Biochemical Pharmacology, Vol. 25, pp. 1035–1038. Pergamon Press, 1976. Printed in Great Britain.

THE PERMEABILITY OF CHROMAFFIN GRANULES TO NON-ELECTROLYTES

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(*Received* 13 *June* 1975; *accepted* 10 *October* 1975)

Abstract—Chromaffin granules isolated from bovine adrenal glands were incubated in solutions of non-electrolytes, and the release of catecholamine from these granules was measured. Chromaffin granules are rapidly lysed upon incubation in hypotonic sucrose, or during incubation in isotonic solutions of low molecular weight non-electrolytes such as methanol, ethanol, ethylene glycol, glycerol, formamide, or urea. Lysis of chromaffin granules in isotonic solutions of larger non-electrolytes occurs more slowly. At 30°, the time required for the release of 50 per cent of the osmotically releasable catecholamine is approximately 0.6 min in isotonic erythritol, 4 min in arabitol, and 9 min in mannitol or glucose. The lysis of chromaffin granules is markedly temperature dependent. Below 16–19°, the rate of catecholamine release from granules incubated in isotonic erythritol, arabitol, mannitol or glucose is greatly decreased. Above 20°, the granules behave as though their membranes are permeable to hexoses, hexitals, and smaller non-electrolytes.

Chromaffin granules are the organelles within which catecholamines are stored, and from which they are secreted. Because the membranes of chromaffin granules may play an important role in the synthesis, storage and secretion of catecholamines, an understanding of the properties of these membranes is of interest. Considerable information is available concerning the chemical composition of chromaffin granule membranes. These membranes are characterized by a high cholesterol to phospholipid ratio, and by a high content of lysolecithin [1]. Relatively little information is available concerning the permeability of chromaffin granule membranes. Hillarp and Nilson have shown that chromaffin granules are osmotically active particles [2]. These granules are stable in isotonic solutions of sucrose or of salts, but are lysed in hypotonic solutions. Lysis consists of the release of catecholamines, ATP, and soluble proteins from the chromaffin granules [3], and is associated with a decrease in the turbidity of chromaffin granule suspensions [4, 5]. I have studied the permeability of chromaffin granules to non-electrolytes by measuring the lysis of these granules in isotonic solutions of these compounds. This paper presents the results of these studies.

METHODS

Chromaffin granules were isolated from bovine adrenal glands by differential centrifugation. Adrenal medullas were homogenized in 5 ml/g of STE buffer (0.27 M sucrose–10 mM TES*–1 mM EDTA, pH 7.0), in a Potter–Elvehjem homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 800 *g*, and the supernatant obtained after this centrifugation was centrifuged for 10 min at 20,000 *g*. The 20,000 *g* pellet was washed once with STE, and was then resuspended in this buffer and used as the chromaffin granule fraction for all experiments. The chromaffin granules were analyzed for catecholamines by the method of Shore and Olin [6]. Protein was measured by the method of Lowry *et al.* [7], after precipitation with 10% trichloroacetic acid. Typical preparations contained about 0.5 μmoles of catecholamine/mg protein. All steps in the isolation procedure were performed at 4°, and the granules were kept at 0–4° until use.

Chromaffin granules (containing about 10 nmoles of catecholamine) were incubated with the substances to be tested, as described in the text. Unless otherwise indicated, incubations were for 10 min at 30°, in a vol of 0.5 ml; all incubation mixtures contained 10 mM TES–1 mM EDTA, pH 7.0, in addition to the compounds mentioned. Incubations were terminated by the addition of 2 ml of ice-cold STE, followed by centrifugation at 20,000 *g* for 10 min at 4°. The supernatants were assayed for catecholamines by the method of Shore and Olin [6]. Catecholamine release was estimated after the subtraction of the small amount of catecholamine found in samples maintained at 4° in STE, and was expressed as the percentage of the osmotically releasable catecholamine content of the granules. For these calculations, catecholamine release in samples incubated for 10 min at 30° in H_2O was taken as 100 per cent. In most experiments, incubation of the chromaffin granules in water resulted in the release of between 85 and 95 per cent of the total catecholamines in the granules. All chemicals were reagent grade, and were obtained from commercial sources. Glass-distilled water was used throughout.

