Effects of inflammatory mediators on the glomerular localization of intravenously administered ferritins

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Summary. Effects of inflammatory mediators such as serotonin, histamine, and bradykinin upon glomerular localization of i.v. administered ferritins have been investigated. These mediators appear to enhance accumulation of the i.v. injected ferritins in the glomerular capillary walls of rats.

It has been reported that inflammatory mediators of vasoactive amines may enhance glomerular localization of i.v. injected colloidal carbons[1] as well as immune complexes[2]. These reports, however, did not include ultrastructural examination of glomeruli using ferritin as a tracer. We have undertaken an ultrastructural examination of rat glomeruli to see the effects of such inflammatory mediators as serotonin, histamine, and bradykinin upon the glomerular localization of intravenously administered ferritins.

Materials and methods. 9 rats (Holtzman, males) with an average weight of 250 g were used. Serotonin (5-hydroxytryptamine creatinine sulfate, Sigma Chem. Co.), histamine (Fisher Sci. Co.), and bradykinin triacetate (Sigma Chem. Co.) were dissolved in Ringer's solution at concentration of 1 mg/ml, 10 mg/ml, and 0.01 mg/ml, respectively. Groups of 3, 3, and 3 rats were anesthetized by i.p. injection of Nembutal (50 mg/kg of b.wt), and were injected with serotonin, histamine, and bradykinin into the subcapsular space of the left kidney through the perinephric fat pad with 30 gauge hypodermic needle at a dose of 0.3, 1.0, and 0.04 mg per rat, respectively. Immediately following the completion of the injection, 2 ml of cadmium-removed horse spleen ferritin (45.8 mg/ml) was i.v. injected. 3 control rats were injected into the subcapsular space of the left kidney with Ringer's solution only, and were similarly administered with 2 ml of horse spleen ferritin. All rats were killed at 15 min following the injection of ferritin, and the left kidneys were fixed in 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) for 24 h, washed overnight with the buffer, dehydrated, and embedded in Epon-Araldite mixture No. 1 of Mollenhauer[3]. The remainder of the left kidney was fixed in 10% formalin, embedded in paraffin, sectioned, and were stained with Gomori's iron stain for light microscopy. Unstained thin sections as well as those that were doubly stained with uranyl acetate and lead citrate were examined under Philips 300 electron microscope.

Results and discussion. Conspicuously positive iron reaction to Gomori's reagent was present about the glomerular capillary walls of the kidneys that were treated with serotonin or histamine, but a similar reaction product was not demonstrable in those treated with bradykinin. All these 3 inflammatory mediators, however, appeared to enhance accumulation of the ferritin particles in the lamina rara interna and in the lamina densa of the glomerular capillary wall. This enhanced accumulation of ferritin was most

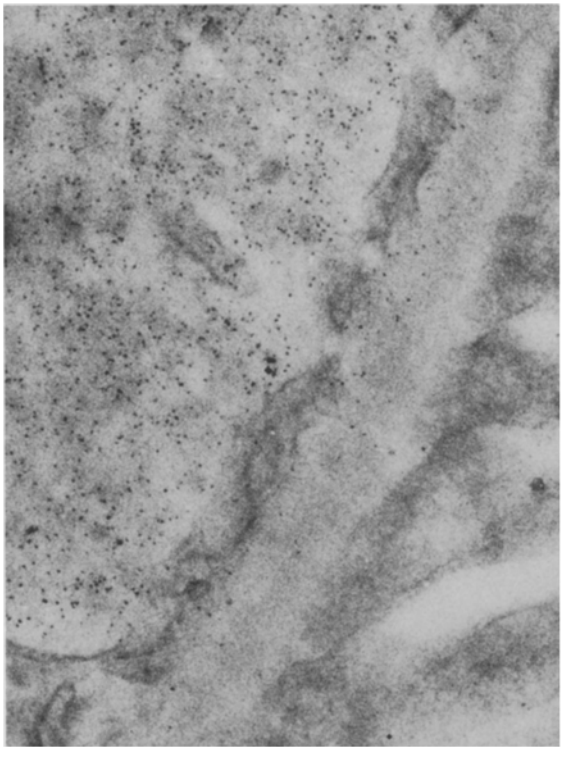

Fig. 1. A control kidney at 15 min following the injection of ferritins. A small number of ferritin particles are localized in the glomerular capillary wall. The ferritin particles are more densely crowded in the capillary lumen than they are in the glomerular basement membrane, an opposite finding to the one shown in figure 2. (Unstained specimen). × 79,800.

Fig. 2. Serotonin-treated kidney at 15 min following the injection of ferritins. Notice the presence of numerous ferritin particles in the lamina rara interna and in the lamina densa. The ferritin particles are more densaly crowded in the glomerular basement membrane than they are in the capillary lumen. Arrows: endothelial fenestrae. (Unstained specimen). × 79,800.

prominent in those treated with serotonin or histamine (figures 1 and 2). The kidneys treated with these 3 mediators revealed that the ferritin particles tended to be more densely crowded in the basement membrane than they were in the capillary lumina. This indicated that most of the ferritin particles that entered into the capillary wall were somehow retained in the glomerular basement membrane. In those kidneys treated with serotonin, but not in others, some ferritin particles apparently passed through the glomerular capillary wall to enter into the Bowman's space. Nonetheless, the majority of the particles was retained at the level of lamina densa.

