Journal of Chromatography, 164 (1979) 205—216
Biomedical Applications
© Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROMBIO. 376

DETECTION OF ENDOGENOUS SALSOLINOL IN NEONATAL RAT TISSUE BY A RADIOENZYMATIC—THIN-LAYER CHROMATOGRAPHIC ASSAY

CHRISTINE A. NESTERICK* and RALF G. RAHWAN**

Division of Pharmacology, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210 (U.S.A.)

(First received February 14th, 1979; revised manuscript received May 23rd, 1979)

SUMMARY

A sensitive radioenzymatic—thin-layer chromatographic assay for the quantitative analysis of the tetrahydroisoquinoline alkaloid, salsolinol, in plasma and neonatal rat tissue is described. The assay involves the enzymatic O-methylation of salsolinol by catechol-O-methyltransferase in presence of [^{3}H]S-adenosylmethionine, and subsequent separation by thin-layer chromatography of the resultant [^{3}H]O-methyl-salsolinol from the O-methylated derivatives of dopamine, epinephrine and norepinephrine. The method allows the detection of as little as 100 pg salsolinol per g tissue, and the accurate quantitation of as little as 100 pg/ml plasma and 500 pg/g tissue. This assay permitted the detection of trace amounts of endogenous salsolinol in neonatal rat tissue (< 500 pg/g tissue).

INTRODUCTION

Recent evidence in both laboratory animals and in humans suggests that tetrahydroisoquinoline (TIQ) and tetrahydro-β-carboline (TBC) alkaloidal metabolites, formed endogenously from interactions between acetaldehyde and catecholamines or serotonin during alcohol consumption, may play a role in the development of physical dependence to ethanol (for reviews see refs. 1 and 2). Since a number of TIQs and TBCs have psychotomimetic activity, and the TIQ alkaloid, tetrahydropapaveroline, is a precursor of morphine in plants, it has further been speculated that physical dependence to ethanol or opiates may share a common biochemical basis.

In in vivo studies involving acute or chronic administration of ethanol [2],

*Dr. William E. Weiss Memorial Fellow of the American Foundation for Pharmaceutical Education.
**To whom correspondence should be addressed.

formation of TIQs in the brain could only be demonstrated under pharmacological conditions which enhanced the biosynthesis of TIQs or inhibited their metabolic degradation. In order to explore the possible formation of the TIQ, salsolinol (the condensation product of endogenous dopamine with alcohol-derived acetaldehyde), in brain tissue under conditions of physical dependence to ethanol without intervening pharmacological manipulations, we developed a gas chromatographic—electron capture (GC—EC) assay for salsolinol capable of detecting as little as 8 ng of the alkaloid per g of brain tissue [3]. Using this assay, we were unable to detect the formation of brain salsolinol in alcoholic mice [4], when conversion of 1% or more of endogenous dopamine to salsolinol could not have escaped detection. Our negative findings were subsequently confirmed by other investigators [5, 6] in mice and rats. Nevertheless, it has recently been demonstrated that intracerebral administration of TIQs or TBCs in minute amounts to rats mimics ethanol dependence and increases free-choice ethanol consumption over water [7—11]. It has thus become evident that the question of in vivo formation of brain TIQs in alcoholic animals (not subjected to pharmacological manipulations which enhance the biosynthesis of TIQs or which delay their degradation) needs re-examination with more sensitive analytical methods for detecting minute amounts of TIQs.

Another contemporary aspect of ethanol toxicity concerns its teratogenic action, which has been established in laboratory animals and in humans (for review see ref. 12). Although no mechanism has been proposed to explain the teratogenicity of ethanol, evidence has been presented in one human study indicative of interference with cellular migration in the brain during embryogenesis [13]. We have speculated that the teratogenic anomalies produced by ethanol could be in part the result of interference with embryonal catecholaminergic or serotoninergic mechanisms as a consequence of aberrant biosynthesis of TIQ or TBC alkaloids. Teratogenicity of opiates and psychoactive drugs has already been reported in the literature [14, 15]. A study to validate this hypothesis is in progress in our laboratory, and mandated the development of a sensitive analytical assay for the detection and quantitation of the TIQ, salsolinol.

