Relative Penetration of L-Dopa and 5-HTP Through the Brain Barrier Using Dimethyl Sulfoxide[1]

The idea that dimethyl sulfoxide (DMSO) may aid in the transport of other drug molecules across the blood-brain barrier (BBB) has been reported using 14 C-pemoline[2], but this finding has been challenged as being inconclusive[3]. The following report agrees with the idea that DMSO can act as a partial transport carrier of some drug molecules facilitating their penetration into brain tissue.

Materials and methods. 56 male Sprague-Dawley rats, 200–300 g, were used. All animals were first pretreated with the monoamine oxidase inhibitor (MAOI) nialamide, 250 mg/kg. L-dopa, 75 mg/kg, or 5-hydroxytryptophan (5-HTP), 75 and 100 mg/kg, were dissolved in pure DMSO, 750 mg/kg, and injected into 24 rats. A second group of 20 animals served as drug-treated controls, receiving 2–10 mg in 2 mg increments of Ro 4-4602 (N-[DL-seryl]-N^1-trihydroxybenzyl-hydrazine) a peripheral dopa decarboxylase inhibitor (DCI), followed by a usual dose of L-dopa or 5-HTP. The remaining animals served as normal controls. Animals were sacrificed 3 h after the first injection. Brains were quickly dissected, frozen and processed for histochemical fluorescence according to a method previously described[4].

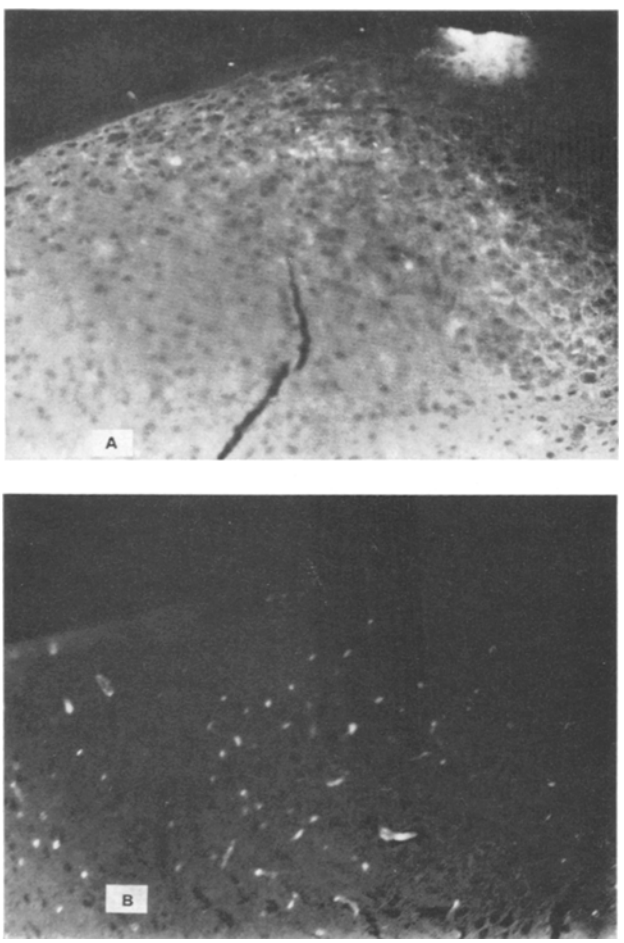
Results and discussion. When MAOI and L-dopa or 5-HTP are administered to rats, a concentration of dopamine or serotonin accumulates in brain capillaries as a result of peripheral decarboxylation[5–8]. The newly formed products remain in the capillary network until they are eventually deaminated by monoamine oxidase. If, however, a DCI is administered after MAOI treatment but before L-dopa (or 5-HTP) is given, then a complete or partial breakdown of the brain barrier for the 2 amino acids will result due to total or partial inhibition of peripheral dopa decarboxylase.

