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The evaluation of interdigitated array electrodes for measurement of catecholamines and indoleamines

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Abstract

The use of Interdigitated Array (IDA) Microelectrodes for detection of low levels of biogenic amines has been demonstrated in stationary solutions and flow systems [M. Morita et al., Electrochemica Acta 42(20–21) (1997) 3177–3183]. This technique is highly sensitive. We have evaluated this technology as applied to High Pressure Liquid Chromatography with Electrochemical Detection (HPLC-EC) for analysis of microdialysate and tissue samples. With this new technology we demonstrated a $\times 10$ fold increase in sensitivity in comparison to our existing technology. We are now able to detect dopamine at a level of 53×10^{-18} moles on column and serotonin at 26×10^{-18} moles on column. This technology now permits analysis of biogenic amines in samples from brain areas not previously amenable to this type of experiment.

Keywords: Interdigitated array microelectrodes; Reversed phase chromatography; Microdialysate samples

1. Introduction

The measurement of low levels of biogenic amines as a function of time after the administration of putative drug substances is of great interest to researchers in the field of neurochemistry. Data obtained from microdialysate samples or brain tissue can provide supportive evidence for functional responses induced by novel chemical entities.

Two of the most widely studied neurochemicals are serotonin and dopamine. Analysis of these substances in microdialysate samples or brain tissue samples can be achieved using High Performance Liquid Chromatography with Electrochemical Detection (HPLC-EC). HPLC is required in order separate the substances of interest from other structurally related substances also present in the sample, this includes dopamine and serotonin precursors and degradation products (Figs. 1 and 2). Electrochemical Detection is required in order to achieve the high level of detection sensitivity required for measurement of the substances of interest.

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Recently a method had been reported [1] for the analysis and detection of dopamine at levels significantly lower then previously obtained. The reported increase in sensitivity is due in part to the use of a new electrochemical cell design incorporating an Interdigitated Array Microelectrode (IDA) [1].

The aims of these current experiments were to reproduce the reported sensitivity for measurement of dopamine in our own laboratories and to evaluate the system for the measurement of serotonin.

2. Experimental

Mixed Standard 1 was prepared as follows. A stock solution containing a mixture of dopamine

