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Different Handling of Parathyrin by Basal-Lateral and Brush-Border Membranes of the Bovine Kidney Cortex

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The two parts of the bovine kidney cortex plasma membrane, the basal-lateral and the brush-border membrane, were simultaneously prepared from the same organ. Both types of membrane bound parathyrin, but only from the basal-lateral fraction was the hormone displaceable by its bioactive *N*-terminal fragment. In parallel, parathyrin-stimulated adenylate cyclase was predominantly found in basal-lateral membranes. The hormone was fragmented by both membrane types. Basal-lateral membranes generated fragments with a rather uniform size distribution (somewhat smaller than the intact peptide) and apparently preferred the hormone itself as a substrate. In contrast, the fragments produced by brush-border membranes were numerous small peptides.

The plasma membrane of the proximal tubular cell consists of two entities: the luminal brush-border membrane and the anti-luminal basal-lateral membrane. Both sites are morphologically and enzymically differentiated (Rhodin, 1958). The brush-border membrane is enriched in hydrolytic and proteolytic enzymes (Maack *et al.*, 1971). This determines a main function of the luminal membrane of the proximal tubule: substances are taken up from the primary urine, converted and transported into the cell, where they are further metabolized (Maack *et al.*, 1971; George & Kenny, 1973). Adenylate cyclase seems to be predominantly present in the anti-luminal basal-lateral membrane (Shlatz *et al.*, 1975), although the enzyme was also found in brush borders (Wilfong & Neville, 1970; Sato *et al.*, 1974). Parathyrin binds to both basal-lateral and brush-border membranes (Shlatz *et al.*, 1975). Because parathyrin undergoes glomerular filtration (Hesch *et al.*, 1978), it seems possible that the hormone directly exerts biological effects at the luminal membrane. The luminal as well as the anti-luminal site of the proximal tubular cells is involved in the metabolism and clearance of parathyrin and its circulating fragments (Martin *et al.*, 1977).

In the present paper we report on the interaction of parathyrin with the kidney cortex plasma membrane. Both types of membranes were simultaneously prepared from the same bovine kidney. We monitored the distribution of parathyrin-sensitive adenylate cyclase and the specificity of parathyrin binding to the membranes. We were further interested in studying the role of basal-lateral and brush-border membranes in the metabolism of the hormone. We found that each site interacts in a specific manner with parathyrin, thus reflecting the different functions of the two parts of the proximal-tubular plasma membrane.

Materials and Methods

Bovine parathyrin (approx. 60% pure immunologically) was a generous gift from Hormon-Chemie, München, Germany. All other chemicals were of reagent grade and obtained from commercial suppliers.

The buffers used were 50mM-Tris/HCl, pH 7.4, containing 1mM-EDTA and 0.25M-sucrose (buffer A), 100mM-ammonium acetate, pH 4.5, containing 0.5% bovine serum albumin (buffer B) and NIRG buffer (50mM-sodium barbitone/HCl, pH 8.0, containing 0.5% NaCl, 0.5% human serum albumin and 0.002% non-immune bovine γ-globulin).

Antisera

Anti-parathyrin antibody S469 is routinely used for radioimmunoassay in our laboratory. It is of *C*-regional specificity with enhanced affinity for the mid-region of the hormone (R. Hehrmann, H.-P. Nordmeyer, H. Mohr & R.-D. Hesch, unpublished work). Antibody AS262 is directed against the

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C-terminal region of parathyrin and was purchased from Burroughs Wellcome, Beckenham, Kent, U.K. The anti-(cyclic AMP) antiserum was raised in a rabbit against bovine serum albumin–succinylated cyclic AMP conjugate by the method of Steiner *et al.* (1972).

Preparation of membranes

Bovine kidneys were obtained from a local slaughterhouse and immediately placed on ice after removal from the animals. All subsequent work was done at 0–4°C. The cortex was sliced off from the organ and suspended in the same amount of buffer A. The tissue was minced by a short burst (5 s) of an Ultra-Turrax homogenizer from Janke & Kunkel KG, Staufen, Germany. The suspension was further homogenized by 10 strokes at 1000 rev./min in a motor-driven Potter–Elvejhem homogenizer from Braun A.G., Melsungen, Germany. The homogenate was diluted with the same volume of buffer A and divided into two parts.

