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Pathophysiologic changes in IA-2/IA-2β null mice are secondary to alterations in the secretion of hormones and neurotransmitters

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Abstract

IA-2 and IA-2β are transmembrane proteins of dense core vesicles (DCV). The deletion of these proteins results in a reduction in the number of DCV and the secretion of hormones and neurotransmitters. As a result this leads to a variety of pathophysiologic changes. The purpose of this review is to describe these changes, which are characterized by glucose intolerance, female infertility, behavior and learning abnormalities and alterations in the diurnal circadian rhythms of blood pressure, heart rate, spontaneous physical activity and body temperature. These findings show that the deletion of IA-2 and IA-2β results in multiple pathophysiologic changes and represents a unique *in vivo* model for studying the effect of hormone and neurotransmitter reduction on known and still unrecognized targets.

Keywords

Type 1 diabetes (T1D); autoantigen; dense-core vesicles (DCV); glucose intolerance; behavior and learning; female infertility; circadian rhythm

Introduction

IA-2 and IA-2β are major autoantigens in type 1 diabetes. Antibodies to these proteins appear years before the development of clinical disease and are widely used as predictive markers [1–4]. Structural analysis revealed that IA-2 and IA-2β are transmembrane proteins of dense core vesicles (DCV) and are widely expressed in neuroendocrine cells throughout the body, particular in pancreatic islets and brain [5, 6]. Our initial experiments showed that knock down or knockout of these genes in cell lines or in mice, respectively, resulted in impaired secretion of insulin [7–9]. Further studies in animals revealed that the deletion of

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Conflict of Interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Ethical standard All studies were performed according to NIH guidelines.

Human and animal rights disclosure This article does not contain any studies with human or animal subjects performed by the any of the authors.

these proteins resulted in multiple pathophysiologic changes. In this review we describe these changes and show that they are the result of decreased secretion of hormones and neurotransmitters due to the deletion of IA-2 and IA-2β.

Structural analysis of IA-2 and IA-2β

IA-2 (PTPRN) encodes a 979 amino acid protein located on human chromosome 2q35 and mouse chromosome 1 [5, 10]. IA-2β (PTPRN2) encodes a 1015 amino acid protein located on human chromosome 7q36 and mouse chromosome 12 [11, 12]. Both proteins consist of a cytoplasmic domain, a transmembrane domain and a luminal domain (Fig. 1). The cytoplasmic domains are 77% identical and both genes have 23 exons. Based on sequence analysis, IA-2 and IA-2β are members of the protein tyrosine phosphate family and evolutionarily conserved [13, 14]. Substitution of two amino acids in the cytoplasmic domain, however, has made both proteins enzymatically inactive [15, 16]. Replacing aspartic acid with alanine at position 911 and alanine with aspartic acid at position 877 of IA-2 restores enzymatic activity [17]. The crystal structure of the cytoplasmic domain of human IA-2 has been determined and reveals a classic protein tyrosine phosphatase architecture that is most similar to PTP1B [18]. The crystal structure highlights the residues responsible for the lack of enzymatic activity. Despite this lack of enzymatic activity, IA-2 and IA-2β play an important role, as described below, in hormone and neurotransmitter secretion.

Impairment of insulin secretion

The importance of the integrity of the DCVs was demonstrated by IA-2 knockdown and knockout experiments which decreased insulin secretion, whereas IA-2 overexpression experiments increased insulin secretion. Electron microscopy revealed that knockout and knockdown experiments resulted in a marked decrease in the number and half-life of DCV (Fig. 2) [19], whereas IA-2 overexpression experiments increased the number of DCV [9]. This showed that changes in the number of DCV vesicles were responsible for the decrease or increase in insulin secretion. These findings were supported by two-photon microscopy, membrane capacitance and changes in Ca^{2+} currents [19].