The relative penetration of L-dopa or 5-HTP into brain tissue can therefore be studied by merely injecting appropriate doses of Ro 4-4602. Previous experiments show that after 4 mg/kg of Ro 4-4602 in MAOI pretreated rats, L-dopa will first penetrate the regions of the ventromedial hypothalamic nucleus, the periventricular hypothalamic nucleus and the arcuate nucleus. Decreased capillary fluorescence is also noted in the caudate, thalamus, cortex and white matter. This progressive breakdown of the BBB for L-dopa continues as Ro 4-4602 is increased until at 50 mg/kg the enzymic barrier is completely suppressed and L-dopa enters all brain regions freely.

By using the histochemical fluorescence method a cerebrovascular network of capillaries is seen to fluoresce throughout the brain following nialamide + L-dopa (or 5-HTP) treatment. As progressive peripheral decarboxylase inhibition is achieved with increased doses of Ro 4-4602, the capillary fluorescence in those areas where the barrier is 'weakest' begins to disappear to be replaced by a diffuse but localized background fluorescence. The reason for this is that peripheral decarboxylase activity has been suppressed by the Ro 4-4602 and the amine precursors are able to penetrate freely into the brain tissue. The precursors remain free or unbound giving off the exogenous fluorescence until they are gradually taken up by the nerve cells (endogenous decarboxylation) several hours later[7,8].

To test the carrier properties of DMSO, L-dopa (Ro 4-4602) omitted, was dissolved in undiluted DMSO and the mixture injected i.p. in rats 1 h after nialamide treatment.

The results show that the combination of nialamide + DMSO:L-dopa gave the same capillary fluorescence picture as when Ro 4-4602 had been used at 8 mg/kg as described above. Nialamide + DMSO resembled normal untreated controls (i.e., no capillary fluorescence). When 5-HTP was used instead of L-dopa, very slight penetration of the amine was noted just dorsally to the arcuate nucleus. The capillary fluorescence otherwise resembled nialamide + 5-HTP treated rats.



Fluorescent microphotographs of rat frontal brain sections at the level of the anterior periventricular hypothalamic nucleus. Treated with: (A) nialamide (250 mg/kg) + DMSO:L-dopa (750:75 mg/kg) solution, and (B) nialamide (250 mg/kg) + L-dopa (75 mg/kg). A light, diffuse fluorescence is seen throughout (A) due to the presence of exogenous dopa in nerve tissue. Fluorescent capillaries (B) and no diffuse parenchymal fluorescence. Third ventricle is seen at top left. Magnification × 120.

[1] Aided by USPHS Grant No. 07376.

[2] J. J. Brink and G. D. Stein, Science *158*, 1479 (1967).

[3] J. J. Kocsis, S. Harkaway and W. H. Vogel, Science *160*, 1472 (1968).

[4] B. Falck and Ch. Owman, Acta univ. lund. *2*, 7 (1965).

[5] A. Bertler, B. Falck and E. Rosengren, Acta pharmac. *20*, 317 (1963).

[6] J. C. de la Torre, Thesis (Univ. of Geneva) Méd. Hyg., *1477*, 5 (1968).

[7] J. Constantinidis, J. C. de la Torre, R. Tissot and F. Geissbuhler, Psychopharmacologia *15*, 75 (1969).

[8] J. C. de la Torre, J. neurol. Sci., in press (1970).

It was thought at first that DMSO may have been acting as weak decarboxylase inhibitor but this idea was discarded in view of the following findings: a) No marked penetration of 5-HTP was seen when the amine was dissolved in DMSO indicating no peripheral decarboxylase inhibition. b) When DMSO is given 1 h prior to L-dopa, no penetration of the latter is seen, indicating no reduction in peripheral decarboxylase activity.

The thought that DMSO may have reacted chemically with L-dopa to produce a dopa analogue capable of partially crossing the BBB was also excluded since a) DMSO is a relatively inert chemical compound, b) increase in tubero-infundibular fluorescence was noted following DMSO:L-dopa, c) dopa analogues do not normally fluoresce in tissue using this technique, d) neostrital fluorescence was also increased following DMSO: L-dopa.

It would appear from the above findings, therefore, that DMSO is able to transport L-dopa across the blood brain barrier to some extent. This carrier activity is probably rapid, since any reasonable amount of L-dopa in the brain capillaries is quickly converted by dopa decarboxylase to dopamine, a substance that does not cross the blood brain barrier.

