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Effect of Ramipril, Valsartan and Candesartan on Thermal and Visceral Pain in Mice

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Abstract: The effect of the angiotensin converting enzyme inhibitor ramipril and the angiotensin II receptor blockers valsartan and candesartan on thermal and visceral pain was studied using the hot plate and abdominal stretching assays in mice. In both tests, ramipril (0.22 and 0.44 mg kg^{-1}, s.c.) and valsartan (6.9 and 13.8 mg kg^{-1}, s.c.), but not candesartan (0.69 and 1.38 mg kg^{-1}, s.c.) produced a dose-related reduction in nociceptive responses. The analgesic effect of ramipril (0.22 mg kg^{-1}, s.c.) in the writhing test was slightly reduced by co-treatment with atropine (1 mg kg^{-1}, s.c.), but almost reversed by propranolol (1 mg kg^{-1}, s.c.) or naloxone (5 mg kg^{-1}, i.p.). Meanwhile, the analgesic effect of valsartan (13.8 mg kg^{-1}, s.c.) was reversed by co-treatment with propranolol or atropine. These results suggest the involvement of beta adrenoceptor mediated mechanism in the visceral analgesic effect of ramipril and valsartan. In addition, results suggest that the visceral antinociceptive effect of ramipril is likely to involve an opioid sensitive mechanism; whilst that of valsartan involves a muscarinic acetylcholine receptor mediated mechanism.

Key words: Angiotensin converting enzyme inhibitor, angiotensin II receptor blockers, visceral nociception, mice, ramipril, valsartan, candesartan

INTRODUCTION

Angiotensins are peptide autacoids derived from the protein precursor angiotensinogen by the sequential actions of proteolytic enzymes. Angiotensinogen is synthesized by the liver and released into the blood, where it is cleaved to form angiotensin I by renin secreted from the juxtaglomerular cells of the kidneys. Angiotensin- Converting Enzyme (ACE) catalyzes the formation of angiotensin II from angiotensin I. This enzyme occurs not only in plasma but also in the kidneys, brain, adrenal glands, ovaries and possibly other tissues. Angiotensin II is a potent vasoconstrictor and the blockade of its synthesis by ACE-inhibitors or blockade of its action by Angio Tensin I receptor (AT1) antagonists are currently the medications of choice in management of hypertension and cardiac failure. Circulating angiotensin II is dispersed to the target tissues of the body, where it exerts a multiplicity of physiological functions via their interactions with specific angiotensin receptors (Matsukawa and Ichikawa, 1997; De Gasparo *et al*., 2000).

There is evidence to suggest a role for angiotensin II in central nociceptive modulation. The repeated oral administration of the ACE inhibitor spirapril (5 mg kg^{-1}) and the angiotensin II antagonist losartan (10 mg kg^{-1}) (though not enalapril) increased hot-plate latency in mice, an effect that was reversed by the opioid receptor antagonist naloxone (Takai *et al*., 1996). Captopril administered i.p. reduced visceral nociception evoked by i.p. acetic acid in mice (Motta *et al*., 2002). Angiotensin II injected intrathecally inhibited the transmission of thermal nociceptive information through an endogenous opioid mechanism and the activation of an AT1 receptor in the rat spinal cord

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(Toma *et al*., 1997). Angiotensin II injected into the Peri-Aqueductal Gray matter (PAG) increased tail-flick latency, an effect inhibited by injecting the specific angiotensin type 1 and type 2 receptor (AT1 receptor and AT2 receptor) antagonists losartan and CGP42,112A, respectively, into the PAG (Pelegrini-da-Silva *et al*., 2005).

