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Distribution of exogenous acetaldehyde in cow adrenal medulla chromaffin granules

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Release of catecholamines from the cow perfused isolated adrenal gland was induced by infusion of acetaldehyde in concentrations between 5×10^{-4} and $3{\cdot}5 \times 10^{-1}$M. There was a significant correlation between the concentration of acetaldehyde and catecholamine release. Analysis of homogenates of medullae from glands stimulated with [^{14}C]acetaldehyde showed that the specific activity (^{14}C mg^{-1} protein) was higher in chromaffin vesicles than in other tissue fractions. The major portion of the ^{14}C of chromaffin vesicles was present as soluble tetrahydroisoquinoline derivatives. There was no correlation between catecholamine release and the amount of tetrahydroisoquinolines formed. A small portion of the vesicle ^{14}C was associated with the membrane, and there was a significant correlation between the membrane-bound ^{14}C and catecholamine release. It is concluded that acetaldehyde can interact with the chromaffin vesicle membrane in a way that alters the ability of the vesicle to retain catecholamines which then diffuse out of the vesicle and into the circulation.

In man and other mammals the immediate product in the oxidation of ethanol is acetaldehyde, which is formed by the action of alcohol dehydrogenase. This aldehyde intermediate reaches concentrations in blood of around 10^{-5}M after ingestion of a moderate amount of alcohol (see Truitt & Walsh, 1971). Higher blood levels are obtained after ingestion of larger amounts of ethanol or after its ingestion in the presence of disulfiram, which inhibits the metabolism of acetaldehyde. An important question is whether acetaldehyde plays a role in the pharmacologic effects of ethanol, either with acute or with chronic ingestion. The pharmacologic actions of acetaldehyde have been summarized by Walsh (1971), and are similar to some of the effects of ethanol. The ability of acetaldehyde to release catecholamines from cardiac tissue (Kumar & Sheth, 1962; Walsh, Hollander & Truitt, 1969; Møller, 1971), adrenal medulla (Akabane, Nakanishi & others, 1965; Schneider, 1971) or brain (see Truitt & Walsh, 1971) may be responsible for some of these effects.

The mechanism by which acetaldehyde releases catecholamines from sympathetic nerves and adrenal medulla chromaffin cells is not known. However, it has been shown that release of catecholamines from the adrenal medulla by acetaldehyde is not through exocytosis, the mechanism by which acetylcholine and its analogues stimulate catecholamine release (Schneider, 1971). It has been suggested that acetaldehyde interacts with the catecholamine-containing storage vesicles in a way which alters their ability to maintain their normally high concentrations of catecholamines (Schneider, 1971). The object of the study reported here was to investigate this possibility further by examining the relation between catecholamine release from the

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adrenal medulla and the distribution of acetaldehyde in adrenal medulla chromaffin vesicles. The cow perfused adrenal gland was used in this study since it is a convenient model for studying catecholamine release.

METHODS

Perfused isolated adrenal gland of cow. Bovine adrenal glands, 10 to 15 g, were obtained approximately 15 min after the animals had been killed and were kept in ice for 30 to 60 min until required. Bovine adrenal glands were perfused in a retrograde fashion through the adrenal vein and stimulated as described previously (Schneider, Smith & Winkler, 1967). Perfusion fluid consisted of mM, NaCl 137, KCl 2·68, $CaCl_2$ 1·80, NaH_2PO_4 0·28, $MgCl_2$ 0·001, $NaHCO_3$ 11·60 and glucose 5·56 and was gassed with 5% carbon dioxide in oxygen. Stimulation of the gland was carried out by infusion (using a Harvard infusion pump) of 10 ml min^{-1} of perfusion fluid to which the drug was added at a point immediately before the perfusion fluid entered the tissue. Perfusates (1·5 ml) were analysed for total catecholamines by the colorimetric method of von Euler & Hamberg (1949) using citrate-phosphate buffer at pH 6·0 (McIlwaine, 1921); catecholamines were expressed as μmol of adrenaline since the absorbance of noradrenaline and adrenaline is similar at pH 6·0.

