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Chip-based biosensor for the detection of low adrenaline concentrations to support adrenal venous sampling

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ABSTRACT

A chip-based amperometric biosensor referring on using the bioelectrocatalytical amplification principle for the detection of low adrenaline concentrations is presented. The adrenaline biosensor has been prepared by modification of a platinum thin-film electrode with an enzyme membrane containing the pyrroloquinoline quinone-dependent glucose dehydrogenase and glutaraldehyde. Measuring conditions such as temperature, pH value, and glucose concentration have been optimized to achieve a high sensitivity and a low detection limit of about 1 nM adrenaline measured in phosphate buffer at neutral pH value. The response of the biosensor to different catecholamines has also been proven. Long-term stability of the adrenaline biosensor has been studied over 10 days. In addition, the biosensor has been successfully applied for adrenaline detection in human blood plasma for future biomedical applications. Furthermore, preliminary experiments have been carried to detect the adrenaline-concentration difference measured in peripheral blood and adrenal venous blood, representing the adrenal vein sampling procedure of a physician.

1. Introduction

Catecholamines such as adrenaline (epinephrine), noradrenaline (norepinephrine) and dopamine belong to a class of chemical neurotransmitters and hormones. They are capable for many profound changes in the body, e.g., regulation of physiological processes and the development of neurological, psychiatric, endocrine and cardiovascular diseases [1,2]. The determination of adrenaline concentrations in blood plasma or urine can help to diagnose different diseases such as hypertension, pheocromocytoma or neuroblastoma [3]. For example, adrenal vein sampling (AVS) is a necessary medical diagnostic test of adrenal gland tumors, where blood from both adrenal glands (left and right) is drained [4,5]. However, adrenal glands are difficult to cannulate which is especially true for the small right adrenal vein (1–15 mm length in cadaver studies) (see Fig. 1). This can become an obstacle in the study and significantly extend the exposure time to radiation and is consuming human resources. It may also lead to a mistakable cannulation of nearby accessory hepatic veins or to dilution of adrenal venous blood with that from other larger veins. Classically, a catheter tip is placed in the right adrenal vein where blood is sampled from the femoral vein collected simultaneously. Thereafter (in view centers at the same time through a second catheter), the left adrenal vein is cannulated, and again, blood specimen from the left adrenal outflow and from the femoral vein are obtained and subjected to hormone analysis. The ratio of the cortisol concentration in the adrenal veins to that of the femoral vein informs the physician on the extent of dilution of adrenal venous blood with peripheral extraadrenal blood and is a measure of selectivity of the study. Since cortisol has a relative long half live and is bound to protein, the ratio of adrenal venous to peripheral cortisol can be as low as 2. However, one possible new approach is the detection of a certain biomarker to confirm the right position of the catheter in adrenal veins. In this case, adrenaline can be used as such biomarker. Since the adrenaline concentration in adrenal vein samples is known to be significantly higher ($\gtrsim$100 nM) than in peripheral samples ($\lesssim$1 nM) [6,7], a presence of high adrenaline concentration of about 100 nM would confirm the right position of the catheter during AVS.

Common methods for the detection of adrenaline in medicine are

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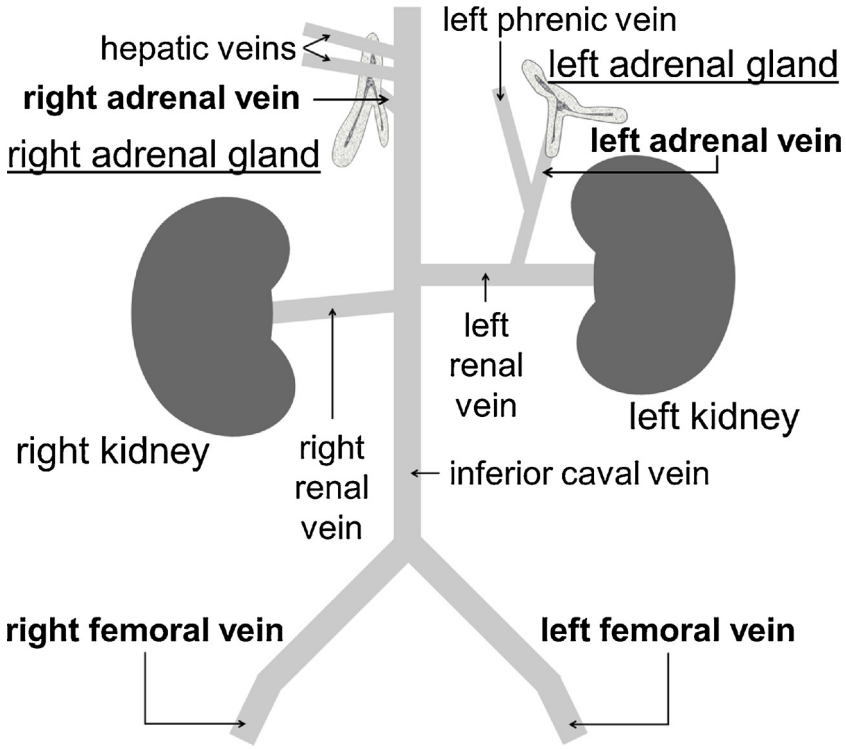

