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Original Research

Inhibition of Adenylyl Cyclase in the Spinal Cord Alleviates Painful Diabetic Neuropathy in Zucker Diabetic Fatty Rats

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ABSTRACT

Objectives: Diabetic neuropathy is the most common complication of both type 1 and type 2 diabetes. In this study, we tested the hypotheses that impaired Gi protein expression/function in the spinal cord is associated with the development of painful neuropathy in people with type 2 diabetes and that reduction of cyclic adenosine monophosphate (cAMP) production by inhibiting adenylyl cyclase in the spinal cord can alleviate diabetic neuropathy.
Methods: To this end, we examined the levels of cAMP, cAMP-dependent protein kinase (PKA) and cAMP response element-binding protein (CREB) in the spinal cord after the development of neuropathic pain in Zucker diabetic fatty (ZDF) rats with type 2 diabetes. We evaluated the effects of intrathecal injections of SQ22536, an adenylyl cyclase inhibitor, on mechanical allodynia and thermal hyperalgesia in rats with painful diabetic neuropathy.
Results: We found that diabetic ZDF rats exhibited mechanical allodynia and thermal hyperalgesia, which are associated with enhanced cAMP production, increased PKA activation and elevated CREB phosphorylation in the spinal cord. Additionally, diabetic ZDF rats exhibited attenuated expression of Giα, but not Gsα, in the spinal cord. Furthermore, intrathecal administrations of SQ22536 dose-dependently alleviated mechanical allodynia and thermal hyperalgesia in diabetic ZDF rats and reduced cAMP production, PKA activation and p-CREB expression in the spinal cord.
Conclusions: Taken together, our study suggested that cAMP-mediated signalling in the spinal cord is likely critical for the development of painful neuropathy in people with type 2 diabetes.

Mots clés :
adénylcyclase
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RÉSUMÉ

Objectifs : La neuropathie diabétique est la complication la plus fréquente des diabètes de type 1 et de type 2. Dans la présente étude, nous avons vérifié les hypothèses selon lesquelles la dégradation de l'expression et du fonctionnement de la protéine Gi dans la moelle spinale est associée au développement de la neuropathie douloureuse chez les personnes atteintes du diabète de type 2, et la réduction de la production d'adénosine monophosphate cyclique (AMPc) par l'inhibition d'adénylcyclase dans la moelle spinale peuvent atténuer la neuropathie diabétique.
Méthodes : Pour ce faire, nous avons examiné les concentrations de l'AMPc, de la protéine kinase AMPc-dépendante (PKA) et de la protéine CREB (acr. angl. pour *cAMP response element-binding protein*) dans la moelle spinale après le développement de la douleur neuropathique chez les rats Zucker obèses et diabétiques (ZDF pour *Zucker diabetic fatty*) atteints du diabète de type 2. Nous avons évalué les effets des injections intrathécales de SQ22536, un inhibiteur de l'adénylcyclase, sur l'allodynie mécanique et l'hyperalgésie thermique chez les rats atteints de neuropathie diabétique douloureuse.
Résultats : Nous avons observé que les rats ZDF diabétiques montraient une allodynie mécanique et une hyperalgésie thermique, lesquelles sont associées à l'amélioration de la production d'AMPc, à l'augmentation de l'activation de la PKA et à l'augmentation de la phosphorylation de la protéine CREB dans la moelle spinale. Par ailleurs, les rats ZDF diabétiques ont montré une atténuation de l'expression de la Giα, mais non de la Gsα, dans la moelle spinale. De plus, l'administration intrathécale de SQ22536 a soulagé de façon proportionnelle à la dose l'allodynie mécanique et l'hyperalgésie thermique chez les rats ZDF diabétiques et a réduit la production d'AMPc, l'activation de la PKA et l'expression de la protéine CREB phosphorylée (p-CREB) dans la moelle spinale.

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Conclusions : Dans l'ensemble, notre étude suggère que la signalisation médiée par l'AMPc dans la moelle spinale est probablement cruciale pour le développement de la neuropathie douloureuse chez les personnes atteintes du diabète de type 2.

Introduction

Diabetes mellitus is prevalent worldwide, and diabetic neuropathy is the most common complication of both type 1 and type 2 diabetes (1,2). It can affect peripheral sensory neurons, and it occurs in a large percent of adult patients with diabetes (2). These patients often experience excessive sensitivity to nociceptive stimuli or perceive normal stimuli as painful stimuli (3–5), which significantly reduces the quality of life measures (6). Thus, it is important to investigate the mechanisms that are critical for the development and persistence of neuropathic pain states induced by diabetes (7–9).

Numerous studies have shown that various G protein-coupled receptors (GPCRs) are critically involved in regulation of pain-signal transmission. The G proteins consist of 3 subunits: alpha (α), beta (β) and gamma (γ) (10,11); activation of G proteins by GPCRs results in dissociation of the Gα subunit from the Gβγ subunits (11,12). On the basis of their G protein-coupling preference, GPCRs can be broadly classified into 4 major categories: $G\alpha_s$-, $G\alpha_i$, $G\alpha_{q/11}$- and $G\alpha_{12/13}$-coupled receptors (10,11). Importantly, previous studies have demonstrated that stimulation of almost all G_i protein-coupled GPCRs in the spinal cord can produce analgesic effects (13). Furthermore, it has been shown that Gi protein-coupled signalling function is impaired in dorsal root ganglia (DRG) neurons in rats with streptozocin-induced diabetic neuropathy (14). However, it is still unclear whether type 2 diabetic neuropathy is associated with altered G_i protein expression in the spinal cord.

