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

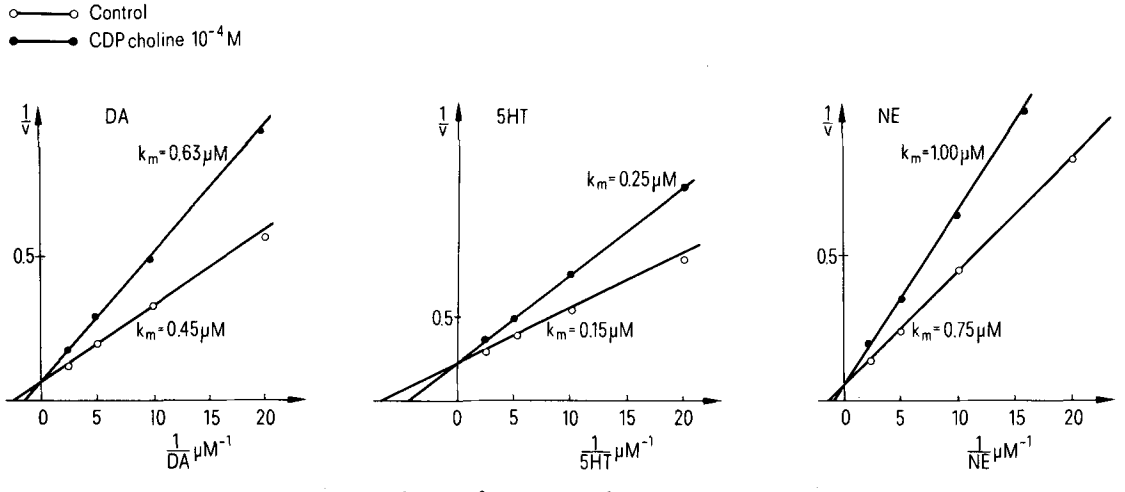

Fig. 1. Graphic analysis of the in vitro inhibition of ^{3}H DA, ^{3}H 5 HT and ^{3}H NE accumulation in corpus striatum synaptosomal homogenates by CDP choline 10^{-4} M. Homogenates were preincubated with CDP-choline in a Krebs Henseleit oxygenated buffer for 5 min before addition of labelled amine 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.

brains were rapidly removed and the corpus striatum dissected out on ice. The tissue was homogenized in 10 vol. ice-cold 0.32 M sucrose. Homogenates were centrifuged at 1000×g for 10 min. The precipitate was discarded and the supernatant fluid was gently stirred to obtain an uniform suspension. The uptake was performed according to Snyder and Coyle[10]. A suspension containing 2 ml of an oxygenated Krebs Henseleit bicarbonate buffer pH 7.5 (1.13 mM $CaCl_2$) containing 1 mM ascorbic acid, 10 mM glucose, 0.18 mM EDTA, 1.25 10^{-5} M nialamide and 500 µl of the synaptosomal homogenate was preincubated for 5 min at 37 °C before adding 100 µl of a Krebs medium containing radiolabelled biogenic amines: 7^3H-norepinephrine 8–20 Ci/mM, ^{3}H-dopamine 5 Ci/mM, ^{3}H 3–5 5-hydroxytryptamine 6 Ci/mM (the radiochemical Center Amersham) ^{3}H 3–5 L tyrosine 56 Ci/mM or ^{3}H L tryptophan 6 Ci/mM (CEA France).

After 5 min incubation at 37 °C for the mediators, or 2 min for the precursor amino-acids, the tubes were centrifuged at 20,000×g for 20 min and the resulting pellets were washed with 3×3 ml ice-cold NaCl 0.9%. The radioactivity was measured by liquid scintillation. Blanks underwent the same experiment but remained at 0 °C.

In the in vivo studies, CDP choline 50 mg/kg/i.v. was injected 1 h before decapitation, to at least 5 rats. After isolating synaptosomes, the incubation was continued, as in the in vitro studies, and the uptake of labelled amines incorporated in the synaptosomes of treated rats was compared with the uptake obtained on proof synaptosomes.