Radioactive acetaldehyde was prepared for infusion into the gland by adding acetaldehyde-1, 2-^{14}C sodium bisulphite (spec. act. 2·6 mCi mM^{-1}; Mallinckrodt) to solutions of acetaldehyde in Tyrode solution to give a radioisotope concentration equivalent to between 3 and 11 $\times$ 10^6 d min^{-1} ml^{-1}. The stock acetaldehyde solutions were kept at 0° and were diluted 1 to 11·5 upon infusion into the adrenal gland. Radioactivity of perfusates was measured by adding 1 ml of the perfusate to 10 ml of Bray's liquid scintillation fluid (Bray, 1960) and counting for radioactivity in a Beckman LS-100 liquid scintillation counter. Counts per minute (counts min^{-1}) were corrected for quenching and counting efficiency and expressed as d min^{-1}.

Preparation of tissue fractions. Homogenates of adrenal medulla prepared with a Potter-Elvehjem glass and Teflon homogenizer in 3 volumes of cold 0·3M sucrose were subjected to differential centrifugation. The homogenate was centrifuged at 550 *g* for 15 min to give the low speed sediment and the cytoplasmic extract (supernatant). The cytoplasmic extract was centrifuged at 12 500 *g* for 20 min to yield the large-vesicle fraction (sediment) and the post large-vesicle supernatant. (All *g* forces are given for the bottom of the tube.)

The large-vesicle fraction was resuspended in 2 vol (w/v) of 0·3M sucrose and centrifuged at 100 000 *g* for 120 min over a discontinuous sucrose gradient, consisting of 1·4 ml each of 1·0, 1·1, 1·2, 1·3, 1·4, 1·5, 1·6, 1·7, 1·8 and 2·0M sucrose, or over a one-step gradient consisting of 7 ml of 1·6M sucrose (Smith & Winkler, 1967). Chromaffin vesicles obtained by either of these methods were washed 3 times with 1·6M sucrose and lysed by 3 freeze-thaw cycles in a volume of distilled water equal to one-third the volume of the cytoplasmic extract. The lysed chromaffin vesicle suspension was centrifuged at 80 000 *g* for 60 min. The resulting supernatant was collected and the sediment was suspended in 1 ml of distilled water and centrifuged again at 80 000 *g* for 60 min. The supernatants were combined to give the soluble lysate and the sediment was washed twice in 1 ml of distilled water. The resulting supernatants were added to the soluble lysate unless indicated otherwise; the washed sediment was suspended in 1 ml of distilled water. Samples were stored at −10°.

Analysis of tissue fractions. Aliquots of the tissue fractions were diluted with Triton X-100 to give a final concentration of 0·9% Triton and assayed for catecholamines by the colorimetric method of von Euler & Hamberg (1949). A 0·1 ml aliquot of the Triton X-100 mixture was counted for radioactivity in 10 ml of Bray's liquid scintillation fluid (Bray, 1960). Protein in a 0·1 ml aliquot of the tissue fractions was precipitated by trichloroacetic acid (final concentration, 5% w/v) and measured by the microbiuret method (Goa, 1953). Protein assays were standardized with bovine serum albumin. Fumarase activity was measured at 23° according to Racker (1950) and β-glucuronidase activity was measured by the procedure of Gianetto & de Duve (1955).

Gel filtration column chromatography was carried out with Bio-Gel P-6, 100–200 mesh (1 × 17 cm) using 0·15M sodium chloride or 0·005M tris-sodium succinate buffer (pH 5·9) as the elution fluid. Protein of column eluate fractions was precipitated with trichloroacetic acid (final concentration, 5% w/v) and assayed by the microbiuret procedure. Aliquots (0·5 ml) of the column fractions were added to 10 ml Bray's scintillation fluid and counted for radioactivity as described above.

