CHAPTER V

DISCUSSION

This study was conducted to investigate the roles of NO and 5-HT on CSD-evoked cerebral hyperemia. CSD is an electrical phenomenon, considered to play an important role in the mechanism generating aura of migraine whereas NO and 5-HT are recognized to play significant roles in the process of headache phase.

CSD cause extensive changes in neurophysiological condition during its propagation across the brain surface (Hansen et al., 1980; Lauritzen, 1987a,b; Sugaya et al., 1975). Cortical cell are depolarized, extracellular K^+ concentration rises, vasoactive metabolites and neurotransmitter released (Krivanek, 1961). In these circumstances, various factors could promote cerebral vascular changes during CSD. In this study, the increasing of NO, a potent vasodilator, evoked by CSD was focus. A series of experiments were conducted to investigate the relationship between NO and the changes of cerebral vessels evoked by CSD.

An animal model was selected in this experiment. Rats were divided into two main groups. That is the control group and the CSD group. Several animal models are probable to trigger CSD (Lauritzen, 1994). In this study, CSD was induced by topical application of solid KCl 3 mg on the parietal brain surface whilst solid NaCl was served as a control (Read and Parsons, 2000). The changes of cerebral vessels were determined by using the laser Doppler flowmetry and the fluorescent microscopic technique.

1. The effect of KCl application on cerebral microvascular changes.

KCl application induced the repeated pattern of cerebral hyperemia. A wave of cortical hyperemia in associated with CSD was firstly suggested by Leao (1944) who demonstrated a brief period of pial dilation in the rabbit. Subsequently, a number of authors using a variety technique have confirmed that CSD results in phase of cortical hyperemia (Lauritzen, 1987a; Lauritzen et al., 1982). The mechanism underlying KCl induced CSD-evoked cerebral hyperemia has been proposed. Grafstein (1963) hypothesized that the increasing of extracellular K^+ concentration results in the desisting of intracellular K^+ efflux. Then the cell membrane potential becomes to depolarization (Grafstein, 1963). The depolarization is associated with dramatic changes in the distribution of ions between intra- and extracellular compartment: K^+ and H^+ leaves the cells, while Na^+, Ca^+ and Cl^- enter to the cell (Nicholsen and Kraig, 1981; Hansen, 1985). These changes induce some transmitter release, especially glutamate and NO. Glutamate is a potent mediator of the propagation of CSD (Curtis and Watkins, 1961) while NO is possible to play roles in the cortical hyperemia evoked by CSD. Therefore, the pattern change in this study is likely to be secondary detector to CSD. The NaCl application group was served as a control which showed no change in rCBF in any period throughout the experiment.

The vasodilation-vasoconstriction cycles were observed in the fluorescent microscopic study. The pattern of these cycles correlated well with the hyperemic cycles recorded by laser Doppler flowmetry. Our findings suggested that an increase in rCBF and pial arteriolar diameter during CSD were closely correlated.

In addition, in contradiction data presented by others (Duckrow, 1993; Goadsby et al., 1992; Lauritzen, 1994), we did not observe a period of oligaemia after CSD. However, some of experiments showed the data which concurred with our results (Colonna et al., 1997). Although the spreading oligaemia was not observed in our experiment, the transient spike activities in rCBF were showed as the unique in CSD phenomenon.

The above data suggested that the changes of neuronal activity evoked by CSD are associated with local circulation changes. Nonetheless, the chemical mediator which is responsible for such hyperemia evoked by CSD has not yet been defined.

2. The effect of NOS inhibitor on CSD-evoked cerebral hyperemia.

As previously mentioned, CSD evoked the release of various factors, that can promote cerebral hyperemia, including NO. In this experiment, the NOS inhibitor, L-NAME, was injected intravenously at the dose of 10 mg/kg BW after the 2^{nd} peak of hyperemic cycles. The amplitude of hyperemic peaks was rapidly attenuated after treatment. These results supported the hypothesis that NO synthesis is increased during CSD and NO is responsible for CSD-evoked cortical hyperemia.

In the present study, it was found that L-NAME administration attenuated CSD-induced cortical hyperemia without altering rCBF in the control group. We found that L-NAME could increase MABP in the control rats. However, these changes did not reach the statistical significant level. This hypertensive effect of L-NAME was likely to be due to its vasoconstriction effect. It is known that CSD induces glutamate release, which subsequently activated the NMDA receptor. Administration of NMDA antagonist could block the propagation wave of CSD (Gorelova et

al., 1987). Activation of NMDA receptor has been shown to stimulate the release of NO in brain via nNOS activity (Faraci et al., 1993; Meng et al., 1995; Northington et al., 1995).

