ELSEVIER

Journal of Chromatography B, 745 (2000) 279–286

JOURNAL OF CHROMATOGRAPHY B

www.elsevier.com/locate/chromb

High-performance liquid chromatographic analysis of muscular interstitial arginine and norepinephrine kinetics A microdialysis study in rats

E.M. Siaghy[a], Y. Devaux[a], H. Schroeder[b], N. Sfaksi[a], D. Ungureanu-Longrois[a,d], F. Zannad[a], J.P. Villemot[a], P. Nabet[b], P.M. Mertes[a,c,*]

[a]*Laboratoire de Chirurgie Expérimentale, UPRES 971068, Faculté de Médecine de Nancy, Université Henri Poincaré, Nancy I, France*
[b]*Laboratoire de Biochimie A, Hopital Central, CHU, Nancy, France*
[c]*Laboratoire de Biologie Cellulaire, Hopital de Brabois, CHU, Vandoeuvre-les-Nancy, France*
[d]*Departement d'Anesthésie et de Réanimation Chirurgicale, Hopital de Brabois, CHU, Vandoeuvre-les-Nancy, France*

Received 12 January 2000; received in revised form 7 April 2000; accepted 11 May 2000

Abstract

Complex interactions between the L-arginine/nitric oxide synthase (NOS) pathway and the sympathetic nervous system have been reported. Methods capable of measuring L-arginine and norepinephrine (NE) have mainly been reported for plasma. We report the use of the microdialysis technique combined with high-performance liquid chromatography (HPLC) for measurement of both L-arginine and NE within the same tissue microdialysis sample. The microdialysis probe consisted of linear flexible probes (membrane length: 10 mm, outside diameter: 290 μm, molecular weight cut-off 50 kDa). The method used for L-arginine measurement was HPLC with fluorescence detection, giving a within-run and a between-day coefficient of variation of 2.9 and 12.8%, respectively. The detection limit was 0.5 pM/20 μl injected for L-/D-arginine. The method used for NE measurement was HPLC with electrochemical detection. The coefficients of variation were 4% for within-assay precision and 7.5% for between-assay precision. The detection limit for NE was 1 fmol/20 μl injected. The microdialysis technique coupled with HPLC system was validated in vivo to measure muscular interstitial concentrations of both arginine and NE under baseline conditions and after intravenous infusion of 500 mg/kg of L-arginine or D-arginine. In conclusion, the microdialysis technique coupled to HPLC allows the simultaneous measurements of both L-arginine and NE within the same tissue microenvironment and will enable the study of the complex interactions between the L-arginine/NO pathway and sympathetic nervous system within the interstitial space of different organs.

Keywords: Microdialysis; Arginine; Norepinephrine

1. Introduction

Nitric oxide (NO) is an important mediator involved in a diverse array of physiological and pathological processes [1–3]. The L-arginine/NO pathway has been shown to interact with the sympa-

*Corresponding author. Tel.: +3-83-15-42-97; fax: +3-83-15-32-13.
E-mail address: pmmertes@chu-reims.fr (P.M. Mertes).

0378-4347/00/$ – see front matter
PII: S0378-4347(00)00284-X

thetic nervous system (SNS) and its neurotransmitter (NE) in various organs such as the central nervous system and the heart [4,5].

Nitric oxide is enzymatically synthesised from the guanidino nitrogen of L-arginine by three isoforms of nitric oxide synthases (NOS) [6,7]. Recent reports have shown that NO can be produced through a nonenzymatic pathway by the reaction of hydrogen peroxide with D- or L-arginine (D-arg, L-arg) [8]. Decreased L-arginine availability as one of the mechanism limiting NO production has been proposed [9]. Pharmacological manipulation of the NOS pathway through increased L-arginine availability has been reported to improve endothelium-dependent vasodilatation in several experimental models and clinical settings [10,11]. In addition, the production of NO from L-arginine has been shown to be stereospecific. Indeed, the D-enantiomer of arginine (D-arginine) has been reported to be ineffective in the production of NO both in vitro and in vivo [12–15]. Therefore, D-arginine may be used as a negative control in this type of experiments. The mechanisms by which administration of L-arginine are responsible for the vasoactive effects are not well understood because the reported plasma and intracellular concentrations of L-arginine are far in excess of the Km of NO synthases for the substrate. In the light of the possible: (1) non enzymatic production of NO from L- or D-arginine; (2) the recently reported interaction between the L-arginine/NO pathway and NE; and (3) the possible manipulation of the L-arginine/NO pathway through increased availability of L-arginine, methods designed to measure tissue interstitial concentrations of both L-arginine and NE within the same tissue are necessary.