RESULTS

Chromaffin granules behave as osmotically active particles. When suspended in isotonic sucrose, the granules are relatively stable; under the conditions of these experiments, chromaffin granules release about 15 per cent of their catecholamine content in

*TES, *N*-tris(hydroxymethyl)methyl-2-aminoethane sulfonic acid.

10 min at 30° in 0.27 M sucrose. Catecholamine release in hypertonic sucrose (0.3 M) is slightly less. However, when the granules are incubated in hypotonic sucrose, they release a larger percentage of their stored catecholamines. Figure 1 illustrates the effect of hypotonic media on catecholamine release. About 50 per cent of the osmotically releasable catecholamine is released in 0.21 M sucrose. Even at 0°, the rate of catecholamine release in hypotonic media occurs too rapidly to be estimated by the methods used in these experiments.

In subsequent experiments, the lysis of chromaffin granules in solutions of non-electrolytes was used as a measure of the permeability of the granules to these compounds. Chromaffin granules are rapidly lysed in 0.3 M solutions of low molecular weight non-electrolytes. Incubation of the granules in 0.3 M solutions of methanol, ethanol, formamide, urea, ethylene glycol, and glycerol results in virtually complete lysis (release of more than 90 per cent of the osmotically available catecholamine) within 1 min at 0° and at 30° (data not shown). When chromaffin granules are incubated in isotonic solutions of higher molecular weight non-electrolytes, they lyse more slowly. Figure 2 illustrates the rate of catecholamine release from chromaffin granules incubated at 30° in isotonic solutions of erythritol, arabitol, mannitol, and lactose. The time required for release of 50 per cent of the osmotically releasable catecholamine is approximately 0.6 min in erythritol, 4 min in arabitol, 9 min in mannitol, and much longer than 10 min in lactose. These differences are reproducible, and are statistically significant ($P < 0.05$ for the difference between incubation in erythritol and arabitol at 2 min, and for the differences between incubations in arabitol and mannitol, and in mannitol and lactose, at 10 min). Experiments of the type illustrated in Fig. 2 were also done in isotonic solutions of other non-electrolytes. These experiments are summarized in Table 1, which presents the percentage of catecholamine released from chromaffin granules incubated for 10 min at 30° in 0.3 M solutions of a number of 5- and 6-carbon containing polyhydroxy alcohols and sugars. Table 1 also presents the effective hydrodynamic radii of some of these substances, as determined by Schultz and Solomon [8]. Incubation in solutions

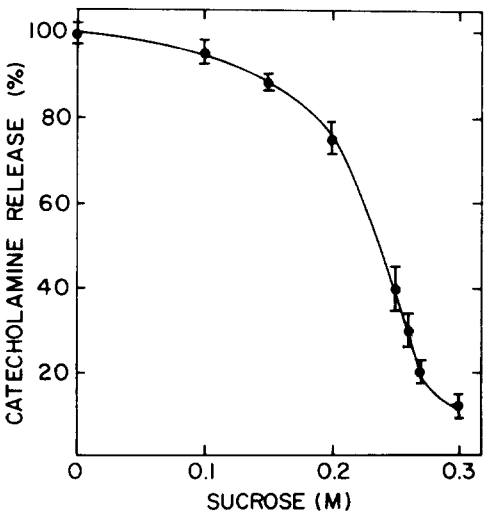

Fig. 1. Lysis of chromaffin granules in hypotonic sucrose. Chromaffin granules were incubated for 10 min at 30° in solutions containing varying concentrations of sucrose, as indicated in the figure. All solutions contained, in addition, 10 mM TES, 1 mM EDTA, pH 7.0. Catecholamine release is expressed as the percentage of the catecholamines which were released during incubation for 10 min at 30° in water. The figure shows the mean ± S.E.M. for three samples.

