Effect of melatonin on exploration and anxiety in normotensive and hypertensive rats with high activity of renin-angiotensin system

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Submitted: February 7, 2007 *Accepted: March 5, 2007*

Key words: **hypertension; renin-angiotensin system; melatonin; exploration; anxiety**

Neuroendocrinol Lett 2007; **28**(3):295–301 NEL280307A11

Abstract

OBJECTIVES: The purpose of the study was to investigate effects of melatonin (MEL) on exploration and anxiety in normotensive Sprague-Dawley (SD) and hypertensive TGR(mREN2)27 (TGR) rats with high activity of renin–angiotensin system.
METHODS: Mature control (n=20) and hypertensive (n=20) rats were used. Half of each group was treated with MEL in drinking water (40 μg/ml) for 3 weeks. The influence of MEL on exploration was measured in the open field test (OF) and on anxiety in the elevated plus maze test (EPM).
RESULTS: Hypertensive TGR rats showed a lower level of ambulation ($p<0.05$) and higher level of urination ($p<0.001$) in OF. In EPM they spent more time in closed arms ($p<0.05$) and showed low frequency of total arm entries ($p<0.01$) than SD rats. MEL treated SD rats exhibited increased ambulation ($p<0.01$), sniffing ($p<0.05$) and decreased creeping ($p<0.05$) in OF than SD controls and did not exhibit differences in behaviour observed in EPM. MEL treated TGR rats exhibited a decrease in creeping, defecation and urination ($p<0.05$) in OF and spent less time in closed arms ($p<0.05$) and increased frequency of total arm entries ($p<0.05$) in EPM than untreated TGR animals.
CONCLUSION: Our results suggest that MEL decreased anxiety related behaviours in hypertensive rats with an up regulated renin–angiotensin system and stimulated active exploration of control animals.

ORIGINAL ARTICLE

INTRODUCTION

Hypertension represents one of the most frequent causes of mortality in adult population in developed countries. Disorders of renin-angiotensin system (RAS) play an important role in development of hypertension. This system is comprised of a variety of peptides including angiotensin II (ANG II), angiotensin III and angiotensin IV. They act through AT1, AT2 and AT4 receptors and control not only blood pressure but modulate also variety of brain functions related to behaviour [13,5,25,35]. RAS is implicated in genesis of stress related disorders, anxiety and depression [2,13,14] that frequently accompany hypertension [14].

The TGR rats are animals with up regulated expression of murine renin-2 gene in several peripheral tissues and the brain [3]. They offer a unique opportunity to study behavioural mechanisms involved in the hypertensive process. These rats show some behavioural changes which parallel those found in hypertensive patients – especially anxiogenic profile [36,33,34]. The additional mouse renin gene affects not only the renin-angiotensin system but results in a phase inversion of the daily rhythm in blood pressure while rhythms in locomotor activity and heart rate preserve the original phase [21]. Disturbances in a control of melatonin biosynthesis were reported in this strain of rats [9,21] and also in hypertensive human patients [37]. Melatonin modulates several behavioural traits [1,16,6,7] and can be useful in anti-anxiety therapy [22,4].

Therefore the aim of our experiment was to investigated effects of melatonin on some behavioural traits in normotensive SD and hypertensive TGR rats with high activity of renin–angiotensin system.

MATERIAL AND METHODS

Male TGR(mREN2)27 rats, carrying an additional mouse *Ren 2* gene and control Sprague-Dawley rats were obtained from the Institute of Clinical and Experimental Medicine (Prague, Czech Republic) and breeding pairs come from the Max-Delbrück-Center for Molecular Medicine (Berlin, Germany). The rats were housed by four in plastic cages (57×37×19 cm) with wood shavings. Temperature in the animal room was kept at 21±2 °C and 12h:12h light/dark cycle (lights from 07.00 h to 19.00 h) was installed. Rats had a free access to water and commercial food pellets in the course of the study. They were allowed to habituate to their housing conditions for 7 days before starting behavioural studies.

Control and hypertensive animals were divided into two groups – without treatment (SD, n=10; TGR, n=10) and with melatonin administration (SD MEL, n=10; TGR MEL, n=10). Melatonin was given in drinking water in concentration (40 μg/ml) and this solution was in disposal to animals only during the dark time. Details about solution preparation and administration are given elsewhere [29].

