used antischistosomal drugs, niridazole and hycanthone, are mutagens[15–17] and, as found recently, are also carcinogenic in mice[18,19]. Furthermore, an antischistosomal nitrofuran (furapromidium, F-30066), widely used in China for the treatment of patients infected with *S. japonicum*[20], has a mutagenic potency of the same order of magnitude as niridazole[21]. When a host-mediated assay system was used, some mutagenic effects on *Salmonella* strain TA-100 were detectable when these bacteria were injected i.p. 2 h after the administration of a dose of the formulated compound exceeding the curative dose by a factor of 25 (i.e. 250 mg/kg). 6 h thereafter the bacteria were washed out from the peritoneal cavity and plated on a histidine-deficient agar. Since the number of mutant colonies was more than twice as great as that of the controls, a mutagenic metabolite must have been produced in the host. This was confirmed by the finding that, following the oral administration of the formulated compound to mice, one of several urinary metabolites was found to be mutagenic for *Salmonella* strain TA-100; none was mutagenic for strain TA-98. A very marked decrease in this mutagenic urinary metabolite, and of the mutant colonies found in the host-mediated assay, was observed when the bacterial flora of the host's intestines was reduced by the oral administration of succinylsulfathiazole (1 g/kg once daily for 3 successive days), preceding the administration of formulated CGP4540. This finding suggests a role of intestinal bacteria in the formation of a mutagenic compound from CGP4540. Preliminary results indicate that reduction of the intestinal bacterial flora by the administration of succinylsulfathiazole or of a mixture of antibiotics does not eliminate the antischistosomal activity of the isothiocyanate derivative, but more studies are required to determine whether the schistosomicidal activity of this compound can be dissociated completely from its metabolism to a mutagen.

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Effects of Physalaemin, a Vaso-Active Peptide from Amphibian Skin, on the Excitability of an Identifiable Molluscan Giant Neurone of *Achatina fulica* Férussac

H. TAKEUCHI, I. YOKOI and A. MORI

Institute for Neurobiology, Okayama University Medical School, 2-5-1, Shikata-cho, Okayama (Japan), 17 November 1975.

Summary. We examined effects of several vasoactive peptides (substance P, physalaemin, neurotensin, bradykinin, angiotensin etc.) on the excitability of molluscan giant neurones identified in the subesophageal ganglia of *Achatina fulica Férussac.* Of these peptides, only physalaemin showed a remarkable excitatory effect on a giant tonically autoactive neurone.

We attempted to elucidate effects of vaso-active peptides, listed in the Table, on neuronal excitability, using 2 spontaneously firing giant neurones (the TAN, tonically autoactive neurone and the PON, periodically oscillating neurone)[2] identified in the subesophagealg anglia of an African giant snail (*Achatina fulica* Férussac). Of the examined peptides, only physalaemin[3], a hypotensive peptide extracted from the skin of a South-American amphibian (*Physalaemus fuscumaculatus*), showed any effect on TAN excitability. All peptides examined had no effect on PON excitability.

We implanted a microelectrode into the cell body of the identified neurone, recorded its biopotential with a pen-writing galvanometer, and counted the number of its spike discharges per min with a spike counter. We applied the peptides to be examined in 2 ways: a peptide dissolved in the physiological solution[4] was directly applied to the dissected ganglia (bath application); or a microdrop (100 ~ 150 μm diameter) of a peptide solution was formed at the tip of a micropipette containing the solution by oil pressure, and placed just on the surface of the identified neurone (microdrop application)[5].

