INVOLVEMENT OF HYPOPHYSIS IN THE STIMULATION OF LIVER ORNITHINE DECARBOXYLASE BY DIBUTYRYL CYCLIC AMP

Terho ELORANTA and Aarne RAINA

Department of Biochemistry, University of Kuopio, P.O. Box 138, SF-70101 Kuopio 10, Finland

Received 13 May 1975

1. Introduction

We have reported earlier that the administration of the dibutyryl derivative of cyclic AMP or theophylline to normal rats results in a sharp increase in the liver ornithine decarboxylase activity [1,2]. This effect, also noticed by Beck et al. [3], was abolished by inhibitors of RNA and protein synthesis, but not by adrenalectomy [1,2]. As discussed earlier [2], several mechanisms may operate in the stimulation of liver ornithine decarboxylase activity by the nucleotide in intact rats. The present results indicate that an intact hypophysis is needed for the stimulation. Furthermore, it was observed that chlorpromazine and vasopressin are potent stimulators of this enzyme in the rat liver.

2. Materials and methods

Where not otherwise indicated, the animals used were female Wistar rats weighing 110–140 g. Hypophysectomized female Sprague-Dawley rats were supplied by the Charles River Breeding Laboratories, Inc., Wilmington, Mass. Hypophysectomy was performed 11 to 13 days before use. All animals were deprived of food for 12 to 14 hr before sacrifice and were killed between 8 and 10 a.m. to avoid diurnal variations in ornithine decarboxylase activity.

$N^6,O^{2'}$-Dibutyryl cyclic AMP (dibutyryl cAMP), synthetic arginine vasopressin and synthetic lysine vasopressin were purchased from Sigma. Chlorpromazine chloride was supplied by Oy Medikalis Ab, Helsinki. Propranolol chloride (Inderal®) was obtained through Imperial Chemical Industries (ICI). L-Dopa (L-3,4-dihydroxyphenylalanine) was a product of E. Merck, Darmstadt. DL-Ornithine-1-^{14}C (spec.act. 2.74 mCi/mmol) was purchased from the New England Nuclear Corporation. Before use it was treated with acid as described earlier [2].

Ornithine decarboxylase activity was assayed from the 100 000 *g* supernatant fraction of a liver homogenate as described earlier [2], using 1 mM L-ornithine-1-^{14}C as the substrate. None of the above-mentioned drugs had any effect on the enzyme activity, when added directly to the assay mixture. The experimental groups were compared by means of the t-test.

3. Results and discussion

Our previous results indicated that the stimulation of liver ornithine decarboxylase activity by dibutyryl cAMP in intact rats represented synthesis de novo of the enzyme, and that the stimulation was probably not mediated by adrenal cortical hormones [2]. It has been reported that the administration of dibutyryl cAMP can release several hormones, such as insulin, glucagon and growth hormone [4], which all are able to increase liver ornithine decarboxylase activity, see [5,6]. To evaluate the role of growth hormone, a very potent inducer of liver ornithine decarboxylase [6], in mediating the nucleotide effect, several other drugs able to release growth hormone in anesthetized rats [7] were tested. As shown in table 1, arginine and lysine vasopressins markedly raised the ornithine decarboxylase activity both in normal and in adrenalectomized rats. This effect could not be abolished by a simultaneous administration of propranolol (400 μg/100 g), a β-adrenergic blocking agent, which has been reported to inhibit growth hormone release by

Table 1
Stimulation of liver ornithine decarboxylase by vasopressins in normal and adrenalectomized rats

Treatment	Ornithine decarboxylase (pmoles/mg protein per 30 min) Unoperated	Adrenalectomized
Saline	70 ± 19	49 ± 10
Arg-vasopressin	384 ± 115[2]	260 ± 252
Lys-vasopressin	1125 ± 311[2]	1194 ± 175[2]

[1] $p < 0.05$; [2] $p < 0.01$ The significance of the differences as compared with the saline-treated controls.
Vasopressins (400 mU/100 g body weight) were injected intraperitoneally in 0.5 ml of 0.9% NaCl at 3.5 hr before killing. Adrenalectomy had been performed 10 days earlier. The values are means ± S.D. of four to five animals.

Table 2
Effect of chlorpromazine on liver ornithine decarboxylase activity in intact rats

Time of treatment (hr)	Ornithine decarboxylase pmoles $^{14}CO_2$/mg prot per 30 min
3 (saline)	18 ± 8
2	87 ± 36[2]
3	121 ± 68[2]
4	160 ± 74[2]
5	223 ± 155[1]
6	149 ± 103[1]

[1] $p < 0.05$; [2] $p < 0.01$ The significance of the differences as compared with the saline-treated controls.
Chlorpromazine (2 mg/100 g body weight) in 0.5 ml of 0.9% NaCl was injected intraperitoneally. The values are means ± S.D. of five animals.

arginine vasopressin in anesthetized rats [7]. Propranolol alone had no effect on liver ornithine decarboxylase activity (data not shown). Table 2 shows that also chlorpromazine treatment increased ornithine decarboxylase activity in the liver. L-Dopa (1 mg/100 g), which is reported to inhibit the chlorpromazine-stimulated release of growth hormone [7], neither changed the basal level of liver ornithine decarboxylase activity nor prevented the stimulation of the enzyme activity by chlorpromazine (data not shown).