All groups of animals were tested at 11 weeks of age (361±9 g mean body weight). The behavioural tests took place between 13.00 h and 19.00 h and the data were registered in protocols by shorthand. The methods and procedures of the presented study have been approved by the local Ethics Committee at the Faculty of Natural Sciences, Comenius University Bratislava, Slovak Republic.

Behavioural tests

Two behavioural tests were used. We used an open field test [20] for measuring exploration behaviour and elevated plus-maze test [15] for measuring anxiety.

Open field test

Each rat was individually tested for 20 min in a wooden testing chamber size 72×34×39 cm (l × w × h). The bottom was demarcated into 32 squares (configuration 8×4 of equal dimensions) by painted dark lines. The testing chamber was cleaned after testing of each animal.

Totally 9 behavioural events were recorded during the observation: ambulation (animals move actively within the observation area), rearing (animals rear up on hind legs), self grooming (self grooming is predominantly demonstrated as washing), defecation, urination (marking), creeping (slow motion, with the body near the ground), sniffing (walls and ground), sniffing the air (inhaling with the head up, whiskers moving) and vocalizations (audible vocalizations, from soft peeps and squeaks to loud shrieks).

Ambulation was estimated on the basis of numbers of squares visited. Rearing, defecation, urination and vocalizations were expressed as frequencies. Creeping, sniffing and sniffing the air were expressed as presence/absence during one minute time interval. The self grooming was expressed as duration in seconds.

Elevated plus-maze

Each rat was individually tested for 5 min in an experimental arena – elevated plus-maze. The experimental arena was cleaned after testing of each animal. The maze was made of wood and consists of four arms in the shape of cross: two open arms (50×10 cm) and two arms of the same size with an open roof but enclosed by walls (40 cm high). The two open arms are opposite to each other and converge into a central platform (10×10 cm).

Rats were placed initially in the central platform facing a corner allowing an equal choice of entering an open or a closed arms. The data were analyzed for parameters of time spent in open and closed arms of maze and total arms entries (frequency).

Statistics

The significance of difference between groups without and with influence of melatonin was estimated by the non-parametrical Mann-Whitney test.

RESULTS

Open field test

TGR rats showed a lower level of ambulation activity ($p<0.01$, Figure 1-a) and higher level of urination ($p<0.001$, Figure 1-b) than SD rats in the open field test. In other behavioural activities we did not observe significant differences between non-treated hypertensive and normotensive rats (Figure 1-b, c, d).

In normotensive SD rats melatonin increased ambulation ($p<0.01$; Figure 2-a) and sniffing ($p<0.05$; Figure 2-c) while decreased creeping ($p<0.05$; Figure 2-c). Normotensive rats did not exhibit differences in rearing, defecation, urination, vocalization, sniffing the air and self grooming after melatonin treatment (Figure 2-b, c, d).

In hypertensive TGR rats melatonin had anxiolytic effects – it decreased defecation ($p<0.05$; Figure 3-b), urination ($p<0.05$; Figure 3-b) and creeping ($p<0.05$; Figure 3-c). In ambulation, rearing, vocalization, sniffing, sniffing the air and self grooming hypertensive rats did not show significant changes after melatonin administration (Figure 3-a, b, c, d).

Elevated plus-maze

TGR rats showed a more anxious profile than SD in the elevated plus-maze test. Hypertensive TGR rats spent more time in closed arms ($p<0.05$; Figure 4-a) and showed lower frequency of total arm entries ($p<0.01$; Figure 4-b) than the SD animals.

Melatonin did not affect recorded behavioural traits in normotensive SD rats (Figure 4-c,d) in EPM. In hypertensive TGR rats melatonin decreased the total time spent in closed arms ($p<0.05$; Figure 4-e) and increased total arm entries ($p<0.05$; Figure 4-f) in comparison with controls.

