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Blockage of HCN Channels with ZD7288 Attenuates Mechanical Hypersensitivity in Rats Model of Diabetic Neuropathy

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ABSTRACT

Background: Diabetes is a chronic condition that affects millions of people worldwide. The disease can have severeimpact on many systems of the human body particularly the nervous system. Indeed, chronic peripheral nerve pain, also known as peripheral neuropathic pain (NP), is the most common complication caused by diabetes. There have existed preclinical researches that were performed using different types of Rodents as models of Chronic Pain. Such studies have proven that hypersensitivity to pain is caused, at least partially, by increased excitability of primary afferent dorsal root ganglion (DRG) neurons that send the sensory information from the periphery to the brain. However, the mechanism of this hyperexcitability is yet to be known.

Aims: The primary aim of the continuing work is to examine the hypothesis that Hyperpolarization-activated Cyclic Nucleotide gated (HCN) channels (that are known to regulate excitability of the neurons) are involved in the pathophysiology of diabetic neuropathic pain (DNP).

Methods and Results: To examine this hypothesis, we used a rat model of diabetic neuropathy by 60 mg/kg, i.p., of (streptozotocin (STZ), a toxin of pancreatic β-cells that release insulin), and tested the effects of blocking the HCN channels with a selective blocker, ZD7288, on two pain behaviours (mechanical hypersensitivity/allodynia, and heat hypersensitivity/hyperalgesia) in these STZ treated rats. The results showed that intraplantar administration of ZD7288 (100 μM) reduced mechanical allodynia but not heat hyperalgesia.

Conclusions: The results are in agreement with previous investigations which used other models of chronic pain, and suggest that HCN channels may be a good target for developing new analgesics (pain killers) for chronic pain.

Keywords: HCN channels , ZD7288 , Mechanical hypersensitivity, Rats model ,diabetic Neuropathy.

INTRODUCTION

Pain is an unpleasant feeling perceived by the brain. The pain signal is normally conduced from the periphery to the CNS via sensory nerve fibers which responds to the painful stimuli. Pain is usually categorized into acute pain or physiological pain which is considered as `good` pain because it plays a protective role, and chronic `bad` pain which plays no useful function and can last for years or even an entire life time. Chronic pain may last for over three to six months or even beyond the point of tissue healing[1]. Thus, chronic pain that affects around 20% of adults worldwide is a major health problem. Chronic peripheral neuropathic pain is associated with many types of injury/diseases, including diabetes which affects over 100 million people worldwide. Indeed diabetic neuropathic pain (DNP) is the commonest diabetic complication. About 50% of diabetic people have neuropathic pain[2]. The clinical symptoms of patients with DNP include allodynia (pain caused by normally painless stimuli), hyperalgesia (increased pain caused by normally painful stimuli) and spontaneous pain (unprovoked pain) [3]. These manifestations frequently occur with an abnormal loss of stimulus-evoked sensation, and they can last for many years or even a life time, and severely impair the quality of life for DNP patients.

Although there are many drugs that are used to control DNP in patients, but effective therapy for DNP is still a clinical challenge due to the available drugs not being effective or having significant adverse side effects. Therefore, there is a need to understand the pathophysiology of DNP in order to develop appropriate alleviative strategies.

Neuropathic pain including DNP is believed to be partly caused by abnormal hyperexcitability of sensory dorsal root ganglion (DRG) neurons. Many ion channels may be involved in this hyperexcitability. These include :

Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels, which produce an inward stimulatory current (termed Ih in neurons) that depolarizes the membrane potential toward the