Brush-border membranes were prepared by the method of Kessler *et al.* (1978). $CaCl_2$ (1 M) was added to the one-half of the kidney cortex homogenate to give a final concentration of 10 mM. After stirring for 30 min the suspension was centrifuged at 3000 ***g*** in a cool centrifuge from MSE, Crawley, Sussex, U.K. The pellet containing intact cells, cell fragments and aggregates of all other organelles except brush-border membranes [this aggregation is induced by Ca^{2+} (Schmitz *et al.*, 1973; Kessler *et al.*, 1978)] was discarded. The supernatant was recentrifuged as above and the resulting pellet was again discarded. The supernatant was then centrifuged at 10000 ***g*** for 15 min in a J2 21 centrifuge from Beckman Instruments, Palo Alto, CA, U.S.A. The resulting pellet containing the brush borders was resuspended in 100 ml of buffer A and centrifuged at 10000 ***g*** for 15 min. The resuspending and centrifugation step was repeated. The pellet was finally suspended in buffer A (to give a protein content of approx. 5 mg/ml), divided into portions and kept frozen at −70°C until use.

Basal-lateral membranes were prepared as follows. The second half of the kidney cortex homogenate was subjected to differential centrifugation as described by Marx *et al.* (1972). The partially purified membranes were suspended in 30 ml of buffer A and then mixed with 20 ml of a solution of 50% Percoll (from Pharmacia Fine Chemicals AB, Uppsala, Sweden), pH 7.4, containing 1 mM-EDTA and 0.25 M-sucrose. The suspension was centrifuged for 45 min at 15000 rev./min in the Beckman centrifuge with a JA 20 rotor. The basal-lateral membranes appeared as a fluffy band near the top of the resulting gradient and were isolated with the aid of a Pasteur pipette. The membrane suspension was diluted to 50 ml with buffer A and centrifuged at 10000 rev./min for 10 min. The pellet was taken up in 50 ml of buffer A and the last centrifugation step was repeated. Finally the pellet was suspended in buffer A (to give a protein content of approx. 5 mg/ml), homogenized by some strokes in a Potter–Elvejhem homogenizer and stored in portions at −70°C.

Enzyme determinations

Alkaline phosphatase (EC 3.1.3.1), $(Na^+ + K^+)$-dependent ATPase (EC 3.6.1.3) and succinate dehydrogenase (EC 1.3.99.1) were measured as described by Rick *et al.* (1972), Hendler *et al.* (1971), Rothstein & Blum (1973) and Tisdale (1967) respectively. Protein was determined by the method of Lowry *et al.* (1951). Adenylate cyclase (EC 4.6.1.1) activity was determined as follows. In a total of 50 μl the reaction mixture contained 100 mM-Tris/HCl (pH 7.5), 4 mM-ATP, 6 mM-$MgCl_2$, 1 mM-isobutylmethylxanthine, 30 mM-phosphocreatine and 50 μg of creatine kinase. Parathyrin (in 20 μl of buffer A, but without sucrose) and membranes (20 μg in 20 μl) were added and the mixture was incubated for 15 min at 30°C. The reaction was stopped by placing the samples into ice-cold water and by adding 50 μl of 3 M-$HClO_4$. After 10 min the suspension was neutralized with 3 M-KOH. After 10 min the precipitate was centrifuged. The supernatant was diluted with 100 mM-sodium acetate buffer, pH 6.2, and its cyclic AMP content was measured by radioimmunoassay by the method of Steiner *et al.* (1972).

Assay techniques

Binding of parathyrin to membranes was measured by the labelled antibody membrane assay by the method of McIntosh & Hesch (1976). Anti-(bovine parathyrin) antiserum AS 262 (used to detect membrane-bound parathyrin) was adsorbed to bovine parathyrin-immunoadsorbent (Miles & Hales, 1968) and labelled with ^{125}I by the chloramine-T technique (Hunter & Greenwood, 1962). Extraction of the high-affinity antibodies from the adsorbent was carried out as described by Addison *et al.* (1971).

The parathyrin content of samples was determined by radioimmunoassay by the method of Hehrmann *et al.* (1976) or by the coated-tubes assay, which utilizes the capability of parathyrin to bind to plastic surfaces (Barrett & Neumann, 1978): 200 μl of the test solution (in NIRG buffer) was incubated for 2 h at room temperature in polyethylene tubes from Sarstedt, Nümbrecht, Germany. The solution was then aspirated and the tubes were washed twice with NIRG buffer. Labelled anti-(bovine parathyrin) solution (200 μl; approx. 5000 c.p.m.) was added and the tubes were incubated overnight at 4°C. The solution was then

aspirated, the tubes washed twice and then counted for radioactivity in a γ-scintillation counter from Berthold KG, Melsungen, Germany. The amount of tube-bound radioactivity is a function of the parathyrin content of the test solution (H. Jüppner, H. Mohr & R. D. Hesch, unpublished work).