More recent studies have confirmed the importance of perivascular nerves in mediating CBF changes during CSD (Bari et al., 2000; Gold et al., 1998; Reuter et al., 1998). Shimizu et al (2002) investigated the role of endothelium in hyperemia during CSD in rats. They used the intravascular administration of phorbal 12, 13-dibutyrate (PDBu) as the acute general pharmacological impairment of endothelial function. The results showed that the impairment of endothelial function did not affect CSD-induced cortical hyperemia. They suggested that endothelium plays a minor role in these responses. As the study by the same group, their results in the insulin resistant rats, the chronic impairment of endothelial-dependent dilator responses, provides further support that endothelium did not mediate vasodilation during CSD (Miller et al., 1998). The results from several studies supported the importance of nNOS activation in mediating cerebral hyperemia during CSD. That is also agreeing with our results and suggestion in the current study.

Here we showed that the minimizing effect of L-NAME on cortical hyperemia depended on its dosage. L-NAME at the dose of 1 mg/kg BW showed the slight effect on CSD-evoked cerebral hyperemia. On the other hand, L-NAME administration at the dose of 10 and 100 mg/kg BW almost completely attenuated cerebral hyperemia evoked by CSD. These data also supported that CSD induced cortical hyperemia by activated NO pathway.

Moreover, the changes of pial arteriolar diameter also were determined in this group. Administration of L-NAME could minimize the

maximum vasodilation of hyperemic peaks after treatment. It also correlated well with the rCBF changes recorded by laser Doppler flowmetry in the same group.

3. The effect of 5-HT_{1B} receptor agonist on CSD-evoked cerebral hyperemia.

It has long been held that 5-HT plays a pivotal role in migraine pathogenesis. This transmitter exerts it various physiological effect via its vast diversity of receptor subtypes. At least 15 subtypes of 5-HT receptors have been characterized. It has been proposed the role of 5-HT type1 and type2 receptor agonist on the CSD phenomenon. Sumatriptan, 5-$HT_{1B/1D}$ receptor agonist could not block or attenuate the CSD propagation wave. On the other hand, sumariptan could minimize the increase in NO level during CSD (Read and Parsons, 2000). It seems likely that sumatriptan, a potent antimigraine drug, may play a role in the mechanism of CSD-activated NO pathway. In 1998, Gold et al investigated whether 5-HT is involved in CSD-associated hyperemia in rat. Intravenous application of 8 mg/kg of the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin, significantly reduced the hyperperfusion amplitude during CSD. They concluded that 5-HT is involved in the modulation of the rCBF increase during CSD, probably via 5-HT_2 receptors (Gold et al., 1998). It is known that 5-$HT_{2A/2C}$ receptor activation elevates calcium concentration in the postsynaptic cells by mobilizing calcium from its intracellular stores and finally increase NO production. As the pervious suggestion, NO plays an important role in the CSD-evoked cerebral hyperemia. These mechanisms could explain the role of 5-HT_2 receptors on CSD-evoke cerebral hyperemia. Furthermore, 311C90, the 5-$HT_{1B/1D}$ receptor agonist, was investigated the effect on CSD-

evoked hyperemia in the same study. They found that 311C90 had no effect in the low dose (100μg/kg BW) whereas the high dose of 311C90 (800μg/kg BW) increased hyperperfusion significantly. They also suggested that the high dose of 311C90 may act via the 5-HT_2 receptors.

Therefore, in this study, naratriptan, 5-HT_{1B} receptor agonist was selected to investigate its effect on CSD-evoked cerebral hyperemia. Naratriptan at the dose of 0.1 mg/kg BW was injected intravenously after the 2^{nd} peak of hyperemic cycles. The results showed that naratriptan had no affect on CSD-evoked cerebral hyperemia both in the signals from laser Doppler flowmetry and the fluorescent microscope. The percent reduction from the naratriptan-treated group was not significantly difference when compare with the control CSD group. It is the same as the effects of naratriptan on MABP or rCBF in the control naratriptan-treated group. It was no changes in any period.

