Biochemical Pharmacology, Vol. 20, pp. 2187–2190. Pergamon Press, 1971. Printed in Great Britain

AROMATIC L-AMINO ACID DECARBOXYLASE IN CALCITONIN-PRODUCING CELLS*

R. HÅKANSON, CH. OWMAN and F. SUNDLER

Departments of Pharmacology, Anatomy and Histology, University of Lund, Lund, Sweden

(*Received* 21 *December* 1970; *accepted* 2 *February* 1971)

Abstract—A common feature of the calcitonin-producing cells (C cells) is their capacity to produce and store arylethylamines. The activity of aromatic L-amino acid decarboxylase (DOPA/5-HTP decarboxylase) was measured radiometrically in the thyroid glands of various species and in the ultimobranchial gland of the chicken. The enzyme activity was well correlated with the number of amine-containing C cells, demonstrated by fluorescence microscopy in these tissues. The ultimobranchial gland had a conspicuously high activity of aromatic amino acid decarboxylase. The follicular cells of the thyroid appeared to have no or only a very low activity of this enzyme.

THE POLYPEPTIDE hormone calcitonin is produced in the parafollicular cells (C cells) of the mammalian thyroid.[1] In birds on the other hand, calcitonin is produced in a separate organ, the ultimobranchial gland.[2] A common feature of the calcitonin-producing cells is their capacity to produce and store arylethylamines.[3–5] In some species, such as sheep, goat and horse, the C cells store 5-HT; in the chicken, the calcitonin-producing cells store dopamine. In other species, such as the mouse, rat, guinea-pig and rabbit, histochemically detectable amines are absent from these cells, although they are capable of producing and storing arylethylamines, such as 5-HT or dopamine, if the immediate precursor amino acid is supplied. The formation of such arylethylamines is catalyzed by aromatic L-amino acid decarboxylase, also referred to as DOPA/5-HTP decarboxylase. There is strong evidence from histochemical studies that DOPA decarboxylase occurs in the C cells of the mouse[6,7] and it has been suggested that the enzyme is a common constituent of all C cells, regardless of species.[3–5] So far, direct chemical evidence for the presence of aromatic amino acid decarboxylase has been obtained only for the bovine thyroid,[8] and the distribution of the enzyme between the various cellular elements of the thyroid gland is not known. The present study was performed with the purpose to compare the DOPA decarboxylase activity of the thyroid of different species and to establish the cellular localization of the enzyme in the gland.

MATERIAL AND METHODS

Animals. The study was performed on adult animals, except chicken (6–8 weeks old, weighing 300–400 g). The thyroid glands were dissected out and freed from adjacent connective tissue. Material from sheep and pigs was obtained from the slaughterhouse, within 30 min after death.

* Abbreviations used: 5-HT, 5-hydroxytryptamine; 5-HTP, 5-hydroxytryptophan; DOPA, 3,4-dihydroxyphenylalanine.

Enzyme assay. Thyroid lobes from seven to ten mice or from two rats were pooled for each enzyme assay. From rabbit, guinea-pig, cat and chicken, one thyroid lobe was taken, while in sheep and pig only the central part of the thyroid lobe was used for the assay. The ultimobranchial glands of three chickens were pooled for each determination. In an attempt to establish the regional distribution of DOPA decarboxylase activity within a thyroid lobe of the mouse, the lobe was divided in three parts of similar size: one cranial, one central and one caudal. Tissue from nine mice were pooled for each determination. Sympathectomy was performed on nine mice by bilateral removal of the superior cervical ganglion 1 week before sacrifice, followed by determination of the thyroid DOPA decarboxylase activity.

The DOPA decarboxylase activity was measured radiometrically.[9] The tissues were homogenized in 0·1 M phosphate buffer, pH 7·0, to a final concentration of 100 mg (wet weight) per ml. After centrifugation at 10,000 *g* for 15 min, 0·1-ml aliquots of the supernatant were incubated with 400 nmoles of 1-^{14}C-DL-DOPA (0·2 mc/mM; New England Nuclear) in the presence of 5 nmoles of pyridoxal-5′-phosphate and 25 nmoles of glutathione. The final incubation volume was made up to 0·5 ml with the phosphate buffer. The $^{14}CO_2$ produced in the reaction was released by acidification, trapped on a filter paper immersed in Digestin^{R}, and quantitated by liquid scintillation counting. Enzyme activity was expressed as nmoles $^{14}CO_2$ produced per mg tissue (wet weight) and hr. Determinations of enzyme activity were made in duplicate.