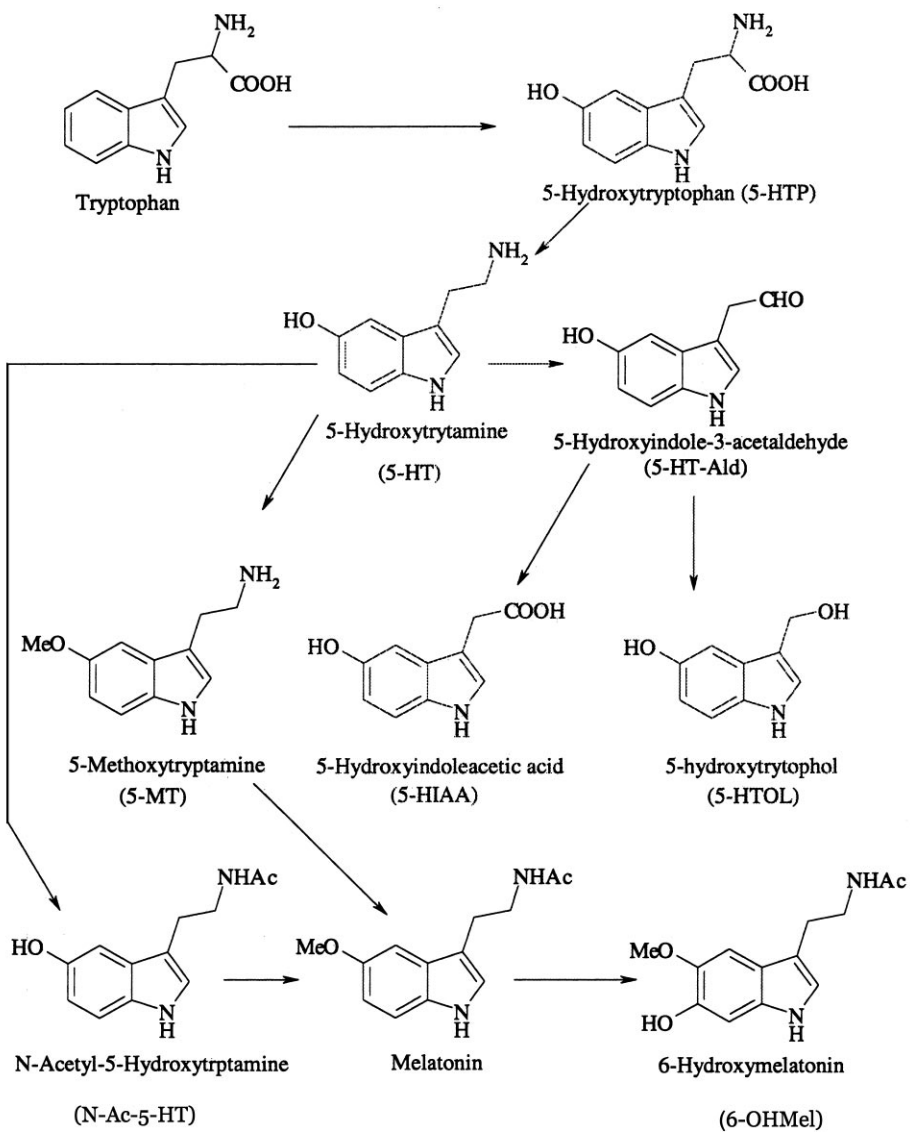

Fig. 1. 5-HT and related structures. Compounds of interest outlined in blue.

