Uptake of Catecholamines into Serotonergic Nerve Cells as Demonstrated by Fluorescence Histochemistry

BARTHOLINI et al.[1] have shown that animals pretreated with a decarboxylase inhibitor which has negligible central nervous system activity exhibit marked accumulation in brain of ^{3}H catecholamines from administered (^{3}H) L-dihydroxyphenylalanine (L-DOPA). Subsequently, CONSTANTINIDIS et al.[2] found a three-fold increase in dopamine content of the striatum after administration of L-DOPA and a peripheral DOPA decarboxylase inhibitor (Ro 4-4602 see below). Furthermore, BARTHOLINI et al.[3] have demonstrated a 50% decrease in brain serotonin (5 HT) after administration of L-DOPA, 200 mg/kg and Ro 4-4602 (N-(DL-seryl)-N'-2, 3-trihydroxybenzyl hydrazine; Hoffmann-La Roche – a peripheral DOPA decarboxylase inhibitor) given in doses which do not inhibit central nervous system decarboxylase activity. These results were verified by BUTCHER and ENGEL[4] using L-DOPA and Ro 4-4602, and by EVERETT and BORSCHERDENG[5] who administered L-DOPA alone. BARTHOLINI et al.[3] have suggested that this decrease in brain serotonin was caused either by displacement of endogenous 5 HT due to cerebral accumulation of dopamine, or by competition of L-DOPA with 5 HT precursors for penetrance into brain. Evidence indicating an active mechanism for uptake of aromatic amino acids into the central nervous system[2,6] supports the latter proposal. Recent histochemical[7] and biochemical observations[8] have suggested that there may be displacement of cerebral 5 HT from binding sites by catecholamines, probably dopamine. The present study uses the histochemical approach to confirm this possibility.

Materials and methods. Albino rats weighing 150–200 g were pretreated with Ro 4-4602, 50 mg/kg i.p. $^1/_2$ h prior to the administration of L-DOPA, 200 mg/kg i.p. Controls were given L-DOPA, 200 mg/kg i.p. alone; Ro 4-4602, 50 mg/kg i.p. alone; or saline, 5 cm^3 i.p. All animals were sacrificed 2 h following their last injection. Other animals were given reserpine, 5 mg/kg i.p. and pargyline, 100 mg/kg i.p., 24 and 5 h respectively before sacrifice according to the method of DAHLSTROM and FUXE[9] for the optimal demonstration 5 HT nerve cell bodies. Two separate but identical experiments were performed using a minimum of 3 animals in each experimental group. Sections of the brain stem were prepared and examined using the histochemical fluorescence technique of FALCK[10].

Results. The brain region examined was that surrounding the pyramids in the medulla where the cell bodies of the nucleus raphe/pallidus and ventral arcuate nucleus (B-1 and B-3 cells of DAHLSTROM and FUXE)[9] are found. These cell bodies normally contain 5 HT, and in untreated control rats they fluorescence faint yellow exhibiting marked fluorescence lability upon UV-exposure (Figure 1). Following treatment with reserpine and pargyline these

[1] G. BARTHOLINI, W. P. BURKARD, A. PLETSCHER and H. M. BATES, Nature *215*, 852 (1967).
[2] J. CONSTANTINIDIS, G. BARTHOLINI, R. TISSOT and A. PLETSCHER, Experientia *24*, 130H (1968).
[3] G. BARTHOLINI, M. DA PRADA and A. PLETSCHER, J. Pharm. Pharmac. *20*, 228 (1968).
[4] L. BUTCHER and J. ENGEL, Brain Res. *15*, 233 (1969).
[5] G. M. EVERETT and J. W. BORCHERDENG, Science *168*, 849 (1970).
[6] A. BERTLER, B. FALCK, C. OWMAN and E. ROSENGREN, Pharmac. Rev. *18*, 369 (1966).
[7] L. BUTCHER, J. ENGEL and K. FUXE, J. Pharm. Pharmac. *22*, 313 (1970).
[8] K. Y. NG, T. N. CHASE, R. W. COLBURN and I. J. KOPIN, Science *170*, 76 (1970).
[9] A. DAHLSTROM and K. FUXE, Acta physiol. scand. *62*, suppl. 232, 1 (1965).
[10] B. FALCK and C. OWMAN, Acta Univ. lund. *II 7*, 1 (1965).

