The pH-Dependent Release of Monoamines from Isolated Platelet, Enterochromaffin and Dopamine Cell Granules in vitro

The cytoplasmic organelles which contain monoamines exhibit a high content of ATP[1,2], of lipids and RNA[3,4], of bivalent ion[4,5], and of soluble and insoluble proteins[6]. The basic amino group of positively charged monoamines is essential for fixing them by granules[7,8]. The complex of ATP and norepinephrine (NE) seems to interact with proteins being held in the granule matrix[6], but only a part of NE is bound by ATP[4,9]. A similar storage form of 5-hydroxytryptamine (5-HT) by enterochromaffin and platelet granules has been suggested[2,10].

The pH of the medium is of importance for the stability of the amine content of granules[11–14] and intact cells[15]. At a pH range of 2–4, 5-HT was readily released from rat peritoneal mast cells with cytoplasmic morphological changes[15].

The preparation of the monoamine-containing granule fraction and release experiments were carried out as described earlier[14]. The cow duodenal mucosa was homogenized in a Potter-Elvehjelm glass and the spleen in an Ultra-Turrax® homogenizer. 5-HT[16] and dopamine (DA)[17,18] were determined spectophotofluorometrically. For electron microscopy (EM) the bottom layer of the pellet obtained at 20,000 *g*, i.e. the granule fraction[14], was fixed in 3% glutaraldehyde in a 0.1 *M* phosphate buffer solution (pH 7.2) for 2 h at 0 °C. The pellet was postfixed in 1% osmium tetroxide for 2 h at 0 °C[19,20], embedded in epoxy resin[21] and the sections were stained by lead citrate[22] and uranyl acetate[23] before viewing in a Philips EM 200 electron microscope.

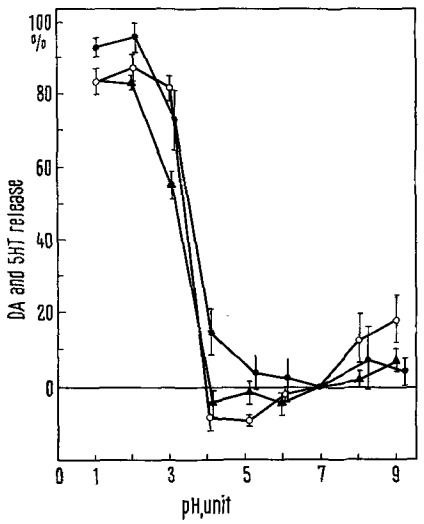

Fig. 1. Release of 5-HT from isolated cow enterochromaffin (○) and platelet (▲) and that of DA from dopamine cell granules (●) in vitro. Release experiments were carried out in a 0.15 *M* phosphate buffer solution (final concentration) for 30 min at 0 °C by using 1.0 ml of the granule fraction (10%, wet weight/volume) and 9.0 ml of the incubation medium. The granule fraction was sedimented by a Sorvall® SS-1 cold centrifuge at 20,000 *g* for 10 min at 2 °C after incubation. The total centrifugation time was 20 min. After addition of 1.0 ml of 0.1 *N* HCl, the granule pellet was frozen and stored at −20 °C for not more than 2 days before 5-HT assay. The incubation mediums below 5.0 were made by adding concentrated HCl in a phosphate buffer at pH 5.0 until the desired pH was obtained. The release of monoamines is expressed on the ordinate, in %. The 5-HT and DA values of the granule pellet at pH 7.0 were set at 0% and the amine content in various pH was determined. Means and standard errors of 7 series of each granule type. One serie consisted of 12 aliquots of the same homogenate studied at various pH.

5-HT was liberated almost quantitatively from isolated platelet and enterochromaffin granules (EG) below pH 4.0 (Figure 1). The plot of DA from dopamine granules was in principle similar to 5-HT plots of platelets and EG. The total DA content of the supernatant and pellet was about the same, regardless of the pH, whereas the total 5-HT of experiments on EG and platelet granules showed a tendency to decrease in the alkaline pH range (Figures 2, a, b, c). This kind of effect of high pH on the 5-HT content was not found when pure 5-HT was incubated instead of granules. Below pH 4.0 the incubation time (0–30 min) had no essential effect and even during the 20 min centrifugation time 5-HT was almost totally liberated from the 2 granule types studied.

