ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
13 (1995) 1339–1348

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

Adsorptive stripping voltammetry of nicardipine at a HMDE; determination of trace levels nicardipine in blood and urine

Dagmar Obendorf *, Gottfried Stubauer

Institut für Analytische Chemie und Radiochemie der Universität Innsbruck, Innrain 52 a, A-6020, Innsbruck, Austria

Received for review 17 March 1995

Abstract

The redox behaviour of nicardipine, a 1,4-dihydropyridine calcium antagonist, has been studied in different media on mercury, glassy carbon, gold and platinum electrodes using various voltammetric techniques. A highly sensitive adsorptive stripping voltammetric method for the determination of nicardipine based on adsorption of the drug onto mercury, followed by differential pulse voltammetric determination of the surface species, is described. All factors (pH, supporting electrolyte, accumulation potential and time, etc.) influencing adsorption as well as voltammetric response are discussed. The application of adsorptive stripping voltammetry at the hanging mercury drop electrode (HMDE) to the determination of trace levels of nicardipine in human urine and blood is illustrated, without an extraction procedure being necessary prior to the voltammetric measurement. A limit of detection of 4.8 ng per ml urine and 34 ng per ml blood is found with a mean recovery of nicardipine in urine and blood of 97%. The mean relative error does not exceed 6.5%.

Keywords: Adsorptive stripping voltammetry; Nicardipine; 1,4-Dihydropyridine calciumantagonist; Determination in biological fluids

1. Introduction

Nicardipine (Fig. 1) (1,4-dihydro-2,6-dimethyl-4-[3-nitrophenyl]-3,5-pyridinecarboxylic acid, methyl-2-[methyl(phenylmethyl)amino]-ethyl ester) belongs to the group of 1,4-dihydropyridine calcium channel antagonists that, owing to their potent vasodilating properties, have become very important in the treatment of heart deseases such as angina pectoris and arterial hypertension.

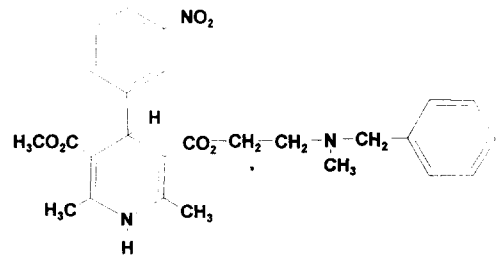

Fig. 1. Structure of nicardipine.

Since the discovery of the vasodilating activity of nifedipine, a calcium channel blocker, many analogues of 1,4-dihydropyridine calcium antagonists have been synthesized with somewhat different pharmacological and therapeutic profiles. As many of these drugs are highly potent and efficient at relatively low doses, analytical techniques of high selectivity and sensitivity are required for therapeutic monitoring and pharmacokinetic studies. Fur-

* Corresponding author.

SSDI 0731-7085(95)01554-X

ther analytical problems arise from extensive metabolism to numerous metabolites. Various methods have been developed for the determination of dihydropyridine calcium channel blockers, including gas chromatography with different detection modes such as mass spectrometry (MS) detection, electron-capture detection (ECD) and nitrogen or flame-ionization detection, as well as high-performance liquid chromatography (HPLC) with spectrophotometric or electrochemical detection [1,2].

The main problems encountered using chromatographic methods are either the need for derivatization, e.g. in the case of gas chromatography, or the need for time-consuming extraction procedures and pretreatment of biological samples prior to the measurement. Therefore, it is still necessary to develop methods for drug determination, even in complex biological matrices that are at the same time sensitive, accurate, quick and easy to use. Electroanalytical techniques have proved to be useful tools in the analysis of a wide range of pharmaceuticals [3–5], with the advantage of being less sensitive to matrix effects than other methods.

However, during the past few years only a few papers on the electrochemical behaviour of nifedipine and some related 1,4-dihydropyridines have appeared in the literature, including voltammetric investigations of the redox mechanism [6,7], polarographic studies of photodegradation products [8,9] and the application of adsorptive stripping voltammetry at the hanging mercury drop electrode (HMDE) for the determination of nifedipine in serum [10]. Wang et al. investigated the redox behaviour of nicardipine and developed an adsorptive stripping method for the determination of nicardipine in diluted urine samples using glassy carbon and carbon paste electrodes [11].

The aim of the present work was to develop a sensitive method for the determination of nicardipine in biological fluids such as blood and urine without any extraction procedure being necessary prior to the voltammetric measurement. For this purpose, the redox mechanism of nicardipine was studied in different media and with different working electrodes to find out the most sensitive instrumental conditions. None of the studies published so far have taken into account that nicardipine can be adsorbed at the mercury electrode. In order to see whether this phenomenon can be used as a preconcentration step prior to a voltammetric measurement, the adsorption properties of nicardipine at the mercury electrode as well as on gold and glassy carbon electrodes were explored. All factors that may influence both the accumulation process and the voltammetric response are presented in this paper. The application of the procedures established to the determination of nicardipine in blood and urine is discussed.

