Biochemical Pharmacology, Vol. 37, No. 3, pp. 551–554, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00

HISTAMINE H_2-RECEPTOR MEDIATED ACTIVATION OF NEONATAL RAT BRAIN ORNITHINE DECARBOXYLASE *IN VIVO**

JOSE RODRIGUEZ,†‡ ALFONSO TOLEDO, REGINA BRANDNER,‡ RICARDO RODRIGUEZ, JOSEFA SABRIA and ISAAC BLANCO

Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad Autónoma de Barcelona, 08193 Bellaterra, Barcelona, Spain

(*Received* 17 *May* 1987; *accepted* 3 *August* 1987)

Abstract—The effect of histamine (HA) administered via intracerebroventricular injection on ornithine decarboxylase (ODC) activity was studied in neonatal rat brain. The HA effect was dose and time dependent. Maximal increase in ODC activity was achieved 2 hr after administration of 10 μg HA (38% over control levels). Impromidine (HA H_2-agonist) mimicked the effect of HA on ODC and ranitidine (HA H_2-antagonist) inhibited the response to HA. Neither 2-thiazolylethylamine (HA H_1-agonist) nor mepyramine (HA H_1-antagonist) modified control ODC activity. The HA-releasers, compound 48/80 and polymixin B sulfate, elicited an increase in brain ODC activity of 35% and 32%, respectively, over the control value.

Ornithine decarboxylase (ODC, EC 4.1.1.17)§ catalyzes the conversion of ornithine into putrescine, the initial and rate-limiting reaction in the polyamine biosynthetic pathway (for review see Refs 1 and 2). Much evidence in the literature suggests that ODC activity is increased in normal, regenerative and neoplasic growth (for review see Refs 2 and 3). During brain ontogenetic development, ODC activity varies depending on the neuronal maturation state of the cerebral area studied [4–6]. Therefore, ODC activity is frequently used as a marker enzyme of neurogenesis (for review see Ref. 3).

Histamine (HA), acting via HA H_2-receptors, has been shown to be involved in cellular division and tissue developmental processes [7–11].

A rise in ODC and histidine decarboxylase (the HA synthesizing enzyme) activity have been described in tumor promotion and rapid cell proliferation [7, 12, 13]. Furthermore, Morris *et al.* have reported that HA administration induced ODC activity in neonatal rat brain [14].

The aim of this work is to study the effect of HA on neonatal rat brain ODC activity and to elucidate the HA receptor mediating this effect. The effect of mast cell HA on the activity of the enzyme has also been investigated. In the present study we have used 6 day-old rats because brain HA levels are highest around this age [15].

MATERIALS AND METHODS

Chemicals. L-[1-^{14}C]-ornithine hydrochloride (52.4 mCi/mmol) was purchased from New England Nuclear Corp. (Boston, MA). L-Ornithine hydrochloride, histamine, mepyramine, pyridoxal 5′-phosphate and compound 48/80 were obtained from Sigma Chemical Co. (St Louis, MO). Dithiothreitol was from Serva Feinbiochemica (Heidelberg, F.R.G.) Impromidine and 2-thiazolylethylamine (2-TEA) were kindly supplied by Smith Kline & French Ltd. (Welwyn Garden City, U.K.) Ranitidine was a generous gift from Lesvi Laboratories (Spain). All other reagents were of analytical grade.

Animals. Six day-old Sprague–Dawley rat pups of both sexes were used. At birth the pups were mixed and randomly distributed in groups of ten per lactating mother, keeping them in maternal care until used. Animals were kept under a normal sequence of 12 hr light and 12 hr dark periods. Food and water were available *ad libitum*.

ODC activity assay. After being treated, the rat pups were killed by decapitation and the brains were rapidly removed and homogenized in 10 vol. of ice-cold 25 mM Tris–HCl (pH 7.4 at 20°), containing 1 mM dithiothreitol and 0.1 mM EDTA. 50 μM pyridoxal 5′-phosphate was added to the homogenate before centrifugation at 30,000 *g* for 30 min at 4°. ODC activity of the supernatant was determined by measuring the release of $^{14}CO_2$ from L-[1-^{14}C]-ornithine, basically as described by Russell and Snyder [16]. The reaction mixture contained 25 μM L-ornithine, 0.25 μCi of L-[1-^{14}C]-ornithine and 500 μl of the 30,000 *g* supernatant fraction, in a total volume of 550 μl. The incubation was carried out in a shaking bath for 60 min at 37° in glass tubes equipped with

* This study was supported by a research grant from the Fondo de Investigaciones Sanitarias de la Seguridad Social (F.I.S.S.) and from the Comisión Asesora de Investigación Científica y Técnica (CAICYT).

† Author to whom all correspondence should be addressed.

‡ Recipients of fellowships from Ministerio de Educación y Ciencia.