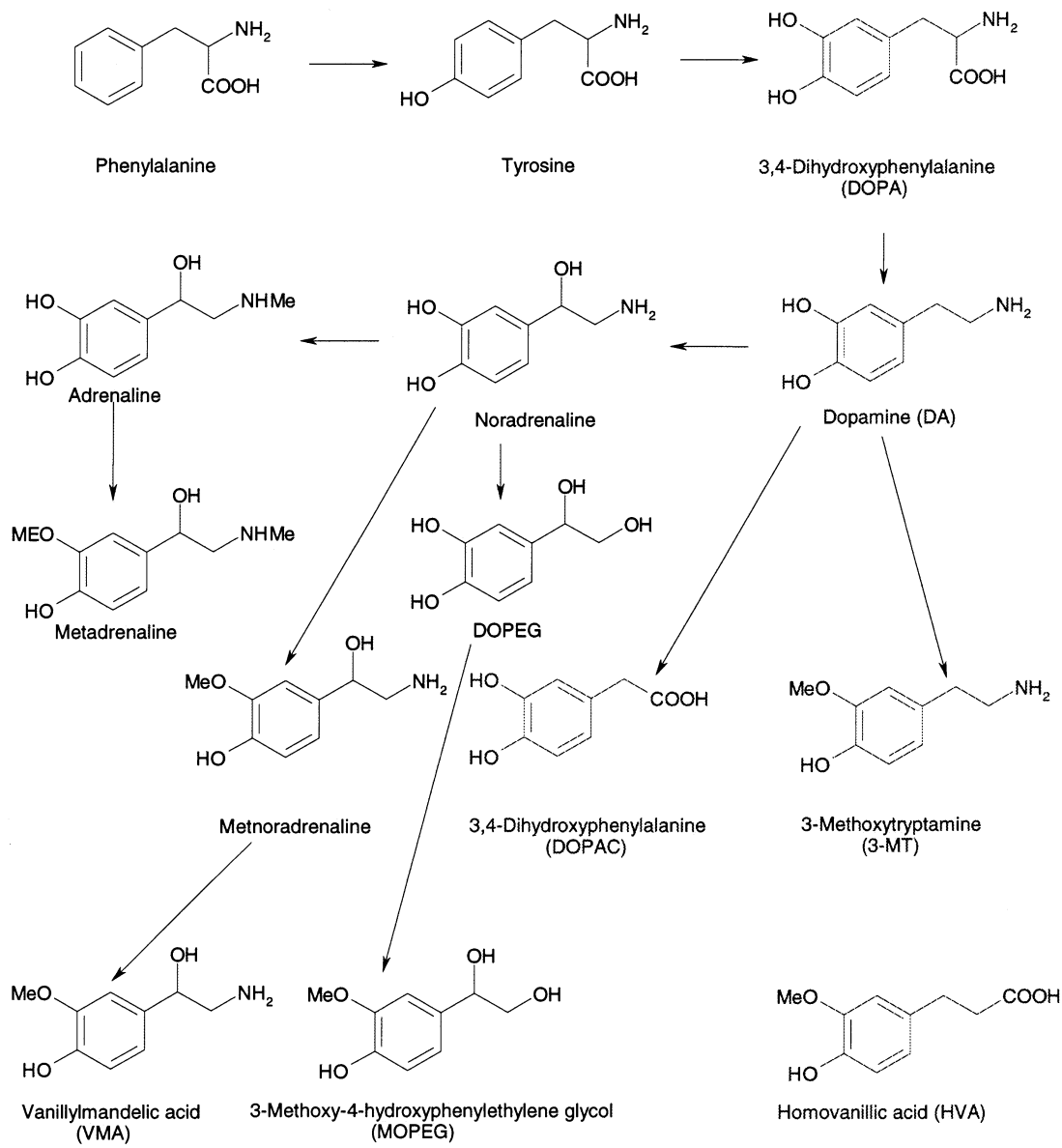

Fig. 2. Dopamine and related structures. Compounds of interest outlined in blue.

(DA), DOPA, 3-MT, 5-HT, DOPAC, 5-HTP, 5-HIAA, HVA (see Figs. 1 and 2) each at a concentration of 0.1 mg/l was prepared in 'homogenising buffer' (0.4 M perchloric acid containing 0.01% w/v EDTA, 0.1% w/v cysteine and 0.1% w/v sodium metabisulfite).

Microdialysate and tissue samples were obtained in house.

The mobile phase buffer was prepared by mixing trifluoroacetic acid (4.45 ml), EDTA (0.0392 mg) and 700 ml deionised water. The pH was adjusted to 1.9 with aqueous sodium hydroxide. The volume was then adjusted to 1 l with deionised water. This buffer was mixed with methanol and THF in a ratio of 94.5:4.5:1.0, v/v to give the mobile phase.

The HPLC system used for this work composed of a Bischoff pump model 2250, a flow splitter fitted with a calibrated tubing to give a ratio of 67:1, a Valco manual injector with a 0.1 μl internal loop and dual channel BAS LC-4C and LC-3C electrochemical detector equipped with a dual cell holder and a interdigitated microelectrode (BAS, Tokyo, Japan).

The IDA electrode consisted of 65 pairs of glassy carbon electrodes with finger widths and gaps of 5 μm and each finger was 2 mm long. The area of one set of band electrode array in the IDA electrode was 6.5×10^{-3} cm^2. The thickness of the gasket was 25 μm. Potentials were applied to each of the two sets of electrodes independently, the first set of electrodes being referred to as the

