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EFFECTS OF LENGTH OF EXPOSURE TO AND CONCENTRATION OF ACETALDEHYDE ON THE RELEASE OF CATECHOLAMINES

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Abstract—The perfused isolated cow adrenal gland was stimulated to release catecholamines by perfusion with Tyrode solution containing acetaldehyde in concentrations between $1{\cdot}5 \times 10^{-5}$ and $2{\cdot}5 \times 10^{-2}$ M. Release per unit time (μmoles/min) of catecholamines by acetaldehyde increased as the length of exposure to the drug was increased. In contrast to carbachol, the concentration–response curve for acetaldehyde was shifted to the left by increasing the stimulation time 3-fold. A delay in reaching the maximum response was observed at 2×10^{-3} M acetaldehyde; this delay increased as the concentration of acetaldehyde was lowered. Catecholamine release returned to its basal level upon terminating the infusion of acetaldehyde. It is concluded that acetaldehyde can release adrenal catecholamines at concentrations similar to those that occur in man after ingestion of alcohol, but a period of time is required for accumulation of an effective concentration of the drug at its site of action.

ACETALDEHYDE can be classed as a sympathomimetic agent, since it can release tissue catecholamines from their tissue storage sites. This releasing effect has been demonstrated for cardiac tissue,[1–3] for adrenal medulla,[4,5] and in brain.[6] However, the lowest concentrations of acetaldehyde reported in these studies to elicit catecholamine release is on the order of 0·3–1 mM, which are higher than those that occur in man after ingestion of ethanol. Although the blood levels of acetaldehyde reported to occur in man are quite variable (0·1–30 μg/ml), the most recent studies have shown that the levels may be as high as 0·56 μg/ml ($1{\cdot}27 \times 10^{-5}$ M) after ingestion of moderate amounts of alcohol.[6] Blood acetaldehyde levels after alcohol administration in animals and in man can be increased several-fold in the presence of disulfiram.[6,7] If neurotransmitter release is a pharmacologically significant factor in determining the effects of acetaldehyde, an effect on release should be observable with concentrations of acetaldehyde that occur in the body. The experiments reported in this paper were designed to evaluate release of catecholamines by acetaldehyde in concentrations equivalent to those that occur in blood after alcohol ingestion. The perfused isolated cow adrenal gland was used for these studies, since it provides a convenient model system with which to study mechanisms of catecholamine secretion.[8]

METHODS

Bovine adrenal glands weighing between 10 and 20 g were obtained approximately 15 min after the animals had been killed and were kept in ice for 30–60 min until

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perfusion was begun. The glands were perfused in a retrograde fashion through the adrenal vein with Tyrode solution at 37°. Slits (1–2 cm) were made to a depth approximately two-thirds of the way through the cortex and the perfusion fluid leaving the gland by way of these slits was collected and kept at 4°. Perfusion fluid containing acetaldehyde or carbachol was infused continuously into the perfusion medium with a Harvard infusion pump at a point immediately before the fluid entered the tissue. The perfusion rate was maintained at 10 ml/min.

Perfusates were analyzed for total catecholamines within 30 min of collection by the colorimetric method of von Euler and Hamberg[9] using citrate-phosphate buffer at pH 6·0.[10] Only total catecholamines were assayed since earlier work showed that there was no preferential release of epinephrine or norepinephrine after stimulation with acetaldehyde.[4] Total catecholamines were expressed as μmoles of epinephrine, since the oxidation products of epinephrine and of norepinephrine have similar absorbances at pH 6. Preincubation of epinephrine with 10^{-3} M acetaldehyde for 60 min at 4° had no effect on the colorimetric analysis of epinephrine over the range of 0·05–1·0 μmoles.

The drugs used in this study were obtained through commercial sources.