Angiotensin-II receptor blockers are a newer class of antihypertensive agents. These drugs are selective for angiotensin II (type 1 receptor); unlike angiotensin-converting enzyme inhibitors, they do not inhibit bradykinin metabolism or enhance prostaglandin synthesis (Kirk, 1999). The aim of the present study was to: (1) evaluate and compare the analgesic effect of systemic administration of the angiotensin converting enzyme inhibitor ramipril and the angiotensin II receptor blockers valsartan and candesartan; (2) examine the possible contribution of opioidergic, adrenergic, cholinergic and adenosine receptors in mediation of the analgesic effect of these drugs. The hot-plate assay of thermal pain and the acetic acid-induced writhing response in mice; a model of visceral pain (Koster *et al*., 1959) were used for this purpose.

MATERIALS AND METHODS

Animals

Swiss male albino mice weighing 22-25 g were used in all experiments. Animals were acclimatized to the laboratory conditions for at least 1 h before testing and were used once during the experiments. The study was done in the department of Pharmacology, National Research Centre, on November 2006. The doses of drugs were based on the human daily dose converted to that of mice according to Paget and Barnes (1964).

Hot-Plate Assay

The hot-plate test was performed according to D'Amour and Smith (1941) by using an electronically controlled hotplate (Ugo Basile, Italy) heated to 52°C (±0.1°C). The cut-off time was 30s. Groups of mice (n = 6/group) were given ramipril (0.22 and 0.44 mg kg^{-1}, s.c.), valsartan (6.9 and 13.8 mg kg^{-1}, s.c.), candesartan (0.69 and 1.38 mg kg^{-1}, s.c.) or saline (control), 30 min prior to testing. The experimenter was blind to dose. Latency to lick hind paws or trials to jump out of the apparatus was recorded for the control and drug-treated groups.

Acetic Acid-Induced Writhing

Groups of 6 mice were administered the vehicle and/or the drugs under study. After the appropriate pretreatment interval (30 min), an i.p. injection of 0.6% acetic acid was administered (0.2 mL/mice) (Koster *et al*., 1959). Each mouse was then placed in an individual clear plastic observational chamber and the total number of writhes made by each mouse was counted for 30 min after acetic acid administration.

In another series of experiments, attempts were made to elucidate the mechanisms by which ramipril or valsartan exert their analgesic effect. Thus, the effects of the following agents on ramipril or valsartan-induced analgesia were examined; alpha-1 adrenoceptor antagonist prazosin (1 mg kg^{-1}, s.c.), alpha-2 adrenoceptor antagonist yohimbine (5 mg kg^{-1}, s.c.), the beta adrenoceptor antagonist propranolol (1 mg kg^{-1}, s.c.), the muscarinic acetylcholine receptor antagonist atropine (1 mg kg^{-1},s.c.), the non-selective opioid receptor antagonist naloxone (5 mg kg^{-1}, i.p.) and the non-selective adenosine receptor antagonist theophylline (20 mg kg^{-1}, s.c.). All drugs were administered 30 min prior to the injection of acetic acid.

Drugs and Chemicals

Atropine sulfate, yohimbine hydrochloride, propranolol hydrochloride, naloxone hydrochloride (Sigma, St. Louis, USA) were used. Ramipril (Aventis), valsartan (Tareg, Novartis), candesartan

(Atacand, AstraZeneca). Analytical-grade glacial acetic acid (Sigma, St. Louis, USA) was diluted with pyrogen-free saline to provide a 0.6% solution for i.p. injection. All drugs were dissolved in isotonic (0.9% NaCl) saline solution immediately before use.

Statistical Analyses

Data are expressed as means±SE Data were analyzed by one way analysis of variance, followed by a Tukey's multiple range test for post hoc comparison of group means. When there were only two groups a two-tailed Student's t-test was used. For all tests, effects with a probability of $p<0.05$ were considered to be significant.

RESULTS

Hot-plate Assay

The mean reaction time on the hot plate was significantly prolonged 30 min after the administration of ramipril (0.22 and 0.44 mg kg^{-1}, s.c.) and valsartan (6.9 and 13.8 mg kg^{-1}, s.c.), compared with basal values, denoting decreased nociception. Candesartan (0.69 and 1.38 mg kg^{-1}, s.c.) failed to alter hot-plate latency (Fig. 1).

