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Full Length Research Paper

Analgesic activity of angiotensin antagonists

Sekar Indumathy* and Subramanian Kavimani

Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences (A Govt. of Puducherry Institution) Puducherry-605006, India.

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Angiotensin Receptor Antagonists (ARAs) are widely used compounds in various cardiovascular disorders like Hypertension, Stroke prophylaxis, Heart failure. In addition, they are approved for the treatment of Diabetic Nephropathy. It is reported to produce analgesia on intracerebro-ventricular administration that could be blocked by naloxone. Angiotensin II has been reported for its pro-nociceptive activity. Angiotensin receptor antagonists block the action of angiotensin II by inhibiting its binding with its receptor hence, they exerts analgesic activity. The analgesic activity of angiotensin antagonists Losartan, Irbesartan and Valsartan evaluated by tail immersion, tail flick and tail clip methods have shown significant increase in basal reaction time. Pentazocine, a kappa receptor agonist exerted a significant analgesic effect ($p<0.001$). In comparison to control, angiotensin antagonists Losartan, Irbesartan and Valsartan show significant reduction in time for onset of writhing and also number of writhing. The % inhibitions of writhing for Losartan, Irbesartan and Valsartan at a dose of 20 mg/kg were 74, 68 and 73% respectively, whereas Aspirin (100 mg/kg) has 83% inhibition. All the three drugs have shown significant p value ($p<0.001$) which is comparable to standard control

Key words: Angiotensin antagonist, Losartan, Irbesartan and Valsartan.

INTRODUCTION

Angiotensin Receptor Antagonists (ARAs) are widely used compounds in various cardiovascular disorders like Hypertension, Stroke prophylaxis, Heart failure (Rohit et al., 2006). In addition, they are approved for the treatment of Diabetic Nephropathy (Goodman and Gilmaan, 2008). It is reported to produce analgesia on intracerebro-ventricular administration that could be blocked by naloxone. (Wang et al., 1992) Angiotensin II has been reported for its pro-nociceptive activity (Fujiyoshi et al., 1989). The aim of this study was to evaluate the analgesic effect of Angiotensin receptor antagonists.

MATERIALS AND METHODS

Albino mice of both sexes, adult (around 16 months old and weighing 25 to 35 g) were selected and used. Animals were procured from the disease free small animal house. They were acclimatized to the laboratory conditions for 5 days. They were kept in sufficient polypropylene cages under controlled temperature and humidity. The animals had free access to food and water and were housed under standard light-dark cycle (12 h each). All the experiments were carried out during day time from 0900 to 1600 h.

Losartan potassium-USP (Simlan Laboratories Ltd., Mumbai), Irbesartan-USP (Hetero labs ltd., Andra Pradesh) Valsartan - USP (Hetero labs ltd., Andra Pradesh), Fortwin (Pentazocine 30 mg/ml-Ranbaxy laboratories Ltd., Ahmadabad.), Ecosprin (Aspirin 150 mg/tab Accent pharma, Jammu). All the drugs were injected intraperitoneally (i.p.) and volume of injection was made 1 ml/100 g body weight of the mouse. All the drugs were dissolved in distilled water except Valsartan (made suspension with 0.5% Carboxy Methyl Cellulose [CMC]) and the analgesic activity was evaluated using the following methods (thermal, physical and chemical methods).

Thermal method

Tail immersion method

Albino mice were selected by immersing the tail in hot water at 55 ± 2°C and the basal reaction time was noted. The animals that show a positive response within 5 s (that is) withdrawal of tail clearly out of water were selected and they were divided into eight groups, five animals in each group. Pentazocine (5 mg/kg i.p.) was used as

*Corresponding author. E-mail: indumathy85@gmail.com. Tel: 9488820614.

positive control, the test drugs Losartan and Irbesartan was given in two doses by i.p. The test drug Valsartan alone made suspension with 0.5% CMC and was given in two doses by i.p. route. Group I served as vehicle control, 0.5% CMC was given. Observations were made up to 90 min after the administration of the test compounds and standard drug (Kulkarni, 2007; Ghosh, 2003; Yu-Ling et al., 2003).

Tail flick method

Albino mice were divided into eight groups of 5 animals in each group. Pentazocine (5 mg/kg i.p.) was used as positive control, the test drugs Losartan and Irbesartan were given in two doses by i.p. The test drug Valsartan made suspension with 0.5% carboxy methyl cellulose (CMC) and given in two doses by i.p. administration. Group I served as vehicle control, 0.5% CMC was given.

