



# Effect of antisense oligodeoxynucleotide for sepiapterin reductase on the viability of PC12 cells in the presence of exogenous carbonyl compounds

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## Abstract

Sepiapterin reductase (SPR) is known as an essential enzyme for the biosynthesis of tetrahydrobiopterin. SPR belongs to the short-chain dehydrogenase/reductase (SDR) family and also reduces various exogenous carbonyl compounds including phenylpropanedione. We found in the present study that phenylpropanedione decreased the rate of proliferation of PC12 cells and that this rate was further diminished by the transfection of the cells with antisense oligodeoxynucleotide for SPR mRNA. When the cells were treated with *N*-acetylserotonin, a specific inhibitor of SPR, in the presence of phenylpropanedione, the cell number decreased to almost the same level as when the cells were transfected with the antisense oligodeoxynucleotide. Thus, the SDR activity of SPR in PC12 cells may serve for detoxification of exogenous carbonyl compounds besides functioning as a specific enzyme for the formation of tetrahydrobiopterin.

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## 1. Introduction

Exogenous carbonyl compounds are generally quite toxic for cells and there are a number of skillfully designed strategies to protect cells against them. Many enzymes belonging to the SDR family are thought to be able to function as protectors in the cell by reducing various carbonyl compounds

with NAD(P)H. PC12 cells have been used as a model of catecholaminergic neurons in culture. For producing catecholamines, these cells require tetrahydrobiopterin (BH4), a natural cofactor synthesized from GTP by three enzymes, one of which is sepiapterin reductase (SPR; Fig. 1). This enzyme, however, can reduce with NADPH various exogenous carbonyl compounds [1–3] besides pteridine derivatives and belongs to the SDR family, based on its molecular weight and amino acid sequence [4–6]. In this study, 1-phenyl-1,2-propanedione (phenylpropanedione), an exogenous carbonyl compound, was added to the cells to

*Abbreviations:* BH4, tetrahydrobiopterin; SDR, short-chain dehydrogenase/reductase; SPR, sepiapterin reductase.

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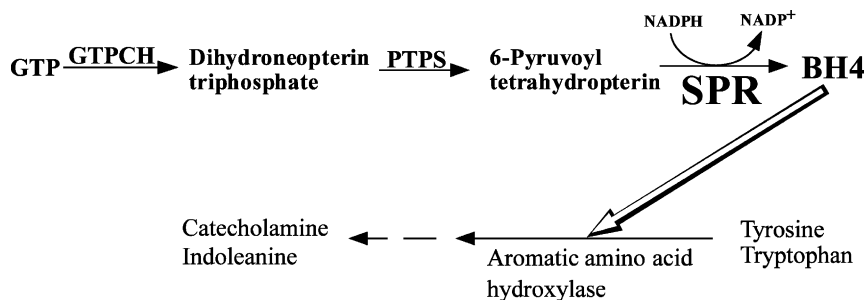


Fig. 1. Functions of sepiapterin reductase and tetrahydrobiopterin. GTPCH, GTP cyclohydrolase I; PTPS, pyruvoyl-tetrahydropterin synthase; SPR, sepiapterin reductase; BH4, tetrahydrobiopterin.

examine the effect of antisense oligodeoxynucleotide for SPR on cell viability.

## 2. Materials and methods

PC12 cells were obtained from the Riken Cell Bank (Tsukuba, Japan) and maintained and sub-cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum and 10% horse serum. Antisense oligodeoxynucleotide (Anti-Oligo) for SPR mRNA, which corresponded to the AUG translational start site and 25 bases 3' to that site; sense oligodeoxynucleotide (Sense-Oligo); and the inverted oligodeoxynucleotide of Anti-Oligo (Inv-Oligo) were synthesized. PC12 cells were plated in six-well plates in DMEM. Then the cells were transfected for 3 h with Anti-Oligo, Sense-Oligo or Inv-Oligo in serum-free medium by use of Lipofectamine, after which the transfected cells were cultured for up to 10 days. Phenylpropanedione was mixed with DMEM and the conditioned medium was replaced with the phenylpropanedione-containing medium after the transfection with the oligodeoxynucleotides. Cell number, total biopterin and BH4 contents [7] and the SPR activity [8] in the cells were monitored every day.

## 3. Results and discussion

PC12 cells were cultured with the medium containing phenylpropanedione (50  $\mu$ M) for up to 10 days (Fig. 2). During 10 days' incubation, the

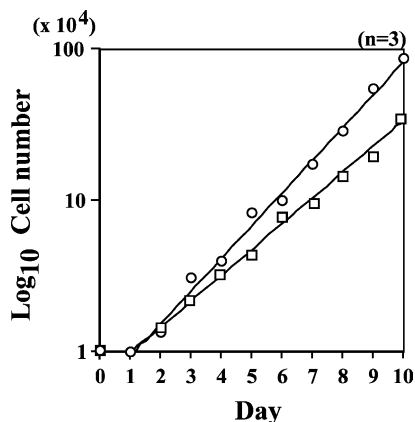


Fig. 2. Effect of phenylpropanedione on the number of PC12 cells. Cells were cultured in the phenylpropanedione-free medium; (open circle) or in the phenylpropanedione-containing medium (open box).

cell number decreased to  $\approx 41\%$  of that in cultures containing the phenylpropanedione-free medium (Fig. 2), suggesting that phenylpropanedione was cytotoxic toward PC12 cells.