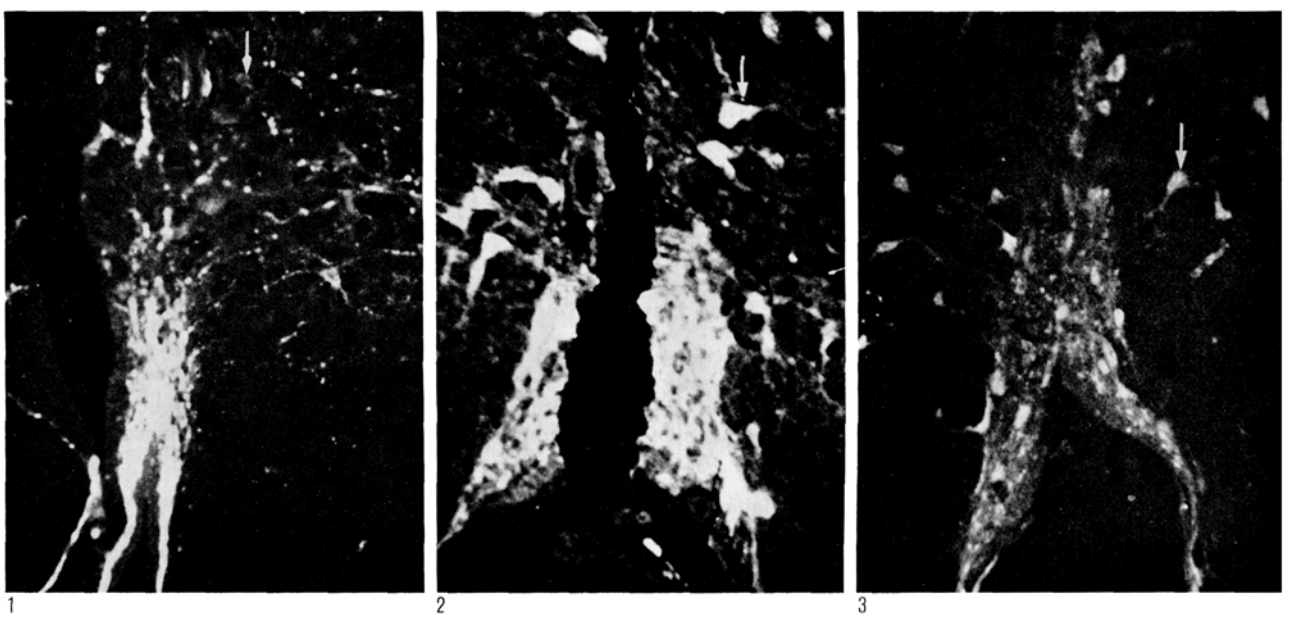

Fig. 1. Nucleus rephe/pallidus in brain stem of untreated control rat. The nerve cell bodies (arrow) fluoresce faint yellow and fade rapidly upon exposure to ultraviolet light (magnification ×250. Prints made from Kodak High Speed Ektrachrome transparencies).
Fig. 2. Nerve cell bodies (arrow) of the nucleus raphe/pallidus after treatment with reserpine and pargyline. The nerve cells and their processes fluoresce intense yellow and exhibit marked ultraviolet lability, but less than the controls (magnification ×250).
Fig. 3. Nerve cell bodies (arrow) of the nucleus raphe/pallidus after treatment with Ro 4-4602 and L-DOPA. The nerve cell bodies exhibit a distinct green fluorescence which has mild lability upon prolonged UV-exposure (magnification ×250).

cells bodies develop and intense yellow fluorescence with similar but less pronounced fluorescence lability (Figure 2). Examination of the corresponding regions in the medulla of rats treated with Ro 4-4602 and L-DOPA reveals a general mild increased fluorescence in the neuropil, however, the neuron cell bodies of the nucleus raphe/pallidus and those in the ventral arcuate nucleus exhibit a distinct green fluorescence with mild fluorescence lability (Figure 3). Rats administered L-DOPA or Ro 4-4602 alone do not reveal significant observable differences from the control group upon examination of the same brain regions. Comparable results were seen in all of the experimental animals.

Discussion. The present histochemical studies show that the administration of a peripheral decarboxylase inhibitor in dosages which exhibit minimal central nervous system activity[1,3] followed by large dosages of L-DOPA results in a change in the fluorescence characteristics of some cell bodies in the brain stem. The change in the fluorescence characteristics from yellow, UV labile to green UV stable indicates that these nerve cell bodies which normally contain 5 HT can contain a catecholamine under the above experimental conditions. These observations are compatible with the suggestion by BARTHOLINI et al.[3] that displacement of endogenous 5 HT may occur as a result of cerebral accumulation of catecholamines. The observations by BARTHOLINI et al.[3] of increased 5-hydroxyindoleacetic acid (5-HIAA), a metabolite of 5 HT, in brain during a 90 min period following the administration of Ro 4-4602 and L-DOPA further strengthen this possibility. EVERETT and BORSCHERDENG[5] confirmed this by showing a two-fold increase of 5-HIAA levels in mouse brain 1 h following the administration of L-DOPA alone. Recent in vitro observations by NG et al.[8] strongly support the displacement hypothesis.

