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COMPARATIVE RESPONSES OF THE CENTRAL ADRENALINE- AND NORADRENALINE-CONTAINING NEURONS AFTER RESERPINE INJECTIONS

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Abstract—The responses of the noradrenaline (NA)- and adrenaline (A)-containing neurons to a reserpine treatment have been studied in the rat brain by using biochemical indices of the neuronal activity. Three days after multiple reserpine injections, tyrosine hydroxylase activity was significantly increased in the locus coeruleus (LC), A_1-C_1 and C_2 regions. No change in this activity was observed in the A_2 region. Furthermore, the NA and A endogenous levels were markedly reduced both in NA and A cell bodies and/or terminals, suggesting a reserpine action on NA and A neurons. The NA turnover was unchanged in all the regions analyzed. Conversely, the A turnover was reduced in the LC, A_2 and C_2 regions and in the nucleus periventricularis of the hypothalamus. This result suggests a different degree of sensitivity and/or response of the NA and A neurons following reserpine administration.

In the central nervous system, the action of reserpine has been studied mainly on serotonin-, noradrenaline- and dopamine-containing neurons. Regarding the noradrenaline (NA)-containing neurons, reserpine injections are known to increase the activity of the tyrosine hydroxylase (EC 1.14.16.2) in various regions (locus coeruleus = LC, nucleus tractus solitarii, A_2 and A_1 groups) of the rat brainstem [1–3]. Furthermore, NA depletion has been reported in the rat brain after reserpine injection [4–6]. On the other hand, the effect of a reserpine treatment on the adrenaline (A)-containing neurons has been less extensively investigated. The only studies performed have shown a central A depletion [5, 7, 8]. However, in these reports, A levels were determined in the whole brain or within hypothalamic regions. No study was performed in other regions of A cell bodies and terminals. In a paper previously published [9], an increase in tyrosine hydroxylase (TH) activity has been reported in the C_2 adrenergic region, suggesting a response from the A-containing neurons to reserpine administration.

The aim of the present study was to compare the responses of NA- versus A-containing neurons to reserpine treatment in several regions using several biochemical indexes of the neuronal activity. For this purpose we have determined after 3 reserpine injections (i) TH activity in four brainstem regions (LC, A_2, C_2 and A_1-C_1 areas), (ii) the changes in NA and A levels in the same brainstem areas and in NA and A terminals hippocampus, tractus intermediolateralis (TIML) of the spinal cord and three hypothalamic nuclei, and (iii) the turnover rates of NA and A in all these regions.

MATERIALS AND METHODS

Animals and treatments. Experiments were performed on male Sprague–Dawley rats (OFA strain, IFFA-CREDO, 69210 Saint Germain sur l'Arbresle, France) weighing 180–200 *g*. The animals were housed 4 or 5 per cage, fed *ad libitum* and maintained in a controlled light-cycled environment (12 hr light and 12 hr darkness) for 7 days prior to the experiments. Four groups of 8 rats were used. Two groups of treated-rats received daily subcutaneous injections of reserpine (1 mg/kg, Serpasil®, Ciba-Geigy) during 3 consecutive days and were sacrificed 3 days after the last injection. Two other groups of control rats received saline in the same conditions. NA and A turnovers were estimated by measuring the disappearance of these amines after inhibition of dopamine-β-hydroxylase (DBH) by FLA 63 [bis(4-methyl-1-homopiperazynil-thiocarbonyl) disulphide]. Three hours before sacrifice, one group of reserpine treated-rats and one group of control rats were injected with FLA 63 (25 mg/kg, i.p.). The two other groups of rats were injected with aqueous solution.

Dissection procedure. The animals were sacrificed by decapitation. Their brains were rapidly removed and sectioned in a vertical plane immediately caudal to the hypothalamus. The posterior and anterior parts of the brain were cut, respectively, into 500 or 300 μm-thick coronal sections. Brainstem and hypothalamic regions were dissected out as previously described [10]. The regions analyzed were (i) in the brainstem: LC, A_2, C_2 and A_1-C_1 regions, (ii) in the hypothalamus, the following nuclei: nucleus periventricularis (NPE), nucleus paraventricularis (NPV) and nucleus dorsomedialis (NDM). Moreover, hippocampus was removed bilaterally from defrozen sections using the atlas of Jacobowitz and Palkovits [11] as a reference. Finally, the spinal cord was cut into 1 mm-thick transversal sections from T_5 to T_{12} and the TIML removed bilaterally from each section with a hollow needle (500 μm) according to Fety *et al.* [10].