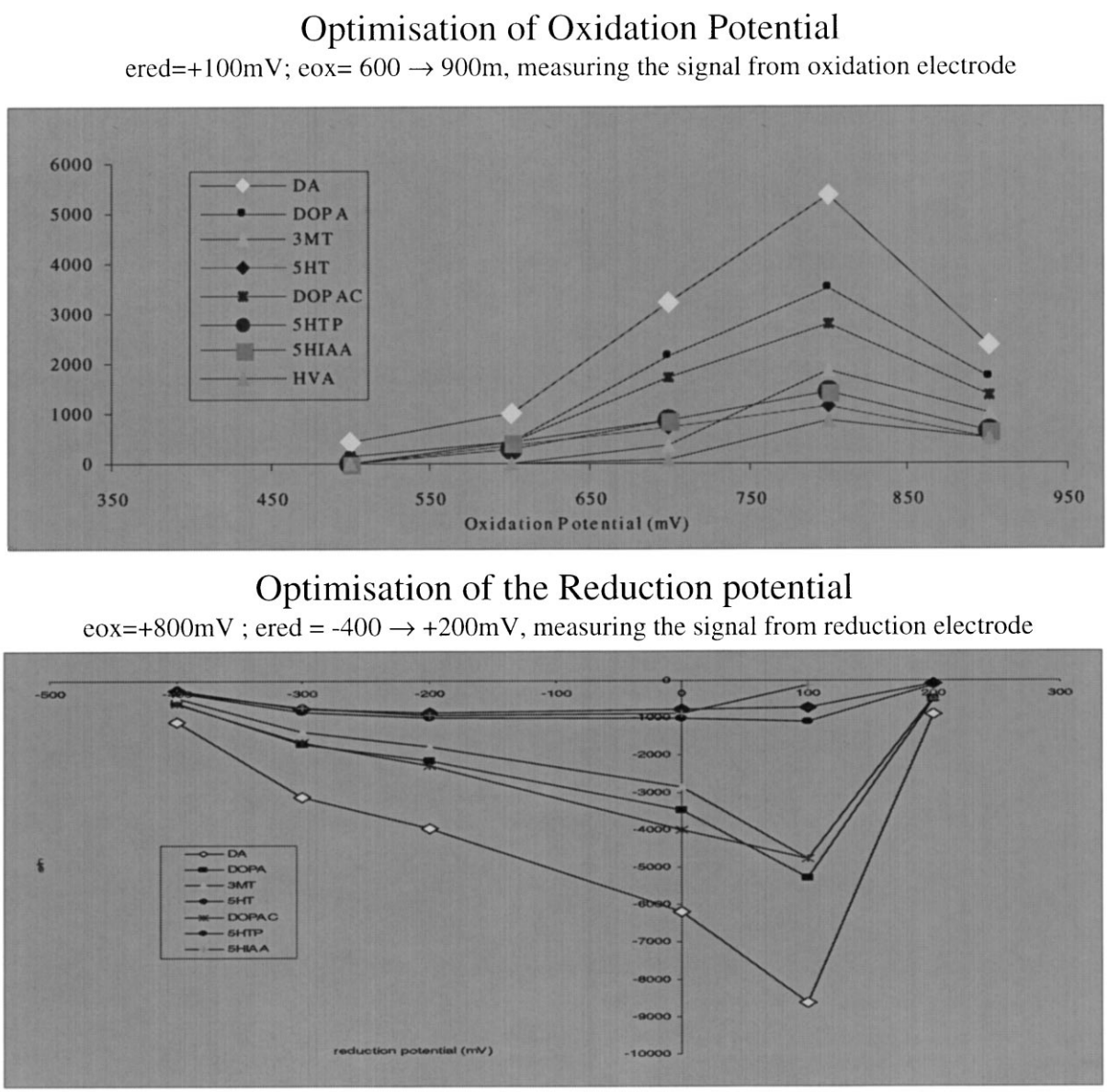

Fig. 3. (a) Optimisation of oxidation potential; (b) Optimisation of reduction potential.

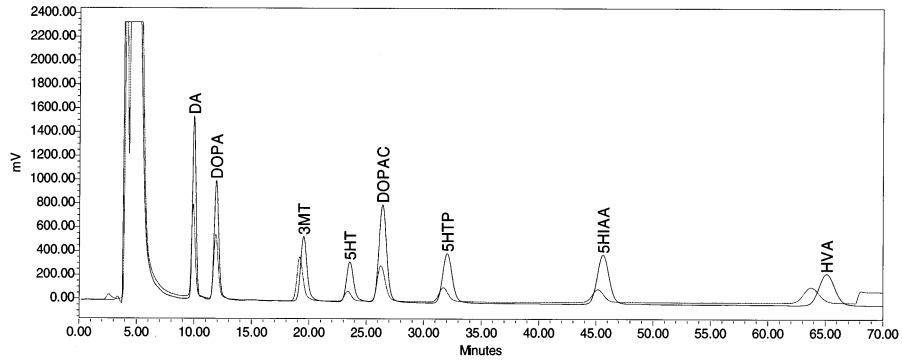

Fig. 4. Chromatogram from analysis of mixed standard 1 with 'reduction' electrode active and inactive.