One of the cellular and molecular functions of G_i protein is to inhibit the cyclic adenosine monophosphate (cAMP)-dependent pathway by inhibiting adenylate cyclase activity, decreasing the production of cAMP which, in turn, results in decreased activity of cAMP-dependent protein kinase (PKA). Although few studies have examined the role of PKA in diabetic neuropathy, previous studies have demonstrated that inflammatory mediator-induced hyperalgesia is dependent on ongoing PKA activity (15). Furthermore, a recent study has shown that continuing activity of adenylyl cyclase and PKA is critical for injury-induced spontaneous activity in the cell bodies of primary nociceptors within DRG, which has been found to make major contributions to chronic pain (16). Importantly, several studies have demonstrated that the levels of cAMP response element-binding protein (CREB) in the spinal cord are increased in animals with streptozocin-induced diabetic neuropathy (17–19), suggesting that cAMP-PKA-CREB signalling is increased in the spinal cord in type 1 diabetic neuropathy. However, it is still unknown whether cAMP-mediated signalling per se is involved in the neuropathic pain associated with type 2 diabetes.

Based on the above information, we hypothesized that impaired Gi protein expression in the spinal cord is associated with the development of painful diabetic neuropathy in type 2 diabetes, and that the reduction of cAMP production in the spinal cord by inhibiting adenylyl cyclase can alleviate diabetic neuropathy. To test this hypothesis, we used Zucker diabetic fatty (ZDF) rats to examine the levels of cAMP, PKA and CREB in the spinal cord after the development of neuropathic pain in patients with type 2 diabetes. We then evaluated the effects of intrathecal injections of SQ22536, an adenylyl cyclase inhibitor, on mechanical allodynia and thermal hyperalgesia in ZDF rats with painful diabetic neuropathy, and we evaluated the effects of SQ22536 on levels of cAMP, PKA and CREB in the spinal cord.

Methods

Animals

We purchased male Zucker diabetic fatty (ZDF; fa/fa) rats and control (Lean; fa/+) rats at the age of 6 weeks from Charles River Laboratories (Beijing, China). After the arrival, rats were acclimated in the Animal Center of The Second Hospital of Shandong University for 1 week before subsequent experiments. All the rats were housed in separated cages in a room with a 12:12 light:dark cycle and were given food and water ad libitum. All animals were maintained in a 12:12 cyclic lighting schedule at 21.0°C to 23.0°C and 50% to 60% humidity. The Institutional Animal Care and Use Committee of The Second Hospital of Shandong University had approved all animal experiments in the present study. The housing and treatment of the rats followed the guidelines of the Guide for the Care and Use of Laboratory Rats (Institute of Laboratory Animal Resources, Commission on Life Sciences, 2011).

Intrathecal catheter implantation

Ketamine/xylazine (80 to 120 mg/kg, 10 to 16 mg/kg, respectively; i.p.) was used to fully anesthetize the rats. In order to expose the L4 to L5 vertebrae, we made a 1 cm midline incision on the dorsal surface and retracted the muscles. Sterile polyethylene tubing (PE-10 catheter) was then inserted into the subarachnoid space and was advanced 3.5 cm rostrally at the level of the enlarged spinal cord lumbar segments. The catheter was secured to the paraspinal muscle of the back and then tunnelled subcutaneously to exit the dorsal neck region, where it was secured to the skin. After the surgery, rats were allowed to recover for 1 week prior to the rest of experiment. To confirm the position of the PE-10 catheter, we conducted intrathecal injections of 2% lidocaine (15 μL) to observe whether there was paralysis of both hind limbs following injections.

Blood glucose and weight monitoring

Starting at the ninth week of rat age, weight was measured daily, and blood glucose measurements (glucose diagnostic reagents; Sigma, St. Louis, Missouri, United States) were recorded every week. All rats were fasted for 3 hours before blood was collected from the tail. The onset of diabetic conditions was defined as blood glucose levels higher than 13.3 mmol/L. Consistent with the literature, the animals in the present study did not develop significant ketoacidosis or prostration during this time period (20,21).

Behavioural analysis

We conducted all the behavioural testing between 1 PM and 3 PM on the Thursday and Friday of each week, starting at week 9 and continuing to week 14 for 6 consecutive weeks. Prior to the first behavioural testing, rats were acclimated to the behavioural apparatus and equipment for a minimum of 2 days. On test days, rats were placed in the behavioural apparatus and allowed to acclimate to the environment for 30 minutes. The von Frey assay was conducted to test rat sensitivity to a mechanical stimulus on Thursdays. To this end, rats were placed in a clear plastic cage on top of a wire mesh grid that allowed rats to access their hind paws for the duration of the analysis. During the test, an ascending series of von Frey filaments with logarithmically incremental stiffness (0.6 to 26 g)