The microdialysis technique has been used to measure concentrations of various putative neurotransmitters such as NE and amino acids such as arginine in different organs in animals and humans [16–22]. Recently, this technique was proposed for the investigation of the pharmacokinetic profile of compounds within tissue because of its better temporal resolution compared to other methods applied so far for the study of tissue kinetics of drugs and metabolites [23].

The aim of this study was to demonstrate that the microdialysis technique permits the measurement of both arginine and NE on the same microdialysis sample, thus enabling the study of the complex physiological and pharmacological interactions between the L-arginine/NO pathway and the sympathetic nervous system within the muscle extracellular space in rat.

2. Experimental

2.1. Chemicals and reagents

L- and D-arginine (free-base, 99% purity grade) were obtained from Calbiochem (Meudon, France) and Sigma (St. Quentin Fallavier, France), respectively. Norepinephrine and octyl sulphate of the highest purity available were purchased from Sigma (St. Quentin Fallavier, France). Sodium dihydrogen phosphate (NaH_2PO_4) and metabisulfite were from Prolabo (Fontenay-sous-Bois, France). Methanol (HPLC grade) was from S.D.S. (ValdonnePeypin, France), acetonitrile and ethylene diamine tetraacetic acid (EDTA) were from Merck (Nogent-sur-Marne, France). Sodium tetraborate and *o*-phtaldialdehyde (OPA) were from Fluka (St. Quentin Fallavier, France). 2-β mercaptoethanol was from BioRad (Ivry sur Seine, France). Ringer–Lactate solutions were purchased from Braun-Fandre (Ludres, France). Deionized water was used throughout the study.

2.2. Arginine measurement

2.2.1. Chromatography

L-/D-arginine dialysate concentration was determined using a method adapted from those used for amino acids measurement in plasma and cerebrospinal fluid [24–26]. The HPLC system consisted of a Waters 510 pump equipped with a Waters WISP 712 autosampler and a 20 μl loop for syringe injection coupled to a Waters 470 fluorescence detector with a 10 μl flow-through cell (St. Quentin, France). A reversed-phase Waters Novapack C_{18} column (150× 3.9, I.D., 4 μm particle size) was used. The column temperature was set at 37°C. The flow rate was 1 ml/min. The excitation and emission wavelengths were 330 and 450 nm, respectively. Peak height was used as a measure of the detector's response. The L- or D-arginine content was determined after comparison with an external arginine calibration curve

(0–500 μ*M*) prepared in Ringer–Lactate buffer supplemented with metabisulfite 0.6% and EDTA 3%. The phosphate buffer, prepared from 0.1 *M* sodium phosphate solution (pH 5.7) and 12.5% acetonitrile was applied as the mobile phase.

2.2.2. Sample preparation

The borate buffer was prepared by dissolving 9.5 g sodium tetraborate in 250 ml of deionized water and the pH was adjusted to 9.5 with 1 *M* sodium hydroxide. The OPA reagent was prepared extemporaneously by dissolving 13.5 mg of OPA in 2.5 ml of sodium borate buffer added with methanol (10%, v/v) and 2-β mercaptoethanol (0.4%, v/v) as previously described [27]. Three microliters of OPA were added to 20 μl of microdialysis sample within the autosampler. After 1-min incubation at room temperature, 20 μl of the reaction mixture were injected into the HPLC system.

2.2.3. Analytical performance

All L- or D-arginine solutions used for the study were prepared in Ringer–Lactate buffer added with metabisulfite (0.6%) and EDTA (3%) because the microdialysate samples were acidified with metabisulfite and EDTA in order to prevent the degradation of NE. The within-run precision of the arginine measurement was evaluated by assaying one sample (10 μ*M*) (aminoacid standard for hydrolysate analysis, Beckman, Strasbourg, France) that contains L-arginine, ten times within the same series. The between-day precision was studied once a day for 10 days by assaying two defined concentrations (10 and 20 μ*M*) of L-arginine.

