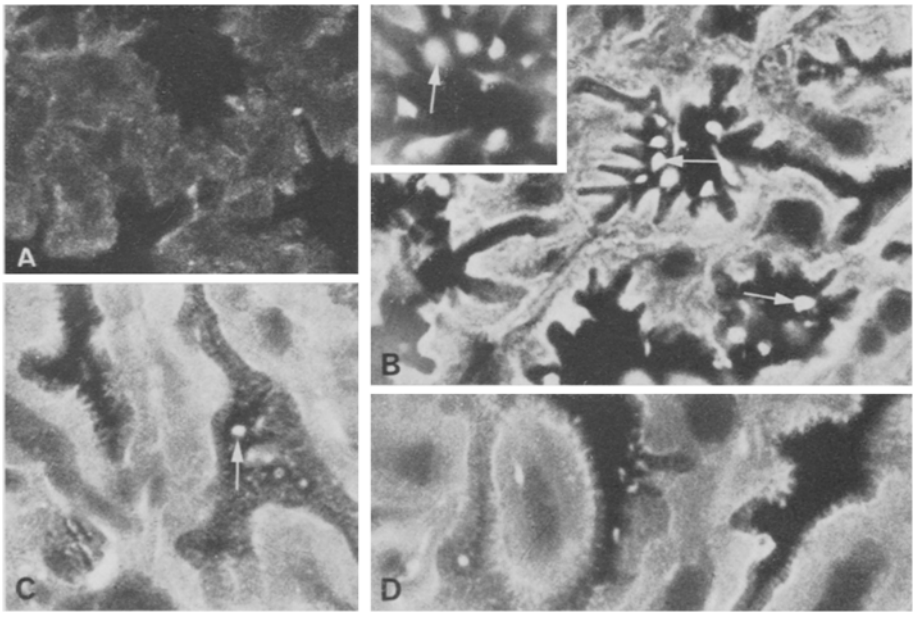
Secretion of L-DOPA Through the Choroid Plexus

Penetration of L-DOPA (L-3, 4-dihydroxyphenylalanine) from the blood into the central nervous system (CNS) is prevented by a special blood-brain barrier, localized in the wall of cerebral capillaries[1–3]. It was reported that the endothelial cells and pericytes of the cerebral capillaries constitute an enzymatic trapping mechanism for the precursors of the monoamines[1–3]. The sequence of events concerning penetration and transportation of L-DOPA into and within the CNS was discussed recently[4,5]. The present experiments furnish further information on this problem, with special respect to the role of the choroid plexus in these processes.

Materials and methods. 25 albino rats of both sexes, weighing 200–250 g, were used. 2 h before the experiment the animals were pretreated with the monoamine oxidase inhibitor nialamide (250 mg/kg body weight i.p.) and then injected with 75 mg/kg L-DOPA by i.v. route. 3, 5, 10 and 20 min after the L-DOPA administration, the animals were killed by decapitation. 15 rats were pretreated with nialamide (250 mg/kg body weight i.p.) and injected with the DOPA decarboxylase inhibitor Ro 4-4602 (50 mg/kg body weight i.p.) 2 h later, followed by the injection of L-DOPA in a similar dose and application as in the other group of animals. The brain was rapidly removed and dissected, quenched in isopentane precooled by liquid air and freeze-dried in a Pearse Speedivac Tissue Dryer. For the histochemical demonstration of the catecholamines, the method of FALCK and HILLARP[6,7] was used. The specimens were investigated under a Zeiss fluorescence microscope equipped with a high pressure mercury lamp (HBO 200). BG 12 primary filter and GG 11 and OG 1 ocular filters were used.

