Biochemical Pharmacology, Vol. 33, No. 12, pp. 1887–1891, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

CHANGES IN TYROSINE HYDROXYLASE AND DOPAMINE-BETA-HYDROXYLASE ACTIVITIES BUT NOT IN PHENYLETHANOLAMINE-*N*-METHYLTRANSFERASE ACTIVITY WITHIN CENTRAL ADRENALINE NEURONS AFTER 6-HYDROXYDOPAMINE ADMINISTRATION

RÉGINE FETY, LAURA LAMBAS-SENAS, GENEVIÈVE CHAMBA and BERNARD RENAUD
Laboratoire de Neuropharmacologie, Faculté de Pharmacie, 8 Avenue Rockefeller, 69373 Lyon Cédex 08, France

(*Received* 1 *August* 1983; *accepted* 16 *December* 1983)

Abstract—By using a new microdissection procedure allowing the noradrenaline (NA) and adrenaline (A) cell groups of the A2–C2 region to be sampled preferentially, it was possible to study the biochemical response of these two neuronal populations after 6-hydroxydopamine (6-OHDA) administration. Five days after an intraventricular 6-OHDA injection, tyrosine hydroxylase (TH) activity increased (+104%, $P < 0.01$) in the adrenergic C2 region, in the locus coeruleus (LC) and in the A1–C1 region, while the NA A2 region exhibited no significant increase. Twenty-one days after 6-OHDA administration, dopamine-beta-hydroxylase (DBH) activity had decreased in both the noradrenergic regions (LC, A1–C1 and A2 regions) and in the C2 adrenergic region. Conversely, phenylethanolamine-*N*-methyltransferase (PNMT) activity was not modified either in the cell bodies or in the terminals located in the tractus intermediolateralis of the spinal cord and in the hypothalamic nuclei. These data suggest: (i) that adrenaline-containing neurons could be sensitive to the neurotoxic action of 6-OHDA since they exhibit changes in TH and DBH activities; and (ii) that the determination of PNMT activity may not be sensitive enough to estimate the functional integrity of the A cell bodies or terminals.

The neurotoxin 6-hydroxydopamine (6-OHDA) is known to induce degeneration of central noradrenaline (NA) terminals in the rat [1]. The loss of these terminals is likely to be responsible for the changes in tyrosine hydroxylase (TH) and dopamine-beta-hydroxylase (DBH) activities occurring in the corresponding cell bodies located in the locus coeruleus (LC), A1–C1 and A2–C2 regions [2, 3]. These changes after intraventricular 6-OHDA administration are represented by an increase in TH activity of the cell bodies 5 days after 6-OHDA. While in the LC, these changes are likely to be due to alterations occurring in the NA cell bodies, it is presently unclear whether the changes occurring in the A1–C1 and A2–C2 regions are due to simultaneous alteration of both NA and adrenaline (A) neurons, or only to a specific alteration of the NA neurons of these regions.

The aim of the present study was to determine if an injection of 6-OHDA into the fourth ventricule induced in the rat medulla oblongata an alteration in the A-containing neurons together with an alteration in the NA-containing neurons. For this purpose, we determined in several areas containing catecholaminergic (CA) cell bodies or terminals the activities of the three major catecholamine-synthesizing enzymes: TH, DBH and phenylethanolamine-*N*-methyltransferase (PNMT). Since we have previously reported [3] that PNMT activity does not exhibit any change after 6-OHDA administration, we sought to find another possibility of performing a biochemical study specific for the A-containing neurons. For this purpose, we used a specific anatomical approach by applying a microdissection procedure, recently developed in our laboratory, which allows preferential sampling of the C2 adrenaline neurons vs the A2 noradrenaline neurons [4]. Thus, the possible changes in TH and DBH activities within the C2 region could give information about the sensitivity of the A neurons in this area.

