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THE CHROMAFFIN GRANULE - PLASMA MEMBRANE INTERACTION AS A MODEL FOR EXOCYTOSIS: QUANTITATIVE RELEASE OF THE SOLUBLE GRANULAR CONTENT.

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Bovine adrenal medullary plasma membranes induce the release of soluble components from chromaffin granules in a Ca^{2+} dependent manner. This interaction can be modulated by changing the temperature. Correlation of the concentrations of four released soluble markers (catecholamines, soluble protein, ATP and dopamine-β-hydroxylase) in samples incubated at different temperatures revealed that those markers were liberated simultaneously. Their ratio did not differ significantly from the ratio measured in chromaffin granule lysates. These results suggest the release of the total granular content upon incubation with plasma membranes. Further analysis of the free catecholamines showed a preferential release of adrenalin.

INTRODUCTION

Incubation of adrenal medullary plasma membranes with chromaffin granules results in the release of catecholamines, ATP and DβH°° from these granules (1). This release can be blocked by addition of EGTA to the incubation medium and is specifically controled by Ca^{2+}. The plasma membrane-induced catecholamine release is stimulated when the $[Ca^{2+}]$ exceeds 2×10^{-7}M with a maximal release at 10^{-5} and a half maximal response around 10^{-6}M Ca^{2+}. Mg^{2+} in contrast does not stimulate the system in the absence, nor inhibit it in the presence, of Ca^{2+} (2). This interaction was proposed as a possible cell-free model for exocytosis.

Exocytotic release implies that all soluble granular components must be discharged in parallel (3). In the present work we investigated the plasma membrane-induced release of four soluble granular

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°°) Abbreviations: DβH, dopamine-β-hydroxylase, EC 1.14.17.1.; EGTA, ethyleneglycol-bis(β-aminoethylether)N.N-tetraacetic acid, a calcium chelator; HPLC, high pressure liquid chromatography.

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markers (ATP, DβH, soluble protein and catecholamines). The ratio of these released compounds was compared with their ratio in the soluble granular matrix in order to determine whether the release of catecholamines, which we use as a marker, reflects the release of the total granular content. The use of an HPLC system enabled us to distinguish between adrenalin and noradrenalin release.

METHODS

Bovine adrenal chromaffin granules and plasma membranes were isolated essentially as described by (4) with the modifications mentioned by (1).

The isolated chromaffin granules and plasma membranes were resuspended in 0.3M sucrose (containing 10mM Tris-HCl, pH 7.4) and the chromaffin granule catecholamine and plasma membrane protein concentrations were determined. After dilution of both suspensions to a concentration adequate for the incubation experiments the plasma membrane suspension or an equivalent blank was incubated for 5 min at 37°C and the chromaffin granule suspension equilibrated at room temperature. The chromaffin granules were added and the incubation mixture shaken for a few seconds. After 1 min this mixture was placed in iced water and 3 volumes of ice-cold buffered 0.3M sucrose were added to stop the incubation. The incubated samples were then centrifuged at 15,000xg for 20 min in a refrigerated centrifuge to pellet the intact chromaffin granules. To avoid any contamination by particle-bound or insoluble material the supernatants were centrifuged at 200,000xg for 45 min before assayed.

Catecholamine concentration was measured by the colorimetric method described by (5).

Proteins were determined according to the methods of (6) and (7) using bovine serum albumin as a standard.

ATP was measured by the luciferin-luciferase method (8) using Sigma FLE firefly extract.

DβH activity was determined as described by (9) with minor modifications (10). One unit of activity represents the formation of 1 nmol octopamine from tyramine per hour at 37°C.

The HPLC system used consisted of the following: a Waters 6000A pump, a Waters U6K injector, a Waters μBondapak C_{18} column and a Bioanalytical Systems LC-4 amperometric detector. The samples were deproteinized with 0.4N $HClO_4$ and diluted with eluens before injection. This eluens contained a 0.07M Na-phosphate buffer, pH 5.8 with 0.1mM Na_2EDTA and 0.5mM heptanesulphonate and was run at a flow rate of 1.5 ml/min.

The soluble material already released before incubation, due to leakage and granule damage caused by resuspending the pellet, was determined by keeping a fraction of the chromaffin granule suspension at 0°C during incubation of the other samples. The "cold" sample was then centrifuged along with the incubated samples and the supernatant assayed for the soluble markers. This value was subtracted from the marker concentration of the supernatant of an incubated sample to find the concentration of that marker released in the sample during the actual incubation. In our experiments the average fractions of material released before incubation were 13.5 ± 1.4, 17.5 ± 1.1, 6.0 ± 1.3 and 17.0 ± 2.0% (mean $\pm$ s.d., n=4) of the total amount of catecholamines, DβH, ATP and soluble protein respectively.

