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0014-4754/91/050457-04$1.50 + 0.20/0

Mastoparan induces hypothermia in mice

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Received 22 May 1990; accepted 13 September 1990

Summary. The polypeptide mastoparan, isolated from the venom of the Oriental Hornet, *Vespa orientalis*, induces hypothermia in white mice 15 minutes after its intraperitoneal injection. The hypothermic effect is induced by mastoparan obtained from different hornet and wasp venoms. The normal murine core temperature is lowered by mastoparan from 38 °C to as far as 33 °C. This lowering lasts for one hour and is reversible.
Key words. Mastoparan; hypothermia; hornet venom.

The Oriental Hornet *Vespa orientalis*, Vespinae, Hymenoptera, is prevalent in the Mediterranean basin as well as in Southeast Asia[1–3]. During the last three decades the contents and pharmacological activities of Oriental Hornet venom sacs have been investigated by several authors[4–10]. The low molecular-weight substances thus far identified in the venom are: 1) volatile compounds, especially ketones, which act as alarm substances[11]; 2) sugars that probably help to fasten the venom droplets to the victim's body[10]; 3) biogenic amines like histamine, 5-hydroxytryptamine, dopamine, adrenaline, noradrenaline or acetylcholine[5,12]; and 4) kinins, which are polypeptides that cause pain and slow contractions of various isolated smooth muscle organs and also raise the vascular permeability[12]. Among the various wasp and hornet kinins isolated to date, one group has lately been identified as mastoparan(s)[13]. Although the various mastoparans present in different hornet and wasp species differ slightly in their amino acid composition, they all induce mast cell degranulation as well as other pharmacological and toxicological effects[13]. From some of the wasp and hornet venoms, several protein components have been fractionated and subsequently identified as enzymes such as hyaluronidase(s), phospholipase(s), trehalase, mono-, di- and polysaccharidases, DNAases, and proteases[7,8,10,12,14–16]. It is an interesting property of hornet venom that shortly after being stung, the victims of a hornet attack usually complain of feeling cold, even if the ambient temperature is as high as 28 °C. The recorded drop in human body temperature in such cases has been of the order of 0.5–1.0 °C. Ishay et al.[17], who studied this hypothermic effect, found that intraperitoneal (i.p.) injection of mice with Oriental Hornet venom sac aqueous extract (VSE) induced in less than an hour a drop in body temperature which, within 3 h, amounted to 8 °C or even 10 °C and was accompanied by a high rate of mortality[17]. Similar results were subsequently obtained in rats, rabbits, cats and dogs following i.p., i.m. or i.v. injection of VSE (Ishay, personal communication). The fall in temperature in all these instances was dose-dependent[17]. However, in mice previously immunized against whole venom, the temperature drop was only by 3 °C to 4 °C and the mice proved to be resistent to $5 \times LD_{50}$ doses of VSE. In the present report we provide evidence that the mastoparan isolated from the venom of *V. orientalis* rapidly reduces body temperature when injected into mice and thus comprises one, if not the only, factor responsible for the observed murine hypothermia. The mode of action whether central or peripheral is discussed.

Materials and methods

Venom of *V. orientalis* was collected from the sting tip by 'milking' the insects. The 'milking' procedure entails extruding the sting with forceps and then applying pressure to the dorsum of the abdomen, whereupon the small drop of venom that appears at the top of the sting is

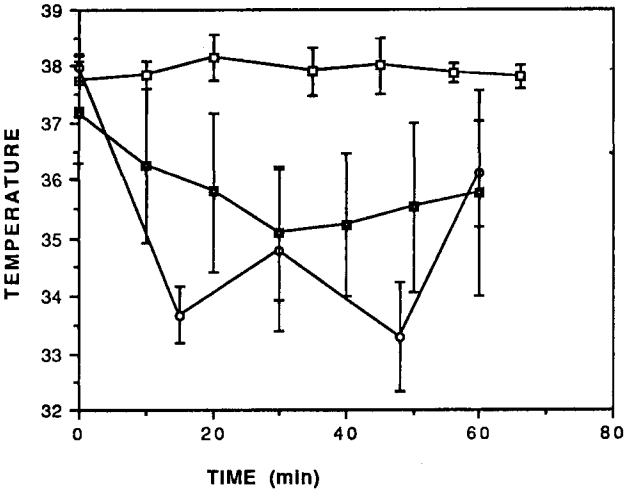
collected with a micropipette. Adult ICR male albino mice (18–22 g) were used. They were housed in plastic cages and received water and food ad libitum in a room kept at 23 °C (± 1). The animals were randomly allocated to groups of six mice each, and placed in new cages for 2 h before the injections. Core temperatures were measured every 10–15 min with a thermistor rectal probe inserted to a depth of about 4 cm.

