Research in Veterinary Science 97 (2014) 204–210

Contents lists available at ScienceDirect

Research in Veterinary Science

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

Accumulation of radioactivity after repeated infusion of ^{3}H-adrenaline and ^{3}H-noradrenaline in the rat as a model animal

M. Lepschy [a,*], T. Filip [b], R.G. Palme [a]

[a] Institute of Medical Biochemistry, Department of Biomedical Sciences, University of Veterinary Medicine, A-1210 Vienna, Austria
[b] AIT Institute of Technology GmbH, Health & Environment Department, Biomedical Systems, A-2444 Seibersdorf, Austria

ARTICLE INFO

Article history:
Received 19 March 2013
Accepted 25 July 2014

Keywords:
Catecholamines
Adducts
Accumulation
Rat
Hair

ABSTRACT

Besides enzymatic inactivation, catecholamines bind non-enzymatically and irreversible to proteins. The physiological impact of these catecholamine adducts is still unclear. We therefore collected basic data about the distribution of catecholamine adducts in the rat after repeated intravenous administration of ^{3}H-adrenaline and ^{3}H-noradrenaline.

In all animals radioactivity in blood increased until the last injection on Day 7 and decreased then slowly close to background values (plasma) or remained higher (erythrocytes). In all sampled tissues radioactivity could be found, but only in hair high amounts remained present even after 3 weeks. Half-life of rat serum albumin loaded with ^{3}H-adrenaline or ^{3}H-noradrenaline was not altered.

This study provides basic knowledge about the distribution of catecholamines or their adducts, but physiological effects could not be demonstrated. However, for the first time deposition and accumulation of catecholamines (adducts) in the hair could be proven, suggesting that hair might be used for evaluating long term stress.

1. Introduction

Challenging and/or ominous situations lead to the activation of the sympatho-adrenal axis and ultimately to the release of catecholamines (CA). Therefore CA are frontline hormones that enable an organism to overcome these stressful situations. Their effects have only very limited duration, as CA are quickly degraded by various enzymes (see Eisenhofer et al., 2004 for a review) and rapidly excreted (El-Bahr et al., 2005; Lepschy et al., 2008; McEwen and Wingfield, 2003; Moberg, 2000; Möstl and Palme, 2002; Palme et al., 2005). But during the past 35 years it has become evident that non-enzymatic binding of CA to serum proteins in mammals and birds represents a further mechanism involved in the transport and/or inactivation of CA. Non-enzymatic binding has been found in several species such as rats (Powis, 1975a), humans (Danon and Sapira, 1972a; May et al., 1974; Mirkin et al., 1966; Powis, 1975b; Sager et al., 1987), dogs (Teixeira et al., 1979), sheep (El-Bahr et al., 2006) and domestic fowl (Powis, 1975a). It has been suggested that binding to plasma proteins (e.g. serum albumin (Danon and Sapira, 1972a; Powis, 1975b; Sager et al., 1987; Teixeira et al., 1979)) occurs due to an interaction with the phenolic hydroxy groups (Danon and Sapira, 1972a), the amino group (Teixeira et al., 1979; Zia et al., 1971), or both (May et al., 1974). Further in *vitro* investigations revealed that binding to blood proteins occurs very rapidly and seems to be irreversible. Several authors produced similar results and showed that almost no variation is attributable to the time of contact of adrenaline (A; Teixeira et al., 1979) or noradrenaline (NA; Boomsma et al., 1991; Branco et al., 1974; Danon and Sapira, 1972a) with plasma. In addition, Powis (1975b) found NA to be highly resistant to removal from proteins by procedures such as equilibrium dialysis. El-Bahr et al. (2006) proposed that CA are even covalently bound to blood proteins since they were unable to separate them from bovine serum albumin by trichloracetic acid precipitation.

In spite of these efforts, the physiological implications of CA adduct formation are still unclear, in *vivo* studies are rare and data in the literature give conflicting information. It was reported that binding to serum albumin reduced the biological potency of CA *in vitro*, and binding to other serum proteins might have similar effects (Powis, 1974). Therefore, hormonal function seems to be lost during binding to proteins, and this mechanism either represents some sort of compensation for an overflow of released CA or it has no beneficial effect at all. Protection or storage of catecholamines, at least, seems to be very unlikely.

Besides these findings in blood plasma, accumulation of A in erythrocytes also takes place and was first reported in cats as early as 1937 (Bain et al., 1937). Since then, it has been shown that other biologically active amines, including NA and dopamine enter erythrocytes and accumulate inside them in several species (Altman et al., 1988; Azoui et al., 1994; Blakeley and Nicol, 1978; Born et al., 1967;

* Corresponding author. Tel.: +43 1 250 77 4115; fax: +43 1 250 77 4190.
E-mail address: michael.lepschy@vetmeduni.ac.at (M. Lepschy).

http://dx.doi.org/10.1016/j.rvsc.2014.07.013
0034-5288/

El-Bahr et al., 2006; Friedgen et al., 1993; Ratge et al., 1991; Roston, 1966; Yoneda et al., 1984). Erythrocytes to plasma ratios of CA in these studies were 3–16 to 1 for dopamine, 2–4 to 1 for adrenaline, and to 1–2 to 1 for noradrenaline, which strongly suggests that accumulation of CA in the erythrocytes is accomplished by an active transport mechanism rather than by passive diffusion (Alexander et al., 1981; Azoui et al., 1996; Bouvier et al., 1987; Danon and Sapira, 1972b). Blakeley and Nicol (1978) concluded that the uneven distribution of CA between plasma and erythrocytes could be the result of some form of intracellular trapping or binding. In addition, only chronically elevated levels of circulating CA lead to changes in the CA content of erythrocytes, while acute changes in plasma are only reflected to a small extent, if at all (Bouvier et al., 1987). The entry of CA shows saturation kinetics and is temperature dependent (Azoui et al., 1997; Blakeley and Nicol, 1978).

