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European Journal of Pharmacology 344 (1998) 149–152

ejp

Short communication

Effects of CDP-choline administration on brain striatum platelet-activating factor in aging rats

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Received 18 November 1997; revised 6 January 1998; accepted 9 January 1998

Abstract

Cytidine 5′-diphosphocholine (CDP-choline) is a precursor in platelet-activating factor (PAF) biosynthesis and it is used in the treatment of diseases of the central nervous system. PAF levels in the striatum of aged (19 months) rats were 67% lower than those found in young (2 months) animals. Chronic treatment of aged rats with CDP-choline (500 mg/kg per day) reduced these PAF levels by more than 65% with respect to those of untreated aged rats after 8 days of treatment. PAF subsequently stabilized at these low levels as treatment continued. These results suggest that some effects of CDP-choline could be mediated by changes in brain PAF levels. © 1998 Elsevier Science B.V.

Keywords: Aging; Brain ischemia; CDP-choline (cytidine 5′-diphosphocholine); PAF (platelet-activating factor); Striatum

1. Introduction

Cytidine 5′-diphosphocholine (CDP-choline) is widely used in the treatment of various diseases of the central nervous system (Weiss, 1995). More recently, evidence has accumulated on its beneficial actions in ischemic brain injury (D'Orlando and Sandage, 1995). Administration of CDP-choline prevents the breakdown of phosphatidylcholine that occurs in some cerebral diseases (Arrigoni et al., 1987) and affects the metabolism and release of certain neurotransmitters (Lopez G.-Coviella et al., 1986) and neurotransmitter receptors (Giménez et al., 1991).

Orally administered CDP-choline is hydrolyzed in the intestine and absorbed rapidly as choline and cytidine, which are then incorporated into their respective cellular pools (Lopez G.-Coviella et al., 1995). These products are precursors in the synthesis of endogenous CDP-choline and hence of phosphatidylcholine and other membrane or intracellular phospholipids (Lopez G.-Coviella and Wurtman, 1992). As CDP-choline is also a direct precursor of PAF (platelet activating factor) synthesis in the brain through the de novo pathway (Snyder, 1995), we investigated if CDP-choline administration could affect ether lipid metabolism and brain PAF levels. This endogenous phospholipid has proinflammatory, hemostatic and vasoactive properties. It also acts as a messenger in the nervous system (Lindsberg et al., 1991) by modulating neurotransmitter release and other aspects of neuronal function and development (Kornecki and Ehrlich, 1988).

2. Materials and methods

2.1. Animals and drug treatments

Female Sprague–Dawley rats (Criffa, Barcelona, Spain) were housed at constant temperature (21°C), relative humidity (60%) under a 12/12-h light/dark cycle, with free access to food and water. The animals were assigned randomly to one of two dietary groups and fed on either regular chow (control) or chow supplemented with 100 or 500 mg/kg per day of CDP-choline (Ferrer Internacional, Barcelona, Spain) for the indicated number of days. In all cases, the animals were killed at 19 months of age. A

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group of untreated 2-month-old rats kept under the same conditions was used for comparison. The experiments were carried out under approval of the institutional ethics committee.

2.2. PAF extraction and assay

For the measurement of PAF levels, the brain striatum was rapidly removed after decapitation. The corpus striatum was immersed in cold methanol:water:acetic acid mixture (49:50:1), weighed and homogenized. Lipid extraction was performed according to the procedure of Bligh and Dyer (1959). Extracts were pooled and evaporated to dryness under a stream of N_2 and stored at $-20°C$ until analysis. To check the overall recovery of PAF, 0.01 μCi of [^{3}H]PAF (132 Ci/mmol, New England Nuclear, UK) was added to the homogenates. The samples were dissolved in chloroform:methanol (2:1) and subjected to thin-layer chromatography by the procedure of Bussolino et al. (1988). The efficiency of lipid extraction was 96%. PAF, lyso-phosphatidylcholine and sphingomyelin (Sigma, St. Louis, MO, USA) were used as standards.

The lipid material of the silica corresponding to PAF in the chromatography plates was extracted three times with chloroform:methanol (1:2) for 20 min at room temperature and the extracts were evaporated and stored as indicated above.

PAF was detected in the presence of indomethacin by aggregation of washed rabbit platelets obtained by the procedure of Lalau-Keraly et al. (1984). The buffer used was a Tyrode's buffer containing 2.6 mM KCl, 1 mM $MgCl_2$, 137 mM NaCl, 12 mM $NaHCO_3$, 5.5 mM glucose, 0.23 mM EGTA and 0.25% gelatin (pH 6.5). Before aggregation, platelets were resuspended in Tyrode's buffer of the same composition but without EGTA (pH 7.4). In aggregation reactions, the following concentrations were used: 1.3 mM for $CaCl_2$ and 10 μM for indomethacin.

The amount of PAF was calculated from a calibration curve of standard PAF (0.01 to 0.15 nM) recorded for each test and routinely expressed in pmol/g of tissue. The specificity of platelet aggregation was confirmed by the inhibitory effect of the specific PAF receptor antagonist CV-6209 (Takeda, Osaka, Japan).

2.3. Data analysis

The results are expressed as means ± S.D. Data were analyzed using a Kruskal–Wallis one-way analysis of variance (ANOVA) followed by Dunnett's test.

