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Biochemical Pharmacology, Vol. 30, pp. 539–541.

0006-2952/81/050539-03 $02.00/0

Activation of soluble striatal tyrosine hydroxylase in the rat brain after CDPcholine administration

(*Received* 30 *May* 1980; *accepted* 30 *September* 1980)

Since its introduction as an antiparkinsonian drug, cytidine 5'diphosphorylcholine (CDPcholine) has been the subject of a number of studies. The pharmacological effect of exogenous CDPcholine may be derived from a biochemical improvement of the impaired lipid metabolism in the brain. Moreover, CDPcholine increases dopamine level and decreases serotonin level in the whole mouse brain, leaving norpinephrine content unchanged [1]. We found that CDPcholine increases the level and synthesis of dopamine in the striatum and decreases the Ceveland synthesis serotonin in the brainstem of the rat brain [2]. The striatal dopaminergic activation induced by CDPcholine is correlated with the specific inhibitory uptake of dopamine in synaptosomal homogenate of rat striatum, observed *in vitro* and *in vivo* [3]. The present paper examines the *in vitro* and *in vivo* effects of CDPcholine on the activity of tyrosine hydroxylase, the rate-limiting enzyme in the synthesis of catecholamines.

Methods

Preparation of tissues. CDPcholine (SIGMA) was dissolved in saline and injected i.v. up to a total volume of 2 ml per kilogram of body weight; rats were administered different doses, and then killed by cervical dislocation at different times after administration; their brains were removed and striatum dissected according to the method of Glowinski and Iversen [4]. Brain tissues were homogenized in 10 vol. (v/w) ice cold 50 mM Tris–HCl buffer pH 6.2 containing 0.2% Triton × 100 using a Glass–Teflon homogenizer (clearance 0.025 cm). The homogenates were centrifuged at 40,000 *g* for 20 min at 4° and the supernatant fluid was gently stirred to obtain a uniform suspension, the source of soluble tyrosine-hydroxylase.

Assay in vitro. Tyrosine-hydroxylase activity was assayed *in vitro* by measuring the formation of tritiated water from 3,5 ditritiotyrosine, as described by Nagatsu *et al.* [5] and modified by Levitt *et al.* [6]; standard incubation mixture contained 0.5 to 1.0 mg protein, 15 μM ^{3}H-tyrosine, 1.1 mM 2-amino-4 hydroxy-6-7-dimethyltetrahydropteridine ($DMPH_4$), 50 mM 2-mercaptoethanol, 0.435 mM $FeSO_4$ in a Tris–acetate 0.11 mM pH 6.1 buffer, and CDPcholine at different concentrations up to a final volume of 300 μl. Incubation was started by shaking at 37° and lasted for a period of 20 min. The reaction was stopped by addition of 50 μl acetic acid; the mixture was centrifuged at 5000 *g* for 5 min and 300 μl of the supernatant were placed on to a Dowex 50 H^+ form column (4 ml of a melanger with water 1/1 v/v). 1 ml of H_2O was run through the column prior to use) and washed with 3 × 600 μl water. The effluent and washings were collected into a scintillation vial and 10 ml Unisolve® solution added. Radioactivity was determined by scintillation counting. Protein in the soluble suspension was estimated by the method of Lowry *et al.* [7] and results of tyrosine hydroxylation determination were expressed as nmoles H_2O formed/hour/mg protein.

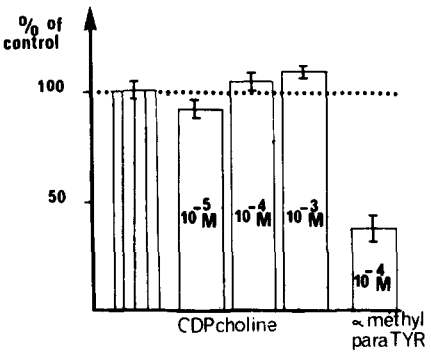

Fig. 1. The *in vitro* effect of CDPcholine on striatal tyrosine hydroxylase measured *in vitro*. CDPcholine (10^{-5} to 10^{-3} M) was added to standard incubation mixture containing 0.5 to 1.0 mg protein of the rat striatum homogenate, 15 μM [^{3}H]tyrosine, 1.1 mM $DMPH_4$, 50 mM 2 mercaptoethanol, 0.435 mM $FeSO_4$, in a Tris–acetate 0.11 mM pH 6.1 buffer. After 20 min incubation at 37° the reaction was stopped by addition of 50 μl acetic acid. The tritiated water formed from 3,5-ditritiotyrosine was separated through a Dowex 50 H^+ form column. α-Methyl paratyrosine (10^{-4} M) was used as the reference inhibitor. Results are expressed as percentage of control values.

Assay in vivo. The *in vivo* effect of CDPcholine on tyrosine hydroxylase activity has been developed according to the combination of *in vitro* and *in vivo* techniques of Levitt *et al.* [6]. CDPcholine was dissolved in saline and injected i.v. into Wistar male rats (180–200 g). After suitable intervals, the animals were decapitated; the brains were removed and dissected and the striatum homogenized as described in section "preparation of tissues". Tyrosine hydroxylase activity was determined as described in section "assay *in vitro*" except that CDPcholine was not added *in vitro*. Protein was estimated by the method of Lowry *et al.* [7].