In Figure 3 a typical EM field of the lower layer of the sediment is obtained at 20,000 *g*. Under identical conditions to those used in present experiments, this fraction contained the highest concentration of monoamines (μg/tissue protein)[14]. After incubation in a 0.15 *M* phosphate buffer solution (pH 7.0) for 30 min, numerous highly osmiophilic particles were seen in the pellet. On the basis of the size, shape, affinity for osmium tetroxide, finely granular inner structure and surrounding membrane, these organelles were interpretated as derived from enterochromaffin and dopamine cells or from platelets. The intact duodenal mucosa and the spleen of the cow served as controls. Some osmiophilic particles of a heterogeneous size, shape and affinity for osmium tetroxide were obviously lysosomes, typical of epithelial cells.

Numerous intact osmiophilic granules were seen after incubation of granules in a buffered 0.3 *M* sucrose solution at pH 7.0 and in 0.15 *M* phosphate buffer at pH 9.0 for 30 min at 0 °C (Figure 4). Only destroyed and distended membranes and membraneous fragments were seen after

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incubation of granules at pH 2.0 (Figure 5). Within the pH range 1–3, only occasional, weakly osmiophilic particles, surrounded by a membrane, were seen (Figure 5). Principally similar observations were made on experiments of the spleen.

The present experiments showed that 5-HT and DA could be released almost quantitatively at 0 °C from isolated enterochromaffin, platelet and dopamine cell granules in the pH range of 1–3. In principle the plots of EG and platelet granules and the DA plot of the dopamine cells were identical.

NE could be released also from isolated bovine splenic nerve and rat heart granules under milder acidic conditions of isotonic salt and sucrose solutions[12, 13]. The

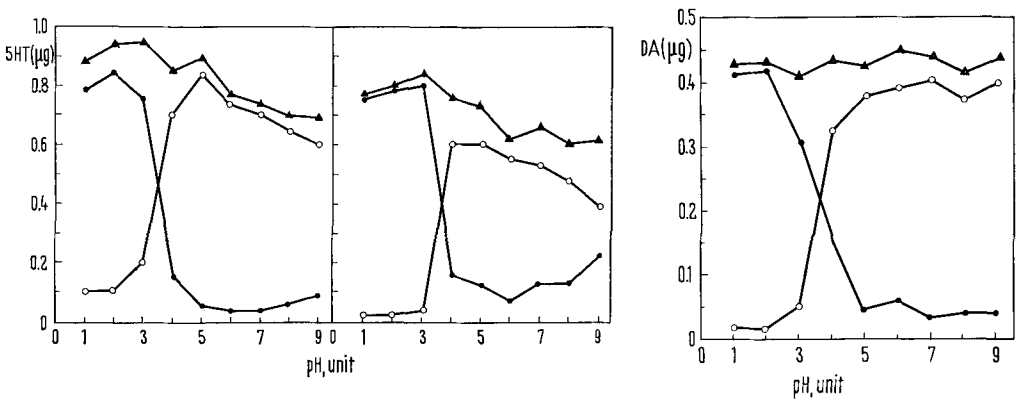

Fig. 2. The amine content of the pellet (○), supernatant (●) and the total amine content (▲) of one experiment, (a) after incubation platelet, (b) enterochromaffin and (c) dopamine granules in identical conditions to those presented in Figure 1.

