A COMPARATIVE ENZYME HISTOCHEMICAL STUDY ON THE PARATHYROID GLANDS OF THE SHEEP, PIG, DOG, RABBIT, RAT, HAMSTER, AND CHICKEN

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Parathyroid glands of seven species were studied by enzyme histochemical reactions for NADH-DH, SDH, \( \alpha \)-GPD, \( \beta \)-HBD, ALPase, ACPase, and AChE.

An intense reaction for NADH-DH was found only in the glands from rabbits while the others were strong to weak. A high intensity for \( \alpha \)-GPD was found in the glands from sheep, pigs, and rabbits while those of the others did not show specificity with the enzyme reaction. The parenchymal cells of the glands of sheep and chickens reacted for ALPase, whereas those of the other species did only in the vascular walls. The ACPase reaction of the glands was intense in chickens, moderate in pigs, and weak in other species.

The C-cells of the internal parathyroid glands within thyroid glands of rabbits reacted moderately for AChE, but the external glands of rabbits and those of the other species did negatively. The parathyroid glands of chickens were different from those of the other species in histochemical properties. Histochemically they showed the characteristics of hyperfunction demanded by increased Ca metabolism.

A number of enzyme histochemical studies have been done on the parathyroid gland by many authors. Alkaline phosphatase was demonstrated at the light microscopic level in the capillary walls of the rat and guinea pig, and in the parenchymal cells of the sheep (1, 7–11, 13, 19, 20, 27, 36). Recently, ultrastructural studies on alkaline phosphatase activity have been performed in the parathyroid glands of the rat (29) and sheep (33).

Studies on acid phosphatase activity have been performed on parathyroid glands of humans, several mammals, and newts by light and electron microscopy (15, 21, 28).

In humans, monkeys, rats and guinea pigs, the activities of several oxidative enzymes of parathyroid glands were studied (2, 6, 15, 31, 32). The enzyme activities in other mammals have not yet been studied histochemically.

We attempted to elucidate the differences in enzyme histochemical properties of the parathyroid glands of 6 mammalian species and chicken.

MATERIALS AND METHODS

The number, sex and age of the animals used are shown in Table 1. After
slaughter, the parathyroid glands of the sheep, pigs, dogs, rabbits, rats, hamsters, and chickens were quickly frozen in a mixture of acetone and dry ice. Cryostat sections (8 µm) were mounted on clean slides and dried at room temperature for 10 min. The methods used for the demonstration of NADH dehydrogenase (NADH-DH) was that of Barka-Anderson (3). Sections were incubated for NADH-DH for 40 min at 20°C. The activity of succinate dehydrogenase (SDH) was demonstrated by the method of Nachlas et al. (21). Sections were incubated for 40 min at 37°C. The activities of α-glycerophosphate dehydrogenase (α-GPD) and β-hydroxybutyrate dehydrogenase (β-HBD) were demonstrated by the method of Pearse (25). Sections were incubated for α-GPD for 40 min and for β-HBD for 60 min at 37°C. The activities of non-specific alkaline phosphatase (ALPase) and acid phosphatase (ACPase) were demonstrated by the method of Burstone (4). Sections were incubated for ALPase for 30 min and ACPase for 40 min at 20°C. The activity of acetylcholine esterase (AChE) was demonstrated by the method of Karnovsky (17). Sections were incubated for 60 min at 20°C.

RESULTS

Fig. 1 shows the seven enzyme histochemical activities in the seven species.

NADH-DH: Enzyme activity was found in the parenchymal cells of parathyroid glands. The activity was intense in the rabbit and sheep, strong in the rat, moderate in the pig, hamster, and chicken, and low in the dog (Figs. 1, 2a–g).

SDH: The enzyme activity was observed in the parenchymal cells of all species examined. It was moderate in the rabbit and low in the other species (Fig. 1).

α-GPD: The enzyme activity in the parenchymal cells was intense in the pig, rabbit and hamster, strong in the sheep, moderate in the dog, and weak in the rat and chicken (Figs. 1, 3a–g).

β-HBD: The enzyme activity was moderate in the pig and hamster, but negative in the other species (Figs. 1, 4a–g).

ALPase: In the chicken, strong enzyme activity was observed in parenchymal cells, whereas it was low in the capillary walls and the connective tissue. In the sheep, the activity in the parenchymal cells was variable, but negative in the capillary walls. In the dog and rat, the activity was positive both in the walls of arteriole and capillary. In the pig and hamster, activity was observed only in the walls of
the arteriole, but negative in the capillary walls and in the parenchymal cells (Figs. 1, 5a–g).

**ALPase**: Enzyme activity was strong in the chicken, moderate in the pig, and low in the other species (Fig. 1).

**AChE**: Enzyme activity was observed in the C-cells in the internal parathyroid glands of the rabbit. It was not observed in those of the other species and in the external parathyroid glands of the rabbit (Fig. 6a–b). In the sheep, pig, rat, dog, and hamster, the enzyme activity was limited to the nerve fibers.

**DISCUSSION**

In humans and monkeys, the parathyroid glands include chief cells and oxyphil cells (26). The latter shows intense oxidative enzyme activities in contrast with the weaker ones in the former (2, 15, 31). In this study, oxyphil cells were not found in the parathyroid glands in seven different species. The enzyme activities were confined to the chief cells in those glands.