RESULTS

Previous studies in this laboratory showing release of catecholamines from the perfused isolated cow adrenal gland in response to stimulation with acetaldehyde were conducted using 2-min stimulation periods.[4] Since the concentrations of acetaldehyde required to induce catecholamine release under these conditions ($> 10^{-3}$ M) were higher than concentrations occurring after ingestion of ethanol, further investigation of release at lower concentrations of acetaldehyde was undertaken. The possibility that exposure of the gland to lower concentrations of acetaldehyde for longer periods of time would lead to catecholamine release was studied first. Figure 1

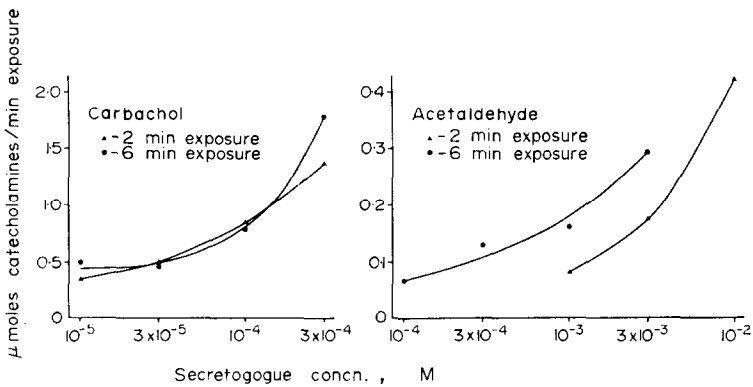

FIG. 1. Concentration-response curves for release of catecholamines from the perfused isolated cow adrenal gland. Catecholamine secretion is expressed as the increase in μmoles above the prestimulation level. In each case the perfusate collected in the 5-min period after stimulation was included in the stimulation period for analysis of catecholamines. The prestimulation period consisted of a period of time equal to the drug exposure period and the 5 min following. Catecholamines released during the prestimulation collection periods ranged between 0·25 and 0·02 μmole/min.

shows a comparison of the effects of the length of stimulation on the concentration–secretion relationships for the cholinergic agonist carbachol and for acetaldehyde. Increasing the stimulation period from 2 to 6 min resulted in a shift of the concentration–response curve to the left for acetaldehyde but did not shift the curve for carbachol. These results indicate that amine secretion does not attain a maximum for these concentrations of acetaldehyde for a 2-min stimulation period, although the responses to carbachol are not increased by increasing the length of exposure 3-fold.

The relationship between the length of time for which the gland was stimulated with acetaldehyde and the amount of catecholamines secreted was studied further at concentrations of 5×10^{-4}, 2×10^{-3} and $6{\cdot}7 \times 10^{-3}$ M (Fig. 2). Catecholamine release, expressed as μmoles/min, increased as the stimulation time increased. However, at these concentrations the amount released per min generally decreased when the stimulation time was extended beyond 6–10 min.