Although some advances could be made in various fields, knowledge especially about long lasting effects of permanently elevated catecholamines and formation and degradation of adducts in the blood and organs is still fragmentary, as most studies focus mainly on short term effects. Basic information (especially *in vivo*) about the distribution and accumulation of radioactively labelled A and NA over an extended period of time is not existent up to now. In this study we therefore collected basic data in the rat as a model animal. We studied the distribution of radioactivity over 28 days in the blood and in organs after repeated intravenous administration of ^{3}H-adrenaline and ^{3}H-noradrenaline. In addition we checked if *in vitro* catecholaminated serum albumin has an altered half-life after i.v. administration. Therefore blood samples were taken frequently and the decrease of radioactivity in plasma samples was compared with an untreated control group.

2. Materials and methods

2.1. Animals and general housing conditions

To investigate the metabolism and excretion of A and NA a total of 24 adult rats were used (Sprague Dawley, Medical University Vienna, Department for Laboratory Animal Science and Genetic). Animals were delivered with a health report according to FELASA recommendation (2002). At the age of 10 weeks and 10 days before starting the experiments, the animals arrived at the laboratory, where they were separated and housed individually in standard Macrolone cage type IV with wire mesh lids (Tecniplast, Buguggiate, Varese, Italy) with aspen wood chips as bedding material. The animal housing room was maintained under standard laboratory conditions (light-dark cycle: 12:12 h, lights on at 6 a.m.; temperature: 22 ± 1 °C; relative humidity: 50 ± 20%). Commercial rat diet and bottled tap water were available ad libitum. Nesting material and nibbling wood bricks were offered for behavioural enrichment. Permission for performing the animal experiments was obtained from the provincial government of Lower Austria (LF1-TVG-34/057–2009).

2.2. Accumulation of CA adducts in the body and distribution of radioactivity over 28 days in the blood

2.2.1. Administration of radiolabelled A and NA

On Day 0 of the experiment at 9:00 in the morning 12 rats (6 males/6 females) received i.v. 244 kBq ^{3}H-A each (special synthesis, WS03DC, E, levo-[ring-7-^{3}H]) and 12 rats (6 males/6 females) received 741 kBq ^{3}H-NA each (NET 678, NE, levo-[ring-7-^{3}H]). Both CA were purchased from Perkin Elmer, Life and Analytical Sciences (Boston, MA, USA) with a specific activity of 40–80 Ci/mmol (purity > 97%) diluted in 180 µl sterile isotonic saline solution intravenously. The injection was repeated at the same time of day on each of the following 6 days (Day 1, 2, 3, 4, 5 and 6; Fig. 1).

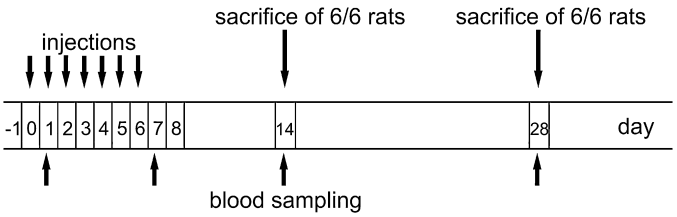

Fig. 1. Time schedule of the first experiment (accumulation of CA adducts in the body and distribution of radioactivity over 28 days in the blood).

The whole procedure of catching, fixation, intravenous injection and transferring the rat into its cage did not exceed 4 minutes per animal.

2.3. Blood sampling and sacrificing schedule

On Days 1 (24 h after the first injection and immediately before the second injection), 7 (24 h after the last injection) and 14 a total of 0.5 ml heparinized blood was taken from all 24 rats. After blood sampling on Day 14, 12 rats (6 males/6 females) were sacrificed and the carcass stored at –20 °C. On Day 28 from the remaining 12 rats (6 males/6 females) 0.5 ml blood were taken before sacrificing the animals and storing carcass at –20 °C.

2.4. Measurement of radioactivity in the blood

Heparinized blood samples were centrifuged (2500 g; 10 min) immediately after sampling and plasma and erythrocytes were separated. Duplicates of plasma samples (0.05 ml) were mixed with 6 ml of scintillation fluid (Quicksafe A, No. 100800, Zinnser Analytic, Maidenhead, UK) and the radioactivity was measured in a liquid scintillation counter (Tri-Carb 2100TR, Packard Instruments, Meriden, CT, USA) for 5 min while running a quench compensation program. Erythrocytes were first haemolysed by deep freezing. After thawing 5 µl erythrocytes in duplicates were mixed with 18 ml of scintillation fluid and measured as described above.

2.5. Dissection of the rats and measurement of radioactivity in organ samples

After defrosting, first 25 mg of hair (in duplicate) of the sacral region were sampled. Afterwards the rats were dissected and 100 mg samples (in duplicate) of the following organs were taken: spleen, liver, kidney, stomach, brain, lunge, muscle, heart and small intestine. In addition 100 mg samples (in duplicate) of abdominal fat, the aorta and skin. Eyes and adrenal glands were completely removed and sampled on the whole. Prior to further processing hair samples were washed with 2 ml 60% methanol at room temperature overnight to remove any possible urinary or faecal contamination. Afterwards methanol was removed and the cleaned hair samples as well as the skin samples were moistened with 0.025 or 0.1 ml distilled water, respectively. The amount of radioactivity was then determined by solubilizing the samples (liver, kidney, spleen, brain, skin, hair, muscle, lung, heart, stomach, small intestine, abdominal fat and aorta) or solubilizing the complete organ in case of the eyes and adrenals for 24 h by adding 1 ml of Biolute S (No. 1310200, Zinnser Analytic). The dissolutions were then neutralized with 1 ml 1M HCl and 18 ml scintillation fluid (Quicksafe A, No. 100800, Zinnser Analytic) for measurement of radioactivity were added. Radioactivity was then measured in a liquid scintillation counter as described before.

