The Veterinary Journal 183 (2010) 228–231

Contents lists available at ScienceDirect

The Veterinary Journal

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

Short Communication

Detection of catecholamines and metanephrines by radio-immunoassay in canine plasma

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ARTICLE INFO

Article history:
Accepted 15 October 2008

Keywords:
Metanephrine
Normetanephrine
Catecholamine
Radio immunoassay
Dog

ABSTRACT

This study investigated the applicability of two human radio-immunoassays (RIA) to detect epinephrine (EPI), norepinephrine (NE), and their *O*-methylated metabolites metanephrine (MN) and normetanephrine (NMN) in canine plasma. The analysis yielded a positive correlation between metabolites and their respective parent compounds: EPI and MN ($r = 0.63$), NE and NMN ($r = 0.47$), as well as between parent compounds, EPI and NE ($r = 0.48$), and between metabolites MN and NMN ($r = 0.71$). Moreover, EPI ($r = 0.99$) and NE ($r = 0.77$) concentrations determined by RIA did correlate positively with high pressure liquid chromatography (HPLC). However, there was limited agreement between both methods. It was concluded that complete validation tests for accuracy, precision and agreement are needed before this RIA can be applied to quantify catecholamines, metanephrine, and normetanephrine in canine plasma. The assay may prove to be a potential alternative to HPLC or tandem mass spectrometry in the work-up of pheochromocytoma and the detection of overall sympathetic activity in dogs.

To date, analytical methods to quantify canine epinephrine (EPI) and norepinephrine (NE) plasma concentrations include high pressure liquid chromatography (HPLC) with electrochemical detection, spectrophotometry or fluorometry. Some of these methods can be complex and time-consuming, and therefore may only be available in highly specialised laboratories. In particular, the quantification of canine EPI and NE *O*-methylated metabolites, metanephrine (MN) and normetanephrine (NMN) (the 'metanephrines'), require elaborate methods of extraction and fluorometry as described by Anton and Sayre (1966), and therefore have rarely been determined in canine plasma.

Metanephrines have a high diagnostic value in the detection of pheochromocytoma and other sympathoneural or adrenomedullary abnormalities (Goldstein et al., 2003; Sawka et al., 2003). In addition, the MN:NMN ratio is useful to differentiate between endogenous and exogenous sources of elevated plasma epinephrine concentrations (Yaworsky et al., 2005). In humans, the quantification of catecholamines and metanephrines by radio-immunoassay (RIA) with a highly specific antibody is a cost and time saving, yet accurate alternative to HPLC or tandem mass spectrometry (LC–MS/MS) (Wassell et al., 1999; Lenz et al., 2006). We therefore investigated whether a human RIA could detect EPI, NE, MN and NMN in canine plasma.

Following approval by the local Animal Protection Committee (G 0084/04), six pure-bred female Beagle dogs (bodyweight (BW) 14.3 ± 0.3 kg) were kept under highly standardised environmental and dietary conditions as described previously (Francis et al., 2006).

Dogs were instrumented with an arterial line (18 G) under local anaesthesia in the right femoral artery to continuously record mean arterial pressure and heart rate. The first blood sample was taken ('instrumentation'). A leak-proof face mask was placed on the dog's nose and connected to a ventilator in continuous positive airway pressure mode (3–4 cm H_2O). The dogs breathed room air and blood samples were taken after 30, 90 and 105 min ('normoxia 30, 90, 105'). Then, a gas mixture of 10% O_2 and 90% N_2 was applied and blood samples were taken after 60 and 120 min ('hypoxia 60, 120'), meaning that each dog was sampled six times.

Blood samples were transferred into potassium–EDTA vials, centrifuged and plasma was stored at −80 °C until analysis. Epinephrine and NE were determined by KatCombi RIA (IBL International) after extraction from the biological sample by use of a cis-diole-specific affinity extraction. Then, the extracted NE and EPI were converted to NMN and MN by pig liver catechol-*O*-methyltransferase and S-adenosyl-L-methionine as coenzyme, and were simultaneously derivatised to N-acylnormetanephrine and N-acylmetanephrine. The RIA procedure was based on the competition between a radioactive and a non-radioactive antigen for a fixed number of antibody-binding sites. Metanephrines were determined by MetCombi RIA (IBL International).

