Chapter 8
Pharmacodynamic study of formulation in rat migraine model

8.1 INTRODUCTION

Migraine headache is thought to result from activation of the trigeminal nerve leading to painful distension of cerebral and meningeal blood vessels. This hypothesis is supported by studies showing that cranial blood vessels are richly innervated by perivascular trigeminal sensory nerves which contain the potent vasodilator calcitonin gene-related peptide (CGRP; Uddman & Edvinsson, 1989; Edvinsson, 1991; Jansen et al., 1992) and that elevated levels of CGRP have been detected in the jugular blood during acute migraine attacks in humans (Goadsby et al., 1990). Clinically effective anti-migraine 5-HT1B/1D receptor agonists such as sumatriptan are thought to abort migraine attacks via direct constriction of distended cranial and meningeal blood vessels (Humphrey & Feniuk, 1991) and/or inhibition of neuropeptide release from trigeminal sensory innervating these blood vessels (Moskowitz, 1992)

Although a complete understanding of the pathogenesis of migraine remains elusive thus far, there seems little doubt that dilatation of cranial blood vessels, including carotid arteriovenous anastomoses, is involved in the headache phase (De Vries et al., 1999). Moreover, evidence is accumulating that a release of vasoactive neuropeptides from the trigeminal sensory nerves may be an important factor in the genesis of migraine (Goadsby et al , 2002) In this respect, a high circulating plasma concentration of calcitonin gene-related peptide (CGRP) has been demonstrated during migraine headache (Goadsby et al., 1990), and these concentrations can be normalized by tryptans in parallel with alleviation of headache (Goadsby et al., 1990; Ashina et al., 2000). Indeed, CGRP is widely distributed in the body, including the central and peripheral parts of the trigeminovascular system (Brain et al., 1985; Van Rossum et al., 1997; Juaneda et al., 2000; Poyner & Marshall, 2001), where it is colocalised with substance P, neurokinin A and/or 5-HTID receptors (Gulbenkian et al., 1995; 2001; Smith et al., 2002). CGRP can mediate neurogenic dilatation of cranial blood vessels, as well as sensory nerve transmission between the first- and second-order afferent input from these vessels during migraine headache (Gulbenkian et al., 2001; Williamson & Hargreaves, 2001; Goadsby et al., 2002; Smith et al., 2002). Migraine headache is thought to be associated with dilatation of cranial vessels and activation of the trigemino-vascular system (Moskowitz & Macfarlane, 1993; Moskowitz, 1984; Goadsby et al., 1991). Moreover, during a migraine

attack levels of CGRP are increased (Goadsby et al., 1990; Gallai et al , 1995). Thus, CGRP has been implicated in the pathogenesis of migraine headache.

Capsaicin, the pungent ingredient in chili peppers, is used in various medicinal treatments to alleviate pain, even though its initial application can cause pain and inflammation (Sterner and Szallasi 1999; Szolcsanyi 1977; Waddell and Lawson 1989). The initial painful sensation after capsaicin application arises from the selective activation of vanilloid receptors in capsaicin-sensitive nociceptors that leads to depolarization and the generation of action potentials (Baumann et al. 1991; Gold et al 1996; Heyman and Rang 1985, LaMotte et al. 1991; Williams and Zieglganberger 1982). Capsaicin causes release of CGRP.

CGRP, a 37 amino acid neuropeptide first identified in 1982 (Amara et al., 1982) belongs to a family of peptides including calcitonin, adrenomedullin and amylin. Localization studies have shown a wide distribution of CGRP- like immunoreactive structures including receptors in the periphery and central nervous system (Sexton, 1991; Brain & Cambridge, 1996) In many tissues, CGRP containing nerves are closely associated with blood vessels. Although CGRP has a number of effects, its most pronounced action is vasodilatation. It is one of the most potent endogenous vasodilators known and its vasodilatory effects have been shown in a variety of vessels, e g CGRP released from sensory fibres originating in the trigeminal ganglia dilates cerebral vessels (Goadsby & Edvinsson, 1993)

Thus, it follows that inhibition of CGRP-mediated cranial vasodilatation and sensory nerve transmission with a potent and selective CGRP receptor antagonist may prove a novel strategy in treating migraine. Using an animal model that seems to be predictive of antimigraine activity the present study in anaesthetized rats was designed (i) to investigate the effects of capsaicin (pungent substance in red chilli pepper), which releases neuropeptides, including CGRP (Alving et al., 1991; Jansen-Olesen et al., 1996; Szallasi & Blumberg, 1999; Eltorp et al , 2000), and (ii) to establish if the sumatriptan formulations is able to attenuate the CGRP release.