Acetic Acid-induced Writhing

Ramipril (0.22 and 0.44 mg kg^{-1}) injected s.c. 30 min before i.p. injection of acetic acid, significantly reduced the number of abdominal constrictions in a dose-dependent manner by 67.8 and 76.3%, respectively (Fig. 2). Significant inhibition of the writhing response by 42.4% was observed after the administration of valsartan 13.8 mg kg^{-1}, s.c.as compared to saline-control group (Fig. 3). The administration of candesartan (0.69 and 1.38 mg kg^{-1}, s.c.) was without effect on the nociceptive response to acetic acid (Fig. 4).

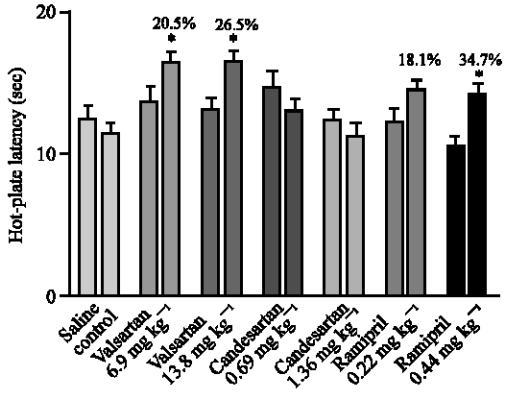

Fig. 1: Hot plate latencies (seconds) of mice treated with saline (control), valsartan, candesartan or ramipril. Basal and 30 min latencies were determined for each treatment group (saline, valsartan, candesartan or ramipril). The first column represents the basal (pre-drug) latencies and the second column represents the 30 min- (post-drug) values of each treated group. Saline or drugs were s.c. administered 30 min prior to testing. Data are expressed as means±SE. Percent increase in hot plate latenices (%) compared to the baseline is also shown an top of bars. Hot-plate latencies comparisons were made between baseline paw withdrawal latencies (pre-drug values) as compared with the respective post-drug values. *$p<0.05$ vs. basal (pre-drug value). Six mice were used per each group

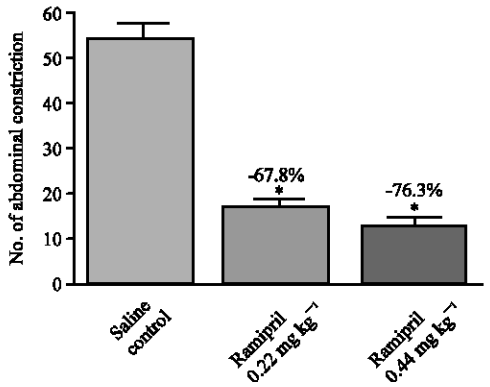

Fig. 2: Effect of ramipril given s.c. on the number of abdominal constrictions caused by i.p. injection of dilute acetic acid in mice. *p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to the control animals is also shown

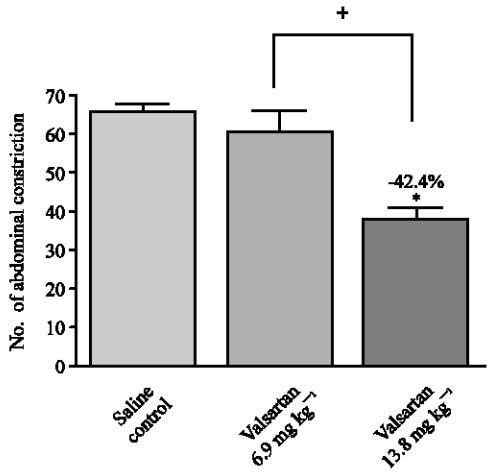

Fig. 3: Effect of valsartan given s.c. on the number of abdominal constrictions caused by i.p. injection of dilute acetic acid in mice. *p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to the control animals is also shown. +p<0.05 comparing the two doses of valsartan