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threshold for production of action potential. HCN channels, first discovered in 1976 in the heart by Noma and Irisawa [8] and characterized by Brown and Difrancesco [2] which are activated when the membrane potential is hyperpolarized. The general structure of HCN channels is similar to that of the voltage-gated K+ channels. There are four subunits HCN channels which have six transmembrane segments [10]. These channels have been implicated in other types of neuropathic pain. Indeed ZD7288, a specific blocker of these HCN channels, has been shown, in a rat model of spinal nerve injury, to decrease spontaneous activity in DRG neurons and to reduce tactile allodynia [5]. Mechanical allodynia was relieved significantly by ZD7288 [7]. While these studies showed that ZD7288 is effective in reducing pain hypersensitivity in nerve injury models of chronic pain. As there has not been, as far as we know, any investigations on the effect of this drug on pain hypersensitivity associated with DNP. Therefore, the aim of the present work is to examine whether blocking these HCN channels with a specific HCN blocker, ZD7288 would alleviate pain hypersensitivity associated with DNP in diabetic rats with neuropathic pain.

METHODS

Materials and methods

Twelve male Sprague Dawley rats (250-300g weight) were used in the present experiments. The rats were used for pain behavioural testing (see below).

Rat model of diabetic neuropathy

We used a rodent (rat) model of diabetes in the present study. The model was induced by an i.p. injection of 60 mg/kg streptozotocin (STZ). After 72 hours the level of glucose in the plasma was measured from blood samples that were taken from the tail vein of the treated rats. Rats were considered to be diabetic if the glucose level in their blood was more than 250 mg/dL.

The drug ZD7288

ZD7288 which is a selective blocker of HCN channels was administered subcutaneously in hind paw of the rats (100 μM), to test the effects of blockading Ih/HCN channels on pain hypersensitivity in the STZ diabetic model. 0.9% physiological saline was used to dissolve ZD7288. Dissolved ZD7288 which was then injected at a volume of 100 μL [12].

Pain behavioural testing

Two types of evoked pain behaviors were used as described previously [12]. These were mechanical allodynia or mechanical hypersensitivity and heat hyperalgesia/hypersensitivity. Before conducting these tests, rats were acclimatized for 7 days; their postures for standing, walking, and resting were monitored daily up to 7 days post operation.

a. Mechanical allodynia

An automated von Frey type system known as a dynamic plantar esthesiometer touch stimulator (Ugo Basile,Comerio, Italy) was used to assess mechanical allodynia (see Figure 1). Mechanical allodynia was indicated by reduced withdrawal thresholds to pressure. This mechanical force was applied to the mid-plantar surface of the hind paw, with a blunt metal filament, through an elevated mesh floor.

The mechanical force that was applied increased from 0 to 50g over 15-seconds period. In order to guard against tissue injury a cut-off of 50 g was used. This force (in grams) was recorded and displayed automatically. An interval of at least 1 minute between each trial was allowed. Each paw was tested 4 times and the average force was calculated and considered as a paw withdrawal threshold.

b. Heat hyperalgesia

Heat hyperalgesia was reported as done in previous studies [12] by decreased paw withdrawal latency to a noxious heat stimulus applied to the planter aspect of the hind paw using a planter (Hargreaves) analgesymeter (Ugo Basil, Comerio, Italy) [4], see Figure (1). The test was done by placing each rat in a chamber on a glass floor (2 mm thickness) under which the heat source was applied. Testing started by clicking a timer which automatically stops upon withdrawal response. An average of four trials were taken. Hyperalgesia was indicated when there was reduced response latency to normally noxious heat stimulus. Also, at least 5 min intervals between sequential trials were allowed to avoid the possibility of sensitization.

A. Heat hypersensitivity

B.