8.2 EXPERIMENTAL

8.2.1 Standardization of Migraine model and pharmacodynamic study in rat

Male Sprague Dawley rats (200±15 gms) were anaesthetized with urethane (1.2g/kg, i.p.), a midline incision was made in neck region. Trachea was cannulated to fascilate breathing. Left jugular vein was cannulated for blood sampling. Capsaicin (10µg/kg, diluted from a 0.1% stock solution of capsaicin in 95% ethanol) was administered intravenously. This capsaicin concentration was used after standardization in pilot experiments, with higher doses causing respiratory depression in rats. Sumatriptan succinate solution was administered intranasally and subcutaneously. Sumatriptan succinate formulations were administered intranasally using previously described method. Blood samples were collected at 0, 15, 30, 45, 60, 90, 120, 180, 240 minutes after capsaicin administration Serum was separated from blood samples and analyzed for CGRP content on the same day using CGRP EIA kits.

8.2.2 Estimation of CGRP by Enzyme Immunoassay Kit

8.2.2a Kit contents· 96 well plates coated with mouse monoclonal antibody ready to use, Anti-CGRP Tracer – lyophilized, EIA buffer-lyophilised, Concentrated wash buffer, Tween 20, Ellman's reagent, Rat CGRP standard.

8.2.2b Procedure: Rinse each well 5 times with wash buffer. Keep wells empty for blank Dispense 100µl of EIA buffer to Non specific binding wells. Dispense 100µl of rat CGRP standard or sample in appropriate wells. Start with lowest concentration till the highest. Dispense 100µl of Anti CGRP ACHe tracer to each well and then cover the plate with a plastic film and incubate for 20 hrs at 4 °C. Empty the plate by turning over and shaking Wash each well three times with wash buffer, shake slightly for 2 min and then rewash 3 times with wash buffer. Dispense 200µl of Ellman's reagent and incubate in dark at room temperature for 45 min. Shake the plate at every 15 min intervals. Before reading shake the plate well and read the plate in micro plate reader at 405 nm.

8.2.2c Standard plot of CGRP:

Rat CGRP standard was used to obtain a standand plot of CGRP. Value of CGRP of test samples was obtained using standard plot of CGRP.

