Pharmacology, Biochemistry and Behavior 96 (2010) 429–437

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Anxiety-related behaviour of low brain angiotensinogen transgenic rats in the canopy test

J.-P. Voigt [a,*], M. Bader [c], B. Bert [b], H. Fink [b], A. Rex [d]

[a] School of Veterinary Medicine and Science, University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, United Kingdom
[b] Institute of Pharmacology and Toxicology, School of Veterinary Medicine, Freie Universität Berlin, Koserstr. 20, D-14195 Berlin, Germany
[c] Max-Delbrück-Center for Molecular Medicine (MDC), D-13122 Berlin-Buch, Germany
[d] Department of Experimental Neurology, Charité-Universitätsmedizin Berlin Klinik für Neurologie, D-10098 Berlin, Germany

ARTICLE INFO

Article history:
Received 21 December 2009
Received in revised form 29 June 2010
Accepted 4 July 2010
Available online 16 July 2010

Keywords:
Anxiety
Transgenic rat
Angiotensin
Canopy test
mCPP
Diazepam
ß-carboline
Stretched attend posture

ABSTRACT

This study investigated risk assessment and anxiolytic/anxiogenic drug effects in "low brain angiotensinogen" transgenic rats (TGR) in comparison to wild-type Sprague–Dawley rats (SD) in the canopy test of anxiety-related behaviour.

TGR showed a higher frequency of the risk assessment behaviour as indicated by performance of stretched attend posture (SAP) compared to SD. Diazepam (0.25 mg/kg) reduced SAP in both strains, whereas FG-7142 had no significant effect. The 5-$HT_{1B/2C}$ agonist mCPP (0.5–2 mg/kg) reduced SAP in both strains.

Diazepam (0.25–1 mg/kg) increased head dips and decreased the time spent under the canopy in SD rats. There were significant anxiogenic effects of both FG-7142 (3–6 mg/kg) and mCPP (0.5–2 mg/kg) on these parameters for SD but not TGR.

Diazepam (1 mg/kg) increased the number of entries into the open zone in both strains. mCPP reduced this parameter in SD (2 mg/kg) and TGR (0.5–2 mg/kg). FG-7142 had a similar effect in SD (3–6 mg/kg) and TGR (6 mg/kg).

This study showed a significant transgenic effect on SAP. The increased number of SAP seen in TGR could be reduced with diazepam. Although both FG-7142 and mCPP are generally anxiogenic, no significant effects of FG-7142 on SAP were observed and mCPP even reduced SAP.

1. Introduction

The octapeptide angiotensin II is a potent vasoconstrictor, it also induces thirst and vasopressin release, increases sodium appetite, and stimulates release of noradrenaline, adrenocorticotrope hormone, and aldosterone. Further to its role as a circulating hormone, angiotensin II may act as a neurotransmitter or neuromodulator (Lind et al., 1985, Ferguson et al., 2001, McKinley et al., 2003), although more investigations are required to establish a detailed picture of the brain neurochemistry of angiotensin (Diz 2006). There is nevertheless accumulating evidence for an involvement of brain angiotensin in different behaviours including anxiety-related behaviour (Gard 2004). This is mainly based on pharmacological studies but receives further support from experiments employing transgenic rodents (Okuyama et al., 1999, Voigt et al., 1999, 2005, Wilson et al., 1996). In a previous study, we described an anxious phenotype in transgenic rats (TGR) with low brain angiotensinogen (Voigt et al., 2005). In these TGR(ASrAOGEN)680 rats, brain angiotensinogen protein concentration is reduced by more than 90% due to a brain-specific expression of transgenic antisense RNA against angiotensinogen mRNA (Schinke et al., 1999). The anxious phenotype of the transgenic rat TGR(ASrAOGEN)680 was revealed by studying the rats in different rodent tests of anxiety-related behaviour including the elevated plus-maze (EPM) and the light/dark box. In the EPM, TGR(ASrAOGEN)680 rats made fewer entries into the open arms, spent less time there, but more time in the closed arms. Head dips were reduced and U-turns into the closed arms were increased. A further behavioural parameter to be shown by rats under these experimental conditions is the stretched attend posture (SAP). SAP as part of the behavioural repertoire of rodents has been described and classified as an approach–avoidance conflict in a social context (Grant and Mackintosh, 1963 in Grewal et al., 1997). Blanchard and Blanchard (1988, 1989) describe this risk assessment behaviour as a defence response to nondiscrete or potential danger. This kind of risk assessment was also monitored during our previous studies, but its incidence as determined upon testing in the EPM was too low for appropriate statistical analysis. By contrast, TGR(ASrAOGEN)680 showed significantly more SAP made from the dark compartment into the illuminated compartment of the light/dark box as compared to SD rats. This went along with an increased latency to the first re-entry

* Corresponding author. Tel.: +44 115 9516408; fax: +44 115 9516440.
E-mail address: peter.voigt@nottingham.ac.uk (J.-P. Voigt).

0091-3057/$ – see front matter.
doi:10.1016/j.pbb.2010.07.001

into the light compartment in TGR(ASrAOGEN)680 and a reduced number of transitions between the two compartments. Whereas the latter parameters are indicative of an increased anxiety, SAP has not been validated as anxiety-related in this particular model.

Grewal et al. (1997) devised a novel and simple method of eliciting high levels of SAP in rodents, the canopy test. The canopy comprised a circular platform elevated from the ground. A smaller "canopy" was supported directly above the platform by a central pillar, thus dividing the platform into an inner, covered zone and an outer, exposed zone. In both the rat and mouse version of this model, vehicle-treated animals exhibited a marked preference for exploring the covered zone. In this test, rats (and mice) showed high baseline levels of SAP, particularly when exploring the outer zone starting out from the protected covered zone (Grewal et al., 1997). This model was pharmacologically validated for both anxiolytic and anxiogenic drug effects on behaviour. Based on these findings, the canopy test appeared to be a useful paradigm to investigate rodent-specific risk assessment behaviour in TGR(ASrAOGEN)680.

This study aimed to investigate risk assessment in TGR(ASrAOGEN)680 in comparison to wild-type Sprague–Dawley (SD) rats, and investigated both the anxiolytic and anxiogenic drug effects in these rats. Diazepam was used to induce anxiolytic effects, whereas the partial inverse benzodiazepine receptor agonist FG-7142 was administered to induce anxiogenic effects (Evans and Lowry 2007, File and Baldwin 1987, for review), although the anxiogenic effects of β-carbolines like FG-7142 could possibly depend on several factors including the animal model in use (Thiebot et al., 1988).

In previous studies, we found that the anxious phenotype in TGR(ASrAOGEN)680 is accompanied by a reduced content of serotonin (5-HT) and its metabolite 5-hydroxyindolic acid (5-HIAA) in the hippocampus, frontal and parietal cortices. HIAA and 5-HIAA/5-HT ratio are reduced in the hypothalamus, striatum and septum (Voigt et al., 2005). Further changes in the brain serotonin system include higher expression levels of hypothalamic 5-HT_{2C} receptor and 5-HT transporter mRNA (Voigt et al., 2008). In addition, and in comparison to wild-type rats, an augmented anxiogenic effect of the anxiogenic 5-$HT_{1B/2C}$ receptor agonist (Kennett et al., 1989, Whitton and Curzon, 1990) mCPP was found in TGR(ASrAOGEN)680 when tested in the open-field. For this reason mCPP was also included in the present study.