The present report outlines the development of a sensitive radioenzymatic assay for salsolinol in plasma and neonatal rat tissue. A future communication will deal with the detection and quantitation of brain salsolinol. Since it has been reported [16] that salsolinol is a substrate for mammalian catechol-O-methyltransferase (COMT) as well as a competitive inhibitor of the O-methylation of catecholamines, the possibility of development of a sensitive thin-layer chromatographic—radioenzymatic (TLC—RE) assay for detection, separation and quantitation of trace amounts of salsolinol and catecholamines became apparent. This endeavor was greatly facilitated by the recent commercial availability of a radioenzymatic assay kit (CAT-A-KIT, Upjohn Diagnostics, Kalamazoo, Mich., U.S.A.) for catecholamines, which is based on the method of Passon and Peuler [17]. The method described below is a modification of the commercial kit, adapted for the incorporation of salsolinol into the assay. Salsolinol (SAL), dopamine (DA), epinephrine (EPI), and norepinephrine (NE) are simultaneously converted to their corresponding *meta* ^{3}H-methoxy

derivatives ([^{3}H]salsoline, [^{3}H]3-methoxytyramine, [^{3}H]metanephrine, and [^{3}H]normetanephrine, respectively) by the catalytic action of COMT in the presence of [^{3}H]S-adenosylmethionine (^{3}H-SAM). The ^{3}H-O-methylated derivatives are extracted and separated by TLC. [^{3}H]Normetanephrine and [^{3}H]metanephrine (but not [^{3}H]salsoline or [^{3}H]3-methoxytyramine) are susceptible to periodate oxidation which converts the former two derivatives into [^{3}H]vanillin; this additional chemical characteristic serves to differentiate between the ^{3}H-O-methylated derivatives of EPI and NE on the one hand and those of DA and SAL on the other.

MATERIALS AND METHODS

Chemicals and equipment

The following principal chemicals were used: CAT-A-KIT (catecholamines radioenzymatic assay kit [^{3}H], Upjohn Diagnostics), salsolinol HBr (Aldrich, Milwaukee, Wisc., U.S.A.), salsoline HCl (ICN Pharmaceuticals, Plainview, N.Y., U.S.A.), and Liquifluor (New England Nuclear, Boston, Mass., U.S.A.).

Vacutainer tubes, containing 100 μl of a solution (pH 6—7) composed of 90 mg EGTA per ml and 60 mg reduced glutathione per ml, were purchased from Upjohn Diagnostics. Glassware was siliconized by immersion for 1 min in a 1% solution of Prosil (VWR Scientific, Columbus, Ohio, U.S.A.), and then rinsed in distilled water and dried at 150°.

Prescored silica gel GF TLC plates, 20 × 20 cm, 250 μm thick (Analabs, North Haven, Conn., U.S.A.) were used in conjunction with a 16-channel TLC Multispotter (Analytical Instrumentation Specialties, Libertyville, Ill., U.S.A.).

Reagents

Reagents 1—9 are CAT-A-KIT reagents [18].

Reagent 1 (catecholamines standard solution): Each ml contains 100 μg each of *l*-NE, *l*-EPI, and DA in acid glutathione solution. Diluted 1:10,000 with demineralized double-distilled water before use.

Reagent 2 (stabilizing solution): Acidic glutathione solution, diluted 1:10,000 with demineralized double-distilled water before use.

Reagent 3 (buffer solution): For buffering the enzyme reaction in the assay. Contains tromethamine, EGTA, and magnesium chloride.

Reagent 4 (^{3}H-SAM, methyl donor): S-Adenosyl-L-methionine (^{3}H-methyl), 5 μCi ^{3}H/10 μl in dilute sulfuric acid—ethanol (acetaldehyde-free).

Reagent 5 (COMT enzyme preparation): Rat liver COMT (in excess of assay needs), tromethamine, glutathione, benzylhydroxyamine HCl, and dithiothreitol.

Reagent 6 (stopping/carrier solution): Contains 4 m*M* each of normetanephrine, metanephrine, and methoxytyramine, in pH 11 borate buffer containing EDTA.

Reagent 7 (oxidizing reagent): Sodium metaperiodate 4% w/v solution.

Reagent 8: Glycerol 10% v/v solution.

Reagent 9 (control human plasma): Contains assayed levels of catecholamines (DA, EPI, and NE), EGTA and glutathione.