The linearity of the method was studied using standard solutions with concentrations ranging from 0 to 500 μ*M* of L-arginine. The detection limit, i.e. the amount that gives a signal twice the peak-to-peak noise level, was determined by successive dilution of a 50 μ*M* L-arginine solution. The correlation between the L- and D-arginine measurement was studied using standard solutions (0–500 μ*M*) of both enantiomers.

2.3. Norepinephrine measurement

2.3.1. Chromatography

Norepinephrine microdialysate concentration was assessed using HPLC and electrochemical detection as described previously [20]. Briefly, the HPLC system was from Kontron Instrument (St. Quentin, France) and consisted of a solvent delivery pump (Model 420), a rheodyne (Model 7125) equipped with a 20 μl loop, a Kontron 405 electrochemical amperometric detector with a plastic cell equipped with a glassy carbon electrode operated at +0.6 V with an Ag/AgCl reference electrode. A reversed-phase Resolve CiS column (150×4 mm I.D., 5 μ*M* paricule size, Interchim, Montluçon, France) guarded with a precolumn was used.

The mobile phase consisted of 0.1 *M* Na_2HPO_4 (pH 5.7), 0.05 m*M* EDTA, 1 m*M* octyl sulfate and 8% methanol (v/v) with a flow rate of 0.8 ml/min. Before use, the mobile phase was filtered and degassed using a Millipore 0.45 μm membrane filter disc and vacuum filtering flask.

2.3.2. Analytical performance

The within-run precision of the NE measurement was evaluated by assaying ten times within the same series two standard samples at two different concentrations (1.2 and 12 n*M*) prepared in Ringer–Lactate solution containing 0.6% metabisulfite and 3% EDTA (v/v). The between-day precision was studied once a day for 10 days by assaying these two defined concentrations of NE. The linearity of the method was investigated using standard solutions ranging from 0 to 30 n*M* of NE dissolved in Ringer–Lactate solution supplemented with metabisulfite and EDTA. The detection limit, i.e. the amount that gives a signal twice the peak-to-peak noise level, was determined by successive dilution of a 30 n*M* NE solution.

2.4. In vivo microdialysis

2.4.1. Dialysis probe

Microdialysis linear flexible probes (membrane length: 10 mm, outside diameter: 290 μm) were custom-made in our laboratory and assembled using a 50 kDa molecular weight cut-off polyacrylonitrile membrane (AN69, HOSPAL, Lyon, France) glued at both ends to thin (75 μm I.D.) inflow and outflow silica tubes. The probes were connected to a microinjection pump (Harvard pump, Cambridge, MA,

USA) and perfused at a flow-rate of 2 μl/min. Under this condition, the in vitro recovery of the probe was found to be 56±3% for arginine and 36±4% for NE.

2.4.2. Surgical procedure

Male Wistar rats (250–350 g) were housed and treated in accordance with accepted practices for humane laboratory animal care. Anaesthesia was induced and maintained with ketamine (Parke-Davis, Courbevoie, France) (90–100 mg/kg i.p. for induction followed by a continuous i.v. infusion of 8 mg/kg/h). Neuromuscular blockade was obtained with pancuronium bromure (Organon Teknika, Fresnes, France) (0.6 mg/kg, i.v.); additional doses of 0.3 mg/kg were administered at 30-min intervals. The trachea was canulated and the lungs were mechanically ventilated with room air using an Harvard Rodent Ventilator model 683 (Harvard Apparatus, MA, USA). The core temperature was maintained at 37°C using a heating pad.

Heart rate and mean arterial blood pressure (left carotid artery canula) were continuously monitored. The right jugular vein was canulated for fluids and L-/D-arginine infusion. The quadriceps muscles were exposed bilaterally, microdialysis probes were inserted as previously described by Lehnmann et al. [28] and continuously perfused as described above.

2.4.3. Experimental protocol

The animals were randomly allocated into two groups: animals receiving L-arginine (L-arg, $n=5$), and animals receiving D-arginine (D-arg, $n=5$).

