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A common error in brain dopamine assays

It is commonly assumed that L-dopa in acute doses penetrates the blood-brain barrier. However, using the Falck-Hillarp histochemical fluorescence technique, it was shown that after nialamide (250 mg/kg) + L-dopa (75 mg/kg) or L-dopa alone, the amino-acid will not pass the blood-brain barrier due to its extraneuronal decarboxylation to dopamine within the brain capillaries (de la Torre, 1968, 1971). The dopamine remains trapped in the capillary endothelial cell layer where it fluoresces brightly. When nialamide + peripheral dopa decarboxylase inhibitor (PDI) + L-dopa are used, the capillary fluorescence is gradually abolished, correlating with the dose of PDI used. Penetration of L-dopa from capillaries to brain tissue is seen as a bright diffuse fluorescence in the area surrounding the capillary. This progressive penetration of the amino-acid can be mapped out in the brain by inactivating the capillary decarboxylase enzyme with the PDI [Ro 4-4602 N^1 (DL-seryl)-N^2-(2,3,4-trihydroxy benzyl) hydrazine] at doses ranging from 2-50 mg/kg (de la Torre, 1968, 1971).

L-Dopa administered to rats at a dose of 50 mg/kg increases brain dopamine 250% above control values within 30 min. Histochemical fluorescence indicates that in similarly treated rats, the brain capillary fluorescence is markedly intense in both the lumen and endothelial cells (Fig. 1a). In fact, the fluorescence around these capillaries is absent and the normal neuronal fluorescence remains unchanged indicating a lack of penetration by the L-dopa into the brain parenchyma.

If the L-dopa remained in the periphery, it was thought that cerebral perfusion might diminish the total values of brain dopamine.

Intracardiac left ventricular perfusion of cerebral vessels appears to wipe out the fluorescence in the lumen but not that of the capillary endothelial cells (Fig. 1b). Furthermore, perfusion at 15, 30 and 60 min after a standard intraperitoneal injection of L-dopa (50 mg/kg) shows that the dopamine "brain increase" as measured biochemically is reduced 11, 42 and 6% respectively compared to non-perfused treated rats (Fig. 2).

Moreover, if dopamine is injected intraperitoneally in rats, a capillary fluorescence restricted to the lumen is observed. Cerebrovascular perfusion with heparanized saline abolishes the capillary fluorescence almost completely in these animals.

These findings indicate that the blood-brain barrier for dopamine lies in the inner or luminal surface of the capillary which is in contact with the blood. On the other hand, L-dopa appears to penetrate this luminal surface where it appears to enter the endothelial cells or inner capillary layer. This and other data (Bertler, Falck & others 1963, 1966) further suggests that administered L-dopa may be rapidly decarboxylated to dopamine within the endothelial cells of brain capillaries where it is shielded from the saline perfusion.

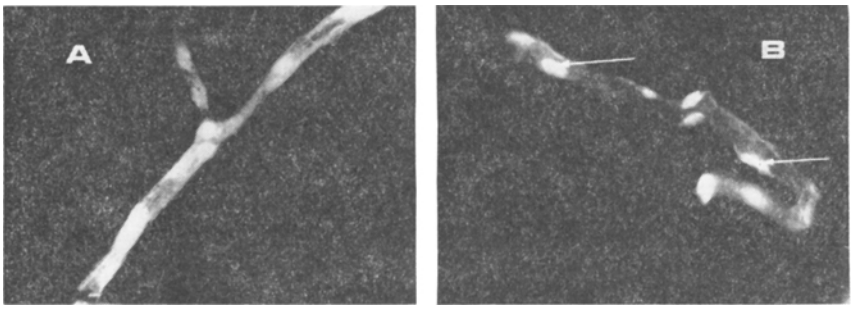

FIG. 1. Cerebral capillaries in the rat at the level of the antero-medial thalamic nucleus. Falck-Hillarp technique. (a) L-Dopa, 50 mg/kg, i.p. Both capillary lumen and endothelial cell layers are brightly fluorescent after 30 minutes. Neuronal tissue remains non-fluorescent. 130 ×. (b) Same as above but perfused with saline through the heart. Only endothelial cell fluorescence (arrows) is observed; capillary lumen remains dark. Neuronal tissue remains non-fluorescent. 130 ×.