2. Material and methods

2.1. Animals

Subjects consisted of 221 adult male transgenic rats TGR(ASrAOGEN)680 (Schinke et al., 1999) and age-matched Hanover Sprague–Dawley (SD) rats (parent strain) (MDC, Berlin-Buch, Germany) weighing 260–300 g. Rats were housed in groups of 5 under standard laboratory conditions (12-h light/dark cycle, room temperature 22 °C) and fed a maintenance diet (Ssniff-R/M-H, ssniff Spezialdiäten GmbH, Soest, Germany). The study was performed according to the guidelines of the German Animal Protection Law and was approved by the Berlin State Authority (LaGeSo).

2.2. Analysis of behaviour

2.2.1. Canopy

The canopy test apparatus used to asses SAP, comprised of an elevated dark grey circular platform (98.5 cm in diameter) elevated 100 cm above the ground. A smaller transparent circular "canopy" (58.5 cm in diameter) was supported directly above the platform by a central pillar, thus dividing the platform into an inner covered zone and an outer exposed zone. The main difference to the original canopy device as described by Grewal et al. (1997) is that the transparency of the canopy used in the present study facilitates a more precise tracking of locomotor activity. The illumination of the apparatus provided by normal room lighting measured 130 lux on the exposed zone.

The following behavioural parameters were measured upon exposure to the canopy:

- Number of stretched attend postures (SAP)
- Time spent under the canopy
- Distance travelled
- Number of head dips (exploratory movement of head/shoulders from the edge of the platform)
- Number of entries into the open zone

2.2.2. Drugs

In the pharmacological experiments, rats were pre-treated with the anxiolytic benzodiazepine receptor agonist diazepam (Ratiopharm, Germany) (0.125–1 mg/kg IP), the anxiogenic inverse partial benzodiazepine receptor agonist FG-7142 (N-methyl-β-carboline-3-carboxamide; Sigma-Aldrich, Germany) the anxiogenic 5-$HT_{1B/2C}$ agonist mCPP (m-(chlorophenyl)piperazine; 0.5–2 mg/kg IP; Sigma-Aldrich, Germany) or saline. Diazepam was administered 15 min before testing, mCPP and FG-7142 30 min before testing. The volume of the substance injected was 1 ml/kg body weight.

2.2.3. Procedure

All experiments were performed in a sound-proofed chamber (180 cm × 180 cm × 230 cm) between 0800 and 1200 h. For all behavioural testing, independent groups of rats that had not been habituated to the test before were used. The behaviour of each rat was recorded by a video camera and analyzed using a computer-driven automatic tracking system with software (VideoMot 2, TSE, Germany). The automated recording system was supplemented by a keyboard-driven recorder to score discrete behavioural events. The parameters 'time spent under the canopy' and 'distance travelled' were directly obtained from the software-assisted analysis. The parameters 'number of stretched attend postures (SAP)', 'number of head dips from the platform' and 'number of entries into the open zone' were all scored manually upon reviewing the video.

2.2.3.1. Experiment 1. In the first experiment, naïve rats of both genotypes were placed onto the edge between the open and covered zone of the platform and allowed to explore the apparatus for a 5 min test period. All behaviours were videotaped.

2.2.3.2. Experiment 2. In independent pharmacological experiments, naïve rats of both genotypes were injected with either saline or test drug and then tested on the canopy as described before. Each rat was only used once.

2.3. Statistics

In the first experiment, between-group comparisons were made using the Student's *t*-test, or where appropriate, the non-parametric Mann–Whitney *U* test. Since the criteria for two-way ANOVA were not always met, data from the second experiment were analyzed locally by one-way ANOVA followed by Dunnett's test or Kruskal–Wallis test followed by Dunn's test, respectively.

Significance was accepted at the $P<0.05$ level. Data are expressed as means ± SEM.

3. Results

3.1. Experiment 1

Untreated TGR(ASrAOGEN)680 made fewer head dips from the platform ($P<0.001$; $t=5.146$ $df=12$), spent significantly more time

under the protective canopy ($P<0.001$; $t=5.057$; $df=12$) and there was a tendency in this group to make fewer entries into the open area of the platform ($P<0.10$). In addition, transgenic rats travelled less than their wild-type counterpart ($P<0.10$). Most importantly, however, the transgenic rats showed a higher incidence of risk assessment as expressed by the significant higher amounts of SAP ($P<0.001$; $t=4.82$; $df=12$) (Fig. 1).

3.2. Experiment 2

3.2.1. DZP

In wild-type SD rats (Fig. 2), diazepam (0.125–1 mg/kg IP) had a significant anxiolytic action. Treatment increased head dips ($P<0.001$, $F(37,3)=9.05$) with 0.25 and 1 mg/kg as effective doses. Diazepam (0.25 mg/kg) also increased the distance travelled ($P<0.05$; $F(37,3)=2.90$). 0.25 and 1 mg/kg diazepam reduced the time the rats spent under the canopy ($P<0.05$; $F(37,3=3.99$). The same dose increased the number of entries into the open area of the platform, although ANOVA showed this to only be a tendency ($P<0.10$; $F(37,3)=2.38$). SAP were reduced after both 0.25 and 1 mg diazepam ($P<0.01$; $F(37,3)=6.125$).

Transgenic rats (Fig. 3) travelled a longer distance following the highest dose of diazepam (1 mg/kg) ($P<0.05$; $H=8.35$). In contrast to SD rats, diazepam did not change the number of head dips in the transgenic group, nor had it an effect on the time spent under the canopy. However, 1 mg/kg diazepam increased the number of entries into the open zone ($P<0.05$; $F(34,3)=3.28$). SAP were also reduced, but this was only significant after the middle dose of 0.25 mg/kg ($P<0.05$; $F(34,3)=3.90$).

3.2.2. FG-7142

FG-7142 (6 mg/kg) reduced the distance travelled in SD rats ($P<0.01$; $F(29,2)=5.77$) and reduced head dips (3 and 6 mg/kg) ($P<0.01$, $F(29,2)=8.52$). FG-7142 (6 mg/kg) increased the time spent under the covered zone ($P<0.05$; $F(29,2)=5.29$) and reduced the number of entries made into the unprotected zone (3 mg/kg and 6 mg/kg) ($P<0.001$; $F(29,2)=9.23$). There was no significant effect of FG-7142 on SAP in SD rats (Fig. 4).

In TGR (Fig. 5), 6 mg/kg FG-7142 reduced locomotor activity as it did in SD rats ($P<0.05$; $F(29,2)=3.88$), whereas there was no significant reduction of head dips. The time spent under the canopy was not affected either, whereas 6 mg/kg reduced the number of entries into the open area ($P<0.05$; $H=6.47$). The effect on SAP is unclear, as data were not significant.

3.2.3. mCPP

The 5-$HT_{1B/2C}$ agonist mCPP (2 mg/kg) reduced the distance travelled in SD rats ($P<0.05$; $F(36,3)=4.39$), reduced head dips ($P<0.001$, $H=16.58$), increased the time spent under the covered zone ($P<0.01$; $H=11.53$) and reduced the number of entries made into the unprotected zone ($P<0.01$; $H=12.61$). Surprisingly, 0.5–2 mg/kg mCPP significantly reduced the number of SAP ($P<0.001$, $H=16.28$) (Fig. 6).

In TGR (Fig. 7), 1 mg/kg and 2 mg/kg mCPP reduced the distance travelled ($P<0.01$; $F(35,3)=5.74$) and 2 mg/kg tended to reduce head dips ($P<0.10$). 0.5–2 mg/kg reduced the entries into the uncovered zone ($P<0.01$; $H=12.53$). As in SD rats, SAP were reduced after 0.5–2 mg/kg mCPP ($P<0.001$; $F(35,3)=13.33$).