2.6. Comparison of the half-life of rat serum albumin (RSA) differing in the extent of catecholamination

RSA at 2.5 g (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in 10 ml isotonic NaCl solution and preincubated with ^{3}H-adrenaline (244 kBq) or ^{3}H-noradrenalinee (742 kBq) for 1 h at 38 °C to enable tracing of the catecholaminated proteins by measuring the radioactivity. Half of the radioactive labelled protein solution was then used for the control group, while the other half was additionally loaded with unlabelled adrenaline and noradrenaline, respectively. Therefore 100 nmol A or NA, freshly dissolved in isotonic NaCl solution was added separately to the radiolabelled protein solution at 38 °C every hour for a period of 5 h (500 nmol in total). After incubation the samples were transferred into dialysis tubes (SERVAPOR dialysis tubing; SERVA Electrophoresis GmbH, Heidelberg, Germany) and the catecholaminated albumin solution was then dialysed against water at 4 °C for 60 h under continuous mild shaking in order to separate the unbound CAs from the RSA. After separation, the dialysed serum albumin solution was lyophilized and suspended in isotonic NaCl solution (concentration of RSA: 70 mg/ml). The control group (total of 16 rats) received 56 mg/0.8 ml of the RSA solution each (8 males/8 females) loaded only with ^{3}H-A or ^{3}H-NA as radioactive tracers. A second group of 16 rats received the same amount of serum albumin (8 males/8 females) loaded with both (tritium-labelled and unlabelled CA). Injected amounts of RSA were chosen not to raise serum albumin levels beyond physiological ranges. For the following 4 weeks, the rats were housed individually and blood samples were taken 18 h, 2 days, 3 days, 7 days, 14 days and 28 days post injection. After separation of erythrocytes and plasma, radioactivity (in duplicate) of plasma samples (0.050 ml) was then measured as described above.

2.7. Statistical analysis

All statistical tests were applied using the software package SigmaStat (3.11). Two independent samples were compared by using the Student's *t*-test (two tailed) when normally distributed, and by using the Mann–Whitney Rank Sum-test (two tailed) when not normally distributed. Differences were considered significant if their probability of occurring by chance was less than 5%.

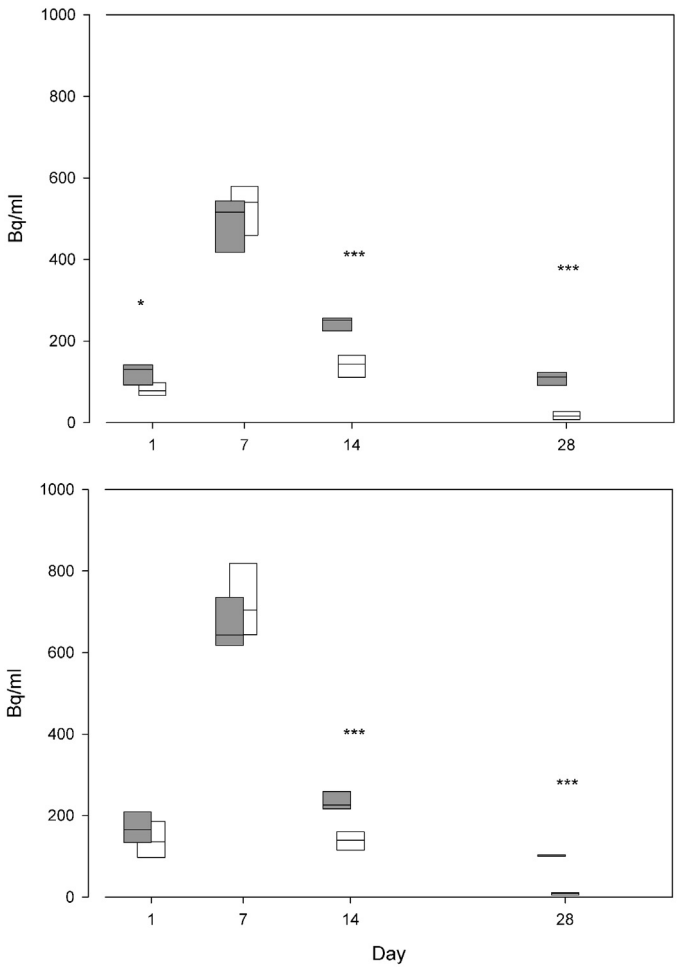

Fig. 2. Time course and distribution of ^{3}H-adrenaline in plasma (white boxes) and erythrocytes (grey boxes) of male (upper panel) and female rats (lower panel) after repeated i.v. administration of ^{3}H-adrenaline. Data are given as box plots (medians: lines in the boxes; 25% and 75% quartiles: boxes). Significant differences (* $p < 0.05$; *** $p < 0.001$) between the erythrocytes and plasma are marked with asterisks, placed on top of the boxes.

3. Results

3.1. Time course and distribution of ^{3}H-adrenaline (Fig. 2) and ^{3}H-noradrenaline (Fig. 3) in the blood

On Day 1, 24 h after the first i.v. injection, a considerable amount of radioactivity could be found ranging from 81 Bq/ml ± 19 in male plasma samples (^{3}H-A) to 313 Bq/ml ± 33 in female erythrocyte samples (^{3}H-NA). Subsequently radioactivity increased over the following 6 days and peaked on Day 7, 24 h after the last injection (lowest values found in erythrocytes of males (^{3}H-A): 490 Bq/ml ± 68; highest values in erythrocytes of females (^{3}H-NA): 1594 Bq/ml ± 207). On Day 14 (7 days post last injection) radioactivity was in male and female blood samples with higher losses in plasma samples (lowest values in female plasma samples: 140 Bq/ml ± 25 (^{3}H-A) and 293 Bq/ml ± 77 (^{3}H-NA); highest values in male ^{3}H-A samples: 242 Bq/ml ± 19 and female ^{3}H-NA erythrocyte samples: 690 Bq/ml ± 139). After 4 weeks (at the end of the experiment) total radioactivity in the blood returned to values close to those from Day 1, although the largest part was now attributable to erythrocytes (lowest values in female ^{3}H-A: 102 Bq/ml ± 2; highest in male ^{3}H-NA: 271 Bq/ml ± 40) while radioactivity in plasma played only a minor role (ranging from 8 Bq/ml ± 3 (^{3}H-A) in females to 40 Bq/ml ± 20 (^{3}H-NA) in males).