Results

In initial experiments we tried to determine whether CDPcholine modifies striatum tyrosine hydroxylase activity when added *in vitro*. The data in Fig. 1 show that CDPcholine is unable to modify tyrosine hydroxylase activity when added *in vitro* at different concentrations (10^{-6} to 10^{-3} M). At a 10^{-4} M concentration, α methyl paratyrosine, the reference inhibitor, causes a 73 per cent inhibition in tyrosine hydroxylase activity. The V_{max} control value was 3.0 nmoles/mg prot/hour and results are expressed as percentage of control values. The time course of tyrosine hydroxylase activity after CDPcholine administration *in vivo* under our experimental conditions is shown in Fig. 2(a). A simple injection of 50 mg/kg/i.v. CDPcholine caused striatal tyrosine hydroxylase activity to increase by 12, 24 and 25 per cent after ½ hr, 1 hr and 2 hr times of action. One hour after administration, CDPcholine 10–30 and 100 mg/kg/i.v. induced a 20 per cent increase in tyrosine hydroxylase activity (Fig. 2(b)). A lower dose (5 mg/kg/i.v.) induced no significant increase, an account of the larger variations observed between the animals of the same groups. The increase in tyrosine hydroxylase activity following CDPcholine administration could not be blocked by treating rat concurrently with atropine sulfate 40 mg/kg/intraperitoneally (Fig. 2(c)) contrary to choline [8]. This muscarinic antagonist had no effect on striatal tyrosine hydroxylase activity when injected alone.

Tyrosine hydroxylase has previously been shown to be readily influenced by numerous substances which compete either with tyrosine or pteridine co-factor. In order to examine the kinetic properties of the striatal enzyme in control and CDPcholine treated animals (50 mg/kg/i.v./1 hour) the K_m and V_{max} of tyrosine and pteridine ($DMPH_4$) were determined. As shown in Fig. 3, the K_m for tyrosine and pteridine are the same in both control and CDPcholine-treated animals. The V_{max} values are for both substrate and co-factor have increased by 20 per cent in the striatum of CDPcholine-treated rats. The use of $DMPH_4$, which is not the endogenous co-factor, gives kinetical parameters different from those obtained with BH_4 (natural co-factor) but allows determination of drug induced alterations.

Discussion

It has been found that CDPcholine is able to stimulate the striatal dopamine synthesis [2] and to inhibit the reuptake of striatal tritiated dopamine *in vitro* and after CDPcholine administration to the animals [3]. Several authors have suggested that the pharmacological effect of exogenous CDPcholine might be derived from a biochemical improvement of the impaired lipid metabolism in the brain [1]. In an attempt to elucidate the biochemical mechanism of action of exogenous CDPcholine, we decided to investigate the *in vitro* and *in vivo* effects of CDPcholine on striatal tyrosine hydroxylase activity in the rat brain. We used a simple method developed for estimating the *in vivo* effect of CDPcholine on tyrosine hydroxylase activity by a combination of *in vitro* and *in vivo* techniques [6]. The validity of this approach was first demonstrated with α methylparatyrosine and with respect to co-factor antagonists.

This study indicates that *in vitro* CDPcholine is unable to modify rat striatal tyrosine hydroxylase activity. Administered to the animals, CDPcholine induces a time dependent striatal tyrosine hydroxylase activation. The high $DMPH_4$ level used could suppress CDPcholine alteration induced *in vitro*; however this does not occur after CDPcholine administration with the same $DMPH_4$ level. So, the hypothetic *in vitro* activation by CDPcholine seems to be very doubtful.

CTP increase striatal tyrosine hydroxylase *in vitro* [9] and choline administration activates rat striatal tyrosine

