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THE MOLECULAR MECHANISM OF NONDEGRANULATIVE RELEASE OF BIOGENIC AMINES

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According to current teaching biogenic amines are released by exocytosis, i. e. by evacuation of amine storing vesicles or granules into the extracellular space. The release of transmitter amines is quantal, i. e. occurs in packs of transmitter molecules. These packs are assumed to be identical with vesicle contents, in other words, the smallest releasable quantum equals the amine content of one vesicle. However, there are experimental observations which do not fit in with this version of an exocytotic release theory. Observed quantitative discrepancies could be explained if the release mechanism allowed a fractional release of transmitter amine from several vesicles instead of the total evacuation of a few. The lack of adequate knowledge about the mechanisms of storage of biogenic amines within the vesicles has up til now rendered it difficult to envisage the machinery behind a fractional release of the amine content of a vesicle.

In extensive in-vitro studies we have found that the matrices of amine storing granules (i. e. from mast cells, chromaffin cells and nerve terminals) show the properties of weak cation exchanger materials, carboxyl groups serving as amine binding ionic sites. When exposed to cations like sodium and potassium ions, the amines are released from their storage sites according to kinetics characteristic of weak cation exchangers. In vivo, amine release from cat adrenals on splanchnic nerve stimulation also occurs according to ion echange kinetics.

Histamine release from mast cells is considered to occur as the result of degranulation, i. e. the expulsion of histamine storing granules to the extracellular space, a typical example of exocytosis. The granules are assumed to loose their histamine by ion exchange, $Na^+ \leftrightharpoons Hi^+$, on exposure to the sodium-rich extracellular medium. However, recent observations on histamine release from superfused mast cells suggest that the release of histamine, although caused by ion exchange, is due to intracellular ion exchange at granule sites between cytoplasmic potassium and activated mast cells as the consequence of intracellular potassium ion flux across the histamine carrying granules, degranulation and extracellular histamine release from expelled granules occurring only as the result of more extensive activation.

The possibility of potassium ions being involved also in an ion exchange process behind the release of other biogenic amines e. g. at nerve terminals will be proposed. The amine release will still be quantal but the size of the released quanta will not depend on the total amine content of a vesicle but on the size of the fractions thereof being released, thereby explaining

many of the quantitative discrepancies attached to the current exocytotic release theory. A fractional release theory may have interesting consequences for our thinking as to the physiology and pharmacology of processes involving storage and release of biogenic amines.

Key words: *Ion exchange, amine release, amine storage, adrenals, chromaffin cells, mast cells, histamine, catecholamines*

The release of biogenic amines is generally assumed to be exocytotic, i. e. the amine is released by evacuation of the contents of the storage vesicles into the extracellular space. The theory stems from observations in the fifties. Katz and his coworkers showed with electrophysiological technique that acetylcholine was released from the cholinergic nerve terminals at the frog motor endplates in multimolecular quanta. Some years later vesicles were discovered in nerve terminals, and vesicles from brain synaptosomes were observed to contain acetylcholine. The idea was born that the multimolecular quanta observed to be released by Katz and coworkers corresponded to the contents of acetylcholine-containing vesicles. In other words, the content of a vesicle was the minimal quantum that could be released by a nerve impulse. During the years this quantal hypothesis has become generalized to include the release of biogenic amines and peptides at all nerve terminals and from biogenic amine-containing cells.

However, there are observations to indicate that a nerve impulse releases only a fraction of the content of a vesicle, and there are difficulties to explain discrepancies between the turnover of biogenic amines and their vesicles. Several other observations could be mentioned which cannot be explained on the basis of the current quantal exocytosis theory. In my presentation I will be heretic enough to argue against the present quantal dogma and propose a theory that will allow a fractional release of biogenic amines from their vesicles. Years ago we observed that mast cell granules, isolated from rat peritoneal mast cells, behaved like cation exchanger particles. When the granules were isolated in distilled water or in deionized sucrose they retained histamine, but the histamine was lost immediately on suspension of the granules in sodium chloride or various balanced salt solutions. When the granules were exposed to inorganic or organic cations (amines) these cations were taken up and retained in a concentration- and pH-dependent way, suggesting the ionic binding sites to be carboxyl groups. All cations competed for the same ionic sites in the granules and could release each other by ion exchange (3).

When we found that also granule materials from chromaffin cells (4) and from nerve terminals showed ion exchanger characteristics, we decided to study further the kinetics of the release of amines.