Biochemical assays Tissues were homogenized in an aqueous solution of dithiothreitol 5.10^{-4} M. A portion of the homogenate was centrifuged and the supernatant fluid was used to determine TH activity as previously described [12]. In this assay, $DMPH_4$ (2-amino-6,7-dimethyl-4-hydroxy-5,6,7,8-tetrahydropteridine) was used as cofactor in a saturating concentration (1.20 mM). The remaining portion of the homogenate was mixed with perchloric acid (concentration of perchloric acid in the final solution = 0.1 M) to precipitate proteins. After centrifugation, the protein-free supernatant fluid was used to determine NA and A concentrations by a modification of the radioenzymatic technique of Da Prada and Zurcher [13]. Protein concentrations were determined on the precipitate using the method of Lowry *et al.* [14]. The statistical analysis was made by using the Student's *t*-test for unpaired data after verification of the equality of variances by the Fisher–Snedecor's *F*-test

RESULTS

Effects of reserpine treatment on brainstem TH activity

TH activity was determined in 4 brainstem regions of rats which had received multiple doses of reserpine (one daily injection, 1 mg/kg, s.c., for 3 days). Three days after the last injection, TH activity was significantly increased (Fig 1) within three of these regions +134% ($P < 0.001$) for the LC, +53% ($P < 0.01$) for the A_1-C_1 region and +51% ($P < 0.01$) for the C_2 region. Conversely, no change in TH activity was observed in the A_2 region (Fig. 1).

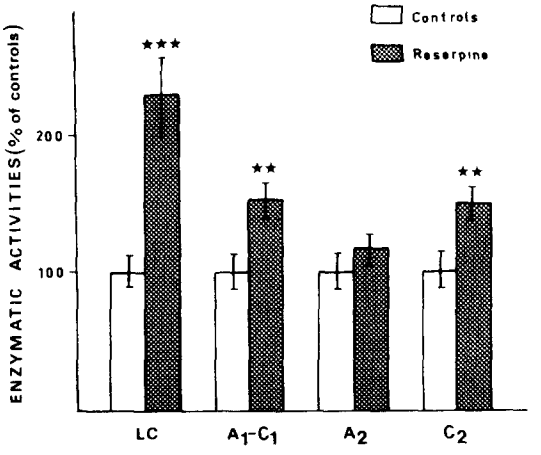

Fig 1 Effects of reserpine injections on the TH activity in the locus coeruleus (LC), A_1-C_1, A_2 and C_2 regions Animals received reserpine (1 mg/kg, s c) daily for 3 consecutive days and were sacrificed 3 days after the last injection TH activities (means ± S E M) of the treated rats (N = 8) are expressed as a percentage of the activities of the corresponding controls (N = 8) The absolute activities ± S E M for the control rats, expressed as pmole of DOPA formed per hr and per mg of protein in different regions, were LC 780 ± 73, A_1-C_1 255 ± 28, A_2 384 ± 33 and C_2 151 ±8 **P < 0 01, ***P < 0 001

Effects of reserpine treatment on regional noradrenaline (NA) and adrenaline (A) levels

In the brainstem nuclei (Table 1) Three days after the last of the 3 consecutive injections of reserpine (1 mg/kg, s c), the endogenous NA levels were markedly reduced (from −46% to −66%) in all the regions analyzed (LC, A_1-C_1, A_2 and C_2 regions). The endogenous A levels were reduced at the same extent (−38% to −68%) in all these regions

In the regions containing NA and A terminals (Table 1) NA and A levels were also significantly decreased (from −63% to −83%) in the three hypothalamic nuclei analyzed (NPE, NPV and NDM) as well as in the TIML (−77%) In the hippocampus, the NA levels were significantly reduced (−77%) while A was not detectable in this region.

Effects of reserpine treatment on regional NA and A turnover

The NA and A depletions obtained three hours after FLA 63 administration were measured concomitantly in various regions of the treated and control rats.

