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Intracerebroventricular administration of a glucocorticoid receptor antagonist enhances the cardiovascular responses to brief restraint stress

Saskia A.B.E. Van Acker, Marc F.J. Fluttert, Rosana M. Sibug, E. Ronald De Kloet *

Division of Medical Pharmacology, Leiden / Amsterdam Center for Drug Research, Leiden University Medical Center, P.O. Box 9503, 2300 RA Leiden, The Netherlands

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Abstract

Intracerebroventricular (i.c.v.) administration of the glucocorticoid receptor antagonist 17β-hydroxy-11β(4-dimethylaminophenyl)17α-(1-propynyl)estra-4,9dien-3one (RU38486) in conscious rats slowly increased systolic blood pressure as assessed with the indirect tail cuff method. However, direct measurement of blood pressure in freely moving rats did not reveal changes in blood pressure after i.c.v. injection of this antagonist either in the light or in the dark phase. In the present study, the hypothesis is tested that aspects of the tail cuff procedure, involving heat (30 min, 32 °C) and brief restraint stress, are necessary conditions to detect the glucocorticoid receptor-mediated cardiovascular effect. Freely moving rats equipped with a telemetric transmitter to directly measure heart rate and blood pressure were injected i.c.v. with either the glucocorticoid receptor or the mineralocorticoid receptor antagonist and were either left undisturbed for 24 h, or were subjected to the tail cuff procedure at 1.5, 6.5 and 23.5 h after injection. Then after 30-min warming and during brief restraint, blood pressure and heart rate showed a rapid increase. The mineralocorticoid receptor antagonist administered i.c.v. did not affect these stress-induced increases in cardiovascular responses. The glucocorticoid receptor antagonist i.c.v. significantly increased the heart rate and pressor response at 24 h. In the undisturbed rats, neither basal heart rate nor blood pressure were affected by either antagonist during the circadian cycle. In conclusion, the blockade of central glucocorticoid receptor causes a long-lasting facilitation of the stress-induced pressor and heart rate response, which does not require a 2-week training to the condition of heat and stress.

Keywords: Glucocorticoid receptor; Mineralocorticoid receptor; Restraint stress; Brain; Blood pressure

1. Introduction

Glucocorticoids and mineralocorticoids exert potent influences on arterial pressure (Yagil and Krakoff, 1988). In addition, the steroid hormones, in particular the mineralocorticoids, have been reported to act on central mechanisms underlying fluid homeostasis and cardiovascular functions (Brody et al., 1980; Gómez-Sánchez, 1991). These actions exerted by the mineralocorticoids and glucocorticoids take place via activation of mineralocorticoid and glucocorticoid receptors.

Previously, it was described that the glucocorticoid receptor antagonist 17β-hydroxy-11β(4-dimethylaminophenyl)17α-(1-propynyl)estra-4,9dien-3one (RU38486), when administered i.c.v., increased systolic blood pressure (SBP) as measured by the tail cuff procedure under conditions that the mineralocorticoid receptor antagonist 3,3-oxo-7propyl-17-hydroxy-androstan-4-en-17yl-propionic acid lactone (RU28318) decreased systolic blood pressure (Van den Berg et al., 1990). Subsequently, it was shown in cannulated rats that the mineralocorticoid receptor antagonist, which decreased the systolic blood pressure, actually did not affect basal blood pressure. Rather, it was the pressor response to the tail cuff procedure involving 2-week training to daily exposure for 30 min at 32 °C and 1.5-min restraint stress, which was significantly attenuated by RU28318 i.c.v.. Without the 2-week training period, the mineralocorticoid receptor antagonist was not effective (Van den Berg et al., 1994). The findings agree with the view that the effects of corticosteroids are conditional. This implies that the molecular and cellular effects exerted by the steroids do not alter basal parameters but only become detectable after stress (De Kloet et al., 1998; Joëls and De Kloet, 1992).

* Corresponding author. Tel.: +31-71-527-6210; fax: +31-71-527-6292.

E-mail address: e.kloet@lacdr.leidenuniv.nl (E.R. De Kloet).

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In the present study, we test the hypothesis that also the increase in systolic blood pressure after RU38486 i.c.v. might be due to the heat and stress exposure during the tail cuff procedure. For this purpose, we measured with telemetry at 2, 7 and 24 h after administration of the glucocorticoid receptor antagonist i.c.v. the effect of 30-min warming and brief restraint as features of the tail cuff procedure to evoke a heart rate and blood pressure response. The results were compared with an administration of the mineralocorticoid RU28318 i.c.v., while telemetric blood pressure and heart rate measurements after administration of both antagonists in freely moving undisturbed rats served as control experiment.

