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Catecholamine containing alterations in the adrenal medulla of the p73 mutant mice

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Resumen

Alteración del contenido de catecolaminas en la medula adrenal del ratón mutante de la p73

La medula adrenal esta compuesta principalmente por células cromafines productoras de hormonas, siendo el órgano principal para la conversión del aminoácido tiroxina en las catecolaminas adrenalina y noradrenalina. Las células la medula adrenal derivan embriológicamente de la cresta neural, como neuronas modificadas. La proteína p73 es un miembro de una familia de factores de transcripción, que también incluye la p53 y p83 y la p73 es necesaria para la supervivencia y el mantenimiento a largo tiempo de las neuronas del sistema nerviosos central, incluyendo el sistema nervioso periférico. El propósito del presente trabajo es estudias la expresión de las enzimas de la biosíntesis de las catecolaminas y la cromogranina A (ChA) in la medula adrenal del ratón mutante de la p73. Se ha usado ratones mutantes de la p73 (KO) y ratones salvajes controles (WT) de 9 días de edad. Anticuerpos contra la tiroxina-hidroxilasa (TH), la dopamina-β-hidroxilasa (DBH), la feniletanolamina-N-metil transferasa (PNMT) y la ChA se usaron como anticuerpos primarios. La expresión TH y la ChA fueron similar en ambos grupos mutante y control. El material inmunoreactivo (IRM) para la DBH y la PNMT estaba incrementado en el ratón mutante con respecto al control. Podríamos concluir que las variaciones en el contenido de catecolaminas células de la medula adrenal del ratón con ausencia de la proteína p73, es probablemente divido a hecho de que la p73 influye en la supervivencia de la neuronas simpáticas.

Palabras clave

Medula adrenal, catecolaminas, ratón mutante p73

Summary

Catecholamine containing alterations in the adrenal medulla of the p73 mutant mice

The adrenal medulla is composed mainly by chromaffin cells producing of hormones, being the main organ for converting the tyrosine aminoacid in the catecholamines adrenaline and noradrenalin. The cells of the adrenal medulla derive embryologically from neural crest, like neurons modified. The protein p73 is a member of a family of transcription factors, which also includes p53 and p63 and p73 is necessary for survival and long-term maintenance of central nervous system neurons, including cells of the peripheral nervous system; such us sympathetic neurons. The aim of present work is study the expression of the catecholamine biosynthesis enzymes and chromogranin A (ChA) in the adrenal medulla of p73 mutant mice. We have used p73 mutant mice (KO) and control wild type mice (WT) of 9 days of age. Antibody against the tyrosine-hydroxylase (TH), dopamine-β-hydroxylase (DBH), phenyl-ethanolamine-N-methyl transferase (PNMT) and ChA, were used as the primary antibodies. TH and ChA expression were similar in both the mutant and control groups. The immunoreactive material (IRM) for DBH and PNMT were increased in the mutant mice with respect to the wild type groups. We could conclude the variations of the catecholamines containing in adrenal medulla cells of the mice lack in p73 protein, is probably owing to the fact p73 influxes the survival sympathetic neurons.

Key words

Adrenal medulla, catecholamines, p73 mutant mice

Introduction

The adrenal medulla is composed mainly by chromaffin cells and derive embryological from neural crest, like neurons modified [1, 3, 7, 12]. Really these cells are postganglionic cells of the sympathetic nervous system that receive the innervations of preganglionic cells [1, 3, 7, 8, 12, and 13]. As the synapse between pre fibres and postganglionic are called autonomous nervous ganglion, the adrenal medulla can be considered as a nervous ganglion of the sympathetic nervous system that producing of hormones, being the main organ of conversion of the tyrosine aminoacid in the catecholamines adrenaline and noradrenalin, and these catecholamines could be altered by different kind of stress, such us the psychosocial stress expressed a differential influence on gene expression

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and protein levels of catecholamine biosynthetic enzymes in the adrenal medulla of adult rats [1,9,12]. The before results indicate a possible adaptation of catecholamine-synthesizing system at the level of TH gene expression in adrenal medulla of chronically isolated animals [9].

