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Original article

Neuregulin 1 improves glucose tolerance in adult and old rats

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Abstract

Aim. – Studies both in vitro and ex vivo of rodent skeletal muscle have highlighted the potential involvement of neuregulin 1 (NRG1) in glucose metabolism regulation, yet nothing is known of the role of NRG1 in systemic glucose homoeostasis. For this reason, it was hypothesized that systemic delivery of NRG1 might improve glucose tolerance and that the effect might be age-dependent.

Methods. – Glucose tolerance tests were performed in 6-month-old (adult) and 22-month-old (old) male Wistar rats 15 min after a single injection of either NRG1 (50 μg/kg) or saline (controls). Skeletal muscle and liver samples were also collected 30 min after the acute NRG1 or saline treatment, while the phosphorylation status of ErbB receptors and AKT was assessed by Western blotting.

Results. – Acute NRG1 treatment decreased the glycaemic response to an oral glucose load in both adult and old rats. NRG1 injection did not activate ErbB receptors in skeletal muscle, whereas phosphorylation of ErbB3 and AKT was markedly increased in the liver of NRG1-treated adult and old rats compared with controls.

Conclusion. – This study shows that NRG1 has a possible glucose-lowering effect in the liver and via an ErbB3/AKT signaling pathway. This NRG1 effect is also maintained in old rats, suggesting that the NRG1/ErbB signaling pathway might represent a promising therapeutic target in insulin resistance states.

Keywords: Ageing; ErbB; Glucose homoeostasis; Liver; NRG1; Skeletal muscle

1. Introduction

Neuregulin (NRG) growth factors belong to a complex family of proteins that is structurally related to epidermal growth factor (EGF). Four *NRG* genes have been identified (neuregulin 1–4), and more than 15 distinct membrane-associated or soluble isoforms result from alternative splicing and differential use of the promoter of the *NRG1* gene, the most studied of the four NRGs [1]. Neuregulin 1 (NRG1) isoforms are expressed somewhat ubiquitously by cells of endothelial and mesenchymal origin, and are critical for cellular proliferation, survival, migration and differentiation [2]. NRG1 is essential for the development and maintenance of the nervous system [3], heart [4], skeletal muscle [5] and liver [6]. Most NRG1 isoforms contain a transmembrane domain, and all have a bioactive extracellular EGF-like domain [1]. Proteolytic cleavage may lead to release of the EGF-like domain, which can bind to and activate the class-I tyrosine kinase receptors called "erythroblastic leukaemia viral

Abbreviations: NRG1, neuregulin 1; ErbB, erythroblastic leukaemia viral oncogene homologue; OGTT, oral glucose tolerance test; AUC, area under the curve; HIRI, hepatic insulin resistance index; MISI, muscle insulin sensitivity index; VEH, vehicle (saline).

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oncogene homologues" (ErbBs). Four receptors make up this family (ErbB 1–4); however, NRG1 only binds to ErbB3 and ErbB4. Phosphorylation of tyrosine residues in the ErbB cytoplasmic domain initiates diverse downstream signaling events, notably the phosphatidylinositol 3-kinase (PI3K)/AKT pathway [7,8].

Although most studies of NRG1 have focused on its myogenic and neurotrophic properties, it has been suggested that NRG1 can also influence glucose metabolism [5], particularly in skeletal muscle, the major site of insulin-stimulated glucose disposal. For instance, NRG1 increases glucose uptake in L6E9 myotubes and isolated strips of soleus muscle [9]. Also, chronic exposure to NRG1 improves insulin sensitivity in L6E9 and C2C12 myocytes [10]. On the other hand, in liver, the main organ involved in blood glucose homoeostasis, insulin and NRG1 appear to have competitive, non-synergistic effects. Indeed, in rat liver, insulin decreases NRG1 expression and impairs its binding to receptors through a PI3K-dependent pathway [11,12]. In addition, in obese ob/ob mice, ErbB transactivation induces hepatic insulin resistance [13]. Therefore, the complex relationship between NRG1 and insulin in muscle and liver raises the question of NRG1 influence on systemic glucose homoeostasis.