Fig. 1. Schematic of the anatomy of the human body, which illustrates the left and right adrenal veins with the corresponding adrenal glands; the catheter has to be pushed through the femoral vein into both adrenal veins.

high-performance liquid chromatography, fluorescence spectroscopy or capillary electrophoresis [8–13]. Although these methods are highly sensitive, they are not suitable for point-of-care testing at the patient due to the complex and labor-intensive procedures [14]. In the last few years, different biosensors have been developed for the detection of adrenaline. One strategy is the application of amperometric biosensors that are based on substrate-recycling principle by combining phenol-oxidizing enzymes such as e.g., tyrosinase, phenoloxidase or laccase in combination with pyrroloquinoline quinone (PQQ)-dependent glucose dehydrogenase (GDH) [15–18]. By using the substrate-recycling principle, the analyte is oxidized by one enzyme into a product, which can be reduced back to the original substrate by the second enzyme. Due to the recycling process, catecholamines can be detected down to the nanomolar concentration range [18–20]. Recently, we have reported on a biosensor based on laccase and PQQ-GDH by using substrate-recycling principle for the detection of adrenaline with a lower detection limit of about 1 nM at pH 7.4 in both phosphate buffer and Ringer's solution [21]. In this case, a genetically modified laccase variant has been applied which is active in a broader pH range, making the sensor applicable for the detection of adrenaline at a pH value in the physiological range. This is beneficial, when the adrenaline biosensor is applied in biological liquids, in particular in blood samples. However, because the sensor signal is proportional to the oxygen consumption due to the enzymatic reaction, small oxygen changes in the surrounding have influence on the sensor response. In addition, the oxygen concentration of real blood samples can vary and hence, the sensor signal could be falsified. Alternatively, the detection of catecholamines can be also performed by applying a monoenzymatic recycling electrode modified by e.g., laccase, PQQ-GDH or tyrosinase [22–24]. In this case, the analyte is oxidized or reduced at the electrode surface by an applied potential and the resulting product is transformed back to the initial analyte catalyzed by an enzyme. One example is shown in [23], where a screen-printed electrode is modified by the enzyme laccase or PQQ-GDH allowing the detection of different catecholamines in the nanomolar concentration range. With this sensor system, dopamine (not adrenaline) could be detected down to a concentration of 50 nM at pH 5.5 by applying the enzyme laccase, or 2 nM at pH 8.5 by applying the enzyme PQQ-GDH. Nevertheless, the described sensors have an insufficient lower detection limit, in particular for the detection of adrenaline, and their pH optimum does not conform to the pH value of biological liquids.

In this study, we report on a high-sensitive thin-film adrenaline biosensor based on the bioelectrocatalytical amplification principle by using a PQQ-GDH, which enables the detection of adrenaline in the nanomolar concentration range at physiological conditions (pH 7.4). The enzyme from company Sorachim was designed to have a pH optimum at about pH 7.0 [25]. With the developed biosensor set-up – to our knowledge – for the first time successful measurements in real blood samples have been performed. The measuring conditions (pH, temperature and glucose concentration) have been optimized to enhance the biosensor performance. In addition, the long-term stability as well as the sensitivity to other catecholamines (noradrenaline, dopamine and dobutamine) has been investigated. Finally, preliminary results obtained with this biosensor could distinguish between peripheral and adrenal venous blood by the different content of adrenaline concentrations.

2. Experimental

2.1. Materials

The quinoprotein GDH was bought from Sorachim SA (Switzerland, 757 U/mg) [25]. The cofactor PQQ was purchased from Wako (Japan). For the preparation of PBS, the components (monosodium phosphate and disodiumphosphate) were obtained from Sigma-Aldrich (USA), as well as glutaraldehyde, bovine serum albumin (BSA), glycerol, $CaCl_2$ and sulfuric acid. Adrenaline solution (1 mg/mL) was purchased from Infectopharm (Germany), noradrenaline (1 mg/mL) was obtained from Sanofi (Germany), dopamine (250 mg/mL) was bought from Carinopharm (Germany) and dobutamine (250 mg/50 mL) from Fresenius