Full Length Research Paper

The influence of verapamil on the inhibitory effects of corticosterone against neuropathic pain behaviors in rats

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There are many evidences which indicate that calcium channel related voltage plays an important role on the mechanism of neuropathic pain, but no strong evidence has been found yet on the role played by type-L calcium channel. The aim of this study was to evaluate this role in using a properly designed experiment. This cohort study was carried out on seven groups (eight animals each) of male Wistar rats (200 to 300 g). Chronic constriction nerve injury was performed on rats by loosely ligating their common sciatic nerve. The impact of corticosteron at 15 mg/kg on neuropathic pain behaviors was assessed in the presence or absence of verapamil as an L-VSC channel blocker at 5, 10 or 20 mg/kg. Standard procedures were employed to evaluate the behavioral pain responses including the thermal hyperalgesia and the thermal and mechanical allodynia. Our findings indicate that peripheral administration of corticosterone suppressed both hyperalgesia, and thermal and mechanical allodynia; however, verapamil pretreatment attenuated the effects of corticosterone on both thermal hyperalgesia and mechanical allodynia. In addition, the administration of verapamil alone at high dose (20 mg) suppressed both thermal and mechanical allodynia. These findings show that the inhibitory effects of glucocorticoids on neuropathic pain behaviors, at least in part, may be mediated through L-type VSC channels; but our findings suggest a potential role for glucocorticoids receptors agonists in combination with L-type VSC channels antagonists in the clinical management of neuropathic pain.

Key words: Corticosterone, L-type, calcium channel, mechanical allodynia, thermal allodynia, thermal hyperalgesia.

INTRODUCTION

Damage on the peripheral and central nerves can cause neuropathic pain which emerge as spontaneous irritating pains at the place of damage. Hyperalgesia is an increased response to a stimulus which is painful in normal state, while allodynia is a response to a stimulus which does not provoke any pain at natural state (Devor, 2001; Bennett, 1997). In neuropathic pain, in addition to damage in the peripheral or central nerves, there are some disorders in the function of sensual and kinetic nerves, damage on the receiving field of nerves and an increase in the feeling of pain. Since the activities of both pain and non-pain (coolness, heat, touching) sensual nerves increase in neuropathy, possibly the production of unnatural impulses and improper discharge of damaged afferent fibers is the main reason for the continuation and provoking of neuropathic pains (Sugimoto et al., 1990, Woolf, 1992; Wall and Gutnick ., 1974; Bridges et al., 2001). The recent studies indicate the interference of glucocorticoids in modifying the neuropathic pains. For example, it has been shown that the peripheral injection of corticosterone in a dose dependent manner controls
the symptoms of neuropathic pains in chronic constriction nerve injury (CCI) model neuropathy (Faryadian et al., 2008). Other studies also showed that the injection of epidural- betamethasone at the time of nerve damage can cause the reduction of hyperalgesia by stimulating the anti-inflammatory cytokines IL-10 and diminished the rate of pre-inflammatory cytokines (Xie et al., 2006). The chronic consumption of prednisolone in people afflicted with neuropathic pains substantially reduces the neuropathic inflammations and also controls the thermal and mechanical hyperalgesia (Takeda et al., 2004). The consumption of Prednisolone also slightly reduced the expression of FOS gene in the damaged side of the posterior horn of spinal cord (Kingery et al. 2001). Recently, it has been shown that after CCI, the expression of glucocorticoid receptors (GRs) in the posterior horn of the spinal cord in the same side of the damaged nerve increased with the intervention of IL-6 and protein kinase C, so that the inter-spinal cord injection of anti-serum IL-6 and controlling protein inase C considerably reduced the GR expression and neuropathy pain behavior (Wang et al., 2004). It was also shown that inter-peritoneal and inter-spinal injection (RU486 glucocorticoids receptors agonist) creates anti-pain impacts, but its core interior injection has no impact on behavioral response towards the pain (Takasaki et al., 2005). These results show that the anti-pain of RU 486 peripheral injection is mainly mediated through spinal cords. Therefore, these studies show that the glucocorticoids receptors play an important role in modifying neuropathic pains and the control of glucocorticoids receptors play the most important role in the treatment of neuropathic pain, but the underlying mechanisms are not clear.