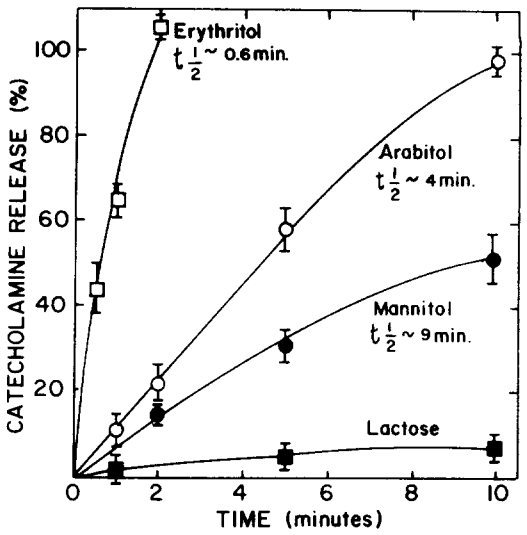

Fig. 2. Time course of chromaffin granule lysis. Chromaffin granules were incubated at 30° for varying periods of time, in solutions containing 0.3 M erythritol, 0.3 M D-arabitol, 0.3 M D-mannitol, or 0.27 M lactose. All solutions contained, in addition, 10 mM TES, 1 mM EDTA, pH 7.0. Catecholamine release is expressed as the percentage of the catecholamines which were released during incubation for 10 min at 30° in water. The figure shows the mean ± S.E.M. for three samples.

Table 1. Catecholamine release by carbohydrates

Compound	Effective hydrodynamic radius	Catecholamine release
	Å	%
Ribitol		89 ± 13
D-Arabitol		91 ± 12
Xylitol		100 ± 10
D-Ribose	3.6	89 ± 9
D-Arabinose	3.8	83 ± 11
D-Xylose		100 ± 10
D-Mannitol	4.2	57 ± 4
D-Sorbitol		61 ± 7
D-Fructose		47 ± 6
D-Galactose	4.2	60 ± 10
D-Glucose	4.2	49 ± 6
L-Glucose	4.2	56 ± 8

Chromaffin granules were incubated for 10 min at 30° at 0.3 M solutions of the compounds listed in the table. All solutions contained, in addition, 10 mM TES, 1 mM EDTA, pH 7.0. Catecholamine release is expressed as the percentage of the catecholamines which were released during incubation for 10 min at 30° in water. The table reports the mean ± S.E.M. for three samples. Effective hydrodynamic radii are taken from Schultz and Solomon [8]; . not reported by these authors.

of pentoses or penitols results in the release of 80–100 per cent of the catecholamine under these conditions. In contrast, only 45–60 per cent of the catecholamine was released during incubation in solutions of hexoses or hexitols. The percentage of catecholamine released from the granules during incubation in solutions of 5-carbon compounds (92 ± 4%) is significantly different from that released during incubation in solutions of 6-carbon compounds (55 ± 3%, $P < 0.001$). There is no evidence for stereospecificity in the lysis of chromaffin granules by non-electrolytes. Thus, L-glucose and D-glucose lyse chromaffin granules at identical rates.

It was of interest to examine the temperature dependence of the lysis of chromaffin granules by non-electrolytes. As mentioned above, chromaffin granules are rapidly lysed in hypotonic media, and in isotonic solutions of low molecular weight non-electrolytes, at 0°. The present methods did not allow measurement of the temperature dependence of chromaffin granule lysis by these compounds. However, there is a profound effect of temperature on the rate of chromaffin granule lysis by the higher molecular weight non-electrolytes. Figure 3 depicts the effect of temperature on the lysis of chromaffin granules in isotonic solutions of erythritol, arabitol, and mannitol. There is a clear temperature transition in the lysis of chromaffin granules by these compounds. Below about 16–19°, the rate of lysis by these compounds is greatly decreased. There is no significant release (less than 10%) of catecholamines from chromaffin granules incubated for up to 1 hr at 0° in 0.3 M arbitol, mannitol, D-glucose, or L-glucose (not shown).

