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Strain differences in the anxiolytic effects of losartan in the mouse

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Abstract

Anxiolytic effects of the angiotensin AT_1 receptor antagonist losartan were studied in the elevated plus maze (EPM) and the light/dark test (LDT) in different mouse strains as were responses to angiotensin II and acetylcholine in isolated ascending colon. There were no significant strain differences in behaviour on the EPM, and diazepam was anxiolytic in C57BL/6, DBA/2 and BKW mice. Losartan was anxiolytic in BKW only. In the LDT, there were significant strain differences, with BKW mice exhibiting greatest anxiety-like behaviour; losartan was ineffective in this test. In vitro responses to angiotensin II and acetylcholine were significantly smaller in BKW than in C57BL/6 and DBA/2. These results indicate that the mouse strain exhibiting least angiotensin receptor function is the most responsive to the anxiolytic effects, suggesting a possible relationship between angiotensin receptor function and anxiolytic response to losartan.

Keywords: Losartan; Strain; Anxiolytic; Elevated plus maze; Light/dark test

1. Introduction

There are several animal models of anxiety-related behaviour in laboratory rodents, but two of the most commonly used are the light/dark test (LDT) and the elevated plus maze (EPM). The EPM was developed for use in rats by Handley and Mithani (1984) following the work of Montgomery (1958) and was subsequently modified for use in the mouse independently by Stephens et al. (1986) and Lister (1987). These various adaptations of the basic method have since been used to study the anxiolytic actions of many drugs such as benzodiazepines (Pellow et al., 1985), a NMDA receptor antagonist, (Stephens et al., 1986) and neuroactive steroids (Wieland et al., 1995). The LDT is a similar exploratory model of anxiety, which was initially described by Crawley and Goodwin (1980) and later developed and validated by Costall et al. (1989). This test has too been used to investigate a range of putative anxiolytic compounds such as neuroactive steroids (Wieland et al., 1995) and losartan (Barnes et al., 1990).

Losartan is an antagonist of angiotensin II, a peptide known predominantly for its effects on the cardiovascular system and as a target for antihypertensive therapies. It is recognised, however, that angiotensin also plays an important role in the brain, being involved in the control of fluid intake, secretion of luteinizing hormone and some aspects of cognition and memory (Phillips, 1987). Angiotensin exerts its effects via two receptor subtypes, namely AT_1 and AT_2 receptors (Chiu et al., 1989). It is the AT_1 receptors that are the most abundant and which mediate most of the physiological responses to angiotensin, with AT_2 receptors being concerned with longer-term effects such as cardiac myocyte proliferation. Both receptor subtypes have been identified in the brain, although AT_1 receptors account for approximately 90% of the population (Hohle et al., 1995). A further receptor subtype, AT_4, has also been identified in the brain, but this subtype appears to be selective for a peptide fragment of the endogenous angiotensin II (Wright and Harding, 1997). Losartan is a nonpeptide, selective AT_1 receptor antagonist (Chiu et al., 1990).

The behavioural effects of losartan have been studied extensively. In rats, for example, it has been shown to antagonise the ability of angiotensin II to improve passive avoidance and object recognition and to reduce anxiety, as determined by the EPM (Kulakowska et al., 1996). In mice, losartan elicits positive responses in the forced swim test, a

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test of potential antidepressant activity (Gard et al., 1999), but the effects of losartan in tests of anxiolytic activity in mice are more equivocal. Barnes et al. (1990) reported that losartan produced anxiolytic-like responses in the mouse LDT at oral doses as low as 0.01 mg kg^{-1}, whilst Shepherd et al. (1996) were unable to confirm these findings using subcutaneous doses of up to 10 mg kg^{-1}. Interestingly, the same workers were also unable to demonstrate an anxiolytic effect of losartan in rats using the elevated zero maze with subcutaneous doses of losartan of up to 10 mg kg^{-1}. The previously mentioned positive responses in the EPM were at an intracerebroventricular dose of losartan of 1.0 μg (Kulakowska et al., 1996). Other than the route of drug administration, the major difference in the mouse method used by Barnes et al. and that used by Shepherd et al. was the strain of mouse studied; Barnes et al. used BKW strain mice, whilst Shepherd et al. used TO strain mice.