The new evidences show that the calcium channels related to voltage play an important role in neuropathic pain. The neurons of spinal cord posterior horn hold different types of these channels (Dobremez et al., 2005; Murakami et al., 2004; Heinke et al., 2004). It seems that these channels are involved in neuropathic pains. For example, it has been shown that subcutaneous injection is a connecting factor to calcium which reduces the thickness of calcium that controls the calcium channels based on the voltage of type N. This determines the control of hyperalgesia caused by mechanical stimulation in the rats afflicted with nerve damage. On the other hand, controlling the calcium channels of type L, Q and P have no impacts (White and Cousins., 1998; Matthews and Dickenson., 2001). The intra-spinal cord injection of Zicontile (an exclusive blocker of calcium channels type N) would have a strong anti-pain impact in the cross damage model of rat feet, and this impact is even stronger and more evident than intra-spinal injection (Wang et al., 2000). Damage of the nerve increases the expression of the sub-unit of a2δ1 of calcium channel in the spinal cord and posterior root ganglion which indicates the role of this sub-unit in behavioral responses of neuropathic pains. The increase of the sub-unit of a2 plays an important role in the development and creation of allodynia (Kim et al., 2001; Abe et al., 2002; Luo et al., 2001). The role of calcium channels of type L in neuropathic pains is not clearly understood. In a recent study, it was shown that after damage was observed on sciatic nerve, the activity and expression of sub-units of this calcium channel in spinal cord posterior horn changes showed the interference of these channels in neuropathic pain (Dobremez et al., 2005). As there are some interactions between the glucocorticoids and calcium channels of type L in the central nerve system (Chameau et al., 2007; Karst et al., 2002), and because these channels are found in the neurons of spinal cord posterior horn (Dobremez et al., 2005), this study was designed to assess the role of these channels in the neuropathic pains and their interaction with the glucocorticoids.

MATERIALS AND METHODS

Experimental animals

Totally, seven groups (eight animals each) of male Wistar rats (200 to 300 g), as the male Wistar rats are used by most researchers, were used for the experiments carried out in the current cohort study. The rats were purchased from the animal house of the Semnan University of Medical Science. Rats were placed in a temperature- and humidity-controlled room and exposed to 12 h light-dark cycles.

The control group received saline and corticosterone carrier, 45 and 30 min before the test, respectively; Cort15 group received saline and corticosterone (15 mg/kg), 45 and 30 min before the test, respectively; Ver5 group received verapamil (5 mg/kg) and corticosterone carrier, 45 and 30 min before the test, respectively; Ver10 group received verapamil (10 mg/kg) and corticosterone carrier, 45 and 30 min before the test, respectively; Vere20 received verapamil (20 mg/kg) and corticosterone carrier, 45 and 30 min before the test, respectively; Gero Ver10+Cort group received verapamil (10 mg/kg) and corticosterone (15 mg/kg), 45 and 30 min before the test, respectively; and Ver20+Cort group received verapamil (20 mg/kg) and corticosterone (15 mg/kg), 45 and 30 min before the test, respectively.

Medications

In this study, the following medications were used for the experiments:

Corticosterone

It was dissolved in propylene glycol and injected into the rats 30 min before starting the behavioral test at 15 mg/kg live weight.

Verapamil (an antagonist of L-type voltage-dependent calcium channel)

This medication (5, 10 and 20 mg/kg) was dissolved in normal saline and injected into the target group 45 min before the behavioral tests. The doses of medications used in the current
study were based on the preliminary findings of the previous reports (Faryadian et al., 2008). All medications were administered to rats intraperitoneally 14 days after surgery.