Two possible explanations exist in regards of these results. First, the dose of naratriptan used was low dose which was not potent enough to act via the 5-HT_1 receptor. However, this is unlikely since naratriptan has high affinity to 5-HT_{1B} receptor. This was evidenced by an observation of Suwattanasophon of the effect of this dose of naratriptan on minimizing NTG-evoked nNOS expression in trigeminal nerves. The second explanation was that, activation of 5-HT_{1B} receptors does not affect the CSD-evoked cerebral hyperemia. It is known that $5HT_{1B}$ receptor agonists have an important role in migraine therapy. This compound acts via constriction-mediating 5-HT_{1B} receptors on cerebral arteries. The 5-HT_{1B} receptors are found at smooth muscle cell of cerebral vessels (Longmore et al., 1997). This receptor is negatively couple to adenylyl cyclase. Naratriptan may act

via constriction-mediating 5-HT_{1B} receptors on cerebral arteries but not blocking neuronal activity within trigeminovascular afferents. As the previous suggestion, the mechanism of CSD-evoked cortical hyperemia was involved in the NO pathway. CSD induced glutamate release and finally activated nNOS activity. The increase in NO production in the perivascular nerves causes vasodilation, and results in cerebral hyperemia. The 5-HT_{1B} receptors agonists may act only in the vascular side as vasoconstrictor. It does not act on the trigeminovascular afferents or cortical nerve cells. Accordingly, its effects only on the cerebral blood vessels may not be potent enough to alter the CSD-evoked vasodilation or cerebral hyperemia.

4. The implication to the pathogenesis of migraine with aura

It has been proposed that CSD may account for neurological symptoms during the aura period of migraine (Lauritzen, 1994) and link excessive cortical excitability with activation of sensory afferent pathways (Moskowitz et al., 1993b; Parsons, 1998). The link between CSD and the generation of headache is not well understood. CSD induces many neurotransmitters release, ion homeostasis disturbances and vasoactive metabolites. Some of neurotransmitters release such as glutamate and NO, are known to be able to activate or sensitize the nociceptor. It has been suggested by Woolf (1996) that CSD stimulation of trigeminal afferent input from sensitized peripheral nerves. Indeed Moskowitz et al (1993b) showed that following induction of CSD there was increased expression of c-fos like immunoreativity in the trigeminal nucleus caudalis, demonstrating a clear association between CSD and nociceptive processing. However, apparently contradictory studies have recently been published (Ingvardsen et al. 1997; Lambert et al., 1999).

The proposed role of NO in migraine headache is based upon several pertinent experimental observations. Infusion of GTN into migraineurs induced a reliable and dose dependent immediate headache, followed by a late migraine attack several hours after termination of GTN infusion. NO is important for the pain phase in both forms of migraine. GTN is disintegrated to NO and is an exogenous source of NO (Feelish and Noack, 1987). NO activates cytoplasmic guanylate cylase, hence causing an increase in cyclic guanosine monophosphate, a decrease in intracellular calcium, and vasodilatation (Ignarro et al., 1981). Dilatation of cerebral arteries is known to cause pain (Nichols et al., 1990) and studies using transcranial Doppler sonography have demonstrated a dilatation of the middle cerebral artery during the pain phase in both migraine subtypes (Friberg et al., 1991; Theomsen et al., 1991). However, simple vasodilatation is not likely to be the only event responsible for spontaneous migraine headache (Thomsen, 1997). A simultaneous sensitization induces by NO, either in the periphery or centrally, has been suggested (Thomsen et al., 1994a,b).

It is interesting that the increase in NO level evoked by CSD may play roles in the pathogenesis of migraine with aura. CSD has recently been identified in human cortical tissue in vitro (Avoli et al., 1991) and in vivo (Mayevsky et al., 1995). NMDA is a potent trigger of CSD (Gorelova et al., 1987), and NMDA receptor activation in turn triggers the synthesis of NO (Faraci et al., 1993; Meng et al., 1995; Northington et al., 1995). Thus, CSD provokes an increase in NO release, which can be blocked by NOS inhibitors (Wahl et al., 1994). These observations have led to the proposal that NO may be the mediator not just of the coupling between CSD and the rCBF changes following CSD (Goadsby et al., 1992), but also as indicated in

this study, the mediator coupling CSD and post-aura headache. An immediate CSD-evoked NO release in pain-associated structure of the brain may explain the phenomenon that migraine headache is experienced almost immediately after the aura during spontaneous attacks. Aura without a subsequent headache may be due to the release of in sufficient amounts of NO from CSD or insensitivity to NO.

In the clinic, NOS inhibition using L-N^G methyl-arginine hydrochloride, has been reported to decrease headache and associated migraine symptoms (Lassen et al., 1998). Accordingly, NO may initiate migraine attacks, hence NOS inhibitor should also be evaluated for this effect as prophylactic agent in migraine. For this purpose, selective inhibitors without systemic circulation effects would clearly be needed.

According to our suggestion, the mechanism of CSD-evoked cerebral hyperemia may involve in the neuronal NO pathway. This mechanism may explain the relation between CSD and the pathophysiology of migraine with aura.