In the brainstem nuclei The reserpine treatment did not change significantly (Fig 2) the FLA 63-induced NA depletion in all the regions analyzed (LC, A_1-C_1, A_2 and C_2), indicating a lack of change in NA turnover Conversely, it was found that the

Table 1 Effects of reserpine injections on regional NA and A levels The values of CA concentrations are given as ng/mg proteins Each value is the mean of 8 values ± S E M The experimental conditions are identical to those indicated in the Fig 1

	NA concentration			A concentration		
Region	Controls	Reserpine	% change	Controls	Reserpine	% change
LC	14 50 ± 0 64	7 83 ± 0 58***	−46	0 17 ± 0 01	0 09 ± 0 01***	−49
A_1C_1	6 81 ± 0 20	2 81 ± 0 15***	−59	0 20 ± 0 01	0 12 ± 0 01***	−38
A_2	20 68 ± 1 19	6 96 ± 0 34***	−66	0 43 ± 0 04	0 14 ± 0 01***	−68
C_2	4 39 ± 0 27	1 76 ± 0 11***	−60	0 16 ± 0 01	0 07 ± 0 01***	−56
NPE	37 47 ± 3 58	6 27 ± 0 90***	−83	1 50 ± 0 13	0 42 ± 0 02***	−72
NPV	146 11 ± 14 85	45 41 ± 4 62***	−69	3 44 ± 0 26	1 04 ± 0 13***	−70
NDM	67 09 ± 6 75	18 15 ± 1 50***	−73	2 45 ± 0 25	0 91 ± 0 05***	−63
TIML	38 51 ± 1 08	8 84 ± 0 94***	−77	0 28 ± 0 02	0 06 ± 0 01***	−77
HC	4 43 ± 0 22	1 00 ± 0 06***	−77	n d	n d	n d

*** P < 0 001

Abbreviations LC locus coeruleus, NPE nucleus periventricularis, NPV nucleus paraventricularis, NDM nucleus dorsomedialis, TIML tractus intermediolateralis, HC hippocampus, n d not detectable

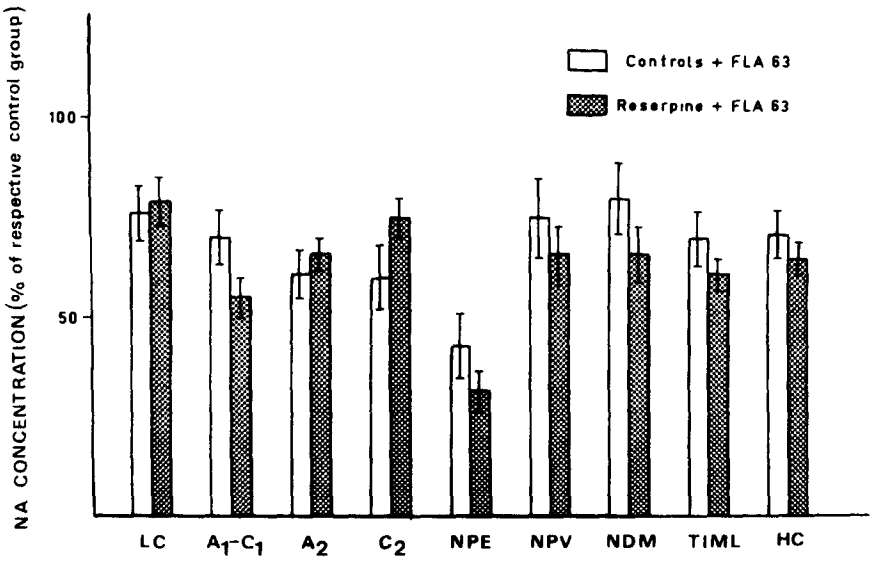

Fig. 2 Effects of reserpine treatment on the FLA 63-induced depletion of NA stores in several brain regions The values of NA concentrations (means ± S E.M) of the control FLA 63-treated rats (N = 8) and the values of reserpine FLA 63-treated rats (N = 8) are expressed as a percentage of the concentrations of the respective control groups (without FLA 63). The NA absolute concentrations (values ± S.E M) of the rats not treated by FLA 63 are indicated in Table 1 The abbreviations are identical to those indicated in the Table 1

A depletion following FLA 63 was less pronounced in three regions (LC, A_2 and C_2) of reserpine-treated rats when compared to control rats (Fig. 3). These results indicate that the A turnover is decreased in these 3 regions of reserpine-treated rats. Conversely, no significant difference was observed in the A_1-C_1 region for the A turnover (Fig. 3).