Separation of O-methylated derivatives of SAL, DA, EPI and NE by TLC

In order to determine whether the O-methylated derivative of salsolinol (salsoline) was separable by TLC from the O-methylated derivatives of EPI, NE, and DA, 5 ml of Reagent 6 were spiked with 4.6 mg of salsoline HBr to provide a 4 m*M* concentration of salsoline. The resulting mixture of salsoline, methoxytyramine, metanephrine and normetanephrine was extracted [19] into 2 ml of toluene—isoamyl alcohol (3:2), and the aqueous and organic phases separated. The O-methylated compounds were extracted from the organic phase into 0.1 ml of 0.1 *N* acetic acid. The acid layer was separated and washed with 1 ml of toluene—isoamyl alcohol (3:2), and the organic wash discarded. Absolute ethanol (0.1—0.15 ml) was added to the acid extract to clear the solution. The ethanolic—acid extract was spotted on the silica gel TLC plates using the TLC Multispotter set at low speed and 60°. The distance between the solvent front and points of application was set at 16 cm. After allowing to cool, the plates were developed in 44 ml of *tert.*-amyl alcohol—toluene—40% methylamine (6:2:3) for approximately 2.5 h in developing tanks lined with Whatman No. 1 filter paper and pre-equilibrated for 10 min with the developing solvent. The plates were allowed to dry, and the four zones were visualized under UV light (254 nm).

Radioenzymatic reaction

The following radioenzymatic assay is a modification of the method of Peuler and Johnson [19]. Aliquots (50 μl) of each sample (e.g. plasma, tissue) to be analyzed for SAL, DA, EPI and NE, were mixed with 10 μl of diluted acidic glutathione stabilizing solution (Reagent 2) and 40 μl of a reagent mixture composed of equal parts (10 μl each) of distilled water, buffer solution (Reagent 3), ^{3}H-SAM (Reagent 4), and COMT (Reagent 5). The mixtures were incubated at 37° for 60 min in a metabolic shaker. To each mixture was then added 50 μl of the buffered carrier solution (Reagent 6) and 5 μl of a salsoline carrier solution (freshly prepared by dissolving 46 mg salsoline HCl in 5 ml of pH 11 borate buffer containing 27.8 mg EDTA per ml). The ^{3}H-O-methylated derivatives and their non-radioactive carriers were then extracted and separated by TLC as described above. The radioactive zones on the TLC plates were scraped into individual scintillation vials.

[^{3}H]Metanephrine and [^{3}H]normetanephrine were eluted from the silica in their respective scintillation vials by vigorous mixing with 1 ml of 0.05 *M* ammonium hydroxide. [^{3}H]Metanephrine and [^{3}H]normetanephrine were then each converted to [^{3}H]vanillin by periodate oxidation as described by Peuler and Johnson [19] and in the CAT-A-KIT Procedures Manual [18]. Following the oxidation, 1 ml of 0.1 *M* acetic acid was added to each vial with vigorous mixing. To each vial were then added 10 ml of toluene—Liquifluor (1000:50, v/v) scintillation cocktail with vigorous mixing, and the radioactivity counted in a Beckman LS-35 liquid scintillation counter with an efficiency for ^{3}H of 58%.

[^{3}H]Salsoline and [^{3}H]methoxytyramine were eluted from the silica in their respective scintillation vials by vigorous mixing with 1 ml of 0.05 *M* ammonium hydroxide. Ten ml of toluene—isoamyl alcohol—Liquifluor (700:300:50, v/v/v) scintillation cocktail were added to each vial with vigorous shaking, and the

radioactivity counted in a Beckman LS-35 liquid scintillation counter with an efficiency for ^{3}H of 39% (38.7—40%).

Aqueous standard curve

A salsolinol HBr stock solution was prepared (10 μg SAL per 10 ml distilled water, with 60 mg reduced glutathione per ml), and various dilutions of this stock solution were made from which 50-μl aliquots were assayed by the radioenzymatic procedure described above. The concentrations analyzed were 0, 50, 500, 1000 and 1500 pg salsolinol per 50-μl sample. Each sample was analyzed in triplicate.

Plasma standard curve

A salsolinol HBr stock solution was prepared (2.5 mg SAL per 10 ml distilled water, with 60 mg reduced glutathione per ml). Various dilutions of this stock solution were made from which 2-μl aliquots were used to spike 50-μl samples of human plasma (Reagent 9) which were subsequently assayed by the radioenzymatic procedure described above. The amounts of salsolinol added to the 50-μl plasma samples were 0, 5, 30, 50, 230, and 500 pg. Each sample was analyzed in duplicate to quadruplicate. An aliquot of an appropriate blank, which consisted of the contents of the vacutainer tubes (see Chemicals and equipment) used for plasma collection diluted with distilled water, was also assayed without salsolinol spiking. In all plasma samples, the ^{3}H-O-methylated derivatives of endogenous DA, EPI and NE were also assayed and compared to the standard values provided with the CAT-A-KIT. Adult rat plasma was analyzed similarly.