§ Abbreviations used: ODC, ornithine decarboxylase; HA, histamine; i.c.v., intracerebroventricular; IMP, impromidine; RAN, ranitidine; 2-TEA, 2-thiazolylethylamine; MEP, mepyramine.

an internal reservoir containing 200 μl of hyamine hydroxide. The reaction was stopped by injecting 500 μl of 0.5 M H_2SO_4 through the rubber cap. After 30 min of additional shaking, to ensure complete absorption of $^{14}CO_2$, the content of each reservoir was transferred into vials containing 3 ml of Unisolve (Koch-Light Ltd. Haverhill, U.K.) for measurement of radioactivity in a Beckman LS800 scintillation spectrometer.

Protein content of the supernatant was measured by the method of Lowry *et al.* [17], using bovine serum albumin as a standard.

Enzymatic activity was expressed as nmoles CO_2/hr/g tissue.

Statistics. The data are presented as means ± SEM. The mean values were compared by analysis of variance (ANOVA). Differences between means were determined using the Student's *t*-test when each value had its own control value or the method of Scheffè when multiple comparisons between a group of means were made.

RESULTS

Histamine-dose effects on neonatal brain ODC activity

The dose–response curve obtained from six-day-old rats treated via intracerebroventricular (i.c.v.) injection with different HA doses (1, 2.5, 5, 10, 25, 50 μg) and killed after 2 hr, shows that a significant increase in brain ODC activity became apparent at 5 μg HA (Fig. 1). Maximal stimulation of enzyme activity was elicited with 10 μg HA and represents an increase of 38% over control ODC activity in neonatal rat brain. Higher doses of HA (25 or 50 μg) did not further increase ODC activity.

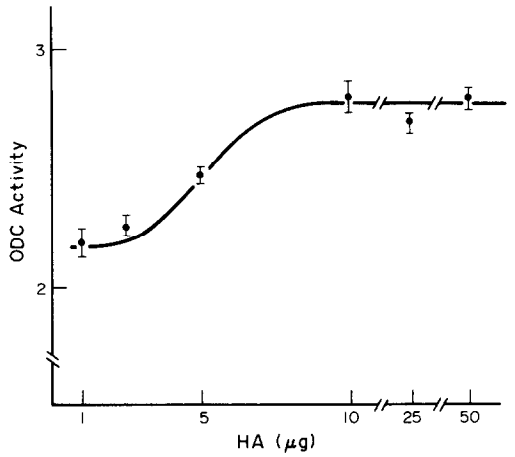

Fig. 1. Dose–response curve for the effect of histamine on brain ODC activity in neonatal rats. Various doses of HA, contained in 10 μl of saline (final pH 7.4), were injected i.c.v. into 6-day-old rats. Rats were killed 2 hr after being treated. Values are means ± SEM (N = 5). Each determination was done in triplicate. Results are expressed in nmoles CO_2/hr/g tissue. The control value of ODC activity was 2.17 ± 0.104 nmoles CO_2/hr/g tissue. Statistically significant differences ($P < 0.001$) became apparent at 5 μg of HA (Scheffè's test).

Time course of ODC activity in response to histamine

The time course of HA action on neonatal rat brain ODC activity is shown in Fig. 2. Administration of 10 μg HA did not elicit a significant increase in ODC activity before the first hour of treatment. Maximal activity was observed at 2 hr (35% over control levels) and declined gradually, reaching the control level at 6 hr.

Two way ANOVA of data indicated that the increase in the neonatal rat brain ODC activity was both dependent on time and on HA ($P < 0.001$), and significant interaction of time × HA ($P < 0.001$) was observed.

Effects of histaminergic drugs on neonatal rat brain ODC activity

In an attempt to identify the HA receptor mediating the increase of brain ODC activity in response to HA, several histaminergic drugs were tested. The

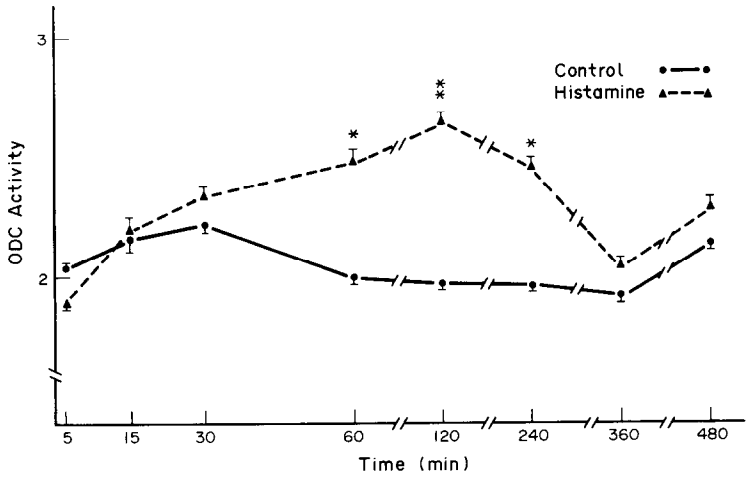

Fig. 2. Time course of the effect of histamine on cerebral ODC activity in neonatal rat. Six-day-old rats were treated via i.c.v. with 10 μg of HA contained in 10 μl of saline (final pH 7.4). Data show means ± SEM (N = 5–8). Results are expressed in nmoles CO_2/hr/g tissue. Each determination was done in triplicate. * $P < 0.05$, ** $P < 0.001$ vs control (Student's *t*-test).

