



Fig. 2. *Epimys norv. var. albina* (Erxl.). a) Animal sacrificed 17 days after removal of left tensor tympani muscle. Ipsilateral mesencephalic nucleus of the trigeminal: chromatolytic nerve cell. b) Animal sacrificed 17 days after removal of left tensor tympani muscle. Contralateral nucleus: chromatolytic nerve cell. Toluidine blue. $\times 600$.

tympani neuromuscular devices were not always present. It is also felt that the absence of nuclear chromatolytic cells should not be considered as a negative finding, since it agrees with the observation of cell migration along the nerve serving the muscle of the malleus¹. The very small number of first-order neurons on the tensor tympani proprioceptive pathways, coupled with such migration, offers a satisfactory explanation of their absence in the central nucleus.

The present study has shown that first-order sensory neurons are situated in the ipsilateral and, though to a lesser degree, in the contralateral mesencephalic nucleus. This result agrees with experimental⁶ and electrophysiological⁷ findings concerning the proprioceptive innervation of the masticatory muscles in the cat.

This demonstration of crossed proprioceptive innervation of the tensor tympani is of practical importance in that it suggests the anatomical basis of a reflex mechanism in the bilateral control of acoustic transmission apparatus adaptability⁸.

Résumé. Chez le rat, *Epimys norv. var. albina* (Erxl.), l'excision unilatérale du muscle du marteau provoque l'apparition de la chromatolyse dans des cellules du noyau de la racine mésencéphalique du trijumeau homo- et contro-latéral, ce qui démontre l'innervation sensitive (probablement proprioceptive) croisée.

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⁶ S. H. DAULT and R. DALE SMITH, *Anat. Rec.* 165, 79 (1969).

⁷ R. DALE SMITH, H. Q. MARCIARIAN and W. T. NIEMER, *J. comp. Neurol.* 133, 495 (1968).

⁸ This work was supported by the Italian Council for Scientific Research (CNR).

Tyrosine Hydroxylase Activity in a Transplantable Islet Cell Tumour of Golden Hamster

A transplantable islet cell tumour in golden hamster originally described by KIRKMAN¹ has recently been found to contain dopa, dopamine, and 5-hydroxytryptamine². Furthermore, enzymatic analyses have shown that tumour cells contain dopadecarboxylase and monoamine oxidase activity³.

To investigate whether the tumour cells also contain an enzyme system catalyzing the conversion of tyrosine to dopa, the following study has been undertaken.

The islet cell tumour was transplanted s.c. to adult golden hamsters of both sexes, and the tumours were allowed to grow for 4-12 weeks. The tumours of 22 animals were analyzed chemically, as can be seen in the Table. To study the tyrosine hydroxylase activity the tumour was gently homogenized in sucrose and then incubated in the presence of L-tyrosine ¹⁴C(U) 80 min as described by NAGATSU et al.⁴. The catechol derivatives present in the incubation medium were isolated on Al₂O₃ according to ANTON and SAYRE⁵, and the labelled catechol derivatives formed during the incubation were finally estimated in a scintillation spectrometer. In each experiment blank values were obtained in the same way as

described above with the exception that the homogenized tissue was boiled for 5 min.

To differentiate between tyrosine hydroxylase and tyrosinase activities⁴ inhibitors or activators of one of these enzymes were added to the incubation medium. Tetrahydrofolic acid was used as activator of tyrosine hydroxylase and 2,2'-bipyridyl and H22/54 (α -propyl-dopacetamide) as inhibitors of tyrosine hydroxylase. Thiourea was used as a tyrosinase inhibitor⁵. The dopa decarboxylase inhibitor (m-hydroxybensylhydrazine)

¹ H. KIRKMAN, *Stanford med. Bull.* 20, 163 (1962).

² L. CEGRELL, B. FALCK and A.-M. ROSENGREN, *Acta physiol. scand.* 77, 23 (1969).

³ L. CEGRELL, B. FALCK and A.-M. ROSENGREN, *Experientia* 25, 969 (1969).

⁴ T. NAGATSU, M. LEVITT and S. UDENFRIEND, *J. biol. Chem.* 239, 2910 (1964).

⁵ A. ANTON and D. SAYRE, *J. Pharm. exp. Ther.* 145, 326 (1964).

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NSD 1015⁷ was also added to the incubation mixture in some experiments. For further identification of the products formed during the incubation, paper chromatographic analyses were performed in phenol: 0.1N HCl (90:10)⁸. The chromatograms were then counted in a strip counter.