Vaso-active peptides examined in the present study

No.	Substance	Amino acid sequence
1	Substance P[a]	Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
2	Physalaemin[a]	Pyr-Ala-Asp-Pro-Asn-Lys-Phe-Tyr-Gly-Leu-Met-NH_2
3	Eledoisin-related peptide[a]	Lys-Phe-Ile-Gly-Leu-Met-NH_2
4	Neurotensin[a]	Pyr-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu
5	Xenopsin[b]	Pyr-Gly-Lys-Arg-Pro-Trp-Ile-Leu-OH
6	Bradykinin[a]	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
7	Lys-Bradykinin[a]	Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
8	Met-Lys-bradykinin[a]	Met-Lys-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
9	Angiotensin I[a]	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu
10	Angiotensin II[a]	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
11	Hypertensin[c]	Asn-Arg-Val-Tyr-Val-His-Pro-Phe
12	Angiotensin III[a]	Arg-Val-Tyr-Ile-His-Pro-Phe

[a] Product of Protein Research Foundation, Osaka; [b] donnated by Eisai Co. Ltd.[1]; [c] donnated by Ciba-Geigy Ltd.

Figure 1 shows the experimental results of 3 peptides including physalaemin at 2×10^{-4} g/ml (bath application) with respect to TAN excitability. This neurone was excited by physalaemin in this concentration, and the frequency of its spike discharges increased to more than twice that of the physiological state. During the excitation caused by the bath application of this peptide, no remarkable synaptic influence on the TAN biopotential was observed. The excitatory effect of physalaemin on the TAN disappeared within 1 or 2 min after washing out this substance with the physiological solution. All peptides examined other than physalaemin (at 2×10^{-4} g/ml in bath application) had no effect on the TAN, and no peptide examined, including physalaemin, had any effect on the PON.

Figure 2 shows effects of physalaemin on the TAN biopotential, when this substance was applied locally on the TAN surface (the microdrop application, totally 3.5 ng). About 10 sec after the application, the TAN biopotential showed a slight hyperpolarization, followed by a remarkable depolarization. Several min later, even without washing out the substance, the biopotential returned to the normal state. During the biopotential change caused by locally applied physalaemin, no notable synaptic influence on the biopotential was observed. We conclude from this that this peptide acts directly on the TAN neuromembrane, rather than by way of synaptic influences.

In the case of the microdrop application of this peptide, 2 phases of TAN biopotential change were observed: the initial hyperpolarization and subsequent depolarization. We assume that excitatory and inhibitory re-

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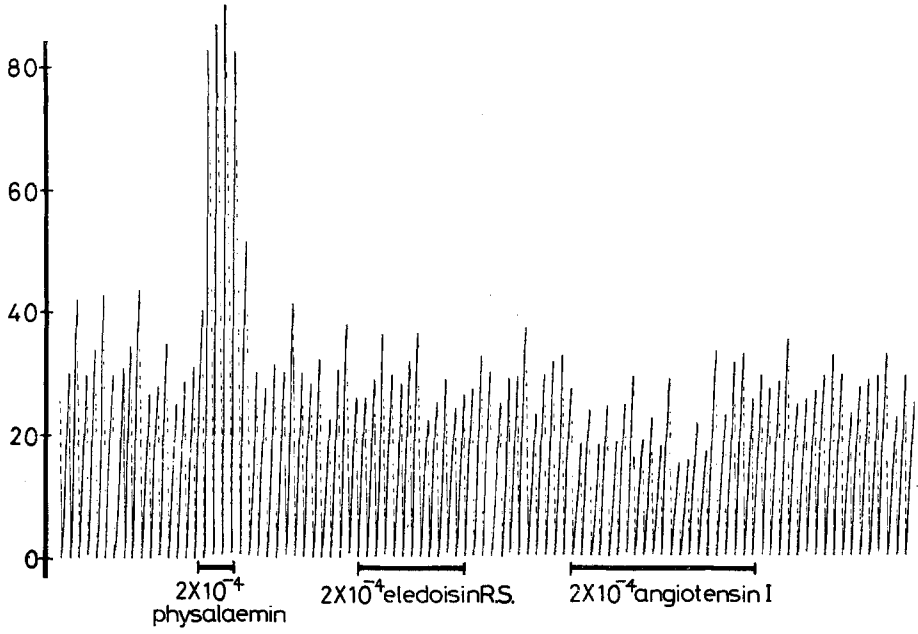

Fig. 1. Effects of some vaso-active peptides on TAN (tonically autoactive neurone of *Achatina fulica*) excitability (bath application). Ordinate, number of spike discharges per min. Abscissa, time course, each histogram is 1 min. We applied physalaemin, eledoisin-related substance and angiotensin I at 2×10^{-4} g/ml. An excitatory effect of physalaemin was observed.