Figure 5 shows that the analgesic effect of ramipril (0.22 mg kg^{-1}, s.c.) in the writhing test was slightly reduced by co-treatment with the muscarinic receptor antagonist atropine, but almost reversed by the beta adrenoceptor antagonist propranolol (1 mg kg^{-1}, s.c.) or the non-specific opioid receptor antagonist naloxone (5 mg kg^{-1}, i.p.). Meanwhile, the analgesic effect of valsartan (13.8 mg kg^{-1}, s.c.) was reversed by co-treatment with the beta adrenoceptor antagonist propranolol or the muscarinic receptor antagonist atropine (1 mg kg^{-1}, s,c.). Naloxone was without effect on valsartan antinociception (Fig. 6). The antinociceptive effect of ramipril was increased by alpha-2 adrenoceptor antagonist yohimbine (5 mg kg^{-1}, s.c.), but unaffected by the alpha 1-adrenoceptor antagonist prazosin (1 mg kg^{-1}, s.c.) or by adenosine receptor antagonist theophylline (20 mg kg^{-1}, s.c.) (Fig. 7). Valsartan antinociception was increased by yohimbine or prazosin, but unaffected by theophylline (Fig. 8).

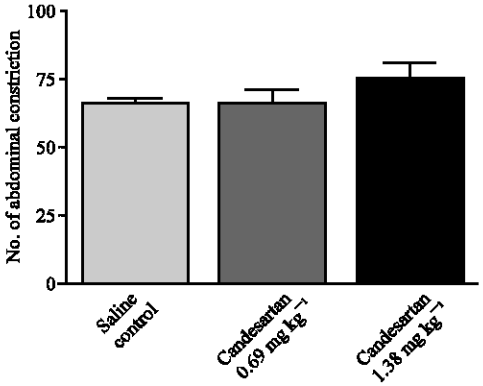

Fig. 4: Effect of candesartan given s.c. on the number of abdominal constrictions caused by i.p. injection of dilute acetic acid in mice

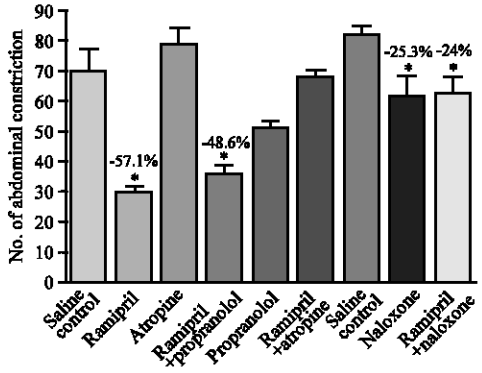

Fig. 5: Effect of atropine (1 mg kg^{-1}, s.c.), propranolol (1 mg kg^{-1}, s.c.) or naloxone (5 mg kg^{-1}, i.p.) on the analgesic effect of ramipril (0.22 mg kg^{-1}, s.c.) in the acetic acid abdominal constriction assay.*p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to control is also shown

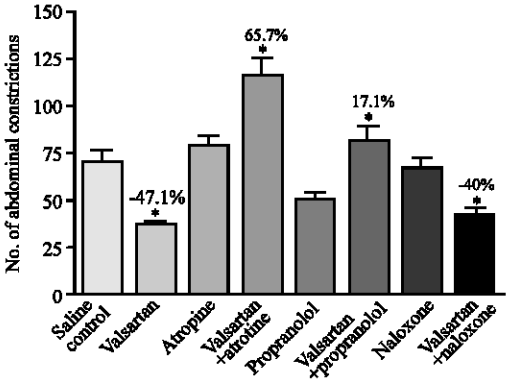

Fig. 6: Effect of atropine (1 mg kg^{-1}, s.c.), propranolol (1 mg kg^{-1}, s.c.) or naloxone (5 mg kg^{-1}, i.p.) on the analgesic effect of valsartan (13.8 mg kg^{-1}, s.c.) in the acetic acid abdominal constriction assay in mice. *p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to control is also shown