Males and females showed significantly higher levels of radioactivity in erythrocytes than in plasma in the first blood sample (24 h after first injection) of the ^{3}H-A experiment (*t*-test, $n_{erythrocytes} = n_{plamsa} = 6$; $p = 0.012$) and the ^{3}H-NA experiment (*t*-test, $n_{erythrocytes} = n_{plamsa} = 6$; $p = 0.013$), respectively. No significant differences could be detected on Day 8, 24 h after the last injection although in all blood samples radioactivity was slightly higher in plasma than in erythrocytes. Subsequently on Day 14 and 28 of the experiments radioactivity in erythrocytes was again significantly higher in all samples (*t*-test, $p < 0.001$).

3.2. Distribution of radioactivity in organs excluding hair (given in Bq/g)

In all sampled organs radioactivity could be found 7 days as well as 21 days after the last injection (Table 1).

One week after the last injection highest concentrations of radioactivity could be found in the adrenals (ranging from 262 ± 72 Bq/g ^{3}H-A in females to 1021 ± 257 Bq/g in ^{3}H-NA in males) and kidneys (ranging from 247 ± 36 Bq/g ^{3}H-A in males to 1372 ± 315 Bq/g in ^{3}H-NA in females). Lowest values were found in the brain (ranging from 270 ± 22 Bq/g ^{3}H-NA in males to 90 ± 20 Bq/g in ^{3}H-A in males) and fat tissue (ranging from 126 ± 13 Bq/g ^{3}H-NA in females to 32 ± 9 Bq/g

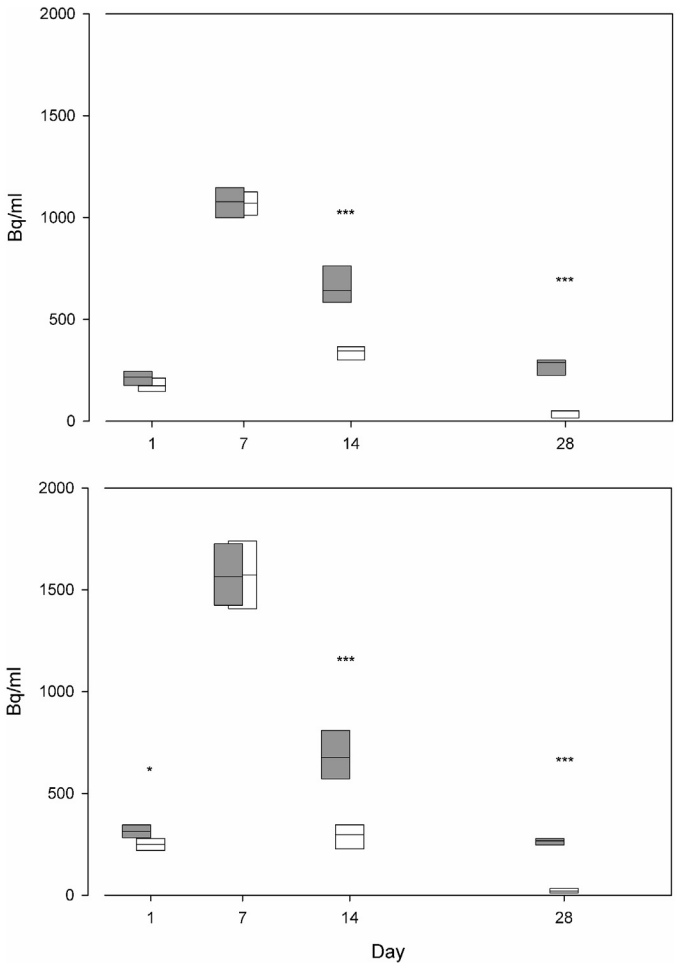

Fig. 3. Time course and distribution of ^{3}H-noradrenaline in plasma (white boxes) and erythrocytes (grey boxes) of male (upper panel) and female rats (lower panel) after repeated i.v. administration of ^{3}H-noradrenaline. For further details see legend of Fig. 2.

in ^{3}H-A in males). Three weeks after the last injection radioactivity in all organs decreased on average 37.2 ± 8.4% in males and 37.3 ± 2.5% in females. Highest decrease could be found in the spleen (ranging from 55.5 ± 9.4% loss of ^{3}H-NA to 74.2 ± 29.9% loss of ^{3}H-A in females).

3.3. Radioactivity in hair (Fig. 4)

In hair radioactivity ranging from 133 Bq/g to 2053 Bq/g could be found 1 week after the last injection (median 582 Bq/g). At the end of the experiment radioactivity in the hair ranged from 88 Bq/g to 4021 Bq/g (median 737 Bq/g). Methanol wash solution contained 43 Bq to 536 Bq (median 128 Bq) 1 week and 31 Bq to 1449 Bq (median 278 Bq) 3 weeks after the last administration.