In the regions containing NA and A terminals. After reserpine treatment, the FLA 63-induced NA depletion was unchanged in all the regions analyzed (hypothalamic nuclei, TIML and hippocampus) (Fig. 2). However, the A depletion after FLA 63 was significantly reduced ($P < 0.01$), i.e. there was a decrease in the A turnover within the NPE (Fig. 3) while no significant difference was noted in the other regions (NPV, NDM and TIML)

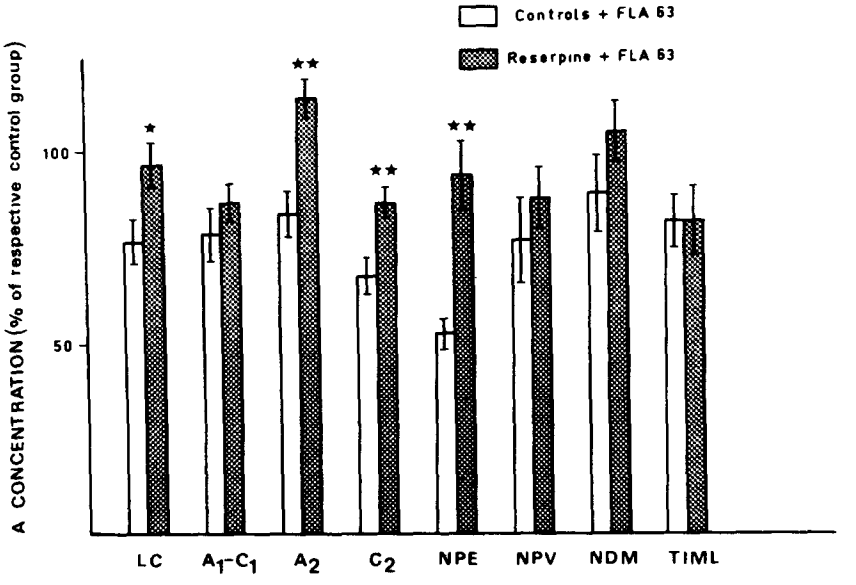

Fig 3 Effects of reserpine treatment on the FLA 63-induced depletion of A stores in several brain regions. The values of A concentrations (means ± S E M) of the control FLA 63-treated rats (N = 8) and the values of reserpine FLA 63-treated rats (N = 8) are expressed as a percentage of the concentrations of the respective control groups (without FLA 63). The A absolute concentrations (values ± S E.M) of the rats not treated by FLA 63 are indicated in Table 1 The statistical differences for the FLA 63-induced depletion in A between the reserpine-treated rats and the control rats are indicated *$P < 0.05$, **$P < 0.01$ The abbreviations are identical to those indicated in the Table 1

DISCUSSION

In a previous study, we have suggested that the A neurons of the C_2 region could be affected by reserpine in the same way as the NA neurons of the rat brainstem [9]. The present study was performed to investigate further the reserpine-evoked response of the A neurons. For this purpose, we have determined after reserpine injections (i) TH activity in areas containing catecholaminergic cell bodies, and (ii) NA and A endogenous levels and turnover rates in areas containing either catecholaminergic cell bodies and terminals.

Changes in TH activities

A dose of 1 mg/kg of reserpine, instead of 10 mg/kg as in our previous studies [2, 9], was used in the present experiment. In these new experimental conditions, i.e. three days after 3 reserpine injections of 1 mg/kg, TH was significantly increased in the LC, A_1-C_1 and C_2 regions although no change was observed in the A_2 region. These changes in enzymatic activities, although of a lesser magnitude, are in accordance with those reported in our previous papers [2, 9]. The smaller increase in TH activity is likely to be due to the lower dose of reserpine used in the present experiment since this enzymatic change is known to be dose-dependent [6, 15]. The increase in TH activity found in the A_2-C_2 region had been previously attributed to a response of the NA neurons of the A_2 group to reserpine [2, 16]. However, by using a microdissection to preferentially sample the NA and A neuronal populations of the A_2-C_2 region [17], we have shown that this increase in TH activity is likely connected to a change in the activity of A-containing neurons of the C_2 group [9]. In order to confirm this result, we measured in the present study (see below) the changes in NA and A levels occurring after reserpine in the A_2 and C_2 regions. The same measurements were also performed in the whole A_1-C_1 region since a preferential microdissection of the two neuronal populations is not possible in this area where A and NA neurons overlap largely [18, 19].