4. Discussion

In this study, a higher score of anxiety-related behavioural measures was found in transgenic rats with low brain angiotensinogen compared to wild-type Sprague–Dawley rats. The higher number of SAP indicates a higher incidence of risk assessment. As the canopy test is a validated rodent model of anxiety focusing on the risk assessing SAP, the results obtained from untreated rats confirm the anxiogenic effect of the transgene (Voigt et al., 2005). In addition to SAP, TGR also made fewer head dips and tended to travel a shorter distance, although there is no evidence for impaired locomotor behaviour in these rats (Voigt et al., 2005). TGR also spent more time in the zone covered by the canopy. In the initial study introducing this model (Grewal et al., 1997), these latter parameters were either not used (head dips) or proved invalid (time spent under the canopy). However, the time spent under the canopy was useful in the present analysis, as diazepam reduced this time in the controls (see below). This might apply to head dips as well and both parameters seem to

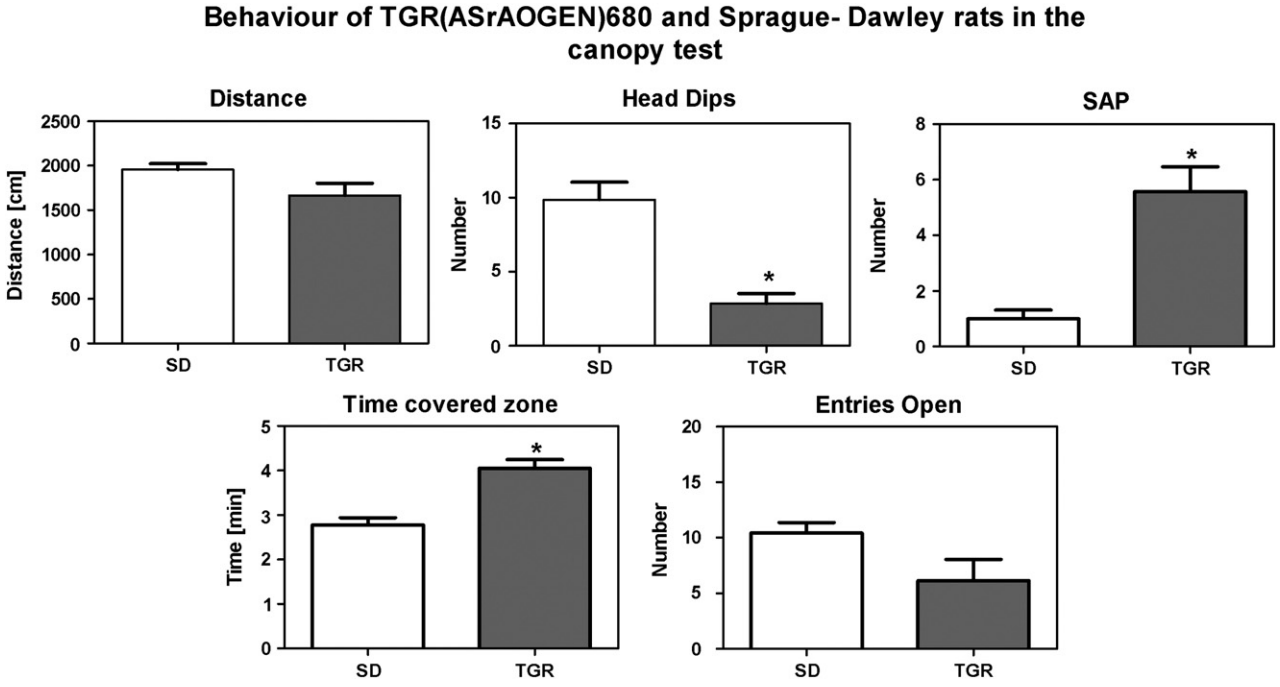

Fig. 1. Canopy behaviour. Comparison between TGR(ASrAOGEN)680 and Sprague–Dawley rats. n = 7/group. Mean ± SEM. *$P<0.05$. Student's *t*-test.

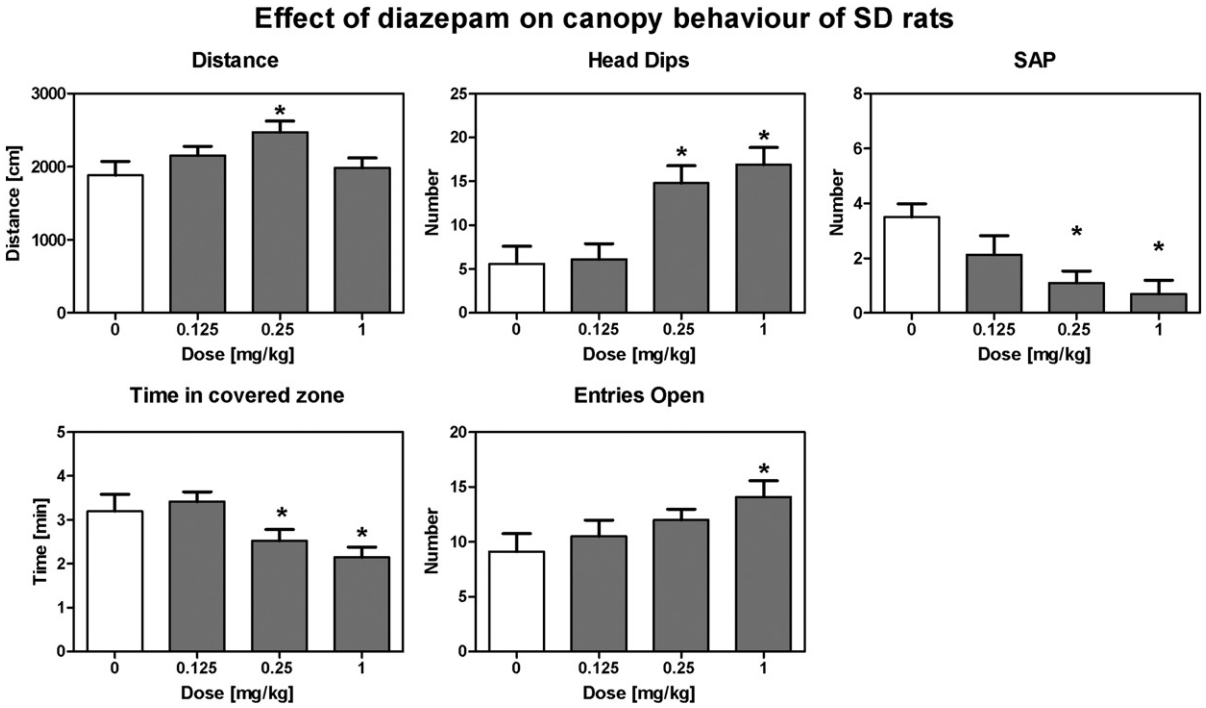

Fig. 2. Canopy behaviour. Effect of diazepam in Sprague–Dawley rats n = 10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test.

support the interpretation of more anxiety-related behaviours in TGR during exposure to the canopy test. For these reasons, we extended the number of parameters to be analyzed in the present study as compared to Grewal et al. (1997).

The total baseline number of SAP in our study, however, is lower when compared to the original report of the canopy test (Voigt et al., 2005). This discrepancy could theoretically be explained by strain differences in anxiety-related behaviours (Rex et al., 1996) although our scores are in line with those obtained from Wistar rats (Ray and Hansen 2005).