In Vitro Studies on Granule Materials from Mast Cells, Chromaffin Cells and Nerves

We developed a perfusion system where in a column granule material was trapped between two millipore filters and could be superfused by a steady flow of inorganic cations. Comparative experiments were made with superfusion of synthetic weak cation exchanger resins, like IRC-50 which has carboxyls as the binding sites. When histamine-containing mast cell granules or histamine-charged IRC-50 were superfused with isoosmotic sodium or potassium solutions, both materials, as could be expected, delivered their histamine to the superfusion fluid. However, especially important for our further studies was the fact that the release of histamine from both materials occurred according to identical kinetics. The release data satisfied not only the Rothmund-Kornfeld equation, characteristic of weak cation exchangers, but also an empirically found equation: $B = B_o e - k_B \sqrt{\sum ml}$ or in its linear form: $\ln B = B_o - k_B \sqrt{\sum ml}$ (where B stands for the content of the releasable amine present in the depot, B_o (previously by us denoted B_{max}) for the estimated maximal binding capacity of releasable amine in the depot, and $\sum ml$ for the cumulated effluent volumes) (5). The importance of these specific release kinetics will be evident from my further presentation, but it should be emphasized already here that two prerequisites are required to demonstrate these release kinetics. Firstly, the granules have to be superfused with an isoosmotic sodium or potassium solution. Secondly, the granules have to be immobilized, as we have done by trapping them between filters. If the granules are allowed to swirl around, the specific kinetics cannot be observed. For the later discussion, it should also be remembered that if an amine is released from a depot by diffusion, such a release occurs as a first order process. For further information on this point see Uvnäs et al. (11).

The observations on the kinetics of histamine release from mast cell granules (8) prompted us to a similar study on chromaffin granules. Chromaffin granules were isolated from ox adrenals by millipore filtration and the granules were then trapped between filters in our column system in the same way as for mast cell granules. When the chromaffin granules were perfused with isotonic sodium or potassium chloride solutions, there was a parallel release of catecholamines and ATP (and ATP metabolites). Also, the release from the chromaffin granules occurred according to the specific kinetics described above for mast cell granules. Together with previous uptake studies the results suggested to us that catecholamines and ATP are stored in the granules ionically linked to carboxyl and NH_3 groups respectively (6).

Corresponding *in-vitro* studies on the storage and release properties of adrenergic granules from bovine splenic nerves and rat vas deferens and other materials such as dopaminergic granules from rat striatum, gave the uniform picture of storage and release characteristics as described above for mast cells and chromaffin granules.

Does Ion Exchange Play a Role in Storage and Release of Biogenic Amines in vivo?

With *in-vitro* observations so unequivocally indicating ion exchange as a general principle in the storage and release of biogenic amines,the unavoidable question arises. Does ion exchange play a role in storage and release processes *in vivo* and if so, which ion (ions) is (are) then in operation, sodium or potassium ions? An important question, since both cations had *in vitro* shown the same capacity to release biogenic amines.

Histamine Release from Mast Cells

We recently made the observation that mast cells released histamine when superfused with isoosmotic salt solutions like sodium chloride or various balanced salt solutions. The cells were trapped between filters in the same way as previously described for granules. Of special interest to us was the fact that the histamine release occurred according to the same kinetics as previously shown for mast cell granules superfused with isoosmotic sodium or potassium chloride solutions. In other words, the histamine release was not simply due to a diffusion from the mast cell but suggested to us a release due to an exposure in some way of the granules in the mast cells to cations. Our first thought was that sodium ions in the perfusion fluid were responsible for the histamine release. However, this was not the explanation, since superfusion with isoosmotic deionized sucrose was also found to release histamine according to ion exchange kinetics. The sucrose did not contain any cations. Therefore the observation suggested to us that the histamine release was due to a cation flux emanating from the mast cells themselves. The only cations existing intracellularly in approximately isoosmotic concentration are potassium ions. Accordingly, we found that superfusion with isoosmotic sucrose induced a concomitant efflux of histamine and potassium (9). The efflux of histamine and potassium might at first sight be explained as a leakage of histamine from cells damaged by the superfusion. However, against this assumption speaks in the first place the specific kinetics of the histamine release, indicating the release to be due to cation exchange. Secondly, no damage of the mast cells was seen in the electron microscope. Thirdly, histamine and potas-