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doi:10.1016/j.tvjl.2008.10.008

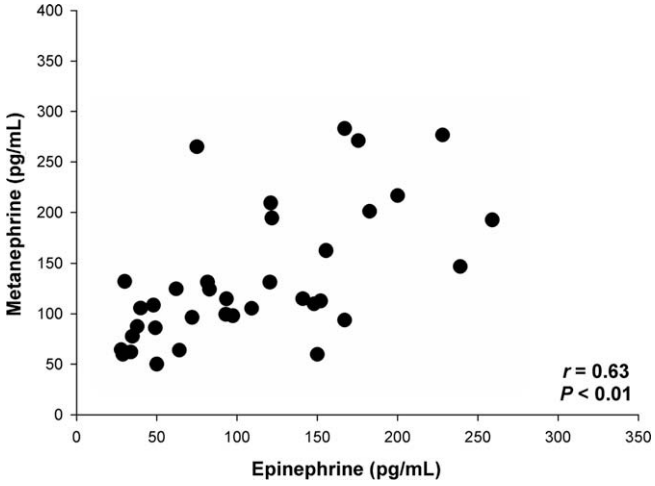

Fig. 1. Correlation of epinephrine and metanephrine plasma concentrations ($n = 36$) in six conscious Beagle dogs during six different conditions: after instrumentation, after 30, 90, and 105 min of normoxia ($F_iO_2 = 21\%$, CPAP 4 cm H_2O), and after 60 and 120 min of hypoxia ($F_iO_2 = 10\%$, CPAP 4 cm H_2O).

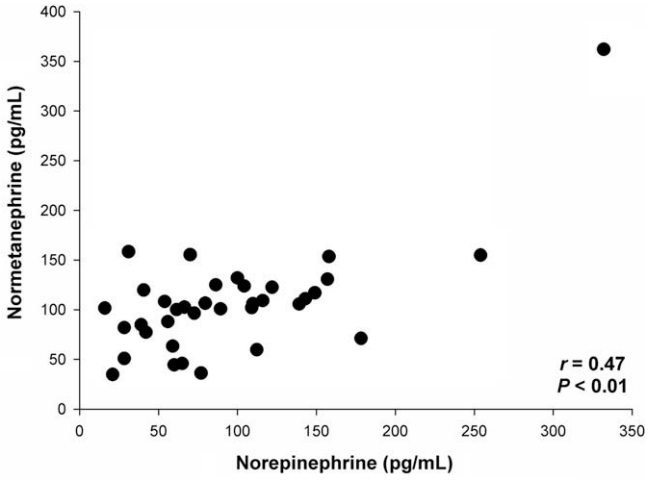

Fig. 2. Correlation of norepinephrine and normetanephrine plasma concentrations ($n = 36$) in six conscious Beagle dogs during six different conditions: after instrumentation, after 30, 90, and 105 min of normoxia ($F_iO_2 = 21\%$, CPAP 4 cm H_2O), and after 60 and 120 min of hypoxia ($F_iO_2 = 10\%$, CPAP 4 cm H_2O).

After extraction of the samples the chemically derivatised metanephrines were measured by RIA. A limited amount of specific antibody reacted with the corresponding ^{125}I-radioisotope labelled antigen. Upon addition of an increasing amount of non-labelled