Cell counting. For the cell counting, thyroid tissue from the mouse, rat, guinea-pig, rabbit, cat, pig and chicken was incubated for 0·5 hr at 37° in Tyrode's solution in the presence of L-DOPA (50 μg/ml), washed in Tyrode's solution for 10 min, and processed for fluorescence microscopy of arylethylamines according to the method of Falck and Hillarp.[10–13] As a result of the incubation, the C cells emitted a strong green, cytoplasmic, formaldehyde-induced fluorescence.[6] Sheep thyroid and chicken ultimobranchial gland were also processed for fluorescence histochemistry, but incubation with L-DOPA was not necessary since the calcitonin-producing cells of these species store large amounts of histochemically demonstrable 5-HT and dopamine, respectively.[5,14]

The specimens were sectioned at 6 μ thickness and cell counting was performed at 312·5 × magnification (eye-piece 12·5 ×, objective 25 ×). The number of fluorescent C cells were counted in ten randomly chosen visual fields from different regions of the thyroids and the ultimobranchial gland. The results were expressed as the mean number of cells per visual field calculated from specimens of five animals of each species.

For the correlation of DOPA decarboxylase activity with the regional distribution of the C cells in the mouse thyroid, each lobe was divided in three parts (see above), and after incubation with L-DOPA, cell counts were made on sections from each of these parts separately.

RESULTS AND DISCUSSION

The C cells of the mouse thyroid have an uneven distribution, being concentrated to the center of the glandular lobes. This characteristic arrangement in the mouse, in contrast to other species, makes it possible to correlate the enzyme distribution with that of the C cells. The regional distribution of thyroid DOPA decarboxylase activity closely paralleled the distribution of the C cells (Fig. 1).

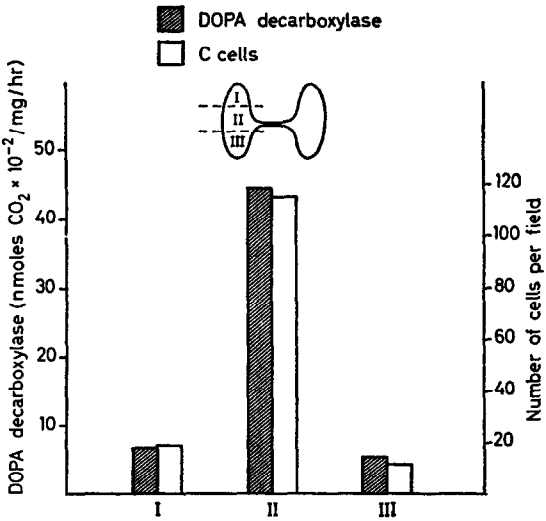

FIG. 1. Distribution of C cells with formaldehyde-induced fluorescence (after incubation with L-DOPA) and DOPA decarboxylase activity in the mouse thyroid. Roman numerals refer to the various regions tested. Three determinations.

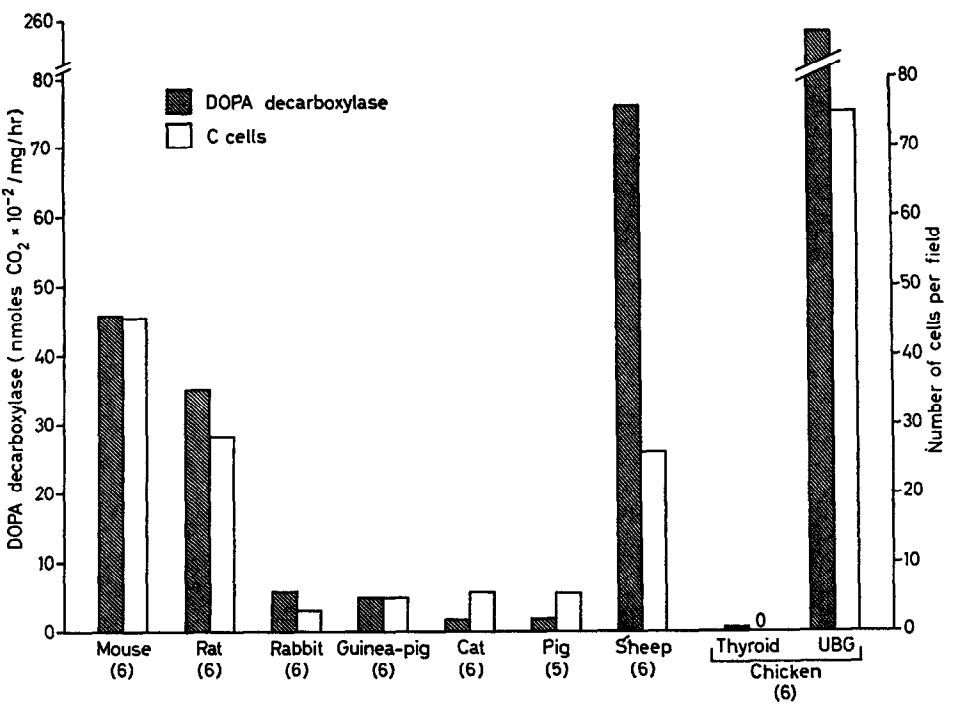

FIG. 2. The number of fluorescent C cells as correlated with the DOPA decarboxylase activity of the thyroid of various mammals and of the ultimobranchial gland (UBG) of the chicken. Note that the two species having endogenous, histochemically detectable amines in the C cells (sheep and chicken) have conspicuously higher enzyme activity. Number of determinations in parenthesis.

