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Influence of different anions on catecholamine release induced by tyramine and amphetamine

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CALCIUM[1–5] is required for release of catecholamines from the adrenal medulla in response to stimulation with acetylcholine, high concentrations of potassium, various biogenic amines and certain polypeptides.[6,7] Two other divalent cations, barium and strontium, can substitute for calcium in the release process, but the monovalent cations, sodium and potassium, will not serve this function.[1,6,7] In contrast to the results from studies with perfused adrenal glands, calcium,[8,9] barium or strontium[9,10] has little effect either on spontaneous release or on ATP-evoked release of catecholamines from isolated chromaffin vesicles. Although much is known about the effects of cations on catecholamine release from chromaffin vesicles, less is known about the effect of anions on this process. ATP-Mg^{2+}-stimulated release of catecholamines from isolated chromaffin vesicles is dependent on the presence of chloride anions in the medium;[9,11,12] sodium and potassium chloride are equally effective.[9] On the other hand, spontaneous release of catecholamines from isolated chromaffin vesicles[8,11] is inhibited by ATP-Mg^{2+} when the incubation is carried out in phosphate buffer. The purpose of this report is to present the results of a comparison of the effects of different anions on tyramine- or amphetamine-induced release of catecholamines from cow adrenal medulla chromaffin vesicles.

The fraction which contained the chromaffin vesicles, mitochondria and lysosomes was prepared from fresh cow adrenal medullae (30 g) that had been dissected free of cortices, minced with a chopper, and homogenized with a loose-fitting Potter–Elvehjem homogenizer in a volume of incubation medium equal to four times their wet weight. The homogenate was centrifuged at 480 *g* for 10 min. The resulting supernatant was centrifuged at 12,100 *g* for 20 min, and the sediment that was obtained was resuspended in incubation medium and centrifuged again at 12,100 *g* for 20 min. The chromaffin vesicle fraction was obtained by suspending the resulting pellet in a volume of incubation medium equal to ten times its wet weight. Release of catecholamines by tyramine hydrochloride (10^{-6} – 10^{-2} M) or by amphetamine sulfate (10^{-4} to 10^{-2} M) was studied by adding a 1-ml suspension of chromaffin vesicles to 2 ml of incubation media containing the appropriate concentrations of tyramine or amphetamine. The media studied included: 0·15 M NaCl, 0·10 M Na_2SO_4, 0·10 M NaH_2PO_4, 0·15 M $NaHCO_3$ and 0·10 M Na_2CO_3. All media were gassed with a mixture of 95% O_2 and 5% CO_2 for 30 min and then adjusted to pH 7 with HCl or NaOH. Spontaneous release of catecholamines in the different media was measured in the absence of tyramine or amphetamine and was arbitrarily set at 100 per cent. The spontaneous release of catecholamines was 6–12 per cent of the endogenous catecholamine content of the chromaffin vesicle fraction, which was measured after disruption of the vesicles with 0·66% (v/v) Triton X-100. Samples were incubated for 12 min at 30° under an atmosphere of 95% O_2–5% CO_2, immediately cooled in ice, and centrifuged at 4° at 27,000 *g* for 15 min. Aliquots of the resulting supernatant were assayed for catecholamines by the colorimetric method of von Euler and Hamberg[13] using citrate–phosphate buffer at pH 6·0; the catecholamine content was expressed as micromoles of epinephrine. Protein in a 1-ml suspension of chromaffin vesicles was precipitated by trichloroacetic acid (final concentration, 5%, w/v) and measured by the microbiuret method of Goa.[14] Catecholamine release was expressed in micromoles of epinephrine per milligram of protein and was then converted to per cent of control spontaneous release in each medium.

Preliminary experiments showed that tyramine (10^{-2} M)-induced or amphetamine (10^{-2} M)-induced release of catecholamines from chromaffin vesicles incubated at 30° in Tyrode solution (137 mM NaCl, 2·68 mM KCl, 1·80 mM $CaCl_2$, 0·28 mM NaH_2PO_4, 0·001 mM $MgCl_2$, 11·60 mM $NaHCO_3$ and 5·56 mM glucose gassed with a mixture of 95% O_2 and 5% CO_2) increased linearly with time up to 24 min. The release of catecholamines from chromaffin vesicles by several concentrations of tyramine (10^{-6} to 10^{-2} M) or of amphetamine (10^{-4} to 10^{-2} M) during a 12-min incubation at 30° in the different anionic media is shown in Fig. 1, A and B respectively. Concentrations of tyramine as high as 10^{-2} M failed to release catecholamines from chromaffin vesicles incubated in Na_2SO_4 or NaCl (Fig. 1A). A slight (122 per cent of control) but significant release by high concentrations of amphetamine occurred in NaCl (Fig. 1B). Tyramine (10^{-2} M) increased catecholamine release to 150 per cent of control (Fig. 1A) and amphetamine (10^{-2} M) increased release to 200 per cent of control (Fig. 1B) when the incubation medium was NaH_2PO_4. Tyramine (10^{-2} M)-induced catecholamine release from chromaffin vesicles in NaH_2PO_4 (150 per cent of control) was comparable to that observed in Tyrode solution (156 per cent of control), whereas that induced by amphetamine (10^{-2} M) in NaH_2PO_4 (200 per cent of control) was less than that observed in Tyrode

(270 per cent of control). In the isolated cow adrenal gland perfused with Tyrode solution, tyramine at $5{\cdot}4 \times 10^{-3}$ M increases catecholamine release to 140 per cent of control, whereas *d*-amphetamine in the same concentration ($5{\cdot}4 \times 10^{-3}$ M) increases release to 384 per cent of control.[15] The addition of phosphate to an NaCl medium resulted in an increase in the release of catecholamines from chromaffin vesicles induced by tyramine (10^{-2} M); release was 133 per cent of control in a 35% $NaHPO_4$–65% NaCl medium and 150 per cent of control in 100% NaH_2PO_4. Tyramine-induced release of catecholamines in Na_2CO_3 was comparable to that in $NaHCO_3$, and was much greater than that observed in the other media (230–280 per cent of control at 10^{-2} M, Fig. 1A), but was less than half that released by amphetamine (10^{-2} M) in these media (540–560 per cent of control, Fig. 1B). Addition of increasing amounts of Na_2CO_3 to a NaH_2PO_4 medium resulted in a progressive increase in the ability of amphetamine to release catecholamines. Amphetamine-induced release of catecholamines in Na_2CO_3 or in $NaHCO_3$ was almost triple that observed in NaH_2PO_4 (Fig. 1B).

