tempting to suggest that the interaction between LHRH and estradiol reported in this paper does play a role in the regulation of the secretion of LH and FSH by the hypothalamus-pituitary system during the ovulatory cycle.

If indeed the estrogen-induced increase of the pituitary LHRH-responsiveness, which occurs during the ovulatory cycle [11] can only occur in the absence of significant concentrations of LHRH in the portal vessel blood, then adequate suppression of the hypothalamic LHRH secretion – by estrogen – is a prerequisite for normal gonadal function. This would imply that LH and FSH are only secreted in aphysiological manner if the effects of estradiol on the hypothalamus and on the pituitary gland are well-tuned to each other, and that any perturbation in the negative feedback of estradiol on the secretion of LHRH by the hypothalamus will also affect the effect of estradiol on the pituitary gland.

In summary, this study shows that 1) LHRH desensitizes the pituitary gland for its own action and that this effect of LHRH is dose-dependent; 2) estradiol potentiates this desensitizing effect of LHRH; 3) estradiol sensitizes the pituitary gland for the LH/FSH-releasing activity of LHRH; 4) LHRH suppresses the sensitizing effect of estradiol.

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## Preliminary observations on the co-existence of regulatory peptides in cells of the baboon endocrine pancreas

S. A. Wolfe-Coote[a], J. Louw[a] and D. F. du Toit[b]

[a] *Research Institute for Medical Biophysics, South African Medical Research Council, P.O. Box 70, Tygerberg 7505 (Republic of South Africa), and* [b] *Department of Surgery, Medical School, University of Stellenbosch, P.O. Box 63, Tygerberg 7505 (Republic of South Africa)*
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*Summary.* Immunocytochemical procedures at ultrastructural and light microscopy level revealed, in the Chacma baboon endocrine pancreas, cells which were immunoreactive for glucagon and pancreatic polypeptide (PP). Some D cells were observed to contain secretory granules with both the appearance and immunoreactivity of A cell secretory granules.
*Key words.* Dual immunoreactivity; baboon; endocrine pancreas; immunolabelling; glucagon; pancreatic polypeptide; somatostatin.

It was proposed over fifty years ago that D cells might represent modified A cells [1]. The advent of ultrastructural studies revealed an intermediate cell type, containing both A and D type secretory granules but with the development of immunolabelling procedures, there was no further mention of intermediate cell types.

More recently, cells containing both glucagon and PP were localized in the pancreas of the frog [2], rat [3, 4] and mouse [5] as well as in human colorectal mucosa [6] and in cat intestine [7]. In the adult primate pancreas they have only been shown to occur separately in distinct cell types.

*Materials and methods.* Pancreas biopsies from 10 Chacma baboons used for transplantation studies were processed as previously described [8, 9]. Semithin sections on slides were labelled, using the Avidin-Biotin technique, in a particular sequence so that any dual immunoreactivity (DIR) could be observed in adjacent sections of the same cell layer. The sequence of primary antisera was as follows: anti-PYY; anti-PP; PP absorbed antiglucagon; glucagon absorbed antisomatostatin and somatostatin absorbed antiglucagon. Antisera absorbed with their homologous antigens and method controls were applied on subsequent slides. The antisera absorbed with heterologous antigens were used to eliminate labelling due to cross reactivity. Antisera were absorbed with homologous and heterologous antigens by incubating antisera for 24 h at 4 °C with 10 nmol of antigen per ml of antiserum diluted optimally for ultrastructural localization or 30 nmol per ml of antiserum diluted for localization at light microscopy. Areas found to contain cells displaying DIR were trimmed and gold sections cut and picked up on gold or nickel grids for immunolabelling with colloidal gold for electron microscopy using higher dilutions of the same antisera [9].

One of the baboons was found to be pregnant and the foetal pancreas was included in the study.

*Results.* Cells which displayed DIR for glucagon and PP (fig. 1 a, b) and for glucagon and somatostatin (fig. 2 a, b) were observed in both the adult and foetal baboon endocrine pancreas.