Five days and 21 days after administration of 6-OHDA into the fourth ventricule, the activities of the CA-synthesizing enzymes (TH, DBH and PNMT) were determined in two areas containing NA cell bodies (LC and A2 regions), in one area containing NA and A cell bodies (A1–C1 region), and in one area containing mostly adrenergic cell bodies (C2 region). In order to determine the neurotoxic action of 6-OHDA not only on cell bodies of the medulla oblongata but also on NA and A terminals, enzymatic activities were also determined in the tractus intermediolateralis (TIML) of the spinal cord and in several hypothalamic nuclei [nucleus periventricularis (NPE), nucleus paraventricularis (NPV) and nucleus dorsomedialis(NDM)].

MATERIALS AND METHODS

Animals and treatment. Experiments were performed on male Sprague–Dawley rats (OFA strain, Iffa Credo, Saint-Germain sur l'Arbresle, France) weighing 200–250 g. The animals were housed 5–7 per cage. They received a standard diet (Entretien UAR, Villemoisson sur Orge, France) and water *ad libitum*.

The animals were anaesthetized with pentobarbital (50 mg/kg i.p. of Nembutal®, Abbot, Saint-Rémy

sur Avre, France) and injected stereotaxically with 250 μg (in 5 μl of vehicle) of 6-OHDA hydrobromide (Sigma, St. Louis, MO) or vehicle only (0.9% NaCl solution containing 1 mg/ml of ascorbic acid) into the fourth ventricule according to the coordinates of Pellegrino and Pellegrino [5].

Dissection procedure. Five days and 21 days after 6-OHDA injection, the animals were sacrificed by decapitation. The brain was rapidly removed and cut in a frontal plane at the level of the hypothalamus. After removing the caudal part of the cerebellum in order to uncover the obex, the hindbrain was quick-frozen on dry ice and cut into 500 μm thick coronal sections caudal or rostral to the obex. The LC and A1–C1 regions were punched out as previously described [6]. Three sections caudal and two sections rostral to the obex were cut into microcubes of 1.0 mm length according to the microdissection procedure recently described [4]. The two (left and right sides) homologous micro-cubes located dorsomedially on the three sections immediately caudal to the obex were referred to as the A2 region. Four microcubes (two on the left and two on the right sides) located dorsomedially on the sections from 500 to 1500 μm rostral to the obex were referred to as the C2 region. The spinal cord was cut into 1 mm thick transversal sections from T5 to T12, i.e. on a length of 2.8 cm. The TIML was removed bilaterally from each section (two punches per section) with a hollow needle (0.5 mm, i.d.). The anterior part of the brain was cut in 300 μm thick frontal sections, and hypothalamic regions (NPE, NPV and NDM) were removed with a hollow needle (0.5 mm, i.d.) under a binocular microscope, using the atlas of Jacobowitz and Palkovits [7] as a reference.

Enzymatic assays. TH activity was measured immediately after homogenization on 50 μl of supernatant by a modification [8] of the radiometric method of Nagatsu *et al.* [9], using 3,5-[^{3}H]tyrosine as substrate. TH activity was expressed as pmole of DOPA formed per hr of incubation and per mg of protein. DBH activity was determined on 10 μl of supernatant by a modification [10] of the method of Molinoff *et al.* [11]. The final concentrations of $CuSO_4$ were 40 μM for the LC, 50 μM for the A1–C1 and A2–C2 regions and for the hypothalamic nuclei, and 180 μM for the TIML. The results were expressed as nmole of octopamine formed per hr and per mg of protein. PNMT activity was measured on 10 μl of supernatant according to a modification [10] of the technique of Saavedra *et al.* [12]. The activity was expressed as pmole of *N*-methylphenylethanolamine formed per hr and mg of protein. The protein concentration of each supernatant was determined by the method of Lowry *et al.* [13] using bovine serum albumin standard solutions. Student's *t*-test for unpaired data was used for statistical analysis of the results.

