EFFECT OF SINGLE TREATMENT WITH THE ANTIHYPERTENSIVE DRUG EPLERENONE ON HORMONE LEVELS AND ANXIETY-LIKE BEHAVIOUR IN RATS

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Objective. The aim of the study was to investigate the effects of single peripheral administration of mineralocorticoid receptor (MR) antagonist eplerenone on: 1. the hypothalamic-pituitary-adrenocortical (HPA) axis; 2. the renin-angiotensin-aldosterone (RAA) system, 3. anxiety-like behaviour.

Methods. Male Wistar rats were injected subcutaneously with eplerenone (100 mg/kg body weight) or vehicle. Two hours following the injections, a half of the animals from each treatment group were decapitated to obtain blood for measurement of hormone levels. The other half of the animals was subjected to behavioural testing in the elevated plus-maze test. To provide comprehensive behavioural profile of eplerenone, standard spatiotemporal and ethologically derived measures of anxiety were assessed.

Results. Single treatment with eplerenone resulted in a significant increase in plasma aldosterone levels. Plasma concentrations of ACTH and corticosterone were not modified by eplerenone injection. Administration of eplerenone failed to alter classical spatiotemporal measures of anxiety (number of entries and time spent in the open arms). However, ethological parameters related to exploration (head dipping) and risk assessment behaviour (stretched attend postures) were significantly affected by eplerenone injection.

Conclusions. Single injection of eplerenone is followed by a reduction of ethological indices of anxiety-like behaviour and by an elevation of plasma aldosterone levels. Acute administration of eplerenone appears to affect the RAA system but not hormones of the HPA axis.

Key words: Eplerenone – Aldosterone - Plasma renin activity – Corticosterone – ACTH - Behaviour

In spite of overwhelming evidence that psychiatric disorders (mainly depression and anxiety) are frequently co-morbid with cardiovascular diseases (IACOVIDES and SIAMOULI 2008), the pathophysiological mechanisms underlying the association remain unclear. There are several factors common to both disorders, which are attractive candidates to account for the high incidence of co-occurrence of these disorders (JOHNSON and GRIPPO 2006).

Dysregulation of the renin-angiotensin-aldosterone (RAA) system belongs to the candidates supposed to be involved in both mentioned diseases. Components of the RAA system have been long recognized mainly for their role in the pathogenesis of cardiovascular diseases. In recent years, there has been a growing interest in the involvement of the RAA system in anxiety disorders. Central or peripheral administration of antihypertensive drugs

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acting on the renin-angiotensin system, including angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors, led to reduction of anxiety in both humans and animals (Kaiser et al. 1992; Srinivasan et al. 2003; Gard 2004; Saavedra et al. 2005, 2006). With regard to hormones related to anxiety, angiotensin II is known to activate the hypothalamic-pituitary-adrenocortical (HPA) axis. Studies using angiotensin II receptor antagonists showed blunted catecholamine responses during stress (Jezova *et al.* 1998).

Interestingly, aldosterone, the last component of the RAA system, has received little attention in relation to anxiety. We have recently shown that the elevation of circulating aldosterone results in increased anxiety-like behaviour (Hlavacova and Jezova 2008). Aldosterone is inducing its effects via mineralocorticoid receptors (MR). It has been observed that the blockade of MRs in the brain by antihypertensive drug spironolactone exerts anxiolytic effects (Bitran et al. 1998; Myers and Greenwood-Van Meerveld 2007). It should be noted that anxiolytic action of spironolactone observed in above mentioned studies was not attributed to aldosterone, as brain MRs bind preferentially glucocorticoids.

Spironolactone is a competitive MR antagonist that has been used clinically in the therapy of hypertension and congestive heart failure for a long time. However, spironolactone was found to be associated with progestational and antiandrogenic adverse effects, such as gynecomastia and impotence, due to its binding to other steroid receptors. Recently, a highly selective MR blocker eplerenone has been introduced and shown to have antihypertensive efficacy (Weinberger 2004) without mentioned side effects. To our knowledge, nothing is known on the effects of eplerenone on hormone levels and anxiety. The aim of this study was to investigate the effects of single peripheral administration of eplerenone on: 1. the hypothalamic-pituitary-adrenocortical (HPA) axis (ACTH, corticosterone), 2. the RAA system (aldosterone, plasma renin activity) and 3. anxiety-like behaviour measured in the elevated plus-maze test. To provide comprehensive behavioural profile of eplerenone, standard spatiotemporal measures of anxiety, as well as ethological parameters related to exploration and risk assessment were assessed.