The present study indicates that serotonergic nerve cell bodies have the capacity under certain conditions to take up and concentrate exogenous catecholamines. That the nerve terminals arising from serotonergic neurons have the same uptake capacity for catecholamines and their precursors has not been demonstrated, however, this possibility seems quite likely on the basis of the observations of BARTHOLINI et al.[3] and NG et al.[8] and has been suggested by BUTCHER et al.[7]. Although the accumulated evidence supports displacement as the mechanism for depletion of cerebral 5 HT following L-DOPA administration, the possibility of blood-brain transport competition of L-DOPA with 5 HT precursors may also be responsible, in part, for some of the biochemical observations.

The implication of these observations is that they may form a basis to understand some of the clinical phenomena observed in patients with Parkinson's disease to whom massive doses of L-DOPA are continuously administered. Patients with this disease commonly experience the onset of choreo-athetoid activity during treatment with L-DOPA[11]. These symptoms are related both to duration of treatment and total daily dosage of L-DOPA. Since the major biochemical deficit in this disease is depletion of brain monoamines (both dopamine and serotonin)[12,13], the susceptibility for either serotonin displacement or occupancy of serotonin binding sites by L-DOPA may be quite pronounced and allow L-DOPA (decarboxylated to dopamine) to act as a false transmitter in such circumstances.

Zusammenfassung. Die histochemische Fluoreszenzmethode wird zum Studium der Nervenzellen des Gehirnstammes der Ratte angewandt. Die erhebliche Zunahme des Dopamins, hervorgerufen durch die Injektion eines peripher wirkenden Dekarboxylase-Hemmstoffes zusammen mit L-DOPA, wird im Gehirn verfolgt und gefunden, dass serotonergische Neuronen imstande sind, Catecholamine aufzunehmen.

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[11] M. D. YAHR, R. C. DUVOISIN, M. SCHEAR, M. M. HOEHN and R. E. BARRETT, Arch. Neurol. *21*, 343 (1969).
[12] H. EHRINGER and O. HORNYKIEWICZ, Klin. Wschr. *38*, 1236 (1960).
[13] H. BERNHEIMER, W. BIRKMAYER and O. HORNYKIEWICZ, Klin. Wschr. *39*, 1056 (1961).
[14] This work has been supported by the Clinical Research Center for Parkinson's and Allied Diseases, grant No. NS05184.

Effect of Polyalkyl Polyphosphonates on Bone Development in Young Rats

Phosphate ions are one of the main constituents of the mineral of bone tissue. These ions are not only incorporated into bone, but also chemisorbed by it, as demonstrated by P^{32} tracer experiments (BAUER et al.[1]). A molecule carrying two PO_x^{n-} groups (e.g., a pyrophosphate ion, $[HOPOPOH]^{2-}$ with O O O O) will show a higher affinity for bone mineral since it is a bidentate complexing agent, capable of being attached to calcium ions through 2 oxygen atoms[2]. The absorption of pyrophosphate onto hydroxyapatite, the mineral form of calcium phosphate in bone, inhibits its crystal growth and dissolution[2,3]. Similar results have been observed with organic diphosphonates, $R_1R_2C(PO_3H)_2^{2-}$ (R = H, OH, or alkyl group), both in vivo and in vitro[4,5]. The latter findings recently encouraged a clinical application of ethane-1-hydroxy 1,1-diphosphonate in the treatment of myositis ossificans[6].

Polymeric alkyl phosphonates, e.g., polyethylene polyphosphonate, which carry many phosphonate groups on

[1] G. C. H. BAUER, A. CARLSON and B. LINDQUIST, *Mineral Metabolism* (Eds. C. L. COMAN and F. BRONER; Academic Press, New York 1961), vol. I B, p. 609.
[2] H. FLEISCH, R. G. G. RUSSELL and F. STRANMANN, Nature, Lond. *212*, 901 (1966).
[3] H. FLEISCH and W. F. NEUMAN, Am. J. Physiol. *200*, 1296 (1961).
[4] H. FLEISCH, R. G. G. RUSSELL and M. D. FRANCIS, Science *165*, 1262 (1969).
[5] M. D. FRANCIS, R. G. G. RUSSELL and H. FLEISCH, Science *165*, 1264 (1969).
[6] G. A. L. BASSETT, A. DONATH, F. MACAGNO, R. PRESIG, H. FLEISCH and M. D. FRANCIS, Lancet *1969*, p. 845.