presynaptic α2 receptors, decreased both locus coeruleus and raphe nuclei activities (11,56). Although these and other results [e.g., (31,51)] suggest that the noradrenergic system could exert a tonic stimulatory role on serotoninergic activity in the brain, several laboratories have observed an inhibitory role of α_2-adrenergic receptors on serotonin release in brain cortex slices (26,38), cortical synaptosomal preparations (13, 41), hippocampal (17,18), hypothalamic (20), or dorsal raphe (19) slices. Therefore, the functional consequences of blocking noradrenergic function on serotoninergic activity in different brain areas are not known.

Chronic stress results in changes in brain noradrenergic and serotoninergic activities (2,3,6,23,45,54). Given the close interrelationship between both systems and the assumed overall opposite role exerted by both neurotransmitters in behaviour and in some brain functions, chronic stress might alter the functional interrelationship between the two systems. In the present experiments this interaction was studied after chronic exposure of rats to immobilization (IMO) stress. Assay of serotonin and 5-HIAA was taken as an index of serotoninergic activity in several brain areas.

METHOD

Adult male Sprague-Dawley rats, 2 mo old upon their arrival at the laboratory, were used. They were maintained (two per cage) in a controlled environment (lights on from 0700–1900 h, temperature 22°C) for 12 days before starting the experiment. Food and water were available ad lib.

The rats were randomly assigned to control or chronic IMO groups. The latter animals were daily subjected to 2 h IMO in the morning by attaching them to wood boards as previously described (35). In the morning of day 12, after chronic IMO rats were stressed, both control and chronic IMO rats were administered either saline or αMpT methylester intraperitoneally (IP), 250 mg/kg (Sigma, St. Louis, MO). On the following day some rats were killed without stress (basal groups); others were killed after being subjected to mild stress caused by 4 min exposure to a novel environment (hole board) plus 5 min exposure to forced swim in water (temperature 25°C); the remaining animals were killed after 2 h of acute exposure to IMO followed by exposure to the behavioral tests. This experimental design allowed us to study the behaviour of the animals in the two tests. The results have been reported elsewhere (21).

After appropriate acute treatments, the rats were decapitated and the trunk blood was collected and centrifuged at 4°C. The brain were immediately removed and frozen at −80°C. Brains were dissected in the following areas: pons plus medulla, midbrain, hypothalamus, hippocampus, and frontal cortex.

These brain regions were homogenized with 10 vol. of a medium containing 8% acetonitrile and 92% monosodium phosphate buffer 0.1 M, disodium EDTA 1 mM, and octane sulfonic acid 0.75 mM (pH 3.2). NA, serotonin, and 5-HIAA were determined by HPLC using an SP8700 XR pump (Spectra Physics), an SPH125 automatic injector (Sparck Holland) fitted to a μBondapak C18 column, and an electrochemical detector (Waters 460) with a working electrode potential of 0.7 V. The mobile phase, the same as the homogenization buffer, was fluxed at a rate of 1 ml/min. In the rat, the main metabolite of NA is MHPG-SO_4. To determine total MHPG, a previous hydrolysis with perchloric acid was carried out by adding 50 μl of a solution of perchloric acid to 650 μl of the homogenate to obtain a final 0.1 N concentration of the acid. After 5 min at 100°C, the supernatant was manually injected into the HPLC apparatus. The mobile phase was composed of 3% acetonitrile and 97% monosodium phosphate buffer 0.1 M and 1 mM disodium EDTA (pH 4.0). The apparatus, column, and conditions were the same as for the other compounds except that the voltage was set at 0.75 V. MHPG-SO_4 was only determined in the hypothalamus because among the regions analyzed, it is the one that most consistently responds to stress (24,27,58). In all cases, external standards were used. The sensitivity of the HPLC determinations was 50 ng/g for all compounds.