**CCI method**

CCI was employed according to the method described by Bennet and Xie (1988). Animals were anesthetized using 50 mg/kg ketamine-rompan (1:8 ratio) followed by hair removal from the upper part of the rats’ thigh. A 2 cm cut was made on the thigh of the left foot by using a B razor. After cutting the muscles of the area and seeing the common part of the three branches of sciatic nerve, using two small glass rods, the tissues around the nerve were detached. Next, four loose knots were made in one millimeter distance before the nerve is turned into three branches using some stitch thread cut 4/0. The knots were made such that they could not make any disruption in the flow of the blood in the nerve. The muscle of rat and the skin were stitched together in order to expose the sciatic nerve more clearly.

**Behavioral tests**

The following behaviors were assessed on the 14th day after surgery.

**Mechanical allodynia**

Animals were placed on a wired network inside a Plexiglas with dimensions of 2020 cm (length and width) and 30 cm height. After the rats were accustomed to the new environment, the various fibers (2 to 60 g) of Von-Frey (Stolting Company) were used to assess the mechanical allodynia. The work was started with using the smallest number of fiber, whereas when there was lack of response, a higher number was selected. Each fiber was pressed three subsequent times in 5 s and each time for a second to the foot palm of the rat and if within the two subsequent times, the response was made (the animal lift his leg), it was considered as the threshold of the response and the test did not continue. In case the rat did not respond to fiber No. 60 too, then the number 60 was considered as the threshold of the response (Faryadian et al., 2008).

**Thermal hyperalgesia**

In this test, animals were placed in the special container of plantar test. After rats were accustomed to the new environment, the source of heat of ultraviolet ray was placed under the feet of the animal and shed with 60 intensity rate. This test was done on both feet of the rat for three subsequent times with 5 min interval. The time of the test cut was 60 s and the animal response was calculated through the following formula (Faryadian et al., 2008).

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\text{average time of the animal forbearance in the left leg} \times \text{average time of the forbearance in the right feet equals the response.}
\]

**Statistical analysis**

The results were presented using descriptive statistics. ANOVA test was used to compare the different groups, and the Tukey test was used to determine the difference between different groups. P<0.05 was considered as the cut-off point for the level of statistical significance. Data were analysed using SPSS version 16 (statistical software was used for this analysis).

**RESULTS**

**Impact of different doses of verapamil on behavioral pain responses resulting from CCI**

**Mechanical allodynia**

Figure 1a shows the impact of different doses of verapamil on mechanical allodynia (Von Frey test). The one-way variance analysis indicates some differences among various groups (F3, P=0.007). The next analysis with Tukey test showed that verapamil at 20 mg was able to reduce the mechanical allodynia in CCI rats (P<0.05).

**Thermal hyperalgesia**

Figure 1b shows the impact of different doses of verapamil on thermal hyperalgesia. The one-way variance analysis indicated some differences among various groups (F3, 28=5/12, P=0.006). The following analysis with Tukey test showed that these responses in CCI at 20 mg and verapamil with the CCI group are solely significant (P<0.01).

**Impact of verapamil on the effects of corticosterone on behavioral responses resulting from CCI**

**Mechanical allodynia**

Figure 2a shows the impact of different doses of verapamil on the effects of corticosterone on mechanical allodynia (Von Frey test). One-way variance analysis indicates the differences between various groups (F5, P=0.0001). The following analysis with Tukey test showed that this response in the CCI receiving CORT with the CCI group is solely significant (P<0.01). However, the difference between groups receiving VER10+CORT with the CCI group receiving CORT was statistically significant (P<0.05), whereas for the other cases, it was not.