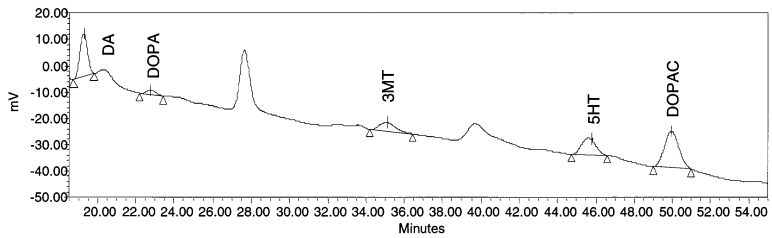

Fig. 5. Chromatogram from analysis of microdialysate sample.

'reduction' electrode and the second set referred to as the 'oxidation' electrode.

Separations were performed on a 200 mm × 0.32 mm i.d Zorbax Stablebond C18, 3.5 μm column, packed in our laboratory and operated at 6 μl min^{-1} flow-rate.

3. Results and discussion

3.1. Optimisation of oxidation voltage

A potential of + 100 mV (all voltages are against the Ag/AgCl working electrode) was applied to the 'reduction' electrode (e_{red}) and the potential applied to the 'oxidation' electrode (e_{ox}) was varied + 600– + 900 mV in steps of 100 mV. At each voltage standard 1 was injected in duplicate and the signal from the 'oxidation' electrode was collected. The data from this experiment is given in Fig. 3a. This data shows as expected an increase in response as a function of increased voltage. The optimum signal to noise is obtained at + 800mV. This value is compatible with data obtained for these analytes on other types of electrochemical detectors.

3.2. Optimisation of reduction voltage

The optimum 'oxidation' potential of + 800 mV was applied to the 'oxidation' electrode and the 'reduction' potential was varied − 400 to + 200 mV in steps of 100 mV. At each voltage standard 1 was injected in duplicate and the signal from the reduction electrodes was collected. The data from this experiment is given in Fig. 3b. This data shows that optimum reduction occurs at + 100mV. This is in agreement with previous work[1].

3.3. Active vs inactive reduction electrodes

In order to validate that the use of dual electrodes does provide a benefit in detection sensitivity a standard mixture was analysed with: (a) $e_{ox} = 800$ mV and $e_{red} = +100$ mV; and (b) $e_{ox} =$

800 mV and the e_{red} disconnected from their voltage supply. In both cases signals were measured from the oxidation electrodes. Data from these experiments are given in Fig. 4. The trace given in black is with the reduction electrodes active and the trace in red is with the reduction electrodes inactive. It can be seen that the sensitivity with the active reduction electrodes is $\times 2$ greater than with inactive reduction electrodes.

3.4. Analysis of microdialysate and brain tissue samples

A chromatogram of data from analysis of a brain microdialysate sample is given in Fig. 5. The limit of detection using this methodology is of the order 53×10^{-18} moles on column for dopamine and at 26×10^{-18} moles on column for serotonin.

4. Conclusions

It has been shown that use of IDA electrodes within HPLC-EC instrumentation can provide increased sensitivity. The limit of detection (measured as ratio of peak height/root mean sqaure of baseline noise) using this methodology is of the order 53×10^{-18} moles on column for dopamine and at 26×10^{-18} moles on column for serotonin.

This permits analysis of microdialysate samples for the presence of neurotransmitters at levels significantly lower than those using conventional HPLC-EC instrumentation. Further validation of methods using the IDA technology would be required.

At present we are unable to fully explain the reasons for enhanced detection sensitivity provided by the IDA and further work is required to gain further understanding of these effects.

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References

[1] M. Morita, et al., Electrochemica Acta 42 (20–21) (1997) 3177–3183.