Material and Methods

Animals. Experiments were performed in forty male Wistar rats (175-200 g) purchased from AnLab (Prague, Czech Republic). They were kept in temperature-controlled animal room (22 °C ± 2 °C) under constant 12:12 h light/dark cycle (lights on at 06.00 h). The animals were housed individually in standard cages with free access to rat chow and tap water. Before testing, the rats were handled daily for 14 days to reduce stress effects associated with experimental manipulations. Experimental procedures were approved by the Animal Health and Animal Welfare Division of the State Veterinary and Food Administration of the Slovak Republic.

Drugs. Eplerenone (Pfizer Inc., Groton, CT, USA) or vehicle was administered subcutaneously. The dose of eplerenone (100 mg/kg body weight) was chosen on the basis of previous studies (Martinez et al. 2002; Susis et al. 2005). To prepare injectable dosage form of eplerenone, ß-cyclodextrin solution was used to aid drug solubilization. Eplerenone was dissolved in 15 % solution of ß-cyclodextrin (Sigma, Germany) in 1 N NaOH by heating at 65 °C in a water bath shaker for 20 min. The pH was adjusted to 7.4 with 2 N HCl. Control rats were injected with vehicle only (15 % ß-cyclodextrin solution).

Experimental groups of animals. Rats were randomly assigned into two treatment groups (n=20 rats/group) and received a single subcutaneous injection of eplerenone or vehicle. Two hours following the injection of their respective treatment, half of the animals from each treatment group (n=10 rats/treatment group) were subjected to behavioural testing in the elevated plus-maze test. The other half of the animals (n=10 rats/treatment group), without behavioural testing, was quickly decapitated and the blood obtained was used for measurement of hormone levels.

Assessment of anxiety-like behaviour in the elevated plus-maze. The elevated plus-maze test was performed as described previously (Hlavacova and Jezova 2008) and was conducted during the light phase between 10.00 and 11.30 h in a room separated from that in which the animals were housed. Animals were subjected to the elevated plus-maze test two hours after administration of their respective treatment. Immediately after the injection of drugs, animals were transported (by the person they were accustomed with) in their home cages from the animal room to the testing room and left undisturbed for two hours before testing. Each rat was placed on the central platform of the maze facing an enclosed arm and allowed 5 min of free exploration. The maze was thoroughly cleaned with water and dried prior the next animal was introduced. All sessions were recorded by a video camera positioned 150 cm above the testing apparatus and the behaviour was analysed by a trained observer using a computer program for registration of behaviour.

Behaviour scored comprised of both conventional spatiotemporal and ethologically derived measures of anxiety. Spatiotemporal measures were the number of open and closed arm entries, total arm entries, and the amount of time spent in each section of the maze (including the central platform), expressed as a percentage of the total test duration. The number of entries and time spent in the open arms as well as the ratio of open to total arm entries (open/total x 100) were used as measures of the anxiety state of the animal. The measurement of total number of arm entries was taken as an indicator of general locomotor activity. An arm entry was defined as all four paws entering the arm and an arm exit was defined as two paws leaving them. Ethological measures included the frequency of stretched attend postures (SAP - exploratory posture in which the rat stretches forward and turns back to its original position without moving forward), head dipping (exploratory movement in which the animal's head is protruding over the side of the open arm and down towards the floor), end-arm exploration (number of times the rat reached the end of an open arm) and rearing. SAP and head dipping were differentiated as protected (occurring in the closed arms or central platform) or unprotected (occurring in the open arms). Increase in exploratory head dipping and reduction in SAP were considered to be an indicator of anxiolysis.

