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Promoting insulin secretion in pancreatic islets by means of bisphenol A and nonylphenol via intracellular estrogen receptors

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Abstract

In this study, we investigated the effects of endocrine disrupters bisphenol A (BPA) and nonylphenol (NP) on insulin secretion from rat pancreatic islets. Following acute exposure to BPA and NP, neither BPA nor NP (0.1, 1, 10, 100 and 1000 μg/l) affected insulin secretion in concentrations of 16.7 mM glucose. However, insulin secretion following long-term exposure to BPA or NP for 24 h in 16.7 mM glucose was significantly higher than without exposure. To determine whether increased insulin secretion resulting from long-term exposure to BPA and NP is induced via intracellular estrogen receptors, we blocked the cytosolic/nuclear estrogen receptors, using actinomycin-D (Act-D), an inhibitor of RNA synthesis, and ICI 182,780 (ICI), an estrogen receptor inhibitor. Following long-term exposure to BPA (10 μg/l) or NP (10 μg/l), Act-D or ICI treatment eliminated the facilitation of insulin secretion. In conclusion, we have demonstrated for the first time that long-term exposure to endocrine disrupters, such as BPA and NP, promotes in vitro insulin secretion from the pancreatic islets, via cytosolic/nuclear estrogen receptors.

Keywords: Endocrine disrupters; Estrogen; Insulin; Pancreatic islets; Rat

1. Introduction

Endocrine disrupters have recently received considerable attention (Sumpter et al., 1996; Mori et al., 2003). Many studies have shown that some endocrine disrupters have estrogenic effects (Stenchever et al., 1981; Soto et al., 1991; Steinmetz et al., 1997; vom-Saal et al., 1997; Sheahan et al., 2002; Mori et al., 2003; Inui et al., 2003; Adachi et al., 2004). Although such xenoestrogens present estrogenic effects, however, their chemical structures do not necessarily resemble those of steroid hormones.

Bisphenol A (BPA), which is a monomer of polycarbonate plastics, and a constituent of epoxy and polystyrene resins that are extensively used in the food-packaging industry and in dentistry (Brotons et al., 1995; Steinmetz et al., 1997; Biles et al., 1999), has been reported to have estrogenic activity (Krishnan et al., 1993). There is concern that BPA may melt when sterilized at high temperatures. Indeed, it has been reported

Abbreviations: Act-D, actinomycin D; BPA, bisphenol A; DMSO, dimethyl sulfoxide; E_2, 17β-estradiol; ER, estrogen receptor; ICI, ICI182,780; KRBB, Krebs-Ringer bicarbonate buffer; NP, nonylphenol; PCR, polymerase chain reaction; RT-PCR, reverse transcription and PCR.

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that BPA is released from polycarbonate flakes during autoclaving (Krishnan et al., 1993). Nonylphenol (NP) has been used in detergents, paints, pesticides, and personal care products (Jobling and Sumpter, 1993; Kvestak and Ahel, 1994; Jobling et al., 1996; Routledge and Sumpter, 1996). NP also has estrogenic activity (Soto et al., 1991; Harries et al., 1997). A comprehensive monitoring study of the measurement of NP and NP ethoxylates levels has been performed (Iguchi et al., 2002; Wenzel et al., 2004).

Estrogenic activity has attracted public attention, because it is related to reproductive tract development, including sexual differentiation. Most studies have been in regard to the reproductive tract (Harries et al., 1997; Nagao et al., 2000; Folmar et al., 2002; Takao et al., 2003). It has been reported recently that insulin secretion from isolated mouse pancreatic β cells increases following exposure to 17β-estradiol (E_2) (Nadal et al., 2000, 2004). It is well known that estrogen can act via the cytosolic/nuclear estrogen receptors ERα (Koike et al., 1987; Malayer and Gorski, 1993; Parker et al., 1993) and ERβ (Kuiper et al., 1996; Mosselman et al., 1996; Kuiper and Gustafsson, 1997; Petersen et al., 1998). However, there has been growing evidence of the non-genomic effects of estrogen, characterized by their rapid time course: from a few seconds to 2–10 min after estrogen application (Nadal et al., 2000, 2004). In the present study, we examined the effects of acute and long-term exposure to endocrine disruptors BPA and NP on insulin secretion from rat pancreatic islets via cytosolic/nuclear and plasma membrane estrogen receptors.

