NONYLPHENOL STIMULATES HYDROXYRADICAL FORMATION IN RAT STRIATUM

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Introduction

Considerable attention has been focused on environmental chemicals which disrupt various tissues including the reproductive systems via steroid receptor. Nonylphenol is a nonionic surfactant widely used in the world. Because of its estrogenic effects on mammalian cells in vitro and in vivo, we hypothesized that nonylphenol may affect on neuronal tissues, especially tissues which express estrogen receptor. Recent reports show that the striatum expresses estrogen receptor(1-3). In the present study we investigated the effect of nonylphenol and bisphenol A on hydroxyradical formation using the microdialysis system.

Materials and Methods

Chemicals. Nonylphenol and bisphenol A were purchased from Kanto Chemicals Co., Ltd. (Tokyo, Japan). Sodium salicylate and its hydroxylated metabolites were purchased from Sigma Chemical Co. (St Louis. MO. USA).

Animals. Adult male Wistar rats (300-400 g) were housed in an environmentally controlled room (20-25 , 50-60 % humidity) with available food and water ad libitum. The rats were anesthetized with chloral hydrate (400 mg/kg i.p.) and prepared for intracranial microdialysis brain perfusion by a method previously used. This study was approved by the Ethical Committee for Animal Experiments, Oita Medical University, Japan.

Analytical Procedures (4-6). The dialysate samples were immediately injected for analysis into an HPLC-EC equipped with a glassy carbon working electrode (EICOM CORP., Kyoto, Japan) and an analytic reverse-phase column on an Eicompak MA-5ODS column (5 mm 4.6 x 150 mm; EICOM). The working electrode was set at a detector potential of 0.75 V. Each liter in the mobile phase contained 1.5 g heptane sulfonic acid sodium salt (Sigma), 0.1 g Na2EDTA, 3 ml triethylamine (Wako, Japan) and 125 ml acetonitrile (Wako) dissolved in H2O. The pH of the solution was adjusted to 2.8 with 3 ml phosphoric acid (Wako). Nonylphenol dissolved in ethanol was dissolved in Ringer's solution containing 147 mM NaCl, 2.3 mM CaCl2 and 4 mM

KCl, pH 7.0 for perfusion (1 ml/min) through a microdialysis probe into the striatum. The microdialysis probe was pre-washed with Ringer's solution for at least 30 min prior to stereotaxical implantation in the striatum (stereotaxic coordinates: AP: 1.0, R/L: 2.5, H: -7 mm from dura matter). Thereafter for trapping _OH radicals in the striatum, sodium salicylate in Ringer's solution (0.5 nmol/ml/min) was perfused by a microinjection pump (Carnegie Medicine CMA/100 Stockholm, Sweden) and basal levels of 2,3-DHBA during a definite period time were determined. Brain dialysate (1 ml/min) was collected every 15 min into small collecting tubes containing 15 ml of 0.1 N HClO4 to prevent amine oxidation and assayed immediately for 2,3-DHBA by an HPLC-EC procedure.

Results and Discussion

The effects of nonylphenol and bisphenol A on hydroxyradical formation in rat striatum were investigated using the microdialysis system. As shown in Figure 1 A, nonylphenol (sample N) significantly stimulated hydroxyradical formation in rat striatum. On the contrary, bisphenol A (sample B) did not significantly stimulate hydroxyradical formation in rat striatum.

Because nonylphenol has an estrogenic activities, we next studied whether tamoxifen inhibits nonylphenol-induced hydroxyradical formation in rat striatum. Tamoxifen concentrations at 1, 10, 50 and 100 uM was preincubated, then nonylphenol (10 uM) was added. As shown in Figure 2, tamoxifen inhibited nonylphenol-induced hydroxyradical formation in a dose-dependent manner.

To our knowledge this is the first report that nonylphenol, but not bisphenol A, induced hydroxyradical formation in nervous tissues by microdialysis system. The effect was inhibited by tamoxifen. The data indicated that the effect was an estrogenic action via estrogen receptor. As recent report showed, the striatum expresses estrogen receptors. The molecular mechanism how nonylphenol induced hydroxyradical formation should be elucidated. Such an experiment is underway in our laboratory.

References

1 . Kuppers E. and Beyer C. (1999) Neurosci Lett 276: 95-98.
2. Lammers CH, D'Souza U, Qin ZH, Lee SH, Yajima S, Mouradian MM (1999) Synapse 34 :222-227.
3. Zhou Y, and Dorsa DM (1994) Horm Behav 28:376-382.
4. Floyd, R.A., Watson, JJ and Wong, PK (1984) J Biochem Biophys Methods. 10 : 221-235.
5. Halliwell, B, Kaur, H, and Ingleman-Sundberg, M (1991) Free Radic Biol Med 10: 439-441.
6. Obata , T and Yamanaka, Y (1995) Neurosci. Lett. 188: 13-16.

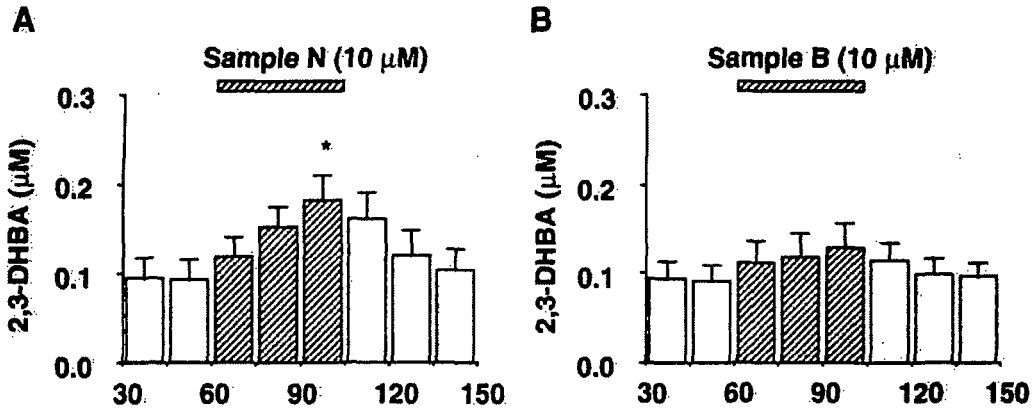

Fig. 1. The effects of nonylphenol and bisphenol on hydroxyradical formation in rat striatum

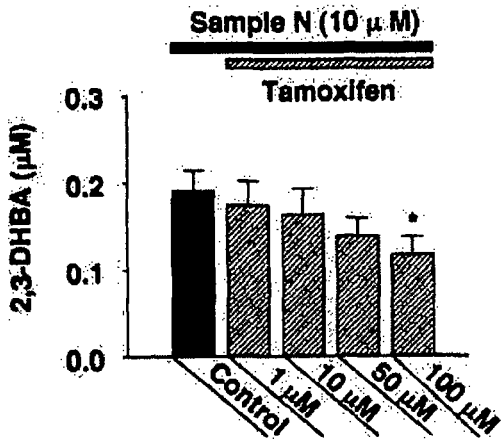

Fig.2 The effect of tamoxifen on nonylphenol-induced hydroxyradical formation in rat striatum