Female infertility

The knockout of IA-2 together with IA-2β had a profound effect on female fertility [20]. As seen in Fig. 3, the female double knockout mice were essentially infertile as compared to the WT mice and the various heterozygous mice. Histologic examination of the ovaries of the DKO female mice revealed very few corpora lutea. Since a surge in the luteinizing hormone (LH) is required for ovulation, we measure the level of LH and found that it was decreased in the serum and pituitary. Treatment of DKO mice with gonadotropin successfully restored corpora lutea formation. In contrast to DKO females, DKO males were fertile. The knockout of both IA-2 and IA-2β most likely decreased the number of DCV in the pituitary resulting in the decreased secretion of LH and other pituitary hormones such as follicle stimulating hormone (FSH).

Changes in behavior and learning

The IA-2/IA-2β DKO mice showed a variety of behavioral changes including a highly significant increase in anxiety-like behavior and impairment of conditioned learning [21, 22], which was associated with a decrease in the brain of norepinephrine, dopamine and serotonin. Similarly, stimulation of synaptosomes prepared from DKO mice also showed a decrease in the secretion of neurotransmitters (i.e., dopamine, GABA and glutamate) as compared to synaptosomes from WT mice [21]. Fractionation studies revealed that IA-2 was primarily associated with DCV, whereas IA-2β was found in both DCV and synaptic vesicles [21]. This may explain why in certain behavior and learning tests IA-2 has a more pronounced effect than IA-2β, whereas in other tests the converse is true. For example (Fig. 4), in the active avoidance learning test, the WT mice showed 60–70% active avoidance responses, whereas the responses in the DKO mice were markedly impaired showing only 10–15% active avoidance [22]. The degree of active avoidance in the IA-2 SKO mice was similar to that of the 10–15% active avoidance in DKO mice, but in marked contrast the IA-2β SKO mice behaved like the WT mice showing 60~70% active avoidance. It should be noted that little is known about the relative density of IA-2 and IA-2β in DCV, the distribution or number of these vesicles in different neuroendocrine cells or the effect of the knockout of IA-2 and/or IA-2β on the storage and secretion of neurotransmitters in various parts of the neuroendocrine system. Each of the tests that are commonly used to study behavior and learning involves complex and often interconnecting neuronal pathways encompassing a variety of different neurotransmitters.

Disruption of circadian rhythms

Circadian rhythmicity is an essential feature of biology, functioning to coordinate daily biological rhythms of multiple organs. The knockout of both IA-2 and IA-2β profoundly disrupted the normal diurnal variation in blood pressure, heart rate, body temperature, spontaneous physical activity (Fig. 5) and neuronal firing, whereas the deletion of either IA-2 or IA-2β alone did not produce a major change [23]. Both the IA-2 and IA-2β transcripts are highly, but nonrhythmically, expressed in the suprachiasmatic nuclei (SCN), the brain’s master circadian oscillator. It is thought that this disruption in circadian rhythm caused by the deletion of IA-2/IA-2β is due to alterations in the release of one or more neurotransmitters (e.g., VIP) within the SCN that is required for the coordination of neuronal signaling within the SCN. The disruption of circadian rhythm leads to significant disruption of the clock genes in the SCN (e.g., Per1, Dbp) and also in several peripheral tissues of the DKO mice but not the single KO mice [24]. These findings add support to the idea that the effect of IA-2 and IA-2β on DCV secretion profoundly influences neurochemical communication among SCN neurons and that although peripheral tissues have a functioning circadian clockwork, the rhythmicity of this clockwork is greatly influenced by the SCN.

