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Biphasic Effects of Losartan Potassium on Immobility in Mice

Pandi VIJAYAPANDI and Anantha Naik NAGAPPA*

Pharmacy Group, Birla Institute of Technology and Science, Pilani–333031, Rajasthan, India

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The effects of losartan potassium, an angiotensin AT_1 receptor blocker on immobility in forced swim test have been studied. Effect of losartan potassium, nortriptyline HCl, fluoxetine HCl and reserpine per se and in combination on forced swimming-induced immobility in mice have also been studied. In mice, losartan potassium elicits biphasic responses i.e. positive responses at lower doses (0.1, 1.0 and 5 mg/kg, i.p.) in the forced swim test, a test of potential antidepressant activity and vice versa at higher dose (20 and 100 mg/kg, i.p.). In chronic studies, enhancement in immobility was observed for losartan potassium (3 and 30 mg/kg, p.o., 21 days). In acute combination studies, losartan potassium (1 and 5 mg/kg) significantly reversed the reserpine-induced immobility, but vice versa at 100 mg/kg. Losartan potassium (0.1 and 5 mg/kg) potentiate antidepressant activity of nortriptyline (30 mg/kg, i.p.) in mice, but vice versa at 100 mg/kg. Likewise, Losartan potassium (100 mg/kg), significantly reversed antidepressant activity of fluoxetine HCl, but at 0.1 and 5 mg/kg, failed to modify fluoxetine HCl induced immobility. The obtained biphasic effect of losartan potassium on immobility in mice might be due to inhibitory effect on AT_1 receptor at lower dose and pronounced effect on AT_2 receptor at higher dose (large concentrations of losartan potassium can displace Angiotensin II (Ang II) from its AT_1 receptor to AT_2 receptor.

Key words——forced swim test; immobility time; losartan potassium; reserpine; nortriptyline; fluoxetine

INTRODUCTION

The presence of a separate renin angiotensin system (RAS) within the mammalian brain complete with the precursors and enzymes necessary for the formation and deactivation of the physiologically active forms of angiotensin (Ang) was supported in many investigations.[1,2] The effector peptide of the RAS Ang II, binds at least to two G protein coupled receptor subtypes, referred to as the AT_1 and the AT_2 receptors. Ang II is known to stimulate catecholamine release[3] mediated by an AT_1 subtype[4] is located on presynaptic nerve terminals. In case of AT_1 receptor blockade, which is supposed to reduce catecholamine release leading to endogenous depression. Since deficiency of aminergic transmission in the CNS might be causative of endogenous depression.[5,6] But on the converse, losartan an AT_1 blocker elicits positive responses in the forced swim test, a test of potential antidepressant activity.[7] These controversial observations lead us to conduct studies on effects of losartan potassium on immobility in mice.

MATERIALS AND METHODS

Male albino mice (Swiss, 20–25 gm) used in these studies were allowed food and water *ad libitum* up to the time of experimentation. Prior to use, the mice were housed in polypropylene cages in groups of six to eight animals under natural light-dark cycle. Institutional Animal Ethics Committee has approved all studies reported here (protocol Number IAEC/RES/5, dated 21/04/2003).

Losartan potassium, fluoxetine HCl (Sun Pharma, India), nortriptyline HCl (Sigma, USA) were dissolved in normal saline and was given i.p. Reserpine (Loba Chemicals, India) was dissolved in a few drops of glacial acetic acid and the volume was makeup with normal saline. The drug solutions were prepared afresh at beginning of each experiment. In acute studies, all the drugs were administered by i.p. in a constant volume of 1 ml per 100 gm of body weight. In chronic studies, required dose of losartan potassium (3 & 30 mg/kg) was dissolved in per day consumption of drinking water (12 ml/100 gm of body weight) and made available for 21 days.

The behavioral despair test has been used as a test of depressive like behavior.[8] The animals were forced to swim individually in a glass cylinder (30 cm high, 22.5 cm in diameter) containing 15 cm water at room temperature. The animals were individually trained in 15 min sessions, using the apparatus described above one day prior to the experimentation. During ex-

e-mail: anantha@bits-pilani.ac.in

perimentation each animal was placed on the cylinder one at a time and left there for 6 min. The duration of immobility for each mouse was recorded. A mouse was judged to be immobile when it ceased struggling and remaining floating motionless in the water making only movements necessary to keep its head above water.