2. Experimental

2.1. Apparatus and reagents

Differential pulse voltammetry and adsorptive stripping experiments were carried out on a (EG&G Princeton Applied Research) PAR 264 A stripping analyzer equipped with a Philips PM 8133 X-Y recorder. For reductions, a PAR 303A static mercury drop working electrode, an Ag/AgCl (saturated KCl) reference electrode and a platinum wire auxiliary electrode were used. Oxidations were performed on a rotating-disk working electrode equipped with different disk materials (Pt, C, Au) (diameter 3 mm, Metrohm) used in non-rotated mode, a glassy carbon counter electrode and a silver/silver chloride/(KCl, Friscolyt®) reference electrode. The cell was covered with aluminium foil to prevent decomposition of the drug in daylight. All measurements were performed at room temperature. Magnetic stirring (reductions) or rotating of the electrode (oxidations) was employed during the preconcentration period.

Stock solutions (either 1.0×10^{-3} M in water or 5.0×10^{-5} M in 10% MeOH) of nicardipine (Sigma) were stored in the dark under refrigeration to avoid decomposition. The compound and the electrolytes were used without further purification. The supporting electrolytes were phosphate, citrate and tris buffer (Fluka, Sigma, p.a.) of different pH and an ionic strength of 0.1 mol l^{-1} prepared in purified water. A small amount of methanol, ethanol or acetonitrile was added whenever modification of the adsorption properties of nicardipine was intended. Blood and urine samples were obtained from healthy volunteers. Blood samples were heparinized to prevent coagulation and kept in the dark at 4 °C.

2.2. Procedures

Adsorptive stripping differential pulse voltammetry

5 ml buffer and 5 ml purified water (or a mixture of water and organic solvent) were added to the polarographic cell (final ionic strength: 0.05 mol l^{-1}) and deaerated by passage of nitrogen or argon for 8 min. A preconcentration potential (−0.5 V or 0 V) was applied for a selected period of time while the solution was stirred. After a 15 s rest, a differential pulse scan (pulse amplitude 50 mV, scan rate 5 mV s^{-1}) towards more negative (reduction) or more positive (oxidation) potential values was started to obtain the stripping voltammograms. Before adding a specific amount of sample to the solution a stripping voltammogram of the blank supporting electrolyte was recorded under the same conditions. Determination of the nicardipine concentration was accomplished by the method of internal standard addition.

Adsorptive stripping voltammetry in whole blood

6 ml supporting electrolyte (buffer pH 11 or mixtures of buffer with organic solvents, ionic strength 0.05 mol l^{-1}) and 0.5 ml whole blood were degassed for 8 min. Increasing amounts of nicardipine were added and the procedure continued as described above. For recovery experiments, various amounts of nicardipine standard solution were added to 0.6 ml blood. An aliquot of 0.5 ml spiked blood was added to 6 ml supporting electrolyte and proceeded as before.

Adsorptive stripping voltammetry in plasma

0.5 ml ethanol and 0.3 ml 5% $ZnSO_4$ were added to 0.3 ml blood, and the mixture was centrifuged for 10 min at 13 000 rpm in a high-speed centrifuge. An aliquot (0.9 ml) of the clear solution was added to a mixture of 6 ml phosphate buffer (pH 11.7, ionic strength 0.1 mol l^{-1}) and 1 ml ethanol and the pH was adjusted to about 12 by addition of 40 µl 2 M NaOH. The solution was deaerated for 8 min, and after a preconcentration period of 10 s at −0.50 V the adsorptive stripping voltammogram was recorded.

Differential pulse palarography in urine

A solution of 5 ml urine, 2 ml ethanol and 1 ml water was adjusted to pH 10.6–11.0 by addition of about 0.5 ml 1 M NaOH, and degassed for at least 8 min. After registration of the voltammogram of the blank supporting electrolyte, calibration plots were obtained by differential pulse polarographic measurements of increasing nicardipine concentrations (scan rate 5 mV s^{-1}, pulse amplitude 50 mV, sensitivity 500 nA).

Adsorptive stripping voltammetry in urine

A mixture of 0.5 ml urine and 0.5 ml ethanol was adjusted to pH 11 by addition of 0.1 ml 1 M NaOH. After addition of 0.5 ml 5% $ZnSO_4$ the solution was centrifuged for 10 min at 13 000 rpm in a high-speed centrifuge. An aliquot (1 ml) of the clear solution was added to a mixture of 6 ml phosphate buffer pH 12 and 1 ml ethanol, and spiked with various amounts of nicardipine. An accumulation potential of −0.60 V was applied for 60 s, and after a 15 s rest a differential pulse scan towards more negative potential values was employed.

For recovery experiments, known amounts of nicardipine were added to urine and blood, and the procedure continued as described before.

3. Results and discussion

4-Nitrophenyl substituted 1,4-dihydropyridines contain at least two electroactive sites: an aromatic nitro group that can be reduced in protic media in a four-electron step to a hydroxylamine derivative [9,12] and a dihydropyridine ring that can be oxidized to the corresponding pyridine ring in a two-electron step. In the case of nicardipine, even a third electroactive group, a tertiary amine, is present in the molecule. The reaction pattern of the anodic oxidation of amines depends strongly on the reaction conditions, e.g. the nucleophilic strength of the solvents. In the presence of water, the anodic oxidation generally brings about dealkylation of the aliphatic amines leading finally to ammonia and the corresponding aldehydes [13]. Since oxidation of the tertiary amine group in aqueous buffer solutions occurs at more positive potential than oxidation of the dihydropyridine-ring, oxidative voltammetric measurements of 1,4-dihydropyridines are in most cases based on oxidation of the dihydropyridine ring.