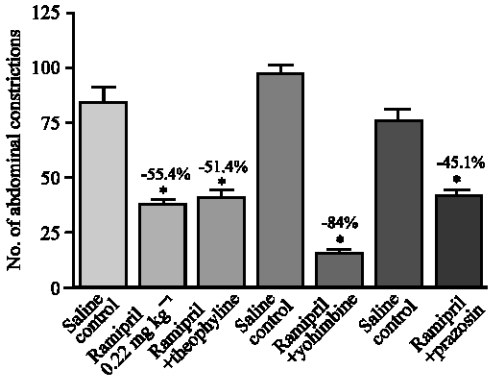

Fig. 7: Effect of theophylline (20 mg kg^{-1}, s.c.), yohimbine (5 mg kg^{-1}, s.c.) or prazosin (1 mg kg^{-1}, s.c.) on the analgesic effect of ramipril (0.22 mg kg^{-1}, s.c.) in the acetic acid abdominal constriction assay in mice. *p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to control is also shown

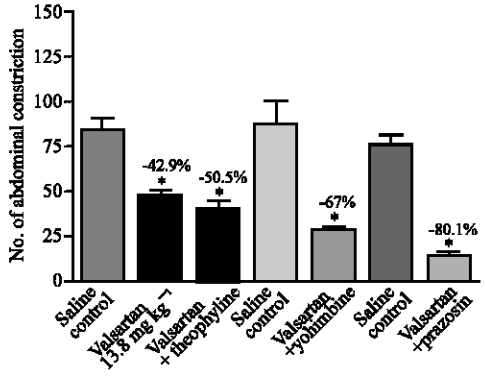

Fig. 8: Effect of theophylline (20 mg kg^{-1}, s.c.), yohimbine (5 mg kg^{-1}, s.c.) or prazosin (1 mg kg^{-1}, s.c.) on the analgesic effect of valsartan (13.8 mg kg^{-1}, s.c.) in the acetic acid abdominal constriction assay. *p<0.05 compared to control. Data represent means of 6 observations (±SE). Percent inhibition (%) compared to control is also shown

DISCUSSION

The present study provides evidence that the ACE inhibitor ramipril and the angiotensin II receptor blocker valsartan decrease thermal nociceptive pain and visceral pain evoked by i.p. acetic acid injection in mice. Candesartan, another angiotensin II receptor blocker, was without effect, at least at the doses used in the present study. These data are in accordance with other studies indicating that blockade of angiotensin II synthesis by ACE-inhibitors or blockade of its action by AT1-antagonists inhibits thermal and visceral nociception in rodents. In this context, it has been shown that the systemic administration of the ACE inhibitor spirapril or the angiotensin II antagonist losartan (though not enalapril, an ACE inhibitor) increases hot-plate latency in mice (Takai *et al*., 1996). Visceral nociception evoked by i.p. acetic acid in mice was also reduced by captopril, a prototype ACE inhibitor (Motta *et al*., 2002). Captopril may in addition act on the central nervous system increasing the beta-endorphin level (Handa *et al*., 1991).

Direct evidence to suggest that angiotensin II is involved in central nociceptive modulation came from studies in which angiotensin II administered by intracerebroventricular route (icv) attenuated

morphine-induced analgesia in the hot plate test in mice (Kaneko *et al*., 1985) and decreased the pain threshold in a paw pressure nociceptive assay in rats (Tchekalarova *et al*., 2003), while its systemic administration, increased hot plate latencies in rats (Irvine and White, 1997). In addition, angiotensin II injected intrathecally (Toma *et al*., 1997) or into periaqueductal gray matter (Pelegrini-da-Silva *et al*., 2005) increased tail-flick latency. Furthermore, visceral antinociception induced by of an adenosine A1 receptor agonist in mice was enhanced by angiotensin II or losartan administered by i.c.v. route (Pechlivanova and Georgiev, 2002).