**Thermal hyperalgesia**

Figure 2b shows the impact of different doses of verapamil on the role played by corticosterone on thermal hyperalgesia (test planter). One-way variance analysis indicated the differences between various groups (F5, P=0/0001). The Tukey test analysis showed that the response in the foregoing for the CCI group which received CORT with the CCI group was significant (P<0.01). However, it was observed that the difference
**Figure 1.** The impact of verapamil on behavioral pain resulting from CCI. (a) mechanical allodynia, (b) thermal hyperalgesia (P<0.01).

**Figure 2.** Evaluation of the impact of verapamil on the role played by corticosterone against the behavioral pain responses that resulted from CCI. (a) Mechanical allodynia, and (b) thermal hyperalgesia (P<0.01 compared to CCI group and P<0.05 compared to CCI + corticosterone group).

between the CCI group which received VER10+CORT and the receptor CCI group was not significant (P> 0.05); while the difference between the CCI group which received VER20+CORT in comparison with the CCI group which received CORT was significant (P<0.05).
It was revealed from the findings of the current study that corticosterone at 15 mg/kg inhibited the thermal hyperalgesia and mechanical allodynia two weeks after the neuropathy. We revealed that glucocorticoids played an important role in neuropathic pain when corticosterone was injected intra-peritoneal in a dose dependent manner. It inhibited the mechanical allodynia and thermal hyperalgesia, though the mechanism behind it was not clear yet. As corticosteron can pass from blood-brain barriers, and since the expression of its receptors within the central nervous system increased particularly inside the spine, it seems that the major impact of corticostron on pain behavior can take place via the spine. These results are preferred over those obtained by the previous studies, in that the injection of corticosterone was intraperitoneal, which is more effective than the intraspinal injection carried out by the previous studies. It was also revealed that verapamil, at 20 mg/kg as a voltage-dependent blocker of the calcium channels, can decrease the mechanical allodynia and thermal hyperalgesia two weeks after the neuropathic pain.

DISCUSSION

The peripheral injection (15 mg/kg) of corticosterone controls mechanical allodynia and thermal hyperalgesia, whereas the peripheral injection of verapamil diminishes the effects of corticosterone on mechanical allodynia and thermal hyperalgesia. Verapamil in high doses was able to control the mechanical allodynia and thermal hyperalgesia. The study of the effects of glucocorticoids on responses resulting from neuropathic pains showed that the intra-peritoneal injection of different doses of corticosterone controls the mechanical allodynia, thermal allodynia and thermal hyperalgesia. Findings of the current study are in agreement with those of a previous study (Faryadian et al., 2008). It seems that the existing glucocorticoids receptors in spine neurons play an important role in the control of pain (Mao, 2005). Studies showed that the intra-spinal cord injection of GR antagonist receptor or antisense oligonucleotides receptor of GR in CCI rats prevents symptoms of neuropathic pains (thermal hyperalgesia and mechanical allodynia) (Dolphin et al., 2006). Removing the adrenal has the same effects, whereas injection of dexamethasone inflamed and developed these symptoms. In addition, it was shown that in CCI rats, in line with the creation of mechanical allodynia and thermal hyperalgesia, the thickness of blood corticosterone increased and the expression of the receptor of GR, which is dependent on time, in the posterior spinal cord on the same side of the injured nerve was seen to a great extent. These sector changes were partly resulting from the increase of IL-6 and protein kinase C after creation of CCI (Wang et al., 2004; Takasaki et al., 2005). Also, intraperitoneal injection of the antagonists which are receptors of glucocorticoids in form of dose-dependent thermal hyperalgesia and mechanical allodynia in the neuropathy model reduced the pain in mouse (Takasaki et al., 2005).