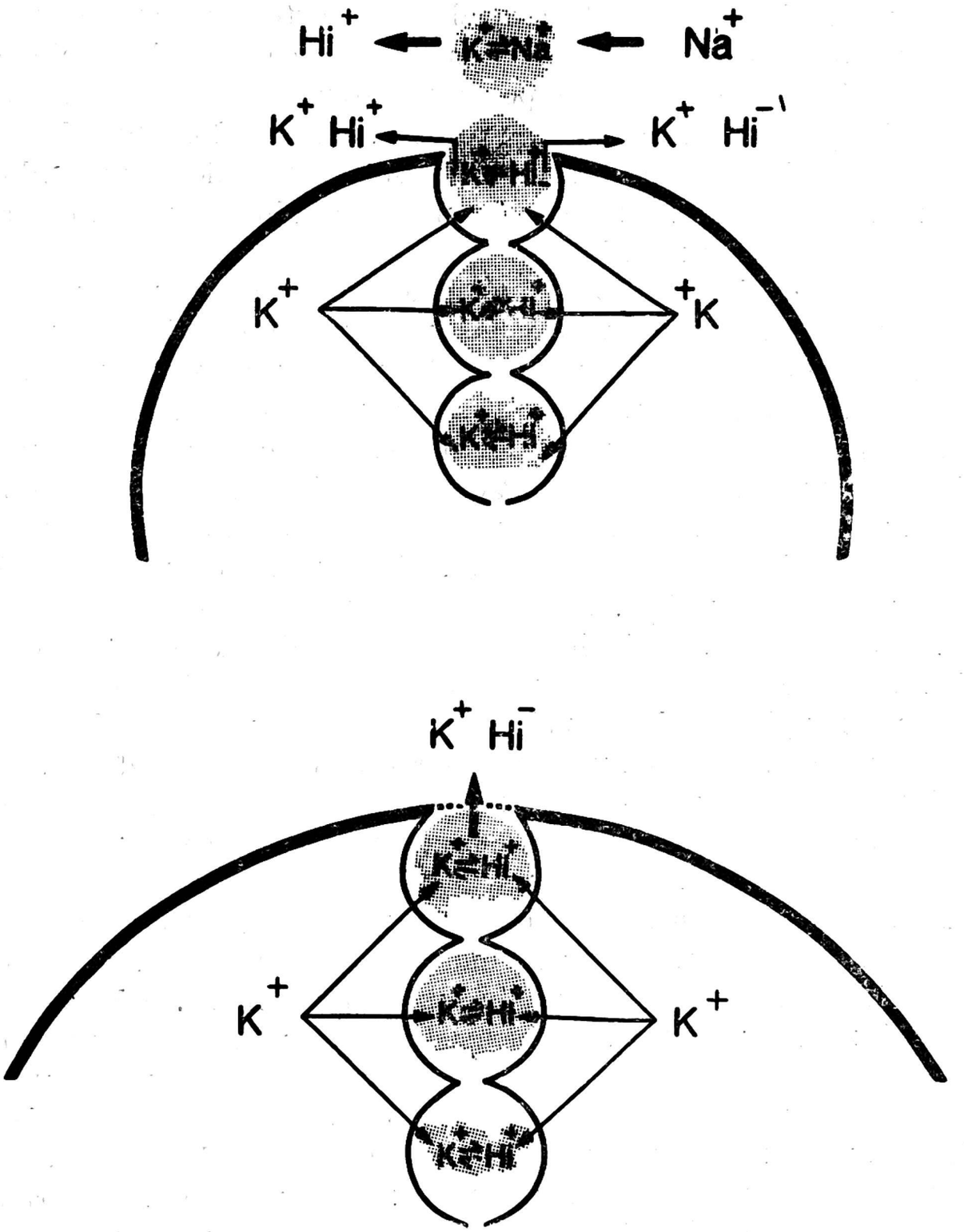

Fig. 1. Release of histamine from mast cells by intra- and extracellular ion exchange (10). (*a*) Nandegranulation phase. Granules are exposed to a flux of cytoplasmic K^+ ions resulting in exchange $K^+ \rightleftharpoons Hi^+$. Released histamine and excess potassium pass out through "pores" at cell surface. (*b*) Degranulation phase. If propulsive forces are strong enough, extrusion of granules occurs. In case extruded granules still contain histamine, Hi will be released by ion exchange $Na^+ \rightleftharpoons Hi^+$.