Thin-layer chromatography (t.l.c.) of 25 μl aliquots of the soluble lysates was carried out on Eastman 6060 silica gel chromatogram sheets. The solvent system consisted of redistilled s-butanol (15 vol), formic acid (3 vol) and water (2 vol). The chromatograms were developed at room temperature (20°) in the dark under an atmosphere of nitrogen. Spots were visualized either under ultraviolet light or by exposure to the colour development system described by Cohen & Collins (1970). The silica gel was removed from the sheet by scraping and extracted five times with 5 ml methanol. The methanol extracts were combined and evaporated to 2 ml under vacuum at room temperature; 1 ml of the concentrated extract was added to 10 ml of Bray's scintillation fluid for measurement of radioactivity by liquid scintillation spectrometry.

Tetrahydroisoquinoline derivatives were prepared from adrenaline and acetaldehyde according to the method of Brossi, Focella & Teitel (1972) for the condensation of L-dopa with formaldehyde. The reaction mixture consisted of 366 mg of adrenaline bitartrate, 5 ml of acetaldehyde, 0·2 ml of 1N sulphuric acid and 5 ml of distilled water and was heated at 50° for 2 h in the dark in a sealed tube. At the end of this time the excess acetaldehyde was removed under vacuum and the solution was stored at 4°. [^{14}C]Tetrahydroisoquinoline derivatives of adrenaline were prepared by including acetaldehyde-1,2[^{14}C]sodium bisulphite (1 μCi) in the reaction mixture. Recovery of [^{14}C]tetrahydroisoquinoline derivatives of adrenaline in the silica gel chromatography and extraction procedures was between 80 and 103% (n = 5).

All drugs were obtained from commercial sources.

RESULTS

Stimulation of cow perfused isolated adrenal glands with acetaldehyde in concentrations between 5×10^{-4} and $3{\cdot}5 \times 10^{-1}$M for varying lengths of time caused catecholamine levels in the perfusates to increase above the pre-stimulation levels. Catecholamine release (% above basal secretion) after stimulation with 5×10^{-4}M for 30 min was 34 and 66% in 2 separate experiments. There was a positive correlation between the concentration of acetaldehyde and the release of catecholamines ($r = 0{\cdot}757$, n = 12; $P<0{\cdot}01$). However, the correlation was higher when the product of concentration and the length of the stimulation period was compared with the release of catecholamines ($r = 0{\cdot}847$, n = 12; $P<0{\cdot}01$).

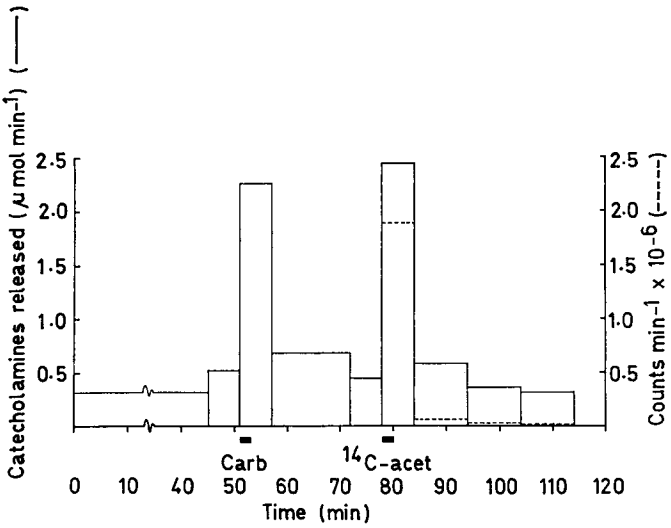

FIG. 1. Secretion of catecholamines from the perfused isolated cow adrenal gland. The gland was stimulated with $3{\cdot}0 \times 10^{-4}$M carbachol (carb) (infused during a 2 min period) and with $3{\cdot}3 \times 10^{-2}$M acetaldehyde [^{14}C]-acet (11 μCi [^{14}C]acetaldehyde + unlabelled acetaldehyde; infused during a 2 min period). The time during which the drugs were infused is indicated by the solid bars below the graph.