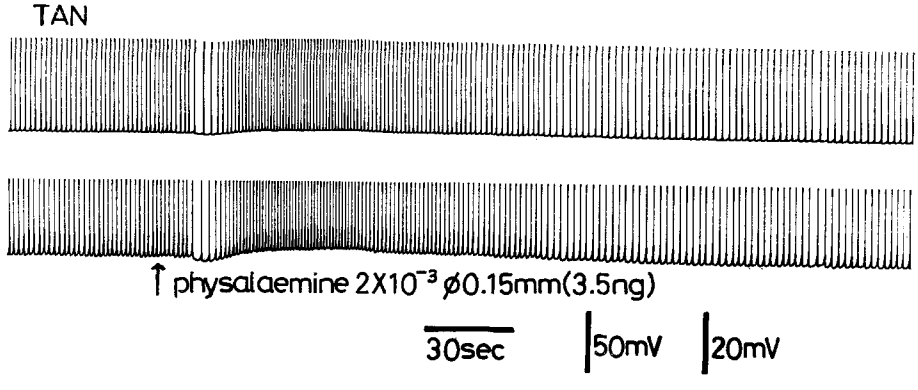

Fig. 2. Effects of physalaemin (microdrop application) on TAN excitability. The upper trace is the fullspike recording by the pen-writing galvanometer. The lower trace is the high amplification recording of the same biopotential of the upper trace (The spike peaks have been cut off by an electronic voltage clipper). A microdrop (150 μm in diameter) of physalaemin solution (at 2×10^{-3} g/ml, totally 3.5 ng) was applied on TAN surface (arrow). Left bar (50 mV) is the amplitude calibration for the upper trace. Right bar (20 mV) is the amplitude calibration for the lower trace. Note that a microdrop of physalaemin produced a slight inhibition followed by a remarkable excitation of the TAN neuromembrane.

ceptors of physalaemin exist on the TAN neuromembrane, and that the majority of the inhibitory receptors is situated more superficially than the excitatory ones. The excitatory effect of physalaemin is clearly predominant to the inhibitory effect. We believe that the excitatory effect masks the inhibitory one, when physalaemin penetrates into the depth of the ganglia and acts on the TAN excitatory receptors. Hence, during bath application of this peptide, we notice only an excitatory effect.

ERSPAMER et al.[3] reported that physalaemin greatly lowered the blood pressure of some mammals when injected i.v. and that this peptide stimulated directly some mammalian smooth muscles (large intestine and ileum), just like eledoisin[6], a peptide extracted from the posterior salivary glands of eledone.

On the central nervous system, KONISHI and OTSUKA[7] reported that a change of the ventral root potential (a mass response of neurones) of the bullfrog spinal cord was similarly caused by the administration of each of the 3 hypotensive peptides, substance P, physalaemin and eledoisin. They assumed that the common C-terminal sequence of these three peptides remarkably excited spinal motoneurones.

We demonstrated in the present study that physalaemin had effects on the excitability of a molluscan giant neurone, the TAN, as well as mammalian smooth muscles or amphibian spinal cord neurones. Of the vaso-active peptides examined, however, the TAN neuromembrane was selectively sensitive to physalaemine, unlike the mass response of the amphibian spinal cord neurones observed by KONISHI and OTSUKA.