Concluding comments

The common denominator that is responsible for the multiple pathophysiologic changes observed in the IA-2 and IA-2β KO mice is the decrease in the number of DCV and the

associated decrease in the secretion of hormones and neurotransmitters (Fig. 6). Since DCV are widely expressed in neuroendocrine cells throughout the body, a number of other pathophysiologic changes would be expected, in addition to the ones described here. For example, in knockout mice, plasma renin and renal renin mRNA levels were reduced by 50% or more [25]. It is thought that this is a secondary effect due to impaired sympathetic transmitter release from the presynaptic vesicles of autonomic nerve terminals. Pilot experiments also have shown changes in the level of norepinephrine and epinephrine in the serum and changes in the number of white blood cells in the peripheral circulation (unpublished data). Because of the high expression of IA-2 and IA-2β in the brain and in the gastrointestinal tract [26, 27], we would expect to find a number of other pathophysiologic changes in these organs if appropriately tested. Thus the alterations in the half-life and number of DCV in the IA-2/IA-2β null mice represent a unique model for studying the effect of hormones and neurotransmitters on known targets and on still unrecognized targets.

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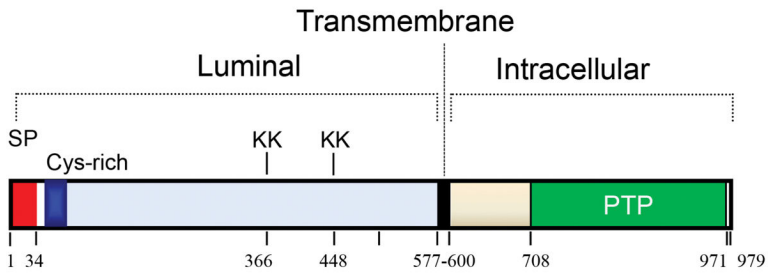

Fig. 1. IA-2 protein structure

Diagrammatic representation of IA-2 protein showing the luminal, transmembrane and intracellular domains of IA-2: SP, signal peptide; KK, dibasic amino acid cleavage sites; PTP, protein tyrosine phosphatase domain. The intracellular PTP domain is located on the cytoplasmic side of the DCV membrane. The luminal domain is cleaved at the KK sites, leaving part of the luminal domain inside the DCV.