Results. The striatal DA, NE and 5 HT uptake exhibited a saturable type of kinetics. For amine concentration ranging from 10^{-8} to 10^{-6} M, the plots obtained gave a Km of 0.45 µM and a Vm of 16.7 nM/g fresh tissue/min for dopamine, a Km of 0.15 µM and a Vm of 4.1 nM/g fresh tissue/min for serotonin and a K_m of 0.75 µM and a V_m of 15 nM/g fresh tissue/min for norepinephrine. For greater concentration, a second kinetic system appeared with DA and 5 HT. IC_{50} (concentration of CDP-choline required to produce half maximum inhibition) was determined for the 3 amines (concentration fixed to 10^{-7} M) and showed more potent inhibition for DA ($5 \cdot 10^{-5}$ M) than NE (10^{-4} M) and 5 HT ($5 \cdot 10^{-4}$ M). In order to evaluate the type of uptake inhibition by CDP-choline, reciprocals of amine accumulation velocity and amine concentration were plotted according to Lineweaver and Burk. A competitive antagonism of the 3 amine uptake (figure 1) was found for CDP-choline concentrations near IC_{50} values. CDP-choline was examined for its in vivo inhibiting effect on the uptake of ^{3}H amines in corpus striatum synaptosomal homogenates. An inhibitory effect on DA uptake only was found in vivo (figure 2), leaving NE and 5 HT uptake unchanged.

Catecholamines and indolamines precursors: tyrosine and tryptophan may be captured by brain synaptosomes. When synaptosomes were incubated in the presence of 10 µM tyrosine or 10 µM tryptophan, the addition of 10^{-4} M and 10^{-3} M CDP-choline respectively increased the accumulation of radioactivity by 25% and 65% for tyrosine and by

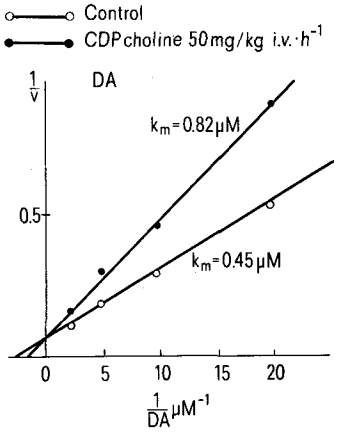

Fig. 2. Graphic analysis of the in vivo inhibition of ^{3}H DA accumulation in corpus striatum synaptosomal homogenates of treated animal by CDP-choline 50 mg/kg/i.v. 1 h before decapitation. Homogenates were preincubated in a Krebs Henseleit oxygenated buffer for 5 min before addition of labelled DA range concentration 0.05–0.4 µM. Amine accumulation is expressed as nmoles/g fresh tissue/min. Each point is the mean of 5 determination. Linear regression for determining kinetic constants were fitted by least square method.

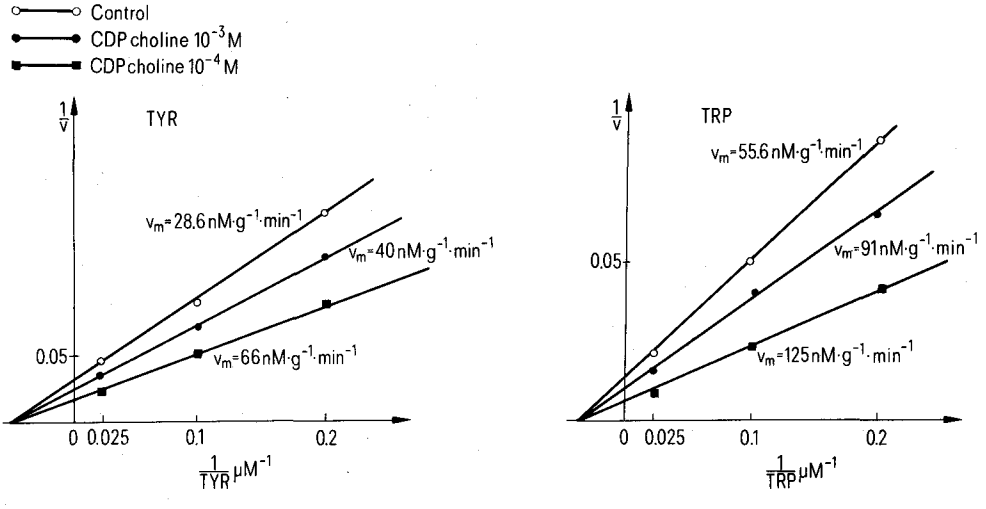

Fig. 3. Graphic analysis of the in vitro activation of ^{3}H TYR and ^{3}H TRP accumulation in corpus striatum homogenates by CDP-choline 10^{-4} M and 10^{-3} M. Homogenates were preincubated with CDP-choline in a Krebs Henseleit oxygenated buffer for 5 min before addition of labelled tryptophan or tyrosine range concentration 5–40 µM. Amino acid accumulation 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.