However, it can be assumed that the adsorption behaviour of nicardipine will be modified

by the presence of the tertiary amine group in the molecule, so that nicardipine will be more lipophilic than nifedipine and most probably adsorb more readily onto stationary electrodes.

3.1. Reduction of nicardipine at the HMDE

The reduction behaviour of nicardipine at the mercury electrode was studied in citrate and phosphate buffers with different pH values using differential pulse polarography. In the pH range between 4.0 and 12.0 only one peak is observed which corresponds to the irreversible reduction of the aromatic nitro group. The reduction peak potential shifts linearly to more negative values as pH increases following the equation: $E_p(V) = -0.12 - 0.054\,pH$. Such a shift is expected whenever protons are involved in the redox process. The intensity of the peak current increases with increasing pH values, reaching a maximum value at about pH 10–12. Calibration plots of higher nicardipine concentrations in neutral or acidic medium showed deviations from linearity, so that for the rest of the experiments basic buffer solutions were preferred.

Normally, it is assumed that all drugs belonging to the family of nitrophenyl substituted 1,4-dihydropyridine calcium antagonists are extremely light sensitive and follow the same photodegradation path: oxidation of the dihydropyridine ring and conversion of the nitrophenyl group into a nitrosophenyl group. Since photodecomposition involves a change in the redox behaviour, the extent of photodegradation of the compound can be studied electrochemically [8]. In general, in case of the 1,4-dihydropyridine calcium antagonists, a decrease in the peak corresponding to the reduction of the nitro group as well as the appearance of several new waves is reported as a consequence of the exposure to light. In the case of nicardipine, no decrease in the reduction peak current after exposure to artificial light or room daylight could be observed within hours. Thus, compared to nifedipine, this drug seems to be relatively insensitive to irradiation by daylight, and in general no special precautions are necessary to prevent decomposition.

In contradiction to literature reports [11], nicardipine can be adsorbed at the mercury electrode and this phenomenon can be used as an effective preconcentration step prior to the voltammetric measurement. Thus, a considerable increase in sensitivity can be achieved by application of adsorptive stripping voltammetry to the determination of nicardipine. Variations in experimental parameters such as pH and composition of supporting electrolyte, accumulation potential and time were performed in order to optimize adsorption conditions. Adsorption, and thus the height of the stripping peak current, are sensitive to the pH value of the supporting electrolyte. The best results concerning peak current enhancement were obtained in citrate and phosphate buffer at pH 11.5 and 12 respectively, whereas calibration plots in tris buffer pH 9.3 showed deviations from linearity and were less reproducible.

Addition of organic solvents (acetonitrile, methanol, ethanol) to buffer solutions of nicardipine affects the voltammetric response. With increasing percentage of organic solvent (Fig. 2(b)), the peak potential is shifted to more negative potential values, and with increasing amount of acetonitrile in phosphate buffer pH 8 (from 0 to 25%) a sharp increase in the peak current is observed (Fig. 2(a)). However, a rapid decrease in the signal occurs when the percentage of acetonitrile exceeds 25%. Almost the same results are obtained with mixtures of methanol, ethanol and other types of buffer solutions (citrate, tris buffer). In more basic buffer solutions, an increase in the peak current can only be observed if small quantities of organic solvents are added. A peak current maximum is obtained at a percentage of about 10% independent of the organic solvent used. Higher concentrations of organic solvent lead to a rapid decrease in and even disappearance of the current signal.

The influence of ionic strength of the supporting electrolyte on the peak height is negligible within 0.04 and 0.10 mol l^{-1}. A higher ionic strength than 0.10 mol l^{-1} leads to a decrease in the peak current.

No great effect of accumulation potential on peak intensity was observed if the preconcentration step was carried out in closed circuit at a preconcentration potential between -0.20 V and -0.50 V. Therefore, in most cases an accumulation potential of -0.50 V was chosen for the analytical process. Fig. 3 shows the influence of preconcentration time on the peak current for lower nicardipine concentrations (20 ppb = 4.2×10^{-8} M). A linear increase in the peak current with accumulation time is observed (Fig. 3). A preconcentration of 5 min, for example, leads to a peak current that is