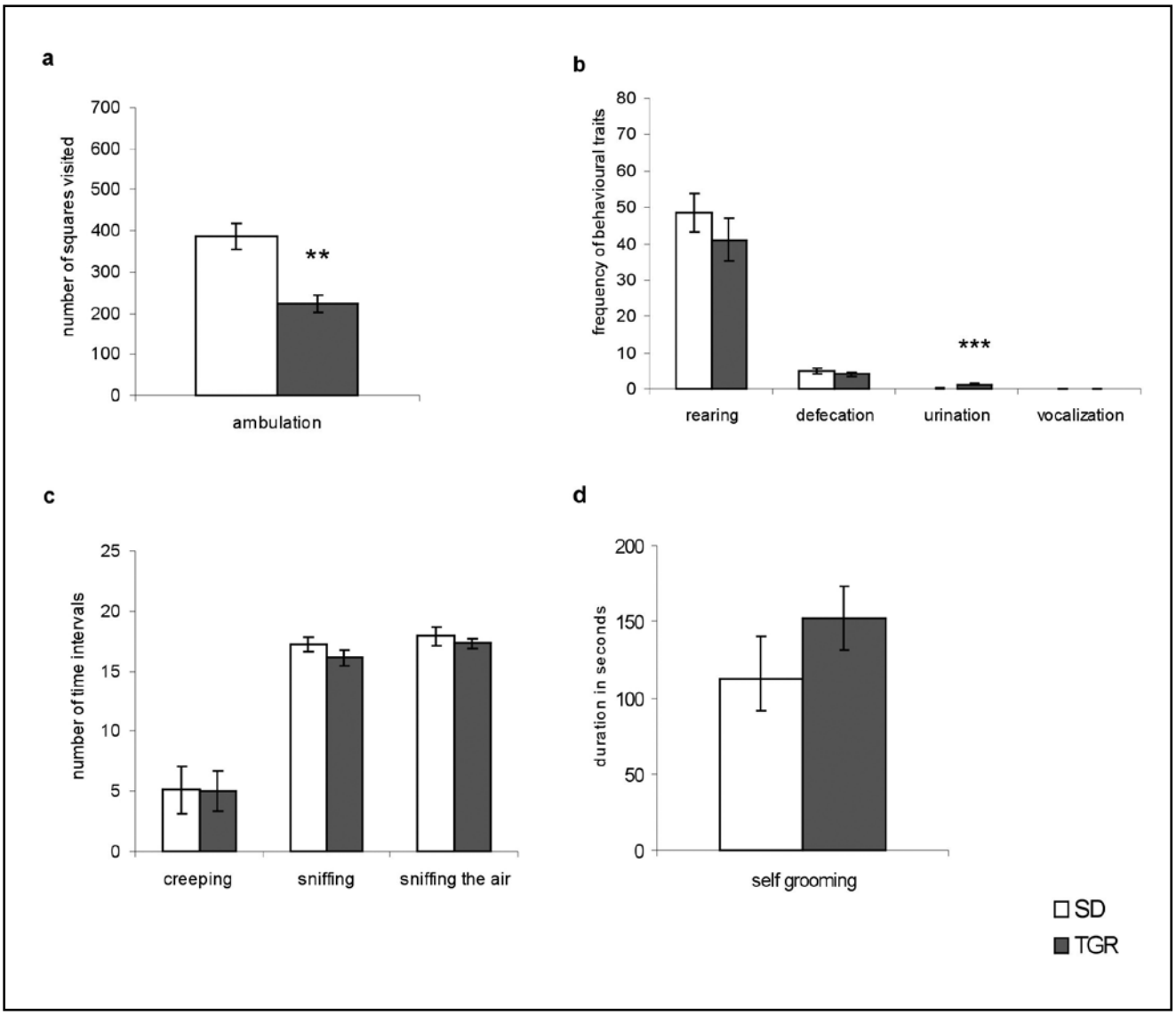

Figure 1 - a, b, c, d. Behaviour of the normotensive SD (n=10) and hypertensive TGR (n=10) rats in open field test. Data are given as means ± S.E.M. per 20 min. Asterisks indicate significant differences between SD and TGR group (** $p<0.01$, *** $p<0.001$).