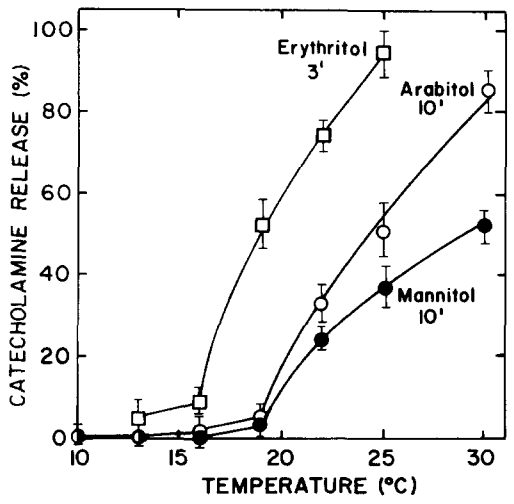

Fig. 3. Temperature dependence of chromaffin granule lysis. Chromaffin granules were incubated at varying temperatures in solutions containing 0.3 M erythritol, 0.3 M D-arabitol, or 0.3 M D-mannitol. All solutions contained, in addition, 10 mM TES, 1 mM EDTA, pH 7.0. Samples in erythritol were incubated for 3 min, whereas those in arabitol and mannitol were incubated for 10 min. Catecholamine release is expressed as the percentage of the catecholamines which were released during incubation for 10 min at 30° in water. The figure shows the mean ± S.E.M. for three samples.

DISCUSSION

In this study, the release of catecholamines from chromaffin granules was used to estimate the permeability of the granules to non-electrolytes. Although it is possible that some of the compounds tested have direct effects on the chromaffin granule membranes, the most reasonable interpretation of these experiments as a whole is that the release of catecholamines from chromaffin granules incubated in isotonic solutions of non-electrolytes is due primarily to the entry of these compounds into the granules, and the subsequent osmotic lysis of the granules. The correlation between molecular size and rate of catecholamine release, and the lack of stereospecificity of this process, are both consistent with this interpretation. At temperatures above 20°, chromaffin granules behave as though they have hydrophilic pores large enough to accommodate hexoses and hexitols, which have radii of about 4.2 Å [8]. However, heterogeneity of the chromaffin granule population, compartmentalization of chromaffin granule water, active transport processes in the chromaffin granule membrane, and non-osmotic mechanisms of catecholamine release are all factors that might complicate any quantitative analysis of chromaffin granule permeability from this data. I have not measured the release of other substances, such as ATP and protein, from the chromaffin granules in these experiments, but preliminary experiments indicate that the release of catecholamines is accompanied by a decrease in the turbidity of chromaffin granule suspensions. Thus, it seems likely that the catecholamine release observed here is associated with lysis of the chromaffin granules. Lloyd has used similar methods to study the permeability of rat liver lysosomes to carbohydrates [9]. The permeability of lysosomes to non-electrolytes is qualitatively similar to that of chromaffin granules; the main difference is that lysosomes are apparently impermeable to hexitols, whereas chromaffin granules are permeable to these compounds. It is not clear whether the slow release of catecholamine from chromaffin granules incubated in isotonic solutions of disaccharides is due to osmotic lysis or to some other process. However, the known stability of chromaffin granules in sucrose solutions is inconsistent with the report of Carlsson and Hillarp that the granules are freely permeable to sucrose [10].

The marked temperature dependence of chromaffin granule permeability is of particular interest. Below 15°, the granules behave as though they are only slightly permeable to erythritol, and are impermeable to 5- and 6-carbon containing non-electrolytes. Because the granules are rapidly lysed both in hypotonic solutions and in isotonic solutions of 1- to 3-carbon containing compounds at 0°, the effect of temperature on chromaffin granule lysis most probably reflects a temperature-dependent change in the permeability of the chromaffin granule membrane, rather than a change in the catecholamine ATP–chromogranin storage complex. Chromaffin granules are permeable to catecholamines and to ATP at 0° [10, 11], but neither of these compounds are incorporated into the intra-granular storage complex at this temperature [12, 13]. The entry of catecholamines and of ATP into chromaffin granules is probably due

to the presence of specific transport carriers for these substances in the chromaffin granule membrane. A catecholamine transport system has been demonstrated in chromaffin granules [13, 14], but a transport system for ATP has not yet been identified.

Acknowledgements—I am grateful to Ms. Michele Carvotta and Ms. Michi Chen for technical assistance. This research was supported in part by research grant AM15135 from the National Institutes of Health. This work was done during the tenure of a research grant-in-aid award from the American Heart Association, Greater Boston Massachusetts Chapter, No. 1256. I am the recipient of a research career development award, AM70648, from the National Institutes of Health.

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