3.4. Comparison of the half-life of rat serum albumin differing in the extent of catecholamination (Fig. 5)

Amounts of used ^{3}H-A and ^{3}H-NA for tracing were not identical and the process of loading RSA with ^{3}H-catecholamines (experimental group and control group) and with unlabelled catecholamines (experimental group) induced variance. In order to preserve comparability between males and females and ^{3}H-A and ^{3}H-NA results are displayed in percent of injected radioactivity per ml plasma. Eighteen hours after the iv injection of the catecholaminated serum albumin only small amounts of radioactivity could be found in the control groups (^{3}H-A males: 0.55 %/ml ± 0.1, females: 0.87 %/ml ± 0.13; ^{3}H-NA males: 0.49 %/ml ± 0.04, females: 1.00%/ml ± 0.32), as well as in the groups additionally loaded with adrenaline (A + ^{3}H-A males: 0.78 %/ml ± 0.17, females: 1.17%/ml ± 0.29) or noradrenaline (^{3}H-NA males: 0.43 %/ml ± 0.13, females: 0.66%/ml ± 0.15), respectively. Subsequently radioactivity decreased over the following 28 days continuously and returned to values close to background. On Day 28 of the experiment values found in plasma ranged from 0.05 %/ml ± 0.01 (found in males additionally loaded with noradrenaline) and 0.13 %/ml ± 0.01 (found in females of the adrenaline control group). Throughout all time points no significant differences could be found between the control groups and the groups loaded additionally with A or NA, respectively (t-test, $n_{control} = n_{experimental} = 4$; $p > 0.05$).

4. Discussion

During the past 35 years it has become evident that non-enzymatic binding of CA to serum proteins and accumulation in erythrocytes in mammals and birds represents a further mechanism involved in the transport and/or inactivation of CA. Nevertheless knowledge about long lasting effects of permanently elevated catecholamines is still fragmentary and basic information especially about the *in vivo* distribution in the body and accumulation of formed adducts over an extended period of time is nonexistent up to now.

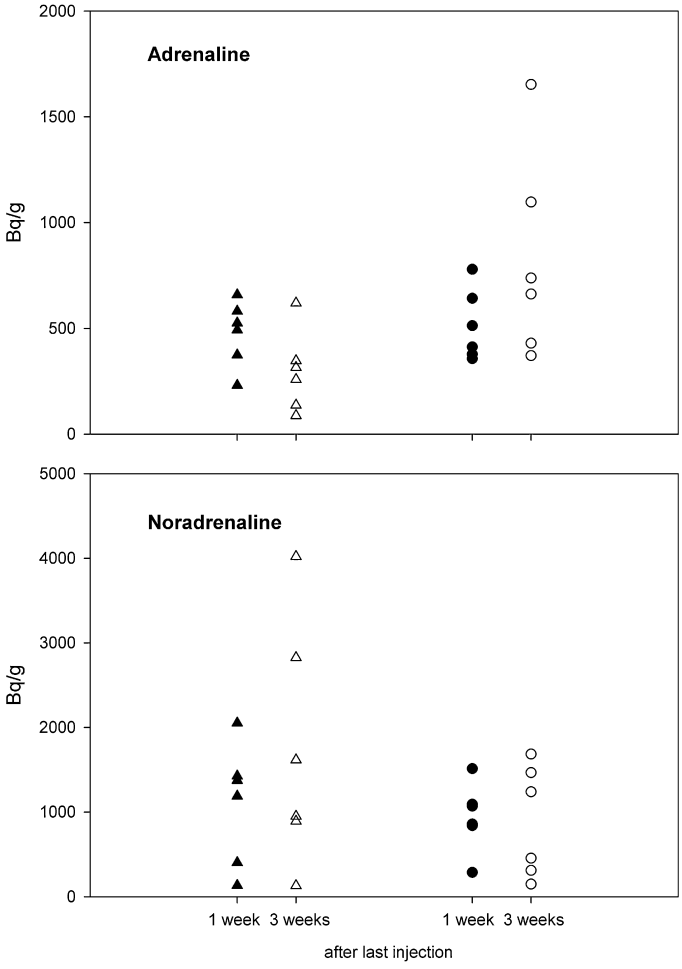

Fig. 4. Radioactivity in hair samples of male (triangles) and female (circles) rats 1 and 3 weeks after the last i.v. administration of ^{3}H-adrenaline (upper panel) and ^{3}H-noradrenaline (lower panel).

Table 1
Measured radioactivity (Bq/g) in organs 1 and 3 weeks after the last i.v. administration of ^{3}H-catecholamines.

	Males				Females			
	1 week post injection		3 weeks post injection		1 week post injection		3 weeks post injection	
	m	sd	m	sd	m	sd	m	sd
^{3}H-Adrenaline								
Spleen	155	27	92	10	223	4	166	68
Liver	97	15	30	8	117	14	30	2
Kidney	247	36	74	19	304	14	98	20
Stomach	112	11	51	6	155	35	49	13
Brain	90	20	21	10	97	13	18	5
Lunge	108	24	43	11	141	9	59	7
Muscle	111	24	37	9	122	16	35	4
Heart	121	14	50	5	131	5	49	3
Small intestine	114	18	41	10	136	14	36	9
Abdominal fat	32	9	19	2	45	12	27	1
Aorta	116	8	48	10	149	4	56	6
Skin	139	39	51	15	148	15	79	32
Eye	106	31	44	14	173	74	40	6
Adrenal glands	453	158	136	86	262	72	82	23
Hair	477	154	294	185	513	81	825	499
^{3}H-Noradrenaline								
Spleen	505	29	308	90	813	124	445	40
Liver	303	22	78	14	314	29	89	17
Kidney	1008	123	329	44	1372	315	487	42
Stomach	317	38	188	166	309	31	170	39
Brain	270	22	53	14	192	18	32	10
Lunge	308	24	118	29	401	26	151	50
Muscle	311	31	79	17	266	19	83	4
Heart	321	11	113	7	388	19	128	18
Small intestine	329	24	91	13	322	32	103	30
Abdominal fat	122	39	28	8	126	13	50	8
Aorta	383	28	148	33	434	32	167	13
Skin	365	27	133	13	373	51	204	12
Eye	328	20	91	9	287	23	112	27
Adrenal glands	1021	257	262	107	792	94	237	66
Hair	1096	726	1739	1458	943	324	883	644

The accumulation in the blood was long known but long term studies *in vivo* were missing. In this study we therefore evaluated the distribution of ^{3}H-CA in the body of the rat as a model animal after repeated i.v. injection and tested whether albumin, a typical blood protein, shows an altered half-life depending on its level of catecholamination.