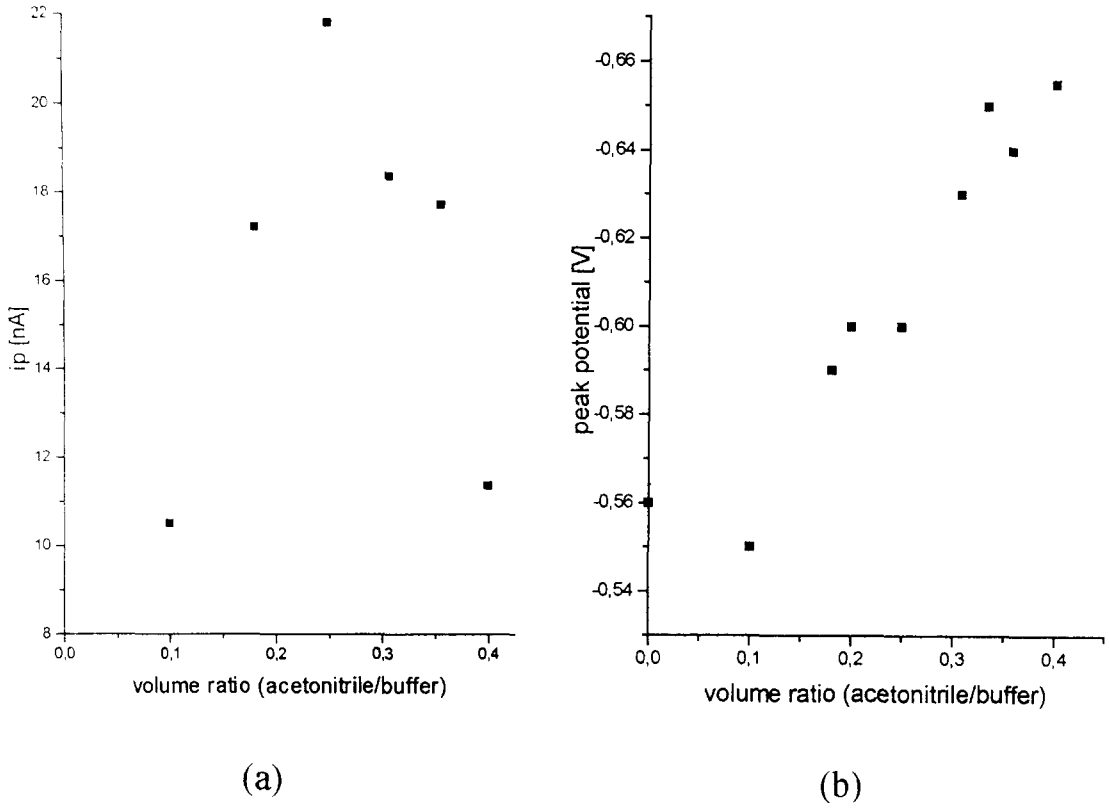

Fig. 2. Dependence of (a) reduction peak current and (b) reduction peak potential on the volume ratio acetonitrile/phosphate buffer pH 8. Nicardipine concentration 5 ppb (1.05×10^{-8} M), $E_{acc} = -0.300$ V, $t_{acc} = 60$ s.

about 25 times larger than that obtained without preconcentration. The influence of nicardipine concentration was studied at three different accumulation times (10, 30 and 60 s) and the resulting calibration plots for the concentration range 10 ppb–1.0 ppm (2.1×10^{-8}–2.1×10^{-6} M) are shown in Fig. 4. It can be seen that the linearity range depends on the accumulation time used. The largest linear range from 10 ppb (2.1×10^{-8} M) to 0.78 ppm (1.6×10^{-6} M) with a correlation coefficient of 0.9992 (slope 0.96 nA/10^{-9} M) is reached with a preconcentration time of 10 s. For a 30 s preconcentration, the response is linear in the range from 10 ppb (2.1×10^{-8} M) to 0.58 ppm (1.2×10^{-6} M) with a correlation coefficient of 0.9988 and a slope of 1.58 nA/10^{-9} M (intercept 49 nA). For an accumulation time of 60 s, surface saturation is reached at concentrations higher than 300 ppb (6.2×10^{-7} M) and deviation from linearity occurs. Thus, the ultimate choice of preconcentration time depends on the concentration range studied. The lowest concentration measured was 0.1 ppb (2.08×10^{-10} M), with a preconcentration time of 180 s and a preconcentration potential of −0.3 V.

Precision was determined by five successive measurements of solutions containing 40, 80 and 380 ppb (8.3×10^{-8}, 1.7×10^{-7} and 7.9×10^{-7} M, respectively) of nicardipine in phosphate buffer pH 11.5 using a preconcentration time of 60 s. The relative standard deviation was found to be lower than 3.5% and the relative error lower than 1.5%.

3.2. Oxidation of nicardipine

Several measurements with different electrochemical techniques using various electrodes and supporting electrolytes were performed in order to obtain information about the oxidation redox mechanism and to find out possible determination alternatives to the method described in Ref. [11]. Nicardipine contains two redox centres that can be oxidized, a dihydropyridine ring and a tertiary amine group. However, cyclovoltammetric studies in nonaqueous solvents (in acetonitrile/0.1 M