The statistical significance of the results was evaluated by two-way or three-way analysis of variance (ANOVA), with previous chronic treatment, drug, and acute stress exposure as the main factors. In the case of noradrenaline and MHPG the effect of the drug was not included in the analysis because in most areas the effect of the drug reduced to undetectable values the brain levels of both compounds. Therefore, in this case two-way ANOVA was used.

RESULTS

Chronic exposure to IMO caused anorexia and reduced body weight gain (data not shown). Figure 1 depicts NA levels in all regions except the hypothalamus; Fig. 2 shows the latter region. The two-way ANOVA of NA data revealed that the overall effect of chronic IMO was an increase in NA levels in pons plus medulla [$F(1, 72) = 29.1$, $p < 0.001$], midbrain

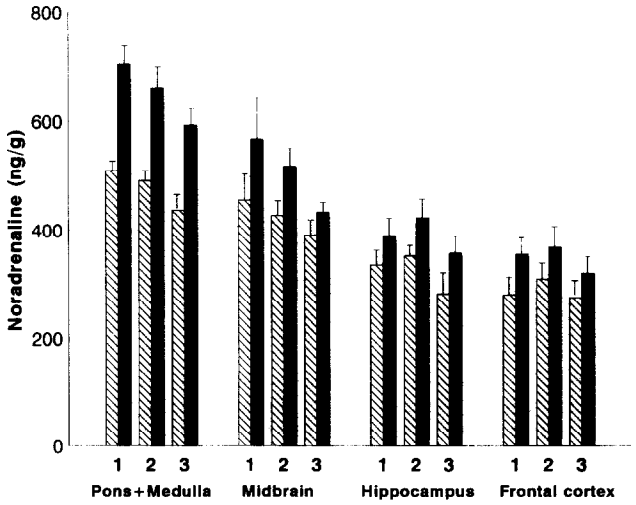

FIG. 1. Effect of chronic and acute stress on noradrenaline levels (ng/g) in various brain regions. Means and SEM are represented. Hatched bars indicate nonchronically stressed rats, and closed bars chronic IMO rats. Numbers under the bars indicate the acute treatments: 1, no acute IMO/no tests ($n = 6$); 2, no acute IMO/tests ($n = 8$); 3, acute IMO/tests ($n = 8$). For statistical analysis, see the text.

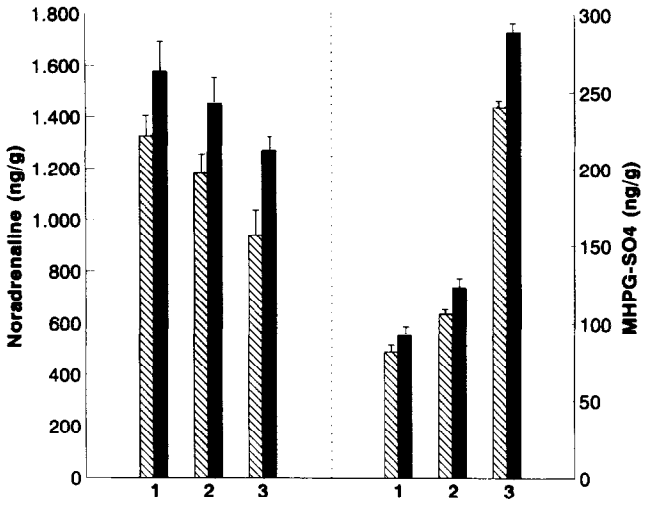

FIG. 2. Effects of chronic and acute stress on noradrenaline and MHPG-SO_4 levels (ng/g) in the hypothalamus. Means and SEM are represented. Hatched bars indicate nonchronically stressed rats and closed bars chronic IMO rats. Numbers under the bars indicate the acute treatments: 1, no acute IMO/no tests; 2, no acute IMO/tests; 3, acute IMO/tests. For statistical analysis, see the text.