We chose the same experimental conditions as in our previous study (Voigt et al., 2005) to allow a direct within-laboratory comparison. In this corresponding previous study, the number of SAP shown upon exposure to the EPM was far too small to allow for statistical analysis. By contrast, the total number of SAP displayed in the canopy test here was high enough to be suitable for statistical analysis. This indicates clearly the "SAP-inducing" effect of the canopy as it was used in our laboratory. The only difference in the design of the canopy apparatus between the original and the present study is the transparency of the canopy itself. However, this did not matter as

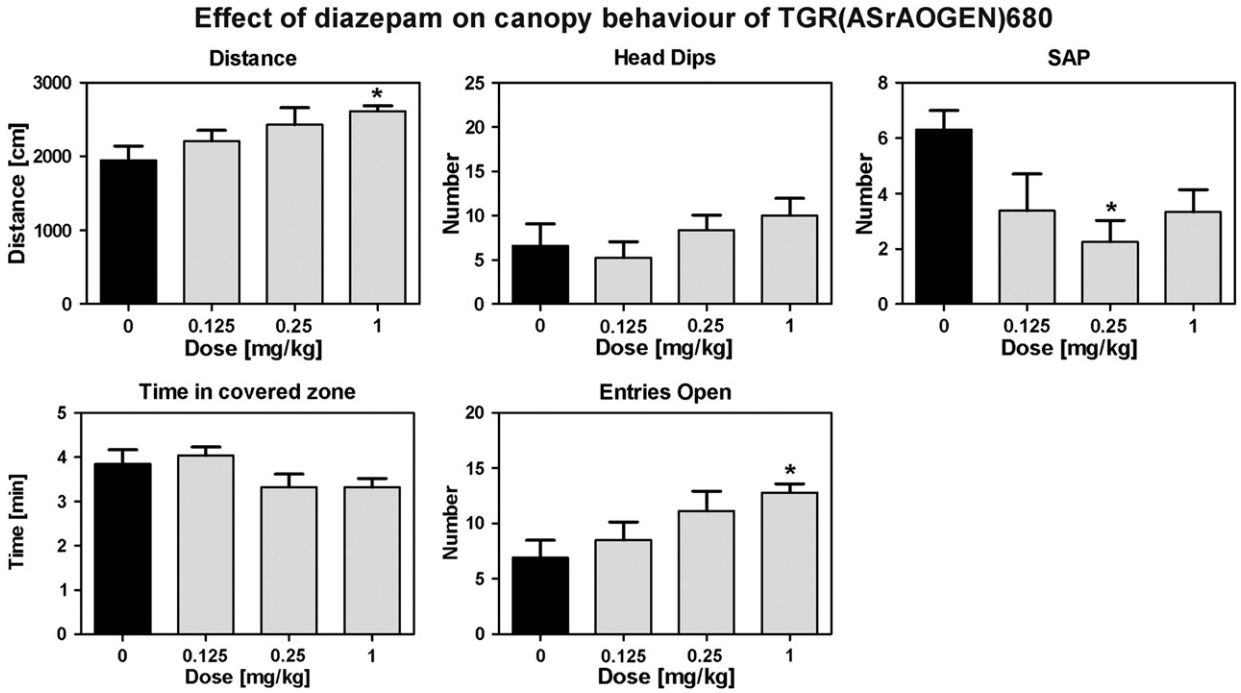

Fig. 3. Canopy behaviour. Effect of diazepam in TGR(ASrAOGEN)680 rats n = 8–10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test or Kruskal–Wallis test followed by Dunn's test.

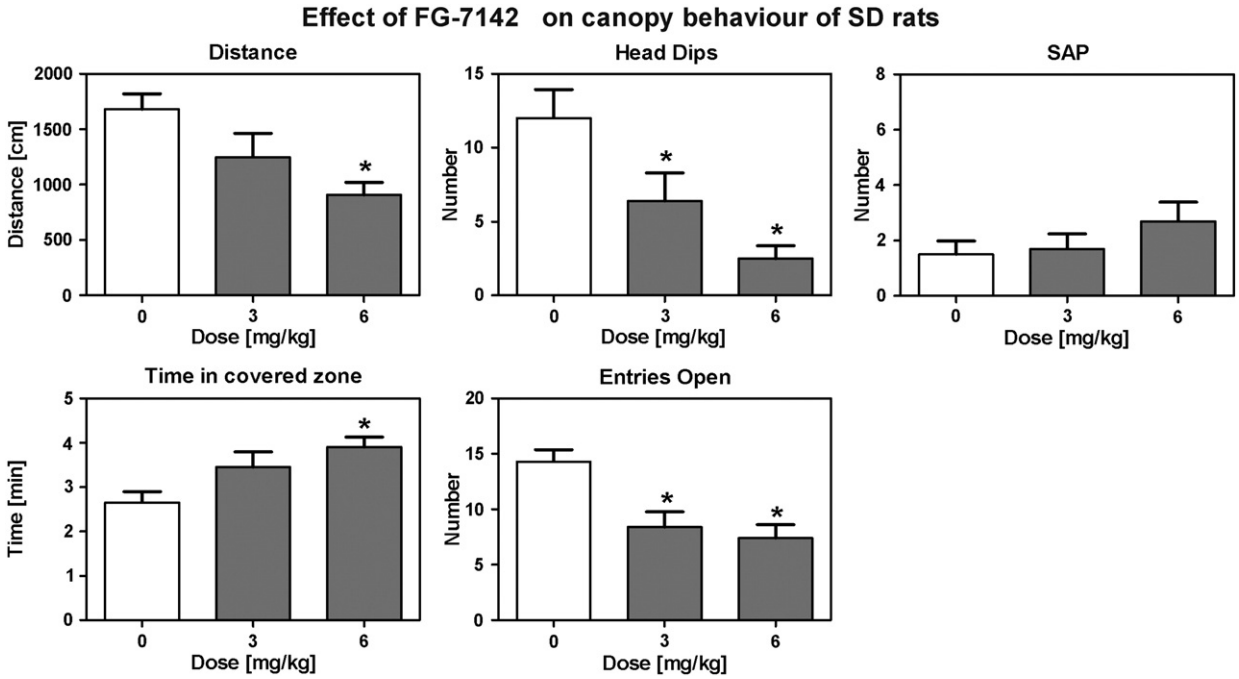

Fig. 4. Canopy behaviour. Effect of FG-7142 in Sprague–Dawley rats n = 10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test.

the rats easily identified the protective effect of the transparent canopy and the anxious TGR spent even more time under its protection. The exposed zone of the canopy is almost twice as large as the protected zone (4932 cm^2 vs. 2688 cm^2). Sprague–Dawley rats spent on average 2.78 ± 0.16 (out of a total of five) min under the canopy. Considering the smaller size of this zone, this measure shows a clear preference for staying under the protecting transparent canopy. The advantage of a transparent canopy is an easier quantification of locomotor activity and other behaviours shown under the canopy. Therefore, it is unlikely that the difference in SAP baseline between the present study and the original description of the model is due to the modification of the design. Recent studies using a canopy similar to that described in the original paper report numbers of SAP that correspond to our data (Ray and Hansen 2005).