In the first experiment we daily injected ^{3}H-CA over a week and collected blood samples before animals sacrificed after 1 and 3 weeks after the last injection. The repeated injection led to a strong increase of radioactivity in the blood. In both, plasma as well as erythrocytes, highest levels of radioactivity were measured in samples collected 24 h after the last injection (Day 8 of the experiment. Afterwards radioactivity declined more rapidly in plasma than in erythrocytes. We observed this in both sexes and both CA. Resulting plasma to erythrocytes ratio changed considerable over time. Lowest ratios were observed 24 h after the last injection of radioactivity (about 1:1 in females and males). This is in line with values after *in vitro* short term exposure of whole blood samples to catecholamines given in the literature, which vary between 1 to 4 and 1 to 1 (Alexander et al., 1981; Azoui et al., 1996; Bouvier et al., 1987; Danon and Sapira, 1972b). Highest ratios were observed 21 days after the last ^{3}H-CA injection (at the end of the experiment). At this time the plasma to erythrocytes ratio was about 1 to 8 and 1 to 13 in males and females, respectively. Reason for these pronounced changes over time might be the longer half-life of erythrocytes (45–75 days; Berlin et al., 1951, Burwell et al., 1953) compared with albumin (1.83–2.66 days; Schreiber et al., 1971). Former studies already showed a delayed excretion of CA in urine and faeces in rats. Lepschy et al. (2008) showed a rather protracted excretion of small amounts of radioactivity over weeks after a single dose injection (*i.v.*) of CA in rats. Taking results of the actual study into consideration low amounts of radioactivity in urine and faeces over an extended period of time (weeks) did presumably originate from the slow tear-down of erythrocytes and plasma proteins loaded with ^{3}H-catecholamines.

Females accumulated more radioactivity in the blood on Day 1 and 7 of the experiment than males. This may be well explained by the smaller body size of the female rats (253 g ± 21) compared with the males (398 g ± 41). Therefore a higher amount of radioactivity was injected per ml blood. On the other hand after 14 and 28 days respectively, there were no more significant differences between the males and females. This is remarkable especially as female rats in general do have a lower metabolism than males and therefore radioactivity is supposed to decrease slower than in males.

Although a remarkable amount of radioactivity could be found in various organs of the rats, it has to be stated that in total only around 1–3% of the injected radioactivity could be found in the processed samples. The majority of the radioactivity was therefore excreted via urine and faeces during the first days after injection.

Differing from distribution profiles in the organs, radioactivity in hair showed high values 1 and 3 weeks after the last injection. This strongly suggests permanent deposition of radioactivity hence catecholamines or catecholamine adducts in hair. Storage of glucocorticoid metabolites – another stress hormone – in hair is already known from guinea pigs (Keckeis et al., 2012). Large amounts of unlabelled glucocorticoid metabolites could be measured in hair although most likely not originating from systemic glucocorticoids. These authors concluded that glucocorticoids are very likely derived from local skin production therefore making measurements of glucocorticoids in hair samples as a parameter of stress rather fault prone. In case of catecholamines or their adducts local production might be less important. As hair cycle dependent changes in