This schematic picture may hold also for amine release from other biogenic amine stores including nerve terminals.

sium were released in equimolar amounts indicating some kind of a functional relationship. In fact the availability of cytoplasmic potassium was a prerequisite for the release of histamine. The release of histamine came to an end when the cytoplasmic potassium store was depleted, in spite of the fact that most of the histamine was left in the cell. Finally, if histamine was added to the superfusion fluid, the histamine release was inhibited in a concentration-dependent way, around 25 μM histamine concentration required for 50 per cent inhibition (10).

If we accept the idea that histamine release from superfused mast cells is due to an intracellular potassium flux across the granules, the question remains by which route does this potassium flux occur? Lacking sufficient knowledge about the ultrastructural organization of a mast cell and its network of granules, a discussion has to be rather speculative. In the degranulating mast cell Padawer (1) has described membrane-free granules located in membraneous sacs which via interconnecting pores form ramified channel systems which in their turn communicate with the extracellular space via openings in the cell surface. The mast cell is rich in contractile proteins which according to Padawer might afford the mechanical force needed for fluid transport across the channels and, if forceful enough, for the actual extrusion of granules into the extracellular compartment (degranulation) as repeatedly described in response to polylysine, compound 48/80, antigens etc. In accordance with this proposal, addition of compound 48/80 was observed to cause a concomitant outflow of histamine and potassium as schematically indicated in (*Fig. 1*).

An intracellular channel system as suggested by Padawer (1) should afford an effective set-up for intracellular histamine release on ion exchange basis with the kinetics observed in our experiments. We have only to assume then that superfusion of the mast cell activates intracellular processes which create the forces required for cytoplasmatic potassium flux across the granules. The result will then be a cation exchange $K^+ \rightleftharpoons Hi^+$ in the granules and a consequent outflow of mast cell histamine and possibly potassium, just as we have found in our superfusion experiments.

The observation that histamine release might result from an efflux of intracellular cytoplasmic potassium across the mast cell granules is a *new* and *important* observation, since it may represent a general mechanism of release of biogenic amines, not only histamine but also other biogenic amines. I will discuss this idea a little more below.

Studies on Catecholamine Release from Adrenals in vivo

Catecholamine release was elicited from adrenals in cat, dog and pig by splanchnic nerve stimulation or by intra-arterial infusion of acetylcholine (7). In order to analyze the kinetics of the catecholamine outflow,

blood was collected from the adrenals every 10 or 20 seconds. To obtain a rapid secretion and minimize enzymatic destruction of the catecholamines we stimulated with high frequencies (10—20 per second). Under these experimental conditions the catecholamine release was observed to occur according to the same kinetics as we had observed previously for release from granules or mast cells in vitro. In other words, the release kinetics suggested to us that the release was due to an exposure of the chromaffin granules to an isotonic flux of cations.

It remains to envisage how such an exposure to ions could occur. If you adhere to the exocytosis theory you should say that the release of catecholamines is due to a cation exchange in the chromaffin granule matrix when this has been expelled by exocytosis. No doubt, catecholamines should be released under such circumstances but in the absence of a directed flow of cations across the granules, the release should, as we understand it, not occur according to ion exchange kinetics, as described above. A more likely alternative should be that, as is the case with mast cells, the activation of the chromaffin cells results in an isotonic potassium efflux directed across the amine-storing granules. The following observation rather supports this assumption. As mentioned above, when the splanchnic nerve was stimulated catecholamines were released according to ion exchange kinetics. However, if the nerve stimulation was stopped during the initial period of high catecholamine release, the efflux continued but the release immediately changed into first order kinetics indicating that the outflow of catecholamines now occurred as a diffusion from an accumulated depot. In other words, catecholamine release runs according to ion exchange kinetics only as long as nerve stimulation keeps the releasing isotonic ion flux going, presumably then a flux of endogenous potassium.

Unfortunately, we have not been able to analyze the release kinetics of biogenic amines at nerve terminals. We can only state that *in vitro* nerve granules behave like weak cation exchanger particles and therefore the presumptions exist for a release by ion exchange also *in vivo*. However, whether endogenous potassium ions play a role as the exchanger ions also here is premature to discuss. But the still unexplained high efflux of potassium from activated nerve terminals (2) should be remembered because it might reflect a flow of K^+ ions in an ion exchange $K^+ \rightleftharpoons$ transmitter amine, as reported for mast cells above.

