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Short Communication

Apparent inter-channel interference in dual-electrode electrochemical detection

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ABSTRACT

During the course of routine high-performance liquid chromatographic analyses of brain catecholamines using dual-electrode electrochemical detection, we encountered an unusual negative peak in the lower-voltage channel. Subsequent investigations suggested that this peak was caused by tyrosine which produced a positive peak in the higher-voltage channel. Our investigations indicate that compounds that generate a peak in one channel appear to be responsible for complex peaks in a second channel set at a lower voltage, close to or below that necessary for oxidation. The complex peaks are biphasic; a sharp negative peak coinciding with the positive peak on the higher-voltage channel, followed by a positive peak. This effect was not specific for tyrosine, but was observed on the lower-voltage channel with all compounds tested that produced signals on the high-voltage channel. The cause of the problem is unknown, but it appears to be an artifact of the electrical coupling of the two electrode channels in a dual-channel system.

INTRODUCTION

We have routinely performed high-performance liquid chromatographic (HPLC) analyses of extracts of brain catecholamines and related compounds with dual-electrode electrochemical detection [1]. The dual-channel system was used because one compound (tryptophan) has a higher redox potential than the catecholamines, and use of a higher voltage on a one-channel detector obscured other peaks of interest. However, we recently encountered a negative peak on the lower-voltage channel. This negative peak caused problems for integration, and it occasionally affected peaks for compounds of interest. Therefore, we investigated the source of the negative peak. This report details our findings, which may be of general applicability to dual-electrode HPLC detection systems.

EXPERIMENTAL

Standard compounds were obtained from Sigma (St. Louis, MO, USA). Brain samples were prepared as described in previous publications [2]. Briefly, samples of frozen brain were homogenized by ultrasonication in 0.1 *M* $HClO_4$ containing 0.1 m*M* EDTA. After freezing and thawing, the precipitates were separated by centrifugation and the supernatants applied directly to the HPLC system.

The HPCL system consisted of an M-6000 dual-piston HPLC pump, WISP 712 autoinjector with the WISP cooling module (Waters, Milford, MA, USA) maintained at 4°C, a 250 × 4.5 mm Spherisorb 5-μm ODS-1 reversed-phase column (Keystone Scientific, Bellefonte, PA, USA) maintained at 35°C with an LC-23A column heater and an LC-22 temperature controller, an LC-17A electrochemical

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cell with an MF-1000 dual glassy carbon working electrode in the parallel configuration, and dual LC-4B amperometric detectors set at 0.78 and 0.95 V relative to a RE4 Ag/AgCl reference electrode (all from Bioanalytical Systems, West Lafayette, IN, USA).

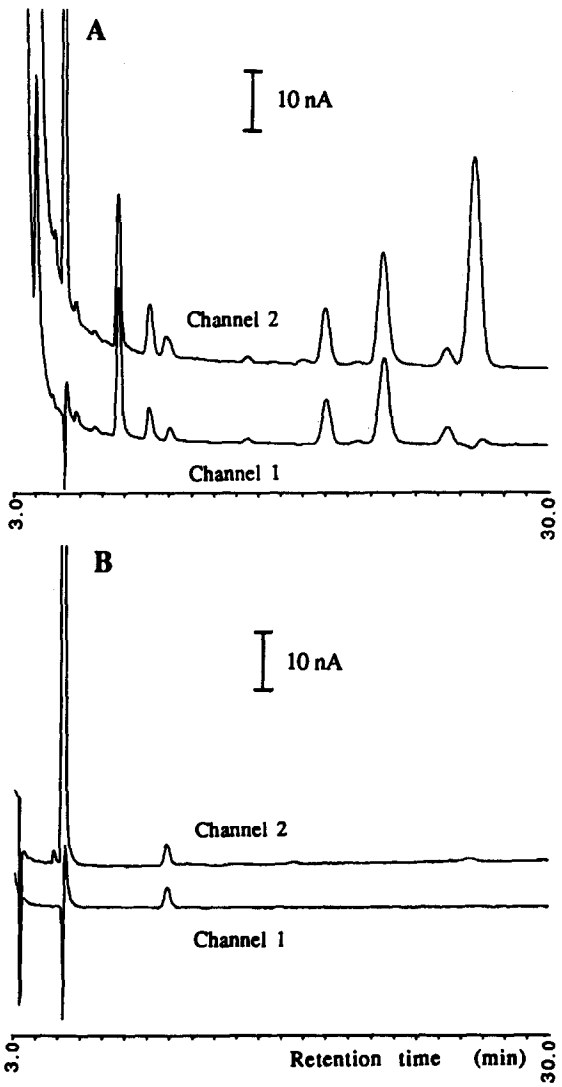

Fig. 1. HPLC chromatograms of a brain sample (A) and standard tyrosine (B) run with channel 1 of the electrochemical detector set to 0.78 V and channel 2 at 0.95 V. Detector sensitivity was set at 10 nA/V. The chromatogram started 3.0 min after sample injection. (A) A 150-ml volume of a perchloric acid extract of mouse brain stem. The retention time (5.5 min) of the sharp negative peak on channel 1 corresponded precisely with that of the large peak in channel 2. (B) A 5-ng amount of L-tyrosine–HCl. The peak at 9.9 min in all traces is the internal standard, N-methyldopamine.