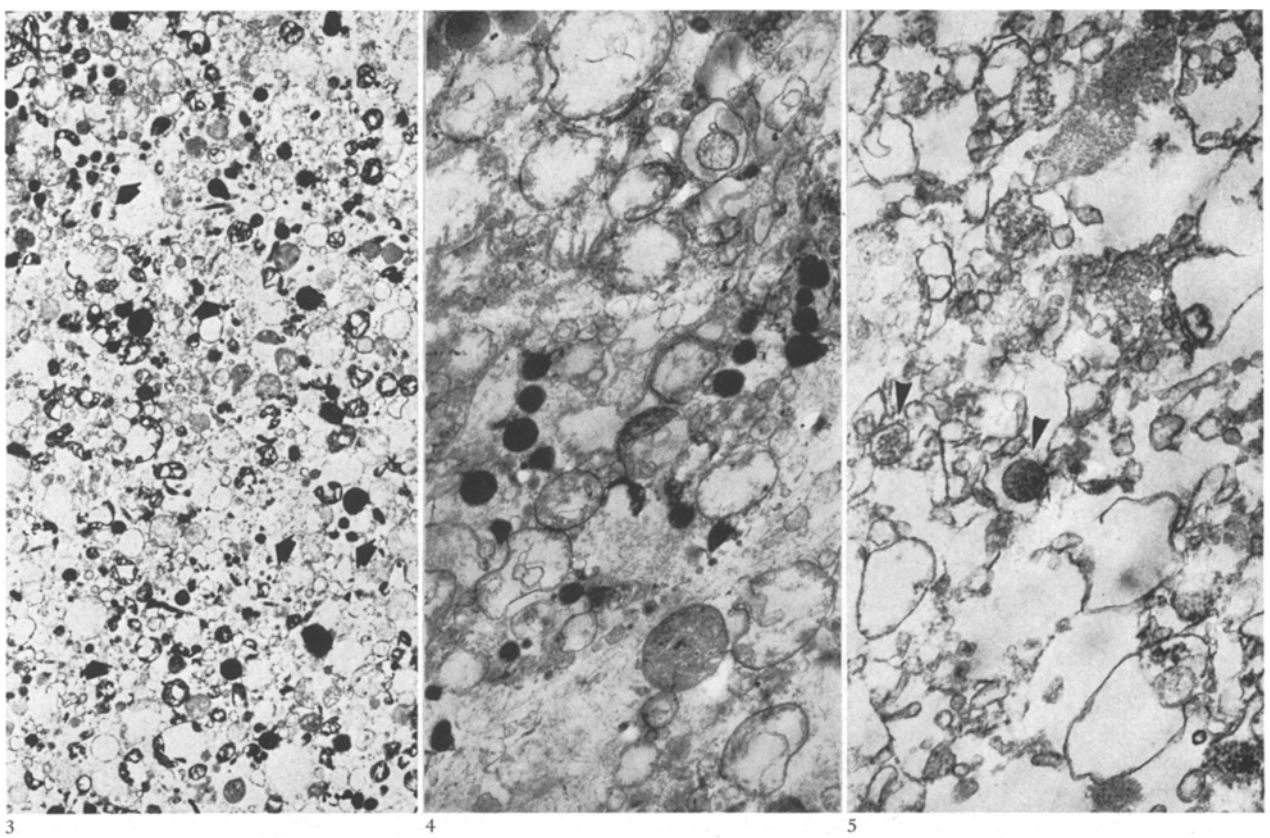

Fig. 3. Electron micrograph of the bottom layer sedimented between 800 and 20,000 *g* for 20 min from the cow duodenal mucosa homogenate after incubation in 0.15 *M* phosphate buffer solution (pH 7.0) at 0 °C for 30 min. The roundish highly osmiophilic granules, interpreted as being monoamine-carrying enterochromaffin and dopamine cell granules, are readily identifiable (arrows). × 13,500.

Fig. 4. The same experimental conditions as for Figure 3 but incubated at pH 9.0. Several highly osmiophilic organelles are to be seen. × 40,000.

Fig. 5. The same experimental conditions as for Figure 3 but incubated at pH 2.0. Numerous destroyed membraneous formations but only a few weakly electron-dense destroyed organelles (arrows) with an identifiable surrounding membrane are to be seen. Note the abscence of highly osmiophilic particles. × 40,000.

similar tendency of all 3 granule types of the present study and of the NE-containing granules towards the acidic reaction is especially interesting in view of the quite different architectures of these various granule types[24-27] and suggests a non-specific effect of acidic medium on these organelles.