[$F(1, 38) = 5.1$, $p < 0.03$], hippocampus [$F(1, 38) = 5.3$, $p < 0.03$], frontal cortex [$F(1, 38) = 7.6$, $p < 0.01$], and hypothalamus [$F(1, 67) = 18.1$, $p < 0.001$]. Acute stress resulted in reduced NA levels in pons plus medulla [$F(2, 72) = 4.6$, $p < 0.02$] and hypothalamus [$F(2, 67) = 11.3$, $p < 0.001$], with no significant changes in the other regions. With regard to hypothalamic MHPG-SO_4 (Fig. 2), the ANOVA revealed that both chronic [$F(1, 38) = 9.2$, $p < 0.004$] and acute stress [$F(2, 38) = 154.7$, $p < 0.001$] increased its concentration in this region.

Tables 1 and 2 respectively show serotonin and 5-HIAA levels in the different brain regions analyzed. The results were analyzed with a three-way ANOVA. With regard to serotonin, only minor significant changes were found: In the pons plus medulla, a significant effect of chronic IMO [$F(1, 76) = 4.0$, $p < 0.05$] and Drug [$F(1, 76) = 10.5$, $p < 0.002$], and a marginally significant effect of chronic treatment × drug [$F(2, 76) = 3.8$, $p = 0.056$] was found; in the frontal cortex the interaction of Drug × Acute Stress [$F(2,76) = 3.4$, $p < 0.05$] was found. With regard to 5-HIAA, a significant positive effect of chronic IMO was observed in the pons plus medulla only [$F(1, 75) = 4.7$, $p < 0.05$]. α-MpT significantly increased 5-HIAA in all brain regions (except in the frontal cortex): pons plus medulla [$F(1, 75) = 35.4$, $p < 0.001$]; midbrain [$F(1, 76) = 13.9$, $p < 0.001$]; hypothalamus [$F(1, 74) = 17.7$, $p < 0.001$]; and hippocampus [$F(1, 75) = 10.5$, $p < 0.002$]. Finally, the positive effect of acute stress exposure on 5-HIAA levels was also highly significant in all brain areas studied: pons plus medulla [$F(2, 75) = 21.4$, $p < 0.001$]; midbrain [$F(2, 76) = 11.8$, $p < 0.001$]; hypothalamus [$F(2, 74) = 5.4$, $p < 0.006$]; hippocampus [$F(2, 75) = 14.9$, $p < 0.001$]; and frontal cortex [$F(2, 76) = 61.3$, $p < 0.001$]. No significant interaction between the main factors was observed in any of the regions studied.

DISCUSSION

In the present experiment, acute exposure to stressors resulted in increased 5-HIAA levels in all brain regions and increased MHPG-SO_4 in the hypothalamus, the only area in which the NA metabolite was determined. The effect was much more marked in response to IMO plus testing than in response to testing alone, probably because testing lasted for 10 min and this period is too small to elicit a detectable neurochemical response. These results are in very good agreement with previously published data using the same measures (2,3,10,12,24,28,43,52). Increases in the concentration of 5-HIAA and MHPG-SO_4 might be considered a reflection of enhanced serotonin and NA release after acute stress, as this conclusion is also supported by other experimental approaches (1,5,8,29,32,33,44,57). Moreover, recent experimental evidence clearly suggests that MHPG-SO_4 and 5-HIAA levels in

TABLE 1

EFFECT OF αMPT ADMINISTRATION ON SEROTONIN LEVELS (ng/g) IN VARIOUS BRAIN REGIONS OF ACUTELY AND CHRONICALLY STRESSED RATS*