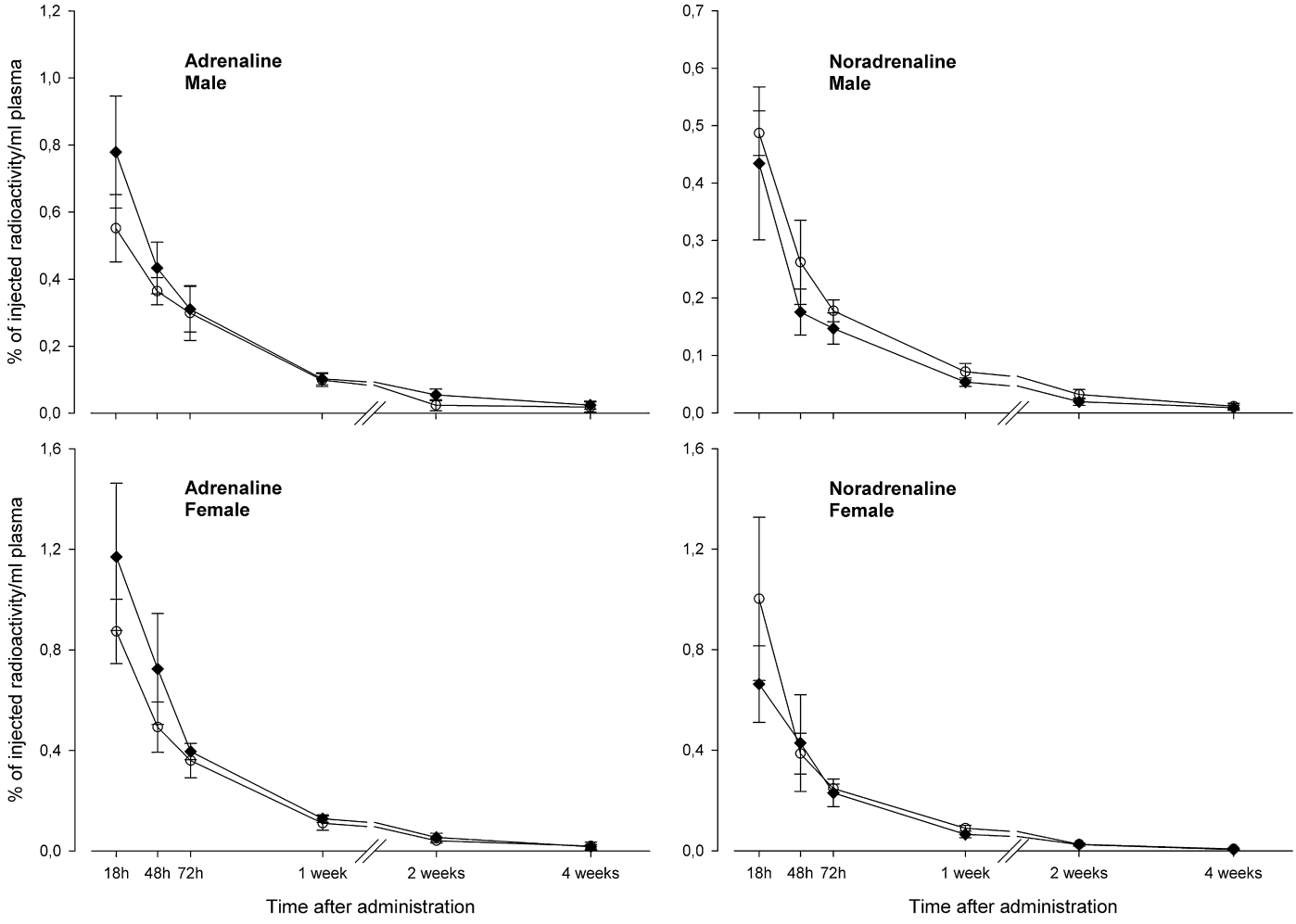

Fig. 5. Time course of radioactivity (mean ± SD) in plasma samples of male rats (upper panels) and female rats (lower panels) after i.v. administration of serum albumin labelled only with ^{3}H-adrenaline (left panels) or ^{3}H-noradrenaline (right panels) as tracer (control group; means given as open circles) and serum albumin loaded with adrenaline and ^{3}H-adrenaline (or noradrenaline and ^{3}H-noradrenaline, respectively; experimental group; means given as filled diamonds).

adrenergic skin innervation were described (Botchkarev et al., 1999) their influence as a possible confounder should be further investigated. Therefore measurement of catecholamine adducts in hair samples might be more promising to non-invasively monitor long term systemic catecholamine levels. On the other hand high variation of radioactivity in hair samples is an issue that has to be investigated in greater detail. Values varied considerable even within samples of an individual. Explanation for this might be different hair growth rates, which leads to different concentration and amounts of radioactivity in the samples. At least, contamination of hair samples as a reason for high variation appears very unlikely. Washing the samples with 60% methanol over 12 h seems to be an efficient procedure to remove external urinary or faecal contamination. The pattern of radioactivity in the methanol washing solution underlines this assumption. Among mostly low values (lowest 31 Bq and median 222 Bq) we also measured some rather high values (up to 1449 Bq) in the washing solution pointing at removed external contamination. Nevertheless more work has to be done to characterize catecholamines or their adducts present in hairs before measurement might be considered as a valid parameter for monitoring long term stress. Besides, in humans hair growth is constant and long term stress might be monitored even over months. This might not be possible in rats as the hair growth period was reported to be only 25 days (Griesemer, 1956).

Only very low levels of the injected albumin (between 1.2%/ml plasma and 0.4%/ml plasma of the injected amount) could be detected in the plasma 18 h after the injection. In the following 4 weeks radioactivity decreased to background levels. As we did not collect any faecal or urinary samples, we can only speculate that the largest parts of the i.v. injected radioactively labelled albumin was metabolized and excreted via the bile or via urine during the first 18 h. Excretion profiles of ^{3}H-catecholamines in rats, mice and chicken after a single-dose injection (Lepschy et al., 2008) support this hypothesis. Against our expectations, we could not detect any significant differences between the control group and the experimental group. Nevertheless, failing to detect any significant differences might be also attributed to the in general low levels of radioactivity in the blood samples. Measurement of low levels of radioactivity induces higher variability and therefore detecting significant differences between the control and the experimental group is more difficult.

This study showed for the first time a clear and pronounced accumulation of radiolabelled catecholamines on or in erythrocytes over an extended period of time *in vivo* in the rat as a model animal. In addition, binding of catecholamines to plasma proteins as well as accumulation in various organs could be proven. Furthermore a considerable amount of radioactivity could be detected in hair samples suggesting hair as a matrix for further investigations for non-invasive measurement of catecholamines as a chronic parameter of stress.

Acknowledgements

Michael Lepschy was funded by a grant of the Austrian Science Foundation (FWF Project No. 21607). In addition all animals, materials and consumables used in the study were funded by the Austrian Science Foundation (FWF Project No. 21607) as well.

References

Alexander, N., Velasquez, M., Vlachakis, N.D., 1981. Red blood cell: in *vivo* site for transport and inactivation of biogenic amines in men and rats. American Journal of Physiology 238, H521–H526.

Altman, R.J., Smith, C.C., Betteridge, J., 1988. Catecholamine content of human erythrocytes. Clinical Chemistry 34, 2120–2122.

Azoui, R., Vignon, D., Safar, M., Cuche, J.L., 1994. Plasma erythrocyte relationship of CA in human blood. Journal of Cardiovascular Pharmacology 23, 525–531.

Azoui, R., Cuche, J.L., Renaud, J.F., Safar, M., Dagher, G., 1996. A dopamine transporter in human erythrocytes: modulation by insulin. Experimental Physiology 81, 421–434.

Azoui, R., Schneider, J., Dong, W.X., Dabire, H., Safar, M., Cuche, J.L., 1997. Red blood cells participate in the metabolic clearance of CA in the rat. Life Sciences 60, 357–367.

Bain, W.A., Gaunt, W.E., Suffolk, S.F., 1937. Observation on the inactivation of adrenaline by blood and tissue. Journal of Physiology 91, 233–253.

Berlin, N.I., Meyer, L.M., Lazarus, M., 1951. Life span of the rat red blood cell as determined by glycine-2-C^{14}. American Journal of Physiology 165, 565–567.

Blakeley, A.G., Nicol, C.J., 1978. Accumulation of amines by rabbit erythrocytes in vitro. Journal of Physiology 277, 77–90.