GENERAL COMMENTS

A release theory on ion exchange basis should circumvent some of the obstacles to the current exocytosis theory according to which one quantum must equal the amine content of a vesicle (granule). It is simple

and attractive. It should allow a selective release of amines, and other ionically bound components, leaving the storage matrix and its enzymatic machineries intact. As a consequence there should be no need for recycling or extensive resynthesis of granules, unless excessive activation of the release process leads to propulsion and extrusion of granule materials. The theory should allow for a partial, concomitant release from multiple granules in a varicosity, instead of total evacuation of a few. The release should still be quantal, although the size of a single quantum should not be determined by the content of a granule but by the intensity of the ion flux across the storage granule induced by the activation of the cell or the nerve. An ion exchange theory could in fact be combined with the current exocytosis theory. As a response to modest (physiological?) activation of the release machinery there would be a selective release of amines (and other ionically bound components) as a result of the initiated potassium ion flux across the granules. The released amine would together with potassium pass out via pores, channel mouths or other hypothetical communications across the cell membrane (nerve terminal membrane). On intense activation, the propulsive forces behind the potassium flux might cause an expulsion of storage material with its remaining stored amine which would then be released by ion exchange with ions in the extracellular fluid, preferably sodium ions as depicted schematically for an activated mast cell (*Fig. 1*). The exocytosis, according to the current views considered as the event in the release process, with complete evacuation of amine-storing vesicles, should according to the present ion exchange theory occur only as the result of intense activation (as usually has been the case under experimental circumstances when exocytosis has been observed). Questions concerning the mechanism of fatigue and faciliatation, inhibition and potentiation, storage and release pools, and many other phenomena in physiology and pharmacology ought to be reexamined with the possibility in mind that these phenomena might be dependent on/or modified by ion fluxes, especially endogenous potassium ion fluxes involved in the ion exchange behind a fractional release process.

REFERENCES

1. Padawer J. The reaction of mast cells to polylysine. J Cell Biol 1970; 47: 352—372.
2. Sykova E. Extracellular K^+ accumulation in the central nervous system. Prog Biophys Mol Biol 1983; 42: 135—189.
3. Uvnäs B. The mechanism of histamine release from mast cells. In Handbook of Experimental Pharmacology, GWR Boen, O. Eichler, A. Farah, H. Herken, AD Welch (eds). vol. XVIII/2, Berlin — Heidelberg-New York, Springer Verlag, 1977, pp. 75—92.

4. Uvnäs B, Åborg C-H. In vitro studies on a two-pool storage of adrenaline and noradrenaline in granule material from bovine adrenal medulla. Acta Physiol Scand 1980; 109: 345—354.
5. Uvnäs B, Åborg C-H. Cation exchange — a common mechanism in the storage and release of biogenic amines stored in granules (vesicles?). Comparative studies on sodium-induced release of biogenic amines from the synthetic weak cation-exchangers Amberlite IRC-50 and Duolite CS-100 and from biogenic (granule-enriched) materials. Acta Physiol Scand 1984: 120: 87—97.
6. Uvnäs B, Åborg C-H. Catecholamines (CA) and adenosine triphosphate (ATP) are separately stored in bovine adrenal medulla, both in ionic linkage to granule sites, and not as a nondiffusable CA-ATP-protein complex. Acta Physiol Scand 1988; 132: 297—312.
7. Uvnäs B, Åborg C-H, Goiny M. The kinetics of adrenal catecholamine secretion elicited by splanchnic nerve stimulation or by acetylcholine is consistent with non-exocytotic, multivesicular release on cation exchange basis. Acta Physiol Scand 1985; 123: 249—259.
8. Uvnäs B, Åborg C-H, Lyssarides L, Thyberg J. Cation exchanger properties of isolated rat peritoneal mast cell granules. Acta Physiol Scand 1985; 125: 25—31.
9. Uvnäs B, Åborg C-H, Lyssarides L, Thyberg J, Danielsson L-G. Rat mast cells superfused with isotonic solutions release histamine, probably via intracellular cation exchange $K^+ \rightleftharpoons Hi^+$ ions. Acta Physiol Scand 1986; 128: 657—658.
10. Uvnäs B, Åborg C-H, Lyssarides L, Danielsson L-G. Intracellular ion between cytoplasmic potassium and granule histamine, an integrated link in the histamine release machinery of mast cells. Acta Physiol Scand 1989; 136: 309—320.
11. Uvnäs B, Åborg C-H, Lyssarides L, Danielsson L-G. Juxtacellular histamine concentration governs histamine release from rat peritoneal mast cells. Acta Physiol Scand 1991; 142: 173—180.

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