INFLUX OF Ca^{2+} INTO ISOLATED SECRETORY VESICLES FROM ADRENAL MEDULLA

Influence of external K^+ and Na^+

Heidemarie KRIEGER-BRAUER and Manfred GRATZL*
Department of Physiological Chemistry, University of Saarland, 665 Homburg/Saar, FRG

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1. Introduction

Secretory vesicles from adrenal medulla contain catecholamines, nucleotides and proteins, all of which are released into the extracellular fluid during exocytosis. Adrenal medullary secretory vesicles also contain high concentrations of Ca^{2+} [1]. The mechanism of the accumulation of Ca^{2+} into the vesicles is largely unknown and the experimental data concerning the uptake of Ca^{2+} into isolated secretory vesicles are contradictory. It has been reported that secretory vesicle membranes are impermeable to Ca^{2+} [2], that secretory vesicles take up Ca^{2+} independently of ATP [3] and that they possess an ATP-stimulated uptake system [4,5].

In earlier work relatively impure and unstable secretory vesicle fractions were used for the determination of Ca^{2+}-uptake. We have developed a method to isolate highly purified and stable secretory vesicles from bovine medulla [6]. With these vesicles we repeated earlier Ca^{2+}-uptake experiments and found that:

(i) The vesicles take up $^{45}Ca^{2+}$ in K^+-containing media;
(ii) $^{45}Ca^{2+}$ uptake is abolished in the presence of Na^+;
(iii) The Ca^{2+} content of isolated secretory vesicles is increased when incubated with Ca^{2+} in media containing K^+, but not in media containing Na^+.

2. Materials and methods

Bovine adrenal medullae were homogenized in a medium containing 20 mM MOPS (pH 7.0) (adjusted with KOH, which gives 15 mM K^+), 1 mM EGTA and sucrose to obtain an osmolality of 420 mOsmol/kg with a Teflon-to-glass homogenizer. After removal of the first pellet (2200 × g_{av}, 10 min) secretory vesicles and mitochondria were sedimented (12 000 × g_{av}, 20 min). Secretory vesicles were separated from mitochondria on a self generating Percoll® gradient [6]. The secretory vesicles were recovered from the gradients and Percoll was removed using a Biogel column (A 150, 100–200 mesh).

Secretory vesicles (1 mg protein/ml) were incubated at 37°C in the above medium containing 100 μM free Ca^{2+}. The total amount of Ca^{2+} to yield the described concentration in the presence of EGTA, at the ionic strength and pH employed was calculated using a computer program as in [7]. For measurements of $^{45}Ca^{2+}$ uptake, the media contained trace amounts of the radioactive isotope (2 nM). In Na^+-containing media, the buffer was adjusted with NaOH and sucrose was replaced by an iso-osmolal amount of NaCl (190 mM). The media were preincubated at 37°C for 3 min, then secretory vesicles were added. Ca^{2+} uptake was stopped by addition of an excess of EGTA (isolation medium as above containing 10 mM EGTA) and the tubes were put on ice. Secretory vesicles were separated from the incubation medium by centrifugation (5700 × g_{max}, 18 min) through a layer of 0.45 M sucrose, and were collected from the interfacial band between 0.45 M sucrose and 2.2 M sucrose. The sucrose solutions for the gradient contained 20 mM MOPS (pH 7.0) and 10 mM EGTA. $^{45}Ca^{2+}$ was determined by liquid scintillation counting, Ca^{2+} was measured by atomic absorption spectroscopy. All other procedures used were carried out as in [6].

* To whom correspondence should be addressed

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3. Results and discussion

Ca^{2+} influx into cells is an essential step in stimulus–secretion coupling. It is generally accepted that the resulting increase in the intracellular free $[Ca^{2+}]$ triggers processes which ultimately result in secretion by exocytosis. Cytosolic free Ca^{2+} can be reduced to resting levels by its transport across the cell membrane or by sequestration in intracellular membrane bound systems such as mitochondria and endoplasmic reticulum.