Fig. 1 shows the patterns of catecholamine release from the cow adrenal gland after stimulation with $3{\cdot}0 \times 10^{-4}$M carbachol and with $3{\cdot}3 \times 10^{-2}$M acetaldehyde (containing 10 μCi of [^{14}C]acetaldehyde). Analysis of the perfusate for ^{14}C shows that the major portion of the acetaldehyde passed through the gland in the first few minutes. Ninety-four percent of the ^{14}C was in the perfusate collected during the stimulation period and the subsequent 4 min. Perfusion of the adrenal gland with [^{14}C]acetaldehyde for times between 1 min 20 s and 60 min resulted in the retention by the tissue of a small portion of the ^{14}C infused. Between 0·3 and 1·4% (mean $0{\cdot}61 \pm 0{\cdot}25$, n = 6) of the infused ^{14}C was recovered in homogenates of adrenal medullae prepared 30 min after terminating infusion of [^{14}C]acetaldehyde at various concentrations and for various lengths of time of infusion.

Distribution of ^{14}C in homogenates of medullae. An initial experiment, in which the adrenal gland had been perfused with $3{\cdot}5 \times 10^{-1}$M acetaldehyde for 5 min, showed that 71% of the radioactivity, 78% of the catecholamines and 53% of the protein of the medulla homogenate was obtained in the low-speed supernatant (cytoplasmic extract). This distribution of catecholamines is similar to that reported previously (Schneider, 1972). Centrifugation of the cytoplasmic extracts from 0·3M sucrose homogenates of medullae obtained from glands perfused with acetaldehyde in concentrations between 5×10^{-4} and $3{\cdot}5 \times 10^{-1}$M containing [^{14}C]acetaldehyde showed that 23 ± 3% (n = 6) of the ^{14}C and 46 ± 7% (n = 6) of the catecholamines of the cytoplasmic extracts were localized in the large-vesicle fractions. There was no correlation between the percentage of the cytoplasmic radioactivity found in the large-vesicle fraction and either the concentration of acetaldehyde ($r = -0{\cdot}337$; n = 6) or the product of the concentration and the length of exposure to acetaldehyde ($r = -0{\cdot}272$; n = 6).

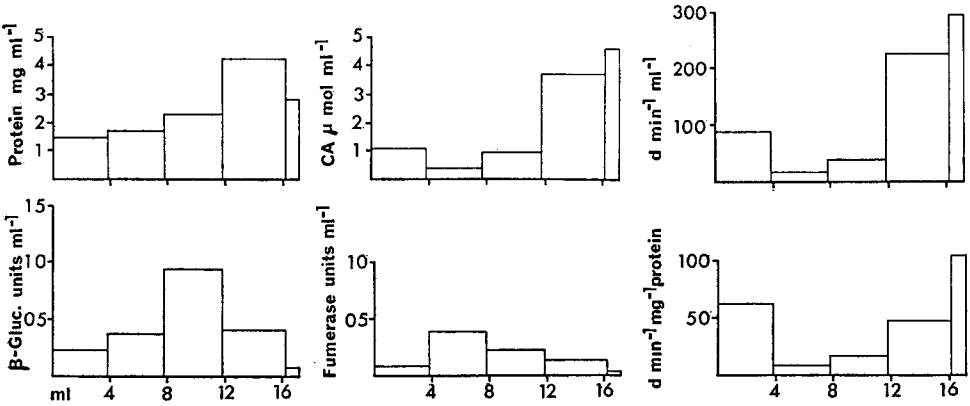

FIG. 2. Sucrose density gradient centrifugation of the large granule fraction obtained from cow adrenal medulla that had been perfused with [^{14}C]acetaldehyde. The medulla was homogenized in 0·3M sucrose and subjected to differential centrifugation (see methods) to obtain the large-granule fraction. The large-granule fraction was resuspended in 0·3M sucrose and an aliquot was layered over a gradient consisting from top to bottom of 1·4 ml each of 1·0, 1·1, 1·2, 1·3, 1·4, 1·5, 1·6, 1·7, 1·8 and 2·0M sucrose. The gradient was centrifuged at 4° for 120 min at 100 000 *g*. After centrifugation the tubes were punctured at the bottom and collected in fractions of 1·0 to 4·9 ml.