2. Methods

2.1. Animals and surgery

Male Wistar rats weighing 180–200 g (Charles River) were acclimated for 1 week. They were equipped with a TL11M2-C50-PXT telemetric transmitter (Data Sciences Intl., St. Paul, MN, USA), which allows simultaneous measurement of systolic, diastolic and mean blood pressure, heart rate and locomotor activity. The transmitter was implanted intraperitoneally under anesthesia with a mixture of N_2O/O_2 (2:1) and isoflurane. Through a midline abdominal incision, the abdominal aorta was exposed and clamped off. A small hole was punctured in the aorta wall just above the bifurcation and the flexible tip of the transmitter cannula was inserted and fixed in place with a drop of tissue glue (Histoacryl). The body of the transmitter was sutured to the inside abdominal wall and all incisions were suture closed. At the same time, an i.c.v. cannula was implanted into the right lateral ventricle according to Brakkee et al. (1979). After surgery, the rats were given a subcutaneous injection with Temgesic as analgesic and allowed to recover in a warm environment until they were completely awake. They were housed individually in macrolon cages under standard conditions, with a room temperature of 21–22 °C and lights on from 7:45 to 19:45 h. All animals received food and tap water ad libitum.

Experiments were performed at least 10 days after surgery, at which time the physiological parameters measured (heart rate and mean arterial pressure) have returned to their normal circadian rhythm, which was then recorded for 24 or 48 h. Animal care procedures were conducted in accordance with the European Communities Council Directive 86/609/EEC. The protocols were approved by the Animal Care Committee of the Faculty of Medicine, University of Leiden, the Netherlands.

2.2. Steroids

Steroids for i.c.v. administration were dissolved in vehicle (2% ethanol in saline). The glucocorticoid receptor antagonist RU38486 and the mineralocorticoid receptor antagonist RU28318 were kindly donated by Roussel-UCLAF (Romainville, France).

2.3. Experiment 1

The rats were handled during the last 5 days of the recovery period. At least 1 day before the actual experiment, a restrainer was left in the home cage of the rat in order to get the animal accustomed to the apparatus. At the same time, sampling of physiological parameters was started to obtain basal values of each rat. Every 5 min, samples were taken using the Data Sciences data acquisition system (Dataquest Labpro, version 3.01, Data Sciences Intl.).

On Day 1 of the experiment, the rats were injected i.c.v. between 9:00 and 9:30 h with either 100 ng RU28318 (mineralocorticoid receptor antagonist) or 100 ng RU38486 (glucocorticoid receptor antagonist) or vehicle (2% ethanol–saline) over a period of 30 s in a maximum volume of 2 μl 1.5, 6.5 and 23.5 h after i.c.v. injection the rats were exposed to heat (30 min, 32 °C) and restraint stress (1.5 min + slight pinching at the base of the tail to mimic the tail cuff blood pressure measurement procedure). During the heat and stress procedure, heart rate and blood pressure were sampled every minute.

2.4. Experiment 2

In order to determine the direct effects of RU28318 and RU38486 on heart rate and blood pressure, a second group of rats was used. These rats were also injected i.c.v. between 9:00 and 9:30 h with 100 ng RU28318 (mineralocorticoid receptor antagonist), 100 ng RU38486 (glucocorticoid receptor antagonist) or vehicle (2% ethanol–saline) over a period of 30 s in a maximum volume of 2 μl. However, these rats were not submitted to the heat and stress procedure, but remained undisturbed in their home cage.

2.5. Statistics

Data are expressed as the means of the absolute values (±S.E.M.). Results were compared with the analysis of variance (ANOVA) and Fisher's least significant differ-

Table 1
Basal values of the three different groups 1 day before treatment

Treatment (*n*)	Mean light	
	Heart rate	Blood pressure
Vehicle (4)	362 ± 13	96.9 ± 4.8
RU28318 (6)	383 ± 7	96.7 ± 3.5
RU38486 (7)	391 ± 7	100.5 ± 2.2