3. Results

The reported age-related decrease of PAF in laboratory animals (Tokumura et al., 1992) led us to establish the PAF levels under our experimental conditions in two groups of rats of 2 and 19 months of age. Our determination of striatum PAF concentration for young rats was 62.8 ± 9.6 pmol/g of tissue ($n = 4$), close to the values reported in the literature (Yue et al., 1990; Tokumura et al., 1992). The concentration of PAF in the striatum of aged rats (19 months old) was statistically different ($P < 0.05$) from the values found in young animals (Table 1).

Table 1
PAF levels in rat brain striatum after oral treatment with different doses of CDP-choline

Animal group	CDP-choline (mg/kg)	PAF levels (pmol/g)
Young	–	62.8 ± 9.6[a]
Aged	–	20.8 ± 7.2
Aged	100	19.5 ± 2.6
Aged	500	3.2 ± 3.0[a]

The data are means ± S.D. obtained from animals ($n = 4$) aged 2 and 19 months (young and aged, respectively). Determinations were performed in duplicate. The CDP-choline was administered daily for two months.
[a] $P < 0.05$ in Dunnett's test vs. untreated aged group.

The effects of CDP-choline on striatum PAF levels were investigated by using two different doses of this compound. With the lower dose of CDP-choline (100 mg/kg), PAF levels were not statistically different from those of the untreated aged animals after long-term (2 months) treatment, whereas with the higher dose (500 mg/kg) a statistically significant decrease ($P < 0.05$) was observed (Table 1). PAF levels were also expressed in terms of the inorganic phosphate or protein content as is normally done in cell culture experiments (Sogos et al., 1990). The inorganic phosphate or protein content in the tissue samples used for PAF analysis did not change significantly during the treatment period (not shown). Consequently, differences in PAF concentration remained un-

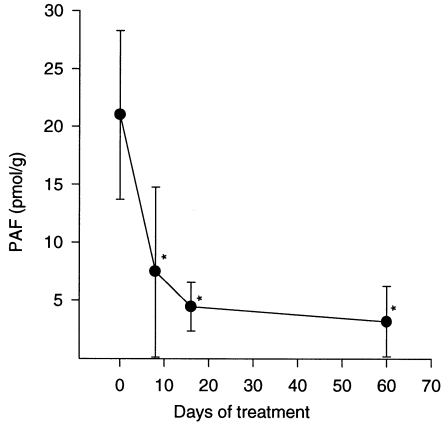

Fig. 1. Time-course of changes in PAF level in the striatum of 19-month-old rats is shown after various days of oral treatment with 500 mg/kg per day of CDP-choline. All data are expressed as means ± S.D. ($n = 4$). Determinations were performed in duplicate. * $P < 0.05$ in Dunnett's test vs. untreated aged group.

changed when the values were expressed as a function of these variables.

The effect of CDP-choline administration on PAF levels was further studied by determining the PAF content at different times after the beginning of the treatment. To this end, a dose of 500 mg/kg was given to the animals at different starting dates and the animals were killed at the same time. Thus, samples were obtained for animals of the same age that had been treated for different periods. Samples were then processed as indicated above for the PAF determination. PAF levels in the striatum of rats showed a rapid and statistically significant decrease (60 to 65%) after 8 days of CDP-choline administration, followed by stabilization at a lower steady-state level as treatment continued (Fig. 1).

4. Discussion

The component phospholipids of neural membranes are important sources of bioactive mediators that participate in formative and reparative processes. One of these mediators has been identified as PAF, which in accordance with its particular role in brain development gradually decreases in an age-related manner to adult levels. The PAF levels determined in our experiments with aged rats are indeed consistent with this change.

The brain distribution of PAF has proved difficult to study and large variations in PAF or its derivative lyso-PAF concentrations have been reported in different brain areas (Tiberghien et al., 1991). For this reason and to reduce ambiguity, our studies were performed with the striatum, a PAF-receptor-rich area of the brain (Domingo et al., 1988) where the increase in lipid labeling upon administration of labeled CDP-choline is particularly relevant (De Medio et al., 1984).

As shown in our experiments, CDP-choline administration already decreased PAF levels after a few days of treatment. Such a short-term effect reinforces the therapeutic potential of CDP-choline. In brain damage secondary to a stroke or to brain trauma, PAF or related compounds accumulate and the decrease caused by CDP-choline may suppress inflammation and reverse the pathological response. It is thus proposed that these modulations of PAF level may participate in the mechanism of some of the pharmacological effects of CDP-choline.

Several phenomena may be invoked to explain the decrease in PAF following the administration of CDP-choline. Increased CDP-choline levels may activate intracellular PAF acetylhydrolase (EC 3.1.1.48), which catalyzes the hydrolysis and inactivation of PAF. Furthermore, PAF transacetylase and lysoPAF transacylase, CoA-independent activities, could also be stimulated, which according to Snyder (1995) could lead to the channeling of PAF to PAF plasmalogen analogues. Alternatively, the increase in CDP-choline may enhance its utilization through other competing pathways. Mechanisms of induction or metabolic activation of choline phosphotransferase (EC 2.7.8.2), leading to the synthesis of phosphatidylcholine or membrane ether lipids (acyl ether lipids) from CDP-choline, could be responsible for such an effect.

Acknowledgements

The authors thank Isabel Guirao Pérez for technical assistance. This work was supported by a grant of the 'Centro de Investigación del grupo Ferrer' of Barcelona, Spain, and by the help of the 'Comissionat per Universitats i Recerca de la Generalitat de Catalunya'.

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