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

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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;

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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).

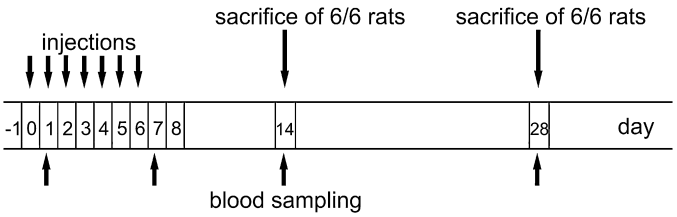

Fig. 1. Time schedule of the first experiment (accumulation of CA adducts in the body and distribution of radioactivity over 28 days in the blood).

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.