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Toxic Substances Produced by *Fusarium*. III. Production and Screening of Phytotoxic Substances of *F. oxysporum* f. sp. *carthami* Responsible for the Wilt Disease of Safflower *Carthamus tinctorius* Linn.

D. K. CHAKRABARTI, K. C. BASU CHAUDHURY and S. GHOSAL[1]

Department of Plant Pathology, Banaras Hindu University, Varanasi-5, (India), and Department of Pharmaceutics, Banaras Hindu University, Varanasi-5 (India), 21 July 1975.

Summary. Fusarium oxysporum f. sp. *carthami*, a causative agent for the wilt disease of safflower (*Carthamus tinctorius* Linn.), has been shown to produce diacetoxyscirpenol, T-2 toxin, fusaric acid and lycomarasmin in artificial media. These substances produced disease syndromes, similar to those seen after the natural infection, when administered in healthy plants. Diacetoxyscirpenol and T-2 toxin have been detected in diseased safflower plants after inoculating with the wilt pathogen. This study is the first demonstration of vivotoxicity of diacetoxyscirpenol.

Safflower (*Carthamus tinctorius* Linn.) is an important oil seed crop cultivated in several States of India. The seeds are edible and are used in culinary purposes; the oil cake is used as a cattle feed. Two new diseases, viz., wilt and dumping off, of safflower, surveyed in the Varanasi and Mirzapur Districts of India, were reported recently[2]. The causative agent for the wilt disease was found here[2] and elsewhere[3] to be *Fusarium oxysporum* f. sp. *carthami*. The nature of substance or substances responsible for the phytotoxic effects has not been evaluated until this investigation. Since food materials infected with fusaria have often been found to contain substances which produce high mammalian toxicity[4], the presence of the title *Fusarium* species in safflower is also important from the public health aspect. The present investigation was designed to isolate and study the phytotoxic substances, produced by the fungus in artificial media and in vivo, and their adverse effects on the host tissues.

The fungus was collected from Varanasi and its identity (IMI-166917) was confirmed by the Commonwealth Mycological Institute, Kew, England. It was grown in Richards solution (200 ml) in still culture flasks (1 l) at 21 °C for 21 days. In a preliminary screening, the effect of the culture filtrate on safflower seedlings was determined. The usual toxic symptoms produced by the natural infection were manifested after administration of the culture filtrate. The phytotoxic substances were extracted from a larger volume of the culture filtrate (5 l) by successive extractions with chloroform (3 l), ethyl acetate (3 l) and *n*-butanol (2 l). The residue from the chloroform extract, containing several trichothecene derivatives[5], produced, in high dilutions, toxic symptoms on safflower seedlings some of which were similar to those showed by the culture filtrate. Clearly, therefore, some more constituents in the culture filtrate are responsible for the total toxic symptoms. The search for these constituents in the ethyl acetate and *n*-butanol extracts resulted in the isolation of fusaric acid and lycomarasmin from these extracts. Additionally, several unidentified nitrogenous components were detected in the latter two extracts. The residue from the EtOAc extract, a pale brown amorphous solid (1.2 g), showed 3 major ninhydrin-positive spots on TLC (silica gel G, E. Merck, thickness, 0.4 mm) and PPC (Whatman No. 1) using *n*-BuOH-AcOH-H_2O (4:1:2) as the developer. The residue was triturated with hot hexane and the hexane-soluble part was filtered. The filtrate was set aside giving straw-coloured crystals (112 mg), m.p. 101–102°; UV: λ_{max} (EtOH) 230 (log ε, 4.02), 272 nm (log ε, 3.64); MS: *m/e* 179 (M^+), significant fragment ion peaks at *m/e* 135, 134, 122. These properties are indistinguishable from those reported for fusaric acid[6]. Processing of the *n*-BuOH extract afforded a brown gum (0.8 g) which also showed a number of prominent and diffused ninhydrin-positive spots on TLC and PPC. The residue

[1] Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India and to whom correspondence should be directed.
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