The volume (in ml) of each fraction is indicated by its width on the graphs. Aliquots of the fractions were assayed by the procedures described in the methods section.

Fractionation of the large-vesicle fraction by sucrose density gradient centrifugation (Fig. 2) in an initial experiment in which the gland was stimulated for 5 min with $3{\cdot}5 \times 10^{-1}$M acetaldehyde for 2 min, showed that the highest specific activity (d min^{-1} mg^{-1} protein) was associated with fraction 5, which also contained the highest concentration of catecholamines. The high catecholamine content and position in the gradient of this fraction reflects the presence of chromaffin vesicles. The high specific activity found in fraction 1, which was localized at the top of the gradient, most likely represents free acetaldehyde. Less radioactivity was associated with lysosomes, indicated by the distribution of β-glucuronidase activity, or with mitochondria, indicated by the distribution of fumarase activity. In later experiments chromaffin vesicles were routinely prepared by centrifugation of the large vesicle fraction over a 1-step sucrose gradient consisting of 1·6M sucrose (Smith & Winkler, 1967). Chromaffin vesicle preparations obtained from medullae of glands stimulated with acetaldehyde in concentrations between 5×10^{-4} and $3{\cdot}5 \times 10^{-1}$M contained 53 ± 10% (n = 9) of the ^{14}C of the large-vesicle fractions. Hypotonic lysis of the chromaffin vesicle preparation obtained from these medullae (followed by 3 distilled water washes of the resulting sediment) yielded two fractions, the soluble lysate, which contains essentially all the chromaffin vesicle catecholamines and 83% of the chromaffin vesicle proteins, and the chromaffin vesicle membrane. The soluble lysate contained 96·2 ± 0·4% (n = 11) of the ^{14}C of the chromaffin vesicle preparation for medullae from glands stimulated with 5×10^{-4} to $3{\cdot}5 \times 10^{-1}$M acetaldehyde.

Characteristics of the ^{14}C associated with the chromaffin vesicle membrane. Radioactivity remained associated with the chromaffin vesicle membrane even after 3 washes in distilled water. The amount of radioactivity in the membrane preparation represented as much as 1·65 μg of acetaldehyde per mg protein. Since a constant amount of [^{14}C]acetaldehyde was added to varying concentrations of cold acetaldehyde

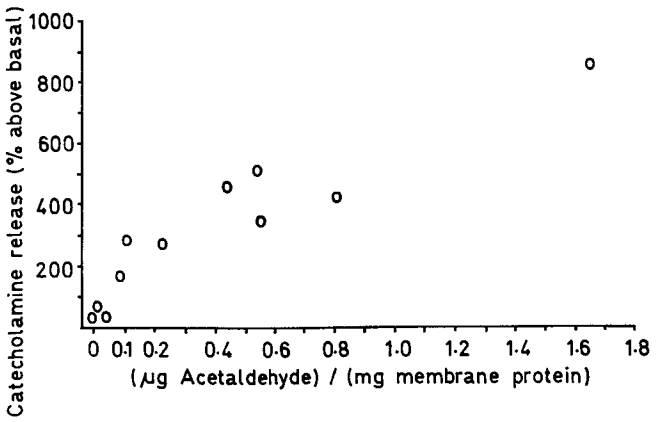

FIG. 3. The relation between catecholamine release from the perfused isolated cow adrenal gland and binding of [^{14}C]acetaldehyde to chromaffin vesicle membrane. Glands were stimulated with 5×10^{-4} to $3{\cdot}5 \times 10^{-1}$M acetaldehyde (containing [^{14}C]acetaldehyde). The membrane fraction was prepared and analysed by methods described in the text.