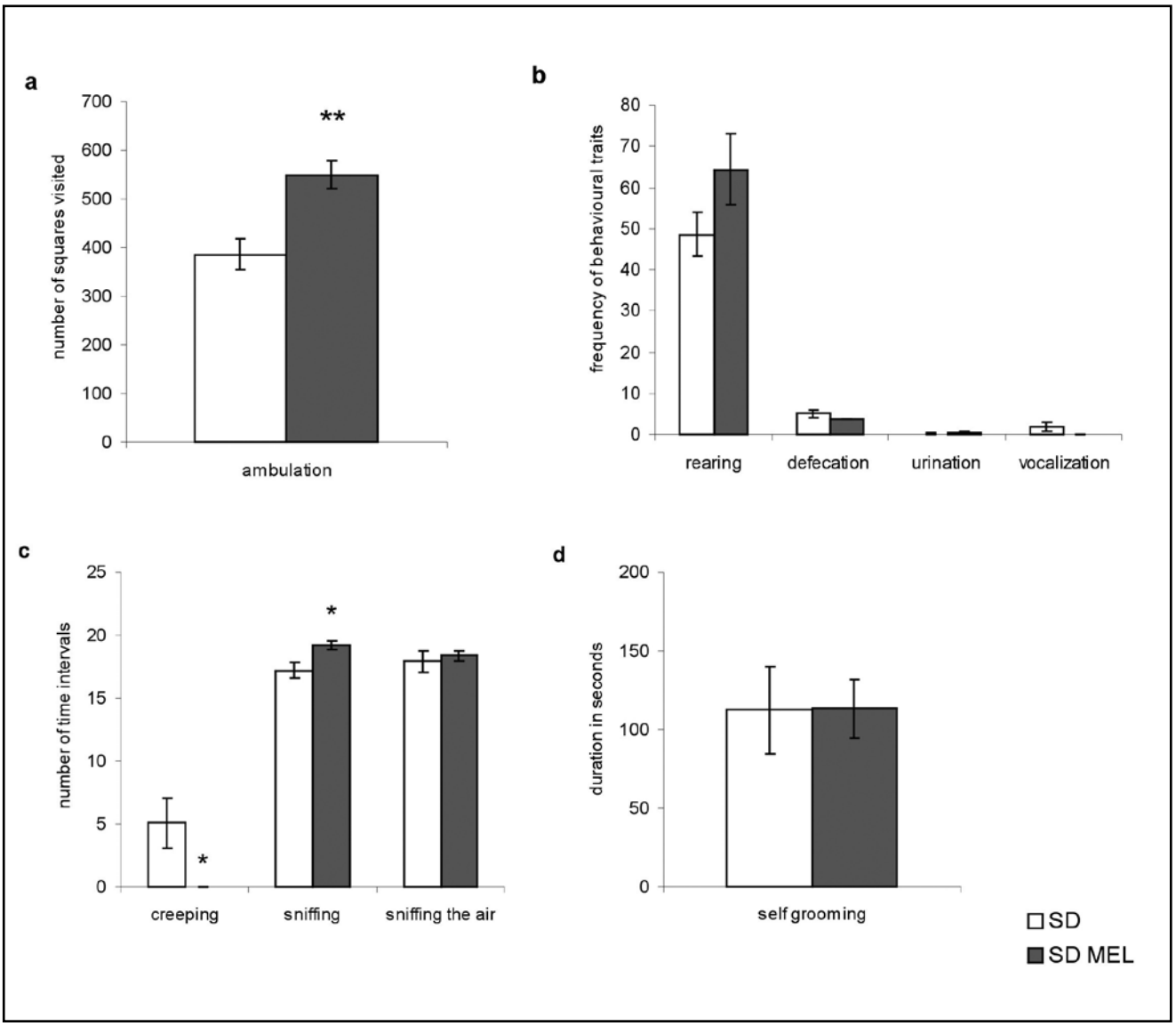

Figure 2 - a, b, c, d. Behaviour of the normotensive rats without (SD, n=10) and with melatonin (40 µg/ml in drinking water) treatment (SD MEL, n=10) in open field test. Data are given as means ± S.E.M. per 20 min. Asterisks indicate significant differences between SD and SD MEL group (* $p<0.05$, ** $p<0.01$).