The protein content of the venom is variable in different groups of hornets. It is approximately 150–180 mg/ml as determined by the method of Lowry et al.[18]. Therefore for electrophoretic analysis the venom was diluted 1:30 in saline, and 100–120 µg protein was loaded per slot. The protein samples were subjected to SDS-PAGE for approximately 3 h at 4 °C. The procedure was according to Laemmli[19] except that the samples were not boiled nor reduced with mercaptoethanol, nor was bromphenol blue added. After the electrophoresis the gel was cut vertically, and only one strip was stained. The unstained gel was cut in accordance with the stained bands, and each band was then chopped into 1-mm^2 pieces and extracted with 2 ml of a solution containing 1 mg/ml bovine serum albumin and 0.1 % Brij 35 (polyoxyethylene 23 Lauryl ether, a non-ionic detergent, Sigma Chemical Co., St. Louis) for 18 h at 4 °C[20]. The extracts were centrifuged for 10 min and the supernatants dialyzed against saline for 72 h at 4 °C in dialysis bags with a MW cut-off at 1000 (Spectrum Medical Industries, Los Angeles). There may have been some loss of peptides, but we have a long experience in using dialysis membranes to retain peptides of this size.

The dialyzed extracts were injected into mice i.p., at a dose of 0.3 ml, in order to assess their hypothermogenic activity. The exact amount of the active principle (mastoparan) could not be assessed due to the small size of the molecule. The remainder of the gel band in which activity was detected was electroblotted onto polyvinyliden difluoride membranes[21] and its amino acid sequence was determined in an Applied Biosystems 477A Protein Sequencer.

Fractionation was also carried out according to Hirai[13]. Venom sacs of 200 hornets were homogenized in 2 ml of 5% trichloroacetic acid, and centrifuged at 7000 r.p.m. for 20 min. The supernatant was chromatographed on a Sephadex G-25 column, and the active principle eluted at 1.5 void volumes of the column was collected, and rechromatographed in a similar manner. CM-cellulose column chromatography was employed for the next step of separation, with linear concentration gradient elution from 0.05 M to 1.0 M ammonium bicarbonate (pH 8.5). The activity emerged when the concentrations of the elution buffer was approximately 0.2 M.

For purposes of comparison we also assessed the hypothermogenic activity of mastoparan prepared from *Vespula lewisii* (Sigma Chemical Co., St. Louis) by dissolving the latter in saline (1 mg/ml) and injecting it in 0.2-ml aliquots i.p. into mice.



The body temperature of mice injected with saline, mastoparan (*V. lewisii* 0.2 mg/mouse) and mastoparan II purified from *V. orientalis*. As can be seen, both mastoparans induced hypothermia, and this effect is reversible. —□—: Saline; —o—: Mastoparan (*V. lewisii*); —■—: Mastoparan II (*V. orientalis*).