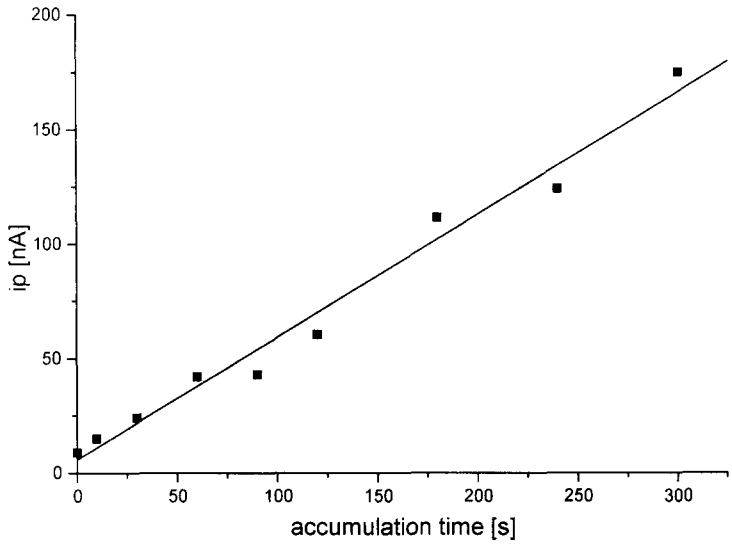

Fig. 3. Influence of accumulation time on the stripping peak current intensity. Nicardipine concentration 20 ppb (4.2×10^{-8} M) in citrate buffer pH 11.5, $E_{acc} = -0.50$ V.

tetrabutylammonium hexafluorophosphate) on glassy carbon and platinum electrodes reveal only one irreversible peak at about 1.37 V (vs. Ag/AgCl) in the potential range from 0 to 2.0 V, which can be attributed to the two-electron oxidation of the dihydropyridine ring via an $EC_{irr}E$ mechanism. All experimental data are in good agreement with the redox mechanism postulated in the literature [6]. No further oxidation peaks that might be attributed to the oxidation of the amino group could be detected within the accessible potential range.

A completely different redox mechanism, however, has to be assumed for the oxidation of nicardipine in acetontrile/0.1 M tetrabutylammonium hexafluorophosphate on a gold electrode. In the range from 0 to 3.0 V, one irreversible wave at about 1.9 V (vs. Ag/AgCl) is observed. Increased peak height and a more symmetrical peak shape indicate catalytic character of the wave. This phenomenon can only be observed in anhydrous acetonitrile, whereas in aqueous solutions of acetonitrile or pure buffer no catalytic waves occur. It seems that nicardipine or intermediates of its oxidation process catalyze the oxidation of acetonitrile, although acetonitrile itself is electroinactive on a gold electrode within a positive potential range from 0 to 3.0 V. Thus, homogeneous reaction of acetonitrile with the postulated intermediate of the nicardipine oxidation, the dihydropyridine radical cation [6], leads to regeneration of the starting material nicardipine and oxidative dimerization of acetonitrile. Nicardipine can be reoxidized, which accounts for the increased peak current of the catalytic wave. This redox mechanism can only occur in anhydrous solvents where the intermediately formed dihydropyridine radical cation is sufficiently stable, which is not the case in aqueous solvents.

Although several important and sensitive electroanalytical assays, for example electrochemical detection of carbohydrates and determination of proteins and amino acids, are based on catalytic processes [3–5,14], catalytical waves are prone to numerous interferences and not easily used for practical quantitative purposes [15]. In the case of nicardipine, all attempts to develop a sensitive method based on the catalytic oxidation process in anhydrous acetonitrile have proved unsuccessful. The main problems arose from insufficient reproducibility of the catalytic wave as well as deviations from linearity between peak current and nicardipine concentration. Moreover, since the appearance of the catalytic wave depends mainly on the stability and the lifetime of the intermediate cation radical, determination of nicardipine in aqueous biological samples would require tedious extraction procedures from the aqueous matrix before the analytical procedure could be performed in anhydrous acetonitrile on a gold electrode.

Oxidative electroanalytical measurements of nicardipine in aqueous media can only be achieved by adsorptive stripping voltammetry on a glassy carbon electrode according to Ref. [11]. The measurement is based on the oxidation of the dihydropyridine ring which occurs

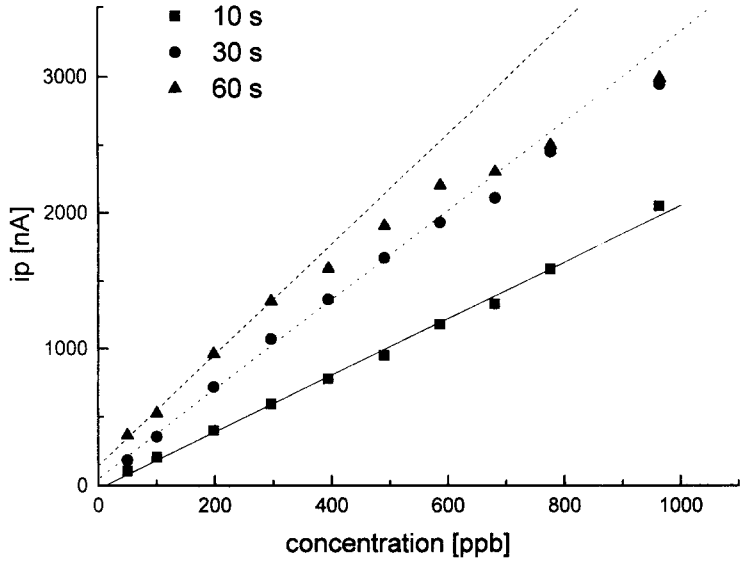

Fig. 4. Calibration plots of nicardipine in citrate buffer pH 11.5 at different preconcentration times. Concentration range of nicardipine from 10 to 1000 ppb (2.1×10^{-8}–2.1×10^{-6} M). $E_{acc} = -0.50$ V, $t_{acc} = 10$, 30 and 60 s.