There have been many reports of strain differences in mouse activity in tests of anxiety-like behaviour. Trullas and Skolnick (1993), for example, studied 16 inbred mouse strains and identified strains with low, medium and high anxiety. Since then, strain differences have been exploited in an attempt to elucidate the biochemical and genetic bases of anxiety (Clement et al., 1997; Hode et al., 2000; Bodkin et al., 1998). The aim of the current study was to investigate the effects of mouse strain on the anxiolytic responses to losartan. For each strain, the contractile response of isolated colon to angiotensin II was also determined to ascertain whether any observed strain differences were related to more generalised differences in functional responses to angiotensin II. The three strains of mouse selected for study were BKW, which is an outbred albino strain; C57BL/6, which is an inbred, black-coated strain; and DBA/2, which is an inbred, brown-coated strain. All of the strains are readily commercially available and differ mainly in their susceptibility to endogenous tumours, although C57BL/6 have been described as a 'low anxiety' strain with significantly lower anxiety-like behaviour than DBA/2 (Bodkin et al., 1998).

2. Methods

2.1. Protocol

The effects of losartan on behaviour in two tests for anxiolytic activity in mice, namely the EPM and the LDT, were studied. Three different strains of mouse were tested on the EPM, with diazepam being used as a positive anxiolytic control. The same strains were subsequently tested in the LDT. Contractile responses to angiotensin II and acetylcholine were also determined in colonic smooth muscle harvested from each of the three strains. All experiments were conducted under the auspices of the Animals (Scientific Procedures) Act 1986, UK.

2.2. Animals

Male mice of BKW (26–31 g, B&K Universal, Hull, UK), DBA/2 (18–22 g, Harlan UK, Bicester, Oxon, UK) or C57BL/6 strain (18–23 g, Charles River Laboratories, Margate, Kent, UK) were housed in groups of up to 24 in a controlled environment (19.5 ± 1.5°C, 50% relative humidity) with a controlled light cycle. The animals had free access to food and water except during the test period and were allowed at least 2 days to acclimatise to the holding room environment before testing in an adjacent dedicated laboratory.

2.3. Elevated plus maze

The EPM was similar to that reported by Stephens et al. (1986). It consisted of an elevated cruciform runway, the four arms of which were 8 × 15 cm. The outer limits of two of the opposing arms were enclose by walls (15 cm) but were open-topped; there was no lip on the open arms. The mouse was placed in the centre of the maze and the behaviour during the 5-min test period was observed remotely using a video camera mounted above the maze. The time spent on the open arms of the maze (expressed as a percentage of the total time spent on either open or closed arms), the entries on to the open arms (expressed as a percentage of the total number of entries), and the total number of entries were recorded; time spent in the centre of the maze was disregarded.

For this test, the mice were housed with a 14 h light:10 h dark cycle, lights on 07:00 h. All experiments were performed between 10:00 and 14:00 h. When investigating drug effects, animals were tested 30 min after intraperitoneal (ip) administration of either diazepam 1 mg kg^{-1} or losartan 10 or 20 mg kg^{-1} and the results obtained were compared with those obtained from animals receiving the appropriate vehicle control.

2.4. Light/dark test

The LDT, as described by Costall et al. (1989), was performed by placing the mouse into the dark area of a divided observational chamber (45 × 27 cm), two-thirds of which was painted white and was illuminated brightly and one-third of which was painted black, screened from ambient light and illuminated by red light. The mouse was able to cross between the areas by movement through a small hole in the dividing partition. The floor of the entire chamber was marked with lines to divide it into boxes of 7.5 × 9.0 cm. The behaviour of the mouse during the 5-min test period was observed remotely by use of a video camera mounted above the chamber. The proportion of time spent in the bright area, the latency to first entry to the illuminated area, the number of line crossings and the number of rears in the bright area were recorded.

For these experiments, mice were housed with a reverse light–dark cycle such that the lights were on 19:00–07:00 h and the experiments were performed between 10:00 and 14:00 h. Animals were tested 45 min after oral administration of losartan 1 mg kg^{-1} and the results obtained were compared with those obtained from animals receiving vehicle control.

2.5. Contractile response of isolated colon

For determination of the contractile responses to angiotensin II, descending colon taken from mice of each of the three mouse strains was suspended in Krebs' solution at 32°C under a resting tension of $0.5 \times g$. Isometric contractile responses to angiotensin II (10^{-12}–10^{-7} M, noncumulative) and acetylcholine (10^{-9}–10^{-4} M, noncumulative) were recorded using a contact time of either 60 (angiotensin) or 20 s (acetylcholine) and a 3-min dose cycle. Results are expressed as $\times g$ tension developed/g tissue (wet weight).