RESULTS

Changes in CA-synthesizing enzymes 5 days after 6-OHDA injection

Within NA cell bodies (Fig. 1). Five days after intraventricular injection of 250 μg of 6-OHDA, a significant increase in TH activity was observed within the LC and the A1–C1 regions (+127%, $P < 0.001$ and +32%, $P < 0.05$, respectively) whereas no significant change was observed within the A2 region. Significant decreases in DBH activity were observed only within the A1–C1 and A2 regions (−25%, $P < 0.01$ and −44%, $P < 0.05$, respectively) while no change was found within the LC. The PNMT activity was found to be unchanged within the LC, the A1–C1 and the A2 regions.

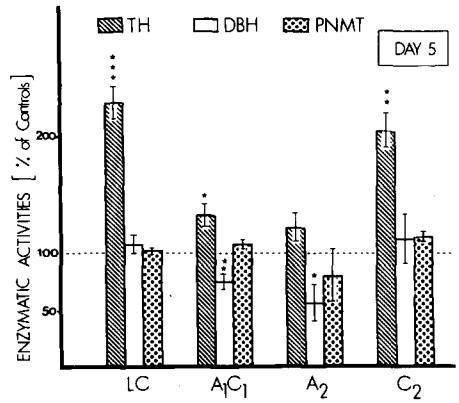

Fig. 1. Changes in CA-synthesizing enzymes activities in the LC, A1–C1 and C2 regions 5 days after injection of 250 μg of 6-OHDA into the fourth ventricule. The activities (mean ± S.E.M.) of the treated rats ($n = 6$) are expressed as a percentage of the activities of the corresponding controls ($n = 8$), which are not shown in the figure. The absolute activities ± S.E.M. for control rats are indicated below and expressed per mg of protein. Tyrosine hydroxylase (TH) activities (pmole of DOPA formed/hr): (a) LC: 1521 ± 67; (b) A1–C1 region: 405 ± 32; (c) A2 region: 532 ± 65; (d) C2 region: 198 ± 24. Dopamine-β-hydroxylase (DBH) activities (nmole of octopamine formed/hr): (a) LC: 11.2 ± 0.4; (b) A1–C1 region: 2.9 ± 0.1; (c) A2 region: 7.1 ± 0.8; (d) C2 region: 1.6 ± 0.2. Phenylethanolamine-*N*-methyltransferase (PNMT) activities (pmole of *N*-methylphenylethanolamine formed/hr): (a) LC: 8.1 ± 1.9; (b) A1–C1 region: 12.2 ± 1.5; (c) A2 region: 22.0 ± 3.0; (d) C2 region: 40.2 ± 5.3. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

Within A cell bodies (Fig. 1). Five days after injection of the neurotoxin, an increase in TH activity was observed in the C2 adrenergic region (+104%, $P < 0.01$). Conversely, DBH and PNMT activities were not significantly altered.

Within NA and A terminals (Table 1). Significant decreases in TH and DBH activities were observed only in the TIML (−55%, $P < 0.05$ and −67%, $P < 0.001$, respectively, for TH and DBH). No change in PNMT activity was observed either in the TIML or in the three hypothalamic nuclei studied.

Changes in CA-synthesizing enzymes 21 days after 6-OHDA injection

Within NA cells bodies (Fig. 2). In contrast with the result obtained 5 days after 6-OHDA injection, a significant decrease in TH and DBH activities was observed in the three regions of NA-containing neurons 21 days after 6-OHDA injection. The decreases for the TH and DBH activities were, respectively, within the LC: −52 and −45% $P < 0.001$; within the A1–C1 region: −31%, $P < 0.01$ and

−50%, $P < 0.001$, and within the A2 region: −37 and −36%, $P < 0.001$. Conversely, no significant change in PNMT activity was observed in the three regions studied.

Within A cell bodies (Fig. 2). Within the C2 region of the medulla oblongata, 21 days after the injection, 6-OHDA induced no change in TH or PNMT activities but a significant decrease in DBH activity was observed (−46%, $P < 0.001$).