Baseline measurements were obtained 20 min after the implantation of the probes. Subsequently, 500 mg/kg L- or D-arginine dissolved extemporaneously in Ringer–Lactate solution were infused over 40 min. Dialysate samples were collected every 20 min for 3 h in tubes containing 50 μl of a mixture of metabisulfite (0.6%) and EDTA (3%) in order to prevent the oxidation of NE and frozen at −80°C until later analysis.

2.5. Statistical analysis

Results are expressed as mean±standard error of the mean (SEM). Correlations between concentrations and peak heights were tested by linear regression analysis. One-way repeated measure analysis of variance (ANOVA) was performed to compare the changes in hemodynamic parameters and peak height for NE, D-arginine or L-arginine over time, followed by Fischer post-hoc test for between group comparisons. The criterion of significance was $P<0.05$.

3. Results

3.1. Analytical performance of arginine measurement

Using an isocratic elution method, OPA derivatives of L- or D-arginine were separated in 8 min. Chromatograms of derivatized L-/D-arginine standard (50 μ*M*) are shown in Fig. 1(A, B) as well as a typical chromatogram of arginine in a microdialysis sample (Fig. 1C). These chromatograms have similar profiles for L- and D-arginine.

The within-run coefficient of variation was 2.9% whereas the between-day precision was 12.8% at 10 μ*M* and 5.3% at 20 μ*M*. The detection limit was 0.5 pM per 20 μl injected for L-/D-arginine. A linear correlation between L-arginine concentrations (0–500 μ*M*) and peak heights was observed ($r=0.99$; $P<0.01$) demonstrating that the method is linear up to 500 μ*M*. The L- and D-arginine measurements correlated well (D-arginine$=0.921\times$L-arginine-5802, $r=0.996$, $P<0.01$). These results demonstrated that this method is suitable for the determination of both arginine enantiomers in microdialysis samples.

3.2. Analytical performance of norepinephrine measurement

Typical chromatograms of NE obtained with standards and microdialysate sample are presented in Fig. 2(A, B). Norepinephrine eluted with a retention time of 4.5 mm. Linear regression analysis of the peak height versus concentration demonstrated linearity for NE in a range from 0 to 30 n*M* ($r=0.99$; $P<0.01$).

The within-run coefficient of variation was 4.3% and 3.3% at 1.2 and 12 n*M*, respectively. Between-day precision was 7.4% and 4.7% for both concentrations. The detection limit of NE was 1 fmol per 20 μl injected.

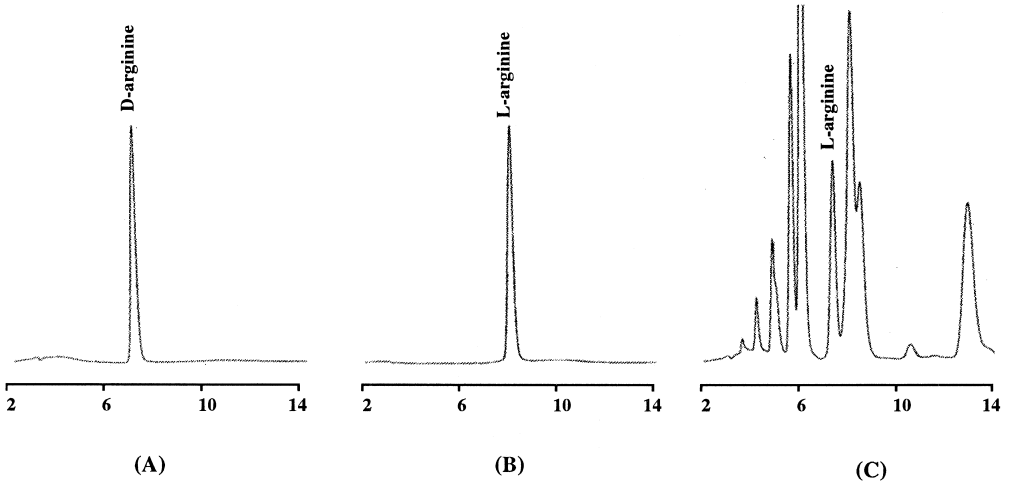

Fig. 1. Chromatograms for arginine. Typical chromatogram of L-arginine standard (A) and D-arginine standard (B). Typical chromatogram of arginine in the rat muscular interstitial fluid microdialysis sample (C).