Boomsma, F., Man in 't Veld, A.J., Schalekamp, M.A., 1991. Not noradrenaline but its oxidation products bind specifically to plasma proteins. Journal of Pharmacology and Experimental Therapeutics 259, 551–557.

Born, G.V., Day, M., Stockbridge, A., 1967. The uptake of amines by human erythrocytes in vitro. Journal of Physiology 193, 405–418.

Botchkarev, V.A., Peters, E.M.J., Botchkareva, N.V., Maurer, M., Paus, R., 1999. Hair cycle-dependent changes in adrenergic skin innervation, and hair growth modulation by adrenergic drugs. Journal of Investigative Dermatology 113, 878–887.

Bouvier, M., Farley, L., de Champlain, J., 1987. Red blood cell catecholamine levels in normotensive and DOCA-salt hypertensive rats. American Journal of Physiology 253, H270–H275.

Branco, D., Torrinha, J.F., Osswald, W., 1974. Binding of exogenous noradrenaline by the proteins of dog plasma. Naunyn Schmiedebergs Archieves of Pharmacology 285, 367–373.

Burwell, E.L., Brickley, B.A., Finch, C.A., 1953. Erythrocyte life span in small animals – comparison of two methods employing radioiron. American Journal of Physiology 172, 718–724.

Danon, A., Sapira, J.D., 1972a. Binding of catecholamines to human serum albumin. Journal of Pharmacology and Experimental Therapeutics 182, 295–302.

Danon, A., Sapira, J.D., 1972b. Uptake and metabolism of catecholamines by the human red blood cell. Clinical Pharmacology and Therapeutics 13, 916–922.

Eisenhofer, G., Kopin, I.J., Goldstein, D.S., 2004. Catecholamine metabolism: a contemporary view with implications for physiology and medicine. Pharmacological Reviews 56, 331–349.

El-Bahr, S.M., Kahlbacher, H., Rausch, W.D., Palme, R., 2005. Excretion of CA (adrenaline and noradrenaline) in domestic livestock. Wiener Tierärztliche Monatsschrift 92, 207–213.

El-Bahr, S.M., Kahlbacher, H., Patzl, M., Palme, R., 2006. Binding and clearance of radioactive adrenaline and noradrenaline in sheep blood. Veterinary Research Communication 30, 423–432.

Friedgen, B., Halbrugge, T., Graefe, K.H., 1993. Plasma clearances and extractions of four CA in the anesthetized rabbit: the role of amine removal by blood cells. Journal of Cardiovascular Pharmacology 21, 21–28.

Griesemer, R.D., 1956. Change in enzyme activity and ribonucleic acid concentration within the epidermal cell of the rat during the growth stage of the hair cycle. Journal of Biophysical and Biochemical Cytology 2, 523–530.

Keckeis, K., Lepschy, M., Schöpper, H., Moser, L., Troxler, J., Palme, R., 2012. Hair cortisol: a parameter of chronic stress? Insights from a radio-metabolism study in guinea pigs. Journal of Comparative Physiology B 182, 985–996.

Lepschy, M., Rettenbacher, S., Touma, C., Palme, R.G., 2008. Excretion of catecholamines in rats, mice and chickens. Journal of Comparative Physiology B 178, 629–636.

May, P., Sanders, F.J., Donabedian, R.K., 1974. Binding of catechol derivatives to human serum proteins. Experientia 30, 304–305.

McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. Hormones and Behavior 43, 2–15.

Mirkin, B.L., Brown, D.M., Ulstrom, R.A., 1966. Catecholamine binding protein: binding of tritium to a specific protein fraction of human plasma following *in vitro* incubation with tritiated noradrenaline. Nature 10, 1270–1271.

Moberg, G.P., 2000. Biological response to stress: implication for animal welfare. In: Moberg, G.P., Mench, J.A. (Eds.), The biology of animal stress. CABI Publishing, Oxon, NY, pp. 123–146.

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domestic Animal Endocrinology 23, 67–74.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and non-invasive measurement in faecal samples. Annals of the New York Academy of Sciences 1040, 162–171.

Powis, G., 1974. Effect of serum albumin upon the response of the rat isolated superfused anococcygeus muscle to catecholamines and to nerve stimulation. Journal of Pharmacy and Pharmacology 26, 344–347.

Powis, G., 1975a. The binding of CA to the serum proteins of the rat and the domestic fowl. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology 52, 85–90.

Powis, G., 1975b. The binding of CA to human serum proteins. Biochemical Pharmacology 24, 707–712.

Ratge, D., Kohse, K.P., Steegmuller, U., Wisser, H., 1991. Distribution of free and conjugated CA between plasma, platelets and erythrocytes: different effects of intravenous and oral catecholamine administrations. Journal of Pharmacology and Experimental Therapeutics 257, 232–238.

Roston, S., 1966. Entrance of epinephrine into the human erythrocyte. Nature 212, 1380–1381.

Sager, G., Bratlid, H., Little, C., 1987. Binding of CA to alpha-1 acid glycoprotein, albumin and lipoproteins in human serum. Biochemical Pharmacology 36, 3607–3612.

Schreiber, G., Urban, J., Zähringer, J., Reutter, W., Frosch, U., 1971. The secretion of serum protein and the synthesis of albumin and total protein in regenerating rat liver. Journal of Biological Chemistry 246, 4531–4538.

Teixeira, F., Branco, D., Torrinha, J.F., 1979. Binding of adrenaline and isoprenaline to plasma proteins of the dog. Pharmacology 18, 228–234.

Yoneda, S., Alexander, N., Vlachakis, N.D., Maronde, R.F., 1984. Role of conjugation and red blood cells for inactivation of circulating normetanephrine. American Journal of Physiology 247, R208–R211.

Zia, H., Cox, R.H., Luzzi, L.A., 1971. *In vitro* binding study of epinephrine and bovine serum albumin. Journal of Pharmacological Sciences 60, 89–92.