Blood sampling and radioimmunoassay. Samples of trunk blood obtained by decapitation were collected in cooled polyethylene tubes containing EDTA as anticoagulant and centrifuged immediately at 4 °C to separate plasma, which was then stored at -20 °C until analysed. Plasma aldosterone levels and plasma renin activity were measured by radioimmunoassay (RIA) using commercially available kits (RIA Aldosterone kit, Angiotensin I RIA kit, Immunotech, France). Plasma concentrations of ACTH were determined by RIA using double antibody technique to separate free and bound fractions as described previously (Jezova et al. 1987). Plasma corticosterone levels were analyzed by RIA after dichloromethane extraction of the steroids from 10 μg aliquots of plasma as described previously (Jezova et al. 1994). Antibodies for ACTH and corticosterone were kindly provided by Prof. G. B. Makara (Budapest, Hungary) and Prof. C. Oliver (Marseille, France), respectively.

Statistical evaluation. Statistical significance was determined by one-way analysis of variance (ANOVA) with factor treatment. Data are expressed as means ± SEM. The overall level of statistical significance was defined as $p<0.05$.

Results

The effects of single eplerenone treatment on hormone levels. The data on hormone concentrations are presented in Fig. 1. Statistical analysis by one-way ANOVA revealed that a single treatment with eplerenone resulted in a significant increase in plasma

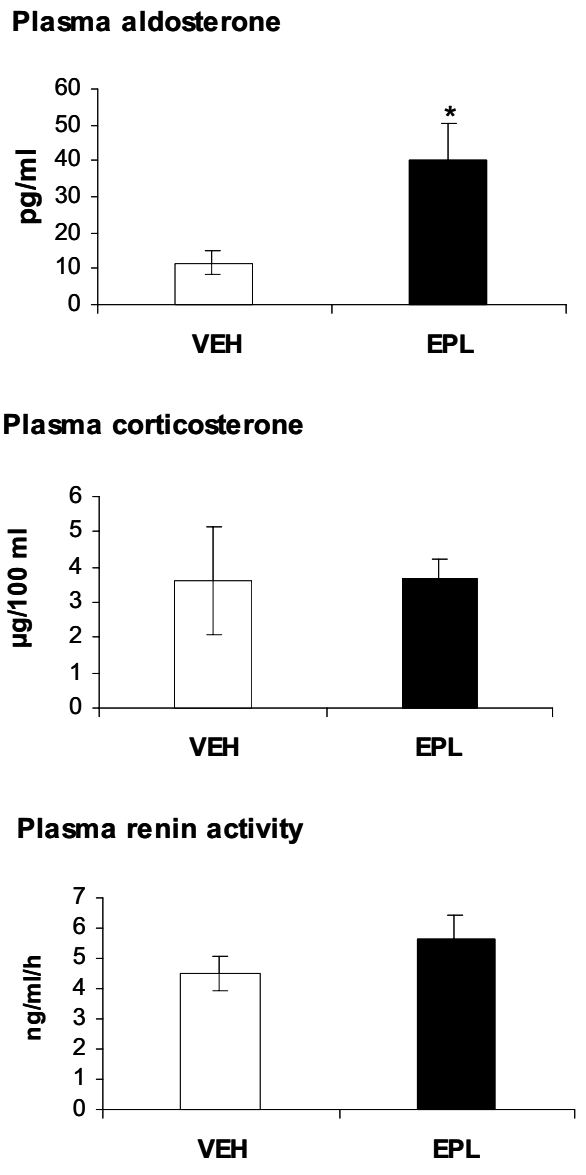

Fig 1 Effects of single treatment with eplerenone on plasma concentrations of aldosterone, corticosterone and plasma renin activity. Data are expressed as means ± SEM (n=10/ treatment group). Statistical significance: *p<0.05 vs. VEH (one-way ANOVA).

