## Augurin: Neuro-endocrine Peptide Increases ACTH, CRF, AVP

Assay specific for long form and C-terminal

# Augurin stimulates the hypothalamo-pituitary-adrenal axis via the release of corticotrophin-releasing factor in rats.

BACKGROUND AND PURPOSE: The functional characterization of secreted peptides can provide the basis for the development of novel therapeutic agents. Augurin is a recently identified secreted peptide of unknown function expressed in multiple endocrine tissues, and in regions of the brain including the hypothalamus. We therefore investigated the effect of hypothalamic injection of augurin on the hypothalamo-pituitary-adrenal (HPA) axis in male Wistar rats. EXPERIMENTAL APPROACH: Augurin was given as a single injection into the third cerebral ventricle (i.c.v.) or into the paraventricular nucleus (iPVN) of the hypothalamus. Circulating hormone levels were then measured by radioimmunoassay. The effect of augurin on the release of hypothalamic neuropeptides was investigated ex vivo using hypothalamic explants. The acute effects of iPVN augurin on behaviour were also assessed. KEY RESULTS: i.c.v. injection of augurin significantly increased plasma ACTH and corticosterone, compared with vehicle-injected controls, but had no effect on other hypothalamo-pituitary axes hormones. Microinjection of lower doses of augurin into the PVN caused a similar increase in plasma ACTH and corticosterone, without significant alteration in behavioural patterns. Incubation of hypothalamic explants with increasing doses of augurin significantly elevated corticotrophin-releasing factor (CRF) and arginine vasopressin release. In vivo, peripheral injection of a CRF(1/2) receptor antagonist prevented the rise in ACTH and corticosterone caused by i.c.v. augurin injection. CONCLUSIONS AND IMPLICA-TIONS: These data suggest that augurin stimulates the release of ACTH via the release of hypothalamic CRF. Pharmacological manipulation of the augurin system may therefore be a novel target for regulation of the HPA axis.



Tadross et al. Br J Pharmacol. 2010 Apr;159(8):1663-71. Epub 2010 Mar 8.



#### Human Augurin Prohormone Schematic and Sequence

#### Alignment of Human, Rat and Mouse Augurin

Alignment Amino acids sequence of rat, mouse, and human Augurin (region)

D4A540	MGTSS&RP&VL&L&GL&LLLLCLGPGDVSGNKLKKMLQKREGPVPSKTNV&VSEHT&KE	60	D4A540_RAT
Q99150	MSTSSARPAVLALAGLALLLLLCLGPDGISGNKLKKHLQKREGPVPSKTNVAVAENTAKE	60	AUGN MOUSE
Q9H1Z8	MAASPARPAVLALTGLALLLLLCWGPGGISGNKLKLMLQKREAPVPTKTKVAVDENKAKE	60	AUGN HUMAN
	*,;*,********;*************************		_
D4A540	FLGGLKRAKRQLWDRTRPEVQQWYQQFLYNGFDEAKFEDDVNYWLNRNQNGHDYYGDYYQ	120	D4A540 RAT
Q99150	FLGGLKRAKFQLWDRTRPEVQQWYQQFLYMGFDEAKFEDDVNYWLNRNRNGHDYYGDYYQ	120	AUGN MOUSE
Q9H1Z8	FLGSLKRQKFQLWDRTRPEVQQWYQQFLYNGFDEAKFEDDITYWLNRDRNGHEYYGDYYQ	120	AUGN HUMAN
	***.*** *******************************		-
D41540	RHYDEDAAIGPRSREGFRHGASVNYDDY 148 D4A540 RAT		
Q99LS0	RHYDEDAAIGPHSRESFRHGASVNYDDY 148 AUGN MOUSE		
Q9H1Z8	RHYDEDSAIGPRSPYGFRHGASVNYDDY 148 AUGN HUMAN		
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### HPLC Analysis of Human Augurin







Human pancreas tissue was stained by rabbit anti-Augurin (71-107) (H) Antiserum (Cat. No.: H-012-22)