The results obtained with vasopressins and chlorpromazine are in line with the possibility that the stimulation of liver ornithine decarboxylase by dibutyryl cAMP in intact rats may be mediated through the growth hormone release. We therefore studied the effect of the cyclic nucleotide derivative on ornithine decarboxylase activity in rats hypophysectomized 11 to 13 days earlier. As shown in table 3, dibutyryl cAMP had no effect on the liver ornithine decarboxylase activity in hypophysectomized rats, although it significantly increased this enzyme activity in unoperated animals 4 hr after the administration. Also the difference in ornithine decarboxylase

Table 3
Effect of dibutyryl cyclic AMP (DBcAMP) on liver ornithine decarboxylase activity in normal and hypophysectomized rats

Treatment	Time of analysis (hr)	Ornithine decarboxylase pmoles $^{14}CO_2$/mg prot per 30 min Unoperated	Hypophysectomized
Saline	4	50 ± 16	22 ± 10
DBcAMP	4	506 ± 369[1]	26 ± 2
DBcAMP	6	58 ± 21	30 ± 2

[1] $p < 0.05$ The significance of the differences as compared with the saline-treated controls.
DBcAMP (5/mg per animal) was given intraperitoneally in 0.5 ml of 0.9% NaCl. At the same time of analysis the body weight was 80–115 g for hypophysectomized animals and 120–160 g for unoperated controls. The values are means of 3 to 5 animals in each group.

activities between the unoperated controls and the hypophysectomized group was statistically significant at 4 hr after the treatment ($p < 0.05$). We therefore conclude that intact hypophyses are needed for the stimulation. Our results are at variance with those reported by Richman et al. [8], who observed increases in the ornithine decarboxylase activities in the livers, kidneys, and adrenals of hypophysectomized rats after treatment with the nucleotide derivative. It appears, however, that in the latter study dibutyryl cAMP was given immediately after hypophysectomy, which might explain differing results. This inference is also in line with the report of Levine et al. [9], demonstrating a progressive fall in the responsiveness of adrenal ornithine decarboxylase to ACTH with increasing time after hypophysectomy.

A number of different stimuli, including several peptide and steroid hormones, are able to enhance ornithine decarboxylase activity in specific target tissues. We have earlier speculated on the possibility that all these agents capable of inducing ornithine decarboxylase might have a common mediator, e.g. cyclic AMP, because treatment with several hormones can increase the level of intracellular cyclic cAMP, and because exogenously administered dibutyryl cAMP is able to stimulate ornithine decarboxylase activity [1,6]. A similar suggestion has also been presented by Beck et al. [3]. Some recent observations, e.g. a stimulation of ornithine decarboxylase activity by exogenous cyclic AMP or its dibutyryl derivative in the oviduct of *Coturnix* quail [10], in the cultured mammary tissue of mice [11], and in BHK cells in cultures [12], could be taken as additional support of the above hypothesis. A role for a cyclic AMP-dependent protein kinase in the regulation of orinthine decarboxylase activity has also been suggested by Byus and Russell [13]. However, estradiol given in vivo has been shown to increase ornithine decarboxylase activity in the rat uterus without changing the concentration of cyclic AMP [14]. Furthermore, the addition of serum or insulin to cell cultures is able to stimulate ornithine decarboxylase, but it decreases the level of cyclic AMP [12]. Large doses of growth hormone have been shown to enhance the adrenal ornithine decarboxylase activity without elevating cyclic AMP levels [8]. These latter observations, taken together with the present results obtained with hypophysectomized rats, render it unlikely that cyclic AMP would act as a common mediator in the hormonal control of orithine decarboxylase activity. A possible role of cyclic GMP in this process remains to be elucidated.

In the light of the present results it appears that exogenous cyclic AMP given to intact rats may induce ornithine decarboxylase by releasing some pituitary factor(s), e.g. growth hormone. On the other hand, some hypophyseal factor(s) may, directly or indirectly, control the synthesis of some undetermined cellular component(s) (e.g. protein kinase) possibly involved in the stimulation of ornithine decarboxylase activity.

Acknowledgements

This work was supported by grants from the Sigrid Jusélius Foundation and from the National Research Council for Medical Sciences, Finland.

References

[1] Hölttä, E. and Raina, A. (1971) Scand. J. Clin. Lab. Invest, Suppl. 116, 54.
[2] Hölttä, E. and Raina, A. (1973) Acta Endocrinol. (Copenhagen) 73, 794–800.
[3] Beck, W. T., Bellantone, R. A. and Canellakis, E. S. (1972) Biochem. Biophys. Res. Commun. 48, 1649–1655.
[4] Hertelendy, F., Todd, H., Peake, G. T., Machlin, L. J., Johnston, G. and Pounds, G. (1971) Endocrinology 89, 1256–1262.
[5] Panko, W. P. and Kenney, F. T. (1971) Biochem. Biophys. Res. Commun. 43, 346–350.
[6] Raina, A. and Hölttä, E. (1972) in: Growth and Growth Hormone (Pecile, A. and Müller, E. F., eds.), pp. 143–149, Excerpta Medica, Amsterdam.
[7] Kato, Y., Dupre, J. and Beck, J. C. (1973) Endocrinology 93, 135–146.
[8] Richman, R., Dobbins, C., Voina, S., Underwood, L., Mahaffee, D., Gitelman, H. J., Van Wyk, J. and Ney, R. L. (1973) J. Clin. Invest. 52, 2007–2015.
[9] Levine, J. H., Nicholson, W. E., Liddle, G. W. and Orth, D. N. (1973) Endocrinology 92, 1089–1095.
[10] Preslock, J. P. and Hampton, J. K., Jr. (1973) Am. J. Physiol. 225, 903–907.
[11] Aisbitt, R. P. G. and Barry, J. M. (1973) Biochim. Biophys. Acta 320, 610–616.
[12] Hogan, B., Shields, R. and Curtis, D. (1974) Cell 2, 229–233.
[13] Byus, C. V. and Russell, D. H. (1974) Life Sci. 15, 1991–1997.
[14] Zor, U., Koch, Y., Lamprecht, S. A., Ausher, J. and Lindner, H. R. (1973) J. Endocrinol. 58, 525–533.