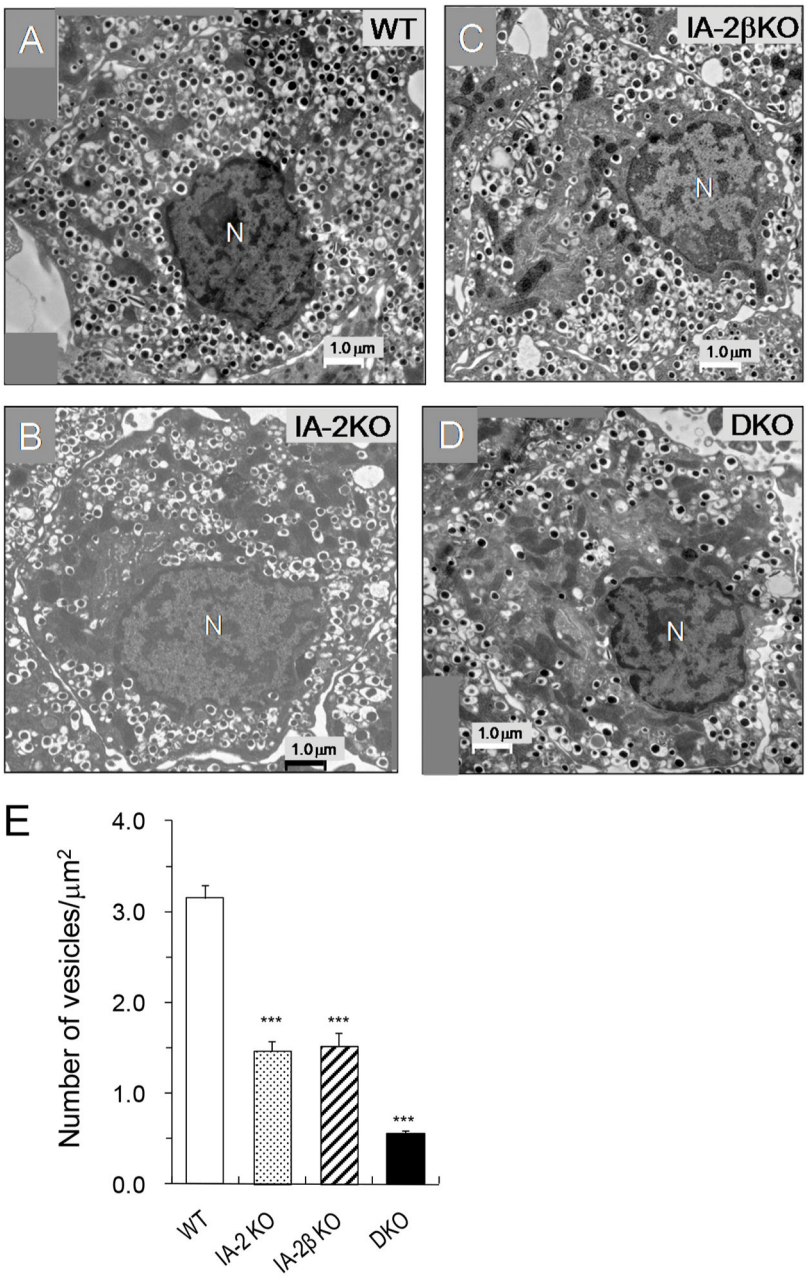

Fig. 2.

Electron micrograph. The number of DCV is dramatically reduced in β cells of (D) double knockout (DKO) and (B–C) single KO mice as compared to (A) WT mice. (E) Average number of DCV from 26 beta cells from each of the four genotypes (three mice per genotype) [19].

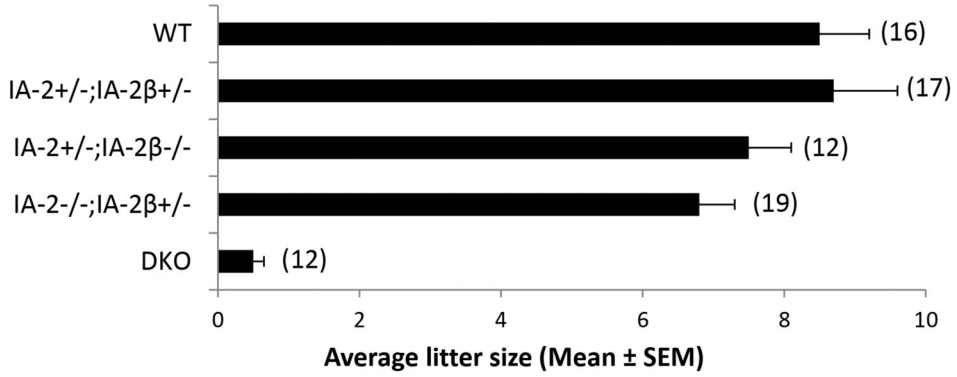

Fig. 3. Infertility in DKO female mice

Litter size (mean ± SEM) in WT, heterozygous, and DKO mice. The number of mating pairs in each group is indicated in parentheses [20].