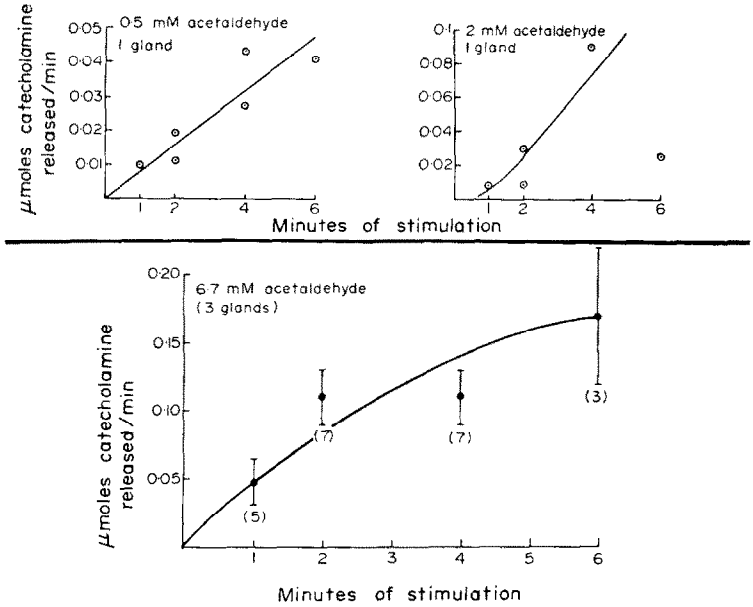

FIG. 2. Relationship between catecholamine release and length of time for which stimulation with various concentrations of acetaldehyde was carried out. In each case, the perfusate collected in the 5-min period after stimulation was included in the stimulation period for analysis of catecholamines. The prestimulation period consisted of a period of time equal to the acetaldehyde exposure period and the 5 min following. Catecholamine release is expressed as μmoles released above prestimulation level/min exposure to acetaldehyde. Catecholamine release during the prestimulation collection periods ranged between 0·28 and 0·01 μmole/min. The bracketed lines in the lower graph represent $\pm$ the standard errors of the analyses, and the number by each point represents the number of determinations for each time analyzed.

A more detailed study of the time course of secretion was carried out by examining the temporal pattern of catecholamine secretion during exposure to acetaldehyde. Figure 3 shows the pattern of catecholamine release in response to stimulation of a single gland with $2{\cdot}5 \times 10^{-2}$ M acetaldehyde for 1 min and with 2×10^{-3} M acetaldehyde for 9 min. The onset of the response is very rapid at the higher concentration, with a delay of only 1 min, whereas at the 12·5-fold lower concentration the onset of

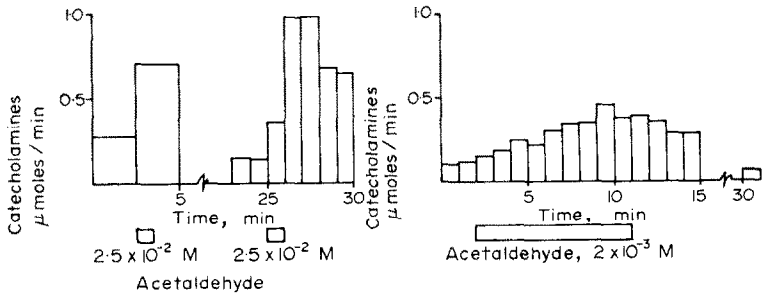

FIG. 3. Release of catecholamines from the perfused isolated cow adrenal gland. Each figure represents a separate perfused gland. Stimulation with acetaldehyde was carried out during the time indicated by the horizontal bars below each graph. The first two vertical bars in the figure on the left represent 2·5-min collection periods; all other represent 1-min collection periods.

the secretion response is slower and reaches its maximum only after 8 min. The reproducibility of the responses to acetaldehyde is evident from the graph on the left in Fig. 3 by comparing the amount of catecholamines released upon the initial stimulation, shown by the 2·5-min vertical bars, with the amount secreted upon the second stimulation, shown by the 1-min vertical bars.

The gradual onset of catecholamine release at the lower concentrations of acetaldehyde suggested that catecholamine release might occur at concentrations much lower than those used previously if the length of the stimulation periods were extended sufficiently. This possibility was studied by exposing perfused glands to concentrations of acetaldehyde as low as 5×10^{-6} M for up to 5 hr. Spontaneous release of catecholamines from glands perfused for prolonged lengths of time, but that were not stimulated with acetaldehyde, gradually decreases in magnitude (the upper graph in Fig. 4). The decrease appears to be linear with some glands and in other cases appears