Degradation experiments

In a volume of 200 μl, 5 μg of parathyrin (in buffer A, but without sucrose) was incubated with membranes at 37°C for the times indicated. The samples were then chilled with ice-cold water. The membranes were centrifuged and, after appropriate dilution, the parathyrin contents of the supernatants were measured by the coated-tubes assay. In other experiments the supernatants were subjected to gel filtration on Ultro Gel AcA 54 (column size 1.5 cm × 100 cm) equilibrated with buffer B. Fractions (2.2 ml) were collected and assayed for their parathyrin content by radioimmunoassay.

Results

Table 1 shows the relative enrichments (compared with the homogenate) of marker enzymes in basal-lateral and brush-border membranes respectively. $(Na^+ + K^+)$-dependent ATPase was enriched in the basal-lateral fraction by a factor of about 9. The last purification step (the self-generating Percoll density gradient) proved to be very effective: when compared with the partially purified membranes, the activity of $(Na^+ + K^+)$-dependent ATPase increased by a factor of about 3. This is similar to that which was obtained by using ultracentrifugation and a preformed sucrose gradient (Zull *et al.*, 1977). The basal-lateral membrane fraction was nearly devoid of contamination by brush borders (the decrease in alkaline phosphate activity was 75%) and the activities of the lysosomal and mitochondrial markers were decreased (enrichment factors were 0.8 and 0.75 respectively).

The brush-border preparation was not contaminated by lysosomes and mitochondria. A small residual $(Na^+ + K^+)$-dependent ATPase activity was still observed [although not as pronounced as reported by Liang & Sacktor (1977)]. The enzyme could not be removed from the membranes by density-gradient centrifugation or by repeated washing. Whether this $(Na^+ + K^+)$-dependent ATPase was derived from contamination by basal-lateral membranes or whether it is an integral part of the brush-border membrane will be discussed below.

Parathyrin-stimulated adenylate cyclase was similar to the enrichment of $(Na^+ + K^+)$-dependent ATPase, i.e. it was predominantly present in the basal-lateral membrane (Table 2, Fig. 1). Adenylate cyclase activity was also found in the brush-border preparations, but overall activity and the response to parathyrin were only weak (Table 2, Fig. 1). The asymmetry in the distribution of the enzyme within the plasma membrane was even more marked if one utilizes the enhancing effect of guanosine 5'-[β,γ-

Table 1. *Enrichment of marker enzymes in basal-lateral and brush-border membranes*
Abbreviation used: n.d., not detectable.

	Enrichment of enzyme (-fold)			
Fraction	$(Na^+ + K^+)$-dependent ATPase	Alkaline phosphatase	Acid phosphatase	Succinate dehydrogenase
Homogenate	1	1	1	1
Partially purified membranes	3.2	0.43	0.55	1
Basal-lateral membranes	8.8	0.25	0.8	0.75
Brush-border membranes	0.7	6.2	n.d.	n.d.

Table 2. *Distribution of adenylate cyclase in basal-lateral and in brush-border membranes*
NaF concentration was 10 mM and that of parathyrin was 1 μg/ml.

	Cyclic AMP (pmol released/mg of protein)		
Fraction	Basal	Stimulation by NaF	Stimulation by parathyrin
Homogenate	150	220	170
Partially purified membranes	140	275	280
Basal-lateral membranes	230	690	720
Brush-border membranes	205	350	370

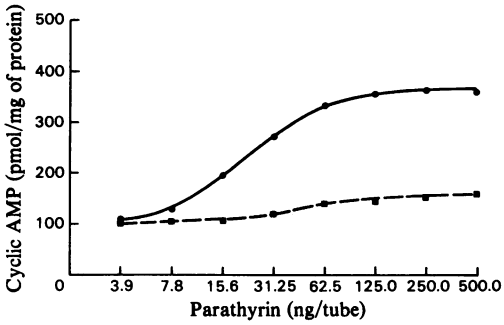

Fig. 1. *Dose–response curves for parathyrin-stimulated adenylate cyclase by basal-lateral* (●) *and brush-border membranes* (■)