Tissue standard curve

Sprague-Dawley neonatal rats were used to prepare the tissue standard curve. The pups were immersed in liquid nitrogen, and stored at —20° until used. At the time of assay, each frozen pup was weighed, minced, and homogenized in 8 ml of 0.1 *N* $HClO_4$ (containing 5 m*M* glutathione) by use of a Polytron tissue shearer. The tissue homogenates were spiked with various concentrations of salsolinol (with a volume not exceeding 150 μl). The concentrations of salsolinol ranged from 100 pg to 48 ng per g tissue. The Polytron was rinsed with an additional 3 ml of 0.1 *N* $HClO_4$ which was then added to the spiked homogenate. Each homogenate was centrifuged at 100,000 *g* at 4° for 1 h. The supernatant was decanted and retained. The pellet was rehomogenized in 6 ml of 0.1 *N* $HClO_4$, recentrifuged at 100,000 *g* at 4° for 1 h, and the supernatant decanted. The two supernatants were combined and stored at —20° until assayed. Upon thawing, the supernatant was subjected to a final centrifugation at 39,000 *g* for 1 h at 4° and any residue discarded. Duplicate aliquots of the supernatants were assayed according to the radioenzymatic procedure described above. Perchloric acid blanks (containing 5 m*M* reduced glutathione) without salsolinol spiking nor tissue were also assayed.

Verification of the identity of trace amounts of tissue salsolinol

Three sets of experiments were designed to verify the identity of [^{3}H]-

salsoline, particularly at the lower limits of sensitivity of the radioenzymatic assay for tissue salsolinol:

(a) The first set of experiments was designed to ascertain that the radioactivity detected on the TLC plate zone corresponding to the R_F of salsoline, at the lower limits of sensitivity of the tissue standard curve, was contributed exclusively by a COMT-dependent end-product (presumably [^{3}H]salsoline). Neonatal rat homogenates were prepared as described above and spiked with salsolinol to give a concentration range of 100—2000 pg salsolinol per g tissue. The samples were subjected to the radioenzymatic assay described above in presence of ^{3}H-SAM but with the omission of COMT. After extraction and development on TLC plates as described above, the zones on the TLC plates corresponding to the R_F of salsoline were scraped, eluted, and counted, in order to determine if a significant amount of radioactivity (above background) could be detected as compared to similar samples which had been O-methylated in presence of COMT.

(b) The second set of experiments was designed to further ensure that the tissue-extracted radioactivity on the TLC plate at the R_F corresponding to salsoline was contributed by a single product ([^{3}H]salsoline). Two-dimensional TLC in two additional solvent systems was performed on two salsolinol-spiked tissue samples (1000 pg salsolinol per g tissue) which were taken through the entire radioenzymatic assay procedure (in presence of ^{3}H-SAM and COMT) as described above. The TLC plates were then developed first in *tert.*-amyl alcohol—toluene—40% methylamine (6:2:3) solution as previously described, and the zones corresponding to the tritiated O-methylated derivatives of SAL, DA, EPI and NE visualized under UV light. The plates were allowed to dry and then turned 90° and re-developed in one of the following solvent systems: isopropanol—*n*-butanol—water—formic acid (60:20:19:1) or 1-butanol—methanol—1 *N* formic acid (60:20:20), and the solvent front allowed to travel a distance of 16 cm. The plates were allowed to dry and again visualized under UV light. The fluorescent spot in the salsoline lane was scraped from each plate, as were additional 1-cm increments of silica below and above the fluorescent spot, from the origin to the solvent front. Elution and counting of radioactivity were performed as described above.

(c) Finally, a third set of experiments was designed to determine if any endogenous salsolinol was present in the neonatal rat, which may contribute to the [^{3}H]salsoline counts resulting from exogenously added salsolinol in the assay. Nine tissue homogenates, with no exogenously added salsolinol, were taken through the entire radioenzymatic procedure (with ^{3}H-SAM and COMT). Duplicate 50-μl aliquots of each tissue homogenate were subjected to the methylation, extraction, and TLC separation procedures as described above.

Presentation of data

All data are presented as gross disintegrations per minute (DPM), as well as gross counts per minute (CPM) ± S.E.M. -- the latter to facilitate comparison with published results for catecholamines [17—19].

RESULTS AND DISCUSSION

Separation of O-methylated derivatives of SAL, DA, EPI and NE by TLC

Fig. 1 illustrates the separation of the O-methyl derivatives of NE, EPI, DA and SAL in four samples, by the TLC method described under Materials and methods. The four distinct spots observed under UV light represent normetanephrine, metanephrine, 3-methoxytyramine, and 7-O-methylsalsolinol (salsoline) with average R_F values of 0.22, 0.35, 0.49, and 0.54, respectively.