2.6. Drugs and chemicals

Losartan potassium (2-butyl-4-chloro-1-[*p*-(*o*-1*H*-tetrazol-5-ylphenyl)benzyl] imidazole-5-methanol monopotassium salt, DuP753), a kind gift from Merck & Co, New Jersey, USA, was dissolved in physiological saline. Diazepam was obtained from Research Biochemicals International (RBI) and suspended in deionised water containing 1 drop polysorbate (Tween)/10 ml.

Angiotensin II and acetylcholine were obtained from Sigma (Poole, UK). Krebs' solution contains 118.41 mM NaCl, 4.63 mM KCl, 0.54 mM $MgSO_4$, 2.53 mM $CaCl_2$, 24.99 mM $NaHCO_2$, 11.0 mM glucose and 1.18 mM KH_2PO_4.

2.7. Data analysis

Data are presented as the mean ± one standard error of the mean. Comparisons between multiple groups were achieved using one- or two-way analysis of variance (ANOVA) as appropriate; significant outcomes were followed by application of Tukey's multiple range test.

3. Results

3.1. Elevated plus maze

Two-way analysis of variance indicated that diazepam caused a significant increase in the percent of time spent on the open arms of the EPM [$F(1,34) = 22.51$, $P < .01$]. There were no significant differences between the different strains or any significant drug × strain interaction. There were no effects of diazepam on the number of entries onto the open arms of the maze or the total number of entries (Table 1).

Losartan (10 mg kg^{-1} ip) caused a significant increase in the percent of time spent on the open arms [$F(1,34) = 4.298$, $P < .05$]. There were no significant effects of strain on the amount of time spent on the open arm but there was a significant drug × strain interaction [$F(2,34) = 2.34$, $P < .05$]. Compared with saline, losartan produced a significant increase in the amount of time spent on the open arm in BKW mice ($P < .05$, Tukey's) but not in either of the other two strains.

At the higher dose of 20 mg kg^{-1} ip losartan, two-way analysis of variance revealed that there were significant strain differences in the number of entries onto the open arms of the maze and the amount of time spent on the arms; C57 mice exhibited significantly fewer entries onto the open arms and spent significantly less time on the open arms [$F(1,20) = 5.10$, $P < .05$ and $F(1,20) = 5.51$, $P < .05$, respectively]. There were no significant effects of the drug or

Table 1
Strain differences in mouse behaviour on the EPM and differential effects of losartan and diazepam

Strain	Treatment	*n*	% Entries onto open arms	% Time on open arms	Total entries
BKW	Saline	8	22.9 ± 7.4	16.7 ± 7.3	12.1 ± 2.1
	Losartan 10 mg kg^{-1}	8	48.0 ± 6.7	55.1 ± 10.1	13.6 ± 2.1
	Diazepam vehicle control	8	25.1 ± 5.4	11.6 ± 3.6	14.25 ± 1.8
	Diazepam 2.5 mg kg^{-1}	8	50.4 ± 11.3	42.8 ± 11.7	17.3 ± 3.2
DBA/2	Saline	6	45.9 ± 9.3	38.8 ± 10.6	14.8 ± 2.8
	Losartan 10 mg kg^{-1}	6	39.3 ± 10.7	30.6 ± 10.7	17.0 ± 2.2
	Losartan 20 mg kg^{-1}	6	45.3 ± 8.3	35.3 ± 9.0	21.8 ± 1.9
	Diazepam vehicle control	6	39.4 ± 6.6	32.9 ± 5.3	20.8 ± 3.0
	Diazepam 2.5 mg kg^{-1}	6	66.8 ± 7.1	54.1 ± 10.4	19.2 ± 4.0
C57	Saline	6	29.8 ± 6.2	18.4 ± 7.9	15.5 ± 1.7
	Losartan 10 mg kg^{-1}	6	34.2 ± 7.5	27.3 ± 7.7	17.5 ± 2.6
	Losartan 20 mg kg^{-1}	6	28.15 ± 4.5	15.7 ± 5.4	18.83 ± 2.9
	Diazepam vehicle control	6	27.1 ± 6.9	14.7 ± 4.4	15.3 ± 1.7
	Diazepam 2.5 mg kg^{-1}	6	57.6 ± 7.1	63.9 ± 10.5	16.5 ± 3.7

Table 2
Strain differences in mouse behaviour in the LDT and differential effect of losartan