Changes in NA and A endogenous levels

After multiple reserpine injections, NA and A endogenous levels were measured in several cell bodies and terminals regions. NA and A levels were markedly reduced in all the regions analyzed (−46% to −83% for NA levels and −38% to −77% for A levels). NA depletions have been previously reported after reserpine in the whole rat brain [4, 6], in the nucleus periventricularis of the hypothalamus [5] and in the hippocampus [20]. These NA depletions are generally attributed to the primary effect of the reserpine, i.e. the blockade of the NA vesicular uptake by this drug [21]. Adrenaline depletions have also been reported in the whole brain [8], in the hypothalamus [20] and in the nucleus periventricularis of the hypothalamus [5]. Our present results show that A is depleted in all of the 8 regions analyzed. Such decreases in central A levels may be due to a direct toxic action of reserpine on the A neurons. This impairment of A neurons by the drug can take place at two different steps of the A metabolism. Firstly, we can hypothetize that reserpine inhibits the vesicular uptake of A in the same manner as for the NA neurons. This first mechanism of action would lead to the decrease observed for the A levels. Secondly, another direct toxic action of reserpine could produce the same effect on A levels: an inhibition of the vesicular uptake mechanisms for NA molecules within the A neurons. This mechanism would lead to a decrease in the NA available for N-methylation by the adrenaline-synthesizing enzyme, the phenylethanolamine-N-methyltransferase (PNMT). In these both hypothesis, the reserpine can be considered to exert a toxic action on the A neurons. However, besides the hypothesis of a direct effect of reserpine on the A neurons, we have to consider the possibility that the decreases observed in A levels may be simply a consequence of the decreases in NA levels, secondary to the toxic action of reserpine on the NA neurons. However, this possibility implies that the NA neurons provide the NA used as precursor by the A neurons. In these conditions, the decrease in the precursor pool of NA would lead to a reduction of A formation by PNMT in undamaged A neurons. Such a possibility is very unlikely since A neurons contain not only PNMT but also TH and DBH [18, 22] and thus, are likely to synthetize the NA needed for the A synthesis. This indirect mechanism of action of reserpine on A neurons through the NA neurons could be important only in the hypothalamus where the presence of PNMT positive neurons lacking TH has been reported [23, 24]. Thus, our results favor the hypothesis of a direct action of reserpine on A neurons, presumably on the vesicular storage mechanisms of NA and/or A.

Changes in NA and A turnover rates

The results obtained in the present study do not show any significant difference in NA turnover between reserpine-treated rats and their controls in the 9 brain regions analyzed. An increase in NA turnover is likely to occur immediately after reserpine administration [4, 25], but another situation may exist several days later. Unfortunately, we have found no study of the NA turnover in brain nuclei as well as in the whole brain of rats treated with reserpine several days before the sacrifice.

The measurement of the disappearance rate of A shows, in 4 out of the 8 brain regions analyzed, a significant decrease in A turnover occurring in the reserpine-treated animals compared to controls. Such a result was not expected since an increase in TH activity occurs in several of these regions. No comparison can be made with the data of the literature since there is no report on the A turnover in brain nuclei of reserpine-treated rats.

Our findings show that the central NA turnover is unchanged in reserpine-treated rats while the A turnover is decreased in several brain regions of the same animals. The differential effect of reserpine on the turnover of these two CA suggests a different degree of sensitivity and/or response of the NA and A neuronal populations of the brainstem. Moreover, these results indicate that some of the functional responses obtained after using reserpine as a pharmacological tool have to be interpreted in taking into

account the action of this drug on the central A neurons as well as on the other central monoaminergic neurons

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