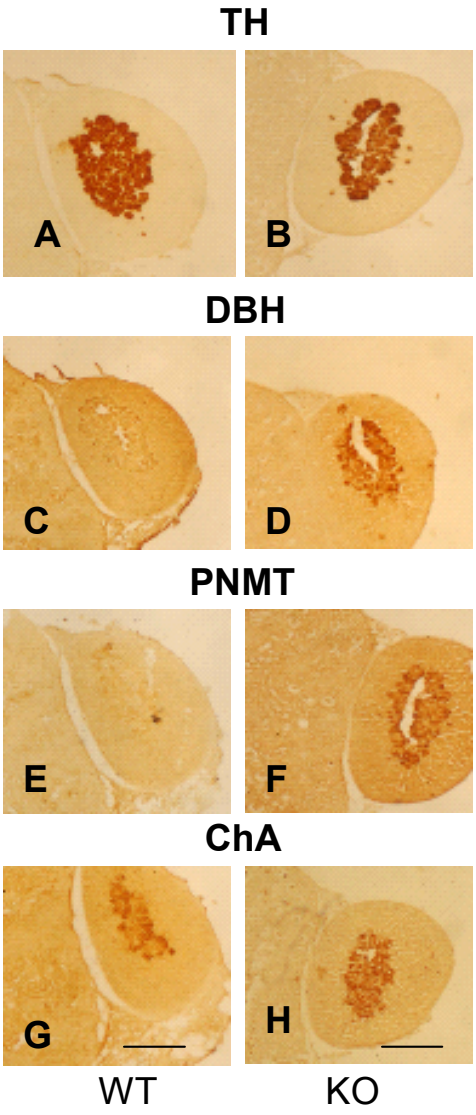

Fig.1

The protein p73 is a member of a family of transcription factors, which also includes p53 and p63, p73 have two main isoform: the transactivating isoforms of p73 (TAp73) are similar to p53 acting as transcription factors that induce cellular apoptosis and the N-terminal truncated isoforms (ΔNp73) can inhibit the transcriptional function of p53 and TAp73 [2,14]. The p73 is present in developing neurons as a truncated isoform whose levels are dramatically decreased when sympathetic neurons apoptosis after nerve growth factor (NGF) withdrawal [2, 14]. Thereafter p73 is necessary for survival and long-term maintenance of central nervous system neurons, including peripheral nervous system [2, 14]. Since the adrenal medulla is considered as part of peripheral nervous system, the purpose of present work is study the expression of the tyrosine-hydroxylase (TH) and dopamine β-hydroxylase (DBH), phenylethanolamine-N-methyltransferase (PNMT) and chromogranin A (ChA) in the adrenal medulla p73 mutant mice.

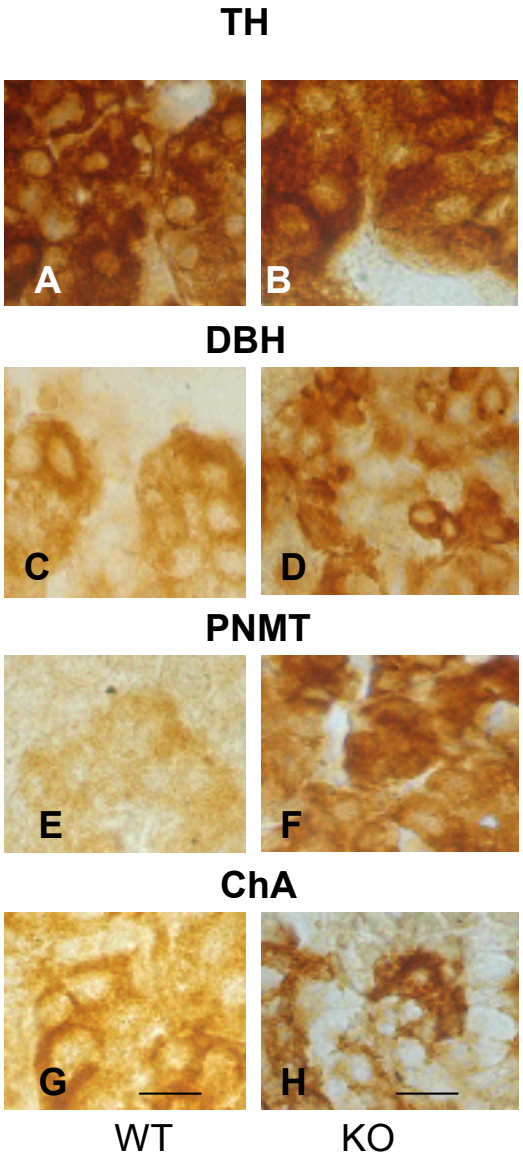

Fig.2

Material and methods

We have used 12 p73 mutant mice (KO) and 12 control wild type mice (WT) of 9, days of age. Antibody against TH, DBH, PNMT and ChA, were used as the primary antibodies. The adrenal medulla of five mice of each groups were fixed by bouin fluid, dehydrated and included in paraffin, the adrenal medulla were cut in series o sections of 10 µm thick. Sections from the WT (+/+) mice and knock-out p73 (KO -/-) mice were incubated simultaneously in the same coupling jar each containing: anti-p73, anti-TH, anti-DBH, anti-PNMT and anti-ChA. Incubation was for 24 h at