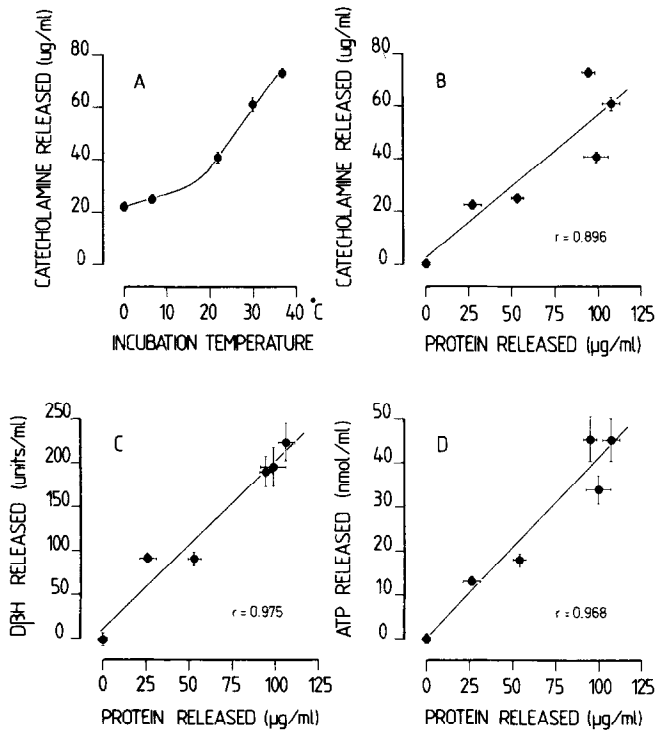

Figure 1. Temperature dependency and mutual correlation of plasma membrane-induced release of soluble chromaffin granular components. Chromaffin granules (200 µg catecholamines/ml) were incubated together with plasma membranes (75 µg protein/ml) in 0.3M sucrose (10 mM Tris-HCl, pH 7.4, 5×10^{-6}M Ca^{2+}) at 37°C for 1 min in a total volume of 400 µl. Each point represents the mean result (± S.E.M.) of four experiments. The concentrations of soluble components which were not granule-bound before incubation have been used as blanks and form the zero-points in B, C and D.

Chromaffin granule lysates were obtained by a tenfold dilution of chromaffin granule suspension samples with 10mM Tris-HCl, pH 7.4. After being frozen and rethawed these samples were also centrifuged for 45 min at 200,000xg.

RESULTS AND DISCUSSION

Figure 1A shows that the plasma membrane-induced catecholamine release from chromaffin granules is a temperature dependent process. Joint incubations of fixed amounts of chromaffin granules and plasma membranes at different temperatures resulted in different concentrations of released catecholamines. These were correlated with the concentrations of soluble protein in the corresponding samples. Figure 1B demonstrates the simultaneous release of catecholamines and soluble protein ($P<0.02$). Further correlation of DβH and ATP release with the protein release (figures 1C and 1D) proves their linked liberation into the extragranular medium ($P<0.001$ and $P<0.01$). The ratio of the com-

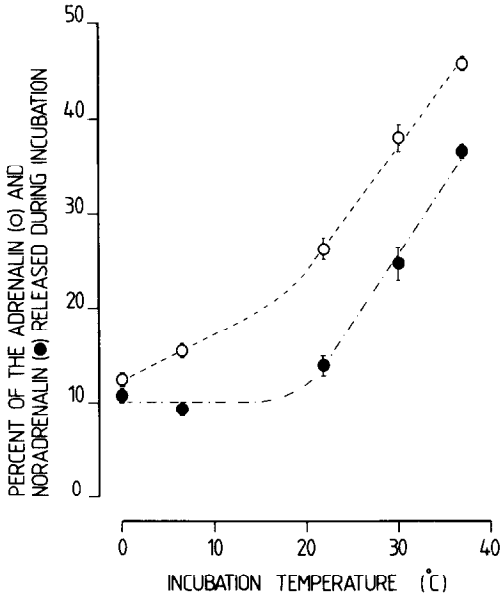

Figure 2. Differential release of adrenalin and noradrenalin from chromaffin granules upon incubation with plasma membranes at different temperatures. The same samples of the experiments specified in figure 1 were assayed for the fraction of adrenalin and noradrenalin released during incubation. The incubation mixtures contained 41.2 μg noradrenalin and 158.8 μg adrenalin per ml of which 7.7 μg noradrenalin and 20.0 μg adrenalin (mean value of four experiments) were not granule-bound before incubation.

pounds released (610 ± 17 μg catecholamines, 412 ± 66 nmol ATP and 2260 ± 620 units DβH per mg soluble protein (mean ± s.d., n=5)) did not differ significantly (Wilcoxon test, $P<0.05$) from their ratio in chromaffin granule lysates (n=4).

It is interesting to remark that we obtained a molar ATP:catecholamine ratio of 1:8.2 in our release experiments. This value corresponds with other recent observations (11, 12) but not with the traditionally accepted value of 1:4.5 (13-17). It is not relevant to compare the specific DβH activity of our samples with the data of other authors since this enzyme has been measured at various, and not saturating, substrate concentrations (18). The value of 610 μg catecholamine per mg soluble protein released falls into the range reported by most laboratories (18). This value however may be subject to seasonal variations (12) and depends on the method of granule preparation due to the existence of differing subpopulations amongst chromaffin granules (19). The most obvious subpopulations are the distinct adrenalin and noradrenalin storing granules (20). We measured the concentrations of released adrenalin and noradrenalin separately in order to detect a possible differential interaction of the two granule-types with the plas-

ma membrane. Figure 2 shows that adrenalin is preferentially released upon incubation of chromaffin granules with plasma membranes.

The concomitant release of catecholamines, soluble protein, ATP and DβH in a ratio which is not different from that in the soluble chromaffin granule matrix indicates the liberation of the total soluble granular content upon incubation with plasma membranes in a Ca^{2+}-containing medium. This finding also affirms that in the present experimental circumstances the determination of the non-granule bound catecholamine concentration is a valid parameter for the exocytosis-like release from chromaffin granules.

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