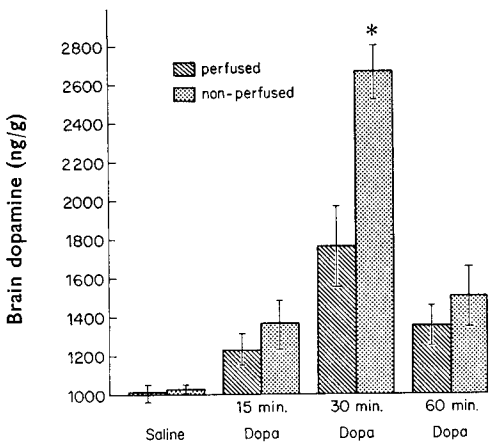

FIG. 2. Effect of intracardiac left ventricular perfusion of cerebral vessels on brain dopamine levels of rats injected intraperitoneally with 50 mg/kg of L-dopa. Non-perfused is a sham procedure. Perfused animals were given with 150 ml of heparinized physiologic saline. N = 100 (at least 6/group). * Only value statistically significant ($P = < 0{\cdot}0025$).

The results indicate that the cerebral values of dopamine after acute injection of L-dopa as measured by biochemical assays of brain tissue, are grossly inflated due to inclusion of (a) circulatory dopamine in the capillary lumen, (b) trapped dopamine in endothelial cells. This occurs because after homogenization and extraction of the amines from brain tissue, the vascular (capillary) tissue dopamine cannot be separated from the neuronal tissue. Such an increase has been incorrectly described in the literature as central or intraneuronal elevation of dopamine by L-dopa.

The fluorescence seen in the lumen may be circulatory dopamine which is formed from the administered L-dopa in extracerebral organs or possibly even in the blood as shown recently by Cohn, Dunner & Axelrod (1971).

The time course involved in the reduction of brain "dopamine increase" as measured by biochemical assays is also evident after fluorescence examination of the treated animal brain tissue. Enzymatic destruction probably reduces the circulatory dopamine fluorescence found in the cerebrocapillary lumen so that after 60 min, the perfused and non-perfused concentrations estimated biochemically from total brain are not markedly different (Fig. 2).

In non-perfused rats, the brain capillary lumen and endothelial cell fluorescence is weak some 60 min following L-dopa after which the luminal fluorescence disappears.

After 90 to 120 min, fluorescence in capillary endothelial cells is no longer seen in either perfused or control animals. We deduce from previous evidence (de la Torre, 1968, 1971) that monoamine oxidase may be localized within the mitochondrial fraction in the endothelial cell layer of brain capillaries and that it is this enzyme which at least in part is eliminating the active dopamine from the capillary endothelium.

It is not yet known how much, if any, of the L-dopa enters the neuronal tissue after its acute administration. Small amounts could conceivably leak through the brain barrier and theoretically alter neuronal transmission resulting in centrally mediated gross behavioral changes. The possibility of significant neuronal penetration by L-dopa after its single administration seems unlikely for two reasons: (*a*) no trace is seen of the diffuse but localized fluorescence around brain capillaries such as occurs after minimal peripheral dopa decarboxylase inactivation with Ro 4–4602 given before L-dopa (de la Torre, 1971). (*b*) if gross behavioral changes are due to the central action of L-dopa or its catabolites, then potentiation of such changes would be more pronounced after PDI + L-dopa since the amino-acid would penetrate the neuronal tissue in greater amounts.

Potentiation of these changes does not occur however, but more significantly, the autonomic signs (piloerection, exopthalmos, salivation, irritability, dyskinesia, etc.) seen in animals after a single dose of L-dopa (100–200 mg/kg) are abolished by pretreating with a PDI (de la Torre. 1968, 1971, 1972; Constantinidis, de la Torre & others, 1969, Butcher & Engel, 1969).

The question of how L-dopa manages to cross blood-brain barrier after heavy doses and chronic administration in parkinsonians is now being investigated experimentally. In man, amelioration of parkinsonian symptoms is usually evident after many weeks of L-dopa therapy in doses averaging 6 g/day (Duvoisin,Yahr & others, 1969). This contrasts with the beneficial effects of L-dopa with prior PDI treatment seen after 24 or 48 h (Tissot, Gaillard & others, 1969, Siegfried, Klaiber, & others, 1969, Barbeau & Gillo-Joffroy, 1969; Barbeau, Gillo-Joffroy & Mars, 1971, all using Ro 4-4602). Clearly, some mechanism in the blood-brain barrier permeability to L-dopa is altered after the amino-acid is chronically administered.

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Division of Neurological Surgery and
Department of Psychiatry,
University of Chicago Hospitals,
950 E. 59th Street, Chicago, Illinois 60637, U.S.A.

J. C. DE LA TORRE
W. O. BOGGAN
R. A. LOVELL

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