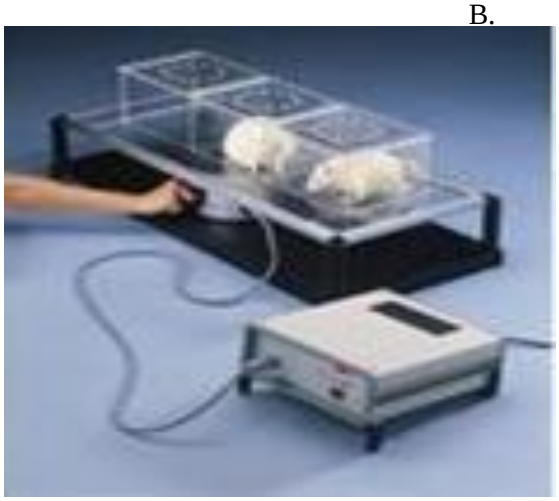

Figure 1. The devices that were used for measuring pain behaviours in STZ rats. Shown in (A) is the planter (Hargreaves) analgesymeter that was used for assessing heat hypersensitivity/ hyperalgesia.
B shows the dynamic plantar esthesiometer touch stimulator that was used for assessing mechanical hypersensitivity/allodynia.

B. Mechanical hypersensitivity

RESULTS

To confirm that rats treated with strepotozotocin (STZ) developed pain behaviours of mechanical allodynia and heat hyperalgesia, we compared pain behaviour values 4 weeks after induction of diabetes with pretreatment (baseline) values. As shown in Figure (2), normal rats responded to noxious heat after about 14.74 seconds, but diabetic rats responded after about 5.85 seconds. This demonstrates that diabetic rats experienced behavioural signs of heat hyperalgesia 4 weeks after induction of diabetes (Figure 2A). Normal rats showed withdrawal response to mechanical stimulus of about 38.5 g, while the diabetic rats responded to a stimulus of about 22.6 g. indicating a reduction in force needed to evoke a response which proves the presence of mechanical allodynia four weeks after induction of diabetes in these rats (Figure 2B). As shown in Figure (2), both the mean withdrawal latency and mean withdrawal threshold in diabetic rats was significantly ($P<0.001$) different from those of baseline (before STZ treatment), indicating that STZ rats do show behavioural signs of mechanical allodynia and heat hyperalgesia.