In the rabbit parathyroid gland, NADH-DH and SDH showed intense activity. This suggests that the chief cells in the glands of the rabbit may include many mitochondria in the cytoplasm. It has not been reported that the chief cells in the glands of rabbit have more mitochondria in number than other species (14, 18, 30). The discrepancy between this study and the previous ones may be due to the difference of age in the rabbits used; the former was at 6–7 months of age and the latter was at 1–2 years of age.

α-GPD is a mitochondrial-bound flavoprotein enzyme and the other half of the α-glycerophosphate shuttle. The parathyroid glands of the sheep, pig, dog, rabbit and hamster had strong to moderate activity, whereas in the chicken and rat was
Fig. 2a–g. NADH-DH activity in seven different species. (a) sheep, (b) pig, (c) dog, (d) rabbit, (e) rat, (f) hamster, (g) chicken. The enzyme activity is intense in the rabbit and sheep, strong in the rat, moderate in the pig, hamster and chicken, and low in the dog. $\times 100$. 
Fig. 3a–g.  α-GPD activity in seven different species. (a) sheep, (b) pig, (c) dog, (d) rabbit, (e) rat, (f) hamster, (g) chicken. The enzyme activity is intense in the pig, rabbit and hamster, strong in the sheep, moderate in the dog, and very weak in the rat and chicken. ×100.
Fig. 4a–g. β-HBD activity in seven different species. (a) sheep, (b) pig, (c) dog, (d) rabbit, (e) rat, (f) hamster, (g) chicken. The enzyme activity is moderate in the pig and hamster, but negative in the other species. ×100.
Fig. 5a–g. ALPase activity in seven different species. (a) sheep, (b) pig, (c) dog, (d) rabbit, (e) rat, (f) hamster, (g) chicken, (T) thymus, (P) parathyroid gland. Arrows and arrow heads indicate arterioles and capillaries. In the chicken and sheep, the enzyme activity is observed in the parenchymal cells. In the other species, the reaction is observed in blood vessels. $\times 100$. 
weak. These findings indicate that the parathyroid glands of 5 species can utilize the α-glycerophosphate shuttle for oxidation of cytoplasmic NADH, but those of the chicken and rat cannot. The parathyroid glands of the chicken and rat may utilize the malate shuttle for oxidation of cytoplasmic NADH.

β-HBD reacted strongly in oxyphil cells of humans and monkeys, moderate to very weak in the normal chief cells of the human and monkey (2, 31, 32). In this study, moderate β-HBD activity was observed in the parathyroid glands of the pig and hamster, but was not in those of the other 5 species. This indicates that keton bodies are highly available in the parathyroid glands of the pig, hamster, and human, but not so in those of the sheep, dog, rabbit, rat and chicken.

Several workers reported that by light microscopy the activity of ALPase was observed in the capillary walls of the parathyroid glands of normal rat (7, 19, 36). Gendec and Danowski observed a decrease of ALPase activity in the endothelial cells of capillaries in an acute anaphylactic shock in the guinea pig (11).

We showed, by electron microscopy, that the activities of the ALPase and adenylate cyclase were only observed in the cell membranes at the intercellular spaces. These spaces play a role not only in exocytosis of hormone secretion, but also in the active transport between external and inner cell membranes in sheep parathyroid gland (33, 34). Setoguti et al. showed that the ALPase activity was found at the capillary walls and parenchymal cells in rat parathyroid gland (29). In the parenchymal cells, it was demonstrated strongly both at the cell membranes facing the pericapillary spaces and at their transitional portions to the lateral membranes and weakly at the lateral membrane. In this study, the chicken ALPase activity was the most intense among the species tested. It was intense in the parenchymal cells, but weak in the capillary walls. These findings suggest that the difference of the localities in ALPase activity is based on the difference of species and may be dependent on the difference of the places of active transport and or hormone secretion.

ACPase activity was demonstrated in the parenchymal cells of parathyroid glands of mouse, rat, rabbit, hamster and pig (24, 28, 29). Setoguti et al. showed that the ACPase activity in the rat parathyroid gland was observed in the Golgi
lamellae and lysosomes (29). They suggested that the ACPase activity was involved in the secretory mechanism or the control mechanism of this gland, or in both. Several workers speculated that the infrequent occurrence of mature secretory granules in chickens was due to the requirement for a high secretory rate of parathyroid hormone to support high rates of bone remodeling (12, 22, 23, 35).

In this study, the ACPase activity in the chicken was the highest among the species used. However, further experimental data may be needed to elucidate whether the ACPase activity is related to the secretory mechanism and/or the control mechanism of this gland.

ACHe positive C-cells were observed in the internal parathyroid glands of the rabbit in this study. The C-cells of external parathyroid glands of the other species were negative to the ACHE reaction. Carvalheira and Pearse found that cholinesterase of the C-cells is classified as a non-specific, or butyryl-cholinesterase in most mammals, except in the case of rabbit thyroid gland and the internal parathyroid gland (5).

Dumont reported that, in rabbit thyroid gland, C-cells are especially associated with the central region and are concentrated in the region close to the internal parathyroid gland (8). Kameda reported that the C-cells were frequently found around the internal parathyroid gland (parathyroid IV) or included within the thyroid gland, but not around the external gland (parathyroid III) (16). These findings suggest that the internal parathyroid gland is adjacent to the central region of the thyroid gland in the rabbit.

In conclusion, the enzyme activities of the parathyroid glands observed in this study showed a wide spectrum. On these various specificity, other explanations are needed.

REFERENCES