Ageing is one of the physiological states associated with glucose homoeostasis impairment [14]. In ageing myocardium, restoration of the NRG1/ErbB pathway has been suggested to mediate the positive effects of caloric restriction, an intervention that improves glucose homoeostasis and insulin sensitivity, and increases the lifespan, too [15,16]. Interestingly, a strong relationship independent of phylogeny has been found between the maximum lifespan of rodent species and NRG1 and ErbB4 expression in the cerebellum [17,18]. This suggests that NRG1, through specific effects on brain function or in the entire body, could be a major determinant of rodent longevity [18]. Ageing also regulates the ErbB pathway in *Caenorhabditis elegans* (a roundworm) [19], while a progressive age-related decline of ErbB4 expression has been observed in the ventral midbrain of rats [17]. Therefore, the NRG1/ErbB signaling pathway could be involved in age-related metabolic impairment.

The aim of the present study was to determine whether acute NRG1 administration affects blood glucose homoeostasis in adult and old rats. To test the hypothesis that NRG1 has a systemic hypoglycaemic effect and that the effect is age-dependent, the effects of an intravenous (i.v.) injection of NRG1 on glucose and insulin responses to an oral glucose challenge were investigated in adult and old rats. NRG1/ErbB signaling was also assessed in liver and skeletal muscle following the acute NRG1 administration to determine the mechanisms involved in NRG1 effects on glucose homoeostasis.

2. Methods

2.1. Animals

Animal maintenance and experimental procedures were in accordance with the current legislation on animal experimentation, and were approved by the local ethics committee for animal experimentation (Auvergne, C2EA-02). Old (22-month-old; $n = 10$) and adult (6-month-old; $n = 6$) male Wistar rats (Janvier Labs [CERJ], Le Genest-Saint-Isle, France) were kept in temperature-controlled (20–22 °C) cages and reversed light–dark cycles (8.00 pm to 8.00 am), and had free access to water.

2.2. Neuregulin-1

To evaluate NRG1 effects, the EGF-like domain of human NRG1 (β2a isoform; residues Ser177 to Glu237) was provided by Zensun Science and Technology Ltd (Pudong, Shanghai, People's Republic of China) in a saline solution at 1.25 mg/mL. Immediately before i.v. injection, the solution was diluted with more saline to 50 μg/mL (final concentration).

2.3. Oral glucose tolerance tests (OGTTs) and metabolic measurements

OGTTs were carried out over 2 weeks. Rats (adult and old) were fasted overnight (12 h) and then received either a saline (VEH) or NRGI injection 15 min before glucose loading by gastric gavage (2 g/kg of body weight). Half the animals (randomly chosen) were treated with NRG1 and the other half with VEH during the first OGTT. The rats in the NRG1 group were then moved to the VEH group, and vice versa, for the second OGTT. Thus, ultimately, all animals received one injection of NRG1 and one injection of VEH. Blood samples were collected from the tail vein at 15, 30, 60, 90 and 120 min after glucose loading, and centrifuged at 6000 G for 5 min for plasma collection. Plasma samples were stored at −80 °C until further use. Plasma insulin was measured with an ELISA assay kit (ALPCO, Salem, NH, USA).

Blood glucose levels were determined, using a glucometer, 15 min before the OGTT (−15) at the time of glucose loading (0), and then at 15, 30, 60, 90 and 120 min after loading (Accu-chek Performa, Roche Diagnostics, Basel, Switzerland). Glucose and insulin responses were expressed as:

- area under the curve (AUC), calculated by the trapezoidal method (total AUC);
- net AUC, calculated after subtracting the baseline concentration.

The homoeostasis model assessment index as a marker of insulin resistance (HOMA–IR) was calculated using the following formula: [fasting plasma insulin (mIU/L) × fasting blood glucose concentration (mmol/L)]/22.5. The product of the net AUC for blood glucose multiplied by the net AUC for plasma insulin during the first 30 min of the OGTT was calculated and used as the hepatic insulin resistance index (HIRI) [20]. The rate of blood glucose concentration decay from its peak value to its nadir divided by the mean plasma insulin concentration during the OGTTs was also calculated and used as a muscle insulin sensitivity index (MISI) [20].