EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Inhibition of Lipid Peroxidation in Synaptosomes from Rat Brain by Peptide and Protein Fraction Isolated from Fetal Human Brain

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 122, No. 8, pp. 233-235, August, 1996
Original article submitted April 6, 1995

Peptide and protein fractions isolated from fetal human brain inhibit with different efficiency nonenzymatic lipid peroxidation in synaptosomes from rat brain. These fractions reciprocally capture the superoxide anion radicals generated in the reaction of 6-hydroxydopamine autooxidation.

Key Words: *rat brain synaptosomes; lipid peroxidation; peptides and proteins of fetal brain*

During the last two decades, human fetal tissues have been beneficially used for therapy of such widespread and severe disorders as Parkinson's disease, insulin-dependent diabetes, and various immunological pathologies [4]. Despite periodic ethical problems, the method of fetal transplantation gains wide acceptance in modern medicine. In light of this a question arises concerning pharmacologically active substances which are present in fetal tissues and are responsible for their therapeutic effect.

Since transplantation of fetal material has a positive effect in diverse pathological states and diseases, one of the active component may be a substance or a group of related compounds affecting the biochemical processes which are common for many diseases. Lipid peroxidaton (LPO), which is known to be activated in various pathologies, may be one of these processes [1,2,5]. In light of this, we explored the ability of peptide (PF) and protein (PrF) fractions from fetal brain to inhibit LPO in membrane preparations and to capture the superoxide anion radical.

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MATERIALS AND METHODS

PF and PrF were isolated from fetal brain as described in Invention Notice No. 95107496 (May 17, 1995). Synaptosomes were isolated from the gray substance of Wistar rats as described elsewhere [6]. The protein concentration was determined after Bradford [3].

LPO was initiated with Fe^{2+} and ascorbate in concentrations of 10 and 200 nmol/mg protein, respectively, and evaluated by the formation of 2-thiobarbituric acid-reactive substances [1]. The superoxide anion radicals were generated in the reaction of autooxidation of 6-hydroxydopamine; the reaction was initiated by adding the greater volume of the solution (pH 7.4) to the lesser volume containing all necessary ingredients (pH 4.0), the concentration of dopamine being 0.1 mM.

6-Hydroxydopamine, superoxide dismutase (activity 3.3 U/μg), and thiobarbituric acid were from

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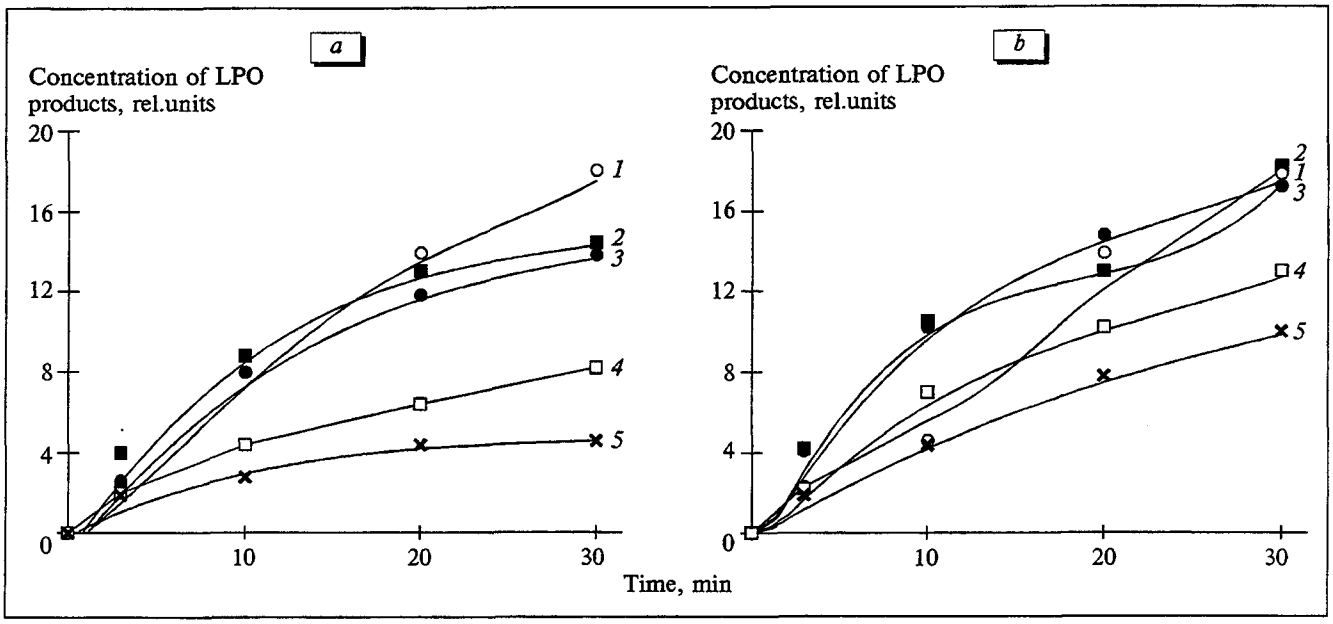