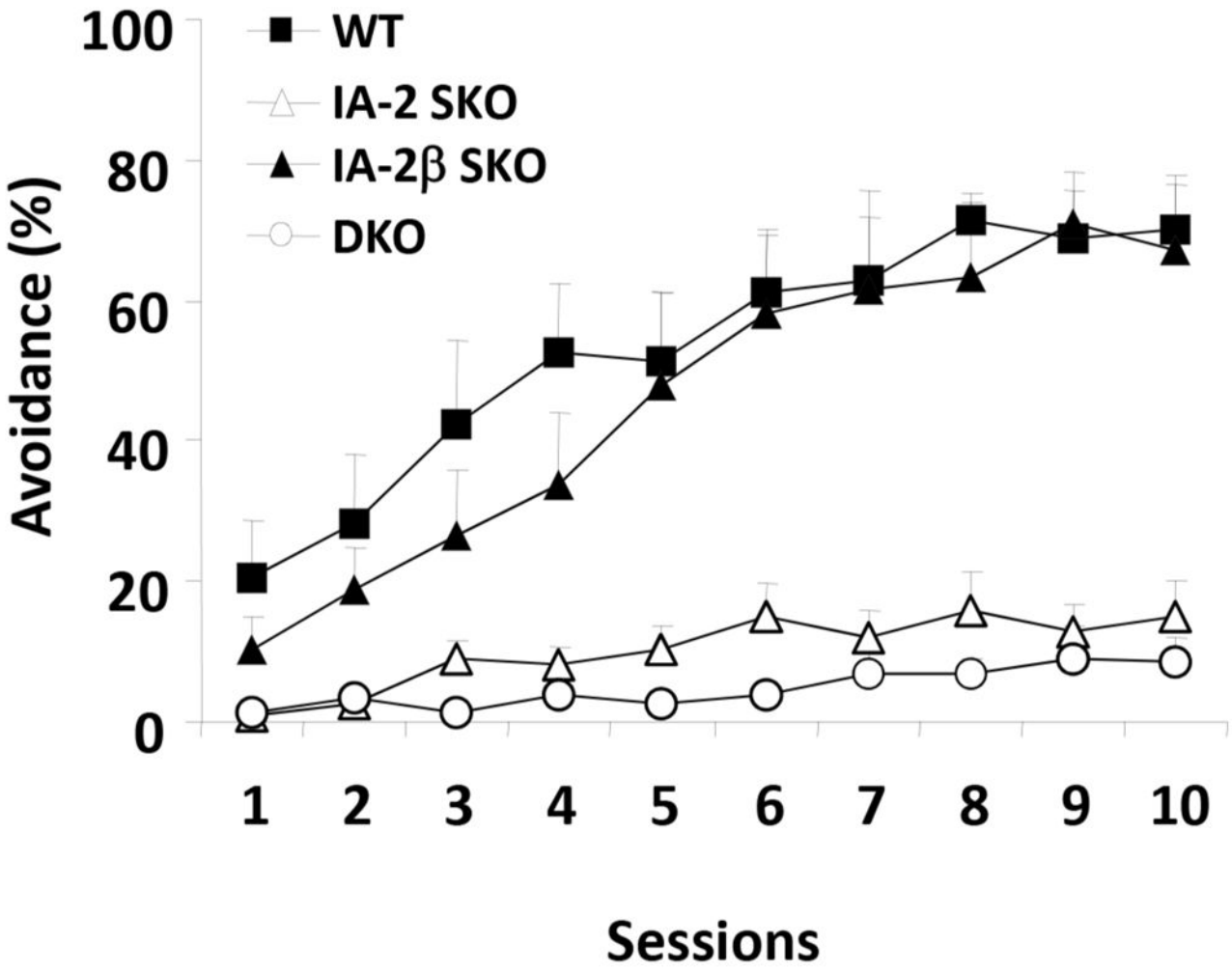

Fig. 4. Impaired active avoidance learning

IA-2 SKO and DKO mice, but not IA-2β SKO or WT mice, show impaired active avoidance learning. Mean percentage of trials showing active avoidance responses during 50 trials (2 sessions per day) over 5 days [22].