	Pons + Medulla	Midbrain	Hypothalamus	Hippocampus	Frontal Cortex
Control-saline					
No acute IMO/no tests (6)	653.5 ± 28.6	996.2 ± 143	882.1 ± 60.0	390.1 ± 17.4	541.3 ± 20.0
No acute IMO/tests (8)	618.3 ± 32.2	928.5 ± 93.0	834.8 ± 43.2	401.7 ± 28.3	550.9 ± 30.0
Acute IMO/tests (8)	625.8 ± 31.8	854.5 ± 26.7	856.8 ± 38.4	361.3 ± 17.9	545.4 ± 19.6
Chronic IMO-saline					
No acute IMO/no tests (6)	672.3 ± 41.4	917.5 ± 74.6	836.6 ± 56.7	384.9 ± 16.0	546.5 ± 35.7
No acute IMO/tests (8)	698.1 ± 30.9	924.8 ± 73.6	845.6 ± 46.7	394.1 ± 15.5	546.3 ± 18.8
Acute IMO/tests (8)	746.1 ± 34.1	981.3 ± 78.4	871.2 ± 45.4	393.4 ± 19.4	583.3 ± 24.3
Control-αMPT					
No acute IMO/no tests (6)	656.0 ± 48.4	901.5 ± 68.2	891.0 ± 59.4	356.9 ± 24.2	536.4 ± 22.3
No acute IMO/tests (8)	768.0 ± 25.3	1057.7 ± 80	956.5 ± 21.1	431.3 ± 30.0	597.8 ± 34.1
Acute IMO/tests (8)	754.8 ± 39.4	976.0 ± 81.4	852.8 ± 30.6	368.5 ± 17.2	547.4 ± 25.5
Chronic IMO-αMPT					
No acute IMO/no tests (6)	721.0 ± 29.4	959.8 ± 103.5	891.1 ± 41.3	424.5 ± 29.5	552.9 ± 26.4
No acute IMO/tests (8)	709.4 ± 17.6	1022.7 ± 11.6	913.8 ± 23.1	413.5 ± 22.1	604.8 ± 24.2
Acute IMO/tests (8)	768.9 ± 42.7	976.8 ± 62.9	867.2 ± 39.6	385.1 ± 24.4	511.1 ± 10.1

*Means ± SEM are represented. The number of animals per group are in parentheses. For statistical analysis see the text.

TABLE 2

EFFECT OF αMPT ADMINISTRATION ON 5-HIAA LEVELS (ng/g) IN VARIOUS BRAIN REGIONS OF ACUTELY AND CHRONICALLY STRESSED RATS*

	Pons + Medulla	Midbrain	Hypothalamus	Hippocampus	Frontal Cortex
Control-saline					
No acute IMO/no tests (6)	672.0 ± 33.9	1012.7 ± 84.3	731.2 ± 75.4	497.0 ± 51.4	466.5 ± 31.8
No acute IMO/tests (8)	705.4 ± 95.6	941.6 ± 78.0	721.3 ± 97.9	477.1 ± 42.9	445.0 ± 22.7
Acute IMO/tests (8)	857.4 ± 60.2	1213.4 ± 84.4	956.2 ± 76.2	651.7 ± 36.5	731.9 ± 53.4
Chronic IMO-saline					
No acute IMO/no tests (6)	697.6 ± 70.3	1018.7 ± 161	787.9 ± 119	469.7 ± 45.7	452.7 ± 54.0
No acute IMO/tests (8)	766.4 ± 51.2	994.0 ± 80.3	835.5 ± 92.4	506.8 ± 49.1	478.1 ± 31.8
Acute IMO/tests (8)	1022.1 ± 57	1319.5 ± 112	936.4 ± 78.9	638.3 ± 32.0	749.8 ± 35.9
Control-αMPT					
No acute IMO/no tests (6)	879.7 ± 59.3	1124.9 ± 119	966.0 ± 87.8	539.7 ± 59.8	489.4 ± 19.2
No acute IMO/tests (8)	929.4 ± 63.7	1220.5 ± 97.0	972.5 ± 48.7	587.7 ± 46.4	535.4 ± 21.3
Acute IMO/tests (8)	1108.0 ± 72	1497.2 ± 77.0	1141 ± 57.4	719.3 ± 17.6	731.0 ± 41.4
Chronic IMO-αMPT					
No acute IMO/no tests (6)	975.3 ± 58.7	1221.6 ± 67.2	988.8 ± 43.0	601.4 ± 53.6	538.6 ± 43.5
No acute IMO/tests (8)	886.5 ± 36.6	1200.0 ± 80.4	981.3 ± 53.4	581.9 ± 63.8	518.1 ± 13.8
Acute IMO/tests (8)	1272.2 ± 64	1445.1 ± 91.5	1071.4 ± 62.5	712.4 ± 32.2	669.0 ± 23.2

*Means ± SEM are represented. The number of animals per group are in parentheses. For statistical analysis see the text.

the rat brain reflect noradrenergic and serotoninergic activities, respectively (39,40).