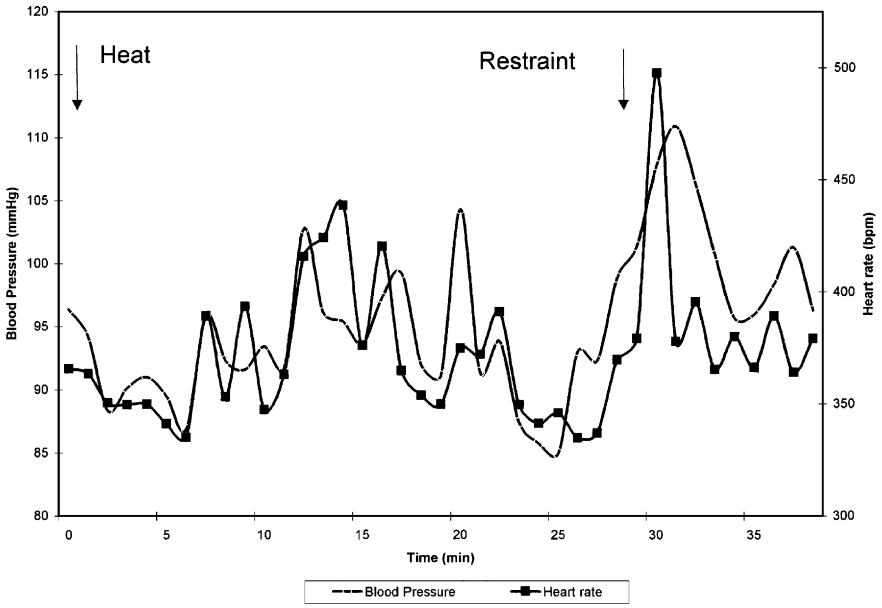

Fig. 1. Typical example of the changes in blood pressure and heart rate during a heat and stress trial. Beginning of heat (30 min, 32 °C) and beginning of restraint (1.5 min, immediately after heat) are indicated by arrows. Data are sampled every minute.

ence (LSD) post hoc test when ANOVA indicated significant differences between groups. Differences were considered significant at $p < 0.05$. For the circadian rhythm experiments, data were averaged over the light and the dark period (mean light and mean dark) per individual rat. Data after treatment were compared with pretreatment data from the same rat in a paired *t*-test. Due to the i.c.v. injection in combination with the heat and stress procedure, some rats developed a very high body temperature (> 39.0 °C), which clearly affected the response to the heat and stress procedure, regardless of the treatment. These rats were excluded from the statistical evaluation.

3. Results

3.1. Basal parameters

In Table 1, the basal values are depicted for all treatment groups from experiment 1 (heat and stress protocol)

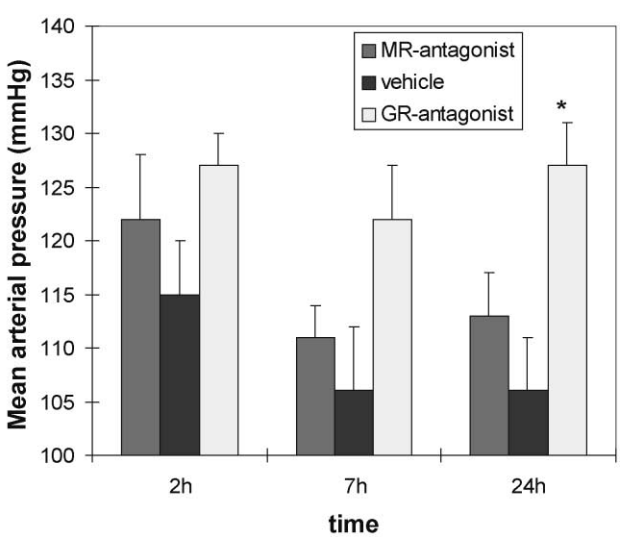

Fig. 2. Maximum increase in blood pressure during restraint at 2, 7 and 24 h after i.c.v. injection of 100 ng RU28318, 100 ng RU38486 or vehicle. Values are expressed as means of four (vehicle) to seven (RU38486) rats per group.