Diazepam reduced SAP both in SD and transgenic rats. The anxiolytic effects of diazepam shown here are not only in keeping with the well established role of this benzodiazepine as an anxiolytic drug, but also with the results shown by Grewal et al. (1997) in their canopy study, although with a slightly lower dose effective in our experiment. Interestingly, we found in SD rats a significant decrease in the time spent under the canopy after diazepam that went along with an increased number of entries into the open zone. This finding seems

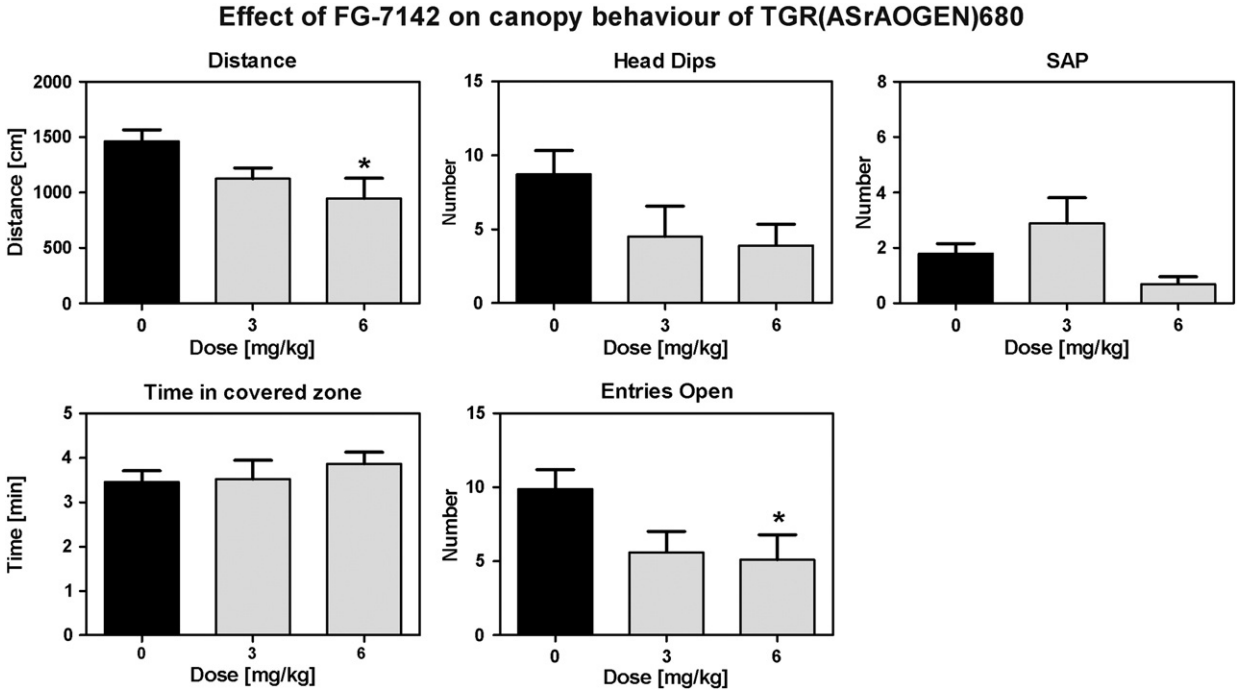

Fig. 5. Canopy behaviour. Effect of FG-7142 in TGR(ASrAOGEN)680 rats n = 10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test or Kruskal–Wallis test followed by Dunn's test or Kruskal–Wallis test followed by Dunn's test.

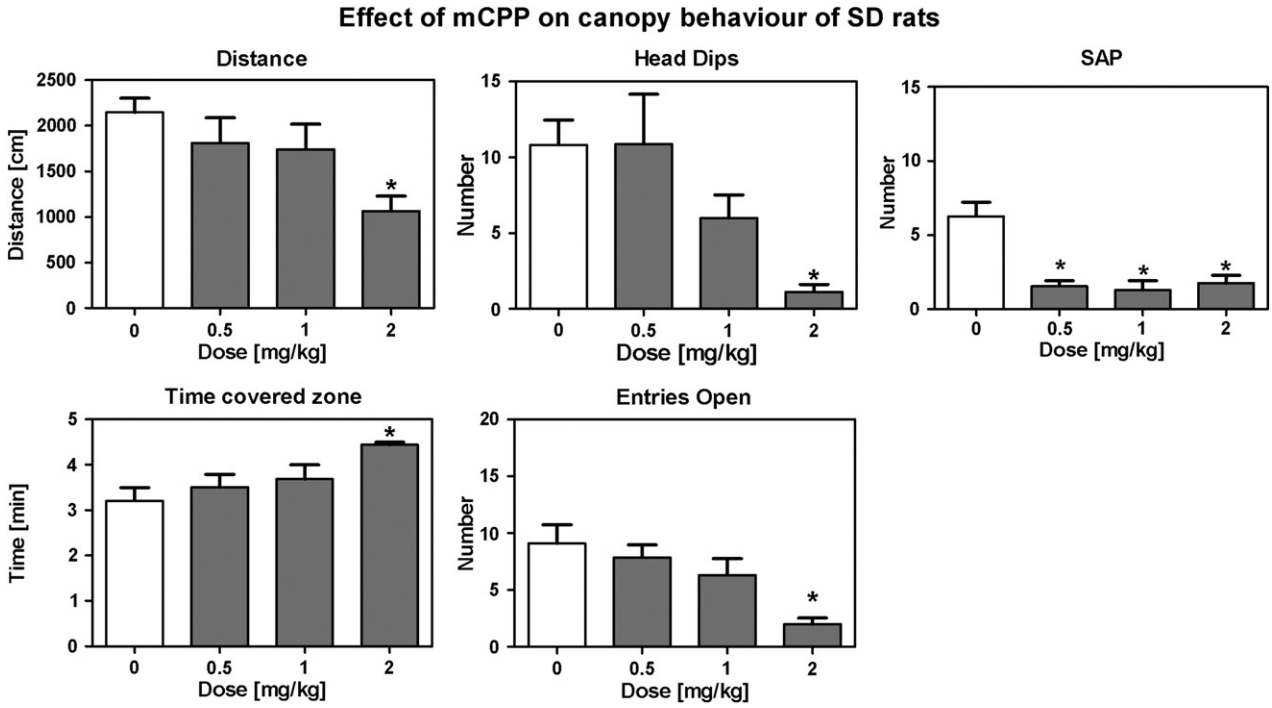

Fig. 6. Canopy behaviour. Effect of mCPP in Sprague–Dawley rats n = 8–10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test or Kruskal–Wallis test followed by Dunn's test .

to contradict the results obtained by Grewal et al. (1997) who conclude that time spent in the open zone is not a reliable parameter. However, in the present study, both the transgenic and the pharmacological effects on the time spent under the canopy suggest that, at least under our conditions, the parameter time and entries might be useful for the interpretation of canopy behaviour.