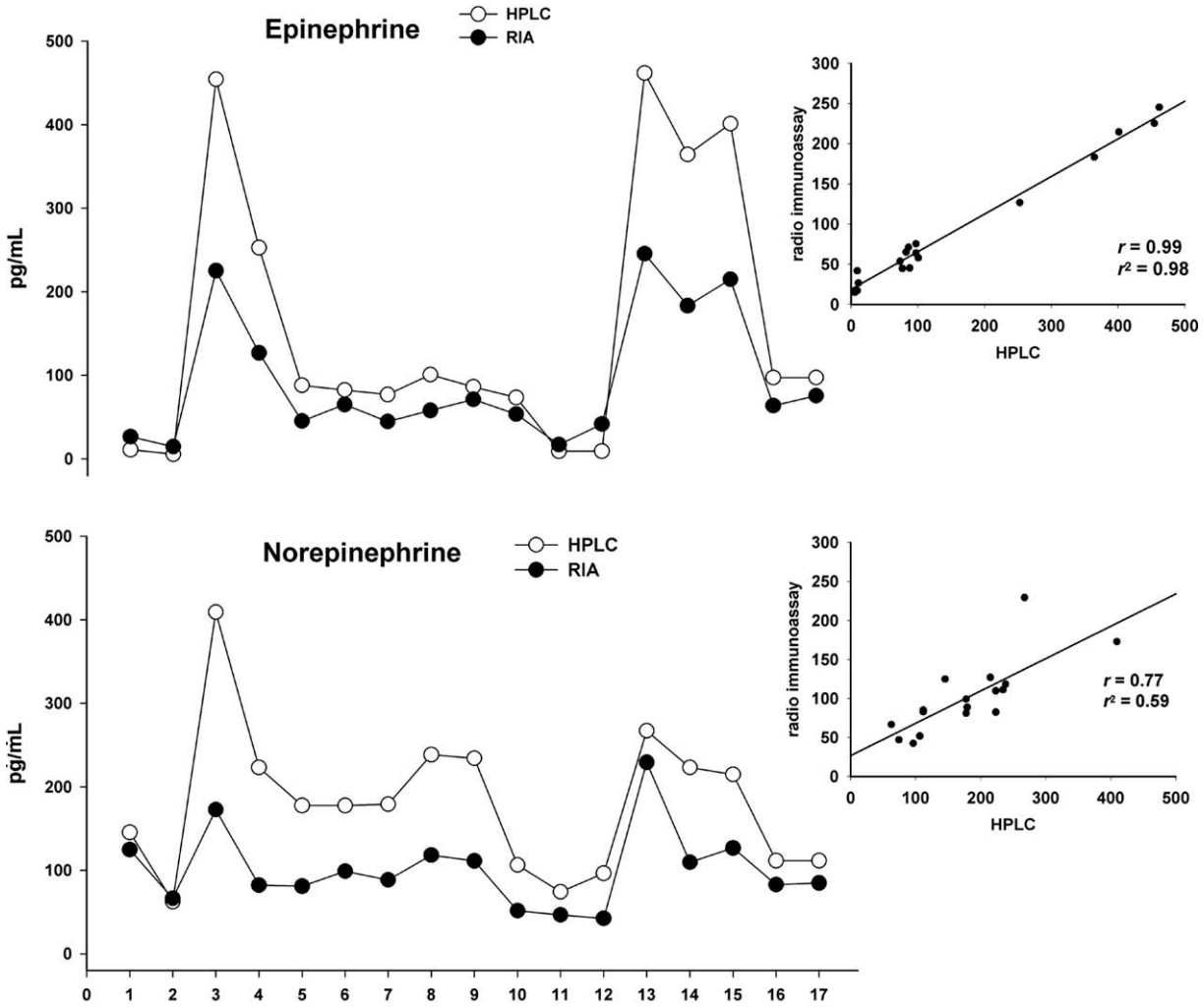

Fig. 3. Comparison of canine plasma concentrations of epinephrine and norepinephrine determined by high pressure liquid chromatography (HPLC) and radio-immunoassay (RIA) in 17 stored plasma samples collected during various conditions, including haemorrhage, hypotension, hypoxia, consciousness and anaesthesia. HPLC and RIA data were correlated and analysed for linear regression. Epinephrine: slope 0.47, intercept 18.8; norepinephrine: slope 0.41, intercept 26.7.