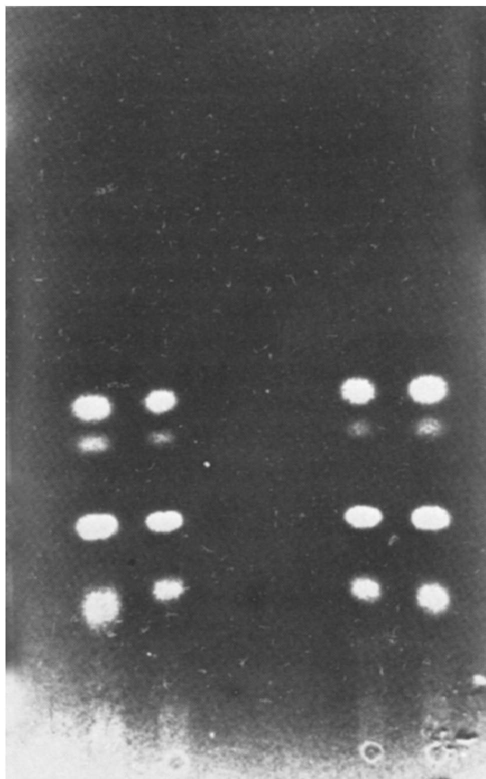

Fig. 1. TLC separation of normetanephrine (R_F = 0.22), metanephrine (R_F = 0.35), 3-methoxytyramine (R_F = 0.49), and salsoline (R_F = 0.54). A total of 20 experiments were performed, including the four shown in the figure. (Reverse negative taken under UV light; the points of application are at the bottom of the photograph.)

Aqueous standard curve for salsolinol

Fig. 2 represents the standard curve for varying concentrations of aqueous salsolinol solutions expressed in terms of DPM and CPM of [^{3}H]salsoline formed. The data demonstrate linearity of the assay from the highest tested concentration of 1500 pg salsolinol per 50-μl sample (30 ng/ml) to the lower limit of sensitivity of less than 50 pg salsolinol per 50 μl (< 1 ng/ml). The aqueous blank (with no salsolinol) averaged 198 ± 4 CPM per 50 μl (508 ± 9 DPM per 50 μl) for the area on the TLC plate corresponding to the R_F of

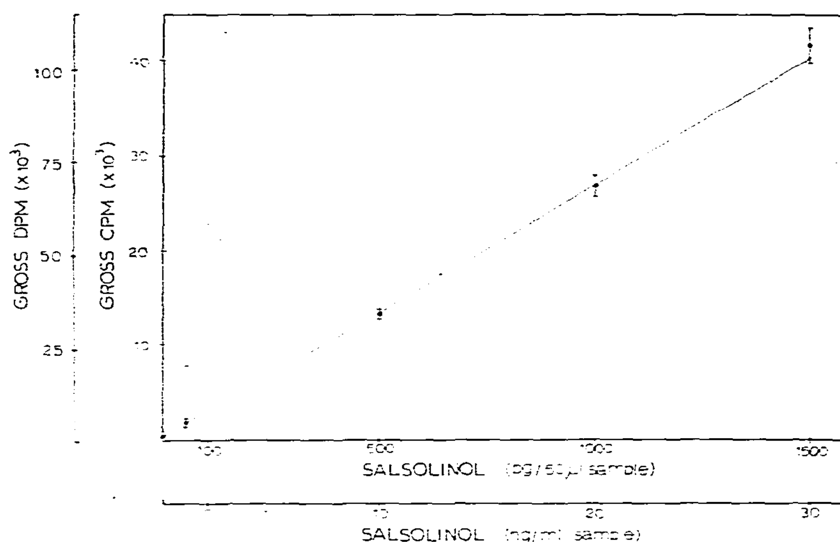

Fig. 2. Aqueous standard curve for salsolinol, expressed in terms of DPM or CPM of [^{3}H]-salsoline formed. Each point represents the mean of three observations. Standard errors are given only for CPMs (those for the DPMs being smaller on the DPM scale).

salsoline. As reported by Upjohn [18] and as observed with our use of the CAT-A-KIT, it is common to obtain a blank value of as much as 185 CPM per 50-μl sample for the area on the TLC-plate corresponding to the R_F of the O-methylated derivative of DA, as compared to a blank of 30—40 CPM per 50-μl sample for the areas on the TLC plates corresponding to the O-methylated derivatives of EPI and NE. This is likely due to the fact that [^{3}H]-methoxytyramine is extracted from a basic solution whereas [^{3}H]metanephrine and [^{3}H]normetanephrine are extracted from acidic solution. Since salsoline is a dopamine derivative, and since [^{3}H]salsoline and [^{3}H]methoxytyramine are extracted by the same procedure and counted in the same cocktail, it was not surprising that the blank for [^{3}H]salsoline resembled that of [^{3}H]methoxytyramine.