Within NA and terminals (Table 2). Twenty-one days after 6-OHDA injection, TH activity decreased only in the TIML (−26%, $P < 0.05$) while no significant decrease was observed in the hypothalamic nuclei. On the other hand, DBH activity significantly decreased in all the terminal areas analysed: TIML: −84%, $P < 0.001$; NPE: −57%, $P < 0.01$; NPV: −54%, $P < 0.01$; NDM: −40%, $P < 0.001$. Conversely, PNMT activity did not exhibit any significant change in any of these regions containing A terminals.

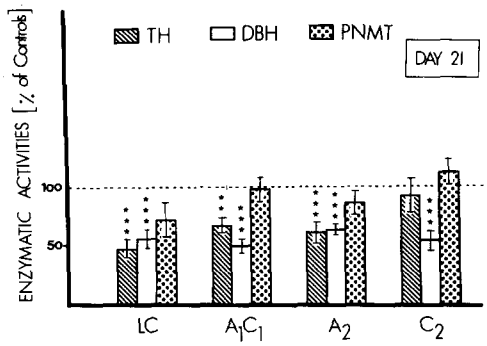

Fig. 2. Changes in CA-synthesizing enzymes activities in the LC, A1–C1, A2 and C2 regions 21 days after injection of 250 μg of 6-OHDA into the fourth ventricule. The activities (mean ± S.E.M.) of the treated rats ($n = 12$) are expressed as a percentage of the activities of the corresponding controls ($n = 10$), which are not shown in the figure. The absolute activities ± S.E.M. for controls rats are indicated below and expressed per mg of protein. Tyrosine hydroxylase (TH) activities (pmole of DOPA formed/hr): (a) LC: 1878 ± 80; (b) A1–C1 region: 341 ± 23; (c) A2 region: 588 ± 34; (d) C2 region: 111 ± 12. Dopamine-β-hydroxylase (DBH) activities (nmole of octopamine formed/hr): (a) LC: 21.2 ± 0.7; (b) A1–C1 region: 3.6 ± 0.1; (c) A2 region: 7.6 ± 0.4; (d) C2 region: 1.5 ± 0.1. Phenylethanolamine-*N*-methyltransferase (PNMT) activities (pmole of *N*-methylphenylethanolamine formed/hr): (a) LC: 8.5 ± 0.1; (b) A1–C1 region: 67.5 ± 3.8; (c) A2 region: 23.0 ± 3.6; (d) C2 region: 45.5 ± 2.1. ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$.

DISCUSSION

In previous studies, it has been reported that intraventricular or intracisternal injection of 6-OHDA induces decreases in the A contents of the hypothalamic [14] and the dorso-caudal part of the medulla oblongata [15]. However, these changes in A were never accompanied by alterations in PNMT activity in any of the cell bodies or terminals analysed [3, 14, 16, 17]. The discrepancy between the former and the latter findings led us to study whether the A-containing neurons were sensitive or resistant to 6-OHDA. For this purpose, we used a specific anatomical approach by applying a new microdissection method [4] allowing the C2 adrenergic and the A2 noradrenergic regions of the medulla oblongata to be separated preferentially. Under these conditions, we assumed that TH and DBH activities could be

Table 1. Effect of 6-OHDA (250 μg) on CA-synthesizing enzymes activities of the TIML and hypothalamic nuclei 5 days after intraventricular administration

	TH activity	DBH activity	PNMT activity
TIML			
Controls (8)	37.7 ± 6.6	0.70 ± 0.01	9.8 ± 0.6
6-OHDA (6)	16.7 ± 4.9*	0.23 ± 0.01***	10.7 ± 0.5
NPE			
Controls (4)	28.0 ± 3.2	4.0 ± 0.7	6.8 ± 0.2
6-OHDA (3)	27.0 ± 5.4	2.6 ± 0.6	5.9 ± 1.0
NPV			
Controls (4)	22.0 ± 1.3	2.5 ± 0.2	5.2 ± 0.9
6-OHDA (3)	23.8 ± 3.2	2.1 ± 0.3	6.8 ± 1.2
NDM			
Controls (4)	33.5 ± 3.6	4.8 ± 0.6	22.4 ± 3.1
6-OHDA (3)	20.8 ± 2.9	2.4 ± 0.5	16.6 ± 28