Statistical Analysis Results are presented as the mean±SEM. Experimental data in Table 1, were analyzed by one way analysis of variance (ANOVA) followed by post hoc comparisons between drug treated at various time intervals and vehicle treated control groups using Dunnett's test. In Table 2, experimental data were analyzed by one way analysis of variance (ANOVA) followed by Student's *t*-test. Statistical significance was set at $p<0.05$.

RESULTS

Effect of losartan potassium, nortriptyline HCl, fluoxetine HCl and reserpine per se and in combination studies on forced swimming-induced immobility in mice is shown in Tables 1 and 2. As shown in Table 1, in comparison to control, losartan potassium (0.1, 1 and 5 mg/kg, i.p.) significantly reduced immobility

Table 1. Effect of Losartan Potassium on Immobility in Mice

Treatment (mg/kg, i.p.)	Duration of immobility (s)	ANOVA values
Control	284.31±4.00	
Losartan potassium (0.1) [1 h prior]	244.33±13.39*	F(4, 25)=3.69
Losartan potassium (0.1) [3 h prior]	271.43±10.65	p=0.0170
Losartan potassium (0.1) [6 h prior]	281.20±7.45	
Losartan potassium (0.1) [24 h prior]	283.82±5.02	
Control	284.21±11.10	
Losartan potassium (1.0) [1 h prior]	181.41±20.39**	F(4, 25)=9.02
Losartan potassium (1.0) [3 h prior]	249.07±19.87	p=0.0001
Losartan potassium (1.0) [6 h prior]	255.59±21.57	
Losartan potassium (1.0) [24 h prior]	286.46±8.42	
Control	274.41±3.41	
Losartan potassium (5.0) [1 h prior]	223.84±5.98**	F(4, 25)=23.07
Losartan potassium (5.0) [3 h prior]	233.09±6.72**	p=0.0001
Losartan potassium (5.0) [6 h prior]	269.76±5.68	
Losartan potassium (5.0) [24 h prior]	277.97±3.65	
Control	296.41±14.55	
Losartan potassium (10) [1 h prior]	307.67±15.29	F(4, 25)=0.11
Losartan potassium (10) [3 h prior]	308.96±14.9	p=0.9759
Losartan potassium (10) [6 h prior]	302.25±14.39	
Losartan potassium (10) [24 h prior]	304.45±8.76	
Control	293.32±7.08	
Losartan potassium (20) [1 h prior]	309.62±9.49	F(4, 25)=10.19
Losartan potassium (20) [3 h prior]	320.10±6.42*	p=0.0001
Losartan potassium (20) [6 h prior]	328.43±4.14**	
Losartan potassium (20) [24 h prior]	283.73±5.81	
Control	284.67±4.2	
Losartan potassium (100) [1 h prior]	312.42±3.05**	F(4, 25)=17.19
Losartan potassium (100) [3 h prior]	321.44±6.85**	p=0.0001
Losartan potassium (100) [6 h prior]	309.90±2.97**	
Losartan potassium (100) [24 h prior]	302.69±3.38**	
Chronic treatment for 21 days		
Control	118.85±2.84	
Losartan potassium (3)	216.39±15.65**	F(2, 15)=34.51
Losartan potassium (30)	259.46±14.10**	p=0.0001

6 min test. Values are means±S.E. of 6 animals in each group. * $p<0.05$, ** $p<0.01$ (one-way ANOVA/Dunnett's test: as compared to control group).

Table 2. Effect of Losartan Potassium upon Nortriptyline HCl, Fluoxetine HCl and Reserpine-induced Immobility on Forced Swimming in Mice