The dissociation constants and the titration curves of the monamines[7,8] suggest that the pH effect in the range of 1–3 is not dependent on the reaction of the terminal amino or ring hydroxyl groups. The electron microscopic observations on the granule pellet showed that in the pH area of 1–3, no identifiable organelles were seen, but only fragments and distended membraneous formations. The absence of highly osmiophilic granules indicates their lysis, apparently preceding the release of 5-HT and DA registered in the same experiments. The mitochondria, lysosomes, osmiophilic particles and various kinds of regular vesicles seen in the pellet at pH 7.0 but not at pH 2.0, suggest a non-specific protein denaturation effect of the low pH. At pH 2.0, the bonds fixing the amine directly to the matrix of specific organelles or the bonds between the intermediate constituent and the granule matrix may be affected. The change of the solubility or the charge of the intragranular proteins may previously be possible explanations of the amine release at highly acidic pH.

Zusammenfassung. 5-Hydroxytryptamin und Dopamin konnten von den Partikeln der enterochromaffinen und dopaminen Zellen und Blutplättchen in den pH-Werten 1–3 gelöst werden. Gleichzeitig wurde die Auflösung und das Verschwinden der Osmiumaffinität der Partikel festgestellt.

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Department of Anatomy of the University, Helsinki 17 (Finland), 3 July 1969.

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Chiasmate Type of Meiosis in the Roach *Nauphoeta cinerea* (Oliver) (Fam: Blattidae)

MATTHEY[1] was the first to report the absence of chiasmata in the roach *Pycnoscellus surinamensis*. Even though SUOMALAINEN[2] also could not observe any 'visible' chiasmata in another roach *Blabera*, he did find clear chiasmata in the genera *Lecucophaea* and *Phyllodromia*; he also gave convincing, though indirect, proofs regarding their occurrence in *Periplaneta australasiae* and *P. americana*. JOHN and LEWIS[3-5], however, strongly ruled out the chiasmate meiosis in certain roaches including *P. americana*. Later JOHN et al.[6] mentioned about the occurrence terminal chiasmata, in *P. americana*, but they did not negate the earlier hypothesis of 'non-chiasmate meiosis' proposed by JOHN and LEWIS for these 'out breeding' animals. Independently, meiosis in *P. americana* was studied in India by RAJASEKARASETTY and RAMAMURTHY[7], and they also reported chiasmata in this roach. In the present article I am describing a case of normal chiasmate-meiosis in another roach *Nauphoeta cinerea* (Oliver).

Males of this roach were sacrificed for their testis squash preparations. Gonial metaphase chromosomes show that some of these are metacentric. The haploid number, *n*, is 19 ($18A + 1X$). The sex determining mechanism is of *X*O type. Pairing is intimate during pachytene, and soon the bivalents start opening out and reveal the chiasmata clearly. At diplotene, of the 18 bivalents 9 (rings) show 2 chiasmata each, while the remaining 9 (rods) show 1 each. And at diakinesis some of the rings open out at one end (Figure 1). From observations on 25 cells at diplotene, the chiasmata frequency works out to be 1.5, while the same at diakinesis is reduced to 1.27 suggesting the opening out of some bivalents at one end, thereby reducing the chiasmata number. Terminalization is almost completed at metaphase I (Figure 2). Anaphase I is a reductional division (Figure 3) while anaphase II is equational.

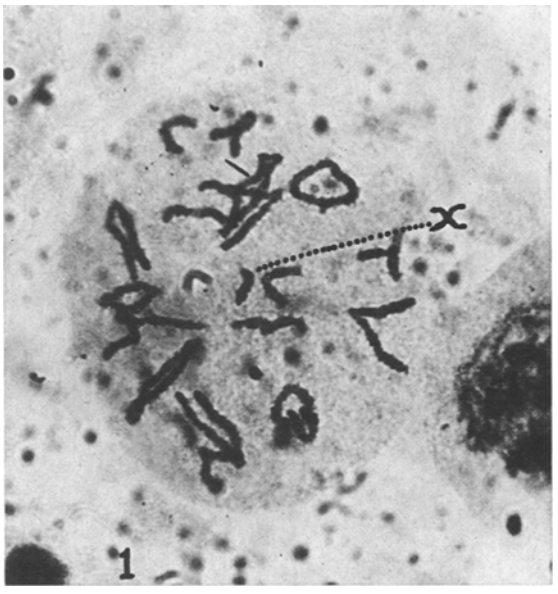

Fig. 1. 18 bivalents and an unpaired sex-chromosome during early diakinesis. × 2000.

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