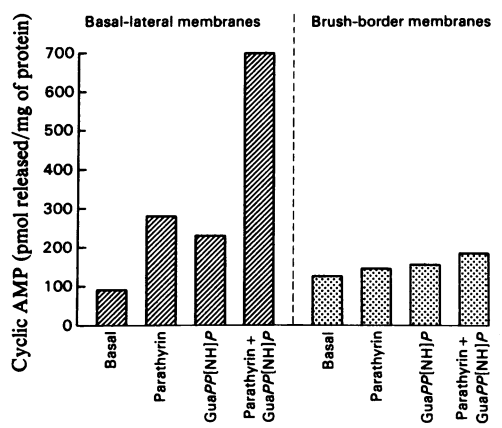

Fig. 2. *Stimulation of adenylate cyclase in basal-lateral and brush-border membranes by parathyrin and guanosine 5'-[β,γ-imido]triphosphate*

The parathyrin concentration was 1 μg/ml and that of guanosine 5'-[β,γ-imido]triphosphate was 10 μM. Abbreviation used: Gua*PP*[NH]*P*, guanosine 5'-[β,γ-imido]triphosphate.

imido]triphosphate on the hormone-stimulated adenylate cyclase (Fig. 2).

Different authors have demonstrated that binding of ^{3}H- or ^{125}I-labelled parathyrin to renal plasma membranes could be suppressed by unlabelled hormone, indicating the presence of hormone-specific receptor sites (Sutcliffe *et al.*, 1973; DiBella *et al.*, 1974; McIntosh & Hesch, 1976; Zull *et al.*, 1977). This is corroborated by our own results as shown in Fig. 3(*a*): about 50% of parathyrin binding to basal-lateral membranes was blocked by the addition of the bioactive hormone fragment (residues

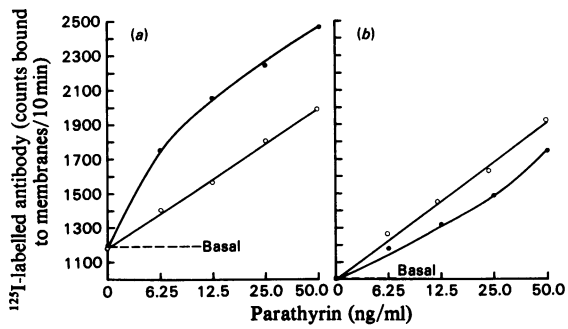

Fig. 3. *Binding of parathyrin to basal-lateral* (*a*) *and brush-border membranes* (*b*) *without* (○) *and in the presence of 1 μg of fragment (1–34) of bovine parathyrin* (●)

Membranes (50 μg in 100 μl of buffer A) + 1% bovine serum albumin were incubated for 60 min with parathyrin. After washing the membranes, bound parathyrin was detected with ^{125}I-labelled anti-(bovine parathyrin) antibody. The procedure is given in detail elsewhere (McIntosh & Hesch, 1976).

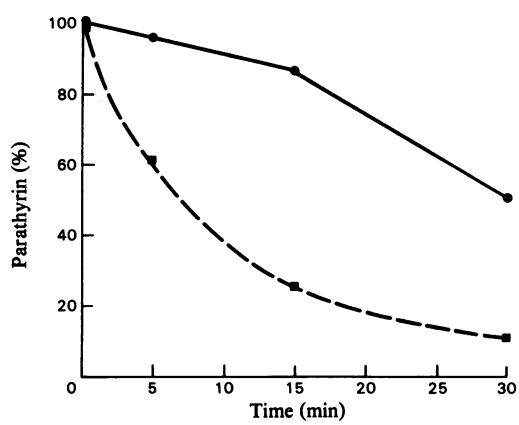

Fig. 4. *Time course of parathyrin degradation by basal-lateral* (●) *and brush-border membranes* (■)

The hormone (5 μg in 200 μl of buffer A) was incubated with 50 μg of membranes at 37°C. The membranes were then centrifuged and the supernatants were assayed for remaining parathyrin by the coated-tubes assay.