The data shows that diazepam has anxiolytic effects in doses below those reducing locomotor activity. Moreover, doses of diazepam that reduced SAP increased head dips and distance travelled. The latter effect indicates that the reduction in SAP is not due to sedation/muscle relaxation and further suggests that the total locomotor activity as expressed by distance travelled seems to be affected by anxiety as

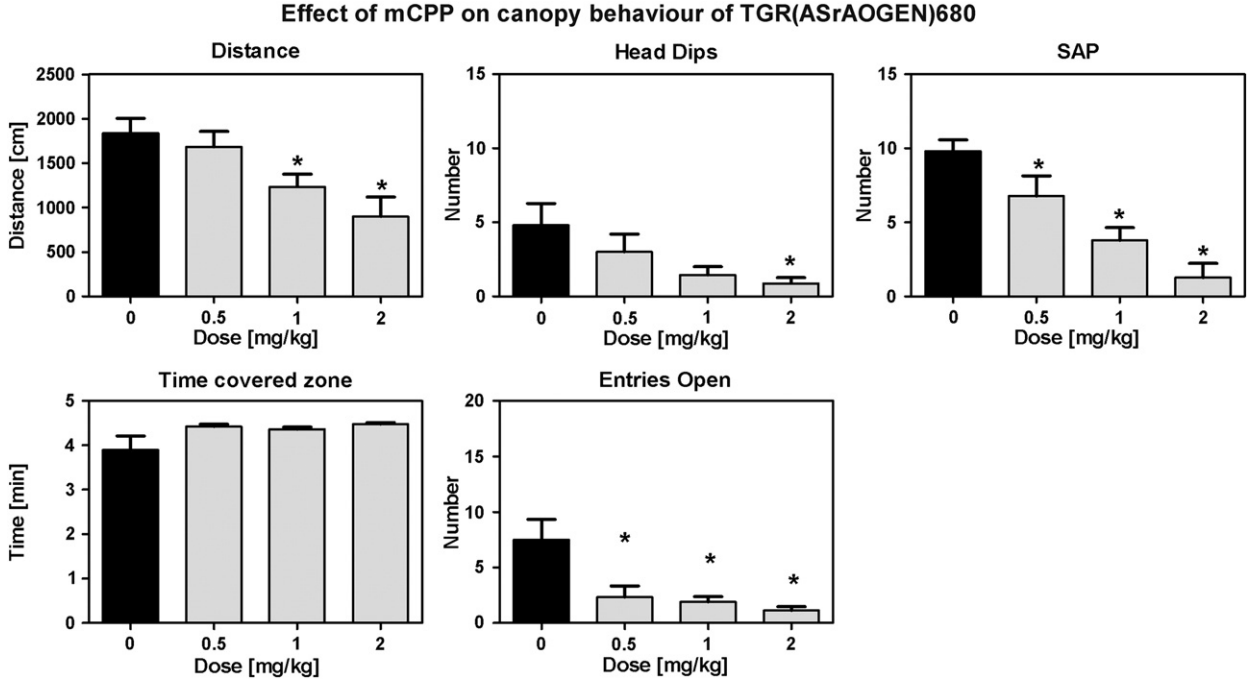

Fig. 7. Canopy behaviour. Effect of mCPP in TGR(ASrAOGEN)680 rats n = 8–10/group. ANOVA followed by Dunnett's test. Mean ± SEM. *P<0.05. ANOVA followed by Dunnett's test or Kruskal–Wallis test followed by Dunn's test or Kruskal–Wallis test followed by Dunn's test.

shown before for the EPM test of anxiety-related behaviour (Cruz et al., 1994). This interpretation is further supported by the slightly lower distance travelled in the anxious transgenics as found in experiment one that could not be attributed to impairments in motor behaviour (Voigt et al., 2005).

Obviously, a slightly higher dose of diazepam is required for anxiolysis in the more anxious transgenic rats. Although 0.25 mg/kg reduced SAP, the higher dose of 1 mg/kg did not. This is unlikely due to any interference with motor behaviour as the same dose increased the distance travelled. A closer examination of individual data showed an increase in SAP in some individuals in the lowest dose group (0.125 mg/kg). "Paradox effects" after low doses of diazepam have been described (Cooper 1983, Desai et al., 1983) and may have occurred here as well. This effect possibly interfered with the statistical analysis as eliminating these individuals would otherwise result in significant effects for all treatment groups. The number of entries in the unprotected area increased after diazepam; but diazepam did not affect the number of head dips. Time spent under the canopy could not be reduced by diazepam in the transgenics either. This partial failure of diazepam has been observed only in TGR and could be down to the high baseline level of anxiety in TGR. A further increase in dose to achieve anxiolysis would interfere with locomotor behaviour and thus obscure any anxiolytic effect.

FG-7142 had an obvious anxiogenic effect in SD rats as shown by the reduction in head dips and the reduced entries into the open area, both after 3 mg/kg. This is in line with earlier findings from the elevated plus-maze as reported by Cruz et al. (1994). These anxiogenic effects were seen below a dose that potentially could interfere with locomotor behaviour. However, contrary to expectation, FG-7142 did not increase the number of SAP significantly in either rat strain. Although there are anxiogenic effects in both groups after doses that are lower than doses reducing locomotor activity, it cannot be decided if the locomotor effect of FG-7142 is reflecting a true anxiogenic effect or rather this is due to sedation. An earlier open-field study, also done with SD rats, shows a reduced distance travelled after 5 mg/kg, but reduced rearing only after 20 mg/kg (Meng and Drugan 1993). This finding would support the interpretation that the reduced locomotor activity as observed in our study, is not necessarily due to sedation.

In summary, diazepam had anxiolytic effects in both SD and TGR, with slightly higher doses required for anxiolysis in TGR. The anxiogenic effect of FG-7142 is more obvious in SD although FG-7142 did not affect SAP. FG-7142 could be potentially less effective in the more anxious TGR due to a ceiling effect related to the higher baseline anxiety, however, changes in brain neurochemistry in TGR interfering with the effects of FG-7142 cannot be ruled out at this stage (Voigt et al., 2005, Hackler et al., 2007).

Surprisingly, mCPP reduced SAP in both genotypes. This decrease in SAP is difficult to explain, since it occurs already at doses that do not affect other motor behaviour at all. In fact, the very same doses increased grooming activity as measured in the open-field test (Voigt et al., 2005). Strain differences and individual differences (Verheij et al., 2009) cannot account for this discrepancy since both studies used Sprague–Dawley rats, and these rats showed increased anxiety after the same doses in our laboratory, an effect that is in keeping with earlier findings (Griebel et al., 1995). A study by Molewijk et al. (1995) found no effects of mCCP towards the anxiogenic stimulus in an approach–avoidance paradigm, but an increase in SAP directed away from the stimulus. Given this complexity, further research into the effects of mCPP on SAP is certainly justified. However, the 5-$HT_{1B/2C}$ agonist mCPP had anxiogenic effects in SD rats as reflected by the reduced number of head dips and increased the time spent under the covered zone. The anxiogenic effects are both in line with earlier studies with the same rat strain (Griebel, 1995, for review), and also with other results from our laboratory (Rex et al., 2004, Voigt et al., 2005) and the original canopy paper (Grewal et al., 1997) as well. The reduced distance travelled after mCPP as shown in both groups would also be in keeping with an interpretation of the present findings as mainly sedative effects. However, this would be in contrast to our previous open-field findings where the same dose of mCPP stimulated grooming activity (Voigt et al., 2005).

We expected a stronger effect of mCPP in TGR, based on previous behavioural experiments showing a potential upregulation of brain 5-HT_{2C} receptors (Voigt et al., 2008). In fact, this was seen for the parameter distance and entries into the open zone. The latter was seen already after the dose of 0.5 mg/kg that had no effect on distance travelled and, therefore, might indicate a rather specific anxiogenic effect.

Considering the anxiogenic effects of FG-7142 on behaviours other than SAP in SD, it becomes obvious the there were less anxiogenic effects measurable in TGR. This is very likely due to the already higher anxiety level in these rats as shown in the first experiment and our previous study (Voigt et al., 2005) that does not allow a further augmentation within the current experimental design.