Since secretory vesicles from different types of tissue contain Ca^{2+} it is possible that an additional physiological function of these subcellular organelles is to remove Ca^{2+} from the cytoplasm and subsequently to extrude it to the extracellular space by exocytosis. Evidence for a parallel release of Ca^{2+} and secretory product has been reported in [8]. An increased content of Ca^{2+} in secretory vesicles isolated from adrenal glands stimulated by acetylcholine has been established [9].

To find out whether adrenal medullary secretory vesicles can take up Ca^{2+} also in vitro (see section 1), we have investigated the influx of Ca^{2+} into highly purified and stable secretory vesicles prepared as in [6]. These vesicles were incubated for different times at 37°C in a medium containing 100 μM free Ca^{2+} plus trace amounts of $^{45}Ca^{2+}$. As shown in fig.1 secretory vesicles take up labelled Ca^{2+} from the medium. The uptake was linear for ~10 min, after this time it levelled off. The influx of $^{45}Ca^{2+}$ into the vesicles was dependent on K^+ (15 mM) in the incubation medium. Further increase of K^+ (up to 190 mM KCl) did not further enhance Ca^{2+} uptake. By contrast, in the presence of Na^+ (190 mM NaCl) no uptake of the radioactive isotope occurred (fig.1).

It is known, that secretory vesicles isolated from adrenal medulla contain Ca^{2+} [1]. We have determined by atomic absorption spectroscopy that the vesicles purified in the presence of EGTA [6], contain 36.8 ±

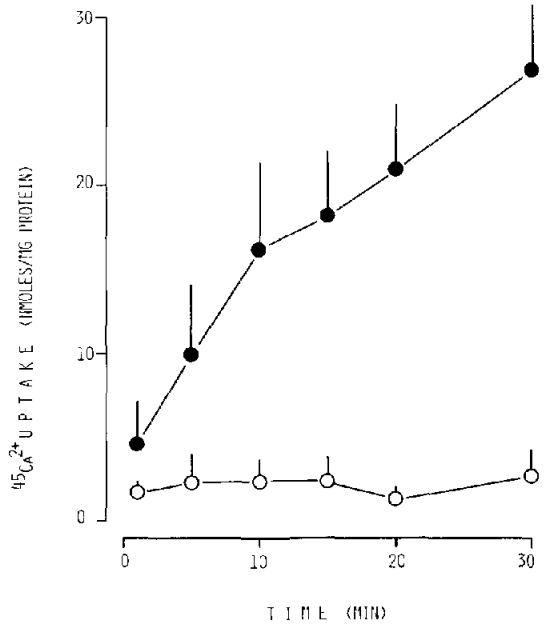

Fig.1. Influence of K^+ and Na^+ on the uptake of $^{45}Ca^{2+}$ in the presence of 100 μM free Ca^{2+} by secretory vesicles isolated from bovine adrenal medulla: (•) incubation medium containing 15 mM K^+; (○) incubation medium containing 190 mM Na^+. The ordinate gives the $^{45}Ca^{2+}$ uptake/mg protein, the abscissa indicates the time of incubation. Values are means ± SD (n = 6).