Treatment (mg/kg, i.p.)	Duration of immobility (s)	ANOVA values
Control	286.69±9.83	
Reserpine (2) [5 h prior]	322.09±8.85*a	F(6, 35)=20.17
Reserpine (2) [24 h prior]	320.80±11.29*a	p=0.0001
Reserpine (2) [5 h prior]+Losartan potassium (100) [3 h prior]	339.43±4.17*b	
Reserpine (2) [5 h prior]+Losartan potassium (20) [3 h prior]	310.97±9.07	
Reserpine (2) [5 h prior]+Losartan potassium (5) [3 h prior]	277.63±13.76*b	
Reserpine (2) [5 h prior]+Losartan potassium (1) [1 h prior]	202.38±19.62*b	
Control	312.42±2.7	
Nortriptyline HCl (10) [1 h prior]	311.53±6.96	F(6, 35)=13.71
Nortriptyline HCl (30) [1 h prior]	244.56±10.40***a	p=0.0001
Nortriptyline HCl (30) [1 h prior]+Losartan potassium (100) [3 h prior]	307.63±17.02**c	
Nortriptyline HCl (30) [1 h prior]+Losartan potassium (20) [3 h prior]	276.94±17.07	
Nortriptyline HCl (30) [1 h prior]+Losartan potassium (5) [3 h prior]	214.39±5.43*c	
Nortriptyline HCl (10) [1 h prior]+Losartan potassium (0.1) [1 h prior]	230.70±18.38*d	
Control	295.02±13.56	
Fluoxetine HCl (10) [1 h prior]	292.96±20.66	F(6, 35)=13.49
Fluoxetine HCl (30) [1 h prior]	203.09±10.89***a	p=0.0001
Fluoxetine HCl (30) [1 h prior]+Losartan potassium (100) [3 h prior]	244.42±9.62*e	
Fluoxetine HCl (30) [1 h prior]+Losartan potassium (20) [3 h prior]	230.13±14.52	
Fluoxetine HCl (30) [1 h prior]+Losartan potassium (5) [3 h prior]	165.35±23.17	
Fluoxetine HCl (10) [1 h prior]+Losartan potassium (0.1) [1 h prior]	315.07±7.26	

6 min test. Values are means±S.E. of 6 animals in each group. p values: *<0.05, **<0.01, ***<0.001 as compared between treatment. (a) Control group, (b) reserpine (2 mg/kg) 5 h prior group, (c) nortriptyline HCl (30 mg/kg) 1 h prior group, (d) nortriptyline HCl (10 mg/kg) 1 h prior group and (e) fluoxetine HCl (30 mg/kg) 1 h prior group

in the mouse forced swim test, but at 20 and 100 mg/kg, i.p. significantly increased immobility. In chronic studies, when compare to control, losartan potassium (3 and 30 mg/kg, p.o., 21 days) significantly enhance immobility in mice (Table 1). As shown in Table 2, losartan potassium (1 and 5 mg/kg) significantly reversed the reserpine-induced immobility in mice, but enhanced in immobility at 100 mg/kg. Losartan potassium (0.1 and 5 mg/kg) potentiated antidepressant activity of nortriptyline (30 mg/kg, i.p.) in mice, but at 100 mg/kg, significantly reversed it. Likewise, Losartan potassium (100 mg/kg), significantly reversed antidepressant activity of fluoxetine HCl, but at 0.1 and 5 mg/kg, failed to modify fluoxetine HCl induced immobility.

DISCUSSION

It has been previously reported, the antidepressant drugs desipramine, fluoxetine and tranylcypromine are able to antagonize the effects of Ang in rats, both *in vivo* and *in vitro*.[9] Giardina and Ebert[10] (1989) showed that captopril elicits antidepressant like effects in an animal model and extend the previous work by showing that losartan has similar actions.[7] Based on these observations, it has been hypothesized that inhibition of Ang function is important in the treatment of endogenous depression. The results of present studies showed biphasic effects of losartan potassium on immobility in mice (i.e.) reduced immobility at lower dose (0.1, 1 and 5 mg/kg) enhanced immobility in higher dose (100 mg/kg, i.p.). The biphasic effect was further confirmed by interaction of losartan potassium with reserpine and antidepressant drugs nortriptyline and fluoxetine (Table 2). Nahmod et al found Ang II to cause 5HT release and accelerate its synthesis in biphasic manner, stimulating at high doses and inhibiting at lower doses.[11] Using the micro dialysis technique, it was demonstrated that stimulation of periventricular AT_1 receptors leads to release of noradrenaline in the paravetricular nucleus and the supraoptic nucleus.[12,13]