in the perfusion fluid, the specific activities of the infused acetaldehyde varied among experiments. For this reason the ^{14}C associated with the tissue fractions is expressed as the equivalent amount of total acetaldehyde for each individual experiment. There was a positive correlation between catecholamine release (% increase above basal level) and the μmoles of acetaldehyde per mg protein associated with the membrane ($r = 0{\cdot}859$, n = 11; $P<0{\cdot}01$) (Fig. 3). Due to the low amount of radioactivity associated with the washed membrane fraction it was not possible to carry out gel filtration studies on the membrane prepared from chromaffin vesicles in order to assess the reversibility of the binding. However, the radioactivity associated with the chromaffin vesicle membrane could be precipitated in acid; 94 and 100% of the ^{14}C of the membrane fractions were recovered from glands stimulated with 32 and 28 mM acetaldehyde respectively after precipitation of the protein in 5% trichloroacetic acid. Treatment of the membrane fraction (obtained from a gland stimulated for 30 min with 5×10^{-4}M acetaldehyde) with 1% Triton X-100 resulted in the solubilization of 75% of the radioactivity.

Characteristics of the ^{14}C associated with the soluble lysate of chromaffin vesicle preparations. There was no correlation between the percentage of the ^{14}C of the chromaffin vesicle preparation which was found in the lysate and the concentration of acetaldehyde with which the gland was stimulated ($r = -0{\cdot}340$; n = 11) or with the product of the acetaldehyde concentration and the length of time for which the gland was stimulated ($r = -0{\cdot}151$; n = 11). Furthermore, there was no correlation between catecholamines released (expressed as the % above basal levels) from the gland upon stimulation with acetaldehyde and the absolute amount of acetaldehyde (determined from the ^{14}C content) found in the soluble lysate ($r = -0{\cdot}15$; n = 11). Fig. 4 shows that the ^{14}C found in the soluble lysate could be completely separated from the chromaffin vesicle soluble proteins by gel filtration chromatography. The radioactivity of the soluble lysate was eluted from the gel filtration columns in the fractions in which adrenaline or [^{14}C]acetaldehyde added to the columns were eluted. The gland from which the data shown in Fig. 4 were obtained was stimulated with $7{\cdot}0 \times 10^{-2}$M acetaldehyde for 4 min; similar results were obtained for glands stimu-

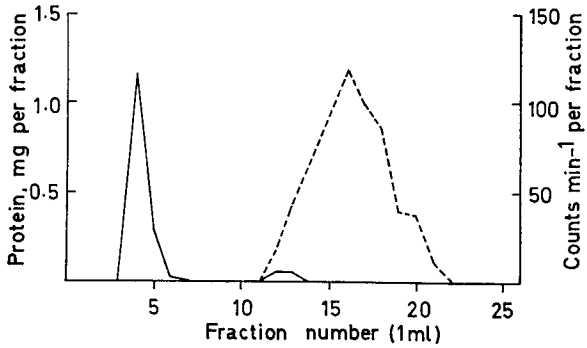

FIG. 4. Gel filtration chromatography of chromaffin vesicle soluble lysate. The vesicles were obtained from a perfused isolated cow adrenal gland which had been stimulated with $7{\cdot}0 \times 10^{-2}$M acetaldehyde for 30 min. The column (1 × 17 cm) consisted of Bio-Gel P-6, 100–200 mesh and was eluted with 0·15M NaCl. Vesicle lysates were added to the columns in a volume of 0·4 ml. (—) Protein. (- -) Counts min^{-1}.

lated with acetaldehyde, $5{\cdot}0 \times 10^{-4}$M, for 30 min (n = 1), 10^{-2}M for 8 min (n = 1) and $3{\cdot}5 \times 10^{-2}$M for 16 min (n = 2). These results are consistent with the finding that only 0·3 to 1·5% (n = 3; stimulation with 10^{-2}, $3{\cdot}5 \times 10^{-2}$ and $7{\cdot}0 \times 10^{-2}$M acetaldehyde) of the radioactivity of the soluble lysate was precipitated in 5% trichloroacetic acid.