Since SPR belongs to the SDR family [4–6] and phenylpropanedione is a good substrate of SPR [1–3], we examined the effect of Anti-Oligo for SPR on the viability of PC12 cells in the presence of phenylpropanedione. About 80% of the total biopterin content in PC12 cells, during 10 days' incubation in the presence of phenylpropanedione, was BH4 (Fig. 3A, B). When the cells were transfected with the Anti-Oligo, both contents of total biopterin and BH4 began to decrease after the transfection (Fig. 3A, B) and the SPR activity was almost completely inhibited at 6–10 days (Fig.

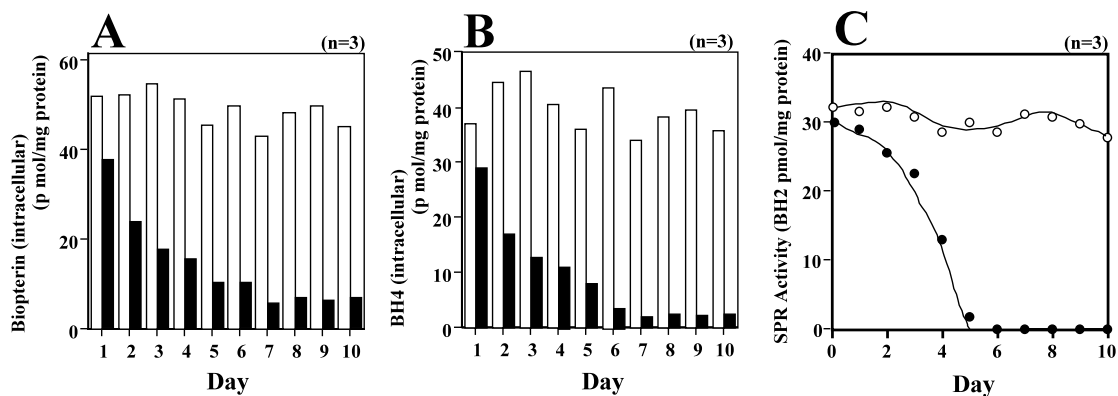


Fig. 3. Intracellular contents of total biopterin and BH4, and SPR activity after the treatment of PC12 cells with Anti-Oligo in the presence of phenylpropanedione. (A) Total biopterin content, (B) BH4 content and (C) SPR activity. White bar, control cells without transfection with Anti-Oligo; black bar, Anti-Oligo-transfected cells. Open circle, control cells without transfection with Anti-Oligo; filled circle, Anti-Oligo-transfected cells.

3C). The cell did not show any significant change either in the content of total biopterin or BH4 or in SPR activity after the transfection with Sense-Oligo or Inv-Oligo (data not shown).

In the phenylpropanedione-containing medium, the cell number significantly decreased as shown in Fig. 2 and it further diminished by the transfection of Anti-Oligo at 3–10 days (Fig. 4). After 10 days' incubation, the cell number decreased to  $\approx 3\%$  of that in cultures not transfected with the Anti-Oligo (Fig. 4), whereas transfection with Sense-Oligo or Inv-Oligo did not significantly affect the viability

(data not shown). The cell number also decreased by the transfection with Anti-Oligo in the absence of phenylpropanedione (data not shown). However, the growth rate of the cells after the transfection in the absence of phenylpropanedione was  $\approx 4$ -fold larger than that in the presence of phenylpropanedione. These results suggest that SPR is available for protecting against the decrease in cell viability caused by phenylpropanedione.

To confirm that SPR functions as a protector in the cell by reducing the cytotoxicity of phenylpropanedione by its SDR activity, we examined the effect of *N*-acetylserotonin (NAS) on the cell number in the phenylpropanedione-containing medium. NAS is a specific inhibitor of SPR [9]. After 10 days' incubation of the cells with NAS (1 mM), the cell number decreased to almost the same level (Fig. 4) as in the case of cells transfected with the Anti-Oligo (Fig. 4).

Besides functioning as a specific enzyme for the formation of BH4 (Fig. 1), SPR in PC12 cells may serve for detoxification of exogenous carbonyl compounds via its so-called carbonyl reductase activity. Some previous reports indicated that exogenous BH4 stimulated DNA synthesis and cell proliferation [10,11]. We also observed in this study, that the cell number significantly decreased by the transfection with Anti-Oligo even when the cells were not treated with phenylpropanedione.

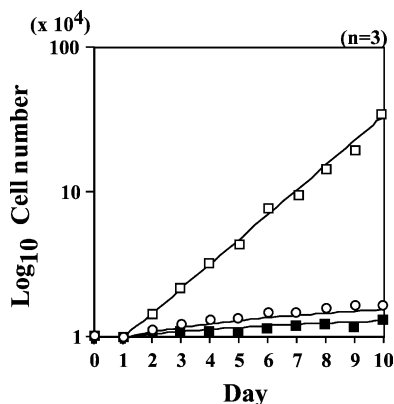


Fig. 4. Effect of Anti-Oligo and NAS on the cell number in the presence of phenylpropanedione. Open box, control cells without Anti-Oligo or *N*-acetylserotonin (NAS); filled box, Anti-Oligo-transfected cells; open circle, NAS added.

The mechanism by which the BH<sub>4</sub> content controls cell proliferation is quite interesting to us and will be studied in the future.

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