1–34) of bovine parathyrin [which binds to the membranes, but is not recognised by the *C*-terminal ^{125}I-labelled anti-(bovine parathyrin) antibody]. It is noteworthy that total displacement of parathyrin from the membranes was not achieved in these experiments. This can be interpreted as demon-

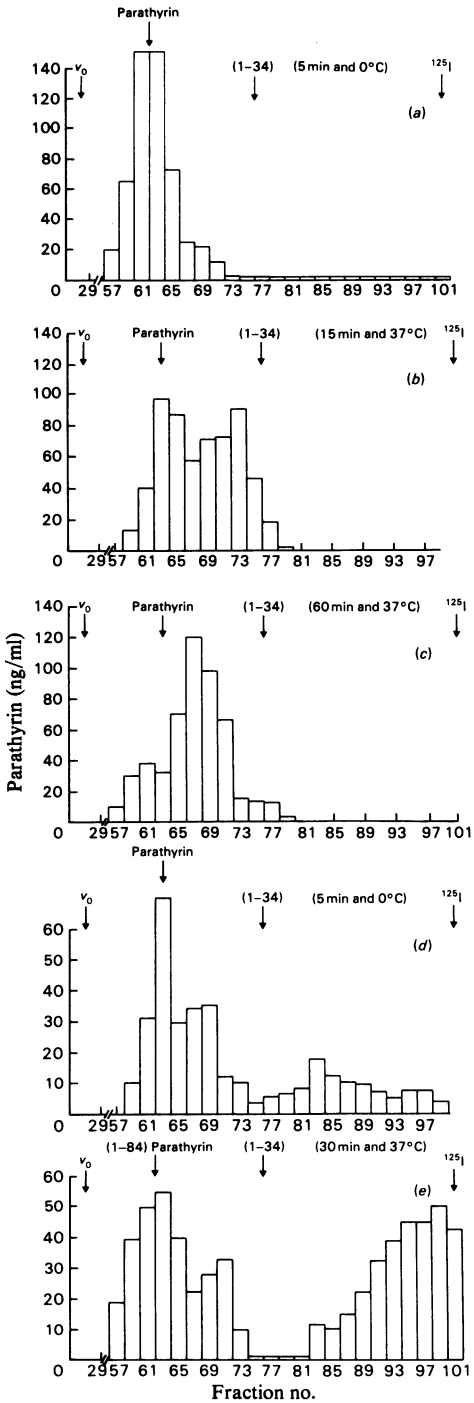

Fig. 5. *Metabolism of parathyrin by basal-lateral (a, b and c) and brush-border membranes (d and e)*
After membrane exposure (see the legend to Fig. 4) the supernatants were subjected to gel filtration on Ultro Gel AcA 54 (column size, 1.5 cm × 100 cm). Fractions (2.2 ml) were collected and assayed for their parathyrin content by radioimmunoassay.

stration of the presence of non-specific binding sites on the membranes. That parathyrin binds to brush-border membranes was suggested by different groups, but not demonstrated (Quirk & Robinson, 1972; Shlatz *et al.*, 1975). Fig. 3(*b*) shows that indeed the hormone adsorbs to the brush borders, although to a lesser extent than to basal-lateral membranes. The most striking difference is that the intact parathyrin cannot be displaced at all by its fragment (residues 1–34). On the contrary, the fragment seems to enhance binding of the intact hormone slightly. This effect was reproducible in numerous experiments. It is not clear whether this 'over-sensitization' is simply an experimental artefact or whether it is a reflection of a change in the membranes. There is some evidence (see below) that parathyrin is rapidly metabolized by the brush-border vesicles. The fragment (residues 1–34) was added in large excess. It might therefore protect the intact hormone against fragmentation and 'displace' it to inert binding sites on the membrane surface. Parathyrin is fragmented by both basal-lateral and brush-border membranes. The time course of parathyrin degradation under the influence of both types of membranes is shown in Fig. 4. There is no doubt that the metabolic activity of the brush-border vesicles is much more pronounced than that of the basal-lateral membranes. This is not surprising because brush borders are known to be enriched in hydrolytic enzymes (Maack *et al.*, 1971; George & Kenny, 1973). The different metabolic responsiveness of parathyrin to basal-lateral and brush-border membranes is further demonstrated in Fig. 5. Parathyrin was incubated with membranes. The supernatants of the reaction mixture were then subjected to gel filtration on Ultro Gel AcA 54. Basal-lateral membranes generate immunoreactive fragments of rather uniform size. After 15 min, about half of the hormone was fragmented and after 60 min nearly all the parathyrin was metabolized (Figs. 5*a*–5*c*). The experiment indicates a certain preference of the proteinase(s) in the contraluminal membrane for the structure of the intact hormone [the fragment peak increases, whereas that of parathyrin disappears]. No production of smaller fragments was detected. Hence, if the first metabolite(s) of parathyrin is further degraded, this would certainly occur very slowly.

