BRUCE D. DAVIES

Determination of Tetrahydroisoquinoline Alkaloids in Biological Materials with High Performance Liquid Chromatography

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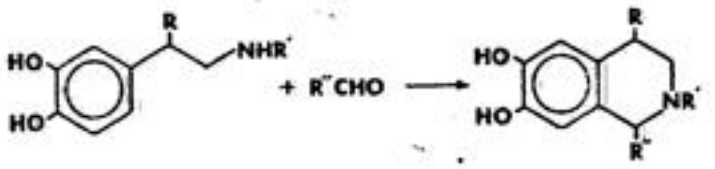
A new approach is described for the selective determination of tetrahydroisoquinolines (TIQ's) formed by condensation of catecholamines with aldehydes in vivo. High performance cation exchange chromatography in combination with amperometric detection permits simultaneous quantitation of tetrahydropapaveroline (THP) and salsolinol (SAL) at 2 ng/g in rat brain and 2 ng/mL in urine. Examples of application of the new method are given for studies pertaining to the possible involvement of TIQ's in alcohol abuse.

The primary metabolic pathways of the catecholamines have been extensively studied and their involvement in neurotransmission and other processes in the peripheral and central nervous systems has long been recognized. There exists, however, another possible metabolic route for these compounds which has only recently received attention. This pathway involves the nonenzymatic condensation of catecholamines with aldehydes to form tetrahydroisoquinoline alkaloids (TIQ's) which can act as "false" neurotransmitters and result in catecholamine depletion. Such a reaction scheme is shown below.



Although it is conceivable that this reaction could occur normally by utilizing endogenous aldehydes (e.g., glyceraldehyde or glyoxylate), these compounds are generally present at low concentrations within the mitochondria or other organelles of the cell and thus never encounter the catecholamines. TIQ formation is believed to have its most probable significance under abnormal circumstances in which the body is presented with an external stimulus which results in significant levels of circulating aldehydes. The most obvious example of such a stimulus is alcohol consumption. During alcohol intoxication, significant levels of circulating acetaldehyde are present and it has been proposed by various workers that the condensation of acetaldehyde with catecholamines to form TIQ's may account in some way for the addicting properties of alcohol. The literature adequately describes the rationale for this hypothesis (*1–4*). It appears as though no definite conclusions have been reached as to the credibility of this idea; however, the balance of evidence reported to date is negative. A recent encouraging report by Myers and Melchior indicates that drinking behavior in rats can be influenced by ventricular perfusion of TIQ's in brain (*5*). There are also reports that TIQ's form during various drug therapies (e.g., L-Dopa treatment of Parkinson's disease) and may play some role in the pharmacological effects of these drugs (*6*).

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It has long been recognized that available analytical methods for catecholamines are quite poor, especially when dealing with endogenous levels in biological samples. Since TIQ levels will always be significantly less than their catecholamine precursors, it is obvious that their assay will be even more difficult. This is indeed the case and has hampered workers in this area to a large extent.

Recently we have described a simple and sensitive assay procedure for urinary and tissue catecholamines (*7, 8*). This method utilizes high performance liquid chromatography (HPLC) coupled to a thin-layer electrochemical detector (LCEC). The advantages of using liquid chromatography as opposed to gas–liquid and other forms of chromatography or fluorometry to assay catecholamines are obvious and have been adequately described by us and others (*7, 9*). The same advantages apply to the present TIQ assay. The fundamental problem with HPLC analysis of TIQ's is the relatively poor sensitivity of the commonly used detectors (e.g., UV-VIS absorption). The use of thin-layer electrochemistry as a detection system has numerous advantages in terms of sensitivity, selectivity, and cost. The sensitivity using electrochemical detection can sometimes be two to three orders of magnitude better than for UV and its inherent selectivity makes it a much more feasible approach for assays of many biological materials.

A fundamental advantage (in many cases a limitation) of LCEC is the fact that only electroactive compounds can be detected. For assay of TIQ's, this turns out to be a great advantage since all the compounds of interest contain at least one phenolic substituent, thus making them electrooxidizable. Figure 1 illustrates the electrochemical behavior of two typical TIQ's. By selecting a detection potential in the range of +0.7 to +0.8 V, one can oxidize the TIQ's (and catecholamines) while not detecting compounds with much larger oxidation potentials.

Using the LCEC technique, we have detected the presence of TIQ's in plant matter and in the urine of individuals following consumption of bananas or cocoa-based products (*10, 11*). This report describes analytical procedures for ppb levels of TIQ's in body fluids and tissues. Several preliminary studies on TIQ formation in vivo are also discussed.

EXPERIMENTAL

The present assay for urinary TIQ's is a modification of our earlier procedure for catecholamines (*7*). Urine was collected over acid (to pH 2) using 6 M HCl and stored at −35 °C prior to analysis. Four milliliters of acid-hydrolyzed (*7*) urine were placed in polyethylene centrifuge tubes with 1.5 g of $(NH_4)_2SO_4$, capped and shaken, and centrifuged to remove solids and precipitated proteins. Acid hydrolysis is necessary to release the alkaloids from sulfate and glucoronide conjugation. The supernate was transferred to 12-mL glass centrifuge tubes and extracted twice with ethyl acetate and once with hexane (5-mL aliquots). The nonaqueous layers were discarded. The aqueous layer was transferred to a 10-mL beaker with 100 μL each of 5% sodium metabisulfite and 10% EDTA. The stirred sample was then adjusted to pH 8.5 and placed in a 5-mL conical vial containing 80 mg of alumina (*12*) and shaken for 12 min on a reciprocating shaker. The urine was aspirated off and the alumina was washed three times with distilled water and dried in vacuo for 3 min at 30 °C.

The compounds were eluted from the alumina with 400 μL of 1 M