Most of the areas of endocrine cells seen in the foetal baboon pancreas contained cells which were immunoreactive for glucagon and PP. In the adult baboon a maximum of 10% of islets in an LM section contained such cells which appeared to be confined to a small number of islets in which they then occurred quite commonly (fig. 1). Ultrastructural labelling revealed that the degree of overlap of glucagon and PP immunoreactivity appears to vary from just one or two granules labelling for PP in a cell with the morphological appearance of an A cell (fig. 3) to a more even content of glucagon and PP. Preliminary evidence suggests that electron dense A-type granules, immunoreactive for glucagon, appear to co-exist with less electron dense granules im-

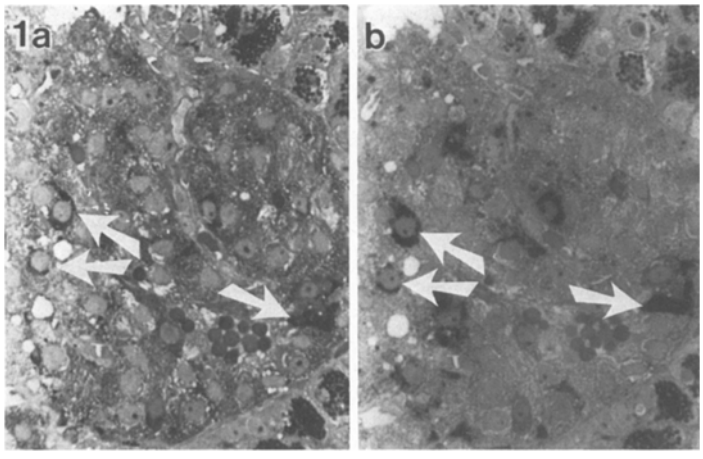


Figure 1. Adjacent sections of adult baboon endocrine pancreas cells immunolabelled using the Avidin-Biotin technique for PP (*a*) and glucagon (*b*). Arrows mark cells displaying dual immunoreactivity. × 384.

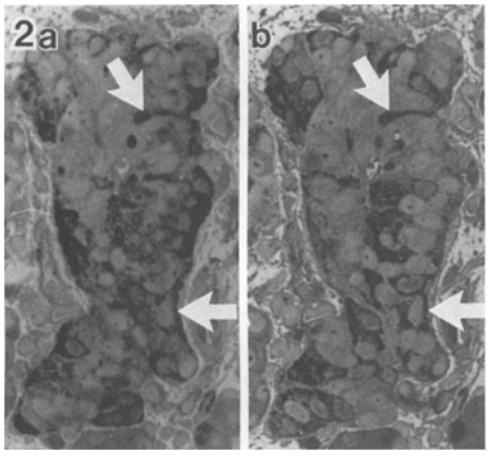


Figure 2. Adjacent sections of foetal baboon endocrine pancreas cells immunolabelled as in figure 1 for somatostatin (*a*) and glucagon (*b*) using antiserum to glucagon absorbed with somatostatin and antiserum to somatostatin absorbed with glucagon. Arrows mark cells displaying dual immunoreactivity. × 384.

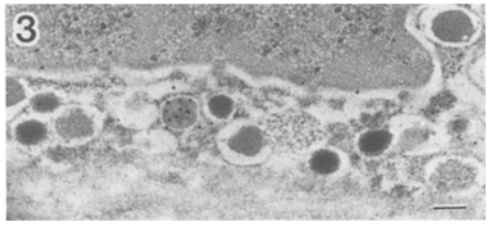


Figure 3. Electron micrograph of an A cell of the adult baboon endocrine pancreas labelled ultrastructurally for PP using colloidal gold. Only one secretory granule in the field of view is labelled for PP. Bar = 200 nm.

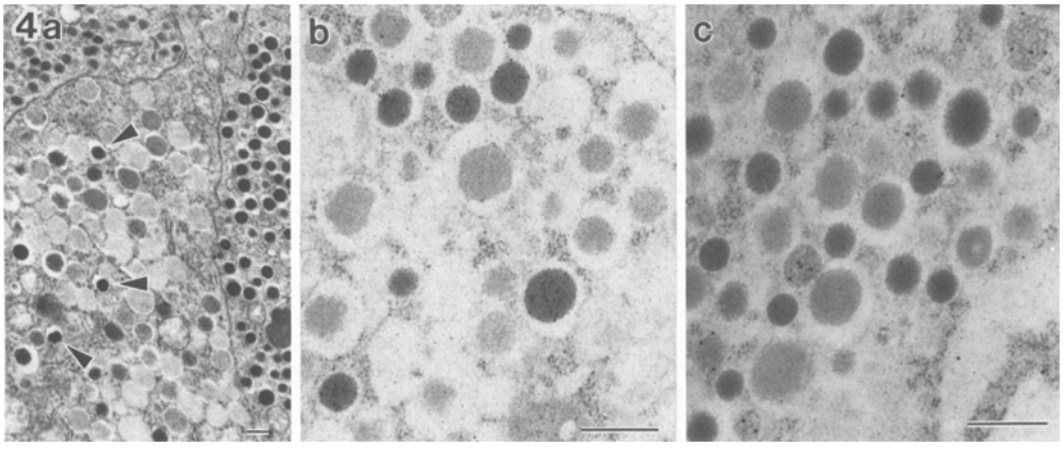


Figure 4. D cells in adult baboon endocrine pancreas illustrating dark staining A type granules in the D cell (*a*) which immunolabel positively for glucagon (*b*) with some of the less electron dense granules immunoreactive for somatostatin (*c*). Bar = 400 nm.

munoreactive for PP. Since secretory granules are active in the proteolytic processing of the peptide and precursor it is possible that the proportions of co-existing peptides will vary so that one peptide may occur at times to the exclusion of the other. Ultrastructural characterization of these cells is in progress.