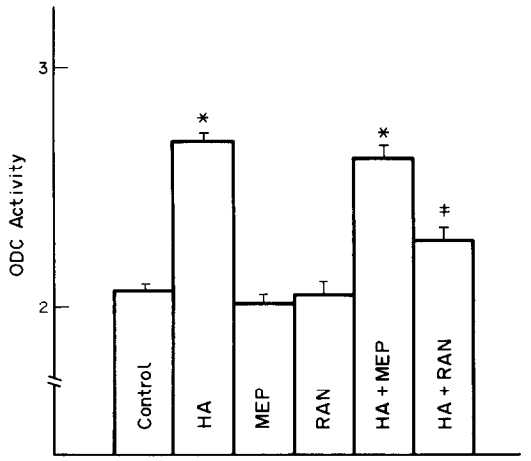

Fig. 3. Effects of histamine antagonists on histamine-induced ODC activity in neonatal rat brain. Six-day-old rats were injected via i.c.v. with HA, 10 μg; mepyramine (MEP), 20 μg; ranitidine (RAN), 25 μg. All drugs were contained in 10 μl of saline (final pH 7.4). After 2 hr, all animals were killed and cerebral ODC activity was determined in triplicate. Values are means ± SEM (N = 5). Control value = 2.06 ± 0.017 nmoles CO_2/hr/g tissue. Results are expressed in nmoles CO_2/hr/g tissue. *,‡ $P < 0.05$ vs control and HA respectively (Scheffè's test).

Table 1. Effects of histamine agonists on ODC activity in neonatal rat brain

	ODC activity	% Control
Control	2.13 ± 0.12	100
2-TEA	2.41 ± 0.09	113 ± 4
IMP	3.10 ± 0.05*	145 ± 2

Six-day-old pups were treated i.c.v. with 2-thiazolylethylamine (2-TEA) 24 μg or impromidine (IMP) 1 μg, both contained in 10 μl of saline (final pH 7.4), and killed 2 hr later. Data are means ± SEM (N = 4). Results are expressed in nmoles CO_2/hr/g tissue. Each determination was done in triplicate. * $P < 0.001$ vs control (Scheffè's test).

results of the pharmacological study are presented in Fig. 3 and Table 1. As shown in Fig. 3, ranitidine (an H_2 receptor antagonist) at a dose of 25 μg blocked the increase of neonatal brain ODC activity induced by an ICV administration of 10 μg HA. In contrast, the H_1 receptor antagonist mepyramine (20 μg), did not modify the HA effect. Neither H_1 nor H_2 receptor antagonists alone affected control ODC activity. On the other hand, 1 μg impromidine (a potent H_2 receptor agonist) mimicked the effect of HA, whereas the H_1 receptor agonist 2-TEA (24 μg), did not modify control ODC activity (Table 1).

These results indicate that HA effect on brain ODC activity is mediated by the H_2 receptor.

Effects of HA-releasers on neonatal brain ODC activity

In order to determine whether mast cell HA was able to elicit an increase in neonatal rat brain ODC activity, animals were treated i.c.v. with HA-releasers (compound 48/80 or polymixin B sulfate). We found that the administration of compound 48/80 (5 μg) or polymixin B (1 μg) caused a rise in control brain ODC activity of 35% and 32% respectively (Table 2).

Table 2. Effects of HA-releasers on brain ODC activity in neonatal rats

	ODC activity	% Control
Control	2.05 ± 0.08 (5)	100
Compound 48/80	2.77 ± 0.04 (5)*	135 ± 2
Control	2.09 ± 0.01 (3)	100
Polymixin B	2.76 ± 0.01 (3)*	132 ± 1

Six-day-old rats were treated i.c.v. with compound 48/80 (5 μg) or polymixin B sulfate (1 μg) contained in 10 μl of saline (final pH 7.4) and killed 2 hr later. Data are means ± SEM (N). Results are expressed in nmoles CO_2/hr/g tissue. Each determination was done in triplicate. * $P < 0.001$ vs control (Student's t-test).

DISCUSSION

The present study shows that HA, administered i.c.v., caused an increase in neonatal rat brain ODC activity, its effects being both dose and time dependent.