The presence of the endogenous dopamine metabolites, 3,4-dihydroxyphenyl acetic acid (DOPAC) and 3-methoxy-4-hydroxyphenylacetic acid (HVA), in the insuloma was determined in 3 and 9 islet cell tumours, respectively. HVA was determined according to ANDÉN et al.⁹ and further identified by electrophoresis¹⁰ and DOPAC was determined according to ROSENGREN¹¹.

The enzymatic activity converting tyrosine to catechol derivatives under the experimental conditions used was in the tumour $20 \text{ nmol} \times \text{g}^{-1} \times \text{hr}^{-1}$, which was calculated on the observation that the tumour contained 35.6 mg tyrosine per g (tyrosine was determined in 4 tumours according to WAALKES and UDENFRIEND¹²; the tumour contained 29.1–40.8 mg/g). Tetrahydrofolic acid increased the enzyme activity, whereas 2,2'-bipyridyl and H22/54 decreased it. No certain effect was obtained when the tumour was incubated with thiourea. The decreasing by NSD 1015 of further metabolism of labelled catechol derivatives into substances not detectable with the above-mentioned method caused an accumulation of labelled material.

When the Al_2O_3 eluate was chromatographed and the papers scanned in a strip counter, a large peak appeared at the position of authentic dopamine. The presence of NSD 1015 in the incubation medium resulted in one more peak corresponding to dopa. In 3 experiments, 2 additional small peaks were obtained; 1 had the same Rf-value as DOPAC, the identity of the second is not known.

The content of HVA in the tumours varied from 0.17 to 0.68 $\mu\text{g/g}$, the mean value being 0.42 $\mu\text{g/g}$. The presence of HVA in the islet cell tumours was confirmed in an electrophoretic study. The concentration of DOPAC in the tumour was between 0.28 and 0.54 $\mu\text{g/g}$.

The enzyme catalyzing the conversion of tyrosine to catechol derivatives in the islet cell tumour is probably tyrosine hydroxylase and not tyrosinase, since there is an inhibition of the activity by 2,2'-bipyridyl and H22/54; thiourea, known to inhibit tyrosinase, did not affect the enzyme activity. This view is further strengthened by the fact that tetrahydrofolic acid increased the activity. The accumulated data in this and a previous report³ indicate that the dopa and the dopamine found in the tumour² have been synthesized by the tumour cells. The occurrence of tyrosine hydroxylase in the tumour is of particular interest, as it is the rate limiting enzyme in the biosynthesis of catecholamines. It has

earlier been found only in brain, adrenal medulla, and sympathetically innervated organs⁴. No adrenergic nerves have been observed in the tumour².

The activity of tyrosine hydroxylase in the tumour seems to be high, even higher than that found in brain and adrenal medulla⁴. In a recent study³ a high dopadecarboxylase activity has been found in the tumour. Thus, in this tissue all the components are available which are necessary for a rapid synthesis of dopamine.

The islet cell tumour was originally found by KIRKMAN¹ in the pancreas of a golden hamster. The morphological similarity with human insuloma¹³ and the presence of insulin in the tumour¹⁴ have further supported the view that the tumour was derived from the pancreatic islet tissue. In the islet cells of many mammalian species, a storage of dopamine has been demonstrated, especially in young animals¹⁵ and recent studies of the golden hamsters have shown that dopamine is present in some islet cells of young animals of this species too¹⁶. The occurrence of dopamine, both in the tumour cells and in normal islet cells, is further evidence that these cells are related.

A rapid turnover of dopamine could explain the occurrence of HVA in the tumour. This substance found in a tumour, usually suggests that the tumour is derived from the neural crest: neuroblastoma, ganglioneuroma or pheochromocytoma¹⁷. On the other hand, neither adrenaline nor noradrenaline has been found in the islet cell tumour². Thus this tumour possesses some properties related to human insuloma and some related to tumours of tissues derivating from the neural crest.

Zusammenfassung. In einem transplantierbaren Inselzelltumor des Goldhamsters ist eine Tyrosinhydroxylase-Aktivität gefunden. Die hohe Aktivität der Enzyme zusammen mit einer starken Konzentration von Tyrosin deutet auf eine schnelle Synthese von Dopa in den Tumorzellen. Die Anwesenheit der Dopametaboliten DOPAC und HVA im Tumor wurde gezeigt.

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The conversion of C-14-tyrosine to catechol derivatives in the islet cell tumour expressed as per cent of control in the presence of different substances

Addition to the incubation mixture	Concentration used (M)	No. of determinations	% of control MV (range)
Tetrahydrofolic acid	10^{-4}	5	340 (260–440)
2,2'-Bipyridyl	10^{-3}	4	18 (14–22)
H22/54	2×10^{-4}	4	40 (24–60)
Thiourea	10^{-4}	4	104 (101–116)
NSD 1015	10^{-4}	5	255 (240–270)

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