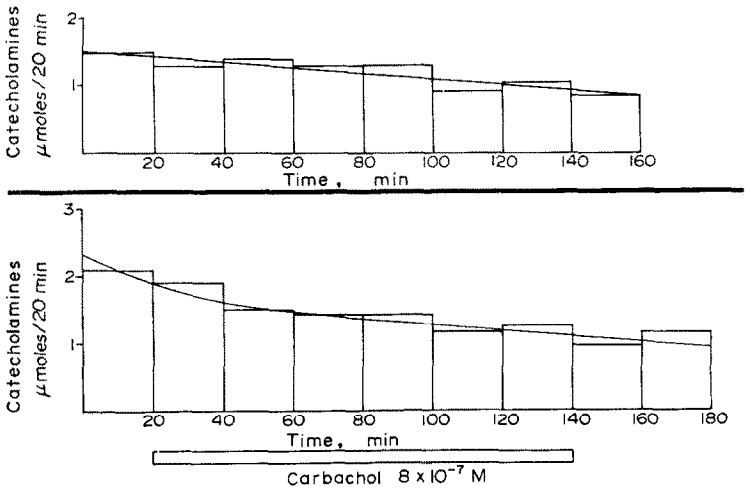

FIG. 4. Catecholamine release from the perfused isolated cow adrenal gland over a prolonged period of time. The top portion of the figure shows release from a gland perfused with Tyrode solution. The results shown in the bottom portion of the figure are from a gland stimulated with carbachol for the time indicated by the horizontal bar below the graph.

to be slightly curvilinear, as indicated by the lines drawn through the secretion responses obtained. Repeated experiments have shown that this method provides an accurate way of examining the gradual decrease in catecholamine release, since the amounts of catecholamines in the perfusates generally follow a very predictable pattern. Seldom does the amount released in a single time period differ by more than 10–15 per cent from the predicted amount. The lower portion of Fig. 4 shows that there was no increase in catecholamine release during a prolonged infusion (120 min) with 8×10^{-7} M carbachol. This concentration is below the threshold concentration of carbachol when infused over a 2-min period, which is approximately 5×10^{-6} M. Exposure of the gland for a prolonged period of time to acetaldehyde in concentrations well below those which induce catecholamine release during a 2-min stimulation period caused an increase in the amount of catecholamines released (Fig. 5). Although there is a tendency toward catecholamine release at 5×10^{-6} and 6×10^{-6} M, the

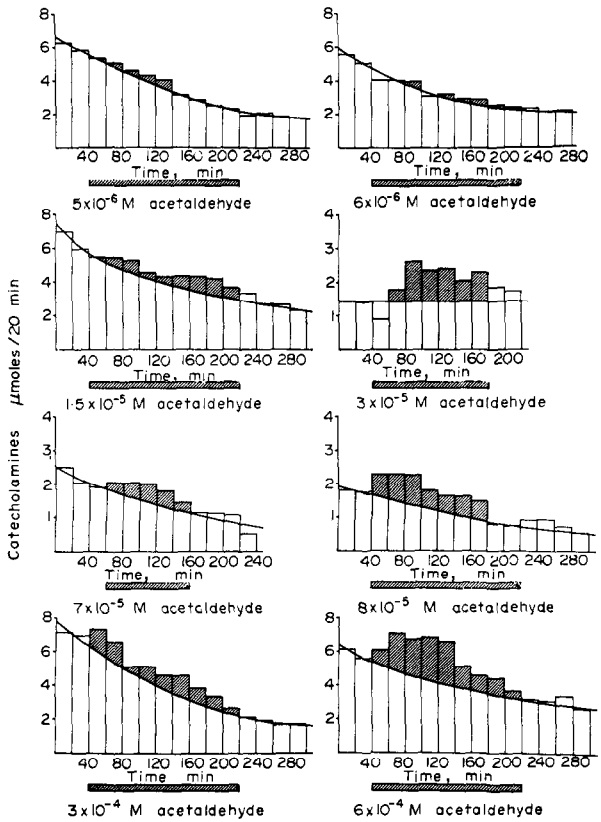

FIG. 5. Catecholamine release from the perfused isolated cow adrenal gland during perfusion with acetaldehyde. Each graph represents a separate gland. Each gland was stimulated with acetaldehyde continuously for the time period represented by the horizontal bars shown below each graph. Concentrations of acetaldehyde represent the concentration in the fluid perfusing the gland. The line drawn through each graph shows a prediction of the control responses based on the initial prestimulation collection periods and on the post-stimulation collection periods. The hatched areas represent responses obtained during stimulation with acetaldehyde that were above the predicted secretion level.

increases above the basal levels are too low to be confidently ascribed to an actual release by acetaldehyde. Release is clearly evident at $1{\cdot}5 \times 10^{-}$ M[5] acetaldehyde. As the concentration of acetaldehyde increases, there is a tendency for the acetaldehyde-induced catecholamine release to drop off even during infusion of the drug. This is especially apparent with the concentration of 6×10^{-4} M acetaldehyde in Fig. 5.