The mobile phase was 0.1 *M* sodium phosphate, 0.1 m*M* EDTA, 0.45 m*M* octanesulfonic acid, pH 3.05, 3.75% acetonitrile, filtered with a 0.45-μm Vericel membrane filter (Gelman Sciences, Ann Arbor, MI, USA) and maintained under helium sparge. Flow-rate was 1.1 ml/min. Data acquisition and integration was with the Rainin (Woburn, MA, USA) Dynamax HPLC system.

RESULTS AND DISCUSSION

Fig. 1A shows the dual-electrode trace of a sample of mouse brain stem. The negative peak at about 5.5 min on channel 1 (set at 0.78 V) clearly corresponded to a large positive peak on channel 2 (set at 0.95 V). Inspection of a large number of runs indicated the consistency of this phenomenon. Therefore, we ran a number of readily oxidizable compounds the presence of which in the brain extracts was suspected. We found that tyrosine ran in approximately the same position relative to the internal standard as the unknown peak in the brain extracts (relative retention time 0.33). A 5-ng injection of tyrosine caused a biphasic peak on channel 1, a sharp negative peak followed by positive one (Fig. 1B). The negative peak exhibited the same relationship to the positive peak in channel 2 as that seen with the unknown peak in brain extracts. The positive component of the channel 1 peak was slightly delayed. Addition of various amounts of L-tyrosine to the brain samples proportionately enlarged both the negative and the positive peaks, such that the compound was indistinguishable from tyrosine.

We next compared the heights of the positive and negative peaks on channel 1 with those of the positive peak on channel 2. Fig. 2A indicates that the height of the positive peak on channel 2 was linearly related to the amount of tyrosine injected and that this amount of sample did not overload either the detector or the integrator. Fig. 2B indicates that there was also a linear relationship between the tyrosine peak on channel 2, and the negative peak on channel 1, suggesting that they were related. Fig. 2C indicates that there was also a linear relationship between the positive and negative components of the peak on channel 1.

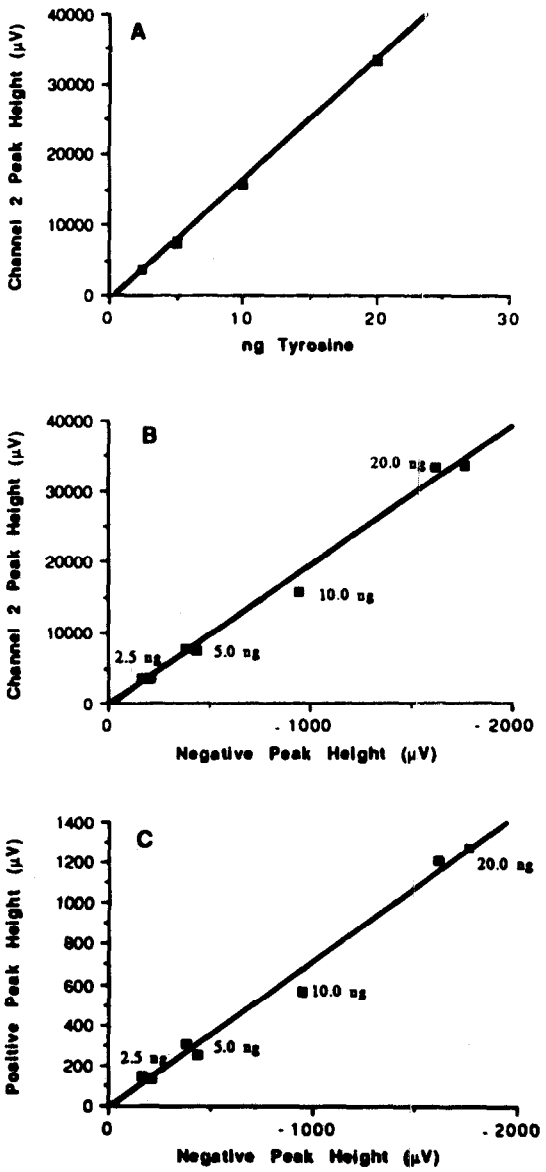

Fig. 2. Analyses of chromatograms of standard tyrosine (2.5–20 ng) run under the conditions of Fig. 1. (A) Peak height on channel 2 *vs.* ng of tyrosine injected; $y = -824.65 + 1711.9x$, $R^2 = 0.999$. (B) Peak height on channel 2 *vs.* negative component of the peak on channel 1; $y = -493.45 + 19.706x$, $R^2 = 0.989$. (C) The height of the positive *vs.* the height of the negative component on channel 1; $y = 17.037 + 0.72531x$, $R^2 = 0.988$.