The absolute activities are expressed as mean ± S.E.M. The number of controls and treated animals is indicated in parentheses. For the hypothalamus, tissue pellets of similar nuclei from two animals were pooled in order to have enough tissue for each enzymatic measurement. Tyrosine hydroxylase (TH) activities are expressed as pmole of DOPA formed/hr per mg of protein. Dopamine-β-hydroxylase (DBH) activities are expressed as nmole of octopamine formed/hr per mg of protein. Phenylethanolamine-*N*-methyltransferase (PNMT) activities are expressed as pmole of *N*-methylphenylethanolamine formed/hr per mg of protein.

Abbreviations: TIML: tractus intermediolateralis; NPE: nucleus periventricularis; NPV: nucleus paraventricularis; NDM: nucleus dorsomedialis.

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 2. Effect of 6-OHDA (250 μg) on CA-synthesizing enzymes activities of the TIML and hypothalamic nuclei 21 days after intraventricular administration

	TH activity	DBH activity	PNMT activity
TIML			
Controls (10)	77.8 ± 5.0	0.95 ± 0.02	11.8 ± 0.5
6-OHDA (12)	57.6 ± 5.0*	0.15 ± 0.01***	12.1 ± 0.5
NPE			
Controls (5)	27.2 ± 0.5	3.0 ± 0.1	7.7 ± 0.3
6-OHDA (6)	23.3 ± 2.5	1.3 ± 0.3**	7.4 ± 1.3
NPV			
Controls (5)	29.1 ± 1.5	2.8 ± 0.3	5.7 ± 0.5
6-OHDA (6)	24.6 ± 3.4	1.3 ± 0.1**	4.3 ± 0.8
NDM			
Controls (5)	25.3 ± 1.1	6.0 ± 0.4	13.1 ± 1.6
6-OHDA (6)	23.8 ± 1.5	3.6 ± 0.2***	12.6 ± 0.9

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.
For experimental conditions and abbreviations, see Table 1.

considered as suitable markers for studying preferentially the response of the A cell bodies in this area.

Changes in CA-synthesizing enzymes within the noradrenergic cell bodies of the medulla oblongata

TH activity exhibited a significant increase five days after injection of 6-OHDA into the fourth ventricule only within the LC and A1–C1 regions, while after 21 days TH activity was found to be significantly reduced in all the noradrenergic areas of the cell bodies studied (LC, A1–C1 and A2 regions). Such results were recently described in the LC by Acheson *et al.* [2] and in two other NA regions of the medulla oblongata (A1–C1 and A2–C2 regions) by our laboratory [3]. In this previous study, TH activity was found to be significantly increased at day 5 and decreased at day 21 in all the regions of cell bodies studied (LC, A1–C1 and A2–C2 regions), while in the present study there was no significant alteration in the TH activity at day 5 in the A2 region. The difference between the former and the latter result could be explained by the fact that the changes in TH activity occurring in the whole A2–C2 region (+24%, $P < 0.01$) could originate within the A cell bodies of the C2 region and not in the NA cell bodies of the A2 region (see below).

DBH activity significantly decreased after 6-OHDA injection within the A2 and the A1–C1 regions at day 5 and in all the areas studied at day 21 (LC, A1–C1 and A2 regions). Thus, this pattern of changes in TH and DBH activities is likely to represent a common characteristic of response of the NA-containing neurons after 6-OHDA injection. As suggested by Acheson *et al.* [2], the initial increase in TH activity could be due to a compensatory response of NA cell bodies to the lesion of their terminals. The decrease in DBH activity of the NA cell bodies could be interpreted as a part of the retrograde reaction [18].

