CHAPTER V

DISCUSSION

In this present study, the experimental were conducted to investigate the effects of N/OFQ on the development of CSD-evoked cortical activity and trigeminal nociception. From our results, it could be discussed as the

Effect of N/OFQ on development of CSD

1.) CSD-evoked depolarization shift from extracellular recording

In our experiment, results demonstrated that topical application of solid KCl 3 mg on surface cortex for 2 hours can generate a series of CSD shift indicated by electrophysiological variables including amplitude, frequency, and AUC from extracellular recording. In contrast, topical application of solid NaCl 3 mg did not effect on cortical activity

The rational for using CSD-evoked depolarization shift in this study was that: 1) to search for a novel approach of migraine treatment, the precise molecular and physiological mechanism of migraine need to be identified. However, it is difficult to perform the experimental in patients during a migraine attack. Development of reliable for detection of CSD in humans will determine the extent to which the large body of experimental finding from animal studies of CSD because can be applied to the investigation and treatment of human brain disease [143]. Therefore, CSD phenomenon in animal has been developed and used as the model of migraine 2) CSD phenomenon was proposed to the mechanism underlying the migraine aura [27] and 3.) This is consistent with the observation that CSD is much easier to elicit in the brain with high neuronal density.

The results from KCl-vehicle group show that the application can generate a series of depolarization shift. Several investigators concluded that CSD is a complex phenomenon resulting from the interaction of various processes. Recently evidences indicated that, underlying the depolarization is a dramatic change in the distribution of ions between extra- and intracellular space. The unparalleled increase in K^+ and H^+ release from the cell, while Na^+, Ca^+, and Cl^- enter together with water [119, 120, 121] causing cells to swell and the volume of extracellular compartment to be reduced. Indeed, excitatory amino acid including glutamate and aspartate have been shown to be release into the extracellular space during CSD [122, 123]. The propagation of CSD is inhibited by pretreatment with the noncompetitive N-methyl-d-aspartate (NMDA) receptor antagonist, MK-801, and therefore is likely to be mediated by glutamatergic signaling through the NMDa receptor [124]. Another study showed that migraine patients during attack had higher CSF concentrations of glutamic acid than in control [125]

2.) Effect of N/OFQ administration on development of depolarization shift

N/OFQ is a signaling molecule that interacts with ORL-1 receptor located both pre- post synaptic terminals. The activation of ORL-1 receptor produces the opening of G-protein-activated inward rectifying K^+ (GIRK) channel [126]. It has been recently reported that N/OFQ is involved extensively in central nervous system pathway, including suppression of the neurotransmitter [127, 128]. These finding suggest that the effect of N/OFQ is mediated of neurotransmitter release of receptor activation. However, the results of present study showed enhancing effect of N/OFQ in CSD elicited by KCl application (Figure 4.2). The electrophysiological variables indicated the induction and propagation of CSD represent by the number of depolarization shift, peak amplitude, and AUC were significantly increased in the KCl-N/OFQ group when compared with KCl-vehicle group (Table 4.1 - 4.3).

The distribution of ORL-1 receptor has been examined at both the mRNA and protein levels. Many of papers reported the initial cloning of the receptor also reported on its distribution as determined by in situ hybridization or Northern blotting results, as have subsequent report. Distinct cellular expression may explicated the

discrepancy in N/OFQ function. In the cerebral cortex, ORL-1 receptor is expressed ubiquitously in presumptive GABAergic interneuron (Golgi type II cells) located layer II [129]. These interneuron terminate axons mainly on a projective glutamatergic neurons (Golgi type I cells) locate layer III. Considering that the activation of ORL-1 receptor induced inhibitory modulation, intrathecal administration of N/OFQ may reduce the GABA release from interneuron during CSD development and then the glutamate activation of adjacent projection neuron is enhance accordingly. On the other hand duration and interpeak latency were not alterd in the KCl-N/OFQ group (Table 4.6, 4.7). These results suggest that N/OFQ may not to be included in the sustainment of development of CSD.

Interestingly, the peak amplitude of depolarization shift after N/OFQ administration was decreased lower than baseline for 2 waves as compared with KCl-vehicle group, but thereafter peak amplitude rise upwards through electrophysiological recording (Figure 4.10). These significantly finding considering that N/OFQ administration may be modulate development of depolarization shift as two phase; 1.) After N/OFQ administration, ORL-1 receptor which expressed in cortex and couple to G_i-mediated intracellular signaling was activated [130] causing induction of hyperpolarization currents via inwardly rectifying K^+ channels [126, 131]. After early phase, peak amplitude rise upwards higher than baseline and KCl-vehicle group at same a time course (Table 4.8). As above discussion, considering that N/OFQ could be control glutamate and GABA release from interneuron in late-phase causing enhance the activity of glutamatergic neuron and enhance peak amplitude accordingly [137].