Although both our previous publication (Voigt et al., 2005) and the present study indicate increased anxiety due to reduced brain angiotensinogen (Schinke et al., 1999), the physiological significance of these findings requires some discussion. There is convincing evidence that angiotensin II plays a role in rodent anxiety-related behaviour. The prevailing view is that an interference with the renin angiotensin system (RAS) will result in anxiolysis. However, the nature of this effect needs to be further clarified due to some conflicting data (Gard 2004, for review and extensive discussion).

There are reports using transgenic or knockout animals that address the role of RAS in anxiety-related behaviour. Studies performed in knockout mice lacking the angiotensin-type-2 receptor found these mice also anxious in the light/dark and the elevated plus-maze test (Okuyama et al., 1999). By contrast, mutant mice lacking angiotensinogen did not show an anxious phenotype during a first behavioural screen (Walther et al., 1999). The latter finding does not relate directly to the present study though, as the knock out occurred both in the periphery and the brain in these mice. Pharmacological studies in this area of research used either angiotensin-type-1 receptor antagonists or angiotensin converting enzyme (ACE) inhibitors which inhibit the formation of angiotensin II. Sometimes angiotensin has been used itself. These pharmacological studies found mostly anxiolytic effects of the drugs after peripheral antagonist or ACE inhibitor administration (Gard 2004 for review). Anxiolysis due to blockade of angiotensin-type-1 receptors seems to be in contrast with our findings. However, the respective contribution of peripheral and central drug effects remains to be determined for those studies. A potential stress reducing effect of angiotensin-type-1 receptor antagonists has been suggested to include both central and peripheral effects (Saavedra et al., 2004). Interestingly, some pharmacological studies performing intracerebral angiotensin II administration show an anxiolytic effect of the peptide in the elevated plus-maze (Tsuda et al., 1992, Belcheva et al., 1997, Holy and Wisniewski 2001). The latter would be in contrast to antagonist studies showing anxiolysis after the peripheral administration of angiotensin receptor antagonists, but in keeping with our present results based on a central transgenic manipulation.

Nevertheless, one could also speculate that the adaptive changes within the brain renin angiotensin system could contribute to the anxiogenic phenotype. An upregulation of angiotensin-type-1 receptors located inside the brain has been reported in TGR(ASrAOGEN)680 (Monti et al., 2001), indicating that the downregulation of astroglial angiotensinogen regulates receptor expression. Although glial angiotensinogen is significantly reduced in these rats, neuronal angiotensin does not appear to be affected (Vinsant et al., 2005). This suggests that TGR(ASrAOGEN)680 could be more sensitive to neuronal angiotensin II than the SD controls. This interpretation would concur with the view of an anxiogenic role of brain angiotensin II.

However, angiotensin-type-1 receptors located in circumventricular organs such as the subfornical organ and the area postrema are downregulated (Monti et al., 2001). As the brain responds to both circulating and tissue angiotensin II (Saavedra 2005, for review), the functional net effect of the observed adaptive changes in receptor expression on anxiety-related behaviour requires further investigation. The need for more research is further supported by the recent finding of an increased stress response in TGR(ASrAOGEN)680 that was rather due to adrenal mechanisms than to a combined hypothalamic and pituitary related mechanism (Müller et al., 2010). Although this increased stress sensitivity in TGR(ASrAOGEN)680 corresponds to the anxious phenotype, this finding also emphasizes the possibility that peripheral mechanism could contribute to the anxiogenic effects of angiotensin II.

Whereas our data support the idea that brain angiotensin has a role in anxiety-related behaviour, it remains to be determined if further neurotransmitter systems are involved in the mediation of this effect. Interactions between angiotensin and other neurotransmitters including catecholamines and 5-HT have been reported. Angiotensin has been shown to stimulate 5-HT synthesis in a biphasic manner (Nahmod et al., 1978). Since 5-HT is involved in many behaviours including anxiety (Lucki, 1998, for review), angiotensin-induced changes in brain 5-HT synthesis (Voigt et al., 2005, 2008) could possibly contribute to the development of the behavioural phenotype in TGR(ASrAOGEN)680.

Biochemical studies provided evidence that the angiotensin-type-1 receptor antagonist losartan decreases 5-HT synthesis outside the blood brain barrier (Baltatu et al., 2002). Possible effects of antagonists on brain 5-HT were not addressed in that study. Another antagonist, candesartan, increased 5-HT content, but inside the blood brain barrier (Jenkins, 2008). The possibility that the pharmacodynamic and/or pharmacokinetic differences between antagonists contribute to apparently contradictory findings cannot be ruled out yet, as the different contributions of central and peripheral effects of these drugs cannot. Interestingly, Mendelsohn et al. (1993), using brain microdialysis, found an increase in the 5-HT metabolite 5-hydroxyindoleacetic acid (5-HIAA) following infusion of angiotensin II into the striatum. Although 5-HT itself was not measured, this finding could also be indicative of an increase in 5-HT (Stenfors and Ross, 2004). This would be in line with the reduced 5-HT content in rats with low brain angiotensinogen (Voigt et al., 2005).

Our previous experiments showing significant transgenic effect on brain 5-HT, and evidence for an adaptive compensatory increase in 5-HT_{2C} receptors (Voigt et al., 2008) suggest a role of 5-HT in mediating the effects of angiotensin on anxiety-related behaviours.

Taken together, the present study provides further evidence for an anxious phenotype in transgenic rats with low brain angiotensin and a dysfunctional brain 5-HT metabolism. In particular, there is a significant transgenic effect on SAP which is also sensitive to the anxiolytic action of diazepam. The anxiogenic effects of the FG-7142 and mCPP are only significant for parameters other than SAP, with even a reduction of SAP as induced by mCPP.

Acknowledgements

The authors thank S. Jacobs for skillful technical assistance and Dr. A. Roshier for discussing the manuscript.