The animals were held in suitable restrainer with the tail protruding out. A cut off period of 10 to 12 s was given to prevent damage to the tail. The tail of the mouse was placed on the hot wire (5.35 ampere) at a distance of 1 cm and the time taken by the mouse to flick its tail from the hot source was taken as the Basal Reaction Time (BRT). For each mouse 3 to 5 Basal reaction times were noted. Basal reaction times were observed at 0, 30, 60 and 90 min respectively. Observations were made up to 90 min after the administration of the test compounds and standard drug and its activities were evaluated (Kulkarni, 2007; Ghosh, 2003; Yu-Ling et al., 2003).

Phsical method

Tail clip method

Albino mice were selected by applying a metal clip to the base of tail. The animals which did not show efforts to dislodge the clip within 15 s were not used for the experiments. Animals were divided into eight groups, five animals in each. Group I served as vehicle control, 0.5% carboxy methyl cellulose (CMC) (10 ml/kg i.p.) was given. Group II served as positive control Pentazocine (5mg/kg i.p.) was given. Groups III and IV received Losartan 10 mg and 20 mg/kg i.p respectively. Groups V and VI received Irbesartan 10 mg and 20 mg/kg i.p respectively. Groups VII and VIII received Valsartan 10 mg and 20 mg/kg i.p. (made suspension with 0.5% CMC) respectively. The tail clip was applied at 0, 30, 60 and 90 min after drug administration and the basal reaction time was noted (Kulkarni, 2007; Ghosh, 2003; Yu-Ling et al., 2003)

Chemical method

Acetic acid induced writhing

Albino mice of both sexes weighing 25 to 35 g were selected and were divided into five groups, each group containing five animals. Writhing is induced by intraperitoneal administration of 1%v/v of acetic acid in 0.9% sodium chloride (1 ml/100 g i.p.). Aspirin 100 mg/kg was used as standard drug. Group I served as vehicle control, 0.5% carboxy methyl cellulose (CMC) (10 ml/kg i.p.) was given. Group II served as positive control Aspirin (100 mg/kg i.p.) (made suspension with 0.5% CMC)was given. Group III received Losartan 20 mg/kg i.p., Group IV Irbesartan 20 mg/kg i.p and Group V received Valsartan 20 mg/kg i.p. (made suspension with 0.5% CMC) respectively. After 30 min of drug administration, animals were challenged with 1%v/v of acetic acid in 0.9% sodium chloride (1 ml/100 g i.p.).

Immediately after injection of algic compound, each animal was isolated in an individual box (25 X 25 X 50 cm). The time of onset of writhing action and the no. writhing were observed for 15 min after the onset of writhing, counted and compared to the response with the control group (Kulkarni, 2007; Ghosh, 2003; Yu-Ling et al., 2003; Ramasamy et al., 1998; Dongmo et al., 2005). “Percentage of writhings inhibition = mean value of control group writhings - mean value of control group writhings/mean value of control group writhings X 100”.

RESULTS

Tail immersion method

The analgesic activity of angiotensin antagonists Losartan, Irbesartan and Valsartan evaluated by tail immersion method showed significant increase in basal reaction time. Pentazocine, a kappa receptor agonist exerted a significant analgesic effect ($p<0.001$) at 30 min. Losartan at both the doses of 10 and 20 mg/kg showed significant analgesic effect ($p<0.001$) at 30 min. Irbesartan and Valsartan showed significant analgesic effect ($p<0.01$) at 60 min. Results were given in Table 1.

Tail flick method

The analgesic activity of angiotensin antagonists Losartan, Irbesartan and Valsartan evaluated by tail flick method showed significant increase in basal reaction time from 0 to 60 min. Losartan and Irbesartan at 10 mg/kg showed p value less than 0.01 ($p<0.01$) at 60 min. Basal Reaction Time (BRT) (mean±SEM) at 0 min is 3.2±0.27, and 5.6±0.49 to 7.4±0.51 and 6.8±0.2 s at 60 min. Valsartan (10 mg/kg) showed $p<0.05$. Losartan, Irbesartan and Valsartan showed very significant effect ($p<0.001$) at a dose of 20 mg/kg at 60 min. BRT (mean±SEM) are increased from 2.8±0.41, 5.4±0.25 and 4.4±0.49 s at 0 min to 8.6±0.49, 8.2±0.37 and 14.8±1.1 s at 60 min. Pentazocine, a kappa receptor agonist exerted a significant analgesic effect ($p<0.001$) at 60 min. The analgesic activity of angiotensin antagonist is comparable to that of standard drug using flick method. Results were given in Table 2.