Fig. 1. Kinetics of generation of LPO products in synaptosomes from rat brain without addition (*1*) and in the presence of peptides (*a*) and proteins (*b*) from fetal brain in concentrations of 45 (*2*), 90 (*3*), 180 (*4*), and 360 (*5*) μg/ml.

Serva. Other chemicals of chemically pure or extra pure grades were manufactured in Russia.

RESULTS

The data presented in Fig. 1 show that both PF and PrF inhibit LPO in rat brain synaptosomes, the inhibiting activity of PF being much higher. Under the same conditions of LPO stimulation PF twofold more effectively inhibits the generation of LPO products than PrF at an equal maximum protein concentration.

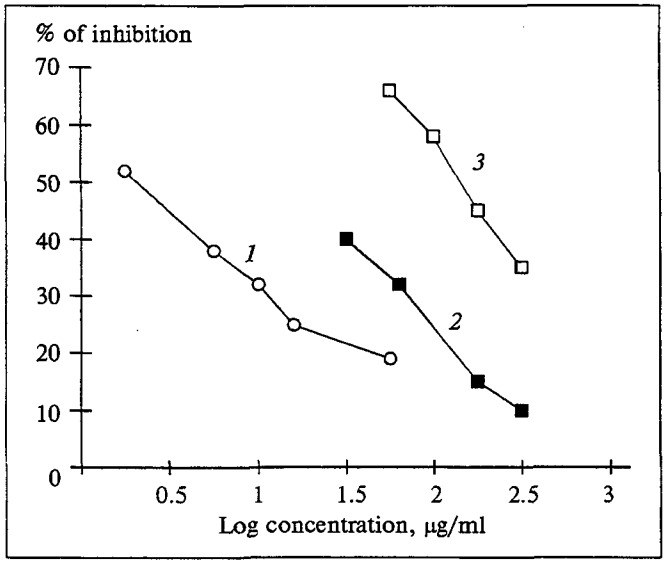

Fig. 2. Inhibition of autooxidation of 6-hydroxydopamine with superoxide dismutase (*1*), protein extract (*2*), and peptide extract (*3*) as a function of concentration.

Minor activation of LPO after the addition of PF and PrF at the early stages of LPO stimulation may result from contamination of the extracts with prooxidants such as transitory metals or reducing agents.

It should be noted that PrF more actively captures the superoxide anion radicals (Fig. 2). Since in our system of LPO-induction the intensity of LPO strongly depends on the generation of reactive oxygen species, including superoxide anion radicals, the mechanisms of LPO inhibition by PF and PrF are different.

The LPO inhibiting ability of PrF may be associated with the scavenging of superoxide anion radicals, while the inhibiting ability of PF is mediated through other mechanisms, in particular, Fe^{2+} chelating.

In any case, it can be concluded that fetal extracts inhibit LPO and capture superoxide anion radicals. Since these processes are universal and activated in various pathological states, it can be assumed that the pharmacological activity of fetal tissues is related to the inhibition of free radical processes.

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