at about 0.5 V (vs. Ag/AgCl). Gold and platinum electrodes are less convenient, because with these electrodes the accessible potential range is much smaller and the oxidation peak for the oxidation of the dihydropyridine ring occurs almost outside the potential window. However, compared to reductive measurements at the HMDE, oxidation on a glassy carbon electrode is less sensitive and much more care has to be taken in the surface renewal and reproducibility of individual measurements. Application of adsorptive stripping voltammetry on a glassy carbon electrode to the direct determination of nicardipine in urine or blood was not possible, and either extraction of the drug from the biological sample prior to the voltammetric measurement or medium exchange as reported by Wang et al. [11] had to be performed. However, direct determination of nicardipine in complex biological matrices such as urine and blood can be achieved by reductive measurements at the mercury electrodes.

3.3. Application of adsorptive stripping voltammetry at the HMDE to the determination of nicardipine in urine and blood

Determination of nicardipine in urine

Without sample preparation other than 1:2 dilution of urine with phosphate buffer pH 11.5, differential pulse polarography can be used for the determination of nicardipine in urine because the interference by urine components during differential pulse polarographic measurements is comparatively small. Only a small peak at -0.7 V is observed in the blank voltammogram. After addition of about 30% ethanol or methanol to the supporting electrolyte, linear calibration plots were obtained for increasing nicardipine concentrations in urine in the range from 96 ppb (2.0×10^{-7} mol l^{-1}) to 1.15 ppm (2.4×10^{-6} mol l^{-1}) (slope 330 nA ml μg^{-1}, correlation coefficient 0.999). The detection limit (3σ) of 30.6 ng nicardipine per ml urine was calculated. Recovery experiments were less satisfying, because relative large differences existed between individual measurements. The mean recovery was found to be 90%.

In order to improve detection limit and recovery, adsorptive stripping experiments were tried. However, without pretreatment of the urine sample, difficulties arose because of the high content of adsorbing substances in urine competing with nicardipine for the adsorption sites on the electrode. The blank urine voltammogram showed a huge interfering peak at about -0.70 V, close to the reduction potential of nicardipine, which can most probably be attributed to disulphide or thiol groups containing proteins, peptides or free amino acids in urine. Those compounds adsorb more readily onto mercury electrodes than nicardipine, forming stable mercury complexes [3] and thus hampering the preconcentration of nicardipine on the electrode surface. Attempts to suppress this peak selectively by variation of experimen-

tal parameters such as accumulation potential, ionic strength and pH of the supporting electrolyte, as well as addition of Cu^{2+} to the solution, were not successful. Further experiments with cystein, as a model substance for the interfering thiol group containing compounds in urine, revealed that the cystein peak at −0.7 V can be suppressed almost completely by addition of a 5% Zn^{2+} solution and appropriate selection of the accumulation potential. This result was applied to the urine samples. Fig. 5 shows the influence of increasing $ZnSO_4$ concentration on the peak height of the interfering stripping peak in urine. After a quick and easy to perform sample preparation (as described in the Experimental section), highly sensitive adsorptive stripping voltammetry can be used for the determination of nicardipine in urine (Fig. 6). A linear dependence of the peak current was obtained for an addition of 55–275 ng nicardipine to 8 ml voltammetric solution. The resulting calibration plot was

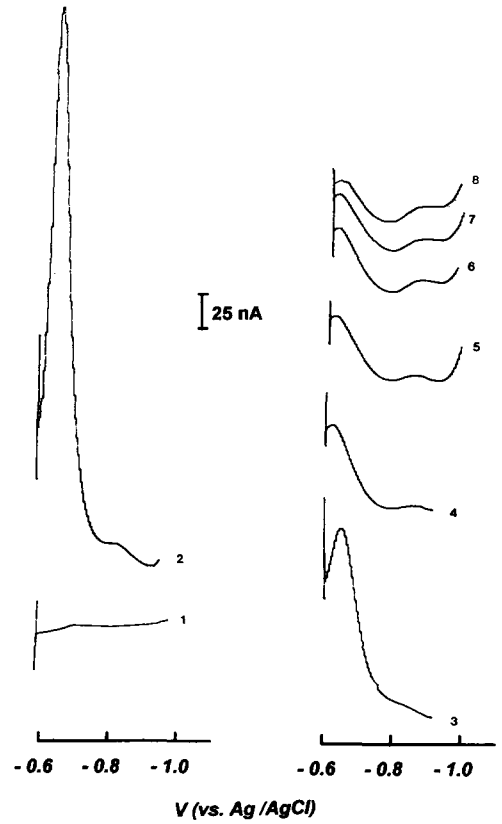

Fig. 5. Influence of increasing Zn^{2+} concentration on the interfering adsorptive stripping peak in urine (E_{acc} = −0.50 V, t_{acc} = 60 s): (1) Blank supporting electrolyte (6 ml phosphate buffer (pH 12, I = 0.1 M) and 1.5 ml ethanol); (2) after addition of 400 µl untreated urine; (3–8) successive addition of 10–80 µl 5%$ZnSO_4$ solution.