Effect of CSD –evoked depolarization shift on trigeminal nociception

Nociceptive (painful) signals originate in nociceptive sensory neuron terminal, where many ion channels and receptors are expressed to transducer various stimuli to neural signals. To test whether central neuron in primary- and secondary order neurons was activated by CSD phenomenon, we examined the expression of TRPV1in TG and c-Fos in TNC as a surrogate maker of neuronal activity.

1.) CSD induced TRPV1 expression in TG

During CSD development, extracellular K^+ rises up to 40-60 mM in the grey matter of the cortex [41]. Thus, elevated K^+ levels may depolarize the primary afferent neurons in the cortex and lead to the perivascular release of various peptides, importantly SP, CGRP, VIP, and NPY, thereafter triggers a C-fiber nociceptor, primary afferent fiber of trigeminal nociceptive system and terminated with central neuron in TG. In our stydy, using immunohistochemical technique to investigated effect of CSD on TRPV1 expression in TG, central neuron of nociceptor. After CSD induction for 2 hours, the number of TRPV1-IR cells on ipsilateral and contralateral side in KCl-vehicle was significantly greater than those of the NaCl-vehicle group (Table 4.8). This finding agrees with various studies, shown that noxious stimuli can cause expression of TRPV1 [132]. TRPV1 is important role in nociceptive pathway because mice lacking this receptor do not develop nociception an dpain sensation [133].

2.) CSD induced c-Fos expression in TNC

The number of Fos-IR cells is useful for mapping functionally related pathway and as a correlative indicator of TNC neuronal activity following noxious stimulation [134]. Results in present study revealed that the number of labeled positive cells in KCl-vehicle group was significantly higher than NaCl-vehicle group. c-Fos protein can be used to identify areas of neuronal activity and to map functionally related neuronal pathways [134, 135]. Within TNC, imuunoreactivity was prominent within ipsilateral laminae I and II in ventrlateral segments of the spinal region corresponding to inputs from the ophthalamic division [135, 136]. More than

likely, trigeminal maningeal afferents stimulated this response, and contralateral TNC (dosomedial as well as ventrolateral segments) would have featured prominently following stimulation by descending somatosensory connections from neocortex

Effect of N/OFQ on CSD –evoked depolarization shift on trigeminal nociception

The results from electrophysiological studies showed that N/OFQ administration potential on cortical activity indicated by electrophysiological variables. Immunohistochemical technique using to test effect of N/OFQ administration on trigeminal nociceptive system indicated by expression of TRPV1 in TG and expression Fos in TNC which is a pathway of nociception respectively.

1.) Effect of N/OFQ on TRPV1 expression in TG

The results from KCl-N/OFQ show that N/OFQ administration significantly increased TRPV1-IR in both ipsilateral- and contralateral side of TG when compared with NaCl-vehicle and KCl-vehicle group (Table 4.8). These finding suggest that ORL-1 receptor in cortex was activated by N/OFQ led to increase of Ca^{2+} current and reduced K^{+} conductance of primary nociceptor on cortex. It may imply that greater activated peripheral nociceptor transmit more nociceptive input to central nociceptive neuron, resulting in more expression of TRPV1.

Moreover, the endogenous peptide N/OFQ modulates the function of brain monoaminergic system via different mechanism and at different neuroanatomical levels. N/OFQ inhibits the firing of the serotonergic and noadrenergic ascending pathway by acting on cell bodies located in the raphe nuclei and modulates 5-hydroxytryptamine (5-HT) release by acting on synaptic terminal in receptive areas, in particular the cerebral cortex [138]. This modulatory action may subserve some of the biological effects of the peptide or its synthetic analogues, such as pain nociception [134, 139]

2.) Effect of N/OFQ on c-Fos expression in TNC

The c-Fos is an early response proto-oncogene and its rapidly and transiently expression to noxious inputs. In the final part, we demonstrated that enhancing effect of N/OFQ on c-Fos expression change as compared with NaCl-vehicle and KCl-vehicle group (Table 4.9). Results from Fos-IR revealed the similar pattern with TRPV1 expression in TG