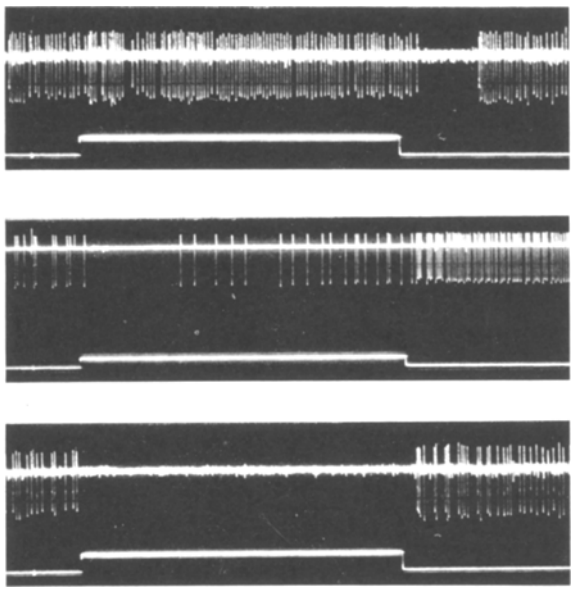
Strychnine-Resistant Inhibition in the Retina

Diffuse illumination of the retina produces transient changes in the maintained activity of retinal ganglion cells and/or fibres of the optic nerve. One type of response consists of a short burst of impulses followed by a silent period. In the other type, illumination produces primary inhibition, which subsides gradually. The responses to the cessation of illumination represent a kind of mirror image of the on-responses, i.e. the same reactions occur in reversed order. As shown by KUFFLER[1], these patterns of activity can be explained by the concentric organization of the receptive field: centre and surroundings act as antagonists. Since the centre responds with a shorter latency, it determines the primary reaction (excitation or inhibition). The latter part of the response is a result of the interaction between the centre and the surroundings. Therefore, it can be interpreted as a balance between post-synaptic excitatory and inhibitory processes[2].



Light responses of three single units in the cat's optic nerve after administration of 0.4 mg/kg strychnine. 1, on-centre unit (dark discharge 89/sec); 2, off-centre unit (dark discharge 46/sec); 3, off-centre unit (dark discharge 58/sec). Duration of light stimuli: 1 sec.

Studying the influence of strychnine on the maintained activity in the optic nerve fibres of the cat, it was necessary to classify the units by light stimulation. The impulses were picked up by microelectrodes from the chiasm[3]. Light stimuli of 3 to 800 nits intensity and 1 to 3 sec duration were used. The maintained activity was drastically changed by intravenous administration of 0.25 to 0.8 mg/kg strychnine hydrochloride, the intervals between the spikes becoming shorter and more regular. However, the typical impulse patterns of on-centre and off-centre units were not changed by strychnine. Surprisingly enough, inhibitory phases in on- and off-effects were preserved or even more marked than in normal preparations (Figure); this has been demonstrated with one exception in 69 units.

The preservation of inhibitory responses in the retina after administration of convulsive doses of strychnine cannot be taken as evidence against the post-synaptic nature of this inhibition. This is proved by the fact that in the brain strychnine-resistant post-synaptic inhibition has been found[4].

Zusammenfassung. Konvulsive Dosen von Strychnin (0,25–0,8 mg/kg) erhöhten die Impulsaktivität von Einzelfasern im N. opticus der Katze; die Hemmungsphasen in den on- und off-Antworten blieben jedoch bei 68 von 69 untersuchten Fasereinheiten erhalten. Die Ergebnisse beweisen die Existenz einer strychninresistenten Hemmung in der Retina.

H. BORNSCHEIN and W. D. HEISS

Institut für Allgemeine und Vergleichende Physiologie und Physiologisches Institut der Universität Wien (Austria), September 18, 1965.

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The Isolation and Amino Acid Sequences of New Pyroglutamylpeptides from Snake Venoms[1]

During the fractionation of the enzymes in the venom of *Agkistrodon halys blomhoffii* (Japanese name 'Mamushi')[2], a non-protein fraction was obtained. From this, two new tripeptides were isolated in pure form. They have tryptophan as a C-terminal, and pyroglutamic acid as an N-terminal amino acid, and they have been identified as follows[3].

$$\text{Pyr-Asp}(NH_2)\text{-Try}, \qquad \text{Pyr-Glu}(NH_2)\text{-Try}$$

Their amino acid sequences were confirmed by chemical synthesis[4].

Isolation procedure and determination of chemical structures of the peptides. A sample of 30.27 g of lyophilized venom of *A. halys blomhoffii* was applied to a DEAE-cellulose column (6.5 · 100 cm). Gradient elution was performed with 0.005 *M* to 0.1 *M* acetate buffer at pH 7.0, as described in the previous paper[2], and the absorbancy of the effluent fractions at 280 nm was measured. A fraction eluted as a third main peak contained low molecular weight peptides and the absorbancy of this fraction at 280 nm was about 10% of that of the unfractionated

[1] Supported in part by a grant from the Ministry of Education (Japan).
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[3] Pyr- = Pyroglutamyl.
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venom. The UV-spectrum of the peptide fraction was quite similar to that of tryptophan. After removing protein contaminants by gel-filtration on a column (7.0 · 3.6 cm) of Sephadex G-25, the eluted peptide fraction was applied to a column (5 · 19 cm) of DEAE Sephadex A-25 (acetate form), and absorbed materials were eluted by 0.5 *M* pyridine acetate buffer at pH 5.0. Final purification was achieved by rechromatography of the partially purified peptide fraction on DEAE cellulose and DEAE Sephadex A-25 and 160 mg dry weight of purified material were obtained.