In the present study, attempts were made to investigate the possible mechanisms by which ramipril or valsartan exert their antinociceptive properties. The acetic acid induced model of visceral inflammatory pain was used in this respect. Different neurotransmitter systems, such as catecholaminergic, opioidergic, cholinergic and purinergic were evaluated. The involvement of an alpha-2 adrenoceptor or adenosine receptor-mediated mechanisms were ruled out since the alpha-2 adrenoceptor antagonist yohimbine or the adenosine receptor antagonist theophylline ailed to inhibit the nociceptive responses.

Present results suggest the involvement of beta adrenoceptor mediated mechanism in the visceral analgesic effect of ramipril and valsartan, since co-administration of the non-selective beta adrenoceptor antagonist propranolol reversed the analgesic effect of ramipril and valsartan. This finding is intriguing since most forms of pain arising from the gastrointestinal tract are mediated by activating the visceral afferent fibres running in sympathetic nerves (Cervero, 1988). ACE inhibitors and angiotensin II antagonists modulate sympathetic neurotransmission. These agents prevent the pressor and sympatho-excitatory responses normally evoked by exogenous or endogenous angiotensin II (Cox *et al*., 1996; Hirooka *et al*., 1997). In rats, angiotensin II (and bradykinin) enhanced noradrenaline release from sympathetic nerves; this effect being inhibited by losartan (Fabiani *et al*., 2001). In addition to its sympatho-inhibitory effect, losartan, exerted a sympatho-excitatory action as shown by the increase in the plasma levels of both noradrenaline and its co-neurotransmitter, neuropeptide Y (Damase-Michel *et al*., 1998). Captopril alone produced an increase in NA release which was inhibited by bradykinin B(2) receptor antagonist (Fabiani *et al*., 2001). Intrathecal administration of norepinephrine and alpha adrenergic agonists in rats with chronic spinal catheters produced a significant elevation of the nociceptive threshold as measured by hot plate and tail flick (Reddy *et al*., 1980). Changes in central sympathetic tone may be one mechanism by which ACE inhibitors or angiotensin II antagonists exert their antinociceptive effects.

Our results in addition suggest that the visceral antinociceptive effect of ramipril, but not that of valsartan, is likely to involve an opioid sensitive mechanism. The non-specific opioid receptor antagonist naloxone reversed the analgesic effect of ramipril on visceral pain. Naloxone, however, was without effect on valsartan antinociception. Other researchers also suggested mediation of thermal antinociceptive effect the ACE inhibitor spirapril or the angiotensin II antagonist losartan by an opioid -dependant mechanism (Takai *et al*., 1996). Toma *et al*. (1997) reported that inhibition of the transmission of thermal nociceptive information in the spinal cord by angiotensin II involved an endogenous opioid mechanism (Toma *et al*., 1997).

Findings in the present study also indicated that the analgesic effect of valsartan in the mouse acetic acid test was reversed by atropine, thereby suggesting that analgesia induced by the drug involves a muscarinic acetylcholine receptor mediated mechanism. The muscarinic receptor antagonist atropine also reduced the analgesic effect of ramipril. Evidence indicates that primary sensory neuronal M2 receptors may represent a viable peripheral target for the treatment of pain and inflammation (Dussor *et al*., 2004). In the mouse acetic acid writhing test, M1-muscarinic agonists produced significant antinociception which was prevented by i.c.v. administration of atropine, a non-selective cholinergic muscarinic antagonist or by the selective M1 antagonist pirenzepine (Bartolini *et al*., 1992), whilst atropine (5 mg kg^{-1}) resulted in hyperalgesia (Ghelardini *et al*., 1990).

In summary, the present study demonstrates an antinociceptive effect for the ACE inhibitor ramipril and the angiotensin II receptor blocker valsartan on thermal pain and on visceral pain caused by i.p. acetic acid injection in mice. The study suggests the involvement of beta adrenoceptor-

dependent mechanism in the visceral analgesic effect of both drugs. Furthermore, that the visceral antinociceptive effect of ramipril involves an opioid-dependent mechanism, while that of valsartan involves a muscarinic acetylcholine receptor-dependent mechanism.

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