Plasma standard curve

Fig. 3 demonstrates the linearity of the human plasma standard curve for salsolinol (measured in terms of [^{3}H]salsoline formed) over the tested range of 5 pg per 50 μl (100 pg/ml) to 500 pg per 50 μl (10 ng/ml). Similar results were obtained with adult rat plasma. The plasma blank (with no salsolinol) averaged 219 ± 13 CPM per 50-μl sample (561 ± 32 DPM per 50 μl) (n = 4) for the area on the TLC plate corresponding to the R_F of salsoline — a value which is not significantly greater than that observed for the aqueous blank (198 ± 4 CPM per 50 μl).

For technical control purposes, the NE, EPI and DA contents of the plasma samples were also determined following the conversion of these catecholamines to their ^{3}H-O-methylated derivatives, and the values obtained were well within the ranges reported by Upjohn [18].

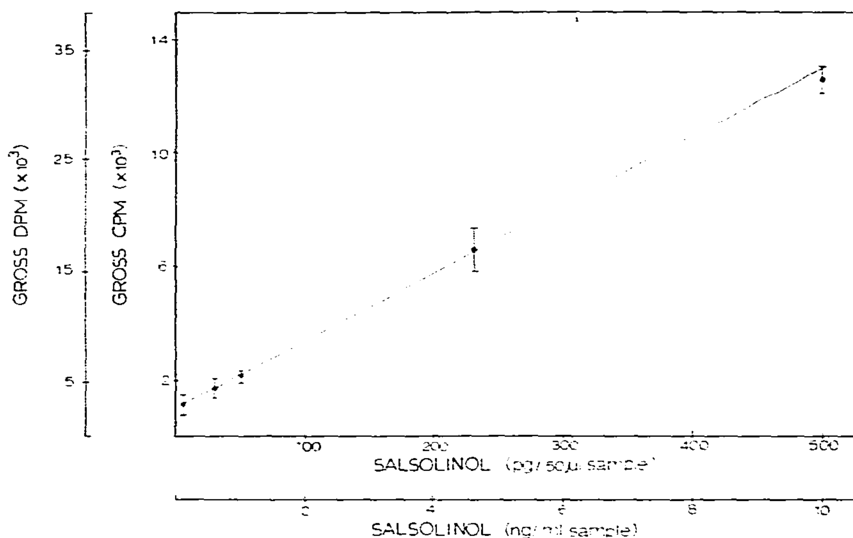

Fig. 3. Plasma standard curve for salsolinol, expressed in terms of DPM or CPM of [^{3}H]-salsoline formed. Each point represents the mean of two to four observations. Standard errors are given only for CPMs (those for the DPMs being smaller on the DPM scale).

Tissue standard curve

Fig. 4 demonstrates the linearity of the tissue standard curve for salsolinol (measured in terms of [^{3}H]salsoline formed) over a range of 500 pg salsolinol per g tissue to 48 ng salsolinol per g tissue. At the lower portion of the curve (< 500 pg salsolinol per g tissue) salsolinol can still be detected but its accurate quantification is no longer evident. The perchloric acid blank (with neither