DISCUSSION

In TGR rats Wilson *et al.* [36] observed a tendency to lower locomotor activity as compared to SD in 10 minute open field test. This tendency was proved significant in our experiment that included 20 minute lasting observations. The significance of differences can be related to longer time of our observation. More intensive urination of hypertensive rats can be related with up regulation of renin–angiotesin system. Angiotensin II induces drinking behaviour [12] and influences renal excretory function [28].

Hypertensive TGR rats showed a more pronounced anxiogenic profile than SD control rats. The differences in anxiety between SD and TGR rats recorded in the elevated plus-maze test in our experiment correspond with observations of other authors [36,33,34].

In both behavioural tests the TGR rats exhibited a lower locomotor activity and more anxiogenic behaviour than the original SD population. These differences do not simply reflect the hypertension of those rats. Spontaneous hypertensive rats (SHR) are more active and reactive [27,31,11] and show higher locomotor activity and exploratory rearing behaviour than control animals [32].

Behavioural differences between TGR and control rats can be related with modification of neurotransmitter metabolism of hypertensive animals [33]. Rat models of acute anxiety are associate with increased release of 5-hydroxytryptamin (5-HT) in the hippocampus [33,24]. Anxiogenic behaviour can reflect modification of brain

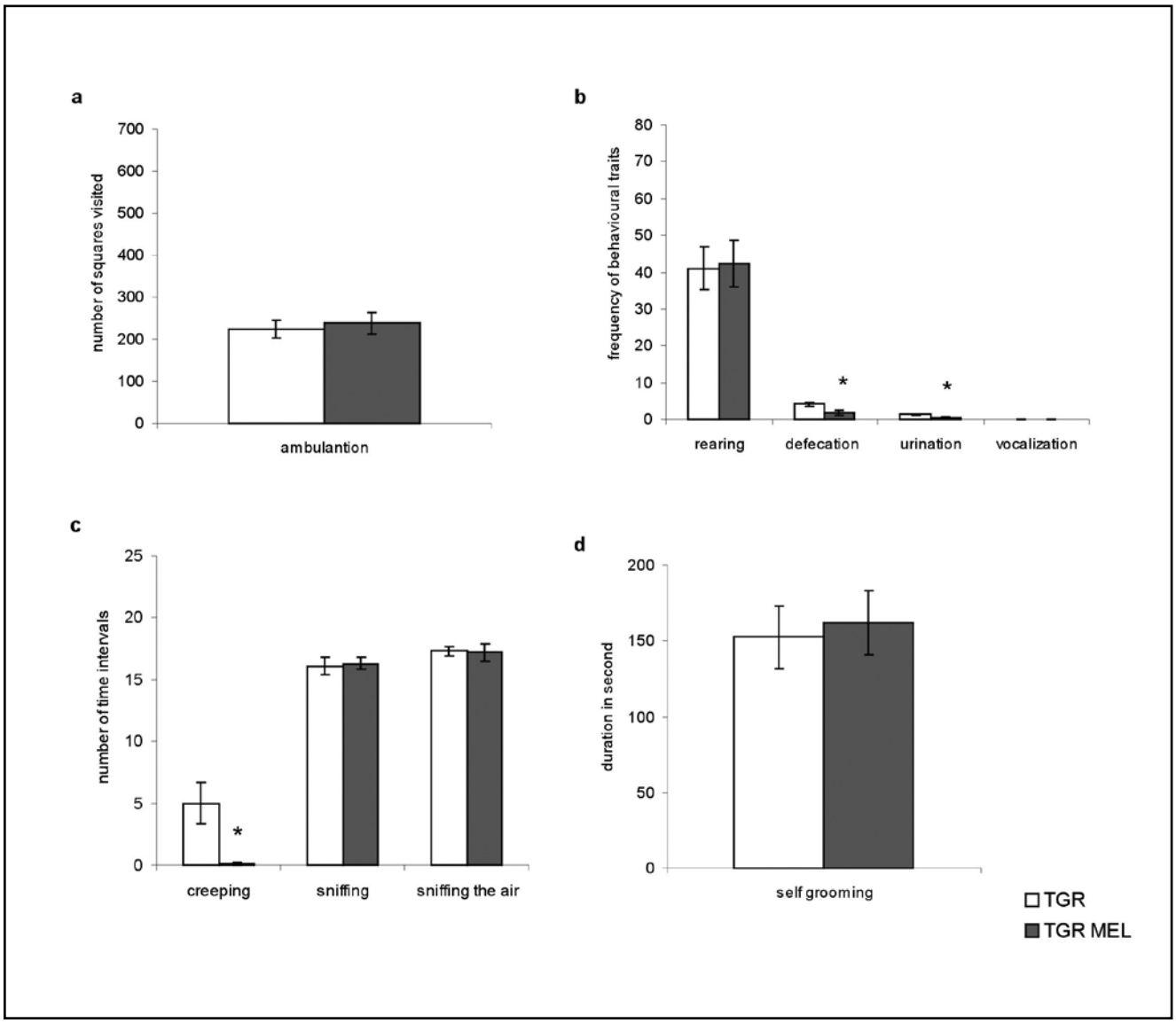

Figure 3 - a, b, c, d. Behaviour of the hypertensive rats without (TGR, n=10) and with melatonin (40 μg/ml in drinking water) treatment (TGR MEL, n=10) in open field test. Data are given as means per 20 min ± S.E.M. Asterisks indicate significant differences between TGR and TGR MEL group (* $p<0.05$).