It has been suggested that the HA H_1 receptor could be involved in ODC activation by HA [14], but our results, using HA antagonists and agonists, provide strong evidence that the HA effect is mediated by H_2 receptors. It is known that the primary action of H_2 receptor stimulation is to increase the intracellular concentration of cyclic AMP [18, 19] and in several situations it has been reported that a rise in cyclic AMP levels is linked to an increase in ODC activity [20–22]. We suggest that the HA effect on brain ODC activity could be mediated by an increase in cyclic AMP levels which may cause an activation of a cyclic AMP dependent protein kinase. This kinase has been reported to mediate in ODC activation [23, 24]. Further work is in progress to test this hypothesis.

Several authors have reported the existence of two different HA pools in rat brain: neuronal and mast cell pools [25–27]. Several studies indicate that brain mast cells contained the major part of the HA found in neonatal rat brain [15, 25]. Moreover, mast cell HA has been associated with cell proliferation [11, 28, 29] and, in some situations, the HA effects on cellular development are mediated by H_2-receptors [7, 9–11]. ODC activity is involved in brain development, and the fact that i.c.v. administration of HA releasers elicited a rise in neonatal rat brain ODC activity suggest that mast cell HA could contribute to brain cellular proliferation through an effect on brain ODC activity.

Acknowledgements—We thank Ms I. Farinas for her help with the preparation of the manuscript.

REFERENCES

1. C. W. Tabor and H. Tabor, *A. Rev. Biochem.* **55**, 749 (1984).
2. D. H. Russell, *Drugs Metab. Rev.* **16**, 1 (1985).

3. T. A. Slotkin and J. Bartolome, *Brain Res. Bull.* **17**, 307 (1986).
4. G. Morris, J. V. Nadler, C. B. Nemeroff and T. A. Slotkin, *Biochem. Pharmac.* **34**, 3281 (1985).
5. T. R. Anderson and S. M. Schanberg, *Biochem. Pharmac.* **24**, 495 (1975).
6. J. M. Bell, W. L. Whitmore and T. A. Slotkin, *Neuroscience* **17**, 399 (1986).
7. J. Bartholeyns and M. Bouclier, *Cancer Res.* **44**, 639 (1984).
8. P. J. M. Tutton and D. H. Barkla, *Cell. Biol. Int. Rep.* **2**, 199 (1978).
9. K. Norrby, *Virchows Arch. B Cell Path.* **34**, 13 (1980).
10. J. O. Armitage and R. D. Sidner, *Lancet* **1**, 882 (1979).
11. K. Norrby, *Agents Actions* **16**, 287 (1985).
12. T. Watanabe, Y. Tagushi, K. Sasaki, K. Tsuyama and Y. Kitamura, *Biochem. biophys. Res. Commun.* **100**, 427 (1981).
13. J. Bartholeyns and J. R. Fozard, *Trends Pharmac. Sci.* **6**, 123 (1985).
14. G. Morris, F. J. Seidler and T. A. Slotkin, *Life Sci.* **32**, 1565 (1983).
15. I. Ferrer, F. Picatoste, E. Rodergas, A. Garcia, J. Sabria and I. Blanco, *J. Neurochem.* **32**, 587 (1979).
16. D. H. Russell and S. H. Snyder, *Proc. natn. Acad. Sci. U.S.A.* **60**, 1420 (1968).
17. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
18. S. Kakiuchi and T. W. Rall, *Molec. Pharmac.* **4**, 379 (1968).
19. L. B. Hough and J. P. Green, in *Handbook of Neurochemistry*, Vol. 6 (Ed. A. Lajtha) p. 145. Plenum Press, New York (1984).
20. C. V. Byus and D. H. Russell, *Science* **187**, 650 (1975).
21. U. Bachrach, *Proc. natn. Acad. Sci. U.S.A.* **72**, 3087 (1975).
22. J. B. Gibbs, C. Y. Hsu, W. L. Terasaki and G. Brooker, *Proc. natn. Acad. Sci. U.S.A.* **77**, 995 (1980).
23. C. V. Byus, M. K. Haddox and D. H. Russell, *J. Cyclic Nucleotide Res.* **4**, 45 (1978).
24. C. V. Byus and D. H. Russell, *Biochem. Pharmac.* **25**, 1595 (1976).
25. F. Picatoste, I. Blanco and J. M. Palacios, *J. Neurochem.* **29**, 735 (1977).
26. M. P. Martres, M. Baudry and J. C. Schwartz, *Brain Res.* **83**, 261 (1975).
27. I. Blanco, E. Rodergas, J. M. Palacios and F. Picatoste, *Agents Actions* **7**, 106 (1977).
28. L. Franzen and K. Norrby, *Cell Tissue Kinet.* **13**, 635 (1980).
29. J. J. Nordlund and P. W. Askenase, *J. invest. Dermatol.* **81**, 28 (1983).