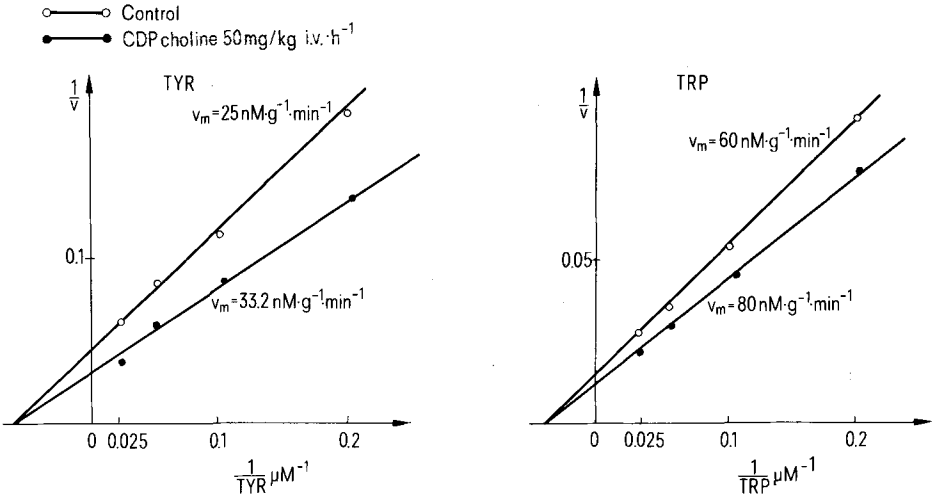

Fig. 4. Graphic analysis of the in vivo activation of ^{3}H TYR and ^{3}H TRP accumulation in corpus striatum homogenates of treated animal by CDP-choline 50 mg/kg/i.v. 1 h before decapitation. Homogenates were preincubated in a Krebs Henseleit oxygenated buffer for 5 min before addition of labelled tryptophan or tyrosine, range concentration 5-40 μM. Amino acids accumulation 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.

18% and 25% for tryptophan. The effect of 10^{-4} M and 10^{-3} M CDP-choline is non-competitive for the tyrosine and tryptophan uptake (figure 3). The in vivo effects of CDP-choline on the uptake of ^{3}H tyrosine and ^{3}H tryptophan were similar to those observed in vitro (figure 4). Both tyrosine and tryptophan uptake were increased non-competitively. The K_m-values were 25 nM/g fresh tissue/min and 50 nM/g fresh tissue/min for tyrosine and tryptophan respectively in control rat homogenates and 32.2 nM/g fresh tissue/min and 80 nM/g fresh tissue/min for tyrosine and tryptophan in treated rat homogenates.

Discussion. By blocking the re-uptake of dopamine, the antiparkinson drugs could potentiate the effect of the limited amounts of this amine remaining in the brain of parkinson patients. Most of the antiparkinson drugs inhibit catecholamine uptake in corpus striatum non competitively[11] and are considerably weaker in inhibiting 5 HT than catecholamine accumulation[12]. In the rat corpus striatum, the uptake of norepinephrine is not specific because of the poor distribution of adrenergic nerve terminals (a very low amount of this amine is found).

Even so, amphetamines which also inhibit catecholamine uptake in the corpus striatum homogenates, but in a competitive fashion[13], and both high and low affinity uptake processes for 5 HT in the whole brain homogenates[14], are effective in the treatment of the rigidity and akinesia, but their central stimulant effect limits dosage.

We have found that CDP-choline inhibits monoamine uptake competively in the rat corpus striatum homogenates in vitro and dopamine uptake in vivo. It could be suggested that the antiparkinson action of CDP-choline is due to its ability to increase dopamine level in the synaptic cleft as amphetamine does, but the less important stimulant effect of CDP-choline provides advantage in therapeutic application.

Tyrosine and tryptophan transport activation by CDP-choline, pointed out in the corpus striatum both in vitro and in vivo may be correlated with dopamine level increase in the same part of the rat brain[15]. Serotonin synthesis is influenced by the activity of brain tryptophan hydroxylase and by the availability of tryptophan, while catecholamine synthesis is not normally markedly influenced by the availability of tyrosine. Although some reports suggest that rat brain catecholamine synthesis responds to changes in brain tyrosine concentration[16], we cannot state whether the increase of tyrosine availability influences the synthesis of dopamine, or the increase in the level of dopamine controls the uptake of tyrosine. It is of interest to notice that amphetamine also increases the level of tryptophan and tyrosine in the brain[17].

1 This research was supported by CNRS grant (ERA No. 560) and Inserm grant (FRA 5).

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