The soluble lysates from glands stimulated with [^{14}C]acetaldehyde were analysed for the presence of tetrahydroisoquinolines, since these compounds have been found in adrenal glands perfused with acetaldehyde (Cohen & Collins, 1970; Cohen, 1971).

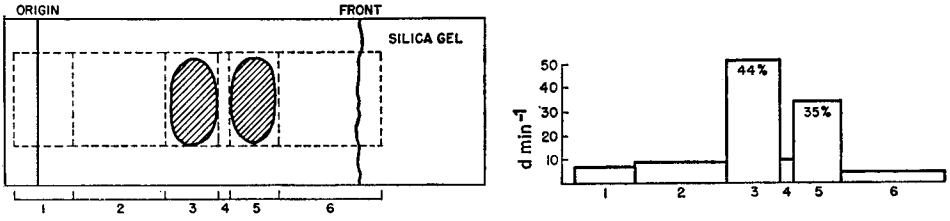

FIG. 5. Silica gel thin-layer chromatography of a chromaffin vesicle lysate obtained from a cow isolated perfused adrenal gland which had been stimulated for 10 min with $2{\cdot}8 \times 10^{-2}$M acetaldehyde containing [^{14}C]acetaldehyde. 25 μg of lysate was applied at the origin. See text for a description of the methods employed.

Silica gel t.l.c. showed that the major portion of the radioactivity of the soluble lysate migrated with tetrahydroisoquinoline derivatives formed from adrenaline and acetaldehyde (Fig. 5). This pattern was observed with lysates obtained from glands stimulated with 5×10^{4}, 10^{-2}, $2{\cdot}8 \times 10^{-2}$, $3{\cdot}2 \times 10^{-2}$ and $3{\cdot}5 \times 10^{-2}$M acetaldehyde; $61 \pm 5\%$ (n = 5) of the radioactivity was associated with the two major tetrahydroisoquinolines R_F values $0{\cdot}44 \pm 0{\cdot}02$ and $0{\cdot}61 \pm 0{\cdot}02$. The remainder of the radioactivity was distributed evenly over the rest of the plate, possibly reflecting oxidation products formed from the tetrahydroisoquinoline derivatives.

DISCUSSION

Release of catecholamines by acetaldehyde has been demonstrated in several systems, including adrenal medulla (Akabane & others, 1965; Schneider, 1971),

heart (Kumar & Sheth, 1962; Walsh & others, 1969; Møller, 1971) and brain (see Truitt & Walsh, 1971). A number of questions remains to be answered pertaining to this observation: 1. What is the mechanism by which acetaldehyde releases catecholamines? 2. Does catecholamine release by acetaldehyde occur *in vivo* after ingestion of ethanol? 3. Does catecholamine release *in vivo* play a role in the pharmacological effects of ethanol or of acetaldehyde? The work here reported was directed toward obtaining information about the first of these questions, the mechanism by which acetaldehyde causes catecholamine release.

Catecholamine release occurred in this study at concentrations of acetaldehyde as low as $5{\cdot}0 \times 10^{-4}$M. Recent studies (Schneider, 1973) have shown that catecholamine release occurs in the cow adrenal medulla after stimulation with concentrations of acetaldehyde as low as $1{\cdot}5 \times 10^{-5}$M. However, a 40 to 60 min exposure of the gland to acetaldehyde at these low concentrations is required to elicit release. The link between concentration and length of exposure is reflected in a higher correlation between catecholamine release and the product of acetaldehyde concentration and length of exposure than with concentration only. It may be that a period of time is required for accumulation of an effective concentration of the drug at its site of action. This period would be prolonged as the concentration of acetaldehyde is decreased.