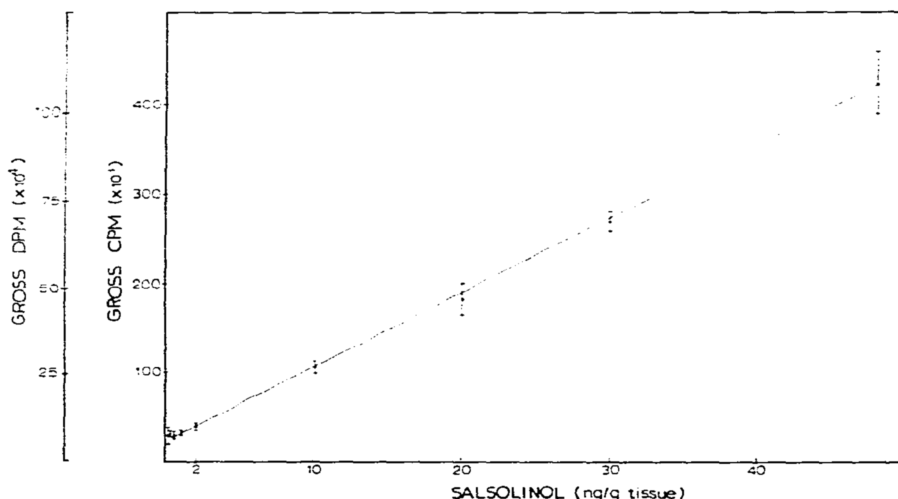

Fig. 4. Tissue standard curve for salsolinol, expressed in terms of DPM or CPM of [^{3}H]-salsoline formed. Each point represents the mean of six observations. Standard errors are given only for CPMs (those for the DPMs being smaller on the DPM scale).

tissue nor salsolinol) averaged 140 ± 7 CPM per 50-μl sample (358 ± 18 DPM per 50 μl) for the area on the TLC plate corresponding to the R_F of salsoline (n = 5).

Verification of the identity of trace amounts of tissue salsolinol

In the Materials and methods section, three sets of experiments were described aimed at verifying the identity of [^{3}H]salsoline, particularly at the lower limits of sensitivity, and at detecting endogenous tissue salsolinol.

(a) Table I shows the results of the first set of experiments, designed to verify the COMT-dependency of the radioactivity detected on the TLC plate zone corresponding to the R_F of salsoline at the lower level of sensitivity of the tissue standard curve (Fig. 4). The results shown in Table I demonstrate that, as compared to tissue samples processed in the same manner in the presence of COMT (Fig. 4), the exclusion of COMT resulted in insignificant radioactivity in the TLC zone corresponding to the R_F for salsoline. It is thus evident that the radioactivity detected at the lower limits of sensitivity in the tissue standard curve (Fig 4) is only contributed by a tritiated O-methylated product (presumably [^{3}H]salsoline) and is not due to the formation of any COMT-independent reaction product which may have utilized ^{3}H-SAM as a ^{3}H-methyl donor. It should also be mentioned that the TLC zones corresponding to the R_F values of methoxytyramine, metanephrine, and normetanephrine were likewise lacking in any significant radioactivity above background values.

TABLE I

RADIOENZYMATIC ASSAY IN THE ABSENCE OF COMT

Concentration of salsolinol added to neonatal tissue (pg per g tissue)	Gross CPM per g tissue*	Gross DPM per g tissue
100	5916 ± 233	15,169 ± 597
500	6069 ± 35	15,561 ± 89
1000	4383 ± 175	11,238 ± 448
2000	5992 ± 264	15,364 ± 677

*Mean value (± S.E.M.) of two observations. These mean values represent 84—120 CPM per 50-μl sample, and correspond to the value of the perchloric acid blank (with neither tissue nor salsolinol).

(b) The second set of experiments, using two-dimensional TLC in two additional solvent systems, was designed to further ensure that the tissue-extracted radioactivity on the TLC plate at the R_F corresponding to salsoline was contributed by a single product ([^{3}H]salsoline). The results of these two-dimensional chromatographic studies demonstrated that radioactivity on the TLC plate in the zone corresponding to the R_F of salsoline was contributed by a single product ([^{3}H]salsoline), since the two-dimensional development in the two additional solvent systems did not resolve the [^{3}H]salsoline spot into any additional components. It should be pointed out that if any [^{3}H]isosalsoline (6-O-methylsalsolinol) was formed along with the [^{3}H]salsoline (7-O-methyl-

salsolinol), then the former must have an identical mobility as the latter in all three solvent systems used in these experiments. It has been reported previously [5, 16] that isosalsoline and salsoline exhibit identical retention times in gas chromatographic procedures.

(c) Finally, in the third set of experiments, which was designed to determine if any endogenous salsolinol was present in the neonatal rat, an average of 19,944 ± 677 CPM/g tissue (51,138 ± 1735 DPM) was found in the zones on the TLC plates corresponding to the R_F of salsoline. Since this value falls on the non-linear lower portion of the tissue standard curve (Fig. 4), the amount of endogenous salsolinol present in neonatal rats cannot be accurately quantitated, but it is significantly less than 500 pg/g tissue ($P < 0.05$ by Student t test) and significantly greater than the values reported in Table I ($P < 0.001$ by Student t test). These findings are indicative of the presence of traces of endogenous salsolinol in neonatal rats who themselves and their parents had never been exposed to exogenous ethanol. An explanation for this finding may reside in the observation that acetaldehyde is endogenously formed in the rat gut [20], and it is possible that, following absorption, this endogenous acetaldehyde may condense with dopamine to form traces of salsolinol. Such reactions could conceivably occur in the fetus or be passively acquired through placental transfer of either acetaldehyde or salsolinol.