The changes in the activity of the voltage-dependent calcium channels can cause fast and transit changes in the thickness of intra-cell calcium provoking cells. Also, in the spinal posterior horn, the activities of these channels play an important role in the processing of pain data. The spinal posterior ganglion neurons have various types of voltage-dependent calcium channels because the units of alpha-1 of these channels are the main determiners of the pharmacologic and biophysical features, whereas sub-units of alpha-2, sigma-1 and gamma along with alpha-1 are recognized as modifiers of the channel performance (Diaz and Dickenson., 1997; Malmberg Yaksh., 1995; Liu et al., 2000). New studies showed that the expression of the sub-units and the type of these channels in DRG neurons and spinal anterior horn increased after injury of the nerve. This increase is seen in the sub-units of alpha-1 and alpha-2. Sigma-1 after nerve damages in sensual neurons, possibly through calcium dependent processes, plays an important role in the processing of the sensual data of neuropathic pain and in the creation and emergence of neuropathic pain behaviors (Matthews and Dickenson., 2001; Dolphin et al., 2006; Kim et al., 2001). The previous studies showed that the local reduction of the calcium thickness at the area of nerve damage through under-the-skin injection, which is a connecting factor to calcium (which reduces the thickness of calcium) or control of the type N calcium channels, can reduce the hyperalgesia resulting from the mechanical stimulus in rates afflicted with damage (White and Cousins., 1998; Matthews et al., 2001). Also, blocking of these spinal channels with intra-spinal exclusive injection of the type N calcium channels can bring about strong anti-pain effects in the pain model resulting from the cut damage of the mouse’s feet. This effect is even stronger and more exclusive than intra-spinal injection of morphine (Wang et al., 2000). These findings show the intervention of the type N calcium channel in the emergence of symptoms of neuropathic pain. Although, the activity and expression of type L calcium channels varies during neuropathic pain (Dobrenez et al., 2005), the role of these channels in the emergence of neuropathic pain symptoms is not clear. The findings of this study show that the injection of verapamil in a dose-dependent manner reduced the symptoms of neuropathic pains, including mechanical allodynia, thermal allodynia and thermal hyperalgesia. However, this finding showed the role played by the type L calcium channels in the symptoms of neuropathic pains. Also, it was observed that verapamil was able to reduce the effects of corticosterone on mechanical allodynia and thermal hyperalgesia. This subject shows the role played by type L voltage-dependent calcium channels in the intervention of the effects of
corticosterone on the emergence of behaviors dependent on neuropathic pain. Previous studies showed that glucocorticoids were able to increase the ion orientation of type L calcium channels and the expression of the calcium channels’ sub-unit. Also, glucocorticoids were able to increase the expression of the calcium channels related genes and their orientation in these channels in Hippocampus and Amygdala neurons (Chameau et al., 2007; Karst et al., 2003). So, it is possible that with increasing the activity of these channels in the neurons of spinal posterior horn, corticosterone causes the emergence of neuropathic pain symptoms such that with the control of such increases, using calcium channels antagonist (verapamil), the effects of corticosterone become weak. The confirmation of this hypothesis demands future studies. In general, the results of this study and our previous studies (Faryadian et al., 2008) show that NMDA receptors and calcium channels play a role in the application of the effects of corticosterone in neuropathic pains. Glucocorticoids apply their own effects through gene or non-gene mechanisms. Gene mechanisms are in need of activation of the core receptors and in increasing the synthesis of the target proteins. The non-gene mechanisms result from activation of membranous receptors (Haller et al., 2008). With regard to the interference of NMDA receptors and calcium channels in mediating the effect of corticosterone (which was injected 30 min before the behavioral test) on neuropathic pain, these effects possibly resulted from non-gene mechanisms. The previous studies in our laboratories showed the intervention of non-genome mechanisms on the effects of glucocorticoids on memory and learning too (Pakdel et al., 2007; Sajadi et al., 2007). So, it seems that the non-gene mechanisms play important roles in the application of the effects of glucocorticoids in different behaviors.

Conclusion

This study showed that intraperitoneal injection of corticosterone in a dose-dependent manner controlled the mechanical allodynia, thermal allodynia and thermal hyperalgesia, and the activation of NMDA receptors played an important role in the application of these effects.

REFERENCES


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