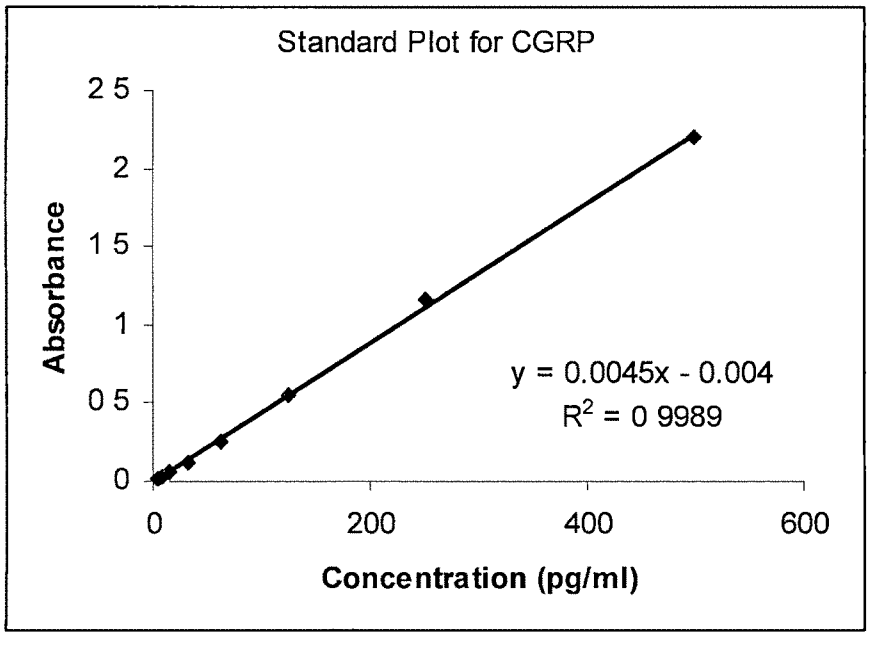

Fig 8.1. Standard plot of CGRP

8.2.3 Statistical analysis

All the data were expressed as mean ± SD(n=4), statistical calculations were done using Graph pad prism software using one way Analysis of Variacne(ANOVA). Results were considered statistically significant when $P<0.05$.

8.3 RESULT AND DISCUSSION

Overwhelming evidence suggests a crucial pathophysiological function of the activation of the trigeminal vascular system in migraine, which involves neuropeptide release and CGRP release in particular (Edvinsson and Goadsby, 1998) The strongest support of this concept is increased concentrations of CGRP during migraine attacks in the external jugular vein, the main venous effuent from the meninges (Goadsby and Edvinsson, 1993). Our study was conducted to validate a rat model that would allow the study of CGRP

release (induced by capsaicin) and its inhibition by sumatriptan succinate administered by subcutaneous and intranasal route (various mucoadhesive formulation and solution form) in a manner closely related to the human situation.

CGRP levels in control (Vehicle) and capsaicin administered rats (Migraine control) are shown in the figure 8.2. CGRP levels were significantly higher in capsaicin administered rats as compared to vehicle administered rats. Administration of capsaicin significantly increased CGRP levels. Migraine headache is thought to result from activation of the trigeminal nerve leading to painful distension of cerebral and meningeal blood vessels. Further elevated levels of a potent vasodilator calcitonin gene-related peptide (CGRP; Uddman & Edvinsson, 1989; Edvinsson, 1991; Jansen et al., 1992) has been detected in the jugular blood during acute migraine attacks in humans (Goadsby et al., 1990). Administration of capsaicin significantly increased CGRP levels in plasma of all the rats and the levels remained high throughout the experimental period. It is well reported that CGRP levels are increased during migraine attacks in humans also (Uddman & Edvinsson, 1989; Edvinsson, 1991; Jansen et al., 1992; Goadsby et al., 1990). Hence administration of capsaicin is believed to induce migraine as evidenced by elevated levels of CGRP in plasma of rats.

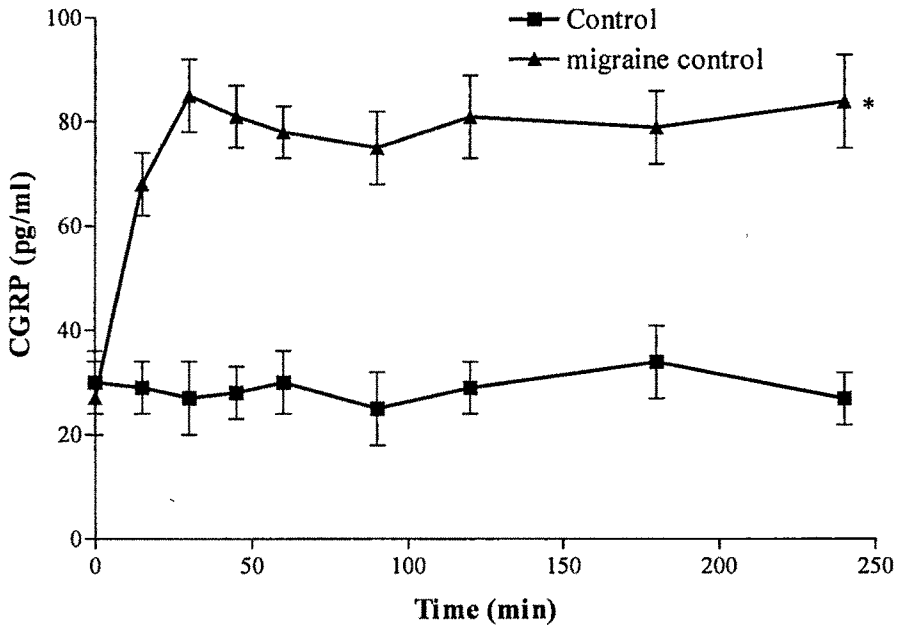

Fig 8.2. Rat CGRP levels in plasma after capsacain (migraine control) or vehicle (Control) administration at various time points. CGRP levels are expressed as pg/ml. Data are expressed as mean ±SD (n=4). * $p < 0.05$

Effect of sumatriptan formulations on rat model of migraine.