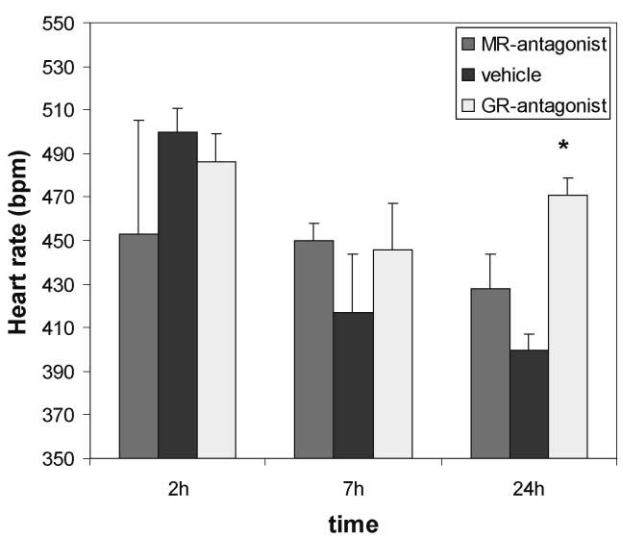

Fig. 3. Maximum increase in heart rate during restraint at 2, 7 and 24 h after i.c.v. injection of 100 ng RU28318, 100 ng RU38486 or vehicle. Values are expressed as means of four (vehicle), six (RU28318) and seven (RU38486) rats per group.

1 day before the i.c.v. injection. Under basal conditions, which is at rest in their home cage, the experimental groups did not show differences in heart rate, mean arterial pressure and locomotor activity.

3.2. Heat and stress

It was reported previously by our group that a 10–100-ng dose of RU38486 i.c.v. increased the systolic blood pressure measured with the indirect tail cuff procedure. The conditions of this tail cuff method include 30-min warming at an ambient temperature of 32 °C followed by a 1.5-min brief restraint of the rat. If these conditions are applied, telemetric blood pressure measurement revealed a pronounced pressor response to the restraint stress (Fig. 1). The glucocorticoid receptor antagonist administered i.c.v. significantly increased the stress-induced pressor response at 24 h after injection (Fig. 2), if compared with the saline injected control animals. No significant effects were observed at 2 and 7 h after injection.

The same effects were observed for heart rate. During restraint stress the heart rate showed an increase, which was significantly larger than in vehicle-treated rats 24 h after administration of the glucocorticoid receptor antagonist (Fig. 3). In addition, for heart rate the peaks after restraint at 2 and 7 h were not affected.

In Figs. 2 and 3, it is shown that i.c.v. administration of the mineralocorticoid receptor antagonist was without effect on the heart rate and blood pressure responses to restraint at any time-point. This was to be expected since with direct blood pressure measurements in cannulated rats, it was shown earlier that the pressor response to restraint becomes mineralocorticoid receptor dependent only in rats, which are trained to the procedure for 14 days.

3.3. Circadian rhythm

As is depicted in Table 2, both the mineralocorticoid receptor and the glucocorticoid receptor antagonist did not affect the basal heart rate and blood pressure of the rats. The effect of the treatment was determined with each rat as its own control in order to account for differences in basal values between treatment groups. In the vehicle group, no differences were observed before and after i.c.v. injection for either parameter (data not shown).

Table 2
Influence of RU28318 and RU38486 i.c.v. on circadian rhythm of heart rate and blood pressure

Treatment (day)	Heart rate		Blood pressure	
	Mean light	Mean dark	Mean light	Mean dark
Vehicle (0)	396 ± 22	439 ± 10	109.7 ± 1.3	111.3 ± 3.6
Day 1	386 ± 10	428 ± 11	106.9 ± 3.0	109.4 ± 4.7
RU28318 (0)	376 ± 10	412 ± 13	94.3 ± 4.9	95.3 ± 5.1
Day 1	376 ± 10	402 ± 13	95.4 ± 5.3	97.4 ± 3.9
RU38486 (0)	391 ± 11	427 ± 11	103.8 ± 2.0	108.8 ± 2.1
Day 1	404 ± 14	424 ± 13	107.7 ± 2.2	109.6 ± 2.4

Data are expressed as means ± S.E.M. of 4–5 rats per group.

4. Discussion

Previously, we have reported that in conscious rats the systolic blood pressure decreased following the i.c.v. administration of the mineralocorticoid receptor antagonist RU28318. Furthermore, it was shown that the i.c.v. administration of the glucocorticoid receptor antagonist RU38486 increased the systolic blood pressure. These observations were based on blood pressure measurements with the indirect tail cuff method. More recently, it was shown that using direct blood pressure measurements in conscious cannulated rats, RU28318 did not affect basal blood pressure and heart rate. The systolic blood pressure-decreasing effect of RU28318 was found to depend critically on the 2-week training period for the tail cuff procedure, which included daily exposure for 30 min to heat followed by brief restraint of 1.5 min (Van den Berg et al., 1994).