A. Heat hypersensitivity

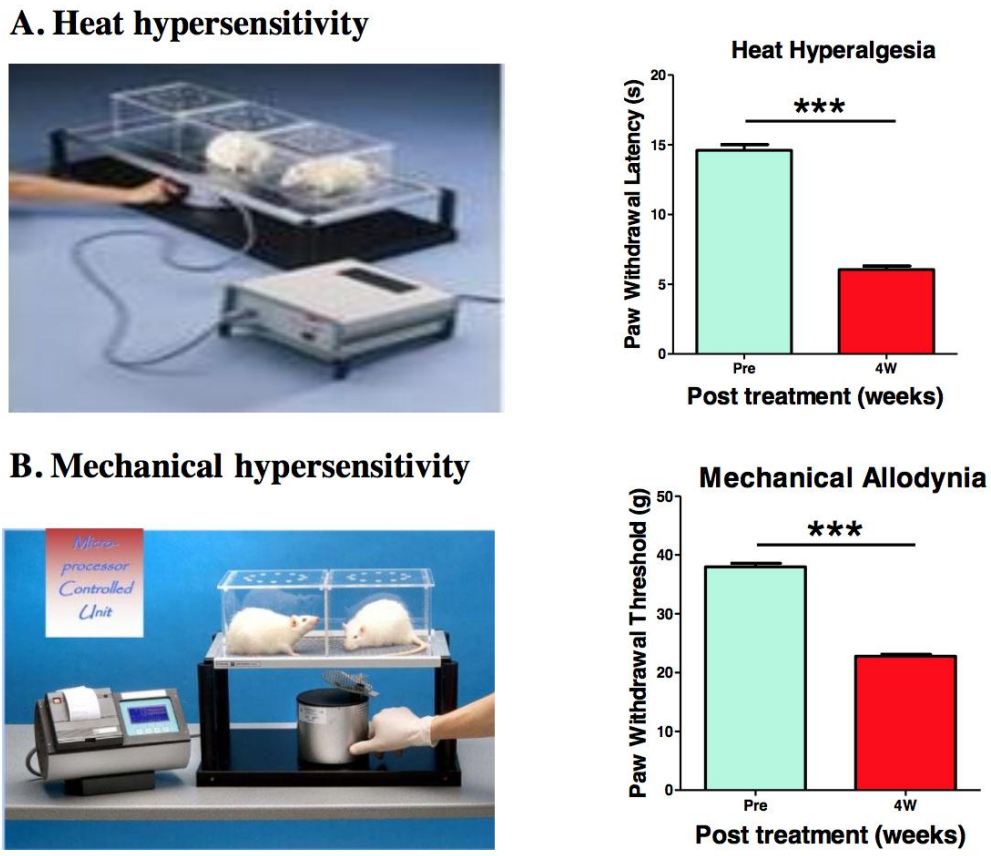

Figure 2: Development of heat hyperalgesia (A, right) and mechanical allodynia (B, right) in STZ rats assessed with the planter (Hargreaves) analgesymeter (A, left) and the dynamic plantar esthesiometer touch stimulator (B, left). Pre= before induction of diabetes; 4W= 4W weeks after STZ treatment.

Effects of ZD7288 on mechanical allodynia and heat hyperalgesia

Intraplantar administration of ZD7288 (100 μM) did not result in an effect on heat hyperalgesia in STZ rats (Figure 3). Comparison of the mean paw withdrawal latencies after vehicle with those after ZD7288 injections showed no significant differences (Figure 3A right). In contrast using paired t-test, the results show that ZD7288 caused a significant increase in the mean paw withdrawal threshold ($P<0.05$) (Figure 3B right), indicating that ZD7288 reduced mechanical allodynia ($P<0.05$).