3.3. In vivo microdialysis

3.3.1. L- and D-arginine measurements

The time course of L- and D-arginine microdialysate concentrations within the rat quadriceps muscle interstitial space is presented in Fig. 3. Endogenous interstitial arginine concentrations rapidly decreased following probe implantation reaching baseline values (40 ± 7 μM) within 40 min. In animals receiving D-arginine, the peak value

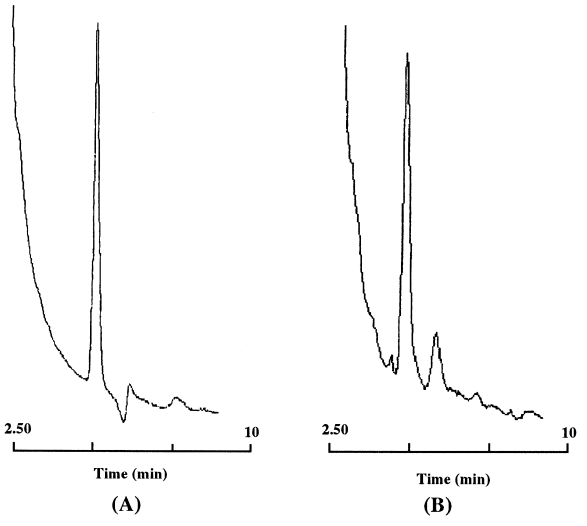

Fig. 2. Chromatograms for norepinephrine. Typical chromatogram of NE standard (A, 1.5 nM). Typical chromatogram of NE in the rat muscular interstitial fluid microdialysis sample (B, 1.4 nM).

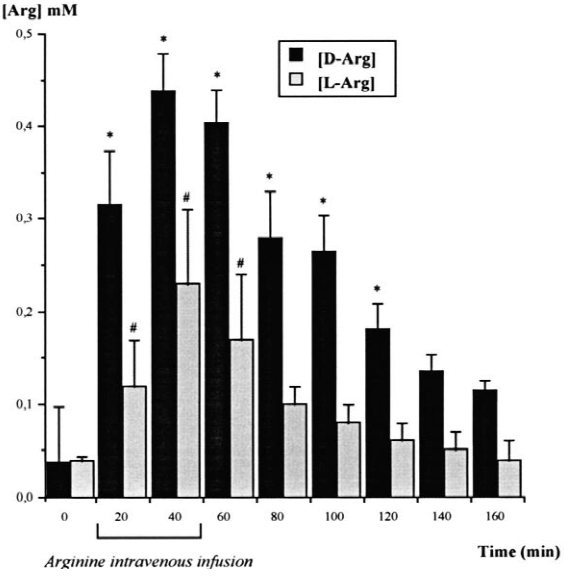

Fig. 3. Time course of arginine microdialysate concentrations in rat muscular interstitial space before and after D-/L-arginine infusion. Values are mean±SEM. L-arg and D-arg designate the groups that received L-arginine and D-arginine intravenously, respectively. Before arginine infusion, only endogenous arginine was detected. *: $P<0.05$ versus baseline value for D-arginine (D-arg, $n=6$), #: $P<0.05$ versus baseline value for L-arginine (L-arg, $n=4$).

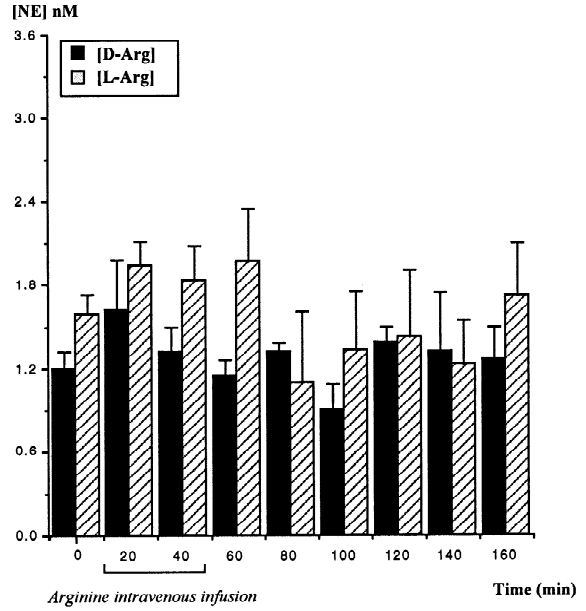

Fig. 4. Time course of norepinephrine (NE) microdialysate concentrations in rat muscular interstitial space before and after D- or L-arginine infusion. Values are mean±SEM. No significant difference was observed in mean norepinephrine microdialysate concentration before, during and after D-arginine (D-arg, $n=6$) or L-arginine (L-arg, $n=4$) infusion in comparison to baseline.