Our data appear to indicate that these inflammatory mediators are capable of enhancing the glomerular localization of i.v. administered ferritins, and they may modulate functions of the glomerular capillary walls. The mechanisms of how these mediators modify the functions of glomerular capillary walls remain to be elucidated.

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Interaction of CDP-choline with synaptosomal transport of biogenic amines and their precursors in vitro and in vivo in the rat corpus striatum[1]

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Summary. Added to a striatal synaptosomal homogenate of rat brain, CDP-choline 10^{-4} M inhibits the uptake of norepinephrine (NE), dopamine (DA) and serotonin (5 HT) in a competitive fashion and enhances the uptake of tyrosine and tryptophan; administered to animals, CDP-choline (50 mg/kg/1 h/i.v.) inhibits only the in vitro uptake of DA but enhances the uptake of precursors.

For over a decade, specific transport systems for neurotransmitter amines and amino acids have been extensively studied, since it is generally accepted that the neuronal reuptake after their release into the synaptic cleft is an inactivation mechanism of neurotransmitter[2]. These studies have shown that many drugs exert their pharmacological action by an interaction with the synaptic uptake. Several reports have demonstrated that uptake of norepinephrine (NE), dopamine (DA) and serotonin (5 HT) in brain slices[3] or in synaptosomes[4] is sodium-dependent, ouabaine-sensitive and a saturable process. The same mechanism seems to occur for catecholamine and indolamine precursors: Tyrosine[5] (TYR) and tryptophan[6] (TRP).

Cytidine-5′ diphophocholine (CDP-choline), an endogenous nucleotide, has been recognized as a brain activator[7]. Moreover, therapeutic effect of CDP-choline has been found in parkinsonism. However, it seems different from classical antiparkinson drugs (benztropine, trihexyphenidyl) in its mechanism of action, since it exerts a facilitory effect on the pyramidal system and an inhibitory effect on the extrapyramidal system[8]. From a biochemical point of view, CDP-choline increases dopamine level and slighty decreases serotonin level, leaving norepinephrine content unchanged in the whole mouse brain[9].

Method. In the in vitro experiments, 5 wistar male rats (150–200 g) were sacrificed by cervical dislocation. Their

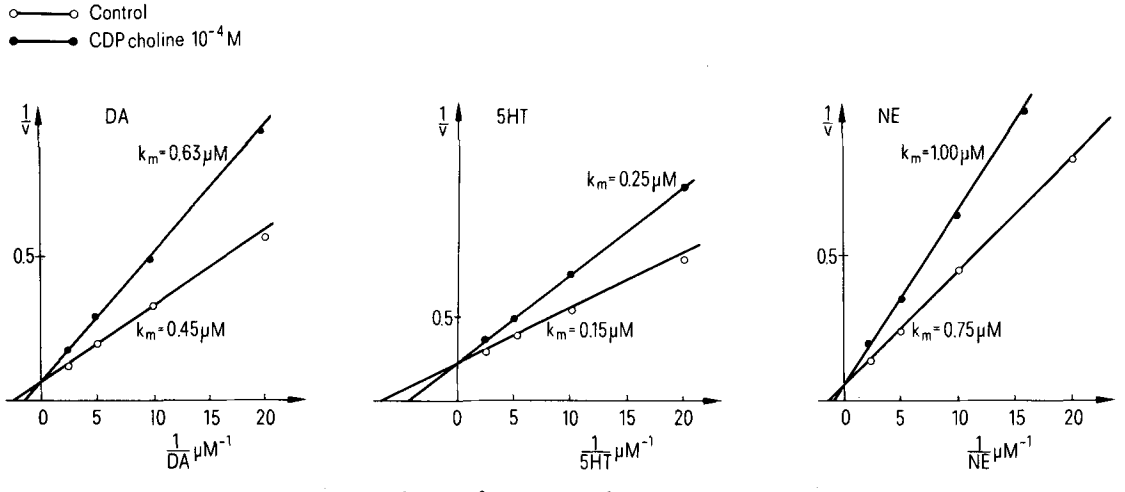

Fig. 1. Graphic analysis of the in vitro inhibition of ^{3}H DA, ^{3}H 5 HT and ^{3}H NE accumulation in corpus striatum synaptosomal homogenates by CDP choline 10^{-4} M. Homogenates were preincubated with CDP-choline in a Krebs Henseleit oxygenated buffer for 5 min before addition of labelled amine range concentration 0.05–0.4 μM. Amine accumulation (V) is expressed as nmoles/g fresh tissue/min. Each point is the mean of 5 determinations. Linear regression for determining kinetic constants were fitted by least square method.