The findings may offer an alternative possibility for increasing dopamine levels in neostrital structures as opposed to massive doses of L-dopa alone or decarboxylase inhibition followed by L-dopa[9]. This increase in brain dopamine is of particular interest in the treatment of Parkinson's disease.

Résumé. Le passage de la dopa à travers la barrière hémato-encéphalique semble être facilité par le diméthyl sulphoxyde (DMSO) tandis qu'aux mêmes conditions le 5-HTP franchit cette barrière seulement dans une région très limitée de l'hypothalamus, chez les rats.

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[9] J. C. DE LA TORRE, Ann. intern. Med., in press (1970).

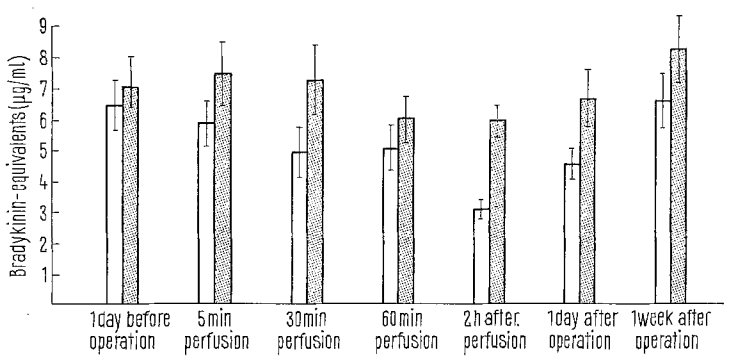
Plasma Kininogen in Extracorporal Circulation and the Influence of a Protease Inhibitor from Bovine Lung

It is well known that, in extracorporal circulation with heart-lung machine, blood clotting system and fibrinolysis are activated. The relationship of blood clotting system and fibrinolysis to the kininogen kinin system (EISEN[1]) suggests also an activation of the kinin forming system in extracorporal circulation. This paper is concerned with the changes of plasma kininogen level in extracorporal circulation and the influence of the kallikrein-trypsin-inhibitor Contrykal®.

Material and methods. The studies have been carried out in 14 patients (5–44 years old). In these patients open heart surgery with heart-lung machine was performed (12 atrial septal defects, 2 stenosis of the pulmonal valvula). The average perfusion time was 65 min in the range of 35–105 min. We used a heart-lung machine developed by STRUSS and SCHOBER[2]. The blood was carried with a pump of the DE BAKEY-Type with a maximal perfusion volume of 6000 ml per min. The oxygenization was performed with a disc-oxygenator of Kay-Cross. The perfusion was carried out in hypothermia (30–32 °C) with the aid of a heat-exchanger of GÜNDEL[3]. The heart-lung machine was filled with heparinized fresh blood (2500 IU Heparin-Richter® Budapest per 500 ml blood), low molecular dextran preparation (Infukoll M 40® VEB Serumwerk Bernburg) and a glucose solution of 5%. Per litre haemodilution (25 ml per kg) 5000 IU Heparin were added. 500 IU Heparin per kg were given to the patient. The action of Heparin was inhibited with protamin-titration after perfusion was finished. The blood conducting parts of the heart-lung machine used by us were not siliconized.

For the determination of plasma kininogen the blood was taken from patients from the cubital vein with a siliconized and heparinized syringe, from the extracorporal circuit into a siliconized tube. The time course of drawing the samples was as follows: 1 day before operation (taken from patients), 5 min of perfusion, 30 min of perfusion, 60 min of perfusion, 2 h after per-

[1] V. EISEN, Br. med. Bull. *20*, 205 (1964).
[2] F. STRUSS and K. L. SCHOBER, Dte GesundhWes. *17*, 1684 (1962).
[3] W. GÜNDEL, Medizintechnik *3*, 208 (1963).



Plasma kininogen during and after extracorporal circulation. Abscissa: time. Ordinate: Kininogen in bradykinin-equivalents µg/ml. White columns: without protease inhibitor. Black columns: with protease inhibitor. Vertical bars are standard error of mean.