(438±40 μ*M*) was achieved at the end of infusion. This was followed by a slow decrease of D-arginine interstitial concentrations. However, 80 min after the end of D-arginine infusion, interstitial concentrations were still significantly higher than basal endogenous arginine concentrations. The time course of L-arginine interstitial concentrations was different. At the end of the infusion, the peak of L-arginine reached a value that was significantly lower (230±80 μ*M*) compared to D-arginine ($P<0.05$). After the infusion, L-arginine interstitial concentrations decreased rapidly to values similar to those of endogenous arginine concentration observed prior to infusion.

3.3.2. Norepinephrine measurement

The time course of microdialysate NE concentrations before and after L- or D-arginine infusion is depicted in Fig. 4. Baseline dialysate NE levels averaged 1.23±0.22 n*M*. No significant difference was observed in NE dialysate concentrations before, during and after D-arginine or L-arginine infusion.

3.3.3. Hemodynamic parameters

Hemodynamic data for both groups are presented in Table 1. No significant difference was observed between groups during the equilibration period. L-arginine infusion resulted in a significant decrease in MAP values during infusion period when compared with group D-arg ($P<0.05$).

4. Discussion

To the best of our knowledge this is the first reported assay for analysis of both interstitial NE and -arginine within the same tissue volume.

The method for arginine analysis described in this paper was adapted from other previously reported methods used for amino acids measurement in plasma and cerebrospinal fluid [24–26]. Our results demonstrate that this method provides a reproducible and sensitive assay for arginine determination in

Table 1
Time course of hemodynamic parameters in L-arg and D-arg groups[a]

Time (min)									
	Baseline	Infusion period		Post-infusion					
	0	20	40	60	80	100	120	140	160
HR									
L-arg	320±8	339±5	341±7	340±2	333±9	328±11	342±8	321±8	319±6
D-arg	355±11	360±10	352±5	361±9	349±10	345±8	359±9	352±11	357±9
MAP									
L-arg	72±3	52±5*	55±2*	59±3	59±2	57±1	60±3	58±4	61±4
D-arg	78±5	72±3	75±4	71±2	72±3	76±5	73±3	75±5	75±3

[a] Results are expressed as mean±SEM, HR, heart rate; MAP, mean arterial pressure. $P<0.05$ versus D-arg.

microdialysate samples. In particular, its high sensitivity (0.5 pM/20 μl injected) is adequate for detecting physiological concentrations of arginine and is comparable with that of previously published HPLC methods with fluorecence detection.

Concerning the analysis of interstitial NE, our results demonstrate that the HPLC method used provides analytical performances that are comparable with those previously published [20,29]. In particular, its high sensitivity (1 fmol per 20 μl injected) allows the detection of physiological NE concentrations within the muscle interstitial space that are ten times lower than those observed in the heart [30,21].

Although analytical techniques based on HPLC have been reported for L-arginine and NE in plasma, their measurement within tissue interstitial space using the microdialysis technique imposes new challenges. Among them are the low sample volume (40 μl in the present study) and the pH/oxidant status of the microdialysate sample. Working with small volumes is possible because of the microdialysis principles per se (no need for previous sample extraction). The problem raised by the pH/oxidant status of the microdialysate sample is more challenging. As previously recommended [31], all microdialysis samples and standards analyzed in the present study were acidified with EDTA and metabisulfite in order to prevent the degradation of NE and allow the long-term storage of biogenic amines in biological samples for subsequent analysis. Under these conditions, in our experiments, a moderate but reproducible decrease in the assay sensitivity for arginine was observed, when standard curves obtained in the presence or absence of EDTA and metabisulfite were compared (data not shown). This decrease was not time-dependent. Moreover, the linear relation between peak heights and standards in the presence of EDTA and metabisulfite was preserved for both L- and D-arginine up to 500 μ*M* ($r=0.99$ $P<0.01$). Therefore, under these conditions, the assay still permits the detection of physiological concentrations of arginine within the interstitial space. The results of the present study demonstrate that the microdialysis technique permits the monitoring of physiological and pharmacological concentrations of arginine and NE in the same microdialysis sample.