References

Baltatu O, Afeche SC, Jose dos Santos SH, Campos LA, Barbosa R, Michelini LC, et al. Locally synthesized angiotensin modulates pineal melatonin generation. J Neurochem 2002;80:328–34.
Belcheva I, Georgiev V, Chobanova M, Hadjiivanova C. Behavioral effects of angiotensin II microinjected into CA1 hippocampal area. Neuropeptides 1997;31:60–4.
Cooper SJ. Suppression of saccharin-induced drinking in the nondeprived rat by low dose diazepam treatment. Pharmacol Biochem Behav 1983;18:825–7.
Blanchard DC, Blanchard RJ. Ethoexperimental approaches to the biology of emotion. Annu Rev Psychol 1988;39:43–68.
Blanchard RJ, Blanchard DC. Attack and defense in rodents as ethoexperimental models for the study of emotion. Prog Neuropsychopharmacol Biol Psychiatry 1989;13 (Suppl):S3-S14.
Cruz AP, Frei F, Graeff FG. Ethopharmacological analysis of rat behavior on the elevated plus-maze. Pharmacol Biochem Behav 1994;49:171–6.
Desai N, Taylor-Davies A, Barnett DB. The effects of diazepam and oxprenolol on short term memory in individuals of high and low state anxiety. Br J Clin Pharmacol 1983;15:197–202.
Diz DI. Approaches to establishing angiotensin II as a neurotransmitter revisited. Hypertension 2006;47:334–6.
Evans AK, Lowry CA. Pharmacology of the beta-carboline FG-7, 142, a partial inverse agonist at the benzodiazepine allosteric site of the GABA A receptor: neurochemical, neurophysiological, and behavioral effects. CNS Drug Rev 2007;13:475–501.
Ferguson AV, Washburn DL, Latchford KJ. Hormonal and neurotransmitter roles for angiotensin in the regulation of central autonomic function. Exp Biol Med (Maywood) 2001;226:85–96.
File SE, Baldwin HA. Effects of beta-carbolines in animal models of anxiety. Brain Res Bull 1987;19:293–9.
Gard PR. Angiotensin as a target for the treatment of Alzheimer's disease, anxiety and depression. Expert Opin Ther Targets 2004;8:7-14.
Grant EW, Mackintosh JH. A comparison of the social postures of some common laboratory rodents. Behaviour 1963;21:246–59.
Grewal SS, Shepherd JK, Bill DJ, Fletcher A, Dourish CT. Behavioural and pharmacological characterisation of the canopy stretched attend posture test as a model of anxiety in mice and rats. Psychopharmacology (Berl) 1997;133:29–38.
Griebel G. 5-Hydroxytryptamine-interacting drugs in animal models of anxiety disorders: more than 30 years of research. Pharmacol Ther 1995;65:319–95.
Hackler EA, Turner GH, Gresch PJ, Sengupta S, Deutch AY, Avison MJ, et al. 5-Hydroxytryptamine2C receptor contribution to m-chlorophenylpiperazine and N-methyl-beta-carboline-3-carboxamide-induced anxiety-like behavior and limbic brain activation. J Pharmacol Exp Ther 2007;320:1023–9.
Holy Z, Wisniewski K. Examination of the influence of 3,5-DHPG on behavioral activity of angiotensin II. Pol J Pharmacol 2001;53:235–43.
Jenkins TA. Effect of angiotensin-related antihypertensives on brain neurotransmitter levels in rats. Neurosci Lett 2008;444:186–9.
Kennett GA, Whitton P, Shah K, Curzon G. Anxiogenic-like effects of mCPP and TFMPP in animal models are opposed by 5-HT1C receptor antagonists. Eur J Pharmacol 1989;164:445–54.
Lind RW, Swanson LW, Ganten D. Organization of angiotensin II immunoreactive cells and fibers in the rat central nervous system. An immunohistochemical study. Neuroendocrinology 1985;40:2-24.
Lucki I. The spectrum of behaviors influenced by serotonin. Biol Psychiatry 1998;44: 151–62.
McKinley MJ, Albiston AL, Allen AM, Mathai ML, May CN, McAllen RM, et al. The brain renin-angiotensin system: location and physiological roles. Int J Biochem Cell Biol 2003;35:901–18.
Mendelsohn FA, Jenkins TA, Berkovic SF. Effects of angiotensin II on dopamine and serotonin turnover in the striatum of conscious rats. Brain Res 1993;613:221–9.
Meng ID, Drugan RC. Sex differences in open-field behavior in response to the beta-carboline FG 7142 in rats. Physiol Behav 1993;54:701–5.
Molewijk HE, van der Poel AM, Olivier B. The ambivalent behaviour "stretched approach posture" in the rat as a paradigm to characterize anxiolytic drugs. Psychopharmacology (Berl) 1995;121:81–90.
Monti J, Schinke M, Bohm M, Ganten D, Bader M, Bricca G. Glial angiotensinogen regulates brain angiotensin II receptors in transgenic rats TGR(ASrAOGEN). Am J Physiol Regul Integr Comp Physiol 2001;280:R233–40.
Müller H, Kroger J, Johren O, Szymczak S, Bader M, Dominiak P, et al. Stress sensitivity is increased in transgenic rats with low brain angiotensinogen. J Endocrinol 2010;204:85–92.
Nahmod VE, Finkielman S, Benarroch EE, Pirola CJ. Angiotensin regulates release and synthesis of serotonin in brain. Science 1978;202:1091–3.
Okuyama S, Sakagawa T, Chaki S, Imagawa Y, Ichiki T, Inagami T. Anxiety-like behavior in mice lacking the angiotensin II type-2 receptor. Brain Res 1999;821:150–9.
Ray J, Hansen S. Temperamental development in the rat: the first year. Dev Psychobiol 2005;47:136–44.
Rex A, Sondern U, Voigt JP, Franck S, Fink H. Strain differences in fear-motivated behavior of rats. Pharmacol Biochem Behav 1996;54:107–11.
Rex A, Voigt JP, Gustedt C, Beckett S, Fink H. Anxiolytic-like profile in Wistar, but not Sprague–Dawley rats in the social interaction test. Psychopharmacology (Berl) 2004;177:23–34.
Saavedra JM, Ando H, Armando I, Baiardi G, Bregonzio C, Jezova M, et al. Brain angiotensin II, an important stress hormone: regulatory sites and therapeutic opportunities. Ann NY Acad Sci 2004;1018:76–84.
Saavedra JM. Brain angiotensin II: new developments, unanswered questions and therapeutic opportunities. Cell Mol Neurobiol 2005;25:485–512.
Schinke M, Baltatu O, Bohm M, Peters J, Rascher W, Bricca G, et al. Blood pressure reduction and diabetes insipidus in transgenic rats deficient in brain angiotensinogen. Proc Natl Acad Sci USA 1999;96:3975–80.
Stenfors C, Ross SB. Changes in extracellular 5-HIAA concentrations as measured by in vivo microdialysis technique in relation to changes in 5-HT release. Psychopharmacology (Berl) 2004;172:119–28.
Thiebot MH, Soubrie P, Sanger D. Anxiogenic properties of beta-CCE and FG 7142: a review of promises and pitfalls. Psychopharmacology (Berl) 1988;94:452–63.
Tsuda A, Tanaka M, Georgiev V, Emoto H. Effects of angiotensin II on behavioral responses of defensive burying paradigm in rats. Pharmacol Biochem Behav 1992;43:729–32.

Verheij MM, Veenvliet JV, Groot Kormelink T, Steenhof M, Cools AR. Individual differences in the sensitivity to serotonergic drugs: a pharmacobehavioural approach using rats selected on the basis of their response to novelty. Psychopharmacology (Berl) 2009;205:441–55.

Vinsant S, Chappell MC, Ferrario CM, Ganten D, Diz DI. Low glial angiotensinogen is not associated with deficits in angiotensin peptides in neuronal pathways in transgenic ASrAogen rats (Abstract). FASEB J 2005;19:A1188.

Voigt JP, Hortnagl H, Rex A, van Hove L, Bader M, Fink H. Brain angiotensin and anxiety-related behavior: the transgenic rat TGR(ASrAOGEN)680. Brain Res 2005;1046: 145–56.

Voigt JP, Raasch W, Hortnagl H, Bader M, Fink H, Johren O. Changes in the brain serotonin satiety system in transgenic rats lacking brain angiotensinogen. J Neuroendocrinol 2008;20:182–7.

Voigt JP, Rex A, Sohr R, Fink H. Hippocampal 5-HT and NE release in the transgenic rat TGR(mREN2)27 related to behavior on the elevated plus maze. Eur Neuropsychopharmacol 1999;9:279–85.

Walther T, Voigt JP, Fukamizu A, Fink H, Bader M. Learning and anxiety in angiotensin-deficient mice. Behav Brain Res 1999;100:1–4.

Whitton P, Curzon G. Anxiogenic-like effect of infusing 1-(3-chlorophenyl) piperazine (mCPP) into the hippocampus. Psychopharmacology (Berl) 1990;100:138–40.

Wilson W, Voigt P, Bader M, Marsden CA, Fink H. Behaviour of the transgenic (mREN2) 27 rat. Brain Res 1996;729:1–9.