The fate of the hormone when it is exposed to brush-border membranes is quite different (Figs. 5*d* and 5*e*). Various small peptides are generated eluting in front of the salt peak of the column. This indicates the more general nature of the proteolytic process mediated by the brush-border membranes. It is of interest that even after short brush-border exposure

Abbreviations used: (1–34), fragment (1–34) of bovine parathyrin; ^{125}I, salt peak of the column.

at 0°C a great deal of the hormone was cleared from the incubation mixture whereas basal-lateral membranes were inert in such conditions.

Discussion

Two different preparation techniques were used in the present study to obtain the two parts of the proximal-tubular-cell plasma membrane. The basal-lateral membranes were prepared by differential centrifugation followed by iso-osmolar centrifugation in a self-generating Percoll gradient. This last purification step proved to be at least as effective as the sucrose-gradient centrifugations described by other authors (Marx *et al.*, 1972; Zull *et al.*, 1977). The Percoll technique offers some advantages in that no ultracentrifuge is needed, no gradient has to be preformed and the vesicles that have to be separated are not exposed to structure-modifying osmolar stress. The purity of the membranes (observed by the increase or decrease of the activities of enzyme markers) was similar or even better than those that were obtained with the aid of a sucrose gradient. Both $(Na^+ + K^+)$-dependent ATPase and parathyrin-stimulated adenylate cyclase were enriched by factors of 8–10 compared with the kidney cortex homogenate. Contamination by brush borders was low and the activities of lysosomal and mitochondrial markers were decreased (enrichment factors 0.7–0.8).

The brush borders that were prepared by the method of precipitation by Ca^{2+} (Kessler *et al.*, 1978) (which is based on the technique described by Schmitz *et al.*, 1973) were enriched, as judged from the brush-border marker alkaline phosphatase by a factor of about 6. Contamination by lysosomes or mitochondria were not detected. $(Na^+ + K^+)$-dependent ATPase was found to be present in the preparations, although it was not enriched when compared with the homogenate. It was not possible to remove the enzyme from the membranes by repeated washing or by density-gradient centrifugation. It was also present in the pellet that was obtained when the first 10000 ***g*** supernatant (see the Materials and Methods section) was recentrifuged. If it were true that Ca^{2+} aggregates basal-lateral membranes together with all other cell constituents except brush-border vesicles (Schmitz *et al.*, 1973; Kessler *et al.*, 1978), then the latter organelles should possess $(Na^+ + K^+)$-dependent ATPase activity. This is the opinion of Liang & Sacktor (1977), who found that the enzyme was enriched 2-fold in brush-border membranes from bovine kidneys. It was found to be enriched 4-fold in brush borders obtained from rabbit kidney cortices (Quirk & Robinson, 1972). These results seem to contradict the results of Kinne's group, demonstrating that by free-flow electrophoresis rat kidney brush borders were separated completely lacking $(Na^+ + K^+)$-dependent ATPase activity (Shlatz *et al.*, 1975). These workers suggest that the residual $(Na^+ + K^+)$-dependent ATPase could be derived from overlapping contamination with membrane fragments derived from the region of the tight junction where the contraluminal and luminal membranes meet. Species differences have also to be taken into account, however. Thus preparing rat kidney cortex brush borders by the method of precipitation by Ca^{2+} as in the present study, we were also unable to detect the enzyme in the vesicles (results not shown).

The distribution of parathyrin-stimulated adenylate cyclase is similar to that of $(Na^+ + K^+)$-dependent ATPase. It was mainly enriched in the basal-lateral membranes, whereas very small amounts were observed in brush borders. This asymmetry, which was also demonstrated by other groups (Shlatz *et al.*, 1975; Liang & Sacktor, 1977), could be brought about more markedly under the influence of the non-hydrolysable GTP analogue guanosine 5′-[β,γ-imido]triphosphate. GTP and its derivatives are known to stimulate adenylate cyclase by binding to its regulatory subunit (Pfeuffer, 1977). The effects of guanosine 5′-[β,γ-imido]triphosphate and parathyrin (when added together) on cyclic AMP formation were more than additive. Thus the differences in the adenylate cyclase activities between basal-lateral and brush-border membranes were potentiated.