Strain	C57		DBA2		BKW	
Treatment	Saline ($n=5$)	Losartan ($n=5$)	Saline ($n=4$)	Losartan ($n=5$)	Saline ($n=5$)	Losartan ($n=5$)
% Time in bright area	33.2±5.3	38.1±1.6	25.9±3.8	26.2±6.9	19.6±3.5	29.1±3.2
Number of transitions between areas	21.9±5.7	20.7±2.0	23.4±3.2	14.5±4.7	17.4±4.4	18.0±2.5
Latency to first transition	13.0±3.2	13.0±4.1	1.9±0.3	18.3±9.8	15.5±2.8	17.4±7.9
Number of rears in bright area	14.3±4.3	21.4±2.1	18.9±4.7	14.2±6.3	2.5±0.8	6.0±5.0
Number of line crossings in dark area	57.9±8.6	65.7±10.0	77.4±6.3	66.3±7.9	115.0±20.0	113.8±19.3
Number of line crossings in bright area	46.4±14.3	55.0±1.4	55.3±6.3	64.7±3.2	40.0±2.0	50.0±3.0

drug × strain interactions. Losartan caused a significant increase in the total number of excursions of the mice onto the arms of the maze [$F(1,20)=4.70$, $P<.05$] but there were no significant strain differences or significant drug × strain interactions.

3.2. Light/dark test

Analysis of the results presented in Table 2 indicated that there were significant differences in the behaviour of the different strains on in the LDT. There were significant strain differences in the amount of time spent in the bright area [$F(2,23)=3.93$, $P<.05$], the locomotor activity (line crossing) in both the bright and dark areas [$F(2,23)=8.69$, $P<.01$ and $F(2,23)=4.71$ $P<.05$, respectively] and the number of rears in the bright area [$F(2,23)=6.21$ $P<.01$]. BKW mice spent significantly less time in the bright area than C57 mice ($P<.05$, Tukey's) and exhibited significantly less locomotor activity in the bright area than DBA2 mice ($P<.05$, Tukey's) and significantly more locomotor activity in the dark area than both C57 and DBA2 mice ($P<.05$, Tukey's). There were no significant strain differences in the number of transitions or the latency to the first transition. Analyses of variance indicated that there were no significant effects of losartan in the light/dark box or were there any significant drug × strain interactions.

3.3. Contractile responses of isolated colon

There were significant differences between the strains in the contractile responses of the isolated colon to angiotensin II [$F(3,360)=24.0$, $P<.01$; Fig. 1]; there was no significant strain × angiotensin concentration interaction. Tissues from BKW mice showed the least response to angiotensin (significantly different from the other two strains; $P<.05$, Tukey's). There were no significant differences between the C57BL/6 strain and the DBA/2.

There were similar significant differences between the strains in the contractile responses of the isolated colon to acetylcholine [$F(2,360)=133.3$, $P<.01$; Fig. 2); there was no significant strain × angiotensin concentration interaction. Tissues from BKW mice showed the least response to acetylcholine (significantly different from the other two strains; $P<.05$, Tukey's). There were no significant differences between the C57BL/6 strain and the DBA/2.

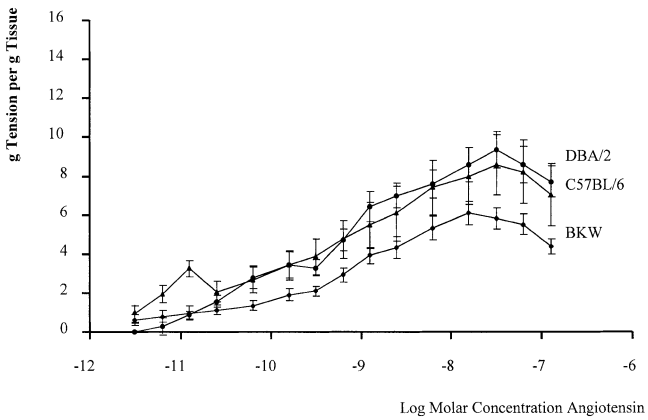

Fig. 1. Mean ± standard error contractile responses of isolated descended colon from mice of different strains to angiotensin II. Analyses of variance indicate that there are significant differences between the strains ($P<.001$). Sample size = 9 for all strains.

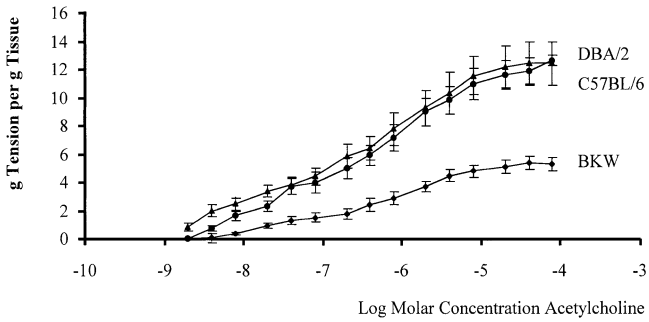

Fig. 2. Mean ± standard error contractile responses of isolated descended colon from mice of different strains to acetylcholine. Analyses of variance indicate that there are significant differences between the strains ($P<.001$). Sample size = 9 for all strains.