Tail clip method

The analgesic activity of angiotensin antagonists Losartan, Irbesartan and Valsartan evaluated by tail clip method did not show very significant increase in basal reaction time comparable to that of tail flick and immersion methods. However, Losartan at a dose of 20 mg/kg shows p value less than 0.01 ($p<0.01$). Pentazocine, a kappa receptor agonist exerted a significant analgesic effect ($p<0.001$) at 30 min. The analgesic activity of angiotensin antagonist is comparable to that of standard drug using clip method. Results were given in Table 3.

Table 1. Analgesic activity of angiotensin antagonists (Tail immersion method*).*

Group	Treatment	Dose (ml/Kg i.p.)	Basal reaction time (s) (mean ± SEM)			
			0 min	30 min	60 min	90 min
Group I	0.5% CMC	10	1.24±0.11	1.12±0.11	1.50±0.12	1.13±0.10
Group II	Pentazocine	5	1.77±0.07	5.31±0.33*	3.11±0.14	1.97±0.11
Group III	Losartan	10	1.53±0.10	3.38±0.19*	3.33±0.48	2.73±0.50
Group IV	Losartan	20	1.65±0.11	3.69±0.12*	3.41±0.25	2.75±0.28
Group V	Irbesartan	10	1.11±0.14	1.88±0.11	2.37±0.09*	1.8±0.08
Group VI	Irbesartan	20	1.75±0.16	3.91±0.69	4.06±0.69**	3.35±0.46
Group VII	Valsartan	10	1.63±0.14	2.08±0.14	3.89±0.57**	3.01±0.4
Group VIII	Valsartan	20	1.57±0.13	2.43±0.48	3.11±0.35**	3.03±0.66

*P<0.001 vs. normal control, **P<0.01 vs. normal control, n = 5.

Table 2. Analgesic activity of angiotensin antagonists (tail flick method).

Group	Treatment	Dose (ml/Kg i.p)	Basal reaction time (s) (mean ± SEM)			
			0 min	30 min	60 min	90 min
Group I	0.5% CMC	10.	5.4±0.51	5±0.45	5±0.45	5±0.310
Group II	Pentazocine	5.	3.4±0.68	9.4±0.24	15.4±1.6*	5.8±0.8
Group III	Losartan	10	3.2±0.37	6±0.32	7.4±0.51**	4.2±0.37
Group IV	Losartan	20	2.8±0.41	7.2±0.49	8.6±0.49*	4.4±0.24
Group V	Irbesartan	10	5.6±0.49	6±0.32	6.8±0.2**	5±0.32
Group VI	Irbesartan	20	5.4±0.25	6.8±1.6	8.2±0.37*	3.8±0.2
Group VII	Valsartan	10	3.8±0.58	7.8±1 $^{\Psi}$*	7.6±1.21	5.2±0.66
Group VIII	Valsartan	20	4.4±0.49	8±0.84	14.8±1.1*	4.6±0.25

*P<0.001 vs. normal control, **P<0.01 vs normal control, $^{\Psi}$*P<0.05 vs. normal control, n = 5.

Table 3. Analgesic Activity of angiotensin antagonists (tail clip method).

Groups	Treatment	Dose (ml/Kg i.p.)	Basal reaction time (s) (mean ± SEM)			
			0 min	30 min	60 min	90 min
Group I	0.5% CMC	10	1.32±0.04	1.34±0.04	1.28±0.02	1.32±0.04
Group II	Pentazocine	5.	1.34±0.05	14.19±2.96	11.43±1.62*	8.94±1.31
Group III	Losartan	10	1.19±0.06	4.68±1.12***	2.87±0.6	1.94±0.2
Group IV	Losartan	20	1.26±0.05	5.49±1.19**	4.18±1.02	2.84±0.59
Group V	Irbesartan	10	2.21±0.29	5.7±2.49	4.1±1.16* $^{\Psi}$	2.69±0.45
Group VI	Irbesartan	20	1.8±0.1	2.7±0.13	3.76±0.15*	2.95±0.23
Group VII	Valsartan	10	1.29±0.5	3.1±1.79	5.6±2.72 $^{\Psi}$	2.31±0.96
Group VIII	Valsartan	20	1.43±0.42	1.71±0.49	2.01±0.5 $^{\Psi}$	1.48±0.43

*P<0.001 vs. normal control, **P<0.01 vs. normal control, $^{\Psi}$*P<0.05 vs. normal control, $^{\Psi}$P<0.5 vs. normal control, ***P<0.02 vs. normal control, n = 5.