In good agreement with the enrichment of parathyrin-stimulated adenylate cyclase in basal-lateral membranes is the fact that displacement of binding of bovine parathyrin by its bioactive *N*-terminal fragment could only be demonstrated in these organelles. This indicates, together with cyclase stimulation, the presence of parathyrin-specific binding sites on basal-lateral membranes. Up to a hormone concentration of 50ng/ml, the membranes were not saturable (Fig. 3*a*). But the parallel slope of the two binding curves (total and non-displaceable) at parathyrin concentrations above 10ng/ml indicates that mainly unspecific binding of the hormone contributed to this non-saturability at higher concentrations.

Shlatz *et al.* (1975) mentioned that ^{125}I-labelled parathyrin bound to brush-border membranes, but the specificity of this process was not clear. We can confirm that the hormone attaches to brush borders, although to a lesser extent than to basal-lateral membranes. The most striking difference is that the hormone cannot be displaced by the fragment (1–34) parathyrin. Since, however, adenylate cyclase is stimulated by parathyrin in the brush-border preparation (although to a low extent, probably due to contamination by basal-lateral membranes), there is evidence for parathyrin receptor sites. But their number seems to be so low that specific binding is

buried in the 'noise' of non-specific membrane attachment. Why the addition of an excess of fragment (1–34) seems to enhance parathyrin binding to the brush borders is not yet understood. If one rules out any experimental artefact, this would indicate that events other than receptor binding are important when parathyrin interacts with the membranes. The enhancement may reflect competition between intact hormone and fragment (1–34) in metabolic and uptake processes. The amount of parathyrin that binds to inert sites could then be enhanced. The phenomenon requires further investigation.

The metabolic behaviour of both membrane types is remarkable. The basal-lateral membranes generated fragment(s) of a rather uniform size, similar to that of the major bulk of *C*-terminal fragments circulating in the peripheral blood (Silverman & Yalow, 1973). The particulate enzyme(s) involved in this fragment production seem to prefer the structure of the intact hormone as a substrate, because this is rapidly degraded whereas the fragment(s) accumulate. Nevertheless, the proteolytic properties of the basal-lateral membranes are not exclusively related to parathyrin. Recently, Duckworth (1978) reported that plasma membranes from rat kidney cortex [enriched 5-fold in the basal-lateral membrane marker $(Na^+ + K^+)$-dependent ATPase] were very active in degrading insulin and glucagon. Whether the same enzyme(s) that cleave parathyrin are involved is at present unclear.

Rapid disappearance of parathyrin and formation of a number of small fragments was observed when the hormone was exposed to brush borders. This indicates the presence of a general proteolytic activity in the membranes. At 0°C a large amount of the hormone was cleared from the incubation milieu within minutes. The same phenomenon was reported by Duckworth (1978) in the above mentioned study concerning the metabolism of glucagon and insulin by rat kidney cortex plasma membranes; he speculated that this could be due to contamination of the membrane preparation by brush borders. If proteinase(s) were responsible for such clearance of parathyrin, the hormone must have been fragmented into peptides with consequent loss of immunoreactivity. This seems to be unlikely. The phenomenon could be explained on the basis of results obtained by Martin *et al.* (1977) and Hruska *et al.* (1977). In perfusion studies with dog kidneys they found that two mechanisms exist for the renal removal of parathyrin from the circulation. (i) The intact hormone (and its bioactive *N*-terminal fragments) is metabolized at the peritubular level. *C*-Terminal fragments accumulate when the glomerular filtration is blocked. This is in good agreement with our finding that the proteinase(s) located in the basal-lateral membrane prefer the intact hormone as a substrate. (ii) Fragments together with intact hormone undergo glomerular filtration and are removed from the filtrate by proximal-tubular degradation and readsorption. The uptake machinery of isolated brush borders is known to be intact (Schmitz *et al.*, 1973; Kinne *et al.*, 1975; Fuss *et al.*, 1977; Kessler *et al.*, 1978). Thus hormone (and fragment) uptake into the membrane vesicles and not only degradation could have caused the rapid disappearance of parathyrin observed during brush-border exposure. This would again indicate that basal-lateral and brush-border membranes have specific functional properties for parathyrin metabolism and clearance.

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