Results and discussion. In all of the time intervals mentioned above, the endothelium and the pericytes of the cerebral capillaries exhibited a marked greenish-yellow fluorescence, reported by earlier investigators[1,2,8] to develop only 20 to 60 min after L-DOPA administration. On the other hand, characteristic changes were observed in the fluorescence reaction of the choroid plexus. 3 min after the i.v. application of L-DOPA the whole choroid plexus of the 3rd ventricle exhibited a marked specific fluorescence, probably due to the presence of L-DOPA. On the ventricular surface of the epithelial cells of the choroid plexus, fluorescent granules measuring 2–5 μm could be observed in high amounts. Under high power it could clearly be seen that some of the granules exhibiting specific fluorescence were still in a close connection with the cytoplasms of the epithelial cells of the choroid plexus, whereas other granules appeared to be located freely within the liquor space. 5 to 10 min after the L-DOPA injection, the number of these granules was strongly reduced; after 20 min, granules were practically absent. L-DOPA is decarboxylated to dopamine in most areas of the brain by virtue of the DOPA decarboxylase located in the capillary wall[1–3]. In the choroid plexus, however, capillaries apparently fail to contain DOPA decarboxylase[1–3]. It could be argued that, in this case, DOPA decarboxylase activity is located within choroid epithelial cells. We found, however, that inhibition of the DOPA decarboxylase does not induce any changes in the fluorescence microscopic pattern described above; therefore, on the basis of these results, the possibility of an active transport of L-DOPA into the

[1] A. BERTLER, B. FALCK and E. ROSENGREN, Acta pharmac. tox. *20*, 317 (1963).
[2] A. BERTLER, B. FALCK, CH. OWMAN and E. ROSENGREN, Phramac. Rev. *18*, 369 (1966).
[3] CH. OWMAN, Bibl. anat., Basel *8*, 46 (1966).
[4] J. CONSTANTINIDIS et F. GEISSBÜHLER, Symposium Bel-Air IV. (Georg et Cie, Genève 1970), p. 99.
[5] J. CONSTANTINIDIS, J. M. GAILLARD, F. GEISSBÜHLER and R. TISSOT, Br. J. Pharmac. *43*, 32 (1971).
[6] B. FALCK, N. A. HILLARP, G. THIEME and A. TORP, J. Histochem. Cytochem. *10*, 348 (1962).
[7] B. FALCK and CH. OWMAN, Acta Univ. lund. II, *7*, 1 (1965).
[8] P. LANGELIER, A. PARENT and L. J. POIRIER, Brain Res. *45*, 622 (1972).



Fluorescence microphotographs of rat choroid plexuses. A) 2 h after nialamide treatment; B–D) treated with nialamide (2 h befor L-DOPA application) and L-DOPA, 3, 10 and 20 min before sacrifice, resp. Strongly fluorescent granules (arrows) can be observed at the ventricular surface of the choroid plexus. For further explanation see text. ×575; inset ×900.

liquor space via the choroid plexus – probably by means of an apocrine secretion – cannot be excluded. Biochemical studies on the monoamine content of the cerebrospinal fluid after L-DOPA administration would give a definite answer to the functional significance of the structural phenomena described above.

Acknowledgment. The author is indebted to Professor B. Csillik for his valuable advice and help in the course of these experiments. Ro 4-4602 was kindly supplied by Professor J. Kiss at L. Hoffman-La Roche.

Zusammenfassung. Bei der Ratte lassen sich nach i.v. Verabreichung von L-DOPA zahlreiche, intensiv fluoreszierende Körnchen an der ventrikulären Oberfläche des Plexus chorioideus beobachten. Morphologische Befunde lassen einen aktiven Transport von L-DOPA in den Liquorraum vermuten.

G. JANCSÓ

Department of Anatomy, University Medical School, P.O. Box 512, H-6701 Szeged (Hungary), 11 June 1974.

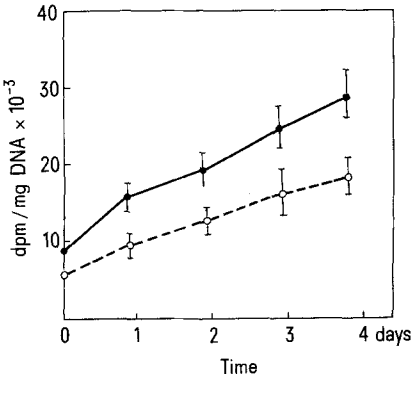
Cell Proliferation in Mouse Kidney by Isoproterenol and the Relationship with the Thyroid Function

Since the adenyl cyclase system is apparently involved in regulating the rate of growth of the salivary glands, it seemed of interest to examine the possibility that the rate of growth of other organs might be influenced by this system. There is general acceptance that isoproterenol stimulates both hyperplasia and hypertrophy in salivary glands[1,2] with an early secretory effect. It is already known that many manifestations of hyperthyroidism are similar to those observed when sympathetic activity is increased, whereas an opposed effect is induced by hypothyroidism[3]. The following experiment was conducted to investigate the effect of isoproterenol on kidney cell proliferation, and the possible relation with thyroid hormone.