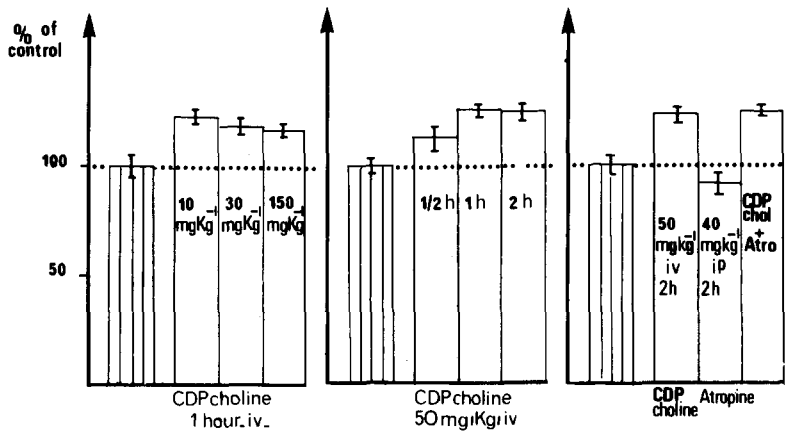

Fig. 2. The *in vivo* effect of CDPcholine on striatal tyrosine hydroxylase measured *in vitro*. (a) Time course of the increase in striatal tyrosine hydroxylase activity after CDPcholine administration (50 mg/kg/i.v.) At different times there after groups of 5 control and CDPcholine treated animals were killed and enzyme activity was assayed as described in Methods. Results are expressed as per cent of respective controls ± SEM (vertical bars). This '100' represents enzyme activity in controls which was as 2.1 ± 0.3 nmoles/hr/mg prot. (b) Activation of striatal tyrosine hydroxylase after administration of different doses of CDPcholine. Results are expressed as per cent of controls ± SEM. (c) Effect of CDPcholine, atropine and CDPcholine + atropine on tyrosine hydroxylase activity. Two hours there after groups of 5 control, CDPcholine treated (50 mg/kg/i.v.), atropine treated (40 mg/kg/ip) or CDPcholine + atropine treated animals were killed and striatal tyrosine hydroxylase activity was assayed as described in Methods. Results are expressed as percentage of respective controls ± SEM.

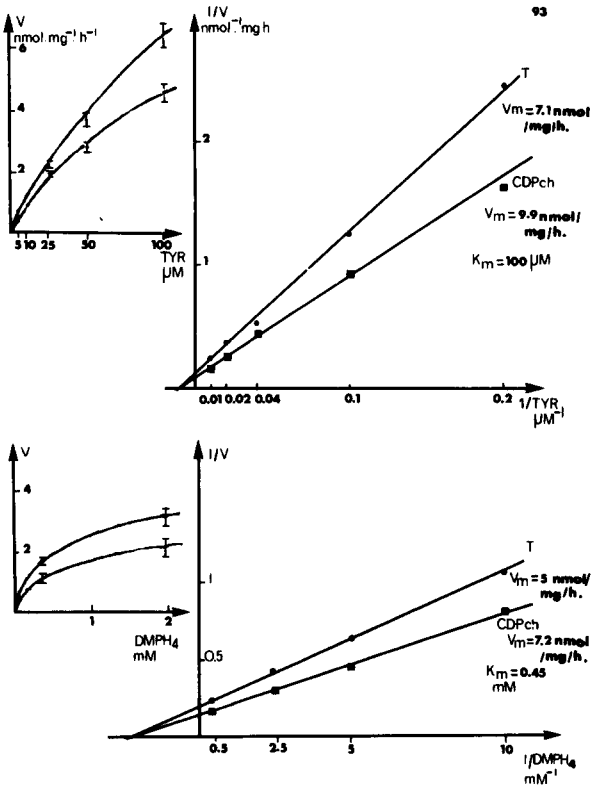

Fig. 3. Kinetic comparison between control and CDPcholine treated rats striatal tyrosine hydroxylase. Two hours thereafter groups of 5 control and CDPcholine-treated animals (50 mg/kg/i.v.) were killed and enzyme activity was assayed in the presence of various concentrations of L-tyrosine in the incubation medium ($DMPH_4$ fixed at 1 mM) or in the presence of various concentrations of $DMPH_4$ in the incubation medium (L-tyrosine fixed at 100 μM).

hydroxylase by elevating the acetylcholine content [8], suggesting that CDPcholine is metabolized *in vivo* and that a metabolite (nucleoside or choline) exercises its activating effects. However the choline induced increase in tyrosine hydroxylase activity is antagonized by atropine (a muscarinic blocker) [8], but not CDPcholine effects. Moreover, the muscarinic induced increase in tyrosine hydroxylase activity is not observed in the striatum, but in the locus coeruleus and lower brainstem, and it needs several days [10]. The activating response is induced by increasing the V_{max} without any change in the K_m for the substrate or the co-factor [11].

It appears that CDPcholine effects are dissociated from a cholinergic action. It is well known that tyrosine hydroxylase according to its localization, exists in two physical forms, a soluble and membrane bound form (particulate); the membrane-bound form predominating in the striatum has a greater affinity for the competitive feedback inhibitors, norepinephrine and dopamine [12]. Phosphorylation of the hydroxylase increases its activity 2-fold and is associated with an increase in V_{max} without any change in K_m for either substrate or co-factor [13]. The authors suggest that the pool of a native tyrosine hydroxylase might be composed of a mixture of enzyme molecules in both active and probably inactive forms. Moreover, they conclude that cholinergic stimulation of central noradrenergic neurons can produce a prolonged increase in the activity of tyrosine hydroxylase as a consequence of a change in the catalytic activity rather than the number of enzyme molecules, suggesting that such mechanisms may be operative in brain.

We propose two types of action for CDPcholine. First a dishinhibition of striatal tyrosine hydroxylase, because CDPcholine increases the release and inhibits the reuptake of dopamine [3]. A second hypothesis may be an activation of striatal tyrosine hydroxylase by inducing an active physical form. The pharmacological impact could be a participation in the lipid metabolism. This vision is conforted by the fact that acetylcholine increases phosphatidylcholine metabolism in the rat brain.

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