SYNOPSIS
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Scorpion sting is painful and even fatal according to species, size and susceptibility, quantity of venom injected, virulence of the venom and the age of the person who has been stung. Information, in varying degrees of completeness, is available about biochemical and biological activities of some species of scorpions but very little is known about the biochemical and biological actions of the scorpion Heterometrus bengalensis common in Bengal. This hinders a clear understanding of the nature of the biological activities of H. bengalensis venom essential for organising rational therapy of scorpion sting.

A previous report from this laboratory indicated that H. bengalensis venom produced fall of blood pressure and smooth muscle contraction; these effects were not blocked by atropine, mepyramine and cyproheptadine. Thus the smooth muscle contractile activity was probably due to substance(s) other than acetylcholine, histamine and 5-hydroxytryptamine.

This led the present worker to the study of unknown smooth muscle contractile material in this venom, its separation, characterisation and pharmacodynamics and other constituents of H. bengalensis venom.

Venom was collected by stimulating the telson with square-wave electrical stimuli. Venom was dried over fused calcium chloride and stored at -20°C until used.

Intravenous administration of crude venom produced a temporary fall of rat and guinea-pig blood pressure. The hypotensive effect of crude venom...
could not be blocked by atropine, mepyramine and methysergide. Crude venom (=CV) increased capillary permeability of guinea-pig skin. CV did not degranulate mast cells. Crude venom was found to contain phospholipase-A activity but not cholinesterase. The venom of *Heterometrus bengalensis* produced a contraction in guinea-pig ileum pretreated with atropine, mepyramine and methysergide and appeared to contain substance(s) other than ACh, histamine and 5-HT. Attempts were made to separate smooth muscle contractile material (=SUM) by solvent extraction, gel filtration and thin layer chromatography. SUM could be extracted in descending order of extractability by methanol (71.6% extraction) butanol, ethanol, acetone, ethyl acetate and ether (nil). SUM could be separated from *Heterometrus bengalensis* venom by gel filtration with Sephadex G-25 though not satisfactorily with Sephadex G-10. SUM activity in gel filtrated material could be extracted by lipid extraction procedure with Bligh and Dyer procedure and also by Folch method. When Bligh & Dyer and Folch extracted materials were subjected to thin layer chromatography (=TLC) with suitable solvents single spot was developed whose RF value was 0.65; eluate from this spot contracted isolated guinea-pig ileum. Elution yield of smooth muscle contractile material was greater with Folch procedure than with Bligh and Dyer procedure. Recrystallization of this active material on another TLC solvent system showed three spots with RF values of RF 0.1, RF 0.2 and RF 0.3, eluates from which showed differing degrees of smooth muscle contractile activity.
Active gel filtration eluates could not be shown to contain protein. With purification of smooth muscle contractile material spasmogenicity in terms of inorganic phosphorus content progressively increased, whether Bligh and Dyer or Folch procedure had been incorporated in the purification procedure. Amino nitrogen content was estimated in different fractions of SMCM. Among the TLC-extracted materials (i.e., SL1, SL2 and SL3) amino nitrogen content, in descending order of magnitude, was found in fractions SL2, SL3 and SL1. The presence of phosphorus and amino nitrogen suggested their chemical nature of being a nitrogenous phospholipid which can be developed with iodine vapour, ninhydrin (0.2% in acetone) or with molybdenum reagent. Different purified materials (designated as K1, K2, K3 and K4 in Section 4) were positive with Molisch test but negative with Benedict reagent which suggested the presence of non-reducing sugar. Anthrone reaction was positive in all the separated (K1, K2, K3 and K4) and re-separated fractions (SL1, SL2 and SL3) of smooth muscle contractile material. When hydrolysis was omitted, Anthrone reaction was negative. This suggested the presence of non-reducing sugar moiety which is freed on hydrolysis. SL3 fraction was found to contain highest amount of carbohydrate. However, pending its chemical characterisation this hitherto-unknown smooth muscle contractile material obtained in solvent extraction combined with gel filtration and thin layer chromatography was provisionally designated as Substance L (L for lipid).

Substance L was found to be relatively thermostable. The contractile
activity of Substance L was found to be reduced about 50% after acid boiling and even further (72,66%) with dilute alkali boiling. On incubation with trypsin, chymotrypsin and lipase there was no change in contractile activity.

Distinction from or identification with prostaglandins of Substance L was considered. For this purpose prostaglandin E₁, E₂ and F₂ were taken into account. It can be distinguished from prostaglandin E₁, E₂ and F₂ by the following findings: polymyxin phosphate blocks the contractile actions of prostaglandins E₁, E₂ and F₂ but not of Substance L on guinea-pig ileum and rat fundal strip. With parallel assay on guinea-pig ileum, rabbit jejunum, rat fundal strip and hamster stomach, Substance L had a high discrimination index vis-à-vis PGE₁, E₂ and F₂. TLC mobility of Substance L differed markedly from that of PGE₁, E₂ and F₂. Adsorption chromatography of Substance L showed marked differences in elution profile from prostaglandin E₁, E₂ and F₂.