Material and methods. Male mice, bred in the animal colony of the Facultad de Farmacia y Bioquimica, were used. The animals were divided into 3 groups: a) control animals, b) injected with 0.5 mCi ^{131}I i.p. 6 weeks before the experiment c) injected with 0.5 mCi ^{131}I and after 6 weeks administered with 15 µg sodium triiodotironine (T_3) i.p. daily for 1 week. Control, ^{131}I and ^{131}I plus T_3 were injected with a total dose of 6 mg of isoproterenol and killed 34 h later. Thymidine-methyl-H^3 was injected i.p. 30 min before killing. Kidneys were removed, weighed and homogenized. DNA was prepared and assayed as described previously[4], and aliquots were counted in a liquid scintillation spectrometer.

Results. In ^{131}I treated animals there was a decrease of organ weight, total DNA and incorporation of thymidine-H^3 (Table, a). After repeated doses of isoproterenol, there was a great difference between the specific activity of control renal and ^{131}I. This divergence appeared to result from an increased sensivity of control kidney and a poor response of hypothyroid mice kidney to different doses of isoproterenol. (Figure). Thyroid hormone has been implicated in the response of catecholamine effects. For this reason, the ability of thyroid hormone to intermediate the effect of isoproterenol-stimulated DNA synthesis was studied in the different groups of controls, ^{131}I or ^{131}I plus T_3 mice. Administration of isoproterenol to control mice significantly increased DNA synthesis compared with normal control (137%). After thyroidectomy, isoproterenol showed no response on kidney DNA synthesis compared with hypothyroid control. When ^{131}I mice were injected with T_3, the effect of β-catecholamines on DNA synthesis and growth response was again similar to the response of control (141%) (Table, b).

Discussion. The effect of thyroid hormone and testosterone on weight and secretory response of rat salivary glands has been described by OHLIN[5]. The similarity observed in the behaviour of thyroid hormone and catecholamines in certain physiological effects, such as increase in systolic pressure, induction of adenyl cyclase synthesis, lipolysis, etc, led to the consideration of a possible interrelation between the thyroid function and the sympathetic function[6,7]. After thyroidectomy, renal growth decreased[8] and columnar and follicles cells became depleted of colloid with a enlarge of thyroid gland after remotion of 1 kidney[9]. These works confirm our evidence that the absence of thyroid hormone affects the hyperplastic response of isoproterenol. From our results, it might be said that the response of β-catecholamines in hypothyroid mice kidney is decreased, and the presence of thyroid hormone (endogenous or exogenous) may increase the sensitivity of receptors and inhibition of their catabolism. Previous studies have indicated a decreased activa-



DNA specific activity in mouse kidney. Mice received 3 mg of isoproterenol per day. Each result is the mean of 6 animals ± S.E. ●—●, control; ○---○, hypothyroid.

[1] T. BARKA, Eur. J. Biochem. *1*, 96 (1967).
[2] D. MALAMUD and R. MALT, Lab. Invest. *24*, 140 (1971).
[3] A. E. DOMINGUEZ, B. E. FERNANDEZ and N. A. VIDAL, Medicina Abstr. *31*, 565 (1974).
[4] H. N. MUNRO and A. FLECK, *Methods of Biochemical Analysis* (Interscience Publisher, New York 1966), vol. 14, p. 161.
[5] P. OHLIN, Q. Jl exp. Physiol. *50*, 446 (1965).
[6] B. B. BRODIE, J. I. DAVIES, G. HYNIE, G. KRISHNA and B. WEISS, Pharmac. Rev. *18*, 271 (1966).
[7] V. PARSON and I. RAMSAY, Postgrad. Med. *44*, 377 (1973).
[8] R. REITER, *Endocrines and Compensatory Renal Enlargment* (Eds W. W. NOWINSKI and R. J. GROSS; Academic Press, New York 1969), p. 183.
[9] R. M. NAKAMURA, D. S. MIYADA, A. T. COCKETT and D. L. MOYER, Experientia *20*, 694 (1964).