The interstitial concentration of endogenous arginine measured in skeletal muscle was approximately 40 μ*M* and is comparable with previously published data in rat cerebral cortex [32]. The dose of arginine perfused in the present study was equivalent to those previously published [33–35] and resulted in L-/D-arginine dialysate concentrations ranging from 200 to 500 μ*M*. However, the results indicate that the pharmacokinetics of L-arginine and D-arginine are different. This may be due to stereospecific differences in their interaction with arginine transmembrane transporters [12–14], NO synthases [15] or other enzymatic systems involved in arginine metabolism [36,37]. These results are consistent with the time-dependent profile of hemodynamic parameters such as the mean arterial pressure that was recorded in the same animals. The mean arterial pressure was lower in rats infused with L-arginine as compared to rats receiving D-arginine probably because L-arginine was metabolized into NO thus enhancing the endothelium dependent vasodilation [38,39]. On the contrary, D-arginine which was probably not metabolized into NO, remained at a higher interstitial concentrations as compared to L-arginine and had no effect on the mean arterial pressure. In the same animals, the endogenous interstitial concentration of NE in rat skeletal muscle was estimated to be 1.38 n*M* which is consistent with previously published results obtained in pig myocardium [20]. All over the experiment, no significant variation of the interstitial NE concentration was observed whatever the arginine enantiomer infused. These results may be related to the fact that this study was performed on rats maintained under physiological conditions without any stimulation of the sympathetic nervous system.

In conclusion, this study demonstrate that microdialysis in combination with simple and specific HPLC assays can be used for the in vivo monitoring of arginine and NE in the same microdialysis sample allowing the study of biological interactions within the same microenvironment.

References

[1] S. Moncada, E.A. Higgs, FASEB. J 9 (1995) 1319.
[2] D. Ungureanu-Longrois, J.L. Balligand, R.A. Kelly, T.W. Smith, J. Mol. Cell. Cardiol 27 (1995) 155.