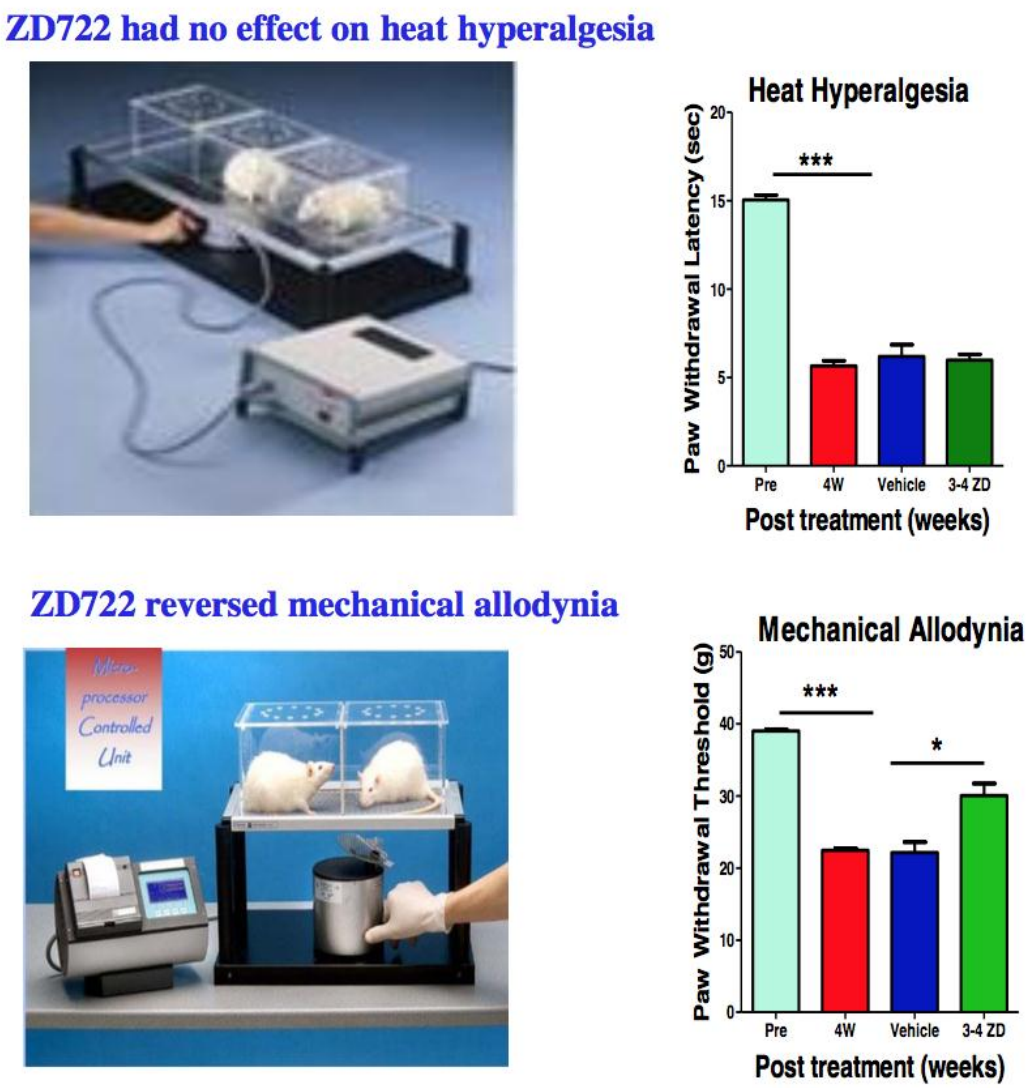

Figure 3: Effects of ZD7288 on mechanical allodynia and heat hyperalgesia in STZ rats assessed with the planter (Hargreaves) analgesymeter (A, left) and the dynamic plantar esthesiometer touch stimulator (B, left). Pre= before induction of diabetes; 4W= 4W weeks after STZ treatment; 3-4 ZD= 3-4 hours after ZD7288 injection. Note that ZD7288 reduced mechanical allodynia ($P<0.05$), but not heat hyperalgesia.

DISCUSSION

The main aim of the present work is to test the effects of blocking HCN channels with a selective blocker (ZD7288) on pain hypersensitivity in diabetic rats with neuropathic pain (DNP). The present findings showed that blocking HCN channels in the periphery reduced behavioural signs of mechanical allodynia, but not hyperlagesia.

As noted in the introduction, Diabetic Neuropathy pain (DNP) is associated with hyperexcitability of primary afferent dorsal root ganglion (DRG) neurons. This hyperexcitability is believed to lead to hypersentivity in chronic pain conditions including DNP. A few animal models of DNP have been developed to investigate its pathophysiology [9] including the widely used STZ rat model (60 mg/ kg, i.p.).

The pain hypersensitivity that is manifested in human patients with DNP is also revealed in this rat model of DNP. Therefore, we used this model to investigate indirectly whether or not HCN channels are involved in mechanisms of DNP. Our results demonstrated that that administration of ZD7288 subcutaneously completely suppressed mechanical allodynia in the STZ diabetic model but not heat hyperalgesia. The present findings are in line with the previous study that used a different model of chronic pain [5]. Those investigators showed that HCN blocker,

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ZD7288, reduced mechanical allodynia in rats with peripheral nerve injury. Our results are also mirrored to those of **Leu *et al.*** [7] who found that intraplantar administration of ZD7288 completely reversed mechanical allodynia, but not heat hyperalgesia, in peripheral neuropathic pain due to ligation of the spinal nerve. Not only that but, our findings are consistent with a recent study that used a rat model with chronic inflammatory pain [12], where the group of researchers similarly found that ZD7288 reduced mechanical allodynia but not heat hyperalgesia. Hence, our results and the findings of the said prior studies and research using various models of chronic pain indicate that the HCN blocker might be a very useful analgesic for chronic pain conditions including DNP.

CONCLUSIONS AND RECOMMENDATIONS

Our recent findings together with those of previous studies suggest that HCN channels may be a good target for developing new analgesics for DNP and other chronic pain conditions. However, further investigations are necessary due to the limited number of model rodents that were used in the present experiments.

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