The research on the Ang receptor subtype characterization in the brain has been reviewed.[14] Both the AT_1 and AT_2 receptors have been localized in the brain RAS[15] together with their mRNAs.[16] In ro-

dents AT_{1A} receptor has been found in brain areas involved in blood pressure and fluid homeostasis. The AT_{1B} receptor is present in glandular tissues, such as anterior pituitary, pineal, adrenal gland, and testes.[17] The AT_2 receptor is densely expressed in the lateral septum, in several thalamic nuclei, in sub thalamic nucleus, in locus cerules and in the inferior olive.[18] Activation of AT_2 receptor seems to induce effects opposite to that of AT_1.[19] AT_2 stimulation inhibits drinking responses and vasopressin release following centrally administered Ang II,[20] promotes differentiation and axonal regeneration, and inhibits proliferation of neuronal cells.[21] Thus the counteracting effects between AT_1 and AT_2 receptors suggest that a negative cross talk exist between the AT_1 and AT_2 receptors,[22] as is the case in catecholaminergic neurons.[23] Most of the central effects of Ang peptides, which are mediated by AT_1 receptor, are under control by AT_2 receptor. They are in accordance with earlier findings from in vitro experiments in endothelial cells where growth promoting effects mediated by AT_1 receptors were counteracted by growth inhibitory actions of AT_2 receptors.[24] Opposite effects of AT_1 and AT_2 receptors on the second messenger phosphatidylinositol have also been described by Gyurko et al.[25] AT_1 selective receptor antagonists are known to bind to AT_2 receptors with low affinity and vice versa. However, the selectivity is not absolute, and large concentrations of AT_1 selective receptor antagonists can displace Ang II from AT_1 receptors to the alternative site (AT_2 receptors).[26] The obtained biphasic effect of losartan potassium on immobility in mice might be due to inhibitory effect on AT_1 receptor at lower dose and pronounced effect on AT_2 receptor at higher dose (large concentrations of losartan potassium can displace Ang II from its AT_1 receptor to AT_2 receptor). In chronic studies with losartan potassium even at lower dose (3 mg/kg, p.o.) potentiated immobility in mice, which might be due to continuous blockade of AT_1 receptor resulting in unopposed AT_2 receptor stimulation. It has also been previously reported that the treatment of Ang II for 4 h has a biphasic effect on Na^+ transport in the primary cultured rabbit renal proximal tubule cells (PTCs); a Pico molar range of Ang II stimulates Na^+ transport, whereas a micro molar range of Ang II inhibits it.[27]

Antagonist IC_{50} ($8.78 \pm 0.11 \times 10^{-9}$ M) and Ki values ($41.57 \pm 5.09 \times 10^{-9}$ M) of losartan from competition binding experiments with [^{125}I]Sar^1-Ilu^8 Ang II on intact Chinese Hamster Ovary Cells expressing the transfected human AT_1 receptor (CHO-hAT_1 cells) was described.[28] Effects of Ang II on AT_1 and AT_2-binding sites and mRNAs in Bovine adrenal fasciculata cells (BAC) have been demonstrated.[29] In these studies, treatment of cells with increasing concentrations of Ang II caused a dose-dependent inhibition of AT_1 and AT_2 binding sites and mRNAs. The maximal inhibitory effect was observed at 10^{-7} M, but the IC_{50} for AT_1 and AT_2-binding sites and AT_1 mRNA ($3 \pm 0.43 \pm 10^{-9}$ M) were significantly higher than that for AT_2 mRNA ($2.8 \pm 0.3 \times 10^{-10}$ M). However, the most striking differences were observed in the time-course effects of Ang II. AT_1-binding sites decreased very rapidly, and by 3 h, more than 50% of the surface receptors disappeared. In contrast, the effects of Ang II on AT_2-binding sites were not significant during the first hours of treatment. Thereafter, the surface receptor declined with an apparent half-life of 14—16 h. Similarly, the effects on AT_2 mRNA were not significant during the first 6 h of treatment, but then the levels dramatically decreased, with an apparent half-life of 2 h.[29] In higher doses of losartan potassium (20 and 100 mg/kg, i.p.) enhancement in immobility was observed even after 3 hs of losartan potassium treatment. The terminal half-life of losartan p.o. is 2.12 h and its active metabolite EXP 3174 (carboxylic acid derivative) about 6 to 9 h.[30] It is reported that the effect of i.p. administration of losartan (20 μmol/kg) on Ang II induced drinking was found to be reduced at 4, 12 and 24 h. EXP 3174 significantly reduced the Ang II induced water intake even at 0.25 h. Therefore, the ability of peripherally administered losartan influencing central Ang mechanisms might be accounted for EXP 3174, which is about 20 times more potent than losartan to inhibit central AT_1 receptors and which apparently crosses the blood-brain barrier rather well.[31]

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