Chronic stress increased NA levels in all brain areas studied, although the effect was more marked in pons plus medulla and hypothalamus. This is consistent with the well-known positive effect of chronic stress on the activity of enzymes involved in catecholamine synthesis, especially tyrosine-hydroxylase (23,45,54), the limiting step in the NA synthesis. That increased NA levels were probably due to enhanced synthesis and not to reduced NA activity or metabolism is supported by the slight increase caused, in the present experiment, by chronic IMO on both resting and acute-stress levels of MHPG-SO_4 in the hypothalamus. Nevertheless, more direct measures of NA turnover could greatly improve the interpretation of the present data.

Whereas chronic IMO appears to enhance NA synthesis, no effect of chronic IMO on either serotonin or 5-HIAA levels was observed, which is in accordance with previous results from our laboratory using the same chronic stress model (48). In the literature, no effect (9,48) or an enhanced (2,3,6) response to some acute, superimposed stressors have been demonstrated. Although we do not know at present the reasons for these discrepancies, genetic differences in the rats used might be taken into account.

The efficacy of αMpT to inhibit catecholamine synthesis is evidenced by the low or even undetectable levels of NA achieved in all brain regions. In αMpT-treated rats, an increase in 5-HIAA levels was observed in all brain regions except in the frontal cortex. Although the possibility might exist that increased 5-HIAA levels after αMpT administration could have not been due to enhanced serotonin release, this explanation is the most likely one. If this was the case, our results would suggest that drastic impairment of noradrenergic function could lead to enhanced serotoninergic activity in most brain areas. The relationship between noradrenergic and serotoninergic functions are at present unclear, but a direct inhibitory effect of α2-adrenergic receptors on serotonin release has been repeatedly reported, especially in the frontal cortex (13, 26,38), and very recently in the hippocampus (60). Surprisingly, the former was the area in which no responsiveness to catecholamine blockade was observed. Although an inspection of the data reveals that in basal conditions the trend was the same as in the other areas (the effect was not seen after exposure to acute IMO), the frontal cortex appears to display a lower degree of sensitivity to the inhibitory effect of the noradrenergic system on serotonin release than other brain regions. In any case, our results show that 5-HIAA levels very consistently increased after inhibition of catecholamine synthesis, with the effect not being homogeneous in all brain regions. The exact physiologic meaning of the present results remains to be established.

The finding that a tonic inhibitory influence of the noradrenergic system on serotoninergic function was not modified as a consequence of the chronic exposure to IMO was important to our purpose. This suggests that the major components of the balance between both systems remained unaltered after chronic stress and that behavioral changes associated with such a model of chronic stress, including chronic IMO-induced modification of the behavioral response to αMpT administration (21), could not be explained by a modification of the noradrenergic-serotoninergic interrelationship. However, the complexity of the relationship between both neurotransmitters within the CNS and the wide range of changes caused by chronic stress in adrenergic and serotoninergic receptors (55) do not allow us to conclude definitively that in some particular brain regions chronic stress could not have altered serotonin-NA interactions.

The present results indicate that acute exposure to stress increased MHPG-SO_4 and 5-HIAA levels. Previous chronic exposure to IMO did not modify this response. In all animals, irrespective of the chronic treatment, inhibition of catecholamine synthesis with α-MpT increased 5-HIAA levels in all regions except in the frontal cortex. It therefore appears that stress enhances both NA and serotonin turnover and that the noradrenergic system exerts a region-dependent tonic inhibitory effect on brain serotoninergic activity; the effect is not modified by previous chronic exposure to a severe stressor such as IMO.

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