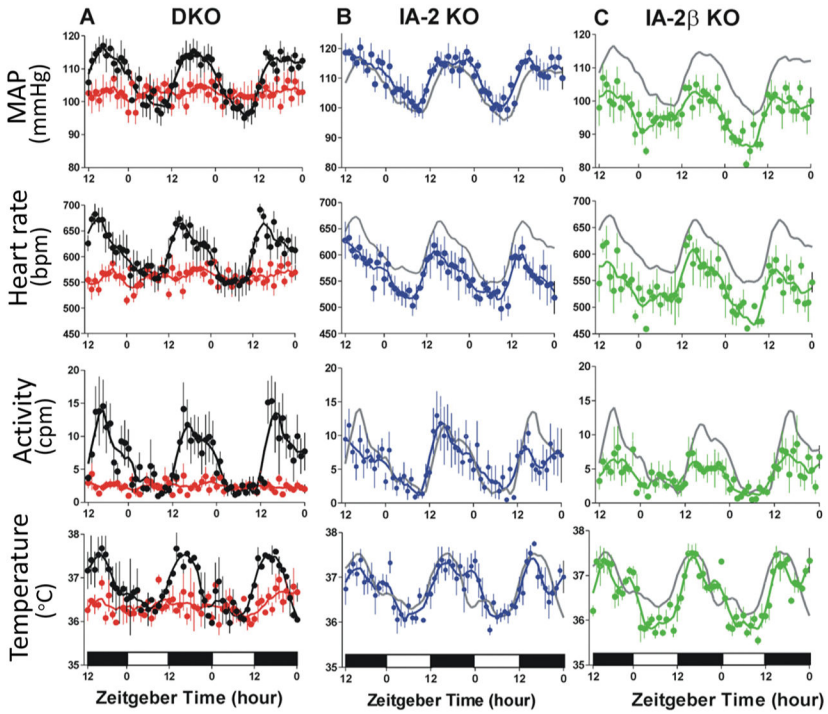

Fig. 5. Loss of circadian rhythm in DKO mice

Mean arterial pressure (MAP), heart rate, spontaneous physical activity, and body temperature in (A) WT (black) and DKO mice (red). Horizontal scale bar, Zeitgeber times 0 and 12 correspond to lights on at 6:00 AM and lights off at 6:00 PM, respectively. Lines represent data smoothing using the weighted average of the 9 nearest points. Circadian rhythms are essentially unaffected in (B) IA-2 (blue) and (C) IA-2β (green) single KO mice as compared to DKO mice [23].

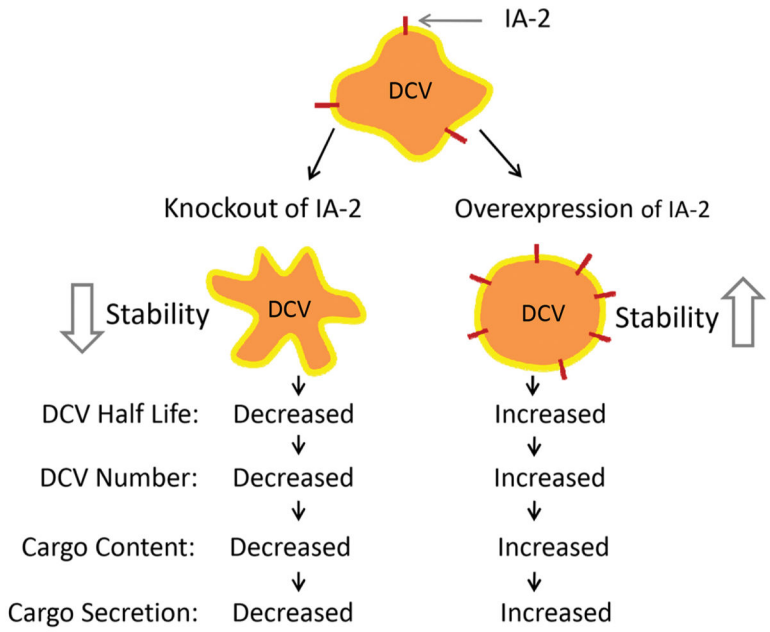

Fig. 6. Effect of IA-2 on the stability of DCV

In mice, the KO of IA-2 destabilizes and decreases the half-life of DCV. In turn, the number of DCV, the amount of insulin in beta cells and its secretion, is decreased. In MIN6 cells, overproduction of IA-2 adds stability to and increases the half-life of DCV. In turn, the number of DCV, the amount of insulin in beta cells and its secretion is increased [19].