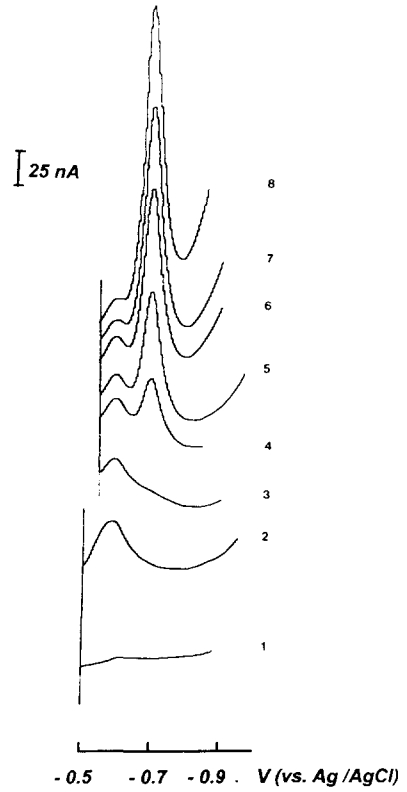

Fig. 6. Determination of nicardipine in urine samples. Adsorptive stripping voltammograms (E_{acc} = −0.50 V, t_{acc} = 60 s): (1) blank supporting electrolyte (6 ml phosphate buffer (pH 12, I = 0.1 M), 1.5 ml ethanol); (2) after addition of 500 µl pretreated urine sample, (3) same as (2) with different stripping conditions, E_{acc} = −0.55 V, t_{acc} = 120 s; (4–8) successive addition of 10 µl of 1.16×10^{-5} M nicardipine standard (55–275 ng). Stripping conditions as in (3).

adjusted to the equation; i_p(nA) = −5.6 + 2.8C (nmol l^{-1}) (correlation coefficient 0.9998). An adjustment of the pH of the urine sample about 11 before centrifugation and addition of ethanol is necessary to prevent co-precipitation of nicardipine. Increasing the amounts of $ZnSO_4$ added to the urine sample leads to an increase in the sensitivity of the measurement as well as an increase in the rate of recovery. The best results were obtained with solutions containing equal volumes of urine, 5% $ZnSO_4$ and ethanol. Fig. 7 shows an example for a recovery experiment of nicardipine in urine. For a 60 s preconcentration at an accumulation potential of −0.6 V, the determination of nicardipine in urine was possible in the range of 42–250 ng ml^{-1} urine (8.8×10^{-8}–5.2×10^{-7} M). The resulting linear calibration plot follows the equation: i_p (nA) = −3.2 + 2.3C (nmol l^{-1}) (correlation coefficient 0.9982). The precision, expressed in

terms of relative standard deviations, was 1.9%. The mean recovery in the range mentioned above was found to be 97% with a mean relative error of 6.5%, and the limit of detection (estimated as the concentration corresponding to a signal-to-noise ratio of 3) was 4.8 ng per ml urine (1.0×10^{-8} M).

Determination of nicardipine in blood

The determination of nicardipine in whole blood diluted with buffer is possible by means of adsorptive stripping voltammetry. However, as in the case of urine, the high content of serum proteins influences the sensitivity of the measurement as well as the recovery of nicardipine. Using more basic buffer solutions (pH > 9.5) in order to work within the pH range for optimal sensitivity of the nicardipine determination leads to the occurrence of a large interfering peak at −0.63 V. This peak increased more readily than nicardipine with increasing accumulation time, rendering more sensitive measurements impossible. Thus, without pretreatment of the blood samples, really satisfying measurements in whole blood were not possible and only comparably high concentrations could be determined. A linear calibration plot was obtained only within a relatively high concentration range of 4–56 μg ml^{-1} blood (8.35×10^{-6}–1.16×10^{-4} M) with a preconcentration period of 60 s at a potential of −0.5 V (sensitivity 2.7 nA/1.0×10^{-7} M, correlation coefficient 0.997). The detection limit was estimated to be 1.14 μg per ml blood (2.38×10^{-6} M).