neurotransmitters involved in the anxiety control, as GABA and serotonin. TGR rats have functionally changed serotonergic system. In both TGR and control SD rats an intrahippocampal 5-HT release increases upon exposure to the elevated plus maze as measured by microdialysis [33]. However, the subsequent decrease observed after returning animals into the home cage was significantly faster in the TGR than SD rats.

Anxiogenic profile of TGR rats characterised by increased brain angiotensin level [36] points to the increased emotionality caused by ANG II. Neuroanatomic sites of these anxiogenic effects of ANG II can be AT1 and AT2 receptors in the amygdala [30] and possibly AT2 receptors in the *locus coeruleus* [23]. Both structures are involved in producing emotional behaviours [30].

After MEL administration we observed stimulation effects of melatonin on locomotor activity of SD rats in the open-field test. According Brotto *et al.* [6] melatonin has a stimulatory effect on open field ambulatory behaviour in normotensive rat. Melatonin increased sniffing and decreased creeping in SD rats suggesting stress alleviated and anxiolytic effects of melatonin [22]. The anti-anxiogenic effect of melatonin was proved in our experiment in TGR rats showing inhibitory effects of the hormone on creeping, defecation and urination.

Melatonin produced different effects on behaviour of SHR and TGR rats in the open field test. SHR offspring prenatal treated with melatonin exhibited less total motor activity and rearing than controls in the open field test [19]. Our TGR animals did not show differences in loco-

