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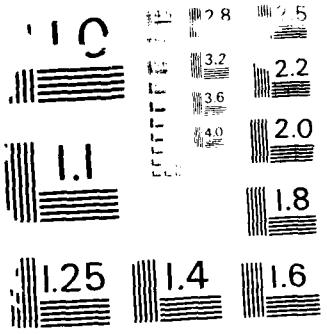

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February 26, 1988

Dr. Michael T. Marron
Scientific Officer, Biotechnology
Department of the Navy
Office of Naval Research
Code 1141 MB
800 North Quincy Street
Arlington, Virginia 22217-5000

Dear Dr. Marron:

A Progress Report for the contract N00014-82-C-0435 is submitted.

JAN. - Dec 1987

Sincerely,

Naftali Primor
Principal Investigator

NP:gh
Encl.

PARDAXIN'S ACTION IN SHARK

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Osborn Laboratories of Marine Sciences

ASSISTED BY Dr. Ychiel, Dr. Jay Fox, Dr. Charles Edwards

WORK UNIT NO. NR CONTRACT N00014-82-C-0435

The objectives of this research were:

OBJECTIVES

1. In Vivo binding study of pardaxin (PX) to gill membranes.
2. Testing for activities of a synthetic pardaxin and N-terminal decapeptide and 26 amino acid peptide. and
3. Permeabilities for monovalent and divalent cations in pardaxin channels in lipid bilayer membranes.

ABSTRACT

1. In experiments in which we tested the effect of proteinase K (PK) and pronase on PX binding and toxicity, the enzyme was first added to the medium for 20 min. Then the fish were washed and ^{3}H-PX with and without unlabelled PX was added to the medium for 30 min. The effect of unlabelled PX on the binding of ^{3}H-PX and toxicity is given in the table below.

	CPM/FISH (mean)	TOXICITY (%)
^{3}H-PX	230	0
^{3}H-PX + PX (25 ug/mL)	212	0
^{3}H-PX + PX (50 ug/mL)	490	30
Treated with PK		
^{3}H-PX	260	0
^{3}H-PX + PX (25 ug/mL)	512	40
Treated with pronase		
^{3}H-PX	230	0
^{3}H-PX + PX (25 ug/mL)	222	0

These results indicate that:

The exposure of fish to PK, but not to pronase, enhanced PX toxicity and binding. Fish treated with PK responded to 25 ug/mL of PX as to 50 ug/ml with no previous exposure to PK.

Upon the addition of unlabelled PX (50 ug/mL) no displacement was observed, indicating that no competitive binding occurred.

2. PX N-terminal decapeptide and the 26 amino acids peptide were synthesized by the solid phase method and were tested for the following activities:

   a. Effect on fish at a concentration of 25 ug/mL PX-33 - causes jumps in a swimming fish. The PX-10 and PX-26 failed to produce such an effect at 400 ug/mL.

   b. Effect on release of neurotransmitter - In frog neuromuscular preparation, at 5-10 ug/mL PX-33 produced an increase in ACH release. The PX-10 and PX-26 were inactive at 200 ug/mL.

   c. Channel formation in liposomes - This effect is produced at nanogram concentrations of PX-33. The PX-10 and PX-26 could not produce channels at 100 times higher concentration.

   d. Aggregation of small liposomes - This is the only effect that the PX-10 and PX-26 peptides elicited, but only at concentrations 10 times greater than that of PX-33.

We concluded that the biological activities of PX-33 are not caused by high hydrophobicity (detergent effect) alone, and that a larger portion then 26 amino acids is needed to retain its activity.

3. We determined the primary sequence of pardaxin:

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                                                    10
NH2 - Gly-Phe-Phe-Ala-Leu-Ile-Pro-Gly-Lys-Ile-Ser-Ser-Pro-Ile-Phe-Lys-
      Thr-Leu-Leu-Ser-Ala-Val-Gly-Ser-Ala-Leu-Ser-Ser-Ser-Gly-Gly-Gln-
      Glu - COOH   20                                              30
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Its composed of two distinct regions: a very hydrophobic region of 26 amino acids and 7 N-terminal hydrophilic acids.

4. PERMEABILITY RATIOS FOR MONOVALENT AND DIVALENT CATIONS IN PARDAXIN CHANNELS IN LIPID BILAYER MEMBRANES

ION	P_I/P_K	RELATIVE HYDRATED SIZE
Rb^+	1.19	0.95
Cs^+	1.15	0.94
NH_4^+	1.03	1.0
Guanidine	1.01	
K^+		1.0
Li^+	0.75	1.90
Methylamine	0.69	
Na^+	0.63	1.46
Dimethylamine	0.45	

	PI/Mg	
Ba++	1.29	0.83
Sr++	1.26	0.88
Mn++	1.18	1.02
Mg++	1.0	1.00
Ethylenediamine	0.99	

LOWEST CONCENTRATION REQUIRED TO INDUCE SINGLE CHANNEL ACTIVITY

PX-33	0.25 ug/ml
PX-26	100 ug/ml
PX-10	no effect at 1750 ug/ml

Published work on the subject:

Renner, P., C. G. Caratsch, P. G. Waser, P. Lazarovici and N. Primor. Presynaptic effects of the pardaxins, polypeptides isolated from the gland secretion of the flatfish Pardachiras marmoratus. Neuroscience 23, 319-325, 1987.

END

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6-1988

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