Acetic acid induced writhing

The vehicle control which was treated with 1% acetic acid i.p. produces onset of writhing at 2.21±0.08 min and no. of writhings were 75±1.92, whereas the group pretreated with Aspirin, angiotensin antagonist Losartan, Irbesartan and Valsartan produces onset of writhing at 12.46±0.8, 6.58±0.19, 4.55±0.25 and 9.52±0.25 min respectively (that is) the time for onset of writhing was increased. At 15 min the number of writhing reduced to 12.4±0.92, 19.4±1.08, 24.2±1.57 and 20.4±0.92 respectively. In comparison to control, angiotensin antagonist Losartan, Irbesartan and Valsartan shows significant reduction in time for onset of writhing and also no. of writhing. The % inhibitions of writhing for Losartan, Irbesartan and Valsartan at a dose of 20 mg/kg were 74, 68 and 73% respectively, whereas Aspirin (100 mg/kg) has 83% inhibition. All the three drugs showed significant p value

Table 4. Analgesic activity of angiotensin antagonists (acetic acid induced writhing).

Groups	Treatment	Dose (ml/kg i.p.)	Acetic acid induced writhing (mean ± SEM)		% inhibition of writhings
			Time for onset of writhing (min)	Total no. of writhings (in 15 min)	
Group I	0.5% CMC	10	2.21±0.08	75±1.92	-
Group II	Aspirin	100	12.46±0.8*	12.4±0.92*	83
Group III	Losartan	20	6.58±0.19*	19.4±1.08*	74
Group IV	Irbesartan	20	4.55±0.25*	24.2±1.57*	68
Group V	Valsartan	20	9.52±0.25*	20.4±0.92*	73

*P<0.001 vs normal control , n = 5.

(p<0.001) which is comparable to standard control. Results were given in Table 4.

DISCUSSION

The present study suggests that, angiotensin receptor blockers Losartan, Irbesartan and Valsartan at the doses of 10 and 20 mg/kg possesses analgesic activity. Pain is the major problem because majority of tissues and organs are innerved by special sensory receptors (nociceptors) connected to primary afferent nerve fibres of different diameters. Various neurotransmitters found in the dorsal horn of the spinal cord may be involved in pain modulation. These include amino acid such as glutamate and γ-aminobutyric acid (GABA), monoamines such as Nor-epinephrine (NA) and 5 –hydroxy tryptamine (5-HT) and certain peptide molecule, of which the opioid peptides are the most important (Walker et al., 2003). Other neurotransmitters include histamine, acetyl choline and prostaglandins. Under normal conditions, pain is associated with electrical activity in small diameter primary afferent fibres of peripheral nerves. These nerves have sensory endings in peripheral tissues, and are activated by stimuli of various kinds (mechanical, thermal, chemical) (Rang et al., 2001).

In this study, the Angiotensin receptor antagonists were evaluated employing some pain models like thermal (tail flick and tail immersion), mechanical (tail clip) and chemical (acetic acid induced writhing). In all models, it possessed significant analgesic activity at high dose (20 mg/kg), in tail flick method it possessed dose dependent increase in analgesic activity. Evidence also exists for the involvement of Ca^{2+} in peripheral mechanisms mediated at the nociceptors level. The intraplantar administration of A23187 evokes hyperalgesia in rats that is potentiated by methylxanthines and antagonized by verapamil, La^{3+} or morphine, thus indicating that the hyperalgesic effect of the Ca^{2+} ionophore depends on the activity of adenylate cyclase on peripheral nociceptors. Angiotensin antagonists may possess analgesic activity by increasing pain threshold (mechanical and thermal model) or by decreasing synthesis of nociceptive neurotransmitter (chemical model). Drugs show significant effect on a viscero somatic model (tonic pain) reflected in a significant reduction of the acetic acid induced abdominal writhes. The abdominal writhing induced by acetic acid involves the production and release of arachidonic acid metabolite via cyclooxygenase (COX) and PG biosynthesis (Ferreira et al., 1979; Elisabetsky et al., 1995; Ito et al., 2001). Activation of AT_1 receptor with Ang II also increases Ca influx; therefore, blockage may possesed analgesic activity.

At low doses, it does not have any effect on nociception at administration while chronic administration produces anti nociceptive effect (Takai et al., 1996), but our findings suggests that, angiotensin antagonists Losartan, Irbesartan and Valsartan at high dose (20 mg/kg) possessed significant analgesic activity. Apart from antihypertensive property, they also possessed analgesic activity and it was proven by various pain models in mice. However, further clinical studies have to be carried out to find its extent of analgesic activity in normal, as well as hypertensive volunteers. Hence, they may prevent the use of multidrug in hypertensive patient for pain relieving purpose.

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