Mysterious inhibitory cell regulator investigated and found likely to be secretogranin II related.

Supplementary IHC Images

A representative collection of mammalian and *Drosophila* IHC images. See article text for methodology and interpretation.

Figs. 1-30 are based on the use of the rabbit polyclonal anti-EPL001 antiserum ER88. Figs. 31-38 involved the use of the goat polyclonal anti-EPL001 antiserum G530.

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Key

‘micrin’ = The unknown mammalian antigen to the anti-EPL001 antisera in IHC is herein denoted thus by way of shorthand, implying no preconceptions as to chemical identity. (Ref: Hart, JE. The body has a brake: micrin is a postulated new gonadal hormone curbing tissue overgrowth and restricting reproduction. Med Hypotheses. 2014; 83: 775-786.)

NE = Neuroendocrine

CgA = Chromagranin A

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The thesis of the paper is that the observed anti-organotrophic hormonal activity is likely secretogranin II related. Fig. 1 is not evidential in this regard but is provided to apprise colleagues of the form in which the array tissues, human normal and tumour, were supplied. Other tissues were sourced non-commercially.
Figure 1: Slides of human tissue array
Figure 2: Tissues stained with micrin on the normal tissue array
Figure 3: Weakly stained with micrin on the normal tissue array
Figure 4: NE cells stained with micrin in prostate and gastrointestinal tract
Figure 5: Serial sections stained with micrin and CgA

[Image of stained sections with annotations]
Figure 6: Tissues +ve stained with micrin on the normal tissue array
Figure 7: Micrin staining with preabsorption on the human prostate samples

a & b x10

c & d x40

Antibody at 1/200

PA = preabsorption with EPL001 peptide at 0.5 mg/ml
invasive follicular ca

colon adenocarcinoma  esophagus squamous cell ca

pheochromocytoma

colon  esophagus  thyroid  adrenal

Figure 8. Tumor tissues stained for min

normal
Figure 9: Immunostaining micrin and CgA on human radical serial sections prostate
Normal Human Kidney: IHC
(ER88 rabbit polyclonal antibody)

No difference seen between ovary intact and ovariectomised ewes in IHC staining of hypothalamic median eminence. Note: 'Terminal' refers to the antibody being raised to the N-terminus of a purified ovine protein.
Immunohistochemistry for Micrin

- Rabbit antibody used at 1/100 to 1/1000
- DAB visualisation
- No staining with pre-immune serum
- Immunochemically stained cells in sheep median eminence (ME), sheep ovary (corpus luteum), rat ovary (theca and granulosa) and human prostate (basal luminal cells)
Figure 18
Figure 19

Micrin in ME 1/1000 x40
Figure 21
Micrin in Rat Ovary x40

Figure 22
Micrin in sheep ovary 1/1000 x10
Micrin in sheep ovary 1/1000 x40
Figure 26

Micrin in the sheep corpus luteum 1/1000 x40
Human prostate
BPH = Benign prostatic hyperplasia
Pca = Prostatic carcinoma
CKHMW = pan cytokeratin (high molecular weight), a marker of epithelial cells in prostate

Figure 27
Micrin cells stained with ER88 in sheep PVN
Micrin positive theca cells in normal human ovary
Micrin positive cells in mouse placenta
Photomicrograph of rat brain perfused with 4% paraformaldehyde and sectioned coronally at 40 µm. Sections blocked with normal rabbit serum; primary goat antibody diluted at 1:1000; incubation at 4 degrees C for 48 hours. Biotinylated rabbit anti-goat secondary (1:500 for 1 hour) and strep-HRP (1:500 for 1 hour). Colour developed using DAB (15min; Roche). Arrow points to immunopositive neurons located in regions of the hypothalamus. The white bay is the 3rd ventricle (‘3V’). Scale bar 50 µm
Figure 32. Epa co-occurs with a subset of glia cells in the Drosophila ventral cord.

Panel (A) shows a view of the ventral cord at stage 16 on a wild type Drosophila embryo stained with antibodies against endocrine pharmaceutical’s goat antibody (Epa) (in green), Elav (in red), and Repo (in blue). Elav is a neural marker expressed in the nucleus of all neural cells. Repo is expressed in all glia. Epa marks the membrane of a subset of cells expressing the Repo nuclear marker. Panels (B, B1, B2, B3, B4) contain zoomed inserts from (A) showing all channels merge and all individual channels. (B2) shows co-occurring Epa and Repo positive cells (shown in insert). Although to a much less extent (B5) shows Elav and Epa co-occurrence (shown in insert). (C1 - C4) is a digitally re-arranged z-stack to show the coronal view of (A). (D1 - D4) shows a lateral view of (A) after digital re-arrangement. Abbreviations V (ventral), D (dorsal), A/P (anterior/posterior).
Figure 33. Lateral View

(A) shows a lateral view of the ventral cord at stage 16 on a wild type Drosophila embryo stained with antibodies against endocrine pharmaceutical’s goat antibody (Epa) (in green), Elav (in red), and Repo (in blue). (B-B⁵) shows zoomed inserts from (A) with all individual channels merge and all individual channels. Abbreviations V (ventral), D (dorsal), A/P (anterior/posterior).
Figure 34. Epa antibody with respect to Eagle expression.

(A) shows a view of the ventral cord at stage 16 on an eg-kinesin lacZ Drosophila embryo stained with antibodies against endocrine pharmaceutical’s goat antibody (Epa) (in green), Elav (in red), and lacZ (in blue). Eg (eagle) is a gene involved in determination of serotonergic neurons. (B) shows a higher resolution picture from an area in (A). Panels (B-B⁴) contain digital re-slicing of (B) where white dotted lines are placed. (C) shows expression of Epa within the Drosophila brain (marked in red by Elav antibody).

Abbreviations V (ventral), D (dorsal), A/P (anterior/posterior)
Figure 35. Epa antibody with respect to Eve and Hb9 expression

(A) shows a view of the ventral cord at stage 16 on a wild type Drosophila embryo stained with antibodies against endocrine pharmaceutical's goat antibody (Epa) (in green), Eve (in red), and Hb-9 (in blue). Panels (A1-A4) consist of a higher resolution image of (A). (B) shows a lateral view of the ventral cord at stage 16 on a wild type Drosophila. (B1-B4) shows a higher resolution image of (B). (B5-B6) insert display a coronal view of the dotted area to show there is no co-localization of the markers. Abbreviations V (ventral), D (dorsal), A/P (anterior/posterior).
Figure 36. Epa antibody with respect to mef2 expression

(A) shows a view of the ventral cord at stage 16 on a wild type Drosophila embryo stained with antibodies against endocrine pharmaceutical’s goat antibody (Epa) (in green) and Mef2 (in white). Mef2 is a transcription factor important during mesoderm and muscle development. Panels (B) and (D) consist of two different z planes of the brain area (not marked). Abbreviations V (ventral), D (dorsal), A/P (anterior/posterior).
Fig. 37 Different views of the same drosophila embryo brain. EPA in green, elav antibody which marks all neural cells in red and repo antibody marking the glial cells in blue. Again EPA co-localizes with repo. A) is a dorsal view of the brain. B) is a more ventral view. C) Is a digital re-slicing of the same image seen in A and B from the lateral view and D) from the transverse view.
Fig. 38 Control experiment using EPL001