[3] D. Ungureanu-Longrois, J.L. Balligand, W.W. Simmons, I. Okada, L. Kobzik, C.J. Lowenstein, S.L. Kunkel, T. Michel, R.A. Kelly, T.W. Smith, Circ. Res. 77 (1995) 494.
[4] P. Schwarz, R. Diem, N.J. Dun, U. Foerstermann, Circ. Res. 77 (1995) 841.
[5] F. Shintani, T. Kinoshita, S. Kanba, T. Ishikawa, E. Suzuki, N. Sasakawa, R. Kato, M. Asai, T. Nakaki, J. Biol. Chem. 271 (1996) 13561.
[6] D.S. Bredt, S.H. Snyder, Annu. Rev. Biochem. 63 (1994) 175.
[7] D.H. Stuehr, O.W. Griffith, Adv. Enzymol. Relat. Areas. Mol. Biol 65 (1992) 287.
[8] S. Nagase, K. Takemura, A. Ueda, A. Hirayama, K. Aoyagi, M. Kondoh, A. Koyama, Biochem. Biophys. Res. Commun. 233 (1997) 150.
[9] P.R. Casino, C.M. Kilcoyne, A.A. Quyyumi, J.M. Hoeg, J.A. Panza, J. Am. Coll. Cardiol. 23 (1994) 844.
[10] A. Calver, J. Collier, P. Vallance, Clin. Sci. 81 (1991) 695.
[11] J.A. Panza, P.R. Casino, D.M. Badar, A.A. Quyyumi, Circulation 87 (1993) 1475.
[12] M.P. Kavanaugh, Biochemistry 32 (1993) 5781.
[13] M.S. Malandro, M.S. Kilberg, Annu. Rev. Biochem. 36 (1996).
[14] W.W. Simmons, E.I. Closs, J.M. Cunningham, T.W. Smith, R.A. Kelly, J. Biol. Chem. 271 (1996) 11694.
[15] T.W. Smith, J.L. Balligand, D.M. Kaye, S.D. Wiviott, W.W. Simmons, X. Han, T. Michel, K. Singh, R.A. Kelly, J. Card. Fail. 2 (1996) S141.
[16] T. Akiyama, T. Yamazaki, I. Ninomiya, Am. J. Physiol. 261 (1991) H1643.
[17] H. Benveniste, P.C. Huettemeier, Prog. Neurobiol. 35 (1990) 195.
[18] B.J. Freda, R.S. Gaitonde, R. Lillaney, A. Ally, Brain Res. 828 (1999) 60.
[19] A. Hallstroem, A. Carlsson, L. Hillered, U. Ungerstedt, J. Pharmacol. Methods 22 (1989) 113.
[20] P.M. Mertes, J.P. Carteaux, Y. Jaboin, G. Pinelli, K. el Abassi, C. Dopff, J. Atkinson, J.P. Villemot, C. Burlet, M. Boulange, Transplantation 57 (1994) 371.
[21] P.M. Mertes, K. el-Abbassi, Y. Jaboin, C. Michel, B. Beck, G. Pinelli, J.P. Carteaux, J.P. Villemot, C. Burlet, J. Mol. Cell. Cardiol. 28 (1995) 1995.
[22] D.O. Scott, L.R. Sorenson, K.L. Steele, D.L. Puckett, C.E. Lunte, Pharmaceut. Res. 8 (1991) 389.
[23] M. Mueller, R. Schmid, A. Georgopoulos, A. Buxbaum, C. Wasicek, H.G. Eichler, Clin. Pharmacol. Ther. 57 (1995) 371.
[24] K.Q. Do, C.J. Lauer, W. Schreiber, M. Zollinger, U. Gutteck-Amsler, M. Cuenod, F. Holsboer, J. Neurochem. 65 (1995) 2652.
[25] R.F. Goldsmith, J.W. Earl, A.M. Cunningham, Clin. Chem. 33 (1987) 1736.
[26] V. Rizzo, A. Anesi, L. Montalbetti, G. Bellantoni, R. Trotti, G.V. Melzi d'Eril, J. Chromatogr. A 729 (1996) 181.
[27] B.A. Donzanti, B.K. Yamamoto, Life. Sci. 43 (1988) 913.
[28] A. Lehmann, J. Neurochem. 53 (1989) 525.
[29] T. Yamazaki, T. Akiyama, T. Shindo, J. Chromatogr. B 670 (1995) 328.
[30] B. Gronlund, A. Astrup, P. Bie, N.J. Christensen, Clin. Sci. 80 (1991) 595.
[31] D.L. Palazzolo, S.K. Quadri, Life. Sci. 47 (1990) 2105.
[32] M. Fabricius, L.H. Jensen, M. Lauritzen, Brain Res. 612 (1993) 61.
[33] A. Kawabata, Y. Fukuzumi, H. Takagi, Eur. J. Pharmacol. 218 (1992) 153.
[34] E. Morikawa, M.A. Moskowitz, Z. Huang, T. Yoshida, K. Irikura, T. Dalkara, Stroke 25 (1994) 429.
[35] K. Nakanishi, J. Vinten-Johansen, D.J. Lefer, Z. Zhao, W.C.d. Fowler, D.S. McGee, W.E. Johnston, Am. J. Physiol. 263 (1992) H1650.
[36] T. Gotoh, T. Sonoki, A. Nagasaki, K. Terada, M. Takiguchi, M. Mon, FEBS Lett. 395 (1996) 119.
[37] M. Takiguchi, M. Mon, Biochem. J. 312 (1995) 649.
[38] P. Clarkson, M.R. Adams, A.J. Powe, A.E. Donald, R. McCredie, J. Robinson, S.N. McCarthy, A. Keech, D.S. Celermajer, J.E. Deanfield, J. Clin. Invest. 97 (1996) 1989.
[39] K. Egashira, Y. Hirooka, T. Kuga, M. Mohri, A. Takeshita, Circulation 94 (1996) 130.