Several potential artifacts were investigated: reversing the voltages on the two channels of the electrochemical detector, substituting a second set of LC4B electrochemical detectors, substituting both

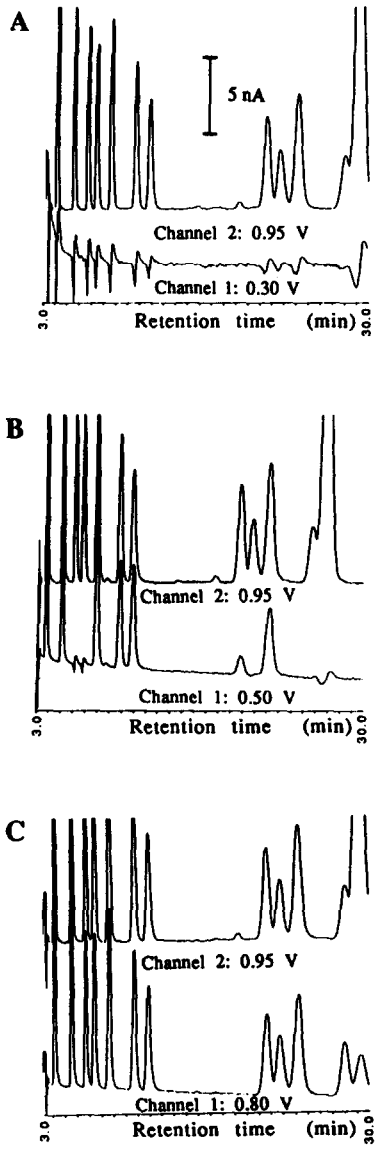

Fig. 3. HPLC chromatograms of mixed standards of catecholamines, indoleamines and related compounds. Peaks in order of elution: norepinephrine, epinephrine, 3-methoxy,4-hydroxyphenylethyleneglycol, (MHPG); normetanephrine (NM), dopamine, 3,4-dihydroxyphenylacetic acid, N-methyldopamine, 5-hydroxyindoleacetic acid, 3-methoxytyramine, 5-hydroxytryptamine, homovanillic acid (HVA), tryptophan (Trp). For all three chromatograms, the channel 2 electrode was set at 0.95 V. (A) Channel 1 at 0.30 V (below the potential necessary to oxidize any of the compounds). (B) Channel 1 at 0.50 V and showing positive peaks for the more easily oxidizable compounds, and baseline disturbances for the others (MHPG, NM, HVA and Trp). (C) Channel 1 at 0.80 V showing positive peaks for all compounds and no baseline disturbances.

working and reference electrodes, turning the MF-1000 dual glassy carbon working electrode so that the two electrodes were in series rather than in parallel [1] and the 0.95 V electrode in both the upstream and downstream positions, and substituting a different integrator. None of these changes significantly altered the presence or the magnitude of the negative peak.

We then tested whether the effect was specific for tyrosine by running our standard mixture of catecholamines, indoleamines and related compounds at different electrode potentials. Fig. 3A shows that when the voltage on the channel 1 electrode was set at 0.30 V, negative peaks appeared corresponding to each of the positive peaks on channel 2. As the voltage on channel 1 was increased, the heights of the negative peaks decreased, and they were replaced by positive peaks. Thus at 0.50 V (Fig. 3B), negative peaks appeared only for 3-methoxy,4-hydroxyphenylethyleneglycol, normetanephrine and homovanillic acid–tryptophan. When channel 1 was set at 0.80 V, close to the potential we normally use on this channel, no negative peaks were observed. The order in which the negative peaks disappeared as the voltage was increased appeared to be related to the order in which positive peaks appeared as the voltage was increased, *i.e.* in the order of their apparent redox potentials. The potential needed to oxidize tyrosine was greater than that needed for any of the other compounds in the standard mixture, except perhaps tryptophan. This may explain why we only observed the phenomenon with tyrosine in our standard runs.

Although the magnitude of the negative peak was related to the magnitude of the positive peak (Fig. 2C), the negative peak appeared with all amounts of sample in the normal range and was not related to overload of the detector or the integrator. Apparently, a negative peak appeared whenever the potential of the electrode was set below the lowest voltage necessary to oxidize the compound (Fig. 3).

We conclude that negative peaks may appear using dual-electrode electrochemical detection whenever the potential of the lower-voltage electrode is set significantly below the redox potential for that compound. The negative peak appears to be an artifact related to the electrical coupling of the two electrode detector channels. We caution chromatographers that it could appear under many different circumstances and is unlikely to be confined to the particular application where we observed it.

ACKNOWLEDGEMENT

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