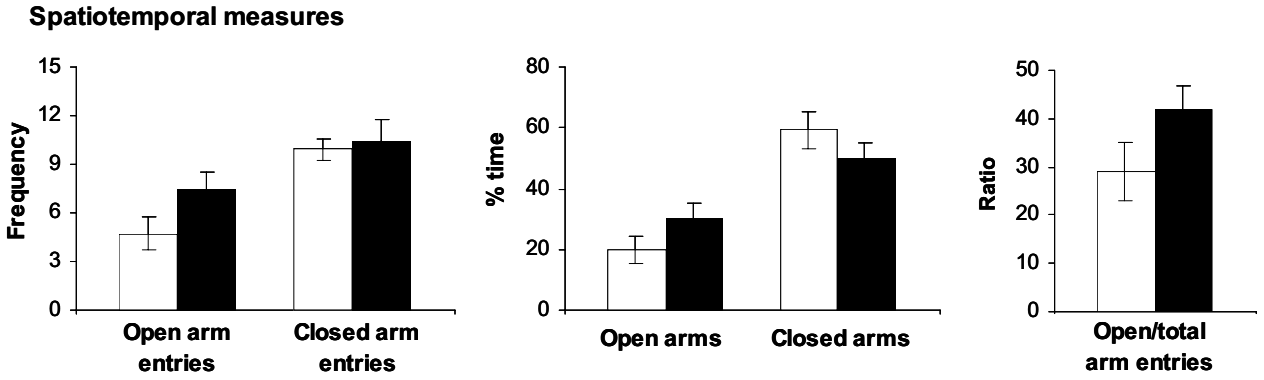

Fig 2 Effects of single treatment with eplerenone on classical spatiotemporal measures of anxiety. Data are expressed as means ± SEM (n=10/treatment group).

aldosterone levels ($F_{(1,16)}$=6.44, p<0.05). There was a trend toward increased plasma renin activity, however, the difference did not reach statistical significance ($F_{(1,16)}$=3.16, p=0.094). Plasma corticosterone concentrations were not affected by the single administration of eplerenone. Similarly, eplerenone failed to modify plasma concentrations of ACTH (data not shown).

The effects of single eplerenone treatment on anxiety-like behaviour. Classical spatiotemporal parameters of anxiety measured in the elevated plus-maze test (Fig. 2) were not significantly modified by single treatment with eplerenone. Although there was a tendency for increased number of open arm entries, the percentage of time spent in the open arms and the ratio of open to total arm entries in eplerenone-treated rats, the differences did not reach statistical significance. No treatment differences were observed in the number of closed as well as total arm entries, indicating that eplerenone had no impact on general locomotor activity. Neither the time spent in the closed arms, nor the time spent on the central platform was significantly affected by eplerenone treatment.

However, in contrast to spatiotemporal measures, ethological parameters related to exploration and risk assessment behaviour (Fig. 3) were significantly affected by a single eplerenone treatment. Statistical analysis revealed a significant reduction in the frequency of protected ($F_{(1,18)}$=6.44, p<0.05), unprotected ($F_{(1,18)}$=7.38, p<0.05) and total SAP ($F_{(1,18)}$=18.99, p<0.001) performed by rats in the eplerenone-treated group. Eplerenone-treated animals showed significantly increased frequency of the unprotected head dips ($F_{(1,18)}$=4.99, p<0.05). There was a marginally significant increase in the number of end arm exploration

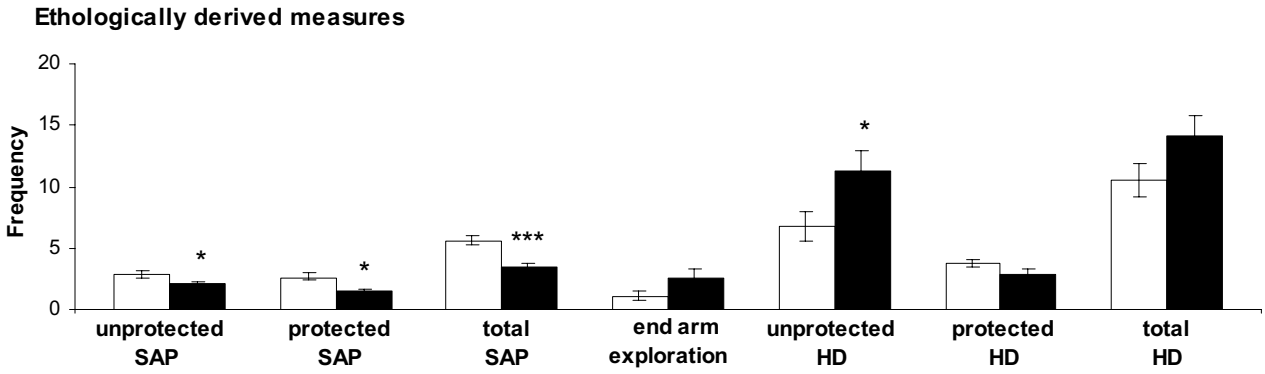

Fig 3 Effects of single treatment with eplerenone on ethologically derived measures of anxiety. Data are expressed as means ± SEM (n=10/treatment group). Statistical significance: ***p<0.001, *p<0.05 vs. VEH (one-way ANOVA). SAP – stretched attend postures, HD – head dipping