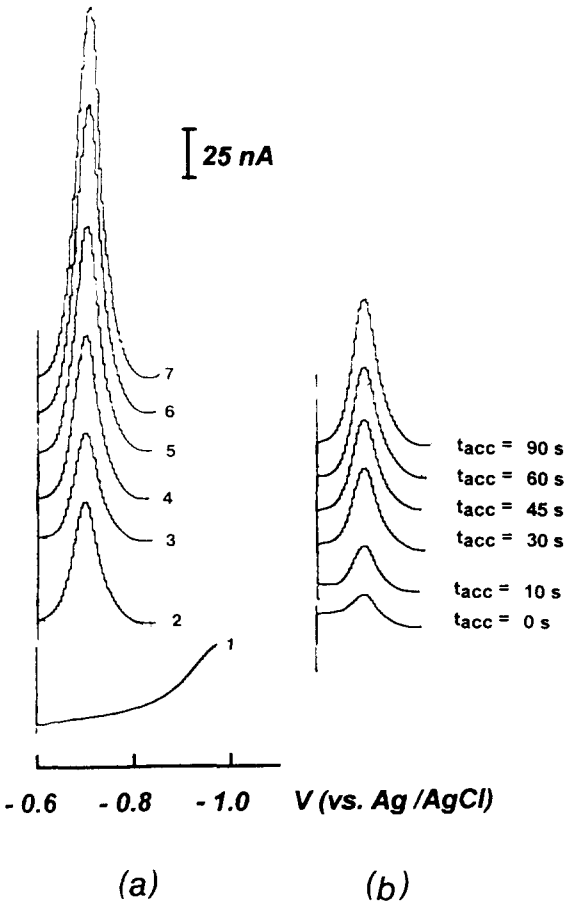

Fig. 7. Recovery experiment of nicardipine in urine samples. (a) Adsorptive stripping voltammograms ($E_{acc} = -0.60$ V, $t_{acc} = 60$ s): (1) blank supporting electrolyte (6 ml phosphate buffer (pH 12, $I = 0.1$ M), 1 ml ethanol); (2) after addition of 1.0 ml pretreated urine sample with nicardipine previously added; (3–7) successive addition of 33 μl of 3.48×10^{-6} M nicardipine standard (56–224 ng). (b) Effect of preconcentration time on the peak height in solution (2).

We tried several sample preparation methods that are generally applied to eliminate the influence of proteins. The application of a similar procedure as described for the urine samples led to the best results and a drastic improvement in the sensitivity of the adsorptive stripping measurement in blood. The best results as to sensitivity and recovery were obtained with a mixture of blood–5%$ZnSO_4$–ethanol (3:3:5, v/v), and adjustment of the pH of the final solution containing supporting electrolyte and an aliquot of the centrifuged blood to 12. An accumulation potential of −0.5 V was applied for 10 s. Although a higher accumulation time had no remarkable effect on the sensitivity of the measurement, the sensitivity of adsorptive stripping voltammetry is much higher than the sensitivity that can be reached by mere differential pulse polarography without preconcentration.

With an optimized procedure (Fig. 8), nicardipine was determined in blood in the concentration range of 0.38–1.44 μg/ml blood (7.9×10^{-7}–3.0×10^{-6} M). A linear dependence of the peak current on concentration was obtained following the equation: $i_p(\text{nA}) = 0.8 + 57C$ (nmol l^{-1}) (correlation coefficient 0.9989). The detection limit (estimated as the concentration corresponding to a signal-to-noise ratio of 3) was 34 ng per ml blood (7.1×10^{-8} M) and the mean recovery was 97% with a mean relative error of 6.5%. The precision expressed in terms of relative standard deviation was 3%.

4. Conclusions

The determination of nicardipine by adsorptive stripping voltammetry at the HMDE is

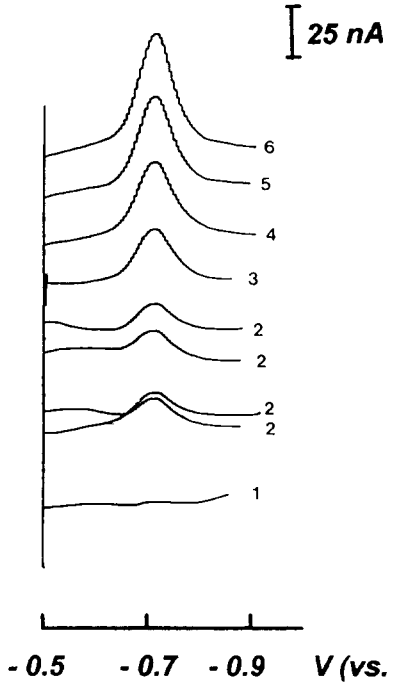

Fig. 8. Determination of nicardipine in blood samples. (a) Adsorptive stripping voltammograms ($E_{acc} = -0.50$ V, $t_{acc} = 10$ s): (1) blank supporting electrolyte (6 ml phosphate buffer (pH 12, $I = 0.1$ M), 1 ml ethanol); (2) after addition of 0.9 ml pretreated blood sample with nicardipine previously added (repeated measurements); (3–6) successive addition of 30 μl of 4.85×10^{-6} M nicardipine standard (70–280 ng).

simple, cheap and not time-consuming, and the results are adequately accurate and precise. With the developed procedure, lower detection limits can be reached compared to adsorptive stripping measurements at carbon electrodes and, moreover, determination of nicardipine in biological samples is possible without an extraction procedure being necessary prior to the voltammetric measurement. However, further studies will have to deal with the investigation of the influence and probable interference of metabolites and the development of a fully validated method. The application of the developed voltammetric method to the determination of other calcium antagonists is in progress, and the results will be presented in a forthcoming paper.

Acknowledgements

The authors thank Professor Dr. H. Glossmann and Doz. Dr. J. Striessnig (Institut für Biomedizinische Pharmakologie, University of Innsbruck) for providing 1,4-dihydropyridine calcium antagonists. Financial support by the Österreichische Nationalbank (project No. 4778) is gratefully acknowledged.

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