Sympathetic denervation of the mouse thyroid failed to affect the DOPA decarboxylase activity. The enzyme activity after denervation (40×10^{-2} nmoles/mg/hr) was not significantly different from that of the controls (45×10^{-2} nmoles/mg/hr). This indicates that in the mouse thyroid, neuronal DOPA decarboxylase is quantitatively insignificant.

The thyroid DOPA decarboxylase activity varied markedly from one species to another (Fig. 2). From counting the C cells in the thyroid gland of various species it could be established that the enzyme activity and the number of C cells in general showed a remarkable agreement. In the chicken, calcitonin-producing cells and thyroid follicular cells occur in separate organs; the dopamine-containing C cells make up the ultimobranchial gland.[5] The DOPA decarboxylase activity of the chick thyroid was almost non-measurable, whereas the ultimobranchial gland had the highest enzyme activity registered. Taken together, these observations suggest that the DOPA decarboxylase of the mammalian thyroid is confined to the calcitonin-producing cells. The finding of a considerable DOPA decarboxylase activity in these cells supports the assumption[6] that the green, cytoplasmic, formaldehyde-induced fluorescence observed after incubation with L-DOPA, is in part, if not entirely, due to the presence of dopamine. The results also suggest that the C cells of different species have somewhat differing DOPA decarboxylase activities. Thus, the C cells of the sheep and chicken, normally storing 5-HT[14] or dopamine,[5] have a higher enzyme activity than those which are devoid of such arylethylamines. This agrees with previous observations on endocrine cells in the gastrointestinal tract, in which the 5-HT-containing enterochromaffin cells of the mammalian gastric mucosa have been found to be rich in DOPA decarboxylase.[15,16] By comparison, gastric "enterochromaffin-like" cells, which lack 5-HT but have the capacity to produce and store arylethylamines if the precursor is supplied, have considerably lower enzyme activity.[15,16]

In conclusion, the C cells are conspicuously rich in aromatic amino acid decarboxylase, while the follicular cells of the thyroid appear to have no or only a low activity of this enzyme. In certain species the C cells store 5-HT or dopamine; these C cells contain more enzyme than the C cells which lack histochemically detectable arylethylamines.

REFERENCES

1. G. BUSSOLATI and A. G. E. PEARSE, *J. Endocr.* **37**, 205 (1967).
2. P. F. HIRSCH and P. L. MUNSON, *Physiol. Rev.* **49**, 548 (1969).
3. B. FALCK and CH. OWMAN, *Adv. Pharmac.* **6**, 211 (1968).
4. A. G. E. PEARSE, *Proc. R. Soc.* B. **170**, 71 (1968).
5. S. ALMQVIST, E. MALMQVIST, CH. OWMAN, M. RITZÉN, F. SUNDLER and G. SWEDIN, *Gen. Comp. Endocr.* in press (1971).
6. B. LARSON, CH. OWMAN and F. SUNDLER, *Endocrinology* **78**, 1109 (1966).
7. A. R. SCHULZ and L. OLINER, *Life Sci.* **6**, 873 (1967).
8. CH. OWMAN and F. SUNDLER, In *Calcitonin. Proceedings of the Symposium on Thyrocalcitonin and the C cells.* (Ed. S. TAYLOR) p. 110, Heinemann, London (1968).
9. R. HÅKANSON, *Acta physiol. scand. Suppl.* **340**, 1 (1970).
10. B. FALCK, *Acta physiol. scand.* **56**, 1 (1962).
11. B. FALCK, N.-Å. HILLARP, G. THIEME and A. TORP, *J. Histochem. Cytochem.* **10**, 348 (1962).
12. H. CORRODI and G. JONSSON, *J. Histochem. Cytochem.* **15**, 65 (1967).
13. B. FALCK and CH. OWMAN, *Acta Univ. Lund. II* **7**, 1 (1965).
14. B. FALCK, B. LARSON, C. v. MECKLENBURG, E. ROSENGREN and K. SVENAEUS, *Acta physiol. scand.* **62**, 491 (1964).
15. D. AURES, R. HÅKANSON and A. SCHAUER, *Europ. J. Pharmac.* **3**, 217 (1968).
16. R. HÅKANSON, CH. OWMAN, N.-O. SJÖBERG and B. SPORRONG, *Histochemie* **21**, 189 (1970).