Migraine was induced in rats as described above by administration of capsaicin. Sumatriptan has been widely used for migraine therapy. Administration of sumatriptan significantly reduces CGRP levels (Arulmani et al, 2004; Juhasz et al, 2005; Buzzi et al, 1991). It is proposed by Moskowitz et al(Moskowitz et al ,1992) that sumatriptan acts at trigeminal 5-HT 1B and 1D receptors to inhibit release of CGRP and the associated neurogenic inflammation. Thus it blocks a deleterious feedback loop in migraine and restore CGRP levels to normal. Formulations were administered intranasally immediately after capsaicin administration. The nasal formulations were compared with sumatriptan solution administration - intranasal and s.c.

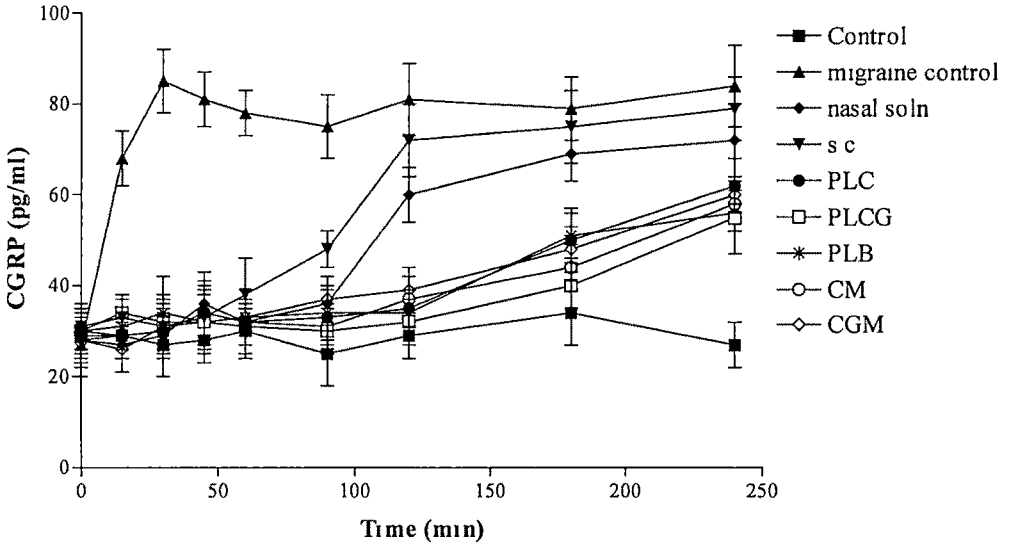

Fig 8.3. Effect of sumatriptan formulations on CGRP levels in rat plasma, after capsaicin administration. All the values are expressed as mean ±SD (n=4). (s.c. = subcutaneous, PLC = Pluronic carbopol gel, PLCG = Pluronic chitosan glutamate gel, PLB = Pluronic gel, CM = Carbopol microspheres, CGM = Chitosan glutamate microspheres)

CGRP levels were significantly ($P<0.05$) higher in migraine control group after administration of capsaicin. Administration of sumatriptan in different forms reduced CGRP levels in plasma When sumatriptan solution was administered subcutaneously or intranasally, CGRP levels were significantly reduced upto 90 min but after 120min CGRP levels were significantly higher as compared to control group. Subcutaneous or intranasal sumatriptan solution was unable to reduce CGRP levels after 120 min as CGRP levels were increased significantly after 120min. During migraine attack, CGRP levels are significantly higher (Goadsby et al , 1990), hence subcutaneous or intranasal sumatriptan solution is able to reduce CGRP thereby alleviate pain in migraine for 90 min but as CGRP levels increases after 2 hrs, migraine attack may reoccur after 2 hr. This increase in CGRP levels may be due to reduction in sumatriptan levels at target sites, sumatriptan nasal solutions and by subcutaneous route has been reported to have half life of 1.4 -2 hrs (Christensen et al, 2003, 2004). Sumatriptan is able to prevent release of CGRP from dural perivascular axon terminals with and without TG (trigeminal) stimulation through $5\text{-}HT_{1D}$ receptors. Under this paradigm immunohistochemical techniques show CGRP