Cells which were immunoreactive for glucagon and somatostatin occurred to a similar extent in foetal and adult tissue. An ultrastructural study on the baboon endocrine pancreas has revealed that D cells contain a few A type granules amongst their normal secretory granules[9]. These granules have been found to be immunoreactive for glucagon (fig. 4). In foetal tissue PYY and PP occurred mainly in separate populations of cells with only an occasional overlap of activity.

Absorption of antisera with their respective homologous antigens prevented immunolabelling and all method controls were negative.

*Discussion.* The antiserum to PP was specifically raised against the C terminal hexapeptide and was shown, using immunoblotting and absorption studies, not to cross react with peptide YY (PYY), NPY or any known gastrointestinal hormone. Using this specific antiserum together with the absorbed antisera it was possible to view the results with a degree of confidence in their specificity. A slight cross reactivity between antiserum to glucagon and somatostatin antigen and between antiserum to somatostatin and glucagon antigen had been indicated in immunoblotting tests. Absorption of these antisera with their heterologous antigens eliminated this problem.

A possible simultaneous occurrence of multiple messengers in certain cell systems has been suggested and disputed for some years[10]. The co-existence of regulatory peptides can either be anticipated, in that it reflects the proteolysis of a known percursor, or it can be unpredictable. The co-existence of PYY and glucagon would be an example of truly unrelated peptides. It has been suggested that PYY and PP share antigenic determinants and that this might explain the reports of the co-existence of glucagon and PP. PYY has been found to co-exist in the large majority, if not all, of glucagon/glicentin containing cells in the mammalian colon[11] and in foetal cells from the pancreas and gut of many species from fish to primate[12]. The antiserum to PP used in our study does not cross react, however, with PYY. Our finding of cells in which glucagon and PP co-exist in both adult and foetus could be yet another case of co-existence of truly unrelated peptides and to our knowledge is the first report of any such cells in the adult primate pancreas.

It is tempting to join Kaung[2–4] in his speculation that at some time in vertebrate evolution, the two peptides were handled simultaneously in their production and that these glucagon-PP cells represent a primitive cell type still existing in small numbers in the rat, the mouse and in the baboon. Further investigation may well reveal their presence in other species. The detection in some D cells of secretory granules, which have the appearance of and are immunoreactive for glucagon, is interesting in that D cells have been described in the past as modified A cells.

Before a definitive identification of cells producing multiple peptide hormones is possible a demonstration of their synthesis is essential[10]. In situ hybridization is virtually the only way that this can be done in such a mixed population of cells and results from the application of this technique will be presented in the near future.

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## Differences in the stimulation by calcium ionophore of juvenile hormone III release from corpora allata of solitarious and gregarious *Locusta migratoria*

J. F. Dale and S. S. Tobe

*Department of Zoology, University of Toronto, Toronto (Ontario, Canada M5S 1A1)*
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*Summary.* Incubation of the calcium ionophore A23187 resulted in an increase in the median rate of juvenile hormone III release by corpora allata (CA) of both gregarious and solitarious adult *Locusta migratoria* females at 3, 5 and 8 days after fledging. At all 3 datapoints, the enhancement of release rates was highly significant for CA from gregarious females but not significant for CA from solitarious females.
*Key words.* Juvenile hormone; corpora allata; locusts; *Locusta*; phase polymorphism; ionophore.

Locust species are characterized by the ability to exist in two phases (solitarious and gregarious) which differ in their biology. In *Locusta migratoria*, the differences in reproductive physiology between the two phases are particularly profound. Solitarious females are more fecund and more fertile[1, 2] and the first wave of oocytes matures much more swiftly in solitarious females[3].

Differences in corpus allatum (CA) activity and juvenile hormone (JH) titre between solitarious and gregarious locusts have been considered to be causative of many of the phenomena associated with phase polymorphism. It has been proposed that the solitarious phase is neotenous and is characterized by a high JH titre[4]. Bioassays of JH titre[5, 6] appeared to confirm this proposal. However, a study using