Substance L could not be separated by Sephadex G-10 Column (15 x 300 mm). The separation by Sephadex G-25 gel filtration suggests a molecular weight between 700 and 5000.

Intravenous administration of Substance L (=SL) produced a considerable but temporary fall in cat and in rat blood pressure which was not tachyphylactic. SL increased cat auricular contraction in situ and did not alter significantly the alteration in blood pressure responses produced by acetylcholine, histamine, 5-HT and bilateral carotid occlusion. The hypotensive response was not blocked by atropine, mepyramine and cyproheptadine.
in cat; in rat use of atropine and methysergide could not be shown to
antagonise SL-induced hypotension. In cat propranolol or spinal transec-
tion did not abolish hypotensive effect of Substance L. It could not be
demonstrated to produce ganglionic blockade on cat nictitating membra
preparation. On isolated toad heart with SL the rate and amplitude was
increased. In isolated guinea-pig heart and guinea-pig auricle, amplitude
of contraction was increased while in guinea-pig heart the rate was slightly
increased. Electrocardiographic examination in rat revealed only brady-
cardia. SL produced vasodilation on rat hind quarter perfusion. It did not
contract rabbit aortic strip, capillary permeability in guinea-pig was
increased.

Substance L increased respiratory rate briefly in rat; with higher
doses apnoea ensued. In guinea-pig, respiratory rate was enhanced. In cats,
except for transient apnoea, there was no change in rate and amplitude.

SL was found to contract a large number of smooth muscle preparations.
As one would expect, the sensitivity of the various preparations vary.
Arranged in ascending order of the (g/ml) concentration (necessary to produce
a contraction, the amplitude of which is 50% of the maximum attainable in
that preparation), the preparations are:

<table>
<thead>
<tr>
<th>Preparation</th>
<th>(g/ml) Concentration</th>
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<tbody>
<tr>
<td>Guinea-pig colon</td>
<td>7.4 x 10^-8</td>
</tr>
<tr>
<td>Guinea-pig ileum</td>
<td>1.3 x 10^-6</td>
</tr>
<tr>
<td>Rat ascending colon</td>
<td>6.3 x 10^-7</td>
</tr>
<tr>
<td>Rat ileum</td>
<td>1.4 x 10^-6</td>
</tr>
<tr>
<td>Rat uterus</td>
<td>1.7 x 10^-6</td>
</tr>
<tr>
<td>Rat fundus</td>
<td>1.2 x 10^-3</td>
</tr>
</tbody>
</table>

Apart from these, moderate-sized contractions can be obtained with hen
rectal caecum (10^-6), chicken rectum (2 x 10^-6), guinea-pig tracheal chain (5 x 10^-6),
rat duodenum (6 x 10^-6), and hamster stomach (2.8 x 10^-5).
Atropine, mepyramine and cyproheptadine did not block SL-induced contraction of ileum, colon and tracheal chain of guinea-pig. Atropine and cyproheptadine did not inhibit SL-induced contraction of ascending colon, ileum, fundal strip and uterus of rat and of chick rectum and hen rectal pouch. It contracted rat duodenum and hamster stomach. Polyphloretin phosphate could not antagonize the contractile activity of Substance L with isolated hamster stomach strip preparation.

No significant release of histamine and 5-hydroxytryptamine could be demonstrated by Substance L with guinea-pig chopped lung and rabbit platelet respectively.

Substance L produced restlessness and itching in mice after i.v. and i.p. injection. The mice appeared to be in severe pain. The effects were very marked for one hour and thereafter it gradually subsided. Intravenous administration of SL increased the spontaneous motility in mice, body temperature or pentobarbitone sleeping time was not significantly altered.

Crude venom was found to possess phospholipase A activity. The phospholipase A activity in crude venom was separated by gel filtration (Sephadex G-50-150). The eluate protein profile showed a peak P with a shoulder S, phospholipase activity being confined to the former. Sephadex G-100 elution yielded similar results, though higher specific activity, which was about 15.13 in tube 11. With polyacrylamide gel electrophoresis of crude venom 5-10 bands were revealed. Out of these, band 5 and 6 exhibited phospholipase activity. Percentage of recovery in polyacrylamide gel was about 68.25%. Between bands 5 & 6, phospholipase enzyme activity was essentially equal. These bands were named P2 and P4 corresponding to the band 5 and 6 respectively (P stands for phospholipase). Molecular weight of phospholipase protein bands P1 and P2.
as assessed in SDS-polyacrylamide gel, was calculated to be about 19,950 and 30,000 respectively. Since the two phospholipase bands have different mobilities on polyacrylamide gel electrophoresis which suggest that they are isoenzymes. The molecular weight of band P1 roughly coincided with the molecular weights of isoenzymes previously reported in *Bothrops newriedii* and *Naja naja* venom and band P2 coincided with the value estimated with the isoenzymes of *C. adamanteus* venom and *Crotalus atrox*.