accumulation within increased varicosities of the dural axon terminals. It has been demonstrated that sumatriptan does not cross BBB however it acts on central structures after disruption of BBB and therefore is available only to lesser extent to inhibit central activation obtained by TG stimulation. There is an ongoing debate whether a prejunctional site of action is sufficient for the anti-migraine effect of triptans (selective 5-HT $_{1B/1D/1F}$ agonists) or a central mechanism is also required. Sumatriptan have shown to inhibit evoked and spontaneous firing of second order trigeminal neurons by direct local application (Storer et al, 1997). These findings demonstrate that the synapse of the trigeminal nucleus in the brain stem and upper cervical cord provide additional sites of action for brain penetrating anti-migraine drugs of the 5HT 1B / 1D class (Storer et al,1997). Thus, when sumatriptan succinate is administered by subcutaneous route, its action is highly limited to prejuctional 5HT receptors as its penetration in brain is highly limited. Thus CGRP levels are evoked after 120 min as sumatriptan succinate is not available for action on centrally located target sites. This data is in correlation with pharmacokinetic data in previous chapter, where t1/2 of sumatriptan succinate in brain and csf was significantly lower for sumatriptan solution administered subcutaneously and intranasally compared to almost all other intranasal formulations. In case of intranasal solution CGRP levels evoked after 120 min as residence time of the solution in the nasal cavity is very less due to mucociliary clearance resulting in limited permeation of sumatriptan in brain by direct olfactory pathway due to rapid clearance of the drug from the site of permeation.

While administration of sumatriptan nasal formulations caused significant decrease in CGRP levels and CGRP levels remained lower for 3 hrs. PLCG formulation effectively reduced CGRP levels in rat plasma and it remained significantly lower (compared to migraine control) for 4 hours, whereas all other formulation did not showed significant reduction in CGRP levels at four hours (compared to migraine control). From the present study the efficacy of sumatriptan formulations is ranked as follows PLCG > CGM > PLB = PLC = CM > nasal soln = s.c. As described in previous chapter all the formulations possessed mucoadhesive potential which will retain the formulations for longer period of time in the nasal cavity and vicinity for direct permeation of sumatriptan succinate to brain via olfactory pathway will increase. This will result in longer duration of action of sumatriptan at centrally located target sites ($5HT_{1B/1D}$ receptors). Thus inhibition of CGRP release is sustained for 3 hours for all mucoadhesive formulations which also have permeation enhancing potential (except pluronic gel). Previously there is not a single

study reported where CGRP levels reduction has been studied in time related manner. We have attempted to study effect of various formulation on attenuating CGRP reduction by more specific central action (which will reduce cardiac side effects) for prolong period of time which will probably prevent repeated dosing often required with present day therapy (particularly intranasal solution and oral dosage form). Intranasal mucoadhesive formulations with permeation enhancing effect seems to be effective antimigraine therapy with more specific and prolonged central action on intracranial target sites, however it needs to be proven clinically.

8.4 CONCLUSION

Thus from the present study it can be concluded that sumatriptan formulations significantly reduced CGRP level in plasma for prolonged period of time compared to both subcutaneous route as well as intranasal solution. This could be attributed to mucoadhesive potential of the formulations associated with permeation enhancing effect, with increased central action on intracranial target sites (5 $HT_{1B/1D}$ receptors),which may be due to direct transport of sumatriptan succinate from the nasal cavity to brain via olfactory pathway.

8.5 REFERENCES.

Alving K, Matran R & Lundberg JM. (1991). Capsaicin induced local effector responses, autonomic reflexes and sensory neuropeptide depletion in the pig. Naunyn-Schmiedeberg's Arch. Pharmacol., 343, 37 – 45.