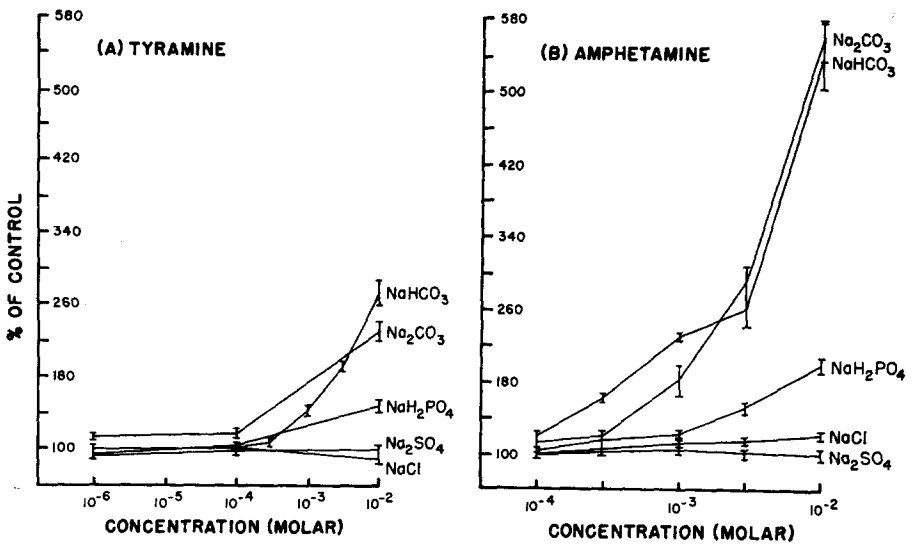

FIG. 1. Catecholamine release from the large granule fraction of the cow adrenal medulla induced by tyramine (A) and amphetamine (B) incubated in media of equal osmolarity. The endogenous catecholamine content of the chromaffin vesicle incubation mixture was $0{\cdot}473 \pm 0{\cdot}007$ μmole ($n = 37$). Ordinates show release as per cent of spontaneous release in each medium; abscissae show molar concentrations of tyramine and amphetamine. Values are mean (±S. E.) per cent release ($n = 3$).

These data indicate that anions have a marked effect on the release of catecholamines from chromaffin vesicles. Neither tyramine nor amphetamine releases catecholamines from the chromaffin vesicles in Na_2SO_4 media, and only amphetamine will release catecholamines in NaCl. The greatest release induced by tyramine and amphetamine occurs in Na_2CO_3 and $NaHCO_3$, and amphetamine is more effective than tyramine in these media. Since the chloride salt of tyramine and the sulfate salt of amphetamine were used in these experiments, the anionic milieu of the incubation media were not strictly those desired. However, the low concentrations of these anions in the media and the fact that release was observed in NaH_2PO_4, $NaHCO_3$ and Na_2CO_3 in the presence of these contaminating anions, which alone did not support amine-induced release, do not negate the qualitatively different effects produced by tyramine and amphetamine. Investigations are in progress to determine whether the differences observed in the various anionic media are due to differences in efflux of the catecholamines from the storage vesicles or reflect differences in uptake or binding of the releasing agents.

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Effect of dietary lipid ingestion on the induction of drug-metabolizing enzymes by phenobarbital

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PREVIOUS reports from this laboratory[1–5] and by others[6,7] have implicated a role for dietary fatty acids in the metabolism of various substrates by liver enzymes of the rat. As little as 3 weeks' feeding of a fat-free diet results in depression of hexobarbital, heptachlor, aniline and ethylmorphine metabolism, decreased levels of cytochrome P-450, decreased binding of aniline and hexobarbital to washed microsomes, and a decrease in the ratio of absorbance peaks when ethyl isocyanide is used as a ligand for cytochrome P-450. Induction of microsomal drug-hydroxylating enzymes by phenobarbital appears to be enhanced in rats whose diets are supplemented with polyunsaturated fatty acids.[6,7] The present research describes the effects of dietary fatty acid consumption and phenobarbital pretreatment on the kinetics of drug hydroxylation by washed microsomes and on substrate binding to microsomal cytochrome P-450. These data plus those provided by the ethyl isocyanide difference spectra suggest a requirement of dietary polyunsaturated fatty acids for the full expresion of microsomal changes produced by the administration of phenobarbital.

Male Sprague–Dawley rats* (50–55 g) were fed a synthetic diet[2] containing 0 or 3 per cent corn oil or 3 per cent coconut oil (substituted for part of the sucrose) for 3 weeks. On four successive days prior to sacrifice each animal received phenobarbital (80 mg/kg i.p.) or saline (1·0 ml/kg i.p.). The animals were decapitated 24 hr after the last injection, and washed microsomes were prepared.[5] Protein content was assayed,[8] and cytochrome P-450 content, rates of metabolism of aniline and

* The Holtzman Company, Madison, Wis.