In the present investigation, freely moving rats equipped with a telemetric transmitter were used. This method enables long-term measurement of heart rate and blood pressure without disturbing the rats. No effects were observed for the glucocorticoid receptor antagonist on basal heart rate and blood pressure during day or night. In addition, the previous results (Van den Berg et al., 1994) showing a lack of effect of the mineralocorticoid receptor antagonist in untrained naive rats were confirmed and extended to the dark phase. In untrained naive but handled rats, RU38486 i.c.v. caused a slow increase in the heart rate and blood pressure response to heat and restraint, which became significantly different from controls at 24 h. Upon the repeated exposure to heat and stress at the three time points, the pressor response remains constant in glucocorticoid receptor antagonist-injected rats, whereas there is a clear decrease in the pressor response in the saline-injected controls. This result shows that already the tail cuff procedure without training is a sufficient condition to reveal the RU38486 effect on heart rate and blood pressure responses. The mineralocorticoid receptor antagonist did not affect pressor and heart rate responses at either time-point, confirming that indeed the complete tail cuff procedure including 14 days of training is essential to detect the pressor decreasing effect of RU28318.

One explanation for the results may be the rise in circulating corticosterone levels after inhibition of negative feedback in the hypothalamic–pituitary–adrenal axis after i.c.v. administration of the glucocorticoid antagonist. Rats given an acute i.c.v. injection with glucocorticoid receptor antagonist show increased peak levels of the adrenocorticotropic hormone (ACTH) (twofold increase) and cortico-

sterone (50% increase) upon exposure to the mild stress of a novel environment 24 h later (Van Haarst et al., 1996, 1997), while the basal a.m. levels of the hormones were not affected. Moreover, the circadian rise of p.m. corticosterone was enhanced in the glucocorticoid antagonist-treated animals within this 24-h period (Van Haarst et al., 1996). During exposure to heat, corticosterone levels rise to about 13 μg/dl in vehicle-treated rats (Van den Berg et al., 1994). It is, therefore, likely that the inhibition of negative feedback on the hypothalamic–pituitary–adrenal axis exerted by RU38486 not only causes an increased corticosterone level during exposure to heat in the present study, but also has produced a state of hypercorticism in the period preceding the 24-h measurement as well. It is known that systemic high circulating levels of corticosterone can influence blood pressure via its effects on the vasculature, increasing sensitivity to pressor agents such as norepinephrine and angiotensin II (Grünfeld and Eloy, 1987;Ullian, 1999).

Alternatively, we have recently shown that the glucocorticoid receptor appears to be involved in the regulation of centrally elicited pressor responses, e.g. by angiotensin II (Van Acker et al., unpublished observation). Removal of the adrenals (ADX) resulted in the loss of a pressor response to angiotensin II and vasopressin administered i.c.v., but only the response to angiotensin II could be restored when the rats were substituted with high circulating corticosterone levels, saturating with mineralo- and glucocorticoid receptors. In correspondence with this observation, one would expect that blocking the central glucocorticoid receptor by i.c.v. injection of RU38486 would decrease the pressor responsiveness if the steroid effect would be exerted solely in the brain. However, in the present investigation we have observed an increased pressor response to heat and restraint stress after i.c.v. RU38486. Therefore, it could be that the peripheral high corticosterone levels rather than the blockade of the central glucocorticoid receptor promotes the pressor and heart rate response to the tail cuff procedure.

Administration of the mineralocorticoid receptor antagonist i.c.v. in the a.m. or p.m. phase of the circadian cycle elevated basal corticosterone levels 1 h later (Bradbury et al., 1994; Oitzl et al., 1995; Ratka et al., 1989). Moreover, the mineralocorticoid receptor antagonist i.c.v. attenuated the pressor response to heat and stress 7 h later, provided the rats experienced 2 weeks training to these conditions. This is in accordance with the observation that here we did not observe any effect of the mineralocorticoid receptor antagonist on any time-point in the absence of training.

In conclusion, the present study has revealed that the central glucocorticoid receptor is involved in the enhanced pressor response to the heat and restraint stress procedure. This enhanced response may be caused by changes in circulating corticosterone levels, due to interference with negative feedback to the hypothalamic–pituitary–adrenal axis after glucocorticoid receptor blockade, rather than be a direct consequence of central glucocorticoid receptor blockade.

Acknowledgements

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