We investigated the estrogenic activity of a bisphenol A (BPA) metabolite (4-methyl-2,4-bis(p-hydroxyphenyl)-pent-1-ene; MBP) in male medaka (Oryzias latipes) using vitellogenin (Vg, Vg1 and Vg2) as a biomarker. Male d-rR medaka were exposed to various concentrations of estradiol-17\beta (E2), MBP and BPA for 3 days, and then the serum Vg concentration was measured using specific chemiluminescent immunooassays. The estimated relative estrogenic activities of MBP and BPA compared with E2 (100%) were 1.3–1.4% and 0.00010–0.00023%, respectively. These findings indicated that MBP has about 104-fold higher estrogenic potency than the parent BPA and about 1/50 that of E2 for Vg synthesis in medaka. This is the first study to show that MBP can act as a highly potent estrogen agonist in living organisms.

Key words —– bisphenol A metabolite, estrogenic activity, vitellogenin, 4-methyl-2,4-bis(p-hydroxyphenyl)-pent-1-ene, medaka

INTRODUCTION

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane; BPA) is a raw material that is used in the worldwide production of polycarbonate and epoxy resins. Large production volumes and consumption of BPA have led to its being detected in human biological fluids and in aquatic environments. The notion that BPA possesses weak estrogenic activity and disrupts the endocrine system is widely accepted. Yoshihara et al. recently found that 4-methyl-2,4-bis(p-hydroxyphenyl)-pent-1-ene (MBP), a BPA metabolite in the rat liver S9 fraction, has several hundred- to several thousand-fold higher estrogenic potency than BPA itself. If BPA is converted to MBP in the environment and if it negatively affects the endocrine system of living organisms, the biological risk of BPA should be reconsidered. However, only one report has described the estrogenic activity of BPA in vitro, and the estrogenic impact of MBP has not been investigated in vivo. Vitellogenin (Vg), a precursor of yolk protein in oviparous animals, is a good biomarker of estrogenomimetic activity because it is synthesized in the liver and is powerfully regulated by estrogen. Here, we evaluated the estrogenic potency of MBP in medaka in vivo using Vg as a biomarker.

MATERIALS AND METHODS

Fish and MBP Exposure —– Adult male medaka (d-rR strain) maintained in 60-l glass aquaria under a 14-hr light : 10-hr dark cycle at 25 ± 1°C were fed daily with a commercial mix of freeze dried, day-old brine shrimp (Artemia Gold; Nagasaki Rikagaku Co., Nagasaki, Japan).

Groups of 3 male fish were transferred to 2-l glass beakers containing dechlorinated tap water. Steroid and chemicals were dissolved in ethanol and added to the water at the following nominal concen-
17β-estradiol (E2; Sigma-Aldrich, St. Louis, MO, U.S.A.), 0.01, 0.05, 0.1, 0.5 and 1 ppb; MBP (synthesized and purified MBP as described by Takao et al.9), 1, 5, 25 and 50 ppb; BPA (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 100, 500, 1000, 5000 and 10000 ppb. Controls contained appropriate volumes of vehicle solvent (0.1 ml/l). The solutions were replaced every day for 3 days and the fish were not fed during exposure to chemicals. The caudal peduncle was severed and blood from the groups was collected into micro-capillary tubes (Microcaps; Drummond Scientific Co., PA, U.S.A.). Serum was separated by centrifugation at 10000 \(\times g\) for 5 min and stored at –30°C.

**Measurement of Serum Vg Concentration**

Serum concentrations of Vg1 and Vg2, were measured using specific chemiluminescent immunoassays10 as follows. Ninety-six well polystyrene luminescence immunoassay plates (LIA plates; Greiner, Frickenhausen, Germany) were coated with either anti-Vg1 or anti Vg2 IgG, and blocked with 1% BSA and 0.1% bovine γ-globulin to prevent non-specific binding. Serum samples or Vg standard were placed into the wells and incubated for 3 hr at 15°C (1st reaction). The plates were washed and acridinium-labeled F(ab')2 of anti-Vg IgG was added (2nd reaction) for 1 hr at 15°C. The chemiluminescent reaction (Reagents 1 and 2; CHIRON, Emeryville, CA, U.S.A.) and luminescence detection were controlled using a Luminescencer-JNR (ATTO, Tokyo, Japan). All assays were performed in duplicate.

**RESULTS AND DISCUSSION**

Figure 1 describes the serum Vg1 and Vg2 concentrations of male medaka exposed to various doses of E2, MBP and BPA. All chemicals at the tested concentrations dose-dependently increased the serum Vg concentration and regression curves of the 3 exposure groups were almost parallel. The LOEC (Lowest Observed Effect Concentration) values for E2, MBP and BPA on the synthesis of both Vgs were 3.7 \(\times 10^{-9}\) M, 9.2 \(\times 10^{-8}\) M and 2.2 \(\times 10^{-5}\) M, respectively. The estimated relative estrogenic activities of MBP and BPA (percentage compared with E2) calculated from the regression curves were 1.3–1.4 and 0.00010–0.00023%, respectively (Table 1). These findings showed that MBP has extremely high estrogenic activity (about 104-fold that of parent BPA, and about 1/50 of that of E2) on Vg synthesis in male medaka.

**Table 1. Estrogenic Potency of MBP on Vg Synthesis**

<table>
<thead>
<tr>
<th>Hormones or chemicals</th>
<th>Effective concentration required to induce 10 mg/ml Vg1 in serum</th>
<th>Effective concentration required to induce 5 mg/ml Vg2 in serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2</td>
<td>2.5 (\times 10^{-9}) M (100)</td>
<td>3.3 (\times 10^{-9}) M (100)</td>
</tr>
<tr>
<td>MBP</td>
<td>1.8 (\times 10^{-7}) M (1.4)</td>
<td>2.6 (\times 10^{-7}) M (1.3)</td>
</tr>
<tr>
<td>BPA</td>
<td>1.1 (\times 10^{-3}) M (0.00023)</td>
<td>3.6 (\times 10^{-3}) M (0.00010)</td>
</tr>
</tbody>
</table>

Regression formula of test chemical concentration-Vg response curve calculated from data described in Fig. 1. From these results, effective concentrations of test chemicals required to induce 10 mg/ml Vg1 and 5 mg/ml Vg2 in serum were calculated. Values in parenthesis indicate percentage of relative estrogenic activity compared with E2.
medaka. The present findings closely correlated with in vitro evaluations of estrogenic MBP activity.\textsuperscript{7)}

A recent report has indicated that not only sunlight but also fluorescent light can photolyze MBP.\textsuperscript{8)} Here, we exposed male medaka to chemicals under white fluorescent light (14 hr/day). Therefore, the estrogenic potency of MBP might be increased by repeating the present study under darkness. Yoshihara et al. considered that MBP might become metabolically activated under conditions of poor glucuronidation capacity as in rat and human fetuses, but not under normal circumstances.\textsuperscript{7)} A similar situation might be extant during the early development of lower vertebrates. Aquatic animals, fish fry or pre-metamorphous amphibians, can easily absorb BPA from water. The present study revealed an extremely potent estrogenic effect of MBP in teleosts in vivo. Therefore, MBP concentrations in these animals should be investigated during early development to enhance understanding of the estrogenic effect of BPA.

REFERENCES