CONCLUSIONS

The radioenzymatic assay for salsolinol described in this report enables detection of trace amounts of salsolinol in tissue (0.1 ng/g), although accurate quantitation of this alkaloid can only be achieved at concentrations of 0.5 ng/g tissue or above. Accurate quantitation of plasma salsolinol can be achieved at concentrations of the alkaloid as low as 0.1 ng/ml. This radioenzymatic method is, therefore, more sensitive than the previous GC—EC assay for salsolinol developed in our laboratory [3], which had a lower sensitivity limit of 8 ng/g tissue. The radioenzymatic method is also more sensitive than the GC—EC procedure of Hamilton et al. [5] which is similar in sensitivity to our previous GC—EC method [3], and is likewise more sensitive than the high-performance liquid chromatographic assay described by Riggin and Kissinger [6] which has a sensitivity of 2 ng salsolinol per g tissue. With the use of the radioenzymatic procedure described in this manuscript, we were able to demonstrate the presence of endogenous salsolinol, in concentrations of < 0.5 ng/g tissue, in neonatal rat tissue in the absence of prior exposure to ethanol.

At the time of submission of this manuscript, Dean et al. [21] reported an apparently similar radioenzymatic assay for salsolinol as the one reported here.

ACKNOWLEDGEMENTS

We are grateful to Dr. Norman J. Uretsky (The Ohio State University) and Dr. Dennis Kane (The Upjohn Company) for helpful suggestions throughout this study. This work was supported by U.S. Public Health Service Grant 1-R01-AA02466 from the National Institute on Alcohol Abuse and Alcoholism.

REFERENCES

1 R.G. Rahwan, Life Sci., 15 (1974) 617.
2 R.G. Rahwan, Toxicol. Appl. Pharmacol., 34 (1975) 3.
3 P.J. O'Neill and R.G. Rahwan, J. Pharm. Sci., 66 (1977) 893.
4 P.J. O'Neill and R.G. Rahwan, J. Pharmacol. Exp. Ther., 200 (1977) 306.
5 M.G. Hamilton, K. Blum and M. Hirst, Alc. Clin. Exp. Res., 2 (1978) 133.
6 R.M. Riggin and P.T. Kissinger, Anal. Chem., 49 (1977) 530.
7 R.D. Myers and C.L. Melchior, Science, 196 (1977) 554.
8 R.D. Myers and C.L. Melchior, Pharmacol. Biochem. Behav., 7 (1977) 381.
9 R.D. Myers and M.M. Oblinger, Drug and Alcohol Dependence, 2 (1977) 469.
10 C.L. Melchior and R.D. Myers, Pharmacol. Biochem. Behav., 7 (1977) 19.
11 C.L. Melchior and R.D. Myers, in R.G. Thurman, J.R. Williamson, H. Drott and B. Chance (Editors), Alcohol and Aldehyde Metabolizing Systems, Vol. 3, Academic Press, New York, 1978, p. 545.
12 S.K. Clarren and D.W. Smith, N. Engl. J. Med., 298 (1978) 1063.
13 K.L. Jones and D.W. Smith, Teratology, 12 (1975) 1.
14 J.L. Schardein, Drugs as Teratogens, CRC Press, Cleveland, Ohio, 1976.
15 T.H. Shepard, Catalog of Teratogenic Agents, Johns Hopkins University Press, Baltimore, Md., 2nd ed., 1976.
16 A.C. Collins, J.L. Cashaw and V.E. Davis, Biochem. Pharmacol., 22 (1973) 2337.
17 P.G. Passon and J.D. Peuler, Anal. Biochem., 51 (1973) 618.
18 CAT-A-KIT (Catecholamines Radioenzymatic Assay Kit [^{3}H]), Upjohn Diagnostics Procedures Manual, The Upjohn Company, Kalamazoo, Mich.
19 J.D. Peuler and G.A. Johnson, Life Sci., 21 (1977) 625.
20 I.R. McManus, A.O. Contag and R.E. Olson, Science, 131 (1960) 102.
21 R.A. Dean, D.P. Henry, R.R. Bowsher and R.B. Forney, Abstracts of the 18th Annual Meeting of the Society of Toxicology, New Orleans, La., March 1979, Abstr. 408.