4. Discussion

In the EPM, time spent on the open arms of the maze and the number of entries onto the open arms are taken as measures of anxiety (Stephens et al., 1986). Anxiolytic drugs increase both of these parameters. The established anxiolytic agent diazepam produced a significant increase in the amount of time spent on the open arms of the maze but had no effect on the total number of entries, indicating that the dose used was eliciting an anxiolytic effect without causing general sedation. There were no indications of any strain differences in the anxiolytic effects of diazepam.

Losartan at the lower dose of 10 mg kg^{-1} ip produced a similar anxiolytic-like effect, but the analysis revealed that this was strain dependent, occurring only in BKW strain mice. In the studies of the higher dose of losartan, it was found that C57 mice exhibited more anxiety-like behaviour than DBA2 mice although there was no significant anxiolytic effect of losartan in either strain. At this higher dose, losartan was seen to increase general locomotor activity in both strains.

In the LDT, another test of anxiolytic activity, the administration of anxiolytic drugs is associated with a shorter latency to transition from the dark to the bright area, a greater percentage of time spent in the bright area, increased rears and line crossing in the bright area and decreased line crossing in the dark area (Costall et al., 1989). Unlike the EPM, this test identified significant differences in behaviour between the BKW, C57BL/6 and DBA/2 mice, with BKW mice showing consistently more anxiety-related behaviour than the other two strains. These results differ slightly from those of Costall et al. (1989) who studied the behaviour of the same three mouse strains in the LDT and reported that C57 mice exhibited less anxiety-like behaviour than either BKW or DBA2 mice; BKW mice were not identified as showing more anxiety-related behaviour. These differences in the findings of the two studies may reflect type II statistical errors due to the small sample sizes used in both.

Costall et al. also demonstrated that anxiolytic drugs induced significant changes in all of the parameters measured in the LDT in all three strains of mouse. The present study, however, found losartan to be inactive at the doses tested, doses that were 100 times greater than those reported as being effective by Barnes et al. (1990).

The combined behavioural evidence from the two tests used in the present study therefore suggests that there are some slight, but probably relatively inconsequential, differences in endogenous anxiety-related behaviour in the mouse strains studied. These differences achieved statistical significance in only one of the two tests and were not consistent with the results of a previous study. There were no marked strain differences in the response to diazepam in the EPM. There were, however, strain differences in the anxiolytic response to losartan in this test, with BKW being the most susceptible strain. In the LDT, losartan was not found to be anxiolytic in BKW mice, although the drug effect may have been confounded by the fact that BKW mice exhibited significantly more anxiety-like behaviour in this test.

Strain differences were also identified in the contractile effects of the isolated descending colon to angiotensin II. Tissues from BKW mice, which was the only strain to exhibit a behavioural response following administration of losartan, were found to have the lowest contractile response to angiotensin II. If colonic angiotensin receptor function is in any way related to brain receptor function, these results may indicate that BKW mice have an endogenous functional deficit in angiotensin activity, which therefore renders them more susceptible to the effects of an angiotensin receptor antagonist, including behavioural effects. Whether the observed decreases in response to angiotensin are due to decreased expression of the angiotensin AT_1 receptor is, as yet, unknown, although the finding that the strain differences in contractile responses to angiotensin are mirrored by strain differences in responses to acetylcholine suggest that the differences may occur down-stream from the receptor, for example, second messenger generation or contractile efficacy of the smooth muscle.

In conclusion, the results of this study in mice highlight the importance of animal strain in both psychopharmacology and angiotensin research by clearly demonstrating that there are strain differences in the behavioural effects of the angiotensin AT_1 receptor antagonist losartan. This finding may explain the contradictory results of the previously published studies in mice (Barnes et al., 1990; Shepherd et al., 1996) and may also offer an explanation of the contradictory results reported in rats. Furthermore, the results suggest that the strain differences in behavioural responses to losartan may be due to differences in the functional activity of angiotensin II. This finding has interesting ramifications for behavioural genetics although the results do indicate that differences in endogenous angiotensin activity do not appear to influence baseline anxiety-related behaviour in mice.

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