Tissue Sample	Human pancreas tissue
Fixative	10% formalin
Embedding	Paraffin
Negative Control	No primary antibody
Pretreatment	N/A
Blocking	3% H2O2, 2% Normal Goat Serum
Primary Antibody	Rabbit Anti-Augurin, Prepro (71-107) (H) Antiserum H-012-22)
Optimal Dilution	1:500, 1 hour at RT
Secondary Antibody	Goat Anti-Rabbit IgG, Biotinylated (1:400), 30 min
Amplification	Streptavidin-HRP (Vector), 1:400, 30 min
Detection System	HRP
Substrate	DAB (Sigma), 3 min
Counterstained	Hematoxylin, 30 sec

Tissue Sample	Mouse Brain Tissue
Fixative	10% formalin
Embedding	Paraffin
Negative Control	No primary antibody
Pretreatment	N/A
Blocking	3% H2O2, 2% Normal Goat Serum
Primary Antibody	Rabbit Anti-Augurin, Prepro (108-132) (H) Antiserum H-012-23)
Optimal Dilution	1:500, 1 hour at RT
Secondary Antibody	Goat Anti-Rabbit IgG, Biotinylated (1:400), 30 min
Amplification	Streptavidin-HRP (Vector), 1:400, 30 min
Detection System	HRP
Substrate	DAB (Sigma), 3 min
Counterstained	Hematoxylin, 30 sec



Mouse brain tissue was stained by Rabbit Anti-Augurin, Prepro (108-132) (Human) Antibody (Cat. No.: H-012-23)



Human pancreas tissue was stained by rabbit anti-Augurin, Prepro (133-148) (H) Antiserum (Cat. No.: H-012-24)

Tissue Sample	Human Pancreas tissue
Fixative	10% formalin
Embedding	Paraffin
Negative Control	No primary antibody
Pretreatment	N/A
Blocking	3% H2O2, 2% Normal Goat Serum
Primary Antibody	Rabbit Anti-Augurin, Prepro (133-148) (H) Antiserum H-012-24)
Optimal Dilution	1:500, 1 hour at RT
Secondary Antibody	Goat Anti-Rabbit IgG, Biotinylated (1:400), 30 min
Amplification	Streptavidin-HRP (Vector), 1:400, 30 min
Detection System	HRP
Substrate	DAB (Sigma), 3 min
Counterstained	Hematoxylin, 30 sec



Peptide	% Cross- Reactivity
Augurin (Human) / Prepro-Augurin (71-148)	100
Prepro-Augurin (133-148)	100
ACTH (Human)	0
ACTH (Rat, Mouse)	0
CRF (Human, Rat, Mouse, Canine, Feline)	0
Vasopressin (AVP) [Arg8] (Human, Rat, Mouse, Ovine)	0

#### **Available Augurin Products**

Catalog No.	Name	Size
012-21	Augurin, Prepro (42-65) (Human)	100 µg
012-22	Augurin, Prepro (71-107) (Human)	100 µg
012-23	Augurin, Prepro (108-132) (Human)	100 µg
012-24	Augurin, Prepro (133-148) (Human)	100 µg
012-25	Augurin (Human)	20 µg
012-25A	Augurin (Human)	100 µg
012-26	Augurin (Human) Short Form	20 µg
012-26A	Augurin (Human) Short Form	100 µg
EK-012-22	Augurin, Prepro (71-107) (Human) - EIA Kit	1 kit
EK-012-23	Augurin, Prepro (108-132) (Human) - EIA Kit	1 kit
G-012-22	Augurin, Prepro (71-107) (Human) - Purified IgG Anti- body	100 µg
G-012-23	Augurin, Prepro (108-132) (Human) - Purified IgG Anti- body	100 µg
G-012-24	Augurin, Prepro (133-148) (Human) - Purified IgG Anti- body	100 µg
H-012-22	Augurin, Prepro (71-107) (Human) - Antibody for Im- munohistochemistry	100 µl
H-012-23	Augurin, Prepro (108-132) (Human) - Antibody for Im- munohistochemistry	100 µl
H-012-24	Augurin, Prepro (133-148) (Human) - Antibody for Im- munohistochemistry	100 µl
RK-012-23	Augurin, Prepro (108-132) (Human) - RIA Kit	1 kit
RK-012-24	Augurin, Prepro (133-148) (Human) - RIA Kit	1 kit
RK-012-25	Augurin (Human) - RIA Kit	1 kit
T-012-25	Augurin (Human) - I-125 Labeled	10 µCi
T-012-26	Augurin (Human) Short Form - I-125 Labeled	10 µCi