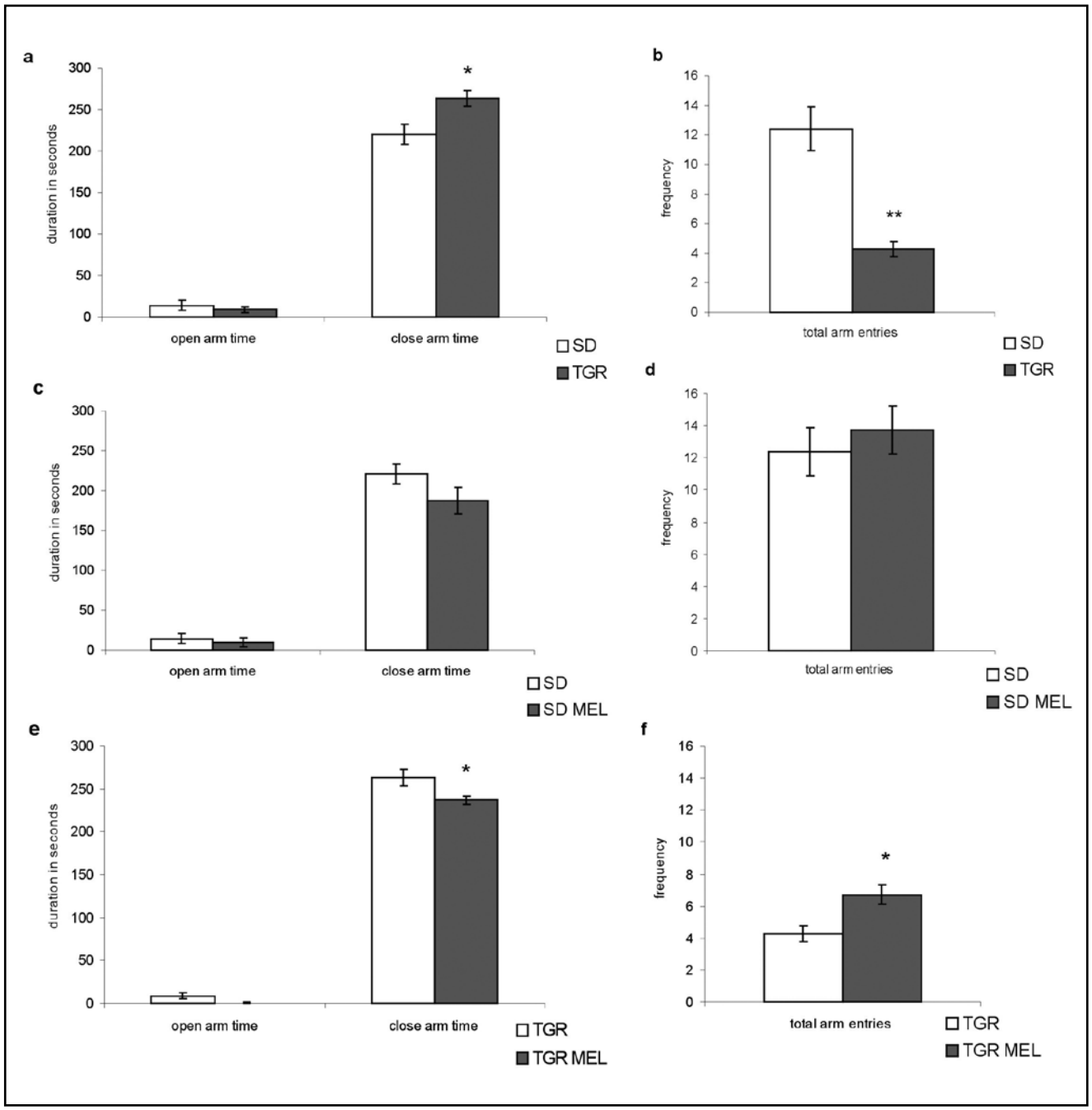

Figure 4 - a, b, c. Behaviour of the normotensive and hypertensive rats without (SD, n=10; TGR, n=10) and with (SD MEL, n=10; TGR MEL, n=10) melatonin (40 μg/ml in drinking water) treatment in the elevated plus-maze test. Data are given as means ± S.E.M. per 20 min. Asterisks indicate significant differences between TGR and TGR MEL group (* $p<0.05$, ** $p<0.01$).

motoric components of exploration but show decreased creeping, defecation and urination after melatonin administration. This finding suggests that differences in behaviour of TGR group after melatonin administration were not simply reflection of hypertension [36] but can reflect specific effects on the up regulated RAS.

Melatonin did not influence behaviour of normotensive SD rats in the elevated plus-maze test. On the other hand, melatonin treated TGR rats spent less time in closed arms and increased frequency of total arms entries. These findings suggest that melatonin reduced anxiety only in rats with developed hypertension. Since TGR rats exhibit an increased brain RAS activity melatonin interacts either directly with this system or normalizes changes at the brain level which were induced by RAS. Stimulatory effect of RAS on adrenergic neurotransmission is well known [10,17,8]. Hypotensive action of melatonin appears to be associated with an inhibition of

basal sympatho-adrenal tone and can be mediated partly by the blockade of postsynaptic alpha1-adrenergic receptor-induced inositol phosphate formation [18]. Alpha1-adrenoreceptor density was significantly increased in TGR rats compared with controls [26]. Therefore, our data in TGR rats can be explained by specific effect of melatonin on up regulated alfa1-adrenoreceptors but interactions with the brain RAS are also possible.

The different effect of melatonin on open field and elevated plus-maze behaviour in normotensive and hypertensive TGR rats demonstrates that a single additional gene is able to disturb the control of behaviour in a highly complex way.

ACKNOWLEDGEMENTS

This work was supported by Science and Technology Assistance Agency under the contract No. APVT-20-022704.

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