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room temperature, followed by "DAKO StreptABCcomplex/HRP Duet, Mouse/Rabbit" procedure. The peroxidase reaction product was visualized using diaminobenzidine reaction. Extract of adrenal medulla were prepared from 7 mice of each group, which were processed by protein electrophoresis (sodium docecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE, 5%-15% gradient). Immunoblotting of the AM extract were used to show bands marked with the primary antibody. The blotted bands were incubated PBS non-fat milk 5% for 45 minutes and then incubated in the primary antibodies against the TH, DBH, PNMT and ChA for 18 h. Anti-mouse IgG labelled with peroxidase (Sigma) was used as the secondary antibody at a dilution of 1:10000 for 2 h at room temperature.

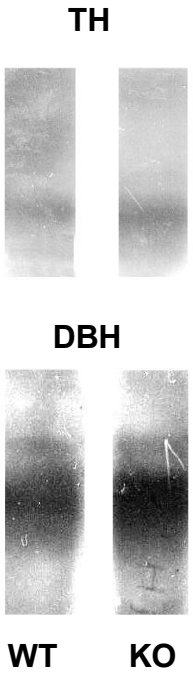

Fig.3

Results

The TH immunoreactive material was found in many cells located mainly in whole adrenal medulla of both of two groups WT and KO in those localization the TH expression was qualitatively similar in WT and KO, quantitatively was lightly decreased in the mutant mice Fig 1,2 A,B, Fig 4. By western blot the IRM was lighter increased in KO group. The IRM for DBH (Fig.1, 2 C, D) and PNMT Fig.1, 2 E, F) were increased as qualitatively as quantitatively (Fig.4) and in the mutant mice with respect to the wild type group, the western blot also showed an increase of the IRM for DBH in the KO group (Fig.3). The ChA (Fig 1, 2 G, and H) expression in the mutant was similar to WT, ChA densitometry (Fig 4) showed a lightly increased in the KO groups

Discussion

Stimulation of chromaffin cells of the adrenal medulla by preganglionic cholinergic neurons releases noradrenalin and adrenalin. The adrenalin synthesis by phenylethanolamine-N-methyltransferase is induced by cholinergic stimulation of the adrenal medulla [15]. While both of these actions of preganglionion nerve activity are controlled by ChA [1,7,8]. Several study, report p73 relations with the sympathetic neurons; possible implication of p73 in neuroblastic differentiation and its presence in the tumours originating from the sympathoadrenal lineage of neural crest [6,10,11,12]. Few work connect the adrenal medulla and p73 so that, experiments on cellular transcription factors derived from the rat adrenal gland have shown that the heat shock protein (HSP) modulate in vitro DNA binding activity of the AP-1 factor of both HSP 70 (p73 and p72) [4,10,11]. In our results we found that there were not differences in the TH and ChA expression in the adrenal medulla between the control and mutant mice, which could be again to the finding described by Pozniak [14], who observed that, the absence of p73 in mice causes enhanced death of developing sympathetic neurons, since the ΔN-p73 has an essential role as an anti-apoptotic protein in neurons that influx the developing brain and sympathetic neurons [14]. But in the present work, we found that the catecholaminergic biosynthesis enzymes, DBH and PNMT, were increased in the mutant mice with respect to the control that could be owing to compensatory mechanism of the underdevelopment of the sympathetic neurons.

We could conclude the variations of the catecholamines containing in adrenal medulla cells, of the mice lack in p73 protein, is probably owing to the fact p73 influxes the survival of the sympathetic cells.

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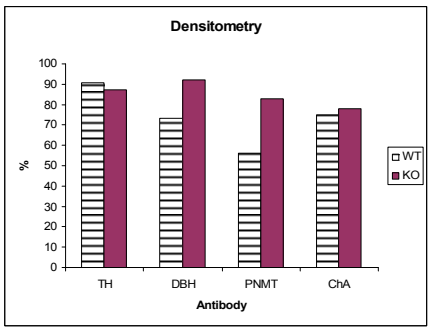

Fig. 4

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