PNMT activity was found to be unaltered after 6-OHDA administration in all the areas containing NA cell bodies. This is in accordance with the data previously obtained by others [16] and by our laboratory [3] showing the lack of any change in PNMT activity in the brain stem after central administration of 6-OHDA.

Changes in CA-synthesizing enzymes within the adrenergic cell bodies of the C2 region

Five days after 6-OHDA injection into the fourth ventricule, TH activity significantly increased in the C2 region (+104%, $P < 0.01$). DBH activity decreased only at day 21 while no change in PNMT activity was observed at days 5 and 21. Such variations, i.e. increases in TH activity associated or followed by a decrease in DBH activity, were also observed in the LC, in the A1–C1 and in the A2 regions as reported above. These changes could be considered as a consequence of the neurotoxic action of 6-OHDA on NA-containing neurons. A similar mechanism could also be responsible for the changes occuring in the adrenergic C2 region and could support the hypothesis of a lesion of A-containing neurons by 6-OHDA. This first hypothesis is supported by the two following series of results obtained after intraventricular 6-OHDA administration: (a) as previously mentioned, the A concentration decreases both in the hypothalamus [14] and in the dorsocaudal part of the medulla oblongata (DCMO) [15]; (b) the PNMT-containing neurons studied by immunohistofluorescence exhibit morphological alterations suggesting that these neurons are affected by 6-OHDA [15] (weak fluorescence of the cell bodies, distorted appearance of the terminals, swollen axons; P. R. C. Howe, personal communication, September 1983). Thus, these biochemical and histological observations are in accordance with the enzymatic changes observed in this paper and favour the hypothesis of a lesion of the adrenergic neurons by 6-OHDA. Another possible explanation for the enzymatic changes observed in the C2 region is that the increase in TH activity could initially originate in the NA cell bodies of the LC which innervate this area [19] and which exhibit 5 days after 6-OHDA an important increase in TH activity. However, under the experimental conditions used for this study, it has already been shown that the noradrenergic terminals are greatly damaged [1, 20], a fact which argues

against an increase in TH activity taking place in noradrenergic terminals. Thus, the most probable explanation for the increase in TH activity occurring in the C2 region remains the hypothesis of an increase in TH activity originating in the C2 A neurons themselves.

Changes in CA-synthesizing enzymes within the adrenergic and noradrenergic terminals

In the TIML, TH and DBH activities were found to be significantly decreased 5 and 21 days after 6-OHDA administration. In the hypothalamic nuclei, TH activity never decreased while DBH activity decreased only 21 days after 6-OHDA. Since these enzymes are markers of A and NA-containing neurons, such an alteration in TH and DBH activities could reflect a lesion of these terminals within the spinal cord or within the hypothalamus occurring after 6-OHDA administration. Conversely, PNMT activity exhibited no significant change at days 5 and 21 both in the TIML and the hypothalamic nuclei. Since PNMT is generally thought to be a specific marker of A neurons, such results favour the hypothesis of resistance of the A-containing neurons to 6-OHDA. However, this hypothesis seems unlikely if we consider the changes in TH and DBH activities found in this study for the C2 A region as well as the decrease in A content previously reported in the hypothalamus by Tessel *et al.* [14]. Since in these two regions (TIML and hypothalamus), it is not possible to distinguish preferentially between adrenergic and noradrenergic terminals, the present results do not allow us to determine if A-containing terminals are sensitive to 6-OHDA as we suggested for the A cell bodies.

The data presented in this paper show that A-containing neurons of the C2 region respond to 6-OHDA injection in the same way as NA-containing neurons of the medulla oblongata, i.e. by an increase in TH activity at day 5 followed by a decrease in DBH activity at day 21. Conversely, the lack of alteration in PNMT activity in the cell bodies as well as in the terminals is at variance with the changes in the other enzymatic activities.

Acknowledgements—We wish to thank Miss E. Lombard for her assistance in the presentation of the manuscript. This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale (I.N.S.E.R.M., contrat de recherche libre 79-1356-6) and the Fondation pour la Recherche Médicale Française (F.R.M.F.).

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