Amara SG, Jones V, Rosenfeld MG, Ong ES & Evans,RM. (1982). Alternative RNA processing in calcitonin gene expression generates mRNA encoding different polypeptide products. Nature, 298, 240 - 244.

Arulmani U, Heiligers JP, Garrelds IM, Sanchez-Lopez A, Willems EW, Villalon CM & Saxena PR (2004). Effects of sumatriptan on capsaicin-induced carotid haemodynamic changes and CGRP release in anaesthetized pigs. Cephalalgia, 24(9), 717-27.

Ashina M, Bendtsen L, Jensen R, Schifter S & Olesen J. (2000). Evidence for increased plasma levels of calcitonin generelated peptide in migraine outside of attacks. Pain, 86, 133 – 138.

Baumann TK, Simone DA, Shain CN & LaMotte RH (1991). Neurogenic hyperalgesia: the search for the primary cutaneous afferent fibers that contribute to capsaicin-induced pain and hyperalgesia. J Neurophysiol, 66, 212–227.

Brain SD & Cambridge H. (1996). Calcitonin Gene-Related Peptide. Vasoactive Effects and Potential Therapeutic Role. Gen. Pharmacol., 27, 607 - 611.

Brain SD, Williams TJ, Tippins JR, Morris HR & Macintyre I. (1985). Calcitonin gene-related peptide is a potent vasodilator. Nature, 313, 54 – 56.

Buzzi MG, Carter WB, Shimizu T, Heath H 3rd & Moskowitz MA (1991). Dihydroergotamine and sumatriptan attenuate levels of CGRP in plasma in rat superior sagittal sinus during electrical stimulation of the trigeminal ganglion. Neuropharmacol., 30(11), 1193-200.

Christensen ML, Mottern RK, Jabbour JT & Fuseau E (2003). Pharmacokinetics of Sumatriptan Nasal Spray in Adolescents J. Clin. Pharmacol., 43, 721-726.

Christensen ML, Mottern RK, Jabbour JT & Fuseau E (2004). Pharmacokinetics of Sumatriptan Nasal Spray in Children J. Clin. Pharmacol., 44(4), 359 - 367.

De Vries P, Villalo´ N CM & Saxena PR. (1999). Pharmacological aspects of experimental headache models in relation to acute antimigraine therapy. Eur. J. Pharmacol., 375, 61 – 74.

Edvinsson L. (1991) Innervation and effects of dilatory neuropep- tides on cerebral vessels. Blood Vessels, 28, 35 - 45.

Edvinsson L, Goadsby PJ (1998). Neuropeptides in headache. Eur J Neurol.,5,329-341.

Eltorp CT, Jansen-Olesen I & Hansen AJ. (2000). Release of calcitonin gene-related peptide (CGRP) from guinea pig dura mater in vitro is inhibited by sumatriptan but unaffected by nitric oxide Cephalalgia, 20, 838 – 844

Gallai V, Sarchielli P, Floridi A, Franceschini M, Codini M, Glioti G,Trequattrini A & Palumbo R. (1995). Vasoactive peptide levels in the plasma of young migraine patients with and without aura assessed both interictally and ictally. Cephalalgia, 15, 384 - 390.

Goadsby PJ & Edvinsson L. (1993). The trigemino-vascular system and migraine: studies characterizing cerebrovascular and neuropeptide changes seen in humans and cats. Ann. Neurol., 33, 48 - 56.

Goadsby PJ, Edvinsson L & Ekman R. (1990). Vasoactive peptide release in the extracerebral circulation of humans during migraine headache. Ann. Neurol., 28, 183 - 187.

Goadsby PJ, Lipton RB & Ferrari MD (2002). Migraine – current understanding and treatment. N. Engl. J. Med., 346, 257 – 270.

Goadsby PJ, Zagami AS & Lambert GA (1991). Neural processing of craniovascular pain: a synthesis of the central structures involved in migraine Headache, 31, 365 - 371.

Gold MS, Dastmalchi S & Levine JD (1996). Co-expression of nociceptor properties in the dorsal root ganglion neurons from the adult rat in vitro. Neuroscience, 71, 265–275.