Our results indicate that acetaldehyde binds to most if not all of the tissue homogenate fractions. Analysis by density gradient centrifugation of the large-vesicle fraction shows that mitochondria (fraction 2), lysosomes (fraction 3) and chromaffin vesicles (fractions 4 and 5) all contain radioactivity, although the specific activity of chromaffin vesicles is considerably higher than that of the others. Some of the radioactivity of fractions 2 and 3 could be due to contamination with chromaffin vesicles. The major portion of the radioactivity associated with the chromaffin vesicles was in the form of tetrahydroisoquinoline derivatives. In fact, over 95% of the radioactivity in the chromaffin vesicle fraction was solubilized upon lysis of the vesicles and appeared by separation on t.l.c. to be tetrahydroisoquinoline derivatives. This finding is in agreement with similar observations by Cohen & Collins (1970) and by Cohen (1971) showing formation of tetrahydroisoquinolines after perfusion of cow adrenal glands with acetaldehyde. These tetrahydroisoquinoline derivatives of catecholamines can be released from the adrenal gland upon stimulation with carbachol or acetylcholine (Greenberg & Cohen, 1973). However, the amount of tetrahydroisoquinolines formed is not related to release of catecholamines by acetaldehyde.

A small portion of the radioactivity found in the chromaffin vesicle was associated with the membrane and this quantity of ^{14}C correlated well with the release of catecholamines after stimulation with acetaldehyde. It may be that acetaldehyde interacts with the chromaffin vesicle membrane in a way that alters the ability of the vesicle to retain catecholamines. As a result the catecholamines, as well as ATP, might diffuse out of the vesicles (Schneider, 1971). However, vesicle soluble protein is not lost upon stimulation of the gland with acetaldehyde. This earlier work also showed that acetaldehyde does not cause release of catecholamines directly into the extracellular space by the process of exocytosis. Release into the cell cytoplasm should allow metabolic inactivation of the catecholamines by mitochondrial monoamine oxidase before diffusing out of the cell. The extent of oxidative deamination will be important in determining the physiological effect of the released catecholamines. The relative proportion of catecholamines released from vesicles that is acted upon by monoamine

oxidase will be higher in brain than in adrenal medulla since much larger quantities of catecholamines are stored and released in the medulla.

The ability of acetaldehyde to bind to the chromaffin vesicle membrane is not surprising in view of the chemical reactivity of the aldehyde group. The ability of aldehydes to react with protein has been demonstrated by several workers (Fraenkel-Conrat & Olcott, 1946; Crawford, Yu & Sinnhuber, 1967; Nakaya, Horinishi & Shibata, 1967). It is possible that the action of low concentrations of acetaldehyde on membranes represents early and reversible stages in protein denaturation. Gardner, Simon & Silver (1969) reported fixation of platelets in 1·8M acetaldehyde, a concentration which is much higher than those required to induce catecholamine release. Acetaldehyde in concentrations of 1 to 3 mM has been shown to alter mitochondrial function (Rubin, Cederbaum & Lieber, 1973). These authors suggested that acetaldehyde exerted its action through interactions with the mitochondrial membranes. Low concentrations ($2{\cdot}5 \times 10^{-3}$M) of formaldehyde have also been reported to have membrane effects that alter cell function (Fozzard & Dominguez, 1968). This 1 carbon aldehyde reduces resting cation conduction in sheep cardiac Purkinje fibres, thereby causing hyperpolarization of the resting potential. Furthermore, the aldehyde of 5-hydroxytryptamine is capable of binding to proteins of brain tissue (Alivisatos & Ungar, 1968).

It appears from this work that acetaldehyde-induced catecholamine release from the adrenal medulla is due to the ability of this aldehyde to interact with the chromaffin vesicle membrane. As a result of this interaction the amines diffuse from the "leaky" vesicles into the cytoplasm of the chromaffin cell and then into the circulation.

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