($F_{(1,18)}$=3.79, p=0.061) in rats treated with eplerenone. No significant treatment differences were observed in the frequency of rearing (vehicle-treated rats: 17.3 ± 1.4, eplerenone-treated rats: 17.0 ± 0.9).

Discussion

Present experiments revealed a stimulatory action of a single injection of the MR blocker eplerenone on hormones of the RAA system, but not on hormones of the HPA axis. Single treatment with eplerenone failed to modify classical parameters of anxiety-like behaviour on an elevated plus-maze, while it resulted in changes in ethologically derived measures of anxiety.

Increased concentrations of aldosterone in response to a single injection of eplerenone have not been described so far. Long time ago, it was reported that depletion of sodium caused by administration of spironolactone gave rise to increased secretion of aldosterone in the rat (Glaz 1971). More recent data mainly concern the repeated or chronic administration of MR antagonists (de Paula et al. 2003; Ortiz et al. 2007). Similarly, it was reported that repeated administration of eplerenone and other MR antagonists activate the renin-angiotensin system (Garthwaite and McMahon, 2004). In the present study only a trend toward the increase in plasma renin activity following a single administration of eplerenone was observed.

Administration of eplerenone apparently did not induce non-specific stress effects, as there were no changes in plasma corticosterone and ACTH two hours following eplerenone injection. The results of previous studies showed either an enhancement or no changes in the activity of the HPA axis after both central and peripheral administration of other MR antagonists in humans and rodents (Cole et al. 2000; Calvo and Volosin 2001; Pace and Spencer 2005; Otte et al. 2007). No data on the effect of eplerenone on HPA axis activity are available. The first administration of various drugs was found to induce stress effects manifested by an activation of the HPA axis (Jezova et al. 1995; Moncek et al. 2003). Present data might suggest that no drug-induced stress effects would appear at the onset of antihypertensive treatment with eplerenone under clinical setting.

Other MR antagonists, such as spironolactone and RU28318, were shown to induce behavioural effects, namely to reduce anxiety-like behaviour in several experimental tests (Korte et al. 1995; Smythe et al. 1998; Bitran et al. 1998; Calvo and Volosin 2001). The present experiments using the elevated plus-maze test failed to reveal any effect of eplerenone injection on frequency of entries and time spent in the open arms of the maze. Nevertheless, a mild anxiolytic action of acute treatment with eplerenone may be suggested on the basis of observed modulation of ethological parameters related to exploration (head dipping) and risk assessment (SAP). To our knowledge, no data on the effects of other MR antagonists on ethological parameters as evaluated in the present study have been reported so far. Risk assessment behaviour represents a complex behavioural pattern connected with decision making and the approach-avoid conflict. Several anxiolytic drugs only affected risk assessment without changing conventional anxiety parameters, and many drugs only acted on spatiotemporal variables (Rodgers and Johnson 1995; Mikics et al. 2005). We have previously shown that chronic treatment with aldosterone affected ethological variables measured in the elevated plus-maze in a manner opposite to that induced by aldosterone blocker eplerenone (Hlavacova and Jezova 2008).

Presented results are in agreement with the findings obtained by blockade of the renin-angiotensin system using other approaches. The finding of a mild anxiolytic action of eplerenone represent additional evidence on the modulation of anxiety-like behaviour by antihypertensive drugs acting on the renin-angiotensin system, including angiotensin II receptor antagonists and angiotensin-converting enzyme inhibitors. Thus, single injection of eplerenone is followed by a reduction of ethological indices of anxiety-like behaviour and by an elevation of plasma aldosterone levels. Further studies are needed to confirm potential anxiolytic properties of eplerenone. Acute treatment with eplerenone is not associated with an activation of the HPA axis.

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