At the beginning of this study, it was considered that only one peptide, which stained with Ehrlich's reagent, was present in this peptide fraction, for only single spots were seen on paper and thin layer chromatography and paper electrophoresis. Acid hydrolysis of the sample in constant boiling HCl in a sealed tube, in vacuo, yielded aspartic and glutamic acids, ammonia and traces of tryptophan. Sanger's technique failed to reveal a free N-terminal residue, and C-terminal analysis by hydrazinolysis gave only tryptophan. Pyroglutamic acid was identified in partial hydrolysates prepared with *N* NaOH or 0.1 *N* HCl. After digestion with carboxypeptidase A, tryptophan and two fragments, namely pyroglutamylasparagine and pyroglutamylglutamine, were separated from the reaction mixture, and these dipeptides were separated from each other by high voltage electrophoresis at pH 3.5 at 3000 V per 60 cm for 120 min. They were identified by comparison with synthetic samples[5].

The above results suggest that there are two peptides of similar electrophoretic mobility in the peptide fraction. Two components, peptide A and peptide B, were separated from the peptide fraction by high voltage electrophoresis at pH 3.5 at 4000 V per 60 cm for 240 min. Using ninhydrin and microbiological methods, the molar ratio of amino acids in the acid hydrolysate of peptide A was established as L-Try:L-Glu = 1:2 and that of peptide B as L-Try:L-Glu:L-Asp = 1:1:1. Thus the sequence in peptide A was deduced to be Pyro-Glu(NH_2)-Try and that of peptide B to be Pyro-Asp(NH_2)-Try.

Presence of peptide A and peptide B in other snake venoms. The venoms of *Crotalus adamanteus, Bothrops jararaca* and *Trimeresurus flavoviridis* contained peptide A and peptide B, and the venom of *Vipera russelli* contained only peptide B. However, in the venom of *Naja naja atra* neither peptide A nor peptide B was found. The significance of peptide A and peptide B in the salivary gland of poisonous snakes is obscure. It is tempting to speculate that peptide A and peptide B originate from precursors of enzymes or biologically active peptides, which are present in especially high concentrations in *Crotalidae and Viperidae* venoms, during activating processes.

Zusammenfassung. Die Strukturaufklärung von zwei neuen tryptophanhaltigen Peptiden im Schlangengift von *Agkistrodon halys blomhoffii* ergeben: L-Pyroglutamyl-L-Glutaminyl-L-Tryptophan und L-Pyroglutamyl-L-Asparaginyl-L-Tryptophan. Diese Peptide sind in Schlangengiften der Viperidae- und Crotalidae-Arten verbreitet.

H. KATO, S. IWANAGA, and T. SUZUKI

Institute for Protein Research, Osaka University, Osaka (Japan), August 3, 1965.

[5] We wish to thank Dr. T. SHIBA (Faculty of Science, Osaka University, Japan) for supplying synthetic pyroglutamylglutamine. Pyroglutamylasparagine was prepared by the action of carboxypeptidase A on synthetic pyroglutamylasparaginyltryptophan, which was synthesized by Dr. S. SAKAKIBARA in our Institute.

Stimulation of Hemin Synthesis in Ehrlich Ascites Tumor Cells by Mouse Liver RNA

It has been reported that treatment of ascites tumor cells with RNA prepared from mouse, rat and calf livers will induce the synthesis of the liver-specific proteins, serum albumin, tryptophan pyrrolase and glucose-6-phosphatase[1,2]. Ehrlich ascites tumor cells utilize Fe^{59} for heme formation[3] and the purpose of the present investigations was to ascertain if the levels of hemin biosynthesis of Ehrlich ascites tumor cells could be altered by incubation with various types of RNA preparations.

Ribonucleic acid was prepared by cold phenol extraction[4] and RNA concentration determined with orcinol[5]. The incubations were performed by culturing Ehrlich ascites tumor cells at a concentration of 8 · 10^6 in 1 ml aliquots of 57% ascites cell-free fluid obtained by centrifugation at 27,000 *g* and 43% Hanks balanced salt solution buffered at pH 7.4. This medium was supplemented with polyvinylsulfate (10 μg/ml) to prevent RNase action and protamine sulfate (100 μg/ml) to aid entry of the RNA into the cells[6]. Carbon-14 labeled δ-aminolevulinic acid (1 μc/ml) was added as a hemin precursor in order to estimate the synthesis of labeled hemin and $FeSO_4$ (140 μg/ml) was present as a co-factor for hemin synthesis. Preparations of RNA from various tissues and tissue-fractions were added to the experimental cultures usually at concentrations of 1 and 2 mg/ml. These cultures were incubated for 1 h at 6°C and then for 6 h at 37°C in an incubator flushed with 95% air-5% CO_2 under humid conditions. At the conclusion of the incubation period the cells were collected by centrifugation at 1000 *g* and washed five times with 0.9% NaCl and then homogenized. The method of LABBE and NISHIDA[7] was used to isolate hemin; 2.5 mg of crystalline hemin was added as carrier. $1^1/_2$ mg of isolated hemin from each sample were suspended in amyl alcohol and plated on a millipore filter

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