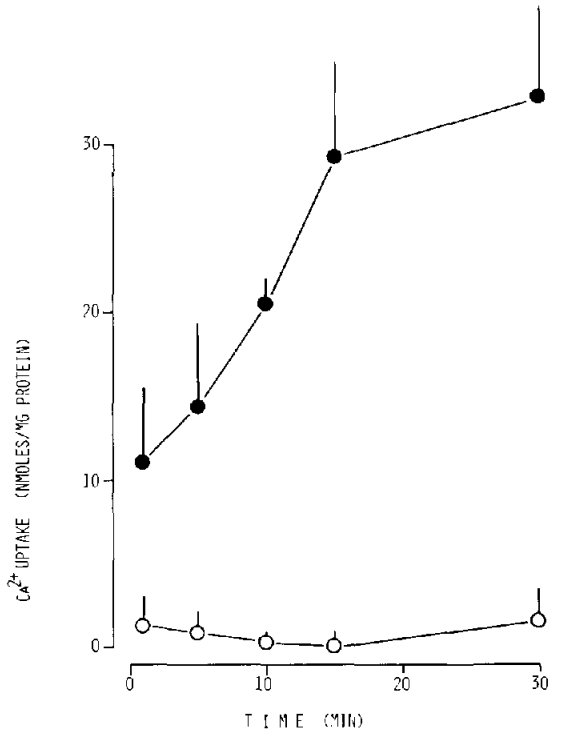

Fig.2. Influence of K^+ and Na^+ on the uptake of Ca^{2+} by the secretory vesicles isolated from adrenal medulla. Uptake is obtained from the difference of Ca^{2+} (determined by atomic absorption spectroscopy) in samples incubated with 100 μM free Ca^{2+} and samples incubated with no Ca^{2+} added. The ordinate gives the Ca^{2+} uptake/mg protein, the abscissa indicates the time of incubation: (•) incubation medium containing 15 mM K^+; (○) incubation medium containing 190 mM Na^+. Values are means ± SD (n = 4).

3.4 nmoles Ca^{2+}/mg protein (n = 10, mean ± SEM). Influx of radioactive isotope into isolated secretory vesicles does not exclude the possibility that the uptake of $^{45}Ca^{2+}$ is due to an exchange of exogenous with endogenous (unlabelled) Ca^{2+}. The demonstration of net accumulation of Ca^{2+} into isolated secretory vesicles from adrenal medulla was impossible with earlier preparations, because they constantly release their contents during incubation. However, the vesicles prepared according to a method introduced recently are stable even after prolongend incubation at 37°C [6]. As shown in fig.2 the amount of Ca^{2+} present in isolated secretory vesicles (see above) was constant during incubation with 100 μM free Ca^{2+} for 30 min at 37°C in the medium containing Na^+ (190 mM NaCl). However, Ca^{2+} is taken up by secretory vesicles from media containing K^+ (15 mM). The extent of uptake as well as the time course was identical to that observed during measurement of $^{45}Ca^{2+}$ influx (compare fig.1 and fig.2). We have also observed Ca^{2+} uptake by adrenal medullary secretory vesicles incubated with 10^{-7} M and 10^{-6} M free Ca^{2+} (unpublished). It can be concluded, therefore, that isolated secretory vesicles are capable of carrying out a net accumulation of Ca^{2+}, and are able to act within the chromaffin cell as a Ca^{2+}-sequestering system.

Some of the disparities in the properties of the Ca^{2+} uptake of adrenal medullary secretory vesicles in [3–5] may arise from the unknown content of Na^+ (which affects strongly the process investigated) in the incubation media used as well as from the presence of other contaminating Ca^{2+}-sequestering systems. Therefore, it seems necessary to reinvestigate the Ca^{2+}-uptake by secretory vesicles isolated from adrenal medulla under these optimized conditions. Further characterization of the Ca^{2+}-influx into secretory vesicles promises progress towards an understanding of the underlying mechanism and the Ca^{2+}-handling of secretory cells.

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References

[1] Winkler, H. and Westhead, E. (1980) Neuroscience 5, 1803–1823.
[2] Johnson, R. G. and Sarpa, A. (1976) J. Gen. Physiol. 68, 601–631.
[3] Kostron, H., Winkler, H., Geissler, D. and König, P. (1977) J. Neurochem. 28, 487–493.
[4] Häusler, R., Burger, A. and Niedermaier, W. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 315, 255–267.
[5] Niedermaier, W. and Burger, A. (1981) Naunyn-Schmiedeberg's Arch. Pharmacol. 316, 69–80.
[6] Gratzl, M., Krieger-Brauer, H. and Ekerdt, R. (1981) Biochim. Biophys. Acta (in press).
[7] Flodgaard, H. and Torp-Pedersen, C. (1978) Biochem. J. 171, 817–820.
[8] Kanagasuntheram, P. and Lim, S. C. (1981) J. Physiol. 312, 445–454.
[9] Serck-Hanssen, G. and Christiansen, E. N. (1973) Biochim. Biophys. Acta 307, 404–414.