DISCUSSION

One of the many pharmacological properties of acetaldehyde is its ability to stimulate release of tissue catecholamines (see introduction). It is conceivable that release of neurotransmitters in the brain may be at least in some part responsible for acetaldehyde-induced alterations in CNS function. Acetaldehyde is known to activate the cortical EEG[11] and to cause central nervous system depression.[12] Although underlying mechanisms of the behavioral alterations associated with alcoholism are unknown, acetaldehyde may play a role in their production through its ability to alter amine storage and release.

Little is known about the mechanism by which acetaldehyde releases amines. It was shown earlier[4] that it differs from carbachol in the manner by which it releases catecholamines. Unlike carbachol, acetaldehyde does not induce exocytosis in the chromaffin cell of the adrenal medulla. A direct interaction with chromaffin vesicles appears to be the mechanism by which acetaldehyde causes release of the stored catecholamines. Proteins contained within the vesicles are not released upon stimulation of the gland with acetaldehyde.

Previous studies have shown that the concentrations of acetaldehyde required for release of catecholamines from the adrenal gland are higher than 1 mM.[4] These observations raise the question of the physiological significance of acetaldehyde-induced catecholamine release. However, Walsh *et al.*[2] reported positive inotropic responses in guinea pig isolated left atria in response to a 24-min exposure to 0·3 mM acetaldehyde. The results reported in the present communication show that responses can be obtained at even lower concentrations of acetaldehyde when the length of exposure time is increased. Secretion of catecholamines was obtained at levels as low as $1{\cdot}5 \times 10^{-5}$ M. This concentration is similar to the concentration of acetaldehyde reported to occur after ingestion of moderate amounts of alcohol.[6] Ingestion of large amounts of alcohol would be expected to result in even higher blood levels of acetaldehyde. Administration of disulfiram to an individual prior to ingestion of alcohol also results in higher levels of acetaldehyde in blood.[6] It is not unlikely that the higher levels of acetaldehyde obtained under these conditions cause release of catecholamines from various tissues in which they are stored.

The slow onset of the response to acetaldehyde at these low concentrations may reflect the fact that its site of action is intracellular, and that the drug must reach a sufficiently high concentration at this site in order to induce catecholamine release. The length of time required to reach an effective concentration decreases as the concentration increases; at $2{\cdot}5 \times 10^{-2}$ M acetaldehyde, the maximum response was reached with 2 min (Fig. 3), whereas approximately 8 min was required to reach a maximum for 2×10^{-3} M. At 3×10^{-5} M acetaldehyde approximately 40 min was required to reach a maximum response after beginning infusion of acetaldehyde. Whether the delay is due to diffusion of acetaldehyde into the cell or to its accumulation at a specific binding site within the cell is not known. It would be of interest to

know if acetaldehyde within the tissue builds up to concentrations higher than those in the perfusate.

The findings reported here suggest that prolonged exposure to low circulating levels of acetaldehyde may allow the accumulation of acetaldehyde at specific sites within the chromaffin cell and as a result cause release of catecholamines. Other pharmacological effects that might occur could include formation of tetrahydroisoquinolines by condensation of acetaldehyde with tissue catecholamines,[13] alteration of the metabolism of biogenic amines by competition between acetaldehyde and aldehyde metabolic intermediates for aldehyde dehydrogenase[14,15] or binding of acetaldehyde to tissue macromolecules.[16,17] It would be of value in understanding the pharmacology of alcohol and of acetaldehyde to know which, if any, of these effects do in fact occur *in vivo* after alcohol intake.

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