Results

The SDS-PAGE fraction of Oriental Hornet venom yielded a pattern of several proteins and polypeptides. One of the fractions caused a rectal temperature drop, of about 3 °C, already 15 min after the injection. The hypothermogenic fraction ran faster on SDS-PAGE than the marker protein of lowest MW (14,000). The amino acid sequence of the active fraction was determined to be: Ile-Asn-Leu-Lys-Ala-Ile-Ala-Ala-Leu-Val-Lys-Lys-Val-Leu-NH_2. This sequence is identical to that of mastoparan II from *V. orientalis*, a 14-amino acid-polypeptide first described by Nasimov[22]. Mastoparan II was then isolated again from *V. orientalis* venom using the technique described by Hirai[13], and it also induced hypothermia in mice when injected in doses of 0.2 mg per mouse (fig.). Moreover, commercial mastoparan from *V. lewisii* was also found to cause hypothermia. It should be of interest to mention that in the low doses injected in mice no sustained toxic effects were observed, and the mice recovered quite easily. No haematuria appeared, and we assume, therefore, that at this dose no haemolysis was induced.

Discussion

The reason that SDS-PAGE was used as an isolation technique was that we suspected that the hypothermia was due to a protein. We were therefore surprised to find that the hypothermogenic activity ran at the very edge of the electrophoresis, but there was enough of the substance involved to show the activity, and it was of enough purity to allow for the successful determination of the primary structure of the peptide. The purification using Hirai's technique was done to confirm our findings, and to obtain larger quantities of the material. Although electrophoretic purification of small peptides is not custom-

ary, it has been used in the past[23]; the technique can be used for peptides with a molecular weight of 2000, which is not far from that of mastoparan (1500). The findings of the present study clearly demonstrate that the tetradecapeptide mastoparan, which is present in hornet venom, exerts a rapid, pronounced and reversible hypothermogenic effect when administered i.p. to mice.

Mastoparan was first isolated and characterized in 1979 by Hirai[13] who found that it caused degranulation in mast cells, much the same as was earlier reported for the fraction in honeybee venom called the mast cell degranulating (MCD) peptide[24]. MCD is known to be composed of 22 amino acids, and both it and mastoparan release histamine from mast cells as part of their various pharmacological activities. There is no sequence homology between the mastoparans from Vespidae and the MCD-peptide from honeybees. In fact, the sequence for mastoparan resembles that of bombinin, a haemolytic peptide isolated from the skin secretions of the European frog *Bombina variegata*[25].

Several biogenic amines have been reported to show thermoregulatory activity mainly when injected intraventricularly or intrahypothalamically[26]. Examples are acetylcholine, noradrenaline, 5-hydroxytryptamine, dopamine, and histamine, all of which occur in Oriental Hornet venom[5, 12]. A group of polypeptides known to induce hypothermia in various animals are the cholecystokinin-like peptides, including cholecystokinin (CCK), an octapeptide, and the decapeptide ceruletide (CER) extracted from vertebrate brain. The s.c. injection into mice of CCK and several analogs of CER dose-dependently produces hypothermia lasting 30–60 min. In this respect the hypothermic activity of these peptides resembles that of mastoparan[27]. But when the amino acid sequences of the two groups of hypothermogenic substances were compared, no homology was found.

When the pattern of hypothermia in mice induced by VSE[16] is compared with that caused by mastoparan, the two are found to be quite different. Mastoparan commences to exert its hypothermic effects in mice 10–15 min after the injection, the effect reaches its maximum effect at 20–30 min and is completely abrogated after some 80 min. On the other hand, VSE lowers the murine temperature only after some 70 min, effects a minimum temperature at about 4 h, and usually causes death. One reason for these differences could be that the more complex VSE, owing to the presence in it of various proteins, penetrates the blood-brain barrier (BBB)[28] and in the process probably introduces not only mastoparan (and venom proteins), but also the biogenic amines always present in hornet venom as well as those released from the mast cells, whereas biogenic amines per se do not usually cross the BBB. On the other hand it was recently proven that neuropeptides, too, do cross the BBB, when injected peripherally[29–31]. We do not know as yet the mechanism by which mastoparan exerts its hypothermic effect, but this is now being investigated.

Acknowledgment. We are very grateful to Dr Ka-Leung Ngai of the Biotechnology Facility, Northwestern University, Evanston, Illinois, for the sequencing of *V. orientalis* mastoparan II.

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32 This paper is part of the Ph.D. thesis by P. Duvdevani to be submitted to the Tel-Aviv University Senate.

0014-4754/91/050460-03$1.50 + 0.20/0