Gulbenkian S, Barroso CP, Cunhaesa M & Edvinsson L. (1995). The peptidergic innervation of human coronary and cerebral vessels. Ital. J. Anat. Embryol., 100, 317 – 327

Heyman I & Rang HP (1985). Depolarizing responses to capsaicin in a subpopulation of rat dorsal root ganglion cells Neurosci. Lett., 56. 69–75.

Humphrey PP & Feniuk W. (1991) Mode of action of the anti- migraine drug sumatriptan. Trends Pharmacol. Sci., 12, 444 - 446.

Jansen I, Uddman R, Ekman R, Olesen J, Ottosson A & Edvinsson L. (1992). Distribution and effects of neuropeptide Y, vasoactive intestinal peptide, substance P, and calcitonin gene-related peptide in human middle meningeal arteries. comparison with cerebral and temporal arteries Peptides, 13, 527 - 536.

Jansen-Olesen I, Mortensen A & Edvinsson L (1996). Calcitonin gene-related peptide is released from capsaicin-sensitive nerve fibres and induces vasodilatation of human cerebral arteries concomitant with activation of adenylyl cyclase. Cephalalgia, 16, 310 – 316.

Juaneda C, Dumont Y & Quirion R. (2000). The molecular pharmacology of CGRP and related peptide receptor subtypes. Trends Pharmacol. Sci., 21, 432 – 438.

Juhasz G, Zsombok T, Jakab B, Nemeth J, Szolcsanyi J & Bagdy G (2005). Sumatriptan causes parallel decrease in plasma calcitonin gene-related peptide (CGRP) concentration and migraine headache during nitroglycerin induced migraine attack. Cephalalgia, 25(3), 179-83.

LaMotte RH, Shain CN, Simone DA & Tsai RJ (1991). Neurogenic hyperalgesia: psychophysical studies of underlying mechanisms. J Neurophysiol 66, 190–211.

Moskowitz MA & Macfarlane R. (1993). Neurovascular and molecular mechanisms in migraine headaches. Cerebrovasc. Brain Metab. Rev., 5, 159 - 177.

Moskowitz MA (1984). The neurobiology of vascular head pain. Ann Neurol., 16, 157 - 168.

Moskowitz MA. (1992). Neurogenic versus vascular mechanisms of sumatriptan and ergot alkaloids in migraine. Trends Pharmacol. Sci., 13, 307 - 311.

Poyner D & Marshall I. (2001). CGRP receptors: beyond the CGRP1 – CGRP2 subdivision? Trends Pharmacol. Sci., 22, 223.

Sexton PM. (1991). Central nervous binding sites for calcitonin and calcitonin gene-related peptide.Mol. Neurobiol., 5, 251 - 273.

Sterner O & Szallasi A (1999). Novel natural vanilloid receptor agonists: new therapeutic targets for drug development. Trends Pharmacol Sci , 20, 459–465.

Storer RJ, Goadsby PJ (1997). Microiontophoretic application of serotonin (5HT)1B/1D agonists inhibits trigeminal cell firing in the cat, Brain. 120 , 2171–2177.

Szallasi A & Blumberg PM. (1999). Vanilloid (capsaicin) receptors and mechanisms. Pharmacol Rev., 51, 159 – 212.

Szolcsanyi J (1977). A pharmacological approach to the elucidation of the role of different nerve fibers and receptor endings in mediation of pain. J. Physiol., 73, 251–259..

Uddman R & Edvinsson L. (1989). Neuropeptides in the cerebral circulation. Cerebrovasc. BrainMetab. Rev., 1, 230 - 252.

Van Rossum D, Hanisch UK & Quirion R. (1997). Neuroanatomical localization, pharmacological characterization and functions of CGRP-related peptides and their receptors. Neurosci. Biobehav. Rev., 21, 649 – 678.

Waddell PJ & Lawson SN (1989). The C-fiber conduction block caused by capsaicin on rat vagus nerve in vitro. Pain 39: 237–242.

Williams JT & Zieglganberger W (1982). The acute effects of capsaicin on rat primary afferents and spinal neurons. Brain Res.,253, 125–131.