2. Materials and methods

2.1. Chemicals

BPA, NP, E_2 and actinomycin-D (Act-D) were purchased from Sigma–Aldrich (St. Louis, MO, USA). ICI 182,780 (ICI) was purchased from Tocris Cookson Limited (Avon-mouth, UK). All other routine reagents were of analytical grade and were obtained from Sigma–Aldrich (St Louis, MO, USA) or Nacalai Tesque (Kyoto, Japan).

2.2. Animals

Wistar rats at eight weeks of age were used for all studies. The rats were housed individually in an air-conditioned room at an ambient temperature of 23 °C, with a 12 h light/dark cycle. The rats were fed a normal diet (CE-2, Crea Japan, Tokyo, Japan) and water ad libitum. All animal care procedures were complied with the Kyoto University Guidelines for Animal Experiments.

2.3. Isolation of pancreatic islets

Twelve male rats were anesthetized with 50 mg/kg pentobarbital. For each experiment, 5000 islets were isolated from the pancreases of rats by the collagenase digestion method (Gotoh et al., 1985), followed by purification, using Ficoll gradients. Five thousand islets were used in each experiment, as described below.

2.4. Expression of estrogen receptor in islets

Total RNA of islets was extracted by means of a commercial kit (RNeasy microkit; Qiagen, Valencia, CA, USA). Reverse transcription of total RNA from islets was performed using reverse-transcriptase (Superscript II, Invitrogen Corp., Carlsbad, CA, USA). Polymerase chain reaction (PCR) was performed using ExTaq DNA polymerase (Takara Bio, Kyoto, Japan). The primer pairs were as follows; estrogen receptor (ER) α, sense primer 5′-GAGATCCTGATGATTGGTCT-3′, antisense primer 5′- GGAGTTCTCAGATGGTGTTGG-3′ (643 bp; 1377-2019) (GenBank ID; S52128); ERβ, sense primer 5′-CATCAGTAACAAGGGCATGG-3′, antisense primer 5′-CACTGAGACT GTAGGTTCTG-3′ (192 bp; 1682-1873) (GenBank ID; U57439). The thermal cycling parameters were 3 min at 94 °C in the initial denaturation step, followed by 30 cycles of 30 s at 94 °C, of 30 s at 60 °C, and of 30 s at 72 °C. The final extension step was for 7 min at 72 °C. PCR products were resolved by 1.5% agarose gel electrophoresis. DNA was stained with ethidium bromide for fluorescence detection.

2.5. Measurement of insulin release from islets by acute and long-term exposure to BPA, NP and E_2, with or without Act-D and ICI

2.5.1. Acute exposure

Islets were preincubated at 37 °C for 30 min in Krebs-Ringer bicarbonate buffer (KRBB), composed of 129.4 mM NaCl, 5.2 mM KCl, 2.7 mM $CaCl_2$, 1.3 mM KH_2PO_4, 1.3 mM $MgSO_4$, and 24.8 mM $NaHCO_3$ (gassed with 95% O_2–5% CO_2), supplemented with 10 mM HEPES, 2.8 mM glucose, and 0.2% (w/v) bovine serum albumin. In the assay for genomic action, the final concentration of 4 μM Act-D or 1 μM ICI was added when islets were preincubated under the conditions described above. Ten islets were then batch-incubated for 60 min at 37 °C in KRBB with the test materials: BPA (0.1, 1, 10, 100, 1000 μg/l), NP (0.1, 1, 10, 100, 1000 μg/l), E_2 (0.01, 0.1, 1, 10, 100 μg/l) and solvent DMSO only (final concentration 0.1%), with glucose (8.3, 16.7 mM). Insulin levels were assayed by radioimmunoassay, using rat insulin (Novo Nordisk, Copenhagen, Denmark) as standard (Tsuji