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TETRAHYDROISOQUINOLINE ALKALOIDS IN THE ADRENAL MEDULLA AFTER PERFUSION WITH "BLOOD CONCENTRATIONS" OF [^{14}C]ACETALDEHYDE

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Abstract—[^{14}C]Tetrahydroisoquinoline alkaloids were formed *in situ* from epinephrine and norepinephrine in the medullae of isolated cow adrenal glands during perfusions with solutions of [^{14}C]acetaldehyde. The radioactive alkaloids were isolated by adsorption onto aluminium hydroxide; they were separated and identified by thin-layer chromatography. The concentration of acetaldehyde used for perfusion (1 μg/ml) corresponds to a blood level seen in man during ingestion of alcoholic beverages.

TETRAHYDROISOQUINOLINE (TIQ) alkaloids were previously identified[1] in the medullae of cow adrenal glands that had been perfused with buffered solutions of dilute acetaldehyde. The TIQs were condensation products of acetaldehyde with endogenous epinephrine and norepinephrine; structures are shown in Fig. 1. TIQ alkaloids possessing an hydroxyl group in the 4-position are not known to occur naturally and have not been studied for pharmacological properties. However, they are related structurally to a group of synthetic and naturally-occurring alkaloids that exhibit a variety of

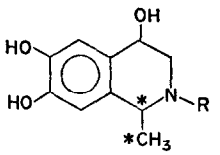

FIG. 1. 1-Methyl-4,6,7-trihydroxy-1,2,3,4-tetrahydroisoquinolines formed by condensation of acetaldehyde with norepinephrine (R=H) and epinephrine (R=CH_3). The radioactive carbon atoms derived from [1,2-^{14}C]acetaldehyde are marked by asterisks.

actions on smooth muscles and nerves and this has led to the suggestion[1] that TIQ alkaloids might be synthesized in the adrenal glands and catecholamine-containing neurones of man during ingestion of alcoholic beverages and that they might contribute to some of the pharmacologic actions of alcohol or its after effects.

In the earlier perfusion experiments,[1] the lowest acetaldehyde concentration for which evidence for TIQ synthesis was obtained was 100 μg/ml. This concentration is roughly 100-fold higher than the acetaldehyde level in the blood of man during ingestion of alcoholic beverages.[2,3] It was important to demonstrate that TIQ synthesis was feasible in intact cells exposed to acetaldehyde at a concentration corresponding to that expected in man. This has now been achieved by perfusions of

cow adrenal glands with [^{14}C]acetaldehyde at 1 μg/ml which resulted in the formation of radiolabeled TIQ alkaloids.

In several unreported experiments, no evidence was obtained for TIQ synthesis in the adrenal glands of rats after *in vivo* administration of ethanol. However, in experiments with methanol, the metabolic percursor of formaldehyde (which reacts rapidly[1] with catecholamines to form TIQs lacking a methyl group in the 1-position), evidence for adrenal TIQ synthesis *in vivo* was obtained both by fluorescence histochemistry after administration of large amounts of non-radioactive methanol[4] and by thin-layer radiochromatographic assay after administration of a tracer dose of [^{14}C]methanol.[5] These latter experiments and the data presented in this manuscript make it appear likely that TIQ synthesis from acetaldehyde (derived from ethanol) is technically feasible in man, although direct evidence for this is lacking at the current time.

METHODS

[1,2-^{14}C]Acetaldehyde (sp.act. 1 μc/μmole, Schwarz BioResearch, Inc., Orangeburg, N.Y.) was prepared at 1 μg/ml in isotonic saline buffered to pH 7·4 with 0·01 M sodium phosphate. The experiments were similar to those described earlier[1] with non-radioactive acetaldehyde. Retrograde perfusions[6] through the adrenal veins of fresh cow adrenal glands were carried out with the buffered acetaldehyde at 37° and with flow rates of 7–12 ml/min. At the conclusion, excess acetaldehyde was removed by brief perfusion with 30–50 ml of buffered saline. The adrenal cortex was stripped and the entire medulla was cut into chunks, mashed with a mortar and pestle in the presence of 2 ml cold 95 per cent ethanol–conc. HCl (99:1) and, finally, homogenized in a total of 10 vol. of cold ethanolic HCl; the ethanolic HCl served to prevent further reaction and to extract the TIQs and catecholamines.[1] The homogenate was stored overnight at −5°, rehomogenized and centrifuged at 700 *g* for 15 min. The supernatant fluid was concentrated 5-fold by flash evaporation; by this process, any remaining [^{14}C]acetaldehyde was removed. The condensed extract was stored at −5°; salts that precipitated on storage were discarded.

Catechol compounds were isolated by extraction with aluminum hydroxide. However, as discussed later, the presence of large amounts of epinephrine in the final extract caused a minor difficulty in subsequent thin-layer chromatography. Therefore, the catecholamines were removed by condensation with non-radioactive acetaldehyde; this procedure simultaneously provided carrier condensation products for chromatography. One ml of tissue extract was mixed with 10 ml of 1 M pH 6·0 acetate buffer, to which was added 0·1 ml non-radioactive acetaldehyde (Eastman Organic Chem., redistilled and stored under nitrogen in sealed ampoules at −5°). After the mixture had been allowed to stand at room temperature for 1 hr, an aliquot was tested by iodine oxidation[1] to ensure that the condensation reaction was complete. The remaining catechol compounds, viz. TIQs (Fig. 1) and two minor condensation products for each catecholamine,[1] were isolated by adsorption on aluminium hydroxide. A modification of the method of Goldenberg and co-workers[7] for isolation of catecholamines from urine was used. The reaction mixture was mixed with 100 ml of 1 per cent (w/v) $Al_2(SO_4)_3$ 18 H_2O (Merck) and $Al(OH)_3$ was precipitated at pH 8·3. The precipitate was washed three times by resuspension in 95 per cent ethanol and recentri-

fugation and, finally, it was dissolved in 3 ml 4 N HCl. In the final stages of this procedure,[7] the purified extract was concentrated by evaporation under a stream of nitrogen. Salts that precipitated were centrifuged, washed once with ethanol and discarded; the ethanol washes were combined with the original extract. Evaporation was continued until 0·5 ml of purified extract remained. Salts that precipitated on storage were discarded. Over 90 per cent of the radioactivity in the condensed extract of the adrenal medulla consisted of contaminants that were eliminated by the purification procedure; thin-layer chromatography showed that the remaining radioactivity was localized over those areas corresponding to the TIQs and minor condensation products. In separate experiments, the aluminum hydroxide extraction procedure recovered 70–80 per cent of the radioactivity in condensates of "cold" acetaldehyde with [^{14}C]catecholamines.

Purified adrenal extracts were analyzed by thin-layer chromatography on Adsorbosil-1 (Applied Science Labs., State College, Pa.) with *sec*-butanol–formic acid–water (15:3:2) in a nitrogen atmosphere. Approximately 50–100 μl of extract was applied as a row of small spots such that a narrow band 15 cm long was formed. After chromatography the plate was sprayed with $K_3Fe(CN)_6$ and $FeCl_3$ to visualize the catechols as Prussian blue bands.[1] The plate was marked off from below the origin to above the solvent front into segments of approximately 1 cm each; slight adjustments were made to permit individual bands to be cleanly separated. The Adsorbosil in each section was scraped from the plate with a razor blade and transferred to a vial containing 15 ml of Bray's solution.[8] Radioactivity in each vial was measured on a Packard Tri-Carb Scintillation Counter.

RESULTS AND DISCUSSION

In thin-layer chromatograms of purified extracts of adrenal medullae (Fig. 2), radioactivity was clearly localized to segments where the carrier TIQs were present (segments 7 and 10). Smaller amounts of radioactivity were associated with the minor condensation products[1] of acetaldehyde with the catecholamines. These data show that TIQs were synthesized in intact tissues during perfusion with buffered solutions of acetaldehyde corresponding in concentration to that seen in the blood of man during ingestion of alcoholic beverages.[2,3]

Initially, tissue extracts were not subjected to the condensation with excess "cold" acetaldehyde prior to purification with aluminum hydroxide. Thin-layer chromatographic radioassay with added carrier condensation products showed a radioactivity distribution similar to that in Fig. 2. Surprisingly, however, a small amount of radioactivity in the vicinity of segment 9 appeared to be associated with epinephrine, which was easily distinguished as a broad pink band after spraying with ferricyanide.[1] To determine whether or not radioactive epinephrine had been synthesized during perfusion with [^{14}C]acetaldehyde, the crude extracts were incubated with excess acetaldehyde in order to redistribute the radioactivity to the products in bands A, B and C. However, despite complete removal of epinephrine (and norepinephrine) as monitored by iodine oxidation,[1] the original radioactivity persisted, now associated with a narrow band (band B). Band B also appeared pink when the ferricyanide-sprayed plate was heated at 110° for about 5 min; since heating was routinely used

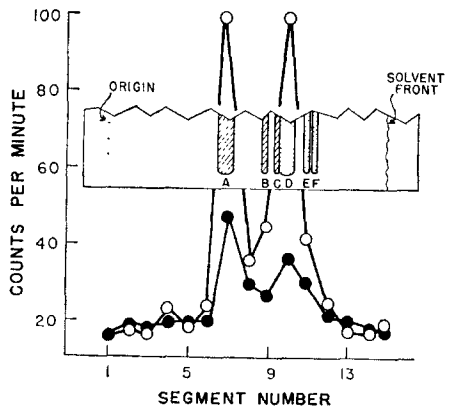

FIG. 2. Thin-layer radiochromatographic analyses of two adrenal glands perfused with 2 l. of solution containing 1 μg [1,2-^{14}C]acetaldehyde per ml. Gland (●), perfusion for 165 min, medulla weight 3·8 g. Gland (O), perfusion for 285 min, medulla weight 2·4 g. In the schematic representation of the thin-layer plate, the wider Prussian blue bands are the TIQs derived from epinephrine (band A) and from norepinephrine (band D); the narrower bands are the minor derivatives of epinephrine (bands B and C) and of norepinephrine (bands E and F). Bands E and F, although clearly separated, were so close to one another that they were removed together (segment 11) for scintillation spectrometry. Band C could not be readily distinguished from band D and these two were removed together (segment 10). Approximately 150 μl of each adrenal extract was analyzed by combining the scrapings from two plates. Background radioactivity was 18 counts/min.

to speed drying prior to respraying with $FeCl_3$, it was apparent that epinephrine and the closely adjacent band B had earlier been removed together.

Another perfusion experiment was performed with 1 μg [^{14}C]acetaldehyde plus 99 μg cold carrier acetaldehyde per ml. Despite the 100-fold dilution in specific activity, large amounts of radioactive TIQs and minor condensation products appeared. A likely interpretation is that the rate of metabolism of [^{14}C]acetaldehyde controlled the extent of the condensation reaction in the gland: At higher acetaldehyde concentrations, the elimination of [^{14}C]acetaldehyde was impeded and large amounts of radioactive condensation products were formed; at lower concentrations, the [^{14}C]acetaldehyde was more rapidly removed and smaller amounts of radioactive products were formed. This explanation is supported by data obtained in earlier experiments[1] where high perfusion rates (16 ml/min) were required to observe TIQs when concentrations of 100 μg acetaldehyde/ml were used, whereas very much slower perfusion rates (0·5–2·0 ml/min) produced excellent results at higher concentrations of 1 mg acetaldehyde per ml. The conditions are quite different when alcohol